GA – 70th Annual Meeting 2022, Thessaloniki, Greece

28–31 August, 2022

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Planta Med 2022; 88: 1385–1588 |

70th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA)

Date/Location:
August 28–31, 2022
Thessaloniki, Greece

Congress president:
Andreana N. Assimopoulou

Thieme

70th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA) August 28–31, 2022 | Thessaloniki, Greece

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The 70th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA) took place in Thessaloniki, Greece, between August 28–31, 2022 with more than 520 participants.

The main scientific topics of the conference were:

1. Ethnobiology – Ethnobotany – Biodiversity
2. Natural compounds from marine organisms, fungi and microorganisms – Endophytes and microbes (incl. Microbiome)
3. Analysis and authenticity – Quality control – Metabolomics
4. Chemistry and bioactivity of natural products
5. Bioinformatics in natural products Drug Discovery
6. Circular economy – Bioeconomy – Green technologies – Sustainable development of agricultural/industrial by-products
7. Botanical products (Herbal Medicinal Products – Food supplements – Cosmetics – Botanical Medical Devices)
8. Natural products against respiratory infections
9. Animal Health care
10. Biotechnology, bioengineering
11. Formulation – Pharmaceutical technology – Drug delivery systems

The symposium started on Sunday, August 28th 2022, with four pre-congress events in hybrid mode:

- the Young Researchers' Workshop,
- the African Research Symposium on "Traditional Nature-based African Medicine",
- the Workshop of the Botanical Safety Consortium (BSC) on "Development of a Toolkit to Evaluate Botanical Safety",
- a Podium Discussion to Commemorate the Legacy of Prof. Otto Sticher, entitled: "Past, Present, and Future of Pharmacognosy and Natural Product Research – A journey through Prof. Sticher's Career".

The scientific program of the Main Conference included:

- 16 Keynote Lectures
- 78 contributed Short Lectures and
- 402 Posters

The meeting was also an excellent platform for exhibitors and sponsors, who presented their latest products and services, as well as a perfect forum for networking and lively scientific discussions and interaction.

We would like to thank everyone who has made this GA meeting possible. All the participants who honoured us with their presence, and of course, the distinguished speakers for kindly accepting our invitation to deliver their inspiring lectures, the members of the Organizing and Scientific Committees, as well as the volunteers. We are also grateful to the Aristotle University of Thessaloniki for providing its auspices, as well as the sponsors for their generous financial support that contributed significantly to the success of the meeting. Last, but not least, we want to thank the Executive Council and the Advisory Board of the Society for Medicinal Plant and Natural Product Research (GA) for all the support and pleasant collaboration before and during the event, as well as Thieme Publishers for publishing the GA2022 conference abstracts in Planta Medica.

On behalf of the GA2022 Organizing Committee
Assoc. Prof. Andreana Assimopoulou

16th Young Researchers' Workshop (YRW 2022)

Sunday, August 28

IPL-YRW Impulse Lecture "Plant Extracellular Vesicles as innovative tools for plant defence and human health"

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Cells produce extracellular vesicles (EVs) with a prominent role in the intercellular and interkingdom communication. Recent studies have shown that also plants produce EVs likely involved in plant immunity as they transport defence proteins and small RNAs (siRNAs) regulating the gene expression of some phytopathogens [1,2]. Moreover, thanks to their natural ability to cross biological barriers, intrinsic biological activities, EVs are ideal candidates for future applications in nanomedicine [3]. For instance, we have reported the proteome and the metabolome of Citrus-derived nano and microvesicles and demonstrated that they exert selective antitumor activities in different cancer cell lines [4,5]. Also, it has been reported that plant-derived vesicles (PDVs) can be bioengineered, such as for the transport of different drugs. More recently, we set up a biotechnological systems based on hairy roots of officinal plants for the production of EVs with proapoptotic activity in pancreatic and breast cancer cell lines.

In this lecture, the most important biological and physiological roles of PDVs will be discussed together with their potential implications in plant protection. Finally, the use of PDVs and EVs as innovative tools in nutraceutical and pharmaceutical sectors will be examined.

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SL-YRW-01 Short Lecture 1 "Adenosine A1 receptor activation by valerian extract Ze 911 measured via β-arrestin 2 recruitment as well as Gαi activation"

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DOI 10.1055/s-0042-1758882

Adenosine receptors play an important role in sleep regulation, particularly adenosine A1 receptor (A1AR) [1]. The inhibitory A1AR is mainly expressed in the brain, specifically in the cortex, cerebellum and hippocampus [2]. Valerian root extract (*Valeriana officinalis* L.) is a well-established mild sleep-inducing agent. It is known that ingredients of valerian are binding to and activating A1AR, leading to inhibition of cAMP formation in cells [3]. The aim of this study is the investigation of a direct cellular impact of valerian on A1AR mediated Gαi subunit activation and G protein-independent β-arrestin 2 recruitment. It is desirable to clarify whether valerian influences both pathways and if one is preferred over the other. Luciferase-based cellular assays were established in HEK 293 cells stably transfected with two plasmids containing A1AR and β-arrestin 2 or Gαi. Dose-dependent β-arrestin 2 recruitment as well as Gαi activation were measured for the specific A1AR agonist CPA, the non-specific AR agonist adenosine as well as for the valerian extract Ze 911. All investigated A1AR ligands follow the same kinetics and show similar effects. The results indicate that both assays are very well suited to measure the influence of plant extracts on both, G protein-dependent and -independent signaling.

The authors declare no conflicts of interest. This study was supported by a research grant of Zeller AG, Romanshorn, Switzerland.

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SL-YRW-02 Short Lecture 2 "The establishment of an analytical database of algal bromophenols and phytochemical characterization of the red alga *Vertebrata lanosa*"

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DOI 10.1055/s-0042-1758883

Since the first description of algal bromophenols a wide array of structurally diverse brominated natural products has been isolated from green, brown and red algae [1]. As very few of these natural products are commercially available, isolation is almost always necessary for biological activity testing. One of the challenges during targeted isolation is the identification of the desired compounds via LC-MS in crude extracts or fractions, justifying the need for a comprehensive database of all known algal bromophenols and their analytical features (UV absorption maxima, MS fragmentation patterns, theoretical and reported adducts, species occurrence). We therefore established a database encompassing all bromophenols of algal origin published until now, including their analytical properties. Based on this database, a phytochemical analysis of a methanolic extract from *Vertebrata lanosa* (L.) T. A. Christensen (Rhodomelaceae) was conducted, a red alga with popular use in cosmetics [2] or as food [3]. This led to the isolation of 18 compounds, four of which have not been reported for V. lanosa previously and five are new natural products. Most of the isolated compounds were common lanosol derived bromophenols, however, also four novel bromotyrosine derivatives (3-bromo-5-sulfotyrosine, 3,5-dibromotyrosine, 3-bromo-6-lanosyltyrosine, 3-(6-lanosyllanosol)-tyrosine) could be isolated. Further, dibromophenylacetic acid methyl ester was isolated from this alga for the first time. In addition to the bromophenols, four acyl glycerogalactosides including one new compound (eicosapentaenoic acid 3′-[(6′′-O-α-galactosyl-β-D-galactosyl)]-1′-glycerol ester) were obtained.

Overall, the newly established database provided a useful tool to identify new bromophenols in *V. lanosa* and may aid future phytochemical studies in red algae.

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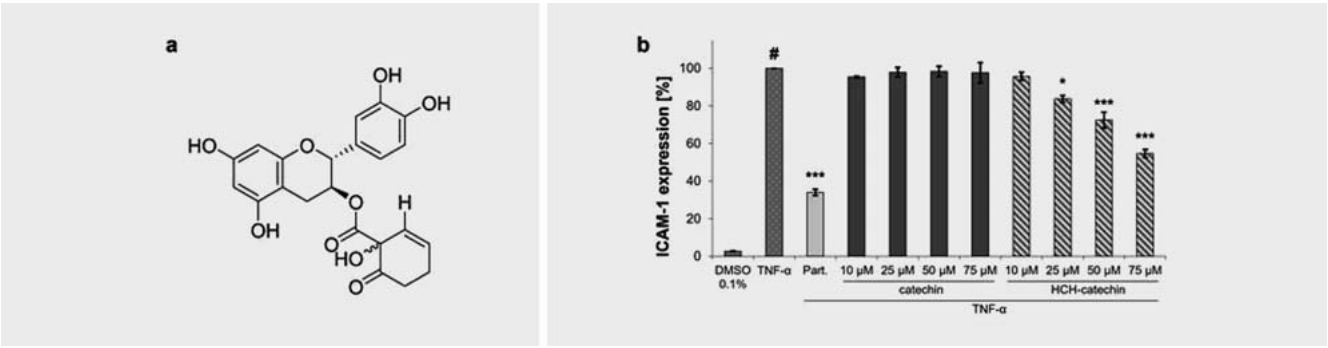

▶ **Fig. 1** (**a**) Structure of catechin-3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester (HCH-catechin). (**b**) Influence of catechin and HCH-catechin on ICAM-1 expression in HMEC-1 cells. (DMSO 0.1 %; untreated control; TNF-α: TNF-α (10 ng/ml) as negative control; Part.: parthenolide (5 µM) + TNF-α as positive control; substance concentrations ranging from 10–75 µM + TNF-α; data presented as mean ± SEM (n = 3); # $p < 0.001$ vs. untreated control; * $p < 0.05$, *** $p < 0.001$ vs. TNF-α).

SL-YRW-03 Short Lecture 3 "HCH-catechin isolated from *Salix cinerea* L. significantly reduces the TNFα-induced ICAM-1 expression in vitro"

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Willow bark is monographed by the ESCOP as herbal drug with analgetic and anti-inflammatory activity [1]. This study was performed to investigate the effect of isolated catechin-3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester (HCH-catechin, Fig. 1a) on intercellular adhesion molecule 1 (ICAM-1) and its contribution to anti-inflammatory effects of willow bark extracts.

LC-, MPLC- and HPLC-fractionation of a methanolic extract from *Salix cinerea* L. led to HCH-catechin which was first isolated from *Salix sieboldiana* [2]. An in vitro-assay was performed to examine the effect of HCH-catechin on expression of ICAM-1 in comparison to catechin in human microvascular endothelial cells (HMEC-1).

HCH-catechin showed significant suppressive effect on ICAM-1, in concentrations from 25 to 75 µM, while catechin had no significant effect in this model (Fig. 1b).

In prior studies, the HCH-moiety containing substance salicortin also showed reduction of ICAM-1 in the same assay due to the formation of catechol from the HCH-moiety [3]. Catechol itself was detected as metabolite in vivo after oral administration of *Salicis cortex* extracts and is already described as anti-inflammatory compound [4, 5].

This could be one step towards a better understanding of willow bark extracts and their therapeutic effect. But in contrast to salicortin, HCH-catechin and other, potentially pharmacologically active, HCH-flavan-3-ols are not quantified with the current method of the European Pharmacopoeia 10 as they are no salicylic alcohol derivatives.

To assess the contribution to overall effects of *Salicis cortex* as anti-inflammatory drug, the occurrence and contents of HCH-moiety containing substances should be focused in future studies.

The authors declare no conflicts of interest.

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SL-YRW-04 Short lecture 4 "An NMR-based metabolite profiling approach: Integration of STOCSY and SHY statistical tools in the identification of honeys' biomarkers"

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DOI 10.1055/s-0042-1758885

NMR-based profiling is a robust approach with high repeatability that is in the foreground regarding authenticity control, quality assessment and biomarker identification in foods. However, the difficulty lies in the identification of relevant compound-markers. This is due to the lack of databases in NMR-based studies, the frequent absence of reference standards and the complexity, high variability and unexpected nature of food matrices. Such a matrix is honey, a high-valued food commodity, which is faced with severe adulteration cases [1]. Following the development of an NMR-based metabolite profiling approach, the goal of this study was to exploit statistical tools in the biomarkers' identification process.

Statistical Total Correlation Spectroscopy (STOCSY) and Statistical Heterospectroscopy (SHY) were applied for the first time in the dereplication process of honey samples. The former generates a pseudospectrum by correlating peaks with the same fluctuation across NMR spectra of the respective samples [2], while the latter can withdraw latent relationships between spectroscopic and spectrometric datasets, in this case NMR and HRMS [3]. Initially, more than 250 Greek honey samples were analysed using both NMR and HRMS approaches. NMR data were then subjected to MVA making possible the discrimination of honey samples with different botanical and geographical origin. The employment of STOCSY and SHY, combined with simpler dereplication methods like literature and 2D spectra, led to the identification of several biomarkers. It seems that both statistical tools could prove valuable in foods' NMR-based profiling.

Conflict of Interest; Funding
The authors declare no conflict of interest; Honey Roads (ID: 2018ΣΕ01 300 000)

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SL-YRW-05 Short Lecture 5 "Anti-atherogenic effects of Rustyback (*Asplenium ceterach* L.) in mice model"

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The fern *Asplenium ceterach* L. (syn. *Ceterach officinarum* Willd.) is used in the traditional medicines for various ailments such as against inflammations, gallstones, as well as for facilitating diuresis [1,2]. In continuation to our previous study on this species [3], we report herein the effects of the traditional *A. ceterach* decoction in an experimental model of atherosclerosis (mice) in order to highlight any beneficial properties for the cardiovascular system. Furthermore, the phytochemical contents of its extracts and decoction were investigated by analytical techniques. The decoction was studied in the experimental model for 8 weeks. 24 adult male mice SV129 wild type and 24 adult male mice PPAR-Alpha knock out SV129 were randomly allocated into three groups: Atheromatosis group (n = 8), Atheromatosis + Rustyback group (n = 8) and Control group (n = 8). Rustyback decoction was given orally as an aqueous solution in a daily dose. Biochemical parameters were determined at baseline, 4 and 8 weeks. Moreover, echocardiography analyses were performed at the baseline and at the endpoint. A histopathological assessment was also carried out at the end of the study. This study is the first report on the investigation of this decoction in an animal model of atheromatosis, examining a potential mode of action through PPARa receptors. Overall, the results demonstrated that the decoction might play a protective role in the progress of the disease. Importantly, this study also confirmed the use of this fern as an anti-inflammatory and diuretic agent in folk medicine.

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SL-YRW-07 Short Lecture 7 "Italian "Vessalico" garlic ecotype: characterization of sulfur compounds and antiviral activity against Tomato brown rugose fruit virus (ToBRFV)"

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Many varieties or cultivars of garlic are available, due to the high environmental adaptative ability of the species, and they can be selected and identified based on morphological, biochemical, and chemical data [1,2]. In this study Vessalico garlic, one of the best-known Italian garlic ecoytpes, was compared with Messidrôme and Messidor, whose cloves are used for sowing, and with the geographically adjacent Caraglio ecotype. UHPLC-Q-trap analyses of the extracts of 52 garlic accessions belonging to these four ecotypes showed similar profiles for sulfur compounds, highlighting the presence of typical molecules of stored garlic. This result was consistent with the usual storage of commercial garlic bulbs at cellar temperature for six-eight months after harvest, to ensure year-round supply for customers. HC, PCA and SOM, applied to the LC/MS data, allowed to separate the four ecotypes, identifying three sub-classes in the accessions of Vessalico ecotype, and confirming similarities between Vessalico and French accessions.
All garlic extracts showed ability to deactivate Tomato brown rugose fruit virus (ToBRFV) and Pepino mosaic virus (PepMV) infectivity, as a possible consequence of disassembly of the virus coat protein (CP). Molecular docking showed a strong interaction of the sulfur compounds characteristic of aged extracts with a high number of residues into ToBRFV CP binding site, interfering with virulence progress. This result could be a good starting point for the possible use of garlic extracts as antiviral agent in organic agriculture, as recovery of waste product or unsold at the season end.

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SL-YRW-09 Short Lecture 9 "Monoesterified analogs of postbiotic metabolites of ellagitannins improve Caco-2 monolayer integrity and increase the bioavailability of urolithin A in vitro"

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Ellagitanin (ET)-rich plants are generally recognized as potent anti-inflammatory agents [1]. The bioavailability of ETs after oral consumption is extremely low; however, thanks to gut microbiota catabolic activity ETs are metabolized into readily absorbed urolithins [2]. Especially urolithin A (UA) exhibits robust anti-inflammatory activity in vitro, yet its beneficial influence on the human organism is strongly limited due to glucuronidation occurring in vivo in the intestinal wall [3]. Therefore, the aim of the study was to synthesize and evaluate the stability of UA derivatives (UADs), monoesterified with non-steroidal anti-inflammatory drugs, which can bypass the glucuronidation process as well as to assess their bioavailability and impact on Caco-2 cells monolayers.

The identity confirmation and stability assays of newly synthesized UADs were conducted using NMR and UHPLC-DAD-MSn methods. Administration of UADs to apical compartments of Caco-2 monolayer resulted in a significantly reduced glucuronidation rate comparing to the administration of UA. Similarly, to UA, UADs increased transepithelial electrical resistance (TEER), indicating improvement of monolayer integrity. Further investigation utilizing Next Generation Sequencing of transcriptome revealed several changes in gene expression between Caco-2 cells exposed to selected UADs compared to control or unmodified UA. These included upregulation of genes participating in phosphatidylinositol signaling, endocytosis, or peroxisomes function.
The presented results highlight the novel strategy for the potential use of synthetic derivatives of postbiotic metabolites as promising health-beneficial agents, overcoming maternal molecule limitations.
Project financially supported by Polish National Science Centre research Grant Preludium Bis No. UMO-2019/35/O/NZ7/00619.

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SL-YRW-10 Short Lecture 10 "Oxone induced oxidation of Oleocanthal and Oleacein tuned to chemoselective conversion to semisynthetic analogues and evaluation of their biological potential"

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DOI 10.1055/s-0042-1758889

The most notable secoiridoids of Olive Oil, Oleocanthal and Oleacein gather intense scientific interest due to their potent biological activities [1]. Recently, their 3-carboxylic forms, named Oleocanthalic and Oleaceinic acids, have been isolated as minor components of OO [2]. This fact has triggered the exploration of various semisynthetic approaches for better understanding of the mechanism of action as well as the discovery of new bioactive compounds. In this context, their semisynthetic preparation was investigated for suitable reaction conditions in order to access these analogues effortlessly. Thus, a reaction using Selenium dioxide and Hydrogen peroxide has been developed to oxidize selectively the aldehyde at position 3 [3]. In a next step, the study of the bis-oxidation of the aldelydic analoques led to the application of Oxone as oxidative reagent. Interestingly, in low oxone consentration the reaction led exclusively to 3-carboxylic analogues while in high oxone concentration, Bayer Villiger mechanism is activated promoting the further oxidation in position 1 and the recovery of corresponding 9-ketonic forms (Oleocanthanonic and Oleaceinonic acids). The reaction mechanism was verified by identifying the intermediate analogues using 1-D and 2-D NMR analysis.
The antioxidant and antigenotoxic properties of the semisynthetic analogues were studied in comparison to initial secoiridoids. The results revealed that Oleocanthanonic acid demonstrated remarkable activity in contrast with the inactive Oleocanthal and Oleocanthalic acid. On the other hand, both Oleacein and its derivatives presented outstanding antioxidant and antigenotoxic activity.
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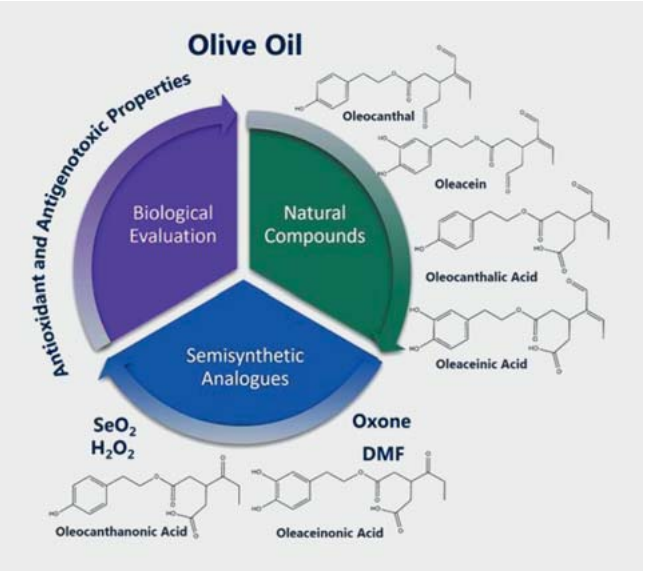

▶ **Fig. 1**

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SL-YRW-11 Short Lecture 11 "Enhancing natural products annotation in dual 13C-NMR and LC-HRMS2 based complex mixtures chemical profiling through custom in silico databases"

Authors Cordonnier J[13], Remy S[1], Kotland A[2], Leroy R[1], Martinez A[1], Borie N[1], Sayagh C[1], Hubert J[2], Aubert D[3], Villena I[3], Nuzillard J-M[1], Renault J-H[1]
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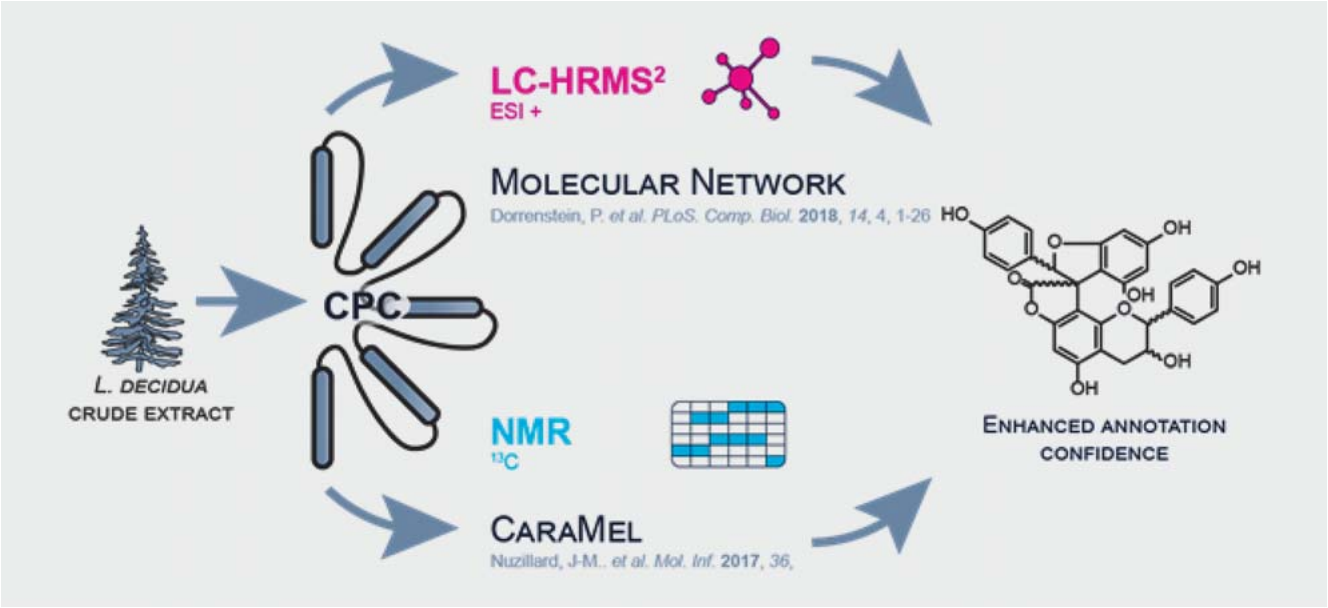
The chemical profiling of plant extract usually involves a dereplication step commonly based on LC-HRMS² or NMR. The high sensitivity of MS provides numerous but sometimes incorrect candidates whereas the low sensitivity and the high universality of NMR lead to less but more accurate annotations. Despite their complementarity, both analytical techniques are rarely used in combination. This study focuses on the chemical profiling of the bark of *Larix decidua* through both LC-HRMS² and NMR data analysis (▶ **Fig. 1**). In a first time AcOEt crude bark extract was fractionated by Centrifugal Partition Chromatography (CPC). In a second time the 12 fractions of decreasing polarity were analyzed both by LC-HRMS² and by ^{13}C NMR, in order to benefit from advantage of both techniques (sensibility, universality resp.). Data were analyzed in parallel workflows. On one hand, pre-treated LC-HRMS² data (MZmine 3) [1], were submitted to the Ion Identity Molecular Network workflow (including NAP and MolNetEnhancer) [2,3] and additionally annotated via SIRIUS4 [4]. On the other hand, ^{13}C NMR data was subjected to the CaraMel workflow [5]. The whole annotation process was realized using in silico spectral database restricted to compound reported in Pinaceae plant family. Databases were generated via an in-house graphical interface based on LOTUS, NMRShiftDB and CFM-ID. Thus, this work shows how the combination of ana-



▶ **Fig. 1** *L. decidua* crude extract chemical profiling workflow.

lytical techniques, and the use of custom database can support chemical profiling of complex mixtures and increase the annotation confidence.

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SL-YRW-12 Short Lecture 12 "Isolation of ecdysteroids from *Cyanotis arachnoidea* using scalable and sustainable centrifugal partition chromatography (CPC)"

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DOI 10.1055/s-0042-1758891

Ecdysteroids are a special group of steroids that play an important role in regulating the molting and development of arthropods. This structurally diverse, non-toxic group of steroids has several beneficial bioactivities in mammals; hence they are widely consumed as dietary supplements. Some derivatives were shown to have cholesterol-lowering [1], antioxidant and neuroprotective [2] effects among others, and a recent in silico study suggests a probable anti-COVID-19 activity of an ecdysteroid (calonysterone) [3].

Our aim was to develop a scalable and cost-effective preparative CPC purification method that can tackle the increasing needs on both consumer and research sides.

After the LC-MS analysis of a commercial *C. arachnoidea* extract, we targeted 20-hydroxyecdysone; (20E) and 5 minor ecdysteroid components for preparation. Afterwards, their partitioning properties were studied in 43 two-phase liquid-liquid chromatographic solvent systems. Then, a laboratory-scale (250 mL rotor volume) and a pilot-scale CPC (2100 mL rotor volume) instrument was used to test 8 selected ternary systems in ascending or descending modes. The optimized method allowed us to achieve a sustainable, economic, and green isolation of 20E on a 10 g scale. Additionally, we performed economic semi-synthesis and purification of the minor ecdysteroid calonysterone.

The developed method is also applicable for the cost-effective and scalable fractionation of several minor natural ecdysteroid other than 20E from a variety of plant starting materials. This is expected to greatly contribute to future R&D activities in the utilization of ecdysteroids.

Acknowledgments
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African Research Pre-Congress Symposium (ARS 2022)

Sunday, August 28

IPL-ARS Impulse Lecture "Development of herbal medicines–example of APIVIRINE, a phytomedicine used for the treatment of COVID-19 in patients without signs of severity"

Authors Ouedraogo D[2], Tarnagda G[1], Ouedraogo JCRP[1], Hema H[2], Tiendrebeogo S[1], Ouedraogo S, Ouedraogo GG[1], Belemnaba L[1], Ouedraogo N[1], Kini FB[1], Tarnagda Z[1], Ouedraogo M[2], Ouedraogo S[1]

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The development of phytomedicines is complex and moves from ethnopharmacological investigations to clinical trials, including preclinical studies and galenic formulations. Since the appearance of the COVID-19 pandemic, several drugs with unsatisfactory therapeutic efficacy have been proposed. Hence the search for solutions based on recipes from traditional medicine to deal with the pandemic. This study aimed to evaluate the clinical safety, efficacy and tolerability of the phytomedicine APIVIRINE in patients with non-severe COVID-19.

Thus, after validated preclinical assessment confirmed quality and tolerance of APIVIRINE, a clinical study included patients followed on an outpatient basis. Vital signs, anthropometric parameters as well as electrocardiographic, hematological and biochemical examinations were measured, and adverse events were recorded.

Clinical signs present at inclusion were mostly cough (44.44%), asthenia (42.22%), headache (40%), and anosmia (35.55%). Dyspnoea and chest pain were less represented in 05 (11.11%) and 06 (13.33%) patients. Cough, dyspnoea, chest pain, sore throat, and nasal obstruction present at inclusion disappeared before treatment day 4. Anosmia and asthenia disappeared before day 7. At the inclusion visit (day 1), CRP, WBC, and blood glucose were abnormal in 15 (33.33%), 13 (28.89%), and 11 (24.44%) patients respectively. In addition, 3 patients (6.66%) had elevated creatinine levels. Transaminases alanine aminotransferase was elevated in 5 patients (11.11%) while aspartate aminotransferase was elevated in 4 patients (8.89%). The cumulative cure rate was 86.67% after 14 days of treatment.

No serious side effects or allergic reactions and no clinical complications were observed during the treatment with APIVIRINE which is in line with the preclinical results.

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SL-ARS-01 Short Lecture 1 "A journey through the compilation of the first South African Herbal Pharmacopoeia"

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DOI 10.1055/s-0042-1758893

According to the World Health Organisation, "A Pharmacopoeia's core mission is to protect public health by making available standards to help ensure the quality of medicines" [1]. There are several known documents containing herbal remedies, such as those that date back to ancient Egypt more than 3000 years ago. However, De Materia Medica dating from the 1st century CE in Greece and Rome is arguably perhaps the first example of a "Pharmacopoeia". Despite the tremendous botanical diversity and widespread use of African Traditional Medicines in South Africa, a compilation of herbal monographs in the form of a Pharmacopoeia acutely focused on the South African flora is lacking. To address this void, we have aimed to collate existing, and generate new data to compile 25 species monographs for botanicals that are currently commercialised or earmarked for commercialisation. In this paper, we discuss the complex workflow required to gain a better understanding of the safety, quality and efficacy of medicinal plants, since these aspects are crucial in monograph development. Several examples will be discussed to illustrate the integration of classic and modern techniques to develop detailed monographs. Ongoing research to document pharmacological activity in an evidence-based ethnopharmacology approach will be presented. Through this project, we aim to provide valuable information for academic research institutions, industrial manufacturers of herbal products, as well as national and international policymakers and regulators, to ensure that products of a desired quality reach the consumer.

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SL-ARS-03 Short Lecture 3 "Anti-biofilm activity of sixteen South African plants against multidrug resistant *E. coli* 0157:H7 associated with outbreaks of diarrhoeal disease"

Authors Lebeloane M[1,2], Famuyide I[1], Adeyemo R[1], Dzoyem JP[3], Kgosana G[2], Elgorashi E[2], McGaw L[1]
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DOI 10.1055/s-0042-1758894

A crucial mechanism of multidrug resistance (MDR) of enterohaemorrhagic *Escherichia coli* 0157:H7 pathogenicity is its ability to form biofilms. The aim of this study was to investigate the antibacterial, anti-biofilm, anti-quorum sensing and anti-motility effects of acetone and methanol extracts of 16 plants against *E. coli* 0157:H7, a major foodborne pathogen. These plants were selected on the basis of preliminary antibacterial activity. Using a broth serial microdilution method, the extracts had minimum inhibitory concentration (MIC) values of 0.90 to 1.25 mg/ml. Interestingly, relatively low cytotoxicity 300<LC50 < 1000 mg/ml for all extracts against Vero cells was reported. The sub-MIC value of 0.195 mg/ml was employed for the biofilm inhibition assays. *Vachellia karroo* and *Salix babylonica* inhibited biofilm formation by approximately 70%, and *V. galpinii* and *V. gerrardii* methanol extracts were effective at eradicating a pre-formed biofilm with 93% and 77% respectively after 24 h. Several plants had good biofilm disruption ability after 48 h incubation. Anti-quorum sensing activity was studied using the biosensor strain *Chromobacterium violaceum. Vachellia gerrardii* acetone and methanol extracts inhibited 89% and 91% of the violacein produced respectively, indicating promising quorum sensing inhibition. Furthermore, *V. gerrardii, V. nilotica* and *V. tortilis* plant extracts showed excellent inhibition of migration of bacteria in semi-solid agar after 72 h compared to untreated bacteria. Finally, a substantial amount of biofilm extracellular polymer substance was reduced by the acetone extracts. This research provides evidence that the selected plants have good activity against biofilms caused by enterohaemorrhagic *E. coli* 0157:H7.

FT-ARS-01 Flash Talk 1 "Quality control of extracts and herbal products containing *Anthocleista nobilis* G. Don. via a validated RP-HPLC method"

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DOI 10.1055/s-0042-1758895

Anthocleista nobilis is a common constituent in numerous conventional medications in West Africa. The stem bark of *A. nobilis* is known to contain brucine and is used to treat intestinal parasites, gonorrhoea, wounds etc [1]. The quality control of herbal material as well as formulations containing this plant is therefore essential due to its extensive use. In this work, some of the ethnomedicinal claims are validated and a validated RP-HPLC method is used to estimate the brucine content of extracts and products containing A. nobilis.

The stem bark of *A. nobilis* was serially extracted with ethyl acetate, ethanol (70%), ethanol (98%) and water by cold maceration. The phytoconstituents were identified using standard methods. Antimicrobial activity against *E. coli, B. subtilis* and *S. typhi* was determined using the broth dilution method [2] with ciprofloxacin as standard. Using brucine as a chemical marker, a validated RP-HPLC method was developed for the assay of brucine in the extracts and herbal medicinal products containing *A. nobilis*.

The common phytochemicals among the various extracts were tannins and alkaloids. The Minimum Inhibitory Concentration of the extracts were be-

tween 2.5–40 mg/mL with the ethyl acetate the most promising extract. The content of brucine in the extracts was 0.0177–0.1259 x10-3 %w/v whereas the herbal products tested had a content of 0.8950–2.5013 x10-3 %w/v. These levels were below the toxicity threshold of brucine. The developed method could be used for the routine quality control of *A. nobilis* extracts and formulations.

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FT-ARS-02 Flash Talk 2 "Antiproliferative activity of *Elephantorrhiza burkei* Benth. and *Cassia abbreviata* Oliv. traditionally used in South Africa, for lung cancer treatment"

Authors Esmear T[1], Twilley D[1], Hlati S[2], Lall N[1,3,4,5]

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DOI 10.1055/s-0042-1758896

In South African traditional medicine, decoctions of *Elephantorrhiza burkei* (EB) and *Cassia abbreviata* (CA) are used to treat lung cancer. This study aimed to validate the traditional use through in vitro investigations. Ethanolic extracts of EB leaves and roots, and CA leaves, seeds and bark, were evaluated for antiproliferative activity against non-small cell lung cancer (A549), small cell lung cancer (SHP-77), human lung fibroblasts (MRC-5) and murine macrophages (RAW 264.7). The EB roots and CA bark extracts showed the highest antiproliferative activity with fifty percent inhibitory concentration (IC_{50}) of 323.35 ± 1.01, 64.61 ± 1.43 and 153.8 ± 2.13 µg/mL (EB roots) and 144.2 ± 2.01, 104.2 ± 1.81, and 115.2 ± 3.4 µg/mL (CA bark), against A549, MRC-5 and RAW 264.7, respectively. The EB and CA leaf extracts showed IC_{50} > 400 µg/mL against each cell lines, however increased the proliferation of RAW 264.7 cells. Selectivity Index (SI) values of the EB root and CA bark extracts, compared to MRC-5 cells, were 0.1 and 0.7 respectively. The combination of CA bark and EB roots (1 : 1) showed IC_{50} values of 81.34 ± 3.21 (MRC-5) and 179.4 ± 3.3 µg/mL (RAW 264.7), respectively, indicating decreased toxicity on non-cancerous cells, and therefore should be investigated against A549 cells. This indicates the potential of EB and CA leaf extracts for potential immuno-modulatory properties and the further investigation of the CA bark: EB roots (1 : 1) combination for its mechanism of action against targets associated with lung cancer. The authors declare no conflict of interest.

FT-ARS-03 Flash Talk 3 "Determination of the mechanistic potential of HF-EtOH through the targeting of factors related to the PI3K/Akt/mTOR cascade"

Authors Maphutha J[1], Twilley D[1], Van de Venter M[2], Venables L[2], Hattingh A[2], Lall N[1,3,4,5]

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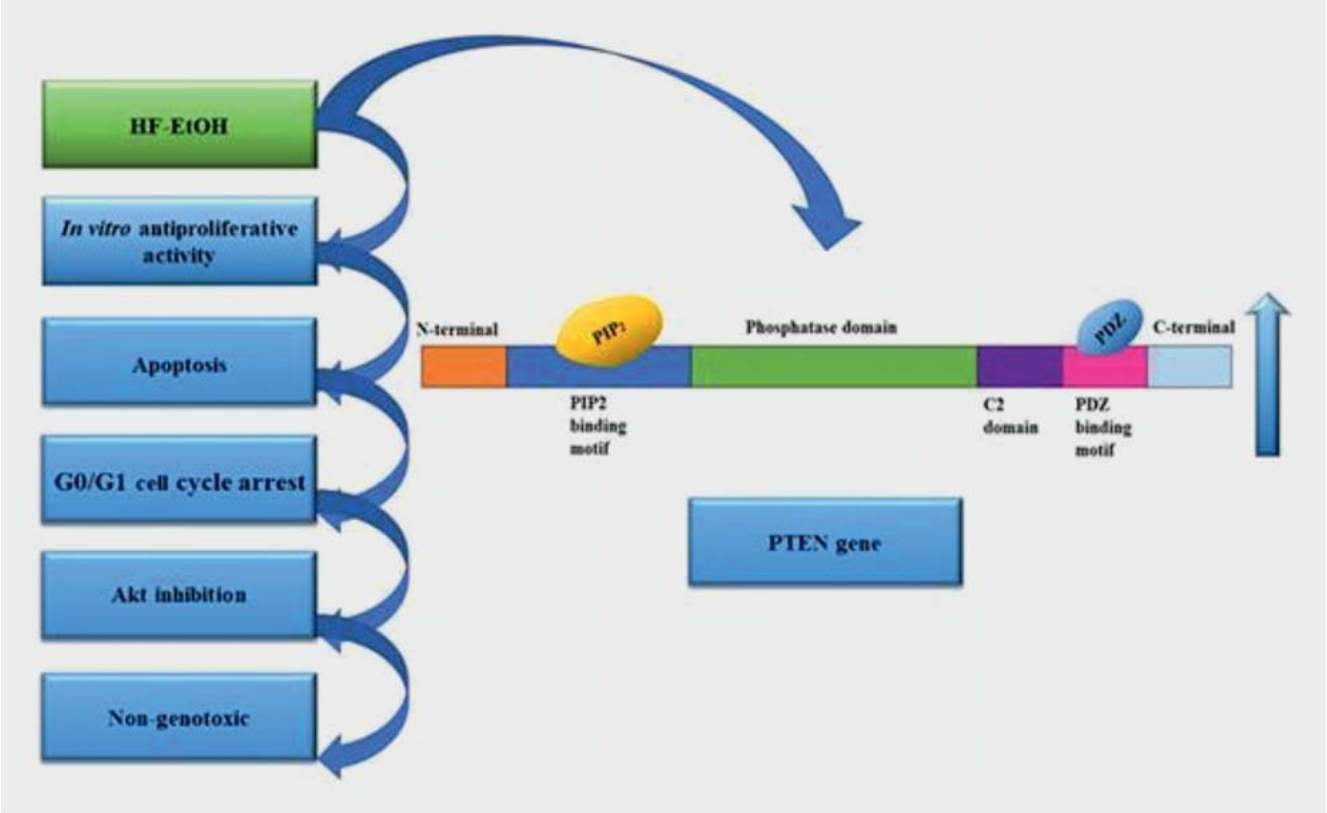
DOI 10.1055/s-0042-1758897

The PI3K/Akt/mTOR cascade is hyperactivated in melanoma which arises due to loss of function mutations in the tumor suppressor, PTEN, which regulates this cascade. The deregulation of the cascade results in the hyperactivation of the precursor protein kinase B (Akt). The aim of this study was to identify the in vitro antiproliferative potential of an ethanolic plant extract (HF-EtOH) against skin cancer and to determine the mechanism of action by targeting various factors related to the PI3K/Akt/mTOR cascade. HF-EtOH, displayed



▶ **Fig. 1**

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high antiproliferative activity against squamous cell carcinoma (A431), human malignant melanoma (RPMI-7951 & A375), and moderate antiproliferative activity against non-tumorigenic kidney cells (Vero) with fifty percent inhibitory concentrations (IC_{50}) of 37.78 ± 4.00, 46.79 ± 2.25, 38.47 ± 0.92 and 68.52 ± 6.00 µg/mL, respectively, resulting in selectivity indexes of 1.81, 1.46 and 1.78, respectively. Mechanistic studies (phosphatidylserine translocation, cell cycle arrest, genotoxicity and Akt modulation) were carried out using RPMI-7951 due to the PTEN-null mutation present in this cell line. HF-EtOH induced apoptotic cell death, G0/G1 and early mitotic cell cycle arrest at 3.10 and 6.25 µg/mL. Furthermore, HF-EtOH did not display genotoxic effects at 0.75 µg/mL on Vero cells. Lastly, HF-EtOH inhibited Akt after four hours at a concentration of 3.00 µg/mL. HF-EtOH upregulated PTEN activity by 18.94 ± 4.56% at 1.50 µg/mL. The collective data highlights the potential use of HF-EtOH as a targeted therapeutic for human malignant melanoma as it upregulates PTEN activity, thus regulating the PI3K/Akt/mTOR cascade (▶ **Fig. 1**). The authors declare no conflicts of interest.

FT-ARS-04 Flash Talk 4 "Harmine and Harmaline of *Peganum harmala* L. Seeds as Promising Hits Against Mycetoma: One of the Most Neglected Diseases"

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DOI 10.1055/s-0042-1758898

Mycetoma, a chronic granulomatous infectious disease caused either by bacteria (actinomycetoma) or fungi (eumycetoma). It is one of the most neglected tropical diseases [1]. Both mycetoma types are treated using different antifungals or antibacterial combinations for extended periods. However, no efficient regimen is yet available for mycetoma treatment, especially eumycetoma [2]. Hence, physicians tend to resort to surgical amputations due to mycetoma deleterious outcomes. *Peganum harmala* L. seeds (Nitrariaceae) contains various secondary metabolites including β-carboline alkaloids. The current study aims to investigate the antimycetomal activity of *P. harmala* seeds with special reference to harmine and harmaline.

P. harmala ethanolic extract and fractions exhibited appreciable antimycetomal activity against two eumycetoma isolates and two actinomycetoma isolates belonging to Madurella mycetomatis, Actinomadura madurae, respectively. Harmine and harmaline were identified using thin layer chromatography in both extract and fractions (▶ **Fig. 1**). Both of them exhibited considerable antimycetomal activity against both mycetoma types. Herewith, we intend to provide evidence that the harmine and harmaline are responsible for the antimycetomal activity of *P. harmala* seeds. The antifungal and antibacterial activity have been previously attributed by total harmala alkaloids and other pure β-carboline alkaloids including harmine and harmaline [3].

In conclusion, the inhibitory properties of *P. harmala* seeds against mycetoma types renders it a promising hit for further optimization to lead molecules that could be further exploited as a treatment against mycetoma types. A discovery that would shorten the diagnosis and hence earlier initiation of treatment before disease progression result to further clinical manifestation requiring surgical intervention.

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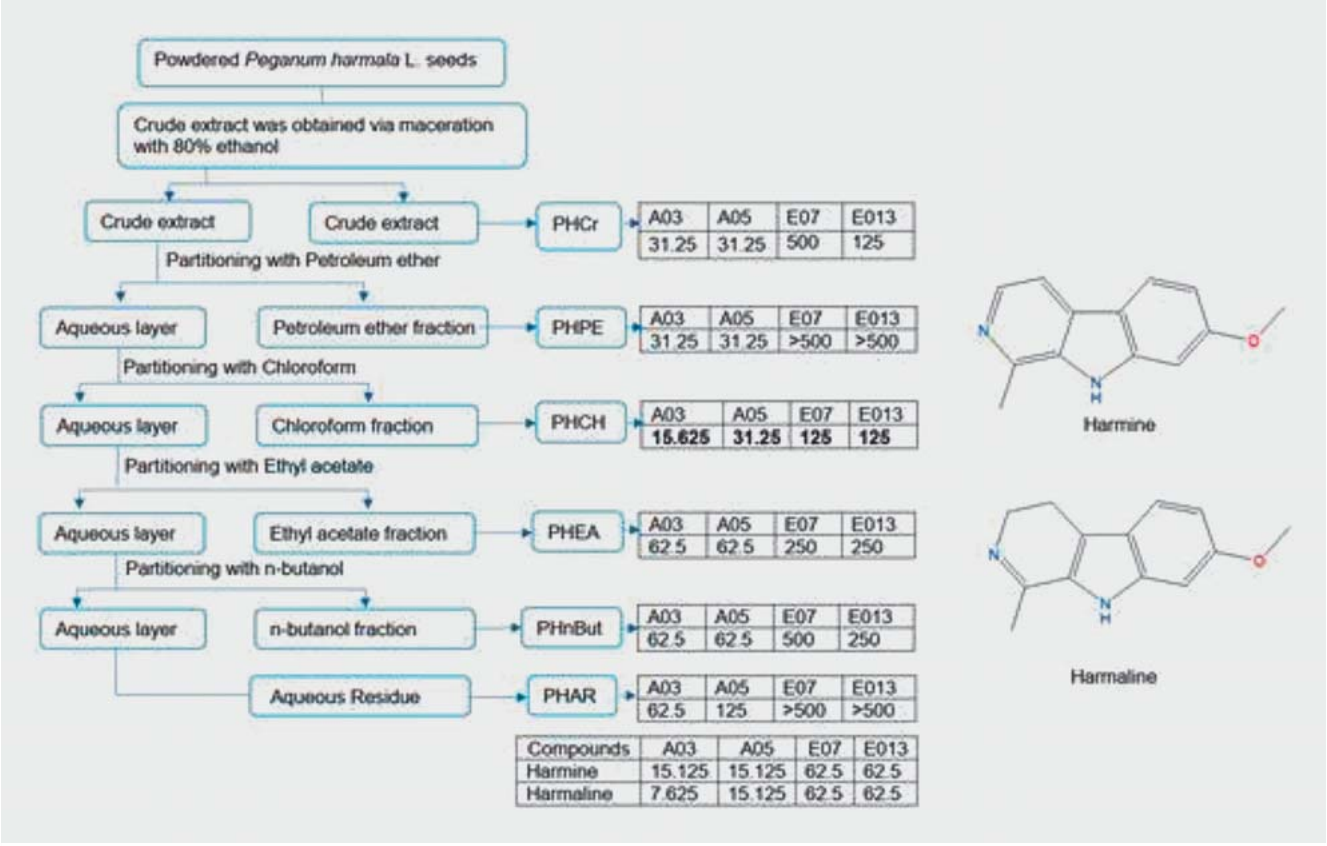

▶ **Fig. 1** Method of extraction and fractionation of *Peganum harmala* seeds along with the screening results of extract, fractions and pure compounds (chemical structures illustrated), represented as MIC in µg/mL. Key. PHCr: Crude extract, PHPE: Petroleum ether fraction, PHCH: Chloroform fraction, PHEA: Ethyl acetate fraction, PHnBut: n-butanol fraction and PHAR: Aqueous Residue.

FT-ARS-05 Flash Talk 5 "Ethnobotanical and ethnopharmacological study of spontaneous medicinal plants used in the treatment of viral respiratory diseases in the Prerif, Morocco"

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DOI 10.1055/s-0042-1758899
Viral respiratory infections (common cold, flu, sinusitis, bronchiolitis etc.) are among the most common infections in the world with severe symptoms. In Morocco, the therapeutic indications of medicinal plants are very present to treat several diseases including respiratory system. The objective of our study is to identify and document medicinal plants used in traditional medicine to treat viral respiratory infections and alleviate their symptoms in order to generate interest for future studies in verifying the efficacy of these traditional medicines and their conservation. The information acquired from 81 questionnaires and the floristic identification allowed us to identify 26 spontaneous species, belonging to 14 families, used as traditional therapies for viral respiratory diseases in the Prerif. The herbs are the most used life form. The results also showed that leaves were the most commonly used plant parts and the most of the herbal medicines which were prepared in the form of infusions and administered orally. Documented data was evaluated using use value (UV), family importance value (FIV) and relative frequency citation (RCF).

SL-ARS-04 Short Lecture 4 "Evaluation of antiproliferative and anti-angiogenic activity of an ethanolic extract of *Helichrysum odoratissimum* (L.) Sweet against skin cancer"

Authors Twilley D[1], Meyer D[2], Langhansova L[3], McGaw LJ[4], Madikizela B[4], Roma-Rodrigues C[5], Baptista PV[5], Fernandes AR[5], Lall N[1,6,7,8]
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Skin cancer is one of the most commonly diagnosed types of cancer, with the number of cases continuing to rise globally. Angiogenesis, which is the formation of new blood vessels, is required for tumor growth and metastasis, including in skin cancer. New blood vessels enhance the supply of oxygen and nutrients needed by cancerous cells to proliferate. Medicinal plants extracts have exhibited the ability to inibit tumor cell angiogenesis, therefore, an ethanolic extract of *Helichrysum odoratissimum* (HO) has been evaluated in this study. HO demonstrated significant antiproliferative activity against squamous cell carcinoma (A431) and human melanoma (A375) with fifty percent inhibitory concentrations (IC_{50}) of 15.5 ± 0.2 and 55.5 ± 6.6 µg/mL, respectively, and a selectivity index of 5.85 and 1.63 respectively, when compared to human dermal fibroblasts (MRHF). HO furthermore modulated interleukins involved in the regulation of angiogenesis. Interleukin (IL)-8 (103 ± 6.1 pg/mL) was significantly inhibited compared to the control (123 ± 3.0 pg/mL), whereas IL-12 (12.4 ± 7.0 pg/mL) was significantly stimulated compared to the control (4.71 ± 0.5 pg/mL). HO (18.5 µg/egg) furthermore, significantly inhibited new blood vessel formation (68.35 ± 12.80% newly formed vessels) as observed in the ex ovo chorioallantoic membrane assay (CAM). In addition, HO (5 mg/mL) did not show a mutagenic effect on Salmonella typhimurium strains TA98 and TA100. These results show the potential of HO for further evaluation in pre-clinical studies. The authors declare no conflict of interest.

SL-ARS-05 Short Lecture 5 "Analytical investigations reveal challenges with the collection of *Combretum mucronatum* (Schumach. & Thonn.) leaves for herbal medicine purposes in Ghana"

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Combretum mucronatum (Schumach. & Thonn.) (CM) leaves are used traditionally in Ghana to treat several disease conditions. They are collected based on the local name, 'hwirɛmoo', which may also loosely apply to other related *Combretum* species, and this could lead to wrong species collection, with attendant safety issues. The plant's phytochemistry may be affected by its geographical origin and season of collection. This could affect its therapeutic effects. These factors were investigated using TLC and UPLC-PDA methods validated following ICH Q2(R1) guidelines. Initial investigations were performed on botanically authenticated representative samples of 9 different *Combretum* species (n = 26). Visual inspection and chemometric evaluation of the TLC profiles developed showed differences among the *Combretum* species. Similarly, the UPLC-PDA profiles demonstrated differences in the fingerprint analysis. 70 samples were then collected in the dry and rainy seasons, by known medicinal plant collectors familiar with CM and its local name from 7 Ghanaian regions, classified under 5 climatic zones. The results showed that 30% (n = 21/70) of the samples were wrongly collected as CM. Univariate and multivariate models were used to analyze all samples (n = 96) and the subset of CM verified samples (n = 49) with respect to the assays of 8 marker compounds. CM and non-CM samples' clusters were distinct, with significant differences in the contents of some markers across the two seasons and different climatic zones. Effectively, local-name-based plant collection practices may be problematic. Also, plant collectors may need to consider geographical effects and seasonal variation on the quality of herbal materials for manufacturing purposes.

SL-ARS-06 Short Lecture 6 "Angolan medicinal plants used in Huambo region for the treatment of neglected tropical diseases – schistosomiasis case study"

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DOI 10.1055/s-0042-1758902
Located in southern Africa, Angola has one of the richest plant diversities in the African continent, with about 6 850 native species. From the Mayombe forest, in the North, to the Namib desert, in the South, the huge diversity of habitats accounts for this relevant biodiversity. Results of ethnobotanical surveys in Angola showed that approximately a hundred plant species are used by traditional medicine practitioners (TMPs) in the treatment of various pathologies, including neglected diseases. However, there is a lack of informa-

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tion about the plants used in traditional medicine in the Huambo region for the treatment of these kinds of diseases, including schistosomiasis. The present study aims to bridge that gap and contribute to the valorization and recognition of the therapeutic value of medicinal plants used in this geographical region for the treatment of schistosomiasis. Results of a semi-structured survey carried out at 30 TMPs in the village of Ndango de Cima, Huambo province, will be presented and discussed. The collected ethnomedical data were treated and compared with the literature available concerning Angolan flora. Results showed that only 10% of the inquirers have higher education and 33% middle education. 17% of the TMPs were aged between 30–40, 33% aged 41–50, 27% aged 51–60 and 23% aged 61–70 years. Twenty traditional recipes involving 20 medicinal plants were identified. One of the most used plants, *Bobgunnia madagascariensis* (Desv.) J.H.Kirkbr. & Wiersema (= *Swartzia madagascariensis* Desv), fruit, was already submitted to in vitro and in vivo molluscicide activity studies and its activity was confirmed.

Keynote Lectures

KL-01 Keynote Lecture 1 "Using complementary approaches for antimicrobial discovery, biosynthesis and interrogation of microbial strain libraries"

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Natural products or specialized metabolites play important roles in the biology and ecology of bacteria, fungi, plants and animals. Microbial natural products in particular account for many therapeutics currently used to treat infectious diseases and cancer. Massive amounts of genome sequencing data have revealed the wealth of new bacterial-derived chemistry that is awaiting discovery. Challenges remain to tap those resources including the re-isolation of known compounds, robust heterologous expression systems to synthesize the compounds of interest, and rapid methods for interrogating the chemical potential of bacterial strains within a microbial collection. Our research focuses in part on the discovery of new antimicrobials, determining the structural basis for how they are biosynthesized, and developing simple but robust methods to evaluate the biosynthetic pathways and chemical potential of in-house strain libraries. In this talk I will share our current progress in these areas. This includes (i) genome-assisted discovery of chryseoviridins, a new class of antitubercular RiPP (Ribosomallly-encoded Post-Translationally modified peptides), and the structural basis for how single and knotted macrolactone and macrolactams are installed in the graspetide family; (ii) a rapid and low-coverage whole genome sequencing approach that combines Oxford Nanopore Flongle technology and HR-MS/MS for strain prioritization and identification of new natural products; and (iii) discovery, biosynthesis and resistance mechanism of a new complex polyketide from a desert-derived *Amycolatopsis* strain. A related aim is to present complementary and affordable methods that can assist with natural products discovery, making it more productive and accessible to diverse scientists.

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KL-02 Keynote Lecture 2 "Opportunities for Natural Product Sciences in Modern Drug Discovery"

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DOI 10.1055/s-0042-1758904
Nature is still a non-exhausted source for new pharmacophores. In recent year several launches of new drugs like Ixempra™, Rydapt™ or Cubicin™ are based on natural products, showing the high impact of natural product research on drug discovery in different therapeutic areas.

To keep the impact of natural products in drug discovery it is crucial to develop and implement new technologies in the research field and to expand the used biological diversity for the identification of bioactive compounds. This includes miniaturized approaches to identify new chemical scaffolds from natural sources, linking of these structures to biological activities and finally assigning the biological target to these highly diverse molecules
In the presentation I will illustrate how to access chemical diversity from different biological sources in an industrial environment, how to use natural product libraries in complex biological assays to identify new bioactive molecules and how to explore and evaluate the potential and the targets of these hits.

KL-03 Keynote Lecture 3 "Polypharmacology-correlated Molecular Networking and other Essential Bioactivity-correlating Techniques for Studying Desert-loving *Eremophila* spp. from Australia"

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Eremophila is a genus of approximately 230 species endemic to Australia and mainly found in the arid regions of Western Australia. *Eremophila* spp. have been used for medicinal and cultural purposes by the Australian Aboriginal people, and *Eremophila* is rich in bioactive diterpenoids and sesquiterpenoids [1,2]. The aim of this study was to apply omics and other state-of-the-art technologies for exploring the chemical and pharmacological properties of more than 300 plant samples collected in a large interdisciplinary project and use these data to select species for further drug lead discovery. For this purpose, we used molecular phylogenetics combined with computational metabolomics to pinpoint intraspecies relationships, high-resolution inhibition profiling as a bioactivity-correlating technique, and polypharmacology-labelled molecular networking for pinpointing metabolites correlated with multiple bioactivities. The results from these explorative studies enabled us to identify *Eremophila* species and/or individual metabolites correlated with (poly)pharmacological bioactivity – and extracts of these species were subsequent analysed by a combination of high-resolution inhibition profiling and hyphenated high-performance liquid chromatography, photodiode-array detection, solid-phase extraction, high-resolution mass spectrometry, and nuclear magnetic resonance spectroscopy, i.e., HR-inhibition inhibition profiling/HPLC-PDA-HRMS-SPE-NMR. This resulted in isolation of more than 120 new and structurally diverse serrulatane, cembrane, and viscidane diterpenoids [3,4] as well as a new class of dimeric branched chain fatty acids formed by Diels Alder reactions [5]. Pharmacological activities included antibacterial, antihyperglycaemic, and cancer resistance-reversing properties. In conclusion, *Eremophila* is a rich source of structurally unique diterpenoids with multiple bioactivities, and thus continues to be an interesting genus for natural-products-based drug discovery.

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KL-04 Keynote Lecture 4 “Biorefineries – Green approaches for the lignocellulosic value chain”

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“Bioeconomy” and “biorefinery” are two buzzwords that are on everyone’s lips these days and are sometimes discussed quite controversially – tension between energy use versus material use or food/feed versus biofuel is just one of them. In many of these discussions, economic or political aspects play a role, the scientific background is not always known (or is perhaps sometimes simply ignored).

In the lecture, basic terms that frequently crop up in these discourses will be illuminated from a scientific perspective and viewed quasi from the eyes of the lignocellulose chemist. What are biorefineries actually – what really distinguishes them from “refineries without bio”? What do biorefineries work with? What is special about lignin and cellulose also with respect to analytics?

After clarifying this basic concept, some important points of the bioeconomy/biorefinery issue will be addressed – again from a wood chemist’s point of view.

There are now numerous (not to say countless) interpretations, economic models, views and opinions on all these aspects. However, some basic questions can be answered quite clearly, and thus corresponding conclusions and representations can either be identified as true or also exposed as or false. The inclusion of the chemical perspective is intended to contribute a little to strengthening the scientific foundation of the bioeconomy discussion and to provide those responsible who move in its field of tension with equipment that makes them less susceptible to seductive bogus arguments in this field and allows them to argue in a scientifically sound manner.

KL-05 Keynote Lecture “Marine Microbiome as Source of Innovative Drugs”

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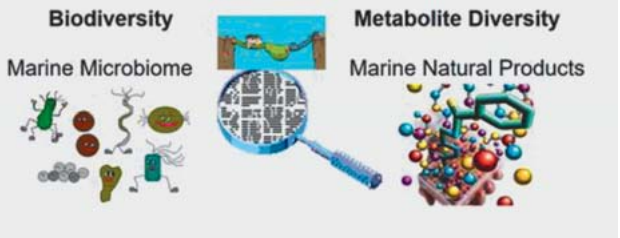
The marine microbiome contains many billions of genes with the ability to express an unimaginable arsenal of chemical structures. Small molecules, as well as proteins, lipids and other classes of products with ecological functions that can be “humanized” for the discovery of new pharmaceuticals, improved enzymes and novel biomaterials, as well as new resources for food and feed stocks, nutraceuticals, diagnostic devices, personal care and cosmetic products and an ever increasing list of marine natural products.

Early studies of the marine environment focused on natural products from invertebrates and tunicates have led to the isolation of several classes of bioactive natural products mainly small molecules such as polyketides and non-ribosomal peptides. However, there is an emerging a rational suspicion, based on marine sponge/tunicate metagenomics that these compounds originally isolated from metazoan organisms are in fact of bacterial origin. The cases of pederin, ecteinascidins (trabectedin, Yondelis©) or didemnins are some examples of the role of marine microbiome as producers of bioactive metabolites with application in human medicine (oncology).



▶ **Fig. 1**

Currently, genomic mining for polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) improves the probability of success in drug discovery using marine microorganisms, both in isolated genomes and metagenomes.

Dr. de la Calle discussed the state of the art in marine biotechnology applied to drug discovery under the experience of PharmaMar, a Spanish company focused in research, development and commercialization of marine derived drugs for the treatment of cancer.

KL-06 Keynote Lecture “Plant biotechnology for pharma, cosmetics and food applications”

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Modern plant biotechnology offers advantages to develop new type of ingredients for industrial applications in an environmental friendly and sustainable way. We have developed industrial-scale production systems for Nordic berry species including their cell cultures with consistent quality and defined chemical composition. Through our proprietary bioprocessing technologies combined with solvent-free extraction, we can enrich and modify natural antimicrobials from the berries and their by-products to obtain multifunctional ingredients with new or improved bioactivities. Ellagitannins, dimeric sanguiin H-6 and sanguiin H-10 isomers, efficiently inhibit the growth of skin pathogens by blocking cell-to-cell signalling in a bacterial community without affecting the beneficial bacteria. In vivo findings show that ellagitannins are very effective against MRSA bacteria thus opening entirely new avenues in wound healing and fighting against antibiotic resistance [1].

Plant cells have conventionally been used to produce single metabolites e.g., pharmaceuticals. However, their use as a whole cell biomass for plant-based food production is a new approach. Our recent investigations concerning the nutritional composition of cultured plant cells revealed very favourable contents of dietary fibre, starch, sugars as well as good quality lipids besides a surprisingly high content of proteins. The cell biomass showed balanced profiles of nutritionally essential amino acids exceeding contents of soy protein isolates and most importantly exhibited differential digestibility, a basis for efficient absorption, depending on species and processing [2]. As a case study, we showed how cultured cells from coffee plant can be processed to obtain coffee [3] and discussed future perspectives of cellular agriculture.

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KL-07 Keynote Lecture “Resveratrol and its analogues – shall we always correct nature?”

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Resveratrol (trans-3,4′,5-trihydroxystilbene), a naturally occurring hydroxystilbene, is considered an essential antioxidative constituent of red wine and many medicinal plants. Moreover, resveratrol, its metabolite piceatannol, and higher hydroxylated analogues were reported to have cytotoxic activities. As a key factor for their activity, the hydroxyl groups are considered [1]. Therefore, one can wonder whether structures with more hydroxyl groups will have a more potent antioxidant and cytotoxic effect. Moreover, is there a link between antioxidant and cytotoxic activity? We have synthesized several other polyhydroxylated resveratrol analogues and studied their pro-/antioxidant and cytotoxicity properties to answer this question. Our experiments suggested that not only the number of hydroxyl groups and their disposition

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in aromatic rings play an important role. Our experiments started from the mitochondrial model and showed that oxidation of ortho-hydroxystilbenes (e.g., piceatannol) results in cytotoxic ortho-semiquinones production. Further investigations revealed that these intermediates undergo redox-cycling, consuming additional oxygen and forming cytotoxic oxygen radicals. In contrast, compounds without such substitution patterns, (e.g., resveratrol) did not show such activity [2]. Following this path, we have performed several in vitro studies employing different cancer cell models showing different cytotoxic effects exerted by resveratrol and analogues [3,4]. In this talk, our results will be confronted with findings from other groups. The results of our investigation suggest that resveratrol has an optimal structure combining antioxidant and cytostatic properties. A further increase in the number of hydroxyl groups may result in pro-oxidative activity that is harmful to cells.

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KL-08 Keynote Lecture "Exploitation of olive oil industry by-products for pilot isolation and semi-synthesis of promising medicinal agents"

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Extra virgin olive oil (EVOO) the main product of *Olea europaea* and key ingredient of Mediterranean diet, is characterized by substantial nutritional and health beneficial value [1]. However, despite olive oil's economic and health impact, olive oil industry must deal with significant environmental problems arising from the vast quantity of by-products such as vegetation waters, olive cake, olive pulp and olive branches and leaves [2]. For instance, the amount of olive leaves, produced every year, exceed 18 million tons, and are used mostly as animal feed, compost production or simply are burned. Recent studies show that burning of olive tree branches is a major organic aerosol source in the Mediterranean basin [3]. Given that olive by-products contain high amounts of added-value compounds such as triterpenoids, secoiridoids, flavonoids, phenolic alcohols, phenolic acids and lignans, known also as olive polyphenols and possessing strong antioxidant profile and other important biological activities (e.g., anti-inflammatory, anticancer) there is an increased industrial interest for their possible nutraceutical and pharmaceutical applications. Among these lines, our research is focused on suggesting alternative strategies to manage olive oil industry residues towards their exploitation for the recovery of polyphenols, structured into two axes. Firstly, the development of liquid/liquid or solid/liquid extraction followed by partition chromatography techniques for the isolation of these compounds in multi gram scale and on the other hand, the use of compounds such as oleoside and EDA as starting material for the hemi-synthesis of new analogues and their evaluation as potential antitumor agents.

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KL-09 Keynote Lecture "Challenges and opportunities in anti-infective discovery from microbial natural products"

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Microbial natural products (NPs) are one of the most prolific sources for the discovery of novel anti-infective drugs to respond to unmet needs in infectious diseases. NPs present a unique chemical space and architectural complexity, and their potency and selectivity is the result of an extended evolutionary selection to create biologically active molecules with the required properties to interact and potentially inhibit microbial and parasite targets. NPs continue to play today a key role in the discovery of new molecules to fill the chemotherapeutic gap and in the last years much attention has been given to less explored and untapped rich sources of new microbial chemical diversity. Microbial genome mining and cutting edge metabolomic approaches are essential tools in the modern NPs drug discovery paradigm, opening new opportunities to identify novel classes of compounds. New integrated NPs discovery approaches involving genome-driven and cultured-based strategies combined to high throughput phenotypic screening platforms are playing a key role in the identification of new molecules to be developed and refill the antibiotic pipeline.

MEDINA is a research organization focused on the discovery of novel bioactive NPs with one of the richest and most diverse NPs collections that are at the origin of our collaborative drug discovery research programs. As a result of these screening programs integrating different approaches to support the discovery and development of novel NPs as potential new leads for drug development, we have identified different novel families of molecules with interesting new chemistry and biological activities that will be discussed in the context of current discovery efforts.

KL-10 Keynote Lecture "Cheminformatics in natural product-based drug discovery"

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DOI 10.1055/s-0042-1758912

Today, cheminformatics offers a versatile toolbox that can provide guidance to researchers in natural product-based drug discovery [1]. The first part of this contribution will provide an overview of the most relevant in silico tools and discuss their scope and limitations in the context of bioactivity, ADME and toxicity prediction. The second part will focus on a recent study in which we tested the capacity of computational methods to predict the macromolecular targets of structurally complex natural products [2]. The third part will be dedicated to the study of natural product ring systems and how computational tools can boost the further exploration of these structural motifs in drug discovery.

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KL-11 Keynote Lecture "Translating and Integrating popular Greek herbs into Rational Phytotherapy, Regulatory system and Clinical practice in EU"

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All over the world the medicinal plants have been used therapeutically for centuries, while many scientific studies are conducted, describing their remarkable healing properties. It is well known that the chemical profile of

plants is influenced by the geographic origin, weather, processing, packaging and storing conditions. Herbal medicines, remain among the oldest forms of human's treatment known worldwide, as the World Health Organization (WHO) estimates that such therapies are used by 70% of the world's population.

The European Union has considered medicinal use of herbal products through mainly the Traditional Herbal Medicinal Products Directive (Directive 2004/24EC amending Directive 2001/83/EC as regards THMPs). The Herbal Medicinal Products Committee (HMPC) at the European Medicines Agency (EMA, London) has drafted and adopted guidelines which are intended to support assessment of THMPs considering their particular characteristics, while has established community monographs of herbal substances, available at EMA's website. In these monographs, the accepted quality, and finally adopted indications among EU countries, together with potential risks, adverse reactions and contraindications in their uses, are reported, based in their longstanding medicinal uses and European experience. In this framework, selected examples will be presented and discussed, in details, based on ethnobotany, Greek history and tradition focused on herbal substances (Olive leaves, Dittany of Crete, ironwort – *Sideritis* species, mastic resin from Chios, rockrose) and their integration into Rational Phytotherapy, Regulatory system and Clinical practice in EU.

KL-12 Keynote Lecture "Amazon Rainforest Hidden Volatiles: Unveiling new minor compounds from Paracress Essential oil"

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Despite the current political views and rapid deforestation, the Amazon rainforest is still one of the greatest biodiversity hotspots in the world [1]. The forest keeps hidden secrets such as undiscovered new compounds with immense pharmacological potential. One of the plants in the Brazilian Amazonian Forest is paracress – *Acmella oleracea* (L.) R.K. Jansen (Asteraceae). Several pharmacological properties [2] have been described for this plant and its extracts, including but not limited to antinociception, anti-inflammatory, antioxidant, immunomodulatory, antimicrobial, antiviral, diuretic and local anaesthetic.

Paracress collected in the state of Amapa-Brazil in 2019 had its essential oil extracted by hydrodistillation. Gas chromatography and gas chromatography coupled to mass spectroscopy were used to analyse the oil. A first look at the oil composition revealed spilanthol and other alkamides as the main constituents in the oil. The oil was chromatographed over silica gel to obtain 13 different fractions of increasing polarities. A close analysis using all those fractions unveiled 30 minor compounds belonging to the class of long-chain α-ketol esters (both saturated and unsaturated). Acmellonate, isolated for the first time in 2006 [3], was the compound that initiated the unveiling of this hidden treasure, with 29 other substances described for the first time in the plant kingdom. A characteristic feature about them is that they are C11, C12 and C13 2-ketols (C10 and C14 are absent) esterified with isobutyric, isovaleric, 2-methylbutanoic, tiglic, angelic and senecioic acids. Acmellonate is known to produce a weak numbing and tingling sensation.

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KL-13 Keynote Lecture "Exploitation of global microbial biodiversity for the discovery and development of novel antiaging molecules"

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DOI 10.1055/s-0042-1758915

In the framework of several national and international projects, our research team in collaboration with industrial and academic partners has established a pipeline for discovery small molecules with antiaging activities. More specifically an innovative scientific and technological platform has been built aiming to the discovery of novel bioactive molecules originating from global terrestrial and marine biodiversity using emerging and state of the art technologies in the field of natural products chemistry, biotechnology, and applied microbiology.

In most cases already existing culture collections have been screened incorporating modern high throughput platforms (in silico & in vitro) for the rational and targeted selection of the most promising strains. Advanced approaches based on LC-HRMS, and molecular networks were used for the rapid dereplication of active extracts. Further analytical techniques for the accelerated fractionation, isolation and identification of natural compounds, were applied. For the evaluation of the antiaging properties of extracts, fractions, and pure molecules, a broad spectrum of bioassays and novel analytical approaches were incorporated. More specifically, it has been evaluated the antioxidant capacity (based on chemical and cell-based assays), the skin-protecting activity (proteasome homeostasis, anti-elastase and anti-collagenase inhibitory potential), and skin-whitening activity (anti-tyrosinase activity). In order to ensure sustainability, attention was given to the selection and optimization of fermentation technologies for the production of final products at pilot scale. Within this frame, several small molecules have been discovered with activities comparable to well established molecules and thus open the potential for new industrial applications.

Acknowledgements

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Planta Med 2022; 88: 1385–1588

KL-14 Keynote Lecture "Formulation development of medicinal products and food supplements. Balancing traditional and innovative solutions"

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DOI 10.1055/s-0042-1758916

The aim of any formulation/process development is to design a product and its manufacturing process to consistently deliver the intended quality. To achieve these goals, a rational formulation development as well as a consistent manufacturing process should be put in place.

In particular a rational formulation development will guarantee the quality and thereafter the safety and efficacy of herbal drug preparations as well as the health-related claims of food supplements.

In this presentation, the technological and biopharmaceutical/bioperformance principles that should guide the development of a product will be reviewed, based on scientific knowledge and the relevant legislation. The focus of the presentation will mainly be on oral solid dosage forms, given their widespread use in the formulation of herbal drugs and herbal drug preparations.

The choice of the dosage form, the role and the properties of the excipients, the criticality of the manufacturing process will be discussed. The advantages of innovative dosage forms will be highlighted as an undisputable means for improving stability and bioperformance of actives. The balance with their formulative complexity as well as the regulatory constraints were addressed.

Examples of development of both conventional and innovative dosage forms (related, among others, to ginkgo, green tea, bilberry, garlic…) were presented.

KL-15 Keynote Lecture "Anti-viral Drug Discovery for Severe Acute Respiratory Syndrome (SARS-CoV-2)"

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Understanding molecular level interactions between the metabolome and proteome, two of the most important classes of molecules in biology, will generate deeper insight into the function of metabolites (natural products) which have a central role in interactions with therapeutic targets.

Drug discovery in today's pharmaceutical environment is driven by high-throughput screening of large chemical libraries. It is now 10 years since we published a paper on the development of natural product fraction libraries with control of LogP properties [1]. We have now turned our attention to using pure natural product libraries to address the timeframe issues associated with isolation and characterization of the active constituent(s).

Coronavirus disease (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome (SARS)-CoV-2 virus [2]. Viral proteins are replicated in the host cell, re-assembled and released as new viral particles. Compounds that bind to viral proteins can interfere with re-assembly by preventing critical protein-protein interactions or by inhibiting the catalytic activity.

We will discuss approaches to use pure natural product libraries to develop antiviral agents. Our strategy is to investigate viral proteomes to identify natural products that can prevent viral assembly thereby blocking release from the host cell.

Natural product-viral protein interactions were identified using native mass spectrometry (nMS) screening [3,4]. Collision-Induced Affinity Selection MS (CIAS-MS) is a second MS technique that can be used for HTS.

The presentation will highlight the value proposition of pure natural product libraries and high-throughput MS methods.

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KL-16 Keynote Lecture "Strategies to prioritize the discovery of bioactive natural products – Chemical space exploration based on massive multi-informative metabolite networks"

Authors Wolfender J-L[1,2], Gaudry A[1,2], Quiros-Guerrero L[1,2], Kirchhoffer O[1,2], Rutz A[1,2], Marcourt L[1,2], David B[4], Grondin A[4], Nothias L-F[1,2], Ferreira Queiroz E[1,2], Allard PM[1,2,3]
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The increasing amount of accurate metabolome data that can be acquired through high resolution mass spectrometry data dependent MS/MS analyses (HRMS/MS), allows mapping of natural extracts at an unprecedented precision level [1]. This potentially allows the construction of virtual chemical libraries based on the combined annotation data set generated from raw extracts for efficient prioritization of valuable NPs for drug discovery, compositional assessment of phytopreparations or correlation in eco-metabolomic or chemotaxonomic studies.

In this context we have pushed forward our applications and further development of UHPLC-HRMS/MS molecular networking (MN) approaches [1,2] to provide enhanced annotation confidence by integrating automated NP class annotations and taxonomically informed scoring. To this end, we have recently set up an online resource for occurrences of NP structures in their source organisms [3].

We have applied this integrated approach to the investigation of a chemodiverse collection of 1,600 plant extracts from Pierre Fabre Laboratories which holds one of the largest plant samples library worldwide with over 17,000 samples (Collection registered in 2020 before the European Commission). For the exploration of the chemical space of such a massive metabolite profile dataset, we have developed new computational tools to prioritize and efficiently target the isolation of valuable bioactive NPs [4,5].

The proof of concept and the exploration of such data in combination with results of various bioassays (anti-infective, anticancer, antiparasitic activities) will be exemplified. The potential and challenges of these approaches to change the paradigms of pharmacognosy in the era of omics and digital science will be addressed.

The authors declare no conflict of interest.

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Monday, August 29 | Short Lectures A
Chemistry and bioactivity of natural products

SL-A02 Short Lecture "Neuroregenerative Potential of Infusions of Different *Sideritis* taxa & Metabolic Fingerprinting"

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In traditional medicine, the infusions of genus *Sideritis* L. (Lamiaceae) are mainly used to alleviate symptoms of the respiratory tract, stomach disorders, and common cold in Cyprus and Greece [1,2]. Over the last years, *Sideritis* plants have been studied for their effects on neurodegenerative disorders such as Alzheimer's disease [3,4]. In particular, two Greek species, namely *Sideritis euboea* Heldr. and *S. scardica* Griseb., have been evaluated for neuroprotective activity and cognitive improvement [3]. Furthermore, a clinical trial showed that *S. scardica* improved picture recognition, speed of attention, and improved state anxiety [4].

Plant extracts and natural compounds of *Humulus lupulus*, acting neurophysiological similar to *Sideritis* [3], were shown to induce the differentiation of neural stem cells in direction to neurons in a neuroregenerative approach [5]. Thus, this study was focused on investigating the neurogenic potential of infusions of different *Sideritis* samples from Cyprus and Greece, using a doublecortin based reporter gene-assay quantifying neuronal differentiation induction in mouse embryonic forebrain cells. In addition, their metabolic fingerprints were explored by GC-MS and LC-UV and MS/MS techniques. Differences and similarities in chemical profiles were determined based on different geographical origins and environmental conditions. Furthermore, all *Sideritis* taxa showed a neurogenic potential similar to retinoic acid, a well-known and widely used inducer of neuronal differentiation. This study is the first report on the neurogenic potential of *Sideritis* taxa.

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SL-A03 Short Lecture "Assisting ^{13}C NMR and MS/MS joint data annotation through on-demand databases"

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Compound identification in complex mixtures by NMR and MS is best achieved through experimental databases (DB) mining. Experimental DB frequently show limitations regarding their completeness, availability or data quality, thus making predicted database (e.g., ISDB, PNMRNP) of increasing common use [1]. Querying large databases may lead to select unlikely structure candidates. Two approaches to dereplication are thus possible: taxonomical filtering (either biological or chemical) of the DB before search or taxonomical scoring of the results after a large-scale search [2]. The present work relies on the former approach. The corresponding dereplication tool involves the selection of the structure set of interest from the largest available structural DB and the prediction of the associated NMR and MS spectral data (▶ **Fig. 1**).

As far as we know, NMRshiftDB2 is the only open-source 13C NMR chemical shift predictor that can be freely operated in batch mode [3]. CFM-ID 4.0 is one of the best-performing open-source tools for ESI-MS/MS spectra prediction [4]. LOTUS is a freely usable and comprehensive collection of secondary metabolites [5]. It can select compounds according to substructure, chemical class, or taxonomical source. Integrating the open-source database and software LOTUS, CFM-ID, and NMRShiftDB2 in a dereplication workflow requires presently programming skills, owing to the diversity of data encoding and processing procedures. A graphical user interface that integrates seamlessly database building and spectral data prediction still does not exist, at the best of our knowledge.

The present work proposes a coherent software tool that assists secondary metabolites specialists to identify mixture components in a simple way.

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▶ **Fig. 1** On-demand natural product databases.

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SL-A04 Short Lecture "Method Development for Pilot Production of Astragaloside VII"

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Based on the promising immunostimulant effect comparable to commercialized adjuvants *Alum* and *Quillaja* saponins (including QS-21) [1–3], our team has been prompted to carry out advance studies for developing Astragaloside VII (AST VII) (▶ **Fig. 1**) as a new vaccine adjuvant or an immunotherapeutic agent. Hence, one of the most critical challenges is establishing efficient isolation and purification processes to obtain AST VII on a large scale. Thus, this study aimed to develop a production methodology for AST VII from Turkish *Astragalus* species.

Factor screening and optimization were performed using experimental designs for lab-scale extraction studies. Then, MeOH as solvent, 1 : 20 (g/mL) as plant : solvent ratio, 0.5–1.0 mm as particle size and 8–10 hours for extraction time were optimum, yielding 0.36% g AST VII/g plant. To enrich AST VII in saponin-rich fractions, pre-purification studies such as liquid-liquid extraction, resin fractionation, and precipitation were performed. The results showed that the resin (D-101) fractionation employing H_2O, 20% EtOH and EtOH was superior. To enrich AST VII up to 85% purity, several chromatographic steps using normal (employing EtOAc : MeOH : H_2O and $CHCl_3$:MeOH : H_2O systems) and reversed phase (C18; employing MeOH : H2O systems) silica gel were used. Lastly, a precipitation method was developed using MeOH and acetone, affording 98% purity. The developed method at lab scale (3.5 g) was successfully transferred to semi-pilot scale (about 100 g) with minor modifications, and a crucial step toward large-scale isolation (kg) of AST VII was accomplished.

Acknowledgment
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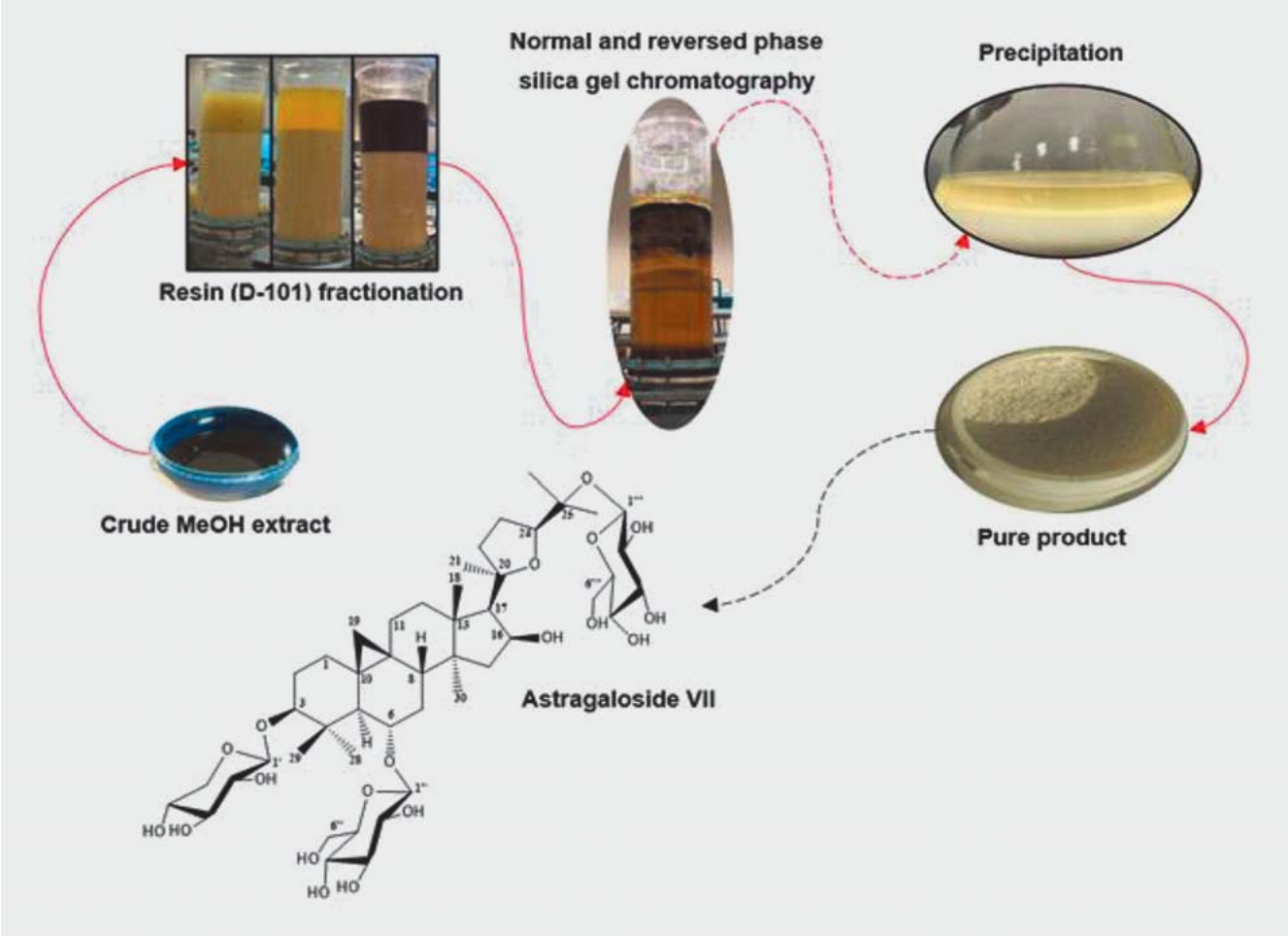

▶ **Fig. 1** Purification methodology of AST VII.

SL-A05 Short Lecture "Essential oils of *Thymus fallax* and *Thymus migricus* from Turkey: Compositions, total phenolic contents, in vitro antidiabetic and antioxidant activities"

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Thymus fallax (TF) and *Thymus migricus* (TM) (Lamiaceae) are naturally grown in east side of Turkey. According to previous studies, the essential oils (EOs) contents were determined in different regions as Turkey, Iran, and Azerbaijan and they were evaluated for some biological activities as antioxidant, antimicrobial, and antispasmodic [1–5]. Aim of our study is determining composition and total phenolic contents of EOs obtained from aerial parts of the plants by hydrodistillation and evaluating in vitro antidiabetic and antioxidant activities of EOs. The major constituents were found as thymol (30.6%), γ-terpinene (26.1%) and carvacrol (15.5%) for TF; γ-terpinen (33.1%), thymol (29.1%) and o-simen (11.3%) for TM. EO of TM exhibited similar α-glucosidase inhibitory activity with an IC_{50} value of 4729 µg/mL compared to positive control acarbose (IC_{50}= 4738 µg/mL), while EO of TF had limited activity with IC_{50} value of 5399 µg/mL. However, both of them had no inhibition against α-amylase. Total phenolic contents were determined as 78.5 µg GAE/mg EO for TF: 110.2 78.5 µg GAE/mg EO for TM. Parallel to antidiabetic activity, EO of TM showed significant ABTS•+ scavenging activity (86.1%), as well as it was 81.4% for EO of TF when compared to trolox (82.6%) and α-tocopherol (37.2%) as standards at 15 µg/mL. According to DPPH• scavenging activity, TM and tF EOs showed potent activity with 60.0% and 57.9%, while trolox and α-tocopherol exhibited 52.7% and 33.6% activity at 25 µg/mL, respectively. The EOs can be used as antioxidant and antidiabetic agents with further studies.

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SL-A06 Short Lecture "Antiadhesive natural products against *Campylobacter*: Recombinant expression of *C. jejuni* FlpA and JlpA adhesins and development of a screening ELISA"

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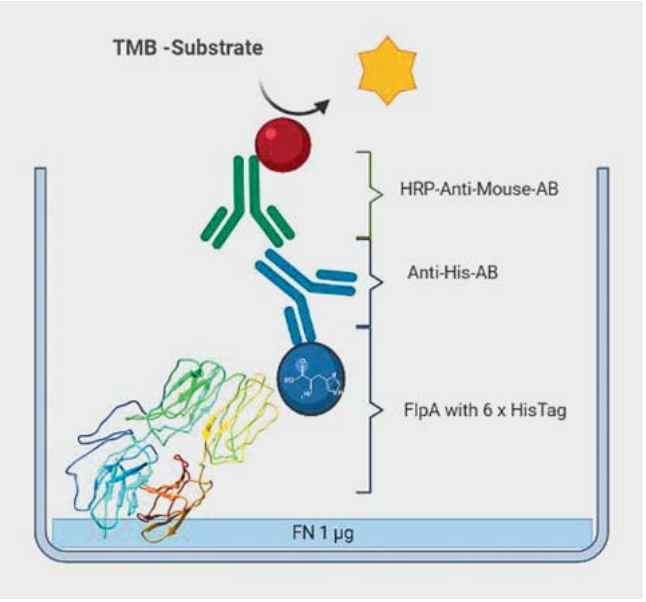
Different species of the genus *Campylobacter* are main causes of acute severe bacterial gastroenteritis [1]. Infections by Campylobacter are initiated by recognition of intestinal epithelial cells by the bacteria, followed by adhesion to host cells, invasion, cellular destruction and subsequent strong inflammation.



▶ **Fig. 1** Setup of FlpA ELISA.

Antiadhesive strategies aim to interfere with the early host-pathogen interaction. *C. jejuni* has a complex adhesion strategy, using mainly three outer-membrane-proteins CadF (*Campylobacter* adhesion protein to Fibronectin), FlpA (Fibronectin like protein A) and JlpA (Jejuni like protein A) for adhesion to host cells. For identification of specific adhesion blockers, FlpA and JlpA were chosen for development of ELISA protocols.
FlpA from *C. jejuni* binds to 45 kDa gelatine binding domain of fibronectin, leading to integrin activation and to invasion [1].
JlpA is a glycosylated lipoprotein, interacting with HsP90α and activating NF-κB-dependent inflammation [2].
FlpA and JlpA from *Campylobacter* DSM 27585 were amplified by PCR and cloned into pCOLD I. Recombinant protein was expressed using ArcticExpress *E. coli* and induction with 1 mM IPTG at 11.5 °C (cold shock). Both His-tagged proteins were purified by Immobilized Metal Affinity Chromatography (IMAC) yielding in 20 mg/L culture. Identity and purity of the proteins was confirmed by MS-sequencing and PAGE.
For screening of FlpA and JlpA inhibitors, specific ELISAs were developed and validated [3]. The plate was coated with FN or HSP90α, coincubated with test compounds and FlpA or JlpA. Bound protein was detected by antibodies, followed by colorimetric readout. Using this system glucosamine-polymers were identified as strong and specific JlpA inhibitors.

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SL-A07 Short Lecture "Deciphering the Anti-Infective Properties of *Peucedanum ostruthium*: Biochemometry Identifies Ostruthin as Pluripotent Anti-Infective Agent"

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To extend the narrow portfolio of resistance-breaking antimicrobial agents, 158 herbal extracts were tested in a phenotypic screening assay using the gram-positive Staphylococcus aureus strain ATCC29213. The traditional Austrian remedy *Peucedanum ostruthium* (L.) Koch was identified as the most promising antimicrobial agent. The root extract (PO-E) tested at 100 µg/mL inhibited the growth of *S. aureus* by 92 %. In a recent study, we further demonstrated that PO-E significantly decreased the survival rate of the nematode *Caenorhabditis elegans* when tested at 25 µg/mL [1]. For the identification of the active principle(s) of PO-E against both *S. aureus* and *C. elegans*, an in-depth investigation using the biochemometric approach ELINA was performed [2]. ^{1}H NMR spectra and LC-MS-CAD data from 31 PO-E-generated microfractions [3] were correlated with their respective bioactivity data (HetCA analysis). ELINA unambiguously identified the coumarin ostruthin as active principle against S. aureus with a minimal inhibitory concentration (MIC) of 12.5 µM. Additionally, ostruthin exerted MIC values of 25 µM against methicillin-resistant-*Staphylococcus aureus* strain ATCC43300 and four *Enterococcus* spp strains. For the nematicidal activity, the biochemometric approach was able to pinpoint the bioactive principle to ostruthin and the furanocoumarin isoimperatorin. Intriguingly, when tested in the *C. elegans* survival and motility assays, a pronounced nematicidal potential could be seen only when ostruthin and isoimperatorin were combined. In sum, ELINA was successfully applied in two anti-infective screenings to identify ostruthin as leading active agent against a set of pathologenic bacteria and a model for anthelmintic drug discovery. The authors declare no competing interests.

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SL-A08 Short Lecture "West Africa medicinal plants with activities against Sars-Cov-2 and other viruses"

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DOI 10.1055/s-0042-1758925

The recent pandemics has highlighted the need for broad-spectrum antivirals against human coronaviruses (HCoVs). Other viruses, such as hepatitis C virus (HCV), are still infecting millions of people worldwide. Before the outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an ethnobotanical survey was carried out in Côte d'Ivoire and aimed at finding anti-HCV products. We selected 15 plants and screened their extracts against HCV. The most active extracts were further studied to specify their IC_{50} and toxicity in vitro, and several tannins were shown to be active [1]. We screened the same 15 crude extracts against HCoV-229E, a coronavirus associated with common cold. The most active extract was selected for bioguided fractionation that successfully led to the identification of a highly active antiviral molecule: pheophorbide a (Pba) [2]. Pba was also shown to be active against highly pathogenic SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV), and its mechanism of action was further assessed. Pba is an inhibitor of coronavirus entry by directly targeting the viral particle. Interestingly, the antiviral activity of Pba depends on light exposure, and Pba was shown to inhibit virus-cell fusion by stiffening the viral membrane. Moreover, Pba was shown to be broadly active against several other enveloped viruses and reduced SARS-CoV-2 and MERS-CoV infection in primary human bronchial epithelial cells. Pba is a natural antiviral agent against SARS-CoV-2 with direct photosensitive virucidal activity that holds potential for COVID-19 therapy or disinfection of contaminated surfaces. The authors declare no conflict of interest.

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SL-A09 Short Lecture "Herbs and Mountain Plants as an Alternative Medication for Anthelmintic Treatment in Livestock Species"

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Managing *Ascaridia galli* remains one of the challenges for poultry production systems, as animals pecking on the ground will be nearly unavoidable infected with this widespread intestinal parasite [1]. Consequently, organic housing (i.e., litter-based housing systems) increases the risk of parasitic infections. Treatments with anthelmintics (AH) such as flubendazole are working; However, the increased occurrence of resistant parasites and the growing awareness of drug residues accumulating in chicken meat and eggs call for effective and organic alternatives.

Based on ethnopharmacological studies, nine plant species growing in the alpine area were selected to explore their potential as AH in the course of the Euregio project HERBAL. The anthelminthic effect caused by the apolar and polar plant extracts was studied utilizing an embryonic development and a worm motility assay. Furthermore, acceptance tests were conducted by feeding Lehmann brown hens with fodder containing the plant extracts (0.1 % or 0.3 % w/w). The results highlighted *Ciceribita alpina, Chicorium intybus*, and *Tanacetum vulgare* as promising anthelminthic species consumed by the chickens. Thus, these plants were submitted to thorough phytochemical studies employing UHPLC-DAD-HRMS² studies and classic activity-guided isolation procedures. The combined results suggest synergistic effects of caffeic acid derivatives and sesquiterpene lactones, such as 11β,13-dihydrolactucin.

In sum, new potent anthelminthic plant extracts being active against *A. galli* were identified, demonstrating urgently needed solutions for the organic production systems of poultry products. In vivo studies utilizing artificial and natural infections are planned to validate and fortify the results.

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SL-A10 Short Lecture "Isolation and in vitro screening of bacterial endophytes from *Arctotis arctotoides* (L. f.) O. Hoffm against *Pythium* spp"

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Bacterial endophytes have been reported to produce antimicrobial compounds against fungal pathogens and metabolites that induce crop self-defence mechanisms [1]. In this study, endophytes isolated from the medicinal plant, *A. arctotoides* were screened as potential antagonists against fungal root pathogens (*Pythium* spp) of maize. Out of 26 isolates, 11 were antagonistic against against the *Pythium* spp. These endophytes significantly ($P \leq 0.05$) reduced the mycelial growth of the pathogens, with inhibition ranging from 8–64%. Using Internal Transcribed Spacers (ITS) sequencing and molecular identification to species level, the most active isolates were identified as *Serratia marcescens* NYS8, *Alcaligenes faecalis* NYS7, *Ralstonia* sp. NYR8, *Bacillus* spp. NYS9, *B. cereus* NYR11 and *Myroides odoratus* 6 G NYL18. The endophytes with inhibitory effects have potential to be used as biological control agents against *Pythium* spp. causing root rot of maize, hence, they were selected for further evaluation under greenhouse conditions.

Reference

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SL-A11 Short Lecture "Essential oil formulations for infectious diseases; a journey from basic research to antimicrobial validation of final product"

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While antimicrobial investigations on essential oils abound within the literature, less attention has been given to neglected diseases, combinations, and the incorporation of antimicrobially active essential oils into formulations. This study focuses on these aspects by presenting data from three focus areas. The first study examines essential combinations against the causative pathogens of gas gangrene. Selected essential oils, were antimicrobially evaluated and *Santalum austrocaledonicum* displayed the lowest minimum inhibitory concentration (MIC) against *Clostridium* spp. (MIC0.02 mg/mL). When tested in combination with *Cymbopogon martinii*, MIC values of 0.01–0.02 mg/mL were observed. A topical hydrogel formulation with essential oil entrapped micelles was validated and noteworthy inhibition was observed. The second study focused on the use of essential oils for acne, whereby the essential oil *Chrysopogon zizanioides* displayed noteworthy antimicrobial activity against dermatological pathogens. Emulsified lotions were developed and these completely inhibited the growth of *Cutibacterium acnes* and demonstrated cidal activity against the other pathogens. The last study focused on the application of essential oils for the prevention of catheter-associated urinary tract infections. The inhibitory and antibiofilm studies revealed several essential oils having noteworthy activity. *Cinnamomum zeylanicum* exhibited the most pronounced antimicrobial activity and was incorporated into a crosslinked film containing. The antimicrobial validation of the formulation inhibited growth of a selection of pathogens. These studies demonstrate the successful starting point of determining antimicrobial activity, followed by the most suitable formulation design and thereafter validation of an essential oil containing formulation.

SL-A12 Short Lecture "Therapeutic Effectiveness of *Arnica* tincture in experimental cutaneous Leishmaniasis caused by Leishmania braziliensis and L. tropica"

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Cutaneous Leishmaniasis (CL) is a disease caused by Leishmania parasites. Pentavalent antimonials are the leading treatment for CL despite their toxicity. In addition, the response of some Leishmania species to pentavalent antimonials is increasingly poorer, and therefore more potent therapeutic alternatives are needed. *Arnica montana* L., is a traditional medicinal plant commonly used for the topical treatment of superficial inflammatory conditions [1]. *Arnica* tincture (AT) and isolated *Arnica* sesquiterpene lactones (STLs) have antileishmanial activity [2,3]. In this work, we studied the in vitro cytotoxicity and antileishmanial activity of AT and STLs against both *L. braziliensis* and *L. tropica*. The in vivo therapeutic effect of AT was studied in hamsters experimentally infected with *L. braziliensis* and L. *tropica*. Furthermore, various semisolid *Arnica* preparations were also evaluated against *L. braziliensis*. The STLs and the AT possess a very high in vitro activity against both Leishmania species with EC_{50} values ranging from 1.9 to 5.9 µg/mL. The AT was not cytotoxic for macrophages, fibroblasts, and hepatic cells. The therapeutic response of hamsters infected with *L. braziliensis* to AT was 87.5% (19.2 µg STL/2 x day/60 d), 72.7% (19.2 µg STL/1 x d/60 d), and 67% (38.4 µg STL/1 x d/60 d). In turn, the response in hamsters infected with *L. tropica* was 100% when treated at 19.2 µg STL/2 x day/60 d and 71% at a dose of 38.4 µg STL/1 x d/60 d. These results are promising and encourage the continuation of clinical trials with AT in CL patients.

Acknowledgement

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Monday, August 29 | Short Lectures B

Analysis and authenticity – Quality control – Metabolomics

SL-B01 Short Lecture "Analysis of volatile constituents in commercial "lavender" products linked to premature thelarche and prepubertal gynecomastia"

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A number of case reports have associated exposure to the essential oil of lavender (*Lavandula angustifolia*, Lamiaceae) to the occurrence of breast enhancement (prepubertal gynecomastia) in 7–11-year-old boys, or premature breast development (premature thelarche) in 1–8-year-old girls [1–4]. The

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link was established based on in vitro estrogenic activities of lavender oil and its main constituents, linalool and linalyl acetate [1,2].
The actual presence of lavender in the products allegedly casing these symptoms was not confirmed in any of the case reports, but the most recent publication [2] listed three commercial products as source of alleged lavender exposure: Crusellas Violet Water Cologne, Mi Tesoro Agua de Violetas, and Baby Magic Calming Baby Bath. Headspace GC-MS analysis of the three products revealed the presence of ionones (6-methyl-α-ionone, isomethyl-α-ionone, t-α-ionone, and t-β-ionone) in all three products, although at vastly differing relative concentrations. Notable concentrations of linalool and linalyl acetate were detected only in the Baby Magic Calming Baby Bath. The data show that neither of the violet water products contained any lavender but are composed mainly of isolates obtained from natural sources or by chemical synthesis. Therefore, the association between exposure to lavender oil and abnormal breast enlargement in children based on these case reports cannot be supported.

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SL-B02 Short Lecture "Regulatory considerations of herbal medicines. New focus for authorization as medical devices"

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DOI 10.1055/s-0042-1758931
Background: In the last decade, there has been a global upsurge in the use of traditional medicine and complementary and alternative medicine in both developed and developing countries. This is one of the main reasons for reinforcing the surveillance of the safety, efficacy and quality control of traditional medicine, complementary and alternative medicines. Important articles concerning to the new initiative about herbal medicines as medical devices were evaluated.
Aims: The objectives of this work are to present the update of regulations of herbal medicines and to show the tools for the evaluation herbal medicines as medical devices.
Results: It is exposed some of regulations about herbal medicines, taking into account the classification of the products, modalities approved, clinical trials quality specifications among others. The WHO strategy for the development of herbal medicinal product is also showed concerning to the strength of the quality, safety and efficacy policy through reglamentation of products, practices and professionals, the importance of clinical trials in order to guarantee the safety, quality and efficacy of Natural Health Product and the main mistakes in Clinical Trials of natural products are explained. Another important result is related with the register of herbal medicines (syrup, tablet etc) as medical devices, these products are characterized by metabolomics techniques, and they have nonpharmacological action for therapeutic, some of these products will be shown. Conclusions: Herbal medicines take special considerations in this moment, for its properties. Drug Regulatory Authorities should ensure the quality, safety and efficacy of traditional medicines.
There is no conflict of Interest.

Reference

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SL-B03 Short Lecture "Consensus statement: The Phytochemical Characterisation of Medicinal Plant extracts (ConPhyMP) – from a needs assessment to the defining best practice"

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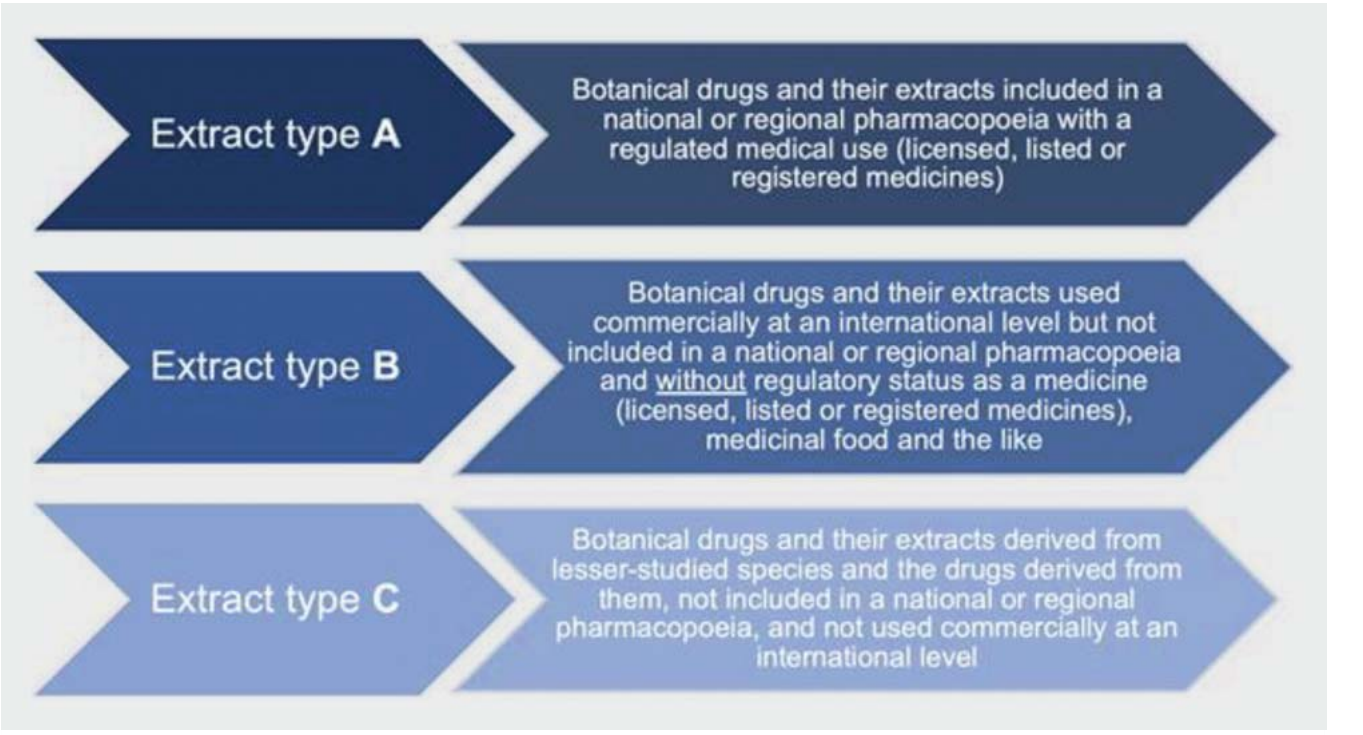
DOI 10.1055/s-0042-1758932
Extracts obtained from plants, fungi or animals pose some unique challenges: they are multicomponent mixtures of active, partially active and inactive substances, and the activity is often not based on a single target. This is a challenge facing all stakeholders, researchers, the relevant industries, and regulators. The chemical profiles of herbal extracts are crucial when considering pharmacological, toxicological, and clinical studies. Numerous analytical methods are available, and researchers may select these based on local availability and expertise. Many researchers have limited choices.
In order to advance the scientific strategy, a survey among researchers was carried out followed by the development of a consensus statement to: (1) Define plant materials, plant extracts, and herbal medicinal products; and (2) Conduct and report the phytochemical analysis of the plant extracts used in these studies enabling transparency in research.
The development of a consensus statement provides a recommendation for phytochemical analysis by classifying extracts into one of three types, capturing species importance and regulatory status. Therefore, rather than chemical criteria alone, the guidelines are based on the importance of a plant as a medicine (as defined by its inclusion in a pharmacopoeia) and, more generally, its importance in international trade (e.g., as a food supplement). For each of these extract types, a different level of detail of phytochemical characterisation is required (▶ **Fig. 1**).
The consensus statement is a 'first of its kind' and it is intended to be an orientation for authors as well as peer reviewers and editors assessing these studies for publication.

Conflict of Interest

None declared.

Funding

This project was funded in part by Dr. Willmar Schwabe GmbH & Co. KG, Germany. The donor had no influence on the design of the strategy including the survey and the interpretation of the data.



▶ **Fig. 1** Classification of medicinal plant extract used in pharmacological, toxicological, and clinical/intervention research – a novel way for guiding the requirements for extract characterisation.

SL-B04 Short Lecture "Metabolization of the herbal preparation BAY 987 204 by human gut microbiota"

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BAY 987 204 (Euphytose®) is a combination of four medicinal herb extracts, *Valeriana officinalis, Passiflora incarnata, Crataegus* sp. and *Ballota nigra*, that is traditionally used for minor anxiety and sleep disorders [1]. With the overall aim to assess the possible role of gut microbiota in mediating the activity of herbal preparations in mental health [2], a short term in vitro colonic batch fermentation model with human fecal microbiota from seven healthy donors has been used to study the microbiome-mediated metabolization, which was performed by ProDigest as previously described [3]. Annotation of the constituents present in the native preparation and of the metabolites formed during anaerobic fermentation was accomplished by UHPLC-HRMS. The results suggest that incubation with human gut microbiota leads to an intensive metabolization of the constituents of the tested product. The majority of the annotated constituents have been catabolized by gut microbiota in all donor samples. Flavonoid C-glycosides showed slower metabolization in comparison to O- and mixed C–O-glycosides, and several intermediate and final metabolites were detected. The mammalian lignans enterolactone and enterodiol were found as the major metabolites in all donor samples, resulting from yet undetected progenitor compounds, such as lignin. It is noteworthy that enterolactone was recently associated with lower prevalence of depressive symptoms and sleep disorders [4,5], suggesting that the newly produced metabolites, may be involved into the observed therapeutic effects.

Conflicts of Interest: The investigations and MEG have been funded by Steigerwald Arzneimittelwerk, Bayer Consumer Health. MRP, RMA, and OK are fully employed by Steigerwald Arzneimittelwerk GmbH.

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SL-B05 Short Lecture "FoodOmicsGR_RI: National research infrastructure for the Comprehensive Characterisation of Foods"

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The national infrastructure FoodOmicsGR_RI is a multidisciplinary consortium that joins forces from eight Greek Universities and Research Centers. Foodomics combines food/nutrition sciences with advanced analytical techniques and bioinformatics, applying a hypothesis-free approach to globally map the composition of foods or biological fluids of food consumers, to elucidate critical questions, and address new challenges of a globalized world. The consortium core (bio)chemical analysis groups provide expertise in bioanalysis, food

analysis, metabolomics, elemental metabolomics, genomics and proteomics offering the know-how and cover the breadth of the Greek agri-food sector. An array of protocols has developed for profiling and quantitative analysis. The implementation plan includes the following research axes: development of a detailed database of Greek food constituents; exploitation of "omics" technologies to assess domestic agricultural biodiversity aiding authenticity-traceability control/certification of geographical/genetic origin; highlighting unique characteristics of Greek products with an emphasis on quality, sustainability and food safety; assessment of diet's effect on health and well-being; creating added value from agri-food waste. To reach these ends FoodOmicsGR_RI has developed more than 80 laboratory protocols. Protocols are available to end-users for the realization and initiation of R&D efforts from research institutes, universities and the private sector to address priority topics including the evaluation and support of the quality of Greek products. FoodOmicsGR_RI provides access to state-of-the-art facilities, unique, well-characterised sample sets, that were generated from precision/experimental farming/breeding (milk, honey, meat, olive oil and so forth) and is open for collaboration with stakeholders and researchers and enterprises from the food industry.

SL-B06 Short Lecture "The Foodomics-GR database initiative. Literature-based Greek food composition database"

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DOI 10.1055/s-0042-1758935
The Foodomics-GR Database is a literature-based public database on food composition of Greek products. It is not actually a classic Food Composition Database (FCDB) [1], but rather a comprehensive database that is focused on individual metabolite values, similar to several recently developed comprehensive databases [2]. It contains concentration values of hundreds of chemical compounds/elements found in more than 12 different types of food of Greek origin, as they are reported in the published articles spanning more than a decade of research.
The database was created using a systematic and reproducible approach where queries containing specific keywords suggested by experts are run on literature platforms to harvest metabolite values from journal articles. Values are then validated and entered automatically in the database. The whole procedure is made possible through an extensive script-based process developed in the Python programming language.
The database is offered through a web interface [3] that allows the user to make queries based on a specific compound/element, the type of food, its region of origin, as well as a combination of the above.
Acknowledgment: This work was supported by the project "Foodomics-GR – National Research Infrastructure for the Comprehensive Characterization of Foods" (MIS5 029 057), funded by the Operational Programme NSRF 2014–2020 and co-financed by Greece and European Regional Development Fund.
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SL-B07 Short Lecture "Increasing the confidence in adulteration and authenticity analysis in food by using Trapped Ion Mobility High Resolution Mass Spectrometry"

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DOI 10.1055/s-0042-1758936
Trapped Ion Mobility Spectrometry (TIMS) is revolutionising the depth of coverage that can be obtained when analysing complex samples. This is true for proteomic, metabolomic, environmental and food samples. Frequently, TIMS can differentiate up to twice as many 'features' within a sample. This is due to TIMS adding an additional separation by cross collision area (CCS) of the molecules in the sample. Thus, samples are being analysed in 4D.
In order for authenticity studies to be undertaken, the first step of finding all 'features' within a sample is more discriminatory if CCS values are included. Once 'feature' sets have been obtained for both authentic samples and samples being tested then statistical analysis can be applied to determine authenticity.
This presentation will describe in greater detail these points and illustrate with field examples.

Monday, August 29 | Short Lectures B
Biotechnology – Bioengineering

SL-B08 Short Lecture "Enzymatically engineered natural products as a source of invaluable bioactive compounds"

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DOI 10.1055/s-0042-1758937
Biotransformation of natural products (NPs) using enzymes represent an alternative to obtain bioactive compounds with original scaffolds and possible new modes of action. The concept of this approach is to start from abundant NPs to generate derivatives using chemoenzymatic reactions. In this context, instead of using a pure enzyme, we have successfully developed an original method based on the use of the enriched pool of enzymes secreted by a phytopathogenic fungus Botrytis cinerea to generate bioactive compounds [1]. The reaction mixtures are monitored by UHPLC-PDA-ELSD-HRMS metabolite profiling to highlight newly generated compounds. We found that the modulation of the reaction conditions, notably by increasing solvent concentration, led to the generation of unusual compounds. Promising reactions were selected and scaled up to generate sufficient amounts of biotransformed compounds. In most cases, it was possible to improve the structural diversity of the genuine NPs resulting in active compounds from inactive scaffolds. To rapidly isolate, characterize and study the biological activities of the generated compounds, the use of high-resolution preparative chromatographic methods was mandatory. For this we develop efficient targeted isolation methods based chromatographic gradient transfer and dry load. Chiral separation was used to check whether stereochemistry could impact biological properties. This approach resulted in a library of more than 170 compounds, some of which show interesting antibacterial properties, and anti Wnt activity

in TNBC cells [2–4]. The possibilities and limitations of this technology will be illustrated by recent investigations performed in our laboratory.

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SL-B09 Short Lecture "High-throughput whole-cell biotransformation approach for fast and efficient chemodiversification of natural products"

Authors Huber R[1,2], Marcourt L[1,2], Schnee S[3], Michellod E[3], Wolfender J-L[1,2], Gindro K[3], Ferreira Queiroz E[1,2]

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DOI 10.1055/s-0042-1758938

Biotransformation is known to be a green, affordable and efficient way to generate chemodiversity. Previous works in our lab have shown that the use of secreted enzymes produced by fungal species ("fungal secretome") was highly effective to generate complex molecules from simple starting materials [1–4]. Despite these successes, the fungal secretome approach suffers from some drawbacks. For example, non-secreted enzymes are not recovered and enzymes requiring a cofactor lose their activity. Conventional whole-cell biotransformation can address these issues, but is time- and substrate-consuming, and therefore not suitable for screening approaches. The present work describes the use of fungal cultures in 96-well plates to perform high-throughput whole-cell biotransformations. This method allows the screening of the biotransformation capacity of a large number of fungal strains with small amounts of substrates. The approach benefits from recent advances in rapid and efficient annotation workflows using UHPLC-HRMS/MS that allow identification of the generated compounds and provide insight into their possible structure. A proof of concept of this screening method was performed with 30 strains of the necrotrophic fungal pathogen *Botrytis* sp. (*B. cinerea* and other close species) to identify those capable of hydroxylation reactions on various substrates. The most promising reactions were performed on a large scale, allowing the isolation of a series of hydroxylated terpene derivatives in a targeted manner by high-resolution chromatography. These preliminary results show that the proposed method allows a fast and efficient screening of biotransformation reactions using living fungi in order to generate libraries of compounds for biological screening.

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[4] Huber R, Koval A, Marcourt L et al. Chemoenzymatic synthesis of original stilbene dimers possessing Wnt inhibition activity in triple-negative breast cancer cells using the enzymatic secretome of Botrytis cinerea Pers. Front Chem 2022; 10: 881298

SL-B10 Short Lecture "In vitro propagation of the medicinal halophyte Polygonum maritimum L. and phenolic composition of produced plants"

Authors Custódio L[1], Ślusarczyk S[2], Matkowski A[2], Fernandes E[1], Castañeda-Loaiza V[1], Pereira C[1], Rodrigues M[1]

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DOI 10.1055/s-0042-1758939

Polygonum maritimum L. is a medicinal halophyte with anti-inflammatory properties, ascribed to flavonoids, such as myricetin and quercetin glycosides [1–3]. Therefore, this study aimed at establishing a micropropagation procedure of *P. maritmum* for potential commercial cultivation, by enhancing shoot multiplication, rooting and acclimatization procedures, followed by the assessment of the phenolic profile of produced plants. The combination of 3 mg/L BA + 0.1 mg/L IAA induced the maximum shoot formation (10.3), which significantly increased in the second cycle (18.3). The best rooting capacity was observed on shoots derived from the control medium (100%), followed by 2 mg/L KIN (97%) and 3 mg/L BA + 0.1 mg/L IAA (90%), however the shoot number in the end of rooting phase was higher on shoots derived from 3 mg/L BA + 0.1 mg/L IAA (6.16). The plant growth regulators used in the multiplication phase influenced survival in the acclimatization process, and plants derived from the control medium had the highest survival percentage (63.1%). Acetone extracts made from aerial organs of micropropagated *P. maritimum* showed a predominance of the flavonoid myricetin-3-O-rhamnoside (8.135 mg/g), ascribed with anti-inflammatory ability. Overall, *P. maritimum* was successfully micropropagated showing their potential as a medicinal crop for extraction of anti-inflammatory molecules.

Funding

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SL-B11 Short Lecture "Novel neuroprotective metabolites produced via biotransformation of cyclocephagenol by *Alternaria eureka* 1E1BL1"

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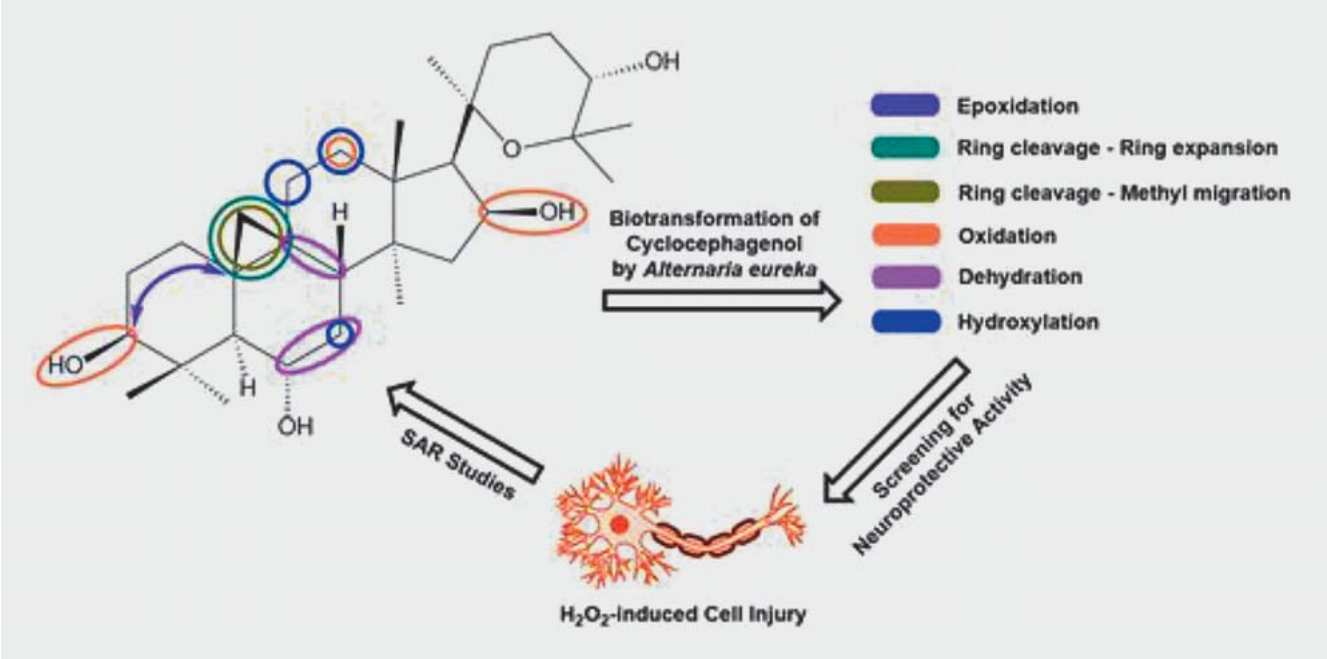
Neurodegeneration refers to the loss of structure/function of neurons leading to neurological diseases including Alzheimer's and Parkinson's. The discovery of novel therapeutics against neurodegenerative diseases has been an area of intense research as neurodegenerative diseases are a huge burden on society and the economy [1]. Numerous studies reported that natural products have



▶ **Fig. 1** Biotransformation of cyclocephagenol by *Alternaria eureka* 1E1BL1 yielded new metabolites with neuroprotective activity.

the potential to prevent and treat neurodegeneration. Among these studies, the neuroprotective activities of cycloartane-type saponins are noteworthy [2,3].

In our preliminary studies, the neuroprotective activity of cyclocephagenol, an aglycone of cyclocephaloside I from *Astragalus microcephalus* [4], was screened for H_2O_2-induced injury in SH-SY5Y cells. Based on the promising bioactivity of cyclocephagenol, the aims of this study were: i) to perform microbial transformation studies on cyclocephagenol using *Alternaria eureka* followed by isolation and structural characterization of the metabolites; ii) to investigate neuroprotective activities of the metabolites; iii) to understand structure-activity relationships towards neuroprotection.

As a result, the biotransformation of cyclocephagenol by *Alternaria eureka* (▶ **Fig. 1**) yielded twenty-one new metabolites with modifications including monooxygenation, dehydration, methyl migration, epoxidation, and ring expansion. In addition to chemical diversity, biotransformation provided several novel compounds having potent neuroprotective activity against H_2O_2-mediated cell death. Further studies revealed that selected compounds reduced the amount of ROS and preserved the integrity of the mitochondrial membrane.

Acknowledgments

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SL-B12 Short Lecture "Biotechnological valorisation of seeds of two halophyte species, *Suaeda vera* and *Arthrocaulon macrostachyum*''

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Plant seeds, including those from salt tolerant (halophyte) plants, such as *Chenopodium quinoa*, can be considered as functional foods if they combine an adequate nutritional profile with health promoting properties [1,2]. Having this mind and targeting the biotechnological valorization of edible halophyte plants in the context of salinisation and climate change, this work aimed to determine the nutritional, biochemical and functional properties of seeds from *Suaeda vera* and *Arthrocaulon macrostachyum*. Proximal composition, fatty acids and minerals were determined, along with the phenolic composition and antioxidant properties of ethanol and water extracts. S. vera seeds had the highest ash and fiber levels. Linoleic acid was the main fatty acid in both species, followed by oleic and palmitic acids. S. vera had the highest level of sodium, potassium, magnesium, iron and copper. The highest content of total phenolics were detected in the ethanol extract of *A. macrostachyum*, and aqueous ethanol (70%) extract of *S. vera*, and the main detected compounds were gallic, cafeic and salicylic acids. *A. macrostachyum* exhibited in general a higher antioxidant capacity. Our results suggest that *S. vera* and *A. macrostachyum* seeds could be further explored as sources of fatty acids, minerals and phenolic compounds with antioxidant properties. The authors declares that there is no conflict of interest.

Funding

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Azul (FA-05-2017-028) and PRIMA (HaloFarMs project). LC was sustained by FCT Scientific Employment Stimulus (CEECIND/00425/2017), and V C–L by a FCT PhD grant (2020. 04541.BD).

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SL-B13 Short Lecture "Cell cultures of *Dracocephalum ruyschiana* and *Juniperus communis* – valuable sources of skin protecting and regenerating compounds"

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DOI 10.1055/s-0042-1758942

Plant cell cultivation is a promising technology for sustainable production of plant secondary metabolites. Extracts containing such compounds are particularly interesting for cosmetics industry. Over the last decades there has been significant increase in use of plant cell culture derived ingredients in cosmetics and the demand continues to grow. Aim of this study was to evaluate various extraction processes to leverage the full potential of *Dracocephalum ruyschiana* and *Juniperus communis* cell cultures. Various solvents (water, ethanol, glycerin, propandiol, pentylene glycol) as well as supercritical fluid extraction was performed to produce extracts. Moreover, remaining by-products after the primary extraction underwent second extraction step to explore the potential of the biorefinery approach for production of various ingredients form the same culture. Chromatography analyses showed the presence of phenolic compounds and flavonoids in *D. ruyschiana* cell biomass extract, with rosmarinic acid, and caffeic acid being the dominating ones. *J. communis* cell culture extracts were rich in procyanidins. Remaining biomass after the primary extraction proved to be a valuable source of amino acids, polysaccharides, glycoproteins. Chemical composition varied depending on the cell cultivation conditions and extraction protocols. In vitro testing showed high safety of the extracts in cytotoxicity and phototoxicity assays. Stimulatory effect on proliferation of skin cells was observed. Quantification of reactive oxygen species by flow cytometry showed high antioxidative activity. Chemical composition and bioactivity data substantiates the application of plant cell cultivation approach for production of *D. ruyschiana* and *J. communis* derived cosmetic ingredients.

Acknowledgments

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Monday, August 29 | Short Lectures C

Ethnobiology – Ethnobotany – Biodiversity

SL-C01 Short Lecture "Implementing the Nagoya protocol: outcomes of a UK-Guatemala collaborative project"

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DOI 10.1055/s-0042-1758943

International partnerships following the Nagoya Protocol (NP) and the Convention on Biological Diversity (CBD) remain a challange. A collaboration requires both the right mix of stakeholders and a transparent process. We tackled this issue by using a multi-stakeholder transdisciplinary platform in Guatemala. A collaborative project, funded by Darwin Initiative, was formed between stakeholders in Guatemala [a Council of Indigenous elders, a government agency (CONAP), a university] and the UK [an industry SME, a university] with the support of other experts.

The aim was creating a collaboration with an international commercial partner to build a model that would provide tools useful for an NP-compliant international partnership for the use of genetic resources (GRs).

Many of the procedural requirements were completed, including a draft ABS agreement with the local community, then a legislative problem was encountered. Recently introduced regulations specify a 50% benefit-sharing, based on a law preceding in date both CBD and NP, rather than allowing stakeholders to agree a figure by mutual discussions. A 50% share is unacceptable to the partners. Lawyers have evaluated alternative solutions, but the problem remains an obstacle to the availability of Guatemala to collaborate in international scientifically developmental projects using GRs, despite its ratification of NP and CBD.

In conclusion, the project has built an international network and enabled indigenous participants to gain direct access to foreign stakeholders but has not yet been able to model an effective collaboration; that will require regulatory changes by the Guatemalan government.

The authors declare no conflicts of interest.

SL-C02 Short Lecture "Medicinal plants from Brazil: the contribution of Giuseppe Raddi"

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DOI 10.1055/s-0042-1758944

Brazil's flora is rich in medicinal plants due to the wide plant biodiversity, which is enriched by millennial Amerindian traditional knowledge. However, all the ecosystems, including Amazon rainforest, have been quickly replaced by monocultures of sugarcane, soybeans, eucalyptus, and livestock. The development of bioproducts from Brazilian plants is currently strongly stimulated. In order to contribute with this, our research group is working for decades in recover data about useful Brazilian plants registered in historical bibliography. Emphasis is done in the work produced by European naturalists, that traveled in the country in 19th century. Among these scientists is the Italian botanist Giuseppe Raddi (1770–1829). Raddi arrived in Rio de Janeiro in 1817 and have identified several useful plants around that city. His work is registered in many publications, being one of the most important the Flora Brasiliana published in 1975/76. In this work Raddi describes important species as *Cariniana estrellensis* (Raddi) Kuntze (Anacardiaceae, jequitibá), *Cyrtopodium glutiniferum* Raddi (Orchidaceae) and the spice *Schinus terebinthifolius* Raddi, known and used in many parts of the world.

▶ **Fig. 1**

SL-C03 Short Lecture "Quroum sensing inhibitory activity by Catalan medicinal plant extracts against *Staphylococcus aureus*"

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DOI 10.1055/s-0042-1758945

Infectious diseases are the leading cause of mortality in the world, and the rise of multidrug-resistant pathogens presents an urgent threat to healthcare across the globe. The Gram-positive pathogen Staphylococcus aureus was responsible for 120,000 bloodstream infections and 20,000 deaths in the US in 2017 [1]. Given the increasingly limited effectiveness of antibiotics, anti-virulence compounds which inhibit quorum sensing (QS) pathways may represent a new path to addressing these urgent health challenges. Medicinal plants are an important source of antimicrobial natural products [2,3], including those exhibiting anti-virulence activity in antibiotic-resistant pathogens [4]. Based on previous ethnobotanical work undertaken in the Catalan linguistic area (Spain) [5], 79 plant extracts representing 77 species were investigated for their potential to inhibit QS in S. aureus. Ethanolic extracts from three medicinal plants (*Cistus clusii* Dunal; *Juniperus oxycedrus* L.; and *Pinus halepensis* Mill.) exhibited a concentration-dependent response indicating anti-QS activity in *S. aureus* reporter strains for agr I–III (IC_{50} 64–256 µg/mL). *Cistus clusii* and *P. halepensis* extracts were well tolerated by human keratinocytes (HaCaTs) for all concentrations tested (IC_{50} 32–1024 µg/mL) and *J. oxycedrus* for concentrations lower than 256 µg/mL. According to the literature, the presence of phenylpropanoid compounds is well-known in *C. clusii*; the chemical composition of *P. halepensis* is characterized by monoterpene hydrocarbons, mainly α-pinene; and the *J. oxycedrus* composition is also characterised by high contents of α-pinene and β-myrcene. Future work will focus on isolation and chemical characterization of the most bioactive compounds from these species.

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SL-C04 Short Lecture "Ethnopharmacological potential of lingonberry (*Vaccinium vitis-idaea* L.) phenolic fractions"

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DOI 10.1055/s-0042-1758946

Lingonberry (*Vaccinium vitis-idaea* L.) raw materials have traditionally been used for urinary tract infections, gastrointestinal or neurodegenerative diseases, and related inflammatory disorders, which are overall related to free radical damage and the existence of triggering pathogenic strains in the human body [1,2]. However, there are not enough scientific data to confirm the predominant specialized metabolites, responsible for the traditional therapeutic use of lingonberries. Taking into account antimicrobial, antioxidant, and anti-inflammatory activities, related to the traditional application of lingonberries and increased demand for natural compounds as antimicrobial and anti-inflammatory drugs with low toxicity and high therapeutic value, special attention in the present study has been directed towards these modes of action. Crude dry extracts of lingonberry leaves and fruits were fractionated by column chromatography using Sephadex LH-20 and analyzed by the validated HPLC-PDA method. For each fraction, potential inhibiting properties against different bacterial strains and hyaluronidase, ability to scavenge hydrogen peroxide, and effect on its production in a macrophage culture J774 were examined. Results displayed higher bioactivities of particular fractions than that of crude extracts and elucidated particular compounds as candidates in pharmaceuticals. Trimeric and dimeric proanthocyanidins-rich fractions had the strongest antimicrobial, antioxidant, and anti-inflammatory potential. Present results suggested that proanthocyanidins could be one of the biomarkers providing strong therapeutic effects described in traditional indigenous medicinal systems. It might be a promising approach for further purification and bioanalysis of proanthocyanidins from lingonberries, thus elucidating single potent molecules, which could be developed into innovative products in the food, cosmetic, and pharmaceutical industries.

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SL-C05 Short Lecture "Developing new approaches for the chemical characterization of *Anacyclus pyrethrum* var. *pyrethrum*"

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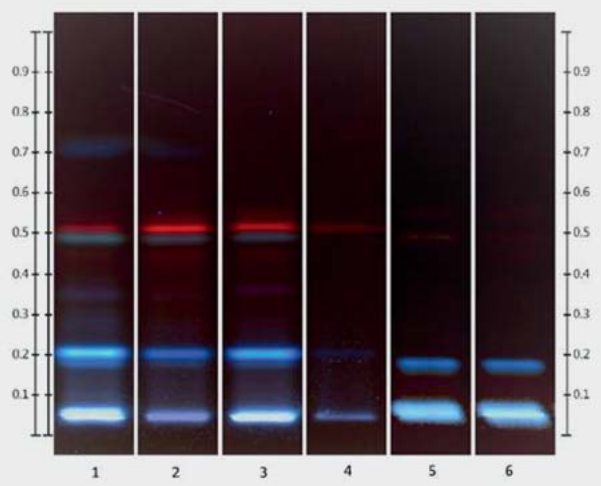
The variety pyrethrum of *Anacyclus pyrethrum* (L.) Lag. is an endemic native to Morocco, Algeria and Spain that is intensively harvested and commercialised across the Middle East and South Asia. The roots are widely used to treat a di-



▶ **Fig. 1** HPTLC image of the developed method for *A. pyrethrum* var. *pyrethrum* field (tracks: 1, 2 and 3) and market (tracks: 5 and 6) samples using Pelitorine (track 4) as a marker compound. Stationary phase: Silica gel 60 F254. Mobile phase: Toluene, ethyl acetate and formic acid (9.5;2;0.5).

verse range of diseases, including dental infections, gum, rheumatism, sickle cell disease and epilepsy [1,2]. With its increasing commercial importance, a better understanding of the species value chains, and the chemical profile of products found in the trade is needed.

An ethnobotanical survey was carried out among 140 informants, including villagers, herbalists, and traditional healers. In parallel, identification phytochemical methods of field collections and market samples (42 samples) were developed using HPTLC and NMR.

The survey identified further uses such as treating stomach ailments and haemorrhoids. Roots were the only part used as maceration using honey (85%), olive oil (6%), water (5%) or toothpaste (4%). Analytical markers were identified, and several chromatographic conditions were optimized resulting in a good separation of alkylamides. For HPTLC, pellitorine, dodeca-2E,4E,8Z,10E, Z-tetraenoic acid isobutylamide, ursolic acid, eugenol, and cinnamic acid were identified as possible marker compounds. Preliminary HPTLC and NMR results showed a slight variation in the profiles obtained, especially for the market samples (▶ **Fig. 1**).

In addition to conventional methods, HPTLC and NMR methods help in a robust quality assessment of *A. pyrethrum* var. *pyrethrum*. The increasing demand for products derived from this species will require a more systematic use of quality control measures as well as an assessment of the trade's sustainability.

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SL-C05B Short Lecture "MicroRNA as a new active substance isolated from *Viscum album* L."

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Recently, the therapeutic potential of plant derived miRNAs has attracted great attention. MicroRNA (miRNA) has been considered as new bioactive ingredients in medicinal plants [1]. Through screening abundant miRNAs in European mistletoe, val-miR218 showed high potential of anti-cancer effects against osteosarcoma. To clarify its molecular mechanism of action, we sequenced val-miR218 associated RNAs as well as its down-regulated RNAs. As a result, a total of 61 genes were considered as the direct targets of val-miR218. The mRNA and protein expression of the targets was confirmed by RT-qPCR and western blot. The interaction between the val-miR218 and miRNA recognition elements (MREs) was validated by dual-luciferase assay. Interestingly, these targets were related to basic cell functions such as cell cycle, DNA replication and cell morphology, suggesting that val-miR218 significantly inhibit cell growth, and arrest osteosarcoma cells in G0/G1 phase through influencing basic cell activities. Mistletoe extracellular vesicles offered val-miR218 effective protection and mediated the uptake of val-miR281 by human cells. Moreover, tests of the efficacy of val-miR218 in vivo showing reduction of tumor volume, confirmed the therapeutic potential.

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Monday, August 29 | Short Lectures C

Natural compounds from marine organisms, fungi and microorganisms – Endophytes and microbes (incl. Microbiome)

SL-C06 Short Lecture "Chemical and biocidal characterization of metabolites from endolichenic fungus *Xylaria* sp."

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The endolichenic fungi are a group of endosymbionts that reside in the intercellular spaces of the lichen thallus in association with the photobiont, without causing any noticeable disease symptoms to their host. The species diversity of endolichenic fungi is hardly known and it is influenced by climate, host lineage and geographic location. These organisms form a diverse group of unexplored mycological flora with a great potential to produce a variety of bioactive secondary metabolites with promising medicinal or agricultural applications [1].

Our aim is the discovery of endolichenic fungal strains and their metabolites that can be used as biocontrol agents. As source material was used the lichen *Hypogymnia tubulosa* that grows as epiphyte on the bark of the endemic tree, *Pinus canariensis*. We selected the strain P6 based on its biopesticide activity and was identified as *Xylaria* sp. based on morphological characteristics and molecular analysis.

The bioguided fractionation of EtOAc extract from liquid culture of P6 on major scale resulted in the isolation of four major metabolites identified with spectroscopic techniques (1D NMR, 2D NMR) as piliformic acid (1) [2], 2-carboxymethyl-3-hexylmaleic acid anhydride (2) [3], 2-hydroxyphenylacetic acid (3) and 4-hydroxyphenylacetic acid (4) (▶ **Fig. 1**).

The extract and the isolated compounds were tested against herbivorous insect pests (*Spodoptera littoralis, Myzus persicae, Rhopalosiphum padi*), phytopathogenic fungi (*Botrytis cinerea, Fusarium oxysporum* and *Alternaria alternata*)

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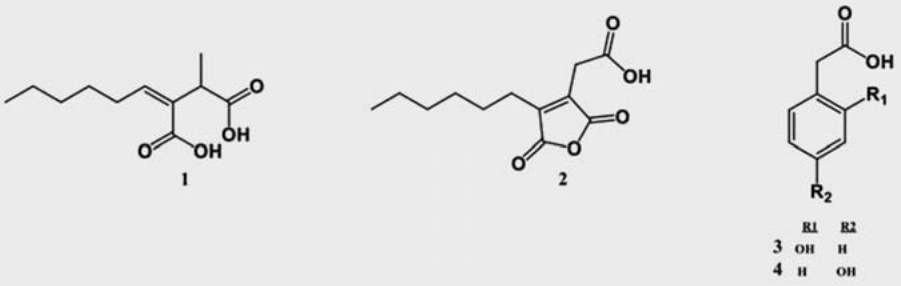

▶ **Fig. 1** Structures of metabolites **1**–**4**.

and plant parasitic nematode (*Meloidogyne javanica*) to assess their potential as biocontrol agents.

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SL-C08 Short Lecture "Astin localization in *Aster tataricus* and astin production by *Cyanodermella asteris*"

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DOI 10.1055/s-0042-1758950

Aster tataricus is a plant used in Chinese Traditional Medicine with antitussive and anticancer activity. Halogenated non-ribosomal cyclopeptides, called astins, have been identified as the main cytotoxic compounds in plant. The astins are a family of 13 compounds (astin A to P), differing e.g., in the presence or absence of hydroxyl groups or halogens [1]. These compounds have been discussed as potential anticancer drugs. Recently, it has been found that some of the astins are not produced by the plant, but by an endophytic fungus called *Cyanodermella asteris* [2], isolated from *A. tataricus*. However, as not all known astins could be detected in the lab-cultivated endophyte, it has been suggested that some plant-fungus interaction is vital for the production of the complete astin series [3]. To study this interaction, we performed cultivation studies with the fungus and plants, and used mass spectrometry imaging (MSI) to visualize the astin distribution in different plant tissues. An *A. tataricus* plant was harvested, and various tissues such as leaves, roots, and stems were embedded, sectioned, and after sample preparation analyzed by AP-MALDI-MSI. We found nonhomogeneous astins distribution in the different sections, and also differing relative abundance in each tissue. In extensive fungus cultivation experiments, using GNPS/Cytoscape to evaluate the data we found in NaCl-supplemented media, all known astins are produced by *C. asteris*. This suggests that the local environment inside the plant results in the production of the whole astin series, rather than a plant metabolic contribution that results in the observed astin diversity.

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Monday, August 29 | Short Lectures C

Circular economy – Bioeconomy – Green technologies – Sustainable development of agricultural/industrial by-products

SL-C09 Short Lecture "Pink berries and lavender extracts from perfume industry wastes, examples of sustainable upcycled cosmetic ingredients"

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DOI 10.1055/s-0042-1758951

The fragrance industry requires a huge amount of raw material and generates substantial wastes in the process of extracting aromatic compounds. When unaltered by the extraction process, they still contain precious non-volatile phytochemicals.

The pink berries (*Schinus terebinthifolia* Raddi), cultivated in Madagascar are extracted by supercritical CO_2 for fragrance industry. The hydro-alcoholic extract of unaltered wastes contains the active biflavonoids (mainly hinokiflavanone, mazasinoflavanone and amentoflavone), with antioxidant and anti-inflammatory properties.

The lavender oil (*Lavandula angustifolia* Mill., Lamiaceae) is a famous essential oil from the south-east of France. Once the stems and flowers are distillated, the spent material accumulate in the fields. Yet, they still contain the famous phenolic acids like rosmarinic acid, well known for their antioxidant and soothing properties.

Each extract has been analyzed by LCMS and chromatograms were compared before and after fragrance extraction to ensure the unaltered state of the waste. The quantification of a specific marker has been developed to check the quality of each produced batch.

Their in vitro and in vivo cosmetic efficacy have been assessed, showing that the upcycled pink berries extract helps to maintain a healthy scalp by protecting it from erythema, flaking and itching in conditions of increased moisture and friction. By strengthening the barrier function and mitigating the oxidative and inflammatory cascades, the upcycled lavender extract shields sensitive skin from indoor pollutants-induced irritation and enhances skin luminosity.

From the fragrance industry waste, a huge potential of opportunities is still available for future launches of sustainable upcycled cosmetic ingredients.

SL-C10 Short Lecture "Asexual propagation and biochemical properties of *Sarcocornia perennis* ecotypes under cultivation in an integrated multi-trophic aquaculture (IMTA) system"

Authors Fernandes E[1], João Rodrigues M[1], Castañeda-Loaiza V[1], Albericio F[2], Custódio L[1]
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DOI 10.1055/s-0042-1758952

Sarcocornia species grow naturally on salt marshes, are extreme salt-tolerant halophytes and considered as a promising vegetable to human consumption due their adequate nutritional profile, levels of bioactive components, including polyphenols, and functional properties, such as antioxidant [1]. Targeting its commercial cultivation, this work aimed at the optimization of the asexual reproduction of *Sarcocornia perennis* (Mill.) A. J.Scott ecotypes, under an IMTA system. Cuttings were collected in the Southern Portugal (Algarve), submitted to hormone rooting treatments, planted on substrate (coir and perlite) and maintained waterlogged on an IMTA system with different salinities. The plant survival was evaluated and collected biomass was evaluated for productivity (amount of produced biomass) and minerals, while total phenolics and radical scaveging activity (RSA) were determined on methanol extracts. Cuttings had higher rooting rates with hormone treatment (87,5%). Plants had better productivity on the lower salinity. Sodium was the major element (> 70 mg/g), followed by K, Mg and Ca. The highest total phenolics content, RSA and copper and iron chelating activity were observed on methanol extracts from plants grown on lower salinity.
The authors declares that there is no conflict of interest.

Funding
Foundation for Science and Technology (FCT), the Portuguese National Budget and operational programmes CRESC Algarve 2020 and COMPETE 2020 (UIDB/04326/2020, PT-IL/0003/2019, PTDC/BAA-AGR/1391/2020, EMBRC.PT ALG-01-0145-FEDER-022121 projects), and PRIMA (HaloFarMs project). LC was sustained by FCT Scientific Employment Stimulus (CEECIND/00425/2017), and EF by a FCT PhD grant (UI/BD/151301/2021).

Reference

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SL-C11 Short Lecture "Effect of saline irrigation on biochemical properties of *Salicornia ramosissima* L. under cultivation in an integrated multitrophic aquaculture (IMTA) system"

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DOI 10.1055/s-0042-1758953

Salicornia ramosissima L. (sea asparagus) is an edible succulent halophyte with organoleptic, nutritional and biological properties that may render potential to be used as a functional food. In this context, and having in mind its sustainable and commercial cultivation, we determined the effect of the production system (IMTA system: aquaponics), under two salinity conditions, in the biochemical properties of produced plants. For this, ethanol and methanol extracts were made from dry biomass from plants cultivated in aquaponics and irrigated with water from a well (conductivity of 20.7 us/cm), and with a diluted effluent from an outdoor tank producing sea bream (conductivity of 27.1 us/cm). Extracts were evaluated for antioxidant activity, (2,2-diphenyl-1-picrylhydrazyl – DPPH- method), anti-inflammatory (nitric oxide -NO- inhibition), and enzymatic inhibition (a-glucosidase and tyrosinase, related with type-2 diabetes and hyperpigmentation, respectively). The extracts exhibited relevant antioxidant activity and high levels of total phenolic compounds, higher at the higher salinity. A high tyrosinase inhibitory capacity was observed, higher at the lowest salinity. Samples had reduced a-glucosidase inhibition and no anti-inflammatory capacity. Our results indicated that sea asparagus can be cultivated in aquaponics, and that the irrigation salinity influences the biochemical properties of sea asparagus.
The authors declares that there is no conflict of interest

Funding
Foundation for Science and Technology (FCT), and Portuguese National Budget (UIDB/04326/2020 and PTDC/BAA-AGR/1391/2020 projects), Fundo Azul (FA-05-2017-028) and PRIMA (HaloFarMs project). LC was sustained by FCT Scientific Employment Stimulus (CEECIND/00425/2017), V C–L by a FCT PhD grant (2020. 04541.BD).

Wednesday, August 31 | Willmar Schwabe Research Scholarship 2021

WSA "A South African secret: an endemic *Plectranthus* sp. targeting the pathogenic factors associated with acne vulgaris"

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DOI 10.1055/s-0042-1758954

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous follicle caused by the Gram-positive bacteria, *Cutibacterium acnes*. The disease affects approximately 9.4% of the world population, making it the eighth most prevalent disease worldwide [1]. In nature, microorganisms rarely exist as planktonic microorganisms but rather as a complex biofilm. Biofilms contribute to antibiotic resistance seen in acne vulgaris and wounds [2]. An endemic South African *Plectranthus* species was selected for further studies based on its traditional use and the lack of research on the plant and its compounds. Traditionally, this plant is used in South Africa by the Zulu and Xhosa communities to treat wounds and other skin maladies [3]. The *Plectranthus* sp. of interest and its compounds were tested and validated for their potential to target the pathogenic factors associated with acne vulgaris, post-inflammatory hyperpigmentation, wounds and antibiotic resistance. These acne pathogenic factors include abnormal keratinisation, sebum production, bacterial proliferation and inflammation [4]. Currently, there is no treatment available that can target all the pathogenic factors associated with acne vulgaris, including antibiotic resistance associated with the disease. In vitro and in vivo studies confirmed the potential of the identified South African *Plectranthus* sp. targeting the pathogenic factors associated with acne vulgaris, including severe forms of acne, without having an adverse effect on the skin. These results support the use of the South African *Plectranthus* sp. of interest, and its novel identified compounds as acne actives that can be used to develop anti-acne technologies.

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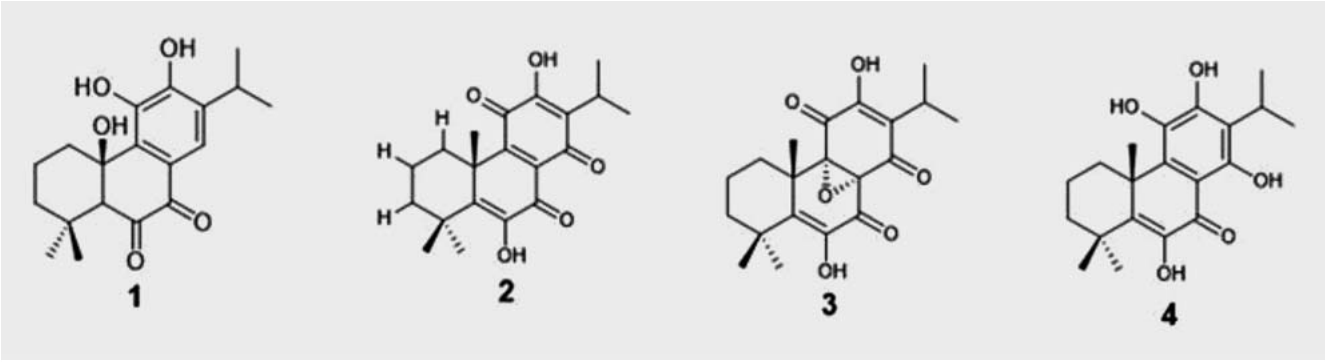
Planta Med 2022; 88: 1385–1588



▶ **Fig. 1** Compound isolation from *P. mutabilis*: new nor-abietane diterpene, (+)-(5S,10R)-10,11,12-trihydroxy-6,7-dioxo-20-nor-abieta-8,11,13-triene (**1**), Coleon-U-quinone (**2**), 8α,9α-epoxycoleon-U-quinone (**3**), and Coleon U (**4**).

Wednesday, August 31 | Egon-Stahl-Award in Bronze

ESA "Isolation and design of diterpenoids from *Plectranthus* species"

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DOI 10.1055/s-0042-1758955

P-gp is one of the major contributors to multidrug resistance (MDR) in cancer. *Plectranthus* plants are a potential source of diterpenoids reported as P-gp modulators [1]. The acetone extracts of sixteen *Plectranthus* spp. were prepared by ultrasound-assisted extraction method (10% (w/v). *P. hadiensis* and *P. mutabilis* extracts were found to be the most bioactive and the compounds responsible for their bioactivity were identified. 7α-acetoxy-6β-hydroxyroyleanon isolated from *P. hadiensis* was cytotoxic against the aggressive triple-negative breast cancer. This compound, its hemisynthetic derivative 7α-acetoxy-6β-benzoyloxyroyleanone (12BzRoy) and 6,7-dehydroroyleanone (DHR) from *P. madagascariensis* were employed as lead molecules for the synthesis of self-assembly nanoparticles using oleic acid (OA) and squalene (sq) as inducers. Roy-OA, DHR-sq, and 12BzRoy-sq drug conjugates were successfully synthesized. Roy-OA NPs released profile was determined. DHR.sq and, Roy-OA NPs were found to have less bioactivity when compared with DHR and Roy respectively. These findings suggest that nanoassemblies serve as prodrugs for the release of cytotoxic lead molecules.

Bio-guided fractionation of *P. mutabilis* extract resulted in the isolation of a new nor-abietane diterpene, (+)-(5S,10R)-10,11,12-trihydroxy-6,7-dioxo-20-nor-abieta-8,11,13-triene (1) alongside three known abietane-type diterpenoids, Coleon-U-quinone (2), 8α,9α-epoxycoleon-U-quinone (3), and Coleon U (4) (▶ **Fig. 1**). Compounds 2, 3, and 4 inhibit P-gp activity in NCI-H460/R cells after 72 h of exposure and revert doxorubicin (DOX) resistance in subsequent combined treatment. All compounds did not influence the ABCB1 expression in NCI-H460/R cells, while the extract significantly increased it. Computational data indicates a biosynthetic relation between 2, 3, and 4 and suggest that both 2 and 3 are formed directly from 4 [2,3].

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Wednesday, August 31 | Botanical Products Session

Quality of natural health products: Status quo and future

SL-BP01 Short Lecture "The reality of food supplements: analytical investigations of different classes of commercial products – exciting claims and frightening food fraud"

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DOI 10.1055/s-0042-1758956

Food supplements are regulated in Europe/U.S. by food law but are often marketed and presented similar to medicinal drug products, which are regulated by the more stringent drug legislation. FS are economically successful, but can harbor risks (poor quality, incorrect declaration).

Plant-based FS ("botanicals") are often marketed with health claims, similar to those used for registered drug products. As standardization is more complicated compared to products containing only one compound, quality problems might occur.

The present study aimed to obtain better insight into the quality of FS. Commercial products from different classes of botanicals (saffron-, bilberry-, broccoli-based products) were investigated for identity and content using ICH-validated product-specific analytical protocols. Fourteen broccoli-based FS (*B. oleracea* var. *italica*) were investigated for >7 glucosinolates, whereby >50% of test samples did not meet specifications [1]. Investigation of 14 bilberry-containing FS (*Vaccinium myrtillus*) revealed major problems for 45% of the products with unacceptable quality (bilberry-free products, anthocyanin/tannin-free products, falsification) [2]. Nine saffron-FS (*Crocus sativus*) were investigated for crocin/picrocin/safranal content. One product was falsified with crocin-containing extracts from *Gardenia jasminoides*.

The present study indicates in part strong quality problems for FS, not acceptable for consumer and patient. Intellectual energy and phytochemical expertise of the producers of falsified products is obvious, making it difficult for control labs to detect such food frauds. Therefore, analytical quality control must be intensified, and methods applied should be capable to authenticate plant material unequivocally. More governmental strategies and efficient regulation of health claim declarations are needed to protect consumer and patients.

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SL-BP03 Short Lecture "HPLC-DAD analysis of CBD content in hemp oils and food supplements"

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DOI 10.1055/s-0042-1758958

Cannabidiol (CBD) has become one of the most popular natural compound applied in various different ways. Although, it has evidence based medical application (i.e., Epidiolex® for seizure disorder), the indications of widely available CBD-containing food supplements are mostly not supported. Furthermore, the frequent or long-term application of cannabidiol without medical supervision has not been investigated. Finally, the loose and uncontrolled legal environment regulating the food supplements market, the Cannabis products CBD-content is not controlled by National authorities in EU. Our aim was to develop a fast, high-throughput analytical method to screen CBD enriched oils and food supplements and to determine the CBD content of the investigated products available on Hungarian market. Robust and reliable HPLC-DAD method for the screening of products with simple sample preparation was developed. The screening process allowed us to check more than 25 products (21 CBD-enriched oils, 4 hemp oils). Hemp oils were CBD-free, while CBD-enriched oils can be categorized into three groups: in case of 4 products the measured CBD-content met the declared value on the label, while 9 products contained up to 63% less CBD and 8 products were with 54% more than it was declared on the labels. Based on our results, it is possible that CBD enriched oils and food supplements may contain higher amount of CBD than the eligible level, thus threatening consumers and patients by increasing the risk of cardiovascular side effects of CBD [1,2].

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SL-BP04 Short Lecture "Enriched Carotenoids based botanical lead and botanical supplement development from Kashmiri Saffron (*Crocus sativus*): Multi-analytical Investigations"

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DOI 10.1055/s-0042-1758959

Carotenoids, with flavonoids, play a crucial role in the biological activity of Saffron. Saffron (stigma of Crocus sativus) is one of the most common spices and food ingredients globally. This holds higher nutritional and medicinal value in supporting human health benefits. The Kashmir saffron is one of the richest aroma precursors and carotenoid derivatives, with the strongest color with sustainable cultivation.

In the present study, multi-analytical Investigations have reduced the timeline for developing botanical and supplements from Kashmiri saffron stigmas. The UHPLC determination of carotenoids and flavonoids was carried out along with an HPTLC-based qualitative analysis of raw materials (stigma, stamen, and tepals) and extracts. The rapid validated method showed excellent linearity ($R^2 > 0.99$) with robust, precise (< 5.0%), and accurate (80–110%) quantification with the combined-expanded uncertainty as per the EURACHEM guide. The ESI-MS/MS analysis provided an untargeted and targeted identification of 36 compounds in this botanical supplement with mass fragmentation. This MS/MS investigation has led to the development of validated bioanalysis of these compounds in-vivo for pharmacokinetics studies for this botanical supplement in rabbit plasma, liver, and brain.

Thus, this multi-analytical approach enables a composite profile of stigma-based extract as a potential supplement, strengthening standardization and bioavailability for health benefits.

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Wednesday, August 31 | Formulation Session

Formulation – Pharmaceutical technology – Drug delivery systems

SL-FOR01 Short Lecture "A new controlled release system of polyphenols from *Vitis* leaves and propolis"

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DOI 10.1055/s-0042-1758960

Agricultural by-products are usually sources of valuable bioactive ingredients the exploitation of which is essential for sustainable agriculture but also for the biological properties it can impart to cosmetics, pharmaceuticals and food. The vine (*Vitis vinifera* L.) is considered one of the most important fruit crops in the world, covering over 7.5 Mha of the world area (FAO 2016) and producing 80 million tons in 2018 (FAOSTAT 2020).

Vine leaves are a bulky by-product that is disposed of and treated as waste in the wine production process, with applications being limited, at best, to a limited number of edible products and at worst to the creation of a soil enhancer. In the present study polyphenols from vine leaves were extracted and simultaneously encapsulated in a new combinatorial system consisting of liposomes and cyclodextrins. In parallel, propolis polyphenols from Mount Olympus was encapsulated in cyclodextrins and partly in the combinatorial liposome-cyclodextrin system resulting in a colloidal suspension that releases polyphenols in a time-controlled way, the rate of which depends on the ratio of the materials. The result is a raw material that exhibits antioxidant and ECM protective effects when administered in cell cultures (HDFs). Treatment of HDFs with the combinatorial delivery system for vine extract and propolis polyphenols promoted collagen end elastin synthesis and deposition in normal conditions and upon induced external stress, as assessed by in vitro transcriptomic and proteomic analysis. Therefore, this liposome-cyclodextrin encapsulated polyphenol complex represents a novel bioactive ingredient with promising skin applications.

SL-FOR02 Short Lecture "Cannabidiol loaded self-microemulsifying drug delivery systems (SMEDDS) for oral administration"

Authors Grifoni L[1], De Donno G[1], Vanti G[1], Bergonzi MC[1], Luceri C[2], Bilia AR[1]
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DOI 10.1055/s-0042-1758961

Cannabidiol (CBD) is a very interesting pleiotropic natural product whose therapeutic use is limited due to the low aqueous solubility, and consequently a poor oral bioavailability (around 6%) [1]. For insoluble drugs such as cannabidiol, the absorption rate from the gastrointestinal lumen is controlled by the dissolution. Among the various approaches that can be used to improve the dissolution rate of these drugs and consequently optimize their bioavailability after oral administration one is represented by self-micro emulsifying drug delivery systems (SMEEDS) which spontaneously form microemulsions in contact with water or gastrointestinal fluid [2].

The aim of the present study was to develop CBD loaded SMEEDS (20 mg/ml), after selection of components with high solubilization capacity for the drug and the evaluation of microemulsion existence by building pseudothernary phase diagram.

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SMEDDS were fully characterized by dynamic light scattering, transmission electron microscopy, and HPLC-DAD in order to evaluate size, homogeneity, morphology, loading capacity. SMEDDS were also characterized in terms of robustness to dilution, using buffers at different pH, and stability in simulated gastrointestinal fluid (SGIF). The droplet size of the formulation did not change significantly in both different medias and SGIF together with a very high chemical stability.

Chemical and physical stability of the formulations were also appreciated over one month period; storage studies were performed at 25 ± 2 °C and far from the light.

Finally, the in vitro permeation studies were assessed to determine the suitability of the developed nanoformulations.

The authors declare no conflict of interest.

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SL-FOR03 Short Lecture "Eco-friendly natural deep eutectic solvents to solubilise and formulate natural products in nanosystems: the case of silymarin"

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DOI 10.1055/s-0042-1758962

Eco-friendly natural deep eutectic solvents (NADES) were used to solubilise and formulate silymarin in nanosized delivery systems. NADES are considered alternative green solvents to extract and solubilise poorly water-soluble molecules [1], such as silymarin, used as a model drug. In this study, NADES were also investigated as a dispersant phase of colloidal systems to reproduce vesicles naturally occurring in plants for pharmaceutical technology purposes and, specifically, for drug delivery.

The eutectic mixtures of choline plus glycerol (NADES1) and choline plus fructose with 20% w/w water (NADES2) were selected for low viscosity and low toxicity to investigate the lipid vesicle's formation in an anhydrous environment [2]. Silymarin solubility, in terms of silybin, obtained after 24 h of dissolution under magnetic stirring at 21 °C, was found to be 37.73 ± 0.5872 mg/mL in NADES1 and 17.77 ± 0.1591 mg/mL in NADES2 by liquid chromatography. Lipid vesicles, made of phosphatidylcholine and cholesterol, were prepared by the thin layer evaporation method using the eutectic mixtures with and without silymarin. Unloaded vesicles, prepared with NADES1 and NADES2, and analysed by dynamic light scattering (DLS), revealed a Size of 98.4 ± 1.60 nm and 89.99 ± 2.194 nm, respectively and a low polydispersity index. By contrast, loaded vesicles were not measurable by DLS, but all formulations observed by scanning and transmission electron microscope showed spherical structures. Preliminary SAXS analyses were also performed to investigate the nanosystem's architecture deeply.

Our results revealed NADES as a highly customisable class of green solvents with remarkable capabilities for formulating natural products in innovative nanosized delivery systems.

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SL-FOR04 Short Lecture "New 3D-printed oral dosage forms"

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DOI 10.1055/s-0042-1758963

In the last decade, additive manufacturing (AM) technologies have revolutionized how healthcare provision is envisioned. The rapid evolution of these technologies has already created a momentum in the effort to address unmet personalized needs in large patient groups, especially those belonging to sensitive subgroup populations (e.g., paediatric, geriatric, visually impaired). At the same time, AM technologies have become a salient ally to overcome defined health challenges in drug formulation development by addressing not only the requirement of personalized therapy, but also problems related to lowering non-specific drug distribution and the risk of adverse reactions, enhancing drug absorption and bioavailability, as well as ease of administration and patient compliance. To this end, drug delivery systems fabricated with the support of AM technologies provide competitive advantages over conventional dosage forms, aiming to entice innovation in drug formulation with special focus on sensitive patient populations.

Wednesday, August 31 | Short Lectures D

Chemistry and bioactivity of natural products

SL-D01 Short Lecture "Protein-Ligand Interactions and in-Cell NMR Spectroscopy in Natural Products Research"

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DOI 10.1055/s-0042-1758964

Recent developments of NMR spectroscopy in investigating protein-ligand interactions and biomolecular interactions of natural products at the cellular level will be presented with emphasis on:

(a) The combined use of saturation transfer difference (STD), Tr-NOESY and INPHARMA (Interligand Noes for PHArmacophore MApping) NMR techniques and docking calculations for specific binding sites and structure elucidation of natural products with non-labelled serum albumin1,2. Interligand NOEs and docking calculations of polyunsaturated fatty acids (FFAs) were interpreted in terms of two orientations in the warfarin binding site, due to two anchoring groups of polar amino acids, which are the reason that the conformational states of the FFAs could not be determined accurately in X-ray structural studies1,2. Prospects for investigating oxidation products of polyunsaturated fatty acids will be discussed3.

(b) Application of in-cell NMR analytical methodology in the monitoring of the interaction of natural products with the anti-apoptotic protein Bcl-2 inside living human cancer cells. STD and Tr-NOESY NMR were employed to evaluate the direct binding of the ligand to the non-labeled Bcl-2 protein intracellularly4, which was further validated in vitro5. This is a very promising strategy for the real-time screening of the interaction and conformational changes of natural products with their targets in living eukaryotic cells.

The research work was supported by the Hellenic Foundation for Research and Innovation (H. F. R. I.) under the "First Call for H. F. R. I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: 2050).

The author declares no conflicts of interest.

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SL-D02 Short Lecture "Rare *Isatis tinctoria* metabolites: Quingdainones overcome multidrug resistance in leukemia cells in vitro"

Authors Riepl H[1,2], Bieringer S[1,2], Urmann C[1,2], Ritter S[1,2], Baas J[3,4], Frias C[3,4], Frias J[3,4], Soehnchen C[3], Prokop A[3,4]
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DOI 10.1055/s-0042-1758965

Isatis tinctoria contains many complicated condensed aromatic compounds – some indigoids and indolochinazolins have shown promising anti-cancer and anti-inflammatory properties, also anti-malaria activity is there [1]. In anti-cancer research, not only the cytotoxic activity of a compound is important, much more counts the therapeutic index and resistance breaking properties. Among Isatis metabolites, quingdainones (Indigo brown) have received less attention. These are cross breeds between tryptanthrines being indoloquinazolines and indoxyl. An active dihydroxyquingdainone AC50 7.5 µmol, has been found and further characterized and compared to synthetic derivatives. No other compound of this family including the unsubstituted quingdainone was active. Finding its apoptosis inducing ability (in a caspase 3 dependent manner) it was further characterized using Nalm-6 cells. Reduced mitochondrial membrane potential was determined using JC-1 staining and flow cytometric measurement, 6.3 µmol dihydroxyquingdainone showed 50% cell number with low mitochondrial membrane potential. BeKa cells (Vincristine resistant Nalm 6 cells), show an increased expression of the p-glycoprotein. Substances can be actively secreted from the cell via this protein [2,3], whereby they become multidrug resistant (MDR) [4], which has also been demonstrated for BeKa cells. BeKa cells show a co-resistance to anthracyclines (Idarubicine, Daunorubicine, Doxorubicine, Epirubicine), Mitoxanthrone, Fludarabine, Vincristine or Vinblastin and Etoposide in vitro. Nalm-6 and BeKa cells had been treated with dihydroxyquingdainone for 72 h. The comparison of the percentage of apoptotic cells in these treated cell lines showed no significant differences. Thus, it is no substrate of the p-glycoprotein and explains a prominent effect in vincristine resistant leukaemia cells.

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SL-D03 Short Lecture "*Aquilaria crassna* leaf extract attenuates high glucose-induced neurotoxicity and Caenorhabditis elegans lifespan/healthspan reduction"

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DOI 10.1055/s-0042-1758966

Hyperglycemia is one of the most important causes of the neurodegenerative disorders such as the development of cognitive impairment, dementia, Alzheimer's disease, Parkinson's disease, and aging. *Aquilaria crassna* Pierre ex Lec (AC) has been widely known as a utilized plant in traditional folk medicine to relieve various health ailments. In this study, the protective effect and anti-aging mechanisms of hexane extract of AC on high glucose-induced neurotoxicity and aging were investigated in human neuroblastoma cells (SH-SY5Y) and *C. elegans*. AC was demonstrated to possess neuroprotective effect by inducing neurite outgrowth and normalizing cell cycle progression. As confirmed by Western blot analysis, these mechanisms were associated with up-regulating GAP-43 and teneurin-4 expression for neurite outgrowth, and down-regulating cyclin D1 and SIRT1 expression for cell cycle progression. AC extract could promote longevity and attenuate the effect of high glucose on body length/size and brood size reduction. As confirmed using mRNA expression, these effects were mediated through the DAF-16/FOXO pathway, and SOD-3 and AQP-1 expression, whereas the expression of SKN-1 was not altered. Furthermore, 27 phytochemicals of AC and their competence on inhibiting insulin/insulin-like growth transmembrane receptor (IGFR) functions were resolved. Molecular docking analysis revealed that top 5 phytochemicals (olean-12-en-3-one, lupenone, stigmasterol, α-amyrin and β-amyrin) were more efficient than the binding of each positive control (EGCG or resveratrol). Together, AC is potentially an interesting natural source including its five phytochemicals as probable active components for the prevention of high glucose-induced neurotoxicity and aging.

SL-D04 Short Lecture "Investigating the potential of *Elegia tectorum* as an anti-ageing agent"

Authors De Canha MN[1], Radebe PG[1], Payne BD[1], Oosthuizen CB[1], Twilley D[1], Verma S[1], Skaltsa H[2], Lymperis P[2], Tomou E-M[2], McGaw L[3], Lall N[1]
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DOI 10.1055/s-0042-1758967

Elegia tectorum, also known as the Cape Thatching Reed (English) is a plant belonging to the Restionaceae family. In South Africa, it is distributed in the Eastern, Western and Northern Cape provinces, populating marshes and deep sandy soils along coastal or lowland flats at altitudes between 10–600 m. The aim of this study was to investigate the anti-ageing potential of E. tectorum (ET) due to the lack of pharmacological activity and ethnobotanical uses available in literature. The ethanolic extract of ET (ETEtOH) inhibited elastase enzyme activity with an IC_{50} of 13.50 ± 1.53 µg/mL. Cytotoxicity of ETEtOH was determined on HT29 cells (human colorectal adenocarcinoma) due to their expression of the KIAA1199 protein, responsible for hyaluronic acid degradation. No toxicity was observed at 400 µg/mL. There was significant reduction of KIAA1199 protein production levels in cells treated with 60 µg/mL and 240 µg/mL ETEtOH, when compared to the untreated HT29 cell control. To determine safety of the ETEtOH, the mutagenic potential of the extract was determined, with 50, 500 and 5000 µg/mL showing no mutagenicity using the TA 98 strain of *Salmonella typhimurium*. A clinical study for irritancy determined the irritancy potential of ETEtOH to be − 30.83, which was less than the negative control (distilled water) characterizing the extract as a non-irritant. An antiwrinkle efficacy study showed that ETEtOH at 10% (w/w) in aqueous cream reduced the appearance of wrinkles after 28 days. The extract of ET is a strong lead for the development of a botanical anti-ageing ingredient.

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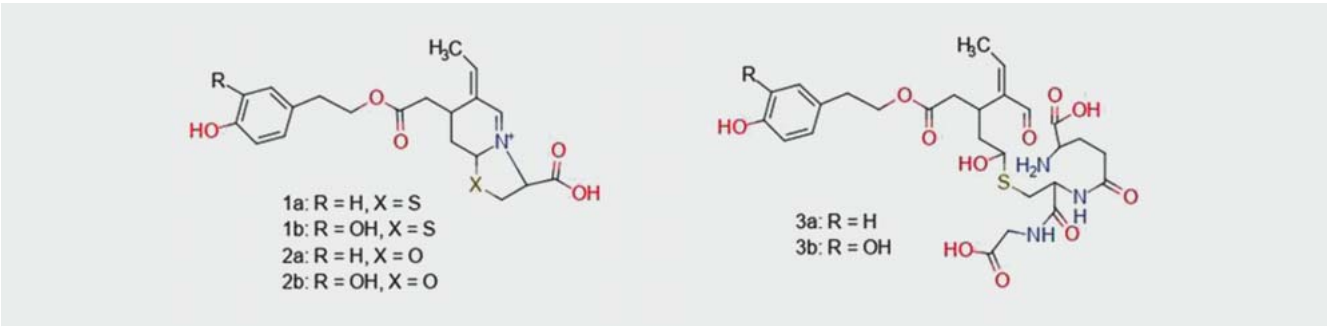

▶ **Fig. 1**

SL-D05 Short Lecture "New insights into the mechanism of action and bioavailability of oleocanthal and oleacein from olive oil"

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DOI 10.1055/s-0042-1758968

Oleocanthal (OC) and oleacein (OL) are highly bioactive secoiridoids found in olive oil at elevated concentrations, especially when it is produced from unripe olives (*Olea europaea* L.). Both compounds have been correlated with strong activities against serious diseases through recent clinical trials. The most important clinical trials have been performed in patients against chronic lymphocytic leukemia [1], against mild cognitive impairment [2] and against platelet aggregation of normal [3] or diabetic patients. Carefully designed nutritional interventions in humans using olive oil with high OC/OL content or olive oil without OC/OL have provided strong evidence about the unique therapeutic role of those compounds. However, both compounds do not actually circulate in the body and cannot be found in any biological fluid. Recent studies have shown that OC and OL react spontaneously with plasma aminoacids like glycine to form new highly bioactive chemical entities like oleoglycine that circulate in the blood and can also reach the brain of experimental animals [4]. During our continuous effort to understand the mechanisms of action of OC/OL, we investigated the reactions of both compounds with aminoacids and peptides found in human plasma. During this study we screened the selectivity and the rate of this reaction, and we discovered new metabolites with unique structures and properties like oleocysteine (1a, b), oleoserine (2a, b) or oleoglutathione (3a, b) (▶ **Fig. 1**). The synthesized metabolites were structurally elucidated using NMR and MS. The obtained results can shed light on the mechanism of action of OC/OL and more significantly explain their bioavailability.

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Wednesday, August 31 | Respiratory Discussion Forum

Natural Products against Respiratory Infections

SL-RDF-01 Short Lecture "Natural products against SARS-CoV-2 or how to catch a butterfly?"

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DOI 10.1055/s-0042-1758969

There is still an unmet medical need for agents to overcome severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this study, we applied an integrated in silico – in vitro approach to explore the potential role of natural products (NP) acting against SARS-CoV-2. The two SARS-CoV-2 viral proteases, the main protease (MPro) and the papain-like protease (PLPro) [1], were selected as targets for the in silico study. Virtual hits (VHs) were determined by molecular docking using GOLD [2] from databases containing > 140,000 molecules from in-house and commercially available natural products. For experimental validation 35 VHs were selected and subjected to enzyme-based assays. The protease inhibitory activity was confirmed for 11 VHs showing > 50 % enzyme inhibition at 20 µM. These target-based hits were further evaluated for their antiviral activity against SARS-CoV-2 in a Caco-2 cell model. The results from the cell-based assay revealed several VHs not only as Mpro inhibitors but also as promising anti-SARS-CoV-2 agents with IC_{50} values in the low µM range without having cytotoxic effects (CC50 > 20 µM). The docking poses of SARS-CoV-2 MPro proposed a butterfly-shaped substrate binding pocket for the bioactive VHs similar to the already known synthetic inhibitor X77 (▶ **Fig. 1**). In addition, saturation transfer difference – nuclear magnetic resonance (STD-NMR) experiments were performed to validate the predicted molecular interactions of in the MPro and support our proposed binding hypothesis.

The authors declare no conflict of interest.

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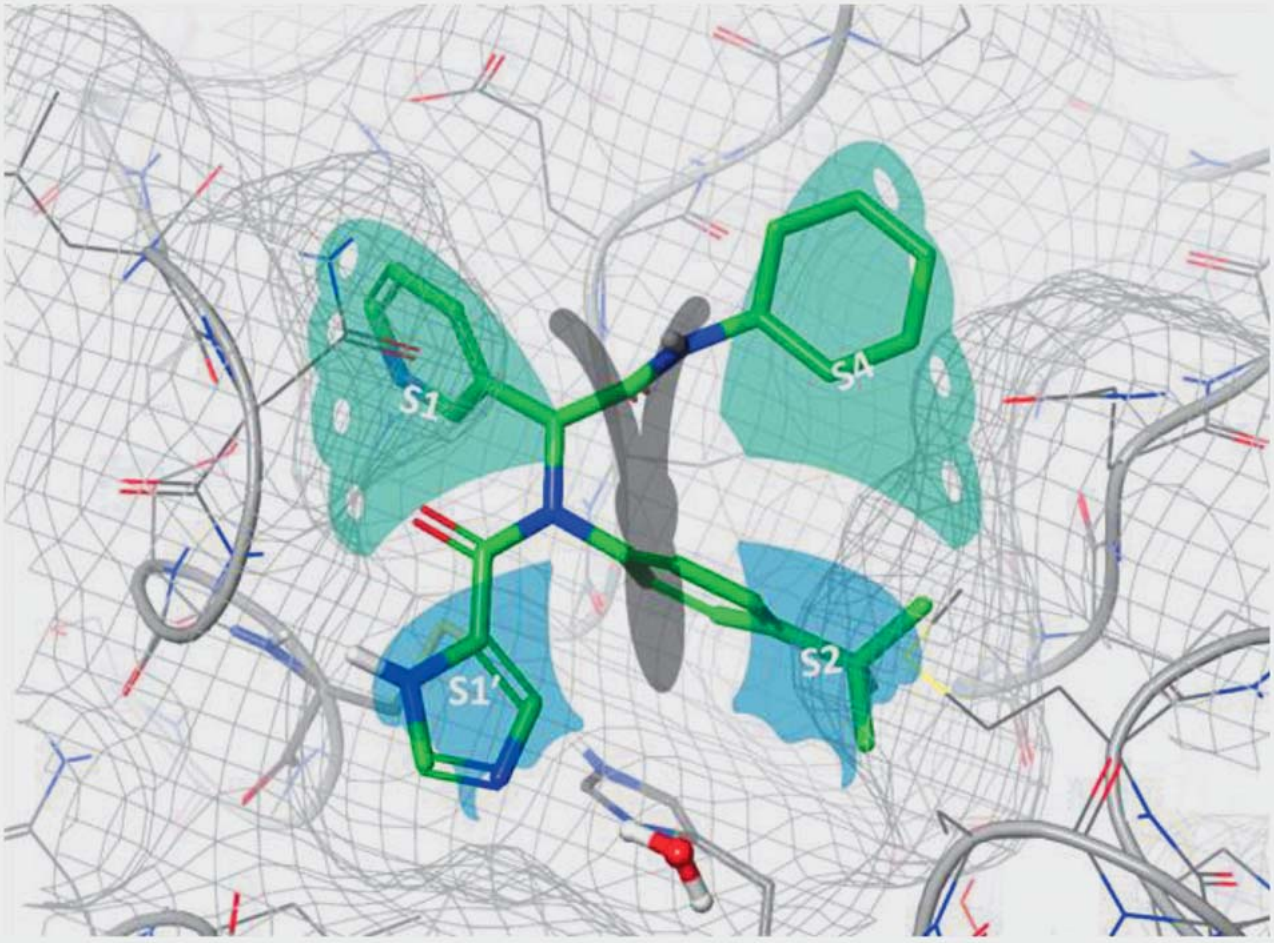

▶ **Fig. 1** Binding pose of X77 in the butterfly-shaped substrate binding pocket of SARS-CoV-2 M^{pro}.

SL-RDF-02 Short Lecture "Isoquinoline alkaloids and their derivatives as a new class of antimycobacterial drugs"

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DOI 10.1055/s-0042-1758970

Tuberculosis (TB) is a widespread infectious disease caused by *Mycobacterium tuberculosis* (Mtb). According to the Global Tuberculosis Report 2021, issued by the World Health Organization (WHO), the latent form of Mtb has infected about a quarter of the world's population, but only a small part (5–10%) will develop this bacterial disease [1]. The increasing incidence of multidrug-resistant (MDR), and extensively drug-resistant (XDR) strains has created a need for new antiTB agents with new chemical scaffolds to combat the disease. Thus, the key question is: how to search for new antiTB and where to look for them? One of the possibilities is to search among natural products.

In order to search for new antiTB drug, we screened isolated alkaloids in our lab within previous phytochemical studies against Mtb H37Ra and four other mycobacterial strains (*M. aurum*, *M. avium*, *M. kansasii*, and M. *smegmatis*). In order to expand portfolio of tested compounds several series of semisynthetic derivatives of selected alkaloids (e.g. berberine, galanthamine, haemanthamine and others) were developed and tested. Derivatization of berberine in position C-9 was connected with a significant increase in antimycobacterial activity against all tested strains (MICs 0.39–7.81 µg/mL). Derivatization of galanthamine in position C-6 was connected with increase of activity against Mtb H37Ra (MICs 1.56–15.625 µg/mL).

The most active compounds were also evaluated for their in vitro hepatotoxicity on a hepatocellular carcinoma cell line (HepG2), exerting lower IC_{50} values than their MIC values, further corroborating their potential as potent and safe antimycobacterial agents.

Reference

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SL-RDF-04 Short Lecture "From farm to pharma: *Euclea natalensis*, a possible adjuvant for TB patients"

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DOI 10.1055/s-0042-1758971

Multi-drug resistant forms of tuberculosis and severe non-compliance is impeding total eradication of this infectious bacterial disease. Many people have

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been relying on traditional remedies together with the current drug regimen for the treatment of associated symptoms of tuberculosis such as coughing and chest related complaints. *Euclea natalensis* A. DC. (EN) has traditionally been used in South Africa for the treatment of tuberculosis and its associated symptoms, stomach ailments and dysentry amongst others. Investigations into the pharmacodynamic properties of EN substantiated the antibacterial, antimycobacterial and hepatoprotective activity of the ethanolic shoot extract. Additional analysis included for the possible product development of EN included cytotoxicity, inhibition and/or stimulation of major CYP P450 enzymes, the nutritional content, heavy metal analysis, microbial content, antimutagenicity and antifibrotic activity of the ethanolic shoot extract. Many other projects have since started looking into the PLGA nanoparticle formulation of the extract and the main active ingredient, propagation trials and a polyherbal combination with other indigenous South African medicinal plant species. The development of EN has had the ability in stimulating the bioeconomy through capacity building and community development. Results obtained from the previous studies conducted and current ongoing investigations can be used to support the further development of the extract and the main active ingredient as a possible adjuvant to be taken in conjunction with conventional treatment.
The authors declare that there are no conflicts of interest.

SL-RDF-05 Short Lecture "*Althaea officinalis* L. root extract and marshmallow cough syrup exert anti-inflammatory properties and improve migration in a model of angiogenesis"

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DOI 10.1055/s-0042-1758972
Introduction: Marshmallow cough syrup (STW42, Phytohustil) containing a root extract of *Althaea officinalis* L. (REA) is used for the treatment of lesioned laryngopharyngeal mucosa leading to dry cough [1–4]. For the healing of such wounds, neovascularization at the site of the injury is central.
Aim: In this regard, the proof of anti-inflammatory/-oxidative properties that improve angiogenesis in the wound could explain the therapeutic effect.
Method: Treatment (24 h) with STW42 or REA was followed by 3 h lipopolysaccharide (LPS) or 1 h H_2O_2. Intracellular reactive oxygen species (ROS) were quantified with dichlorofluorescin (DCFDA) and interleukin- (IL-) 6 release. The migratory capacity of HUVECs was determined by scratch assay.
Results: 100 µg/ml STW42 or REA inhibited the LPS stimulated IL-6 release by 17% and 16%. 500 µg/ml STW42, its excipients or 500 µg/ml REA inhibited the LDL stimulated IL-6 release by 17%, 24% or 22%. After 24 h with STW42 or REA and additionally with H_2O_2, Phyto inhibited the ROS production by 18% (500 µg/ml) or by 16% (1000 µg/ml) as well as after REA by 33% (1000 µg/ml). After 6 h treatment, 500 or 1000 µg/ml STW42 stimulated the wound closure by 10% and 1000 µg/ml REA by 13.5%.
Discussion: STW42 has anti-inflammatory properties, protects against oxidative stress and improves the migratory capacity of HUVECs used as an in vitro model of the vascular endothelium. These properties can explain the therapeutic effects of STW42 in oral and pharyngeal irritation and associated dry cough by a not just only symptomatic, but causal mode of action.

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Wednesday, August 31 | Short Lectures E
Chemistry and bioactivity of natural products

SL-E01 Short Lecture "The standard herbal preparation, STW 5, affects colonization of gut microbiota in ulcerative colitis: in vitro study"

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DOI 10.1055/s-0042-1758973
Dysbiosis of gut microbiota plays an important a role in the pathogenesis of inflammatory bowel disorders. A bidirectional relationship exists between gut microbes and drugs. Gut microbes influence the pharmacokinetics of drugs and drugs affect gut microbe's composition, colonization, and metabolism. Microbial populations colonizing the gastrointestinal tract (GIT) exist as biofilms and thus studying biofilm formation can give an insight into microbe-microbe and drug-microbe interactions. The aim of the current study was to explore the interactions of STW5 with selected members of gut microbiota known to change in UC and to study the effect of STW 5 on bacterial growth using broth microdilution technique and on biofilm formation using crystal violet microtiter plates The gut microbiota studied included *Escherichia coli, Enterococcus faecalis, Lactobacillus acidophilus, Lactobacillus reuteri,* and *Bifidobacterium longum* belonging to Proteobacteria, Firmicutes, and Actinobacteria phyla. Biofilm formation was calculated after normalization to growth of each bacteria. At intestinal concentration of STW5, E. coli growth was significantly decreased while there were no significant effects observed in *E. faecalis, L. acidophilus, L. reuteri* and *B. longum*. STW 5 intestinal concentration increased biofilm formation of *E. coli* and *E. faecalis* but had no effect on the other tested bacteria. STW 5 increased biofilm formation of *E. coli* and *E. faecalis* suggesting possible enhancement of these bacteria colonization of GIT and protection against colitis. The study sheds more light on drug-microbe interactions of STW 5 in the treatment of UC and highlights the role of gut microbiota in UC treatment.

SL-E02 Short Lecture "Neurotrophic Activity of *Ballota nigra* L., *Crataegus oxycantha* L., *Passiflora incarnata* L., *Valeriana officinalis* L. in vitro and in vivo"

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DOI 10.1055/s-0042-1758974
Extract preparations of the plants *Ballota nigra* L. (Bal), *Crataegus oxycantha* L. (Crae), *Passiflora incarnata* L. (Pa) and *Valeriana officinalis* L. (Val) and their combination (combo) modulate the neurothrophic activity, neurotransmitters and hormones involved in the sleep-wake cycle, but not pro-inflammatory cytokines (IL1β, TNF-α) [1], in the neuroblastoma cell line SH-SY5Y.
Gene expression (GE-) profiles from the SH-SY5Y cells – treated or untreated with Bal, Crae, Pa, Val, their combo or Lorezepam – were compared with the GE-profiles of patients suffering from sleep disorders obtained from the GEO-database.
GE-profiles of the parietal lobe (PL) and of the thalamus (T) of patients suffering from fatal insomnia (FFI) (n = 8) as well as GE-profiles from peripheral blood leukocytes of monozygotic twins with short sleep (n = 11 pairs) were identified. Differential gene expression (diseased patients vs. healthy donors) were compared to the GE-profiles obtained from treated SH-SY5Y cells. Expressions of 4.794 genes in the PL and of 5.106 genes in T were significantly

regulated. Of those 459 (PL) and 497 (T) genes were common to all groups. Differential GE-profiles of twins and of treated SH-SY5Y cells had 166 genes in common. These related to the nervous system, to the extracellular matrix as well as to cell proliferation and differentiation. In the context of short sleep most of the identified genes were conversely regulated by the plant extracts compared to the ones of monozygotic twins.

Based on GE-profile comparison an effect of the plant extracts towards insomnia and short sleep can be expected, provided bioavailability is given.

Reference

[1] Ulrich-Merzenich, G, Shcherbakova A, Kolb C. Modulation of the Neurotrophic Activity by Ballota nigra L. Crataegus oxycantha L., Passiflora incarnata L. and Valeriana officinalis L. and their Combination in vitro. Planta Med 2021; 87: 1315–1316

SL-E03 Short Lecture "Flavonolignans in Silymarin Shape Lipid Mediator Profiles and Differentially Modulate the Sensitivity of Cancer and Non-cancer Cells Towards Anti-tumoral Drugs"

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DOI 10.1055/s-0042-1758975

Prolonged exposure to chemotherapeutic drugs adversely affects liver and other organs, thereby causing tissue damage, which is accompanied by local inflammation. Silymarin, a standardized extract from *Silybum marianum* (milk thistle), possesses long-standing tradition for treating hepatobiliary diseases and inflammatory disorders, though the molecular mechanisms are not fully understood. The (pre-)clinical efficacy of silymarin and its major bioactive compound silibinin has been confirmed for the treatment of drug-induced acute liver toxicity, alcoholic- and non-alcoholic fatty liver disease, hepatitis B and C infection and hepatocarcinoma. Here, we investigated whether silymarin and its bioactive flavonolignans modulate the cytotoxic activity of anti-cancer drugs and diverse hepatotoxic agents on cancer and non-malignant cell lines from different origin. Given the close link between low-grade inflammation and tumorigenesis, we explored the impact of silymarin on lipid mediator profiles by targeted metabololipidomics. Focus was placed on prostanoids, leukotrienes, lipoxins, epoxyeicosatrienoic acids, specialized pro-resolving lipid mediators and their precursors, platelet-activating factor and bioactive sphingolipids. We conclude that ingredients in silymarin i) both potentiate and repress the anti-tumoral activity of specific anti-cancer drugs dependent on the cancer type, ii) show partial selectivity for malignant over non-malignant cells, and iii) interfere with pro-inflammatory lipid mediator formation.

The project has been funded by Bionorica SE.

SL-E05 Short Lecture "Unique Molecules from a Bioactive Irish Bog Lichen, *Cladonia portentosa*"

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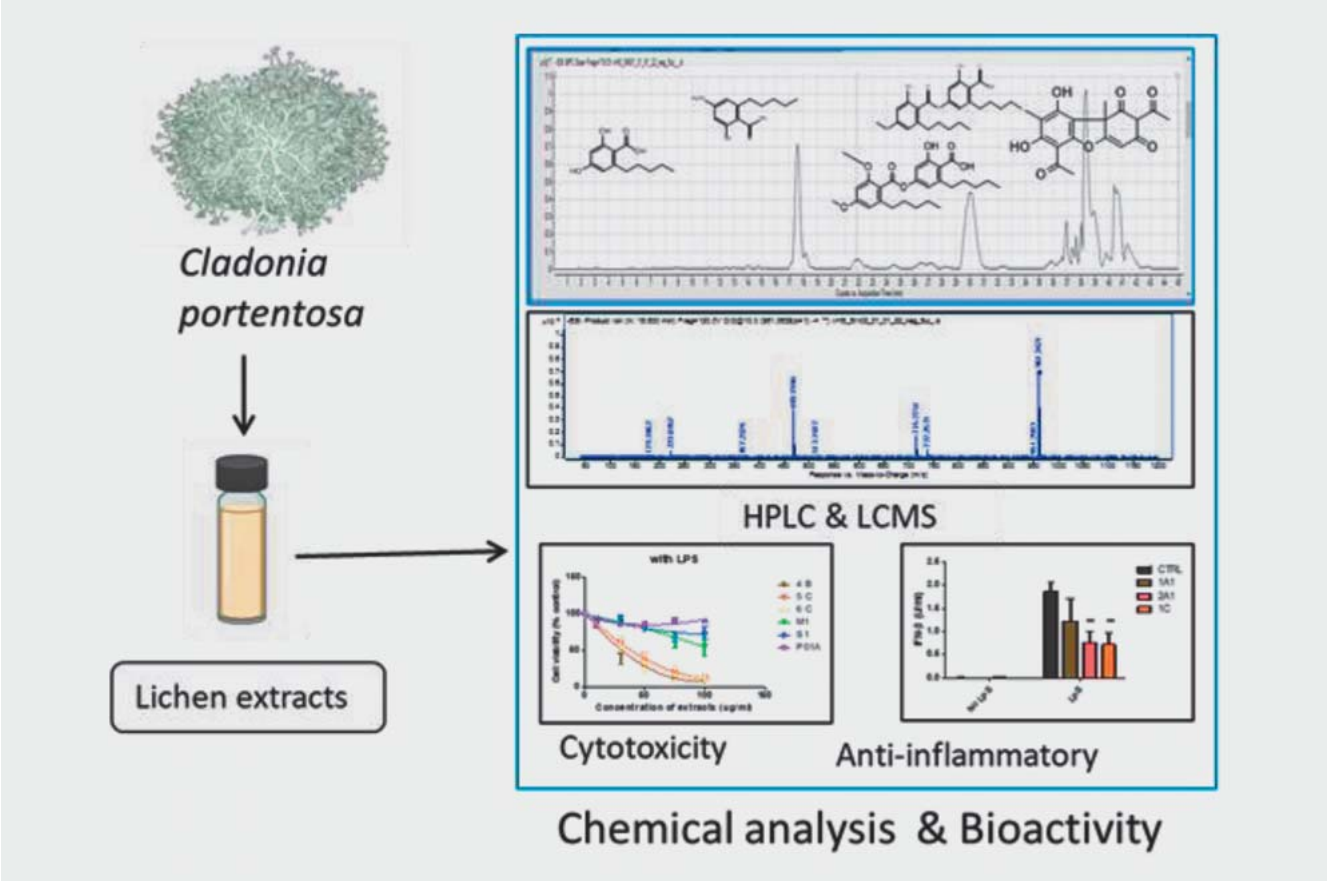
DOI 10.1055/s-0042-1758976

Cladonia portentosa, commonly known as Reindeer lichen, is widely distributed on the diverse Irish boglands. A lichen is a unique symbiotic blend of photobiont (eukaryotic alga and/or cyanobacterium) and mycobiont (filamentous fungus) partners. The former undergoes primary production to fuel the latter, which in return biosynthesise secondary metabolites called lichenic



▶ **Fig. 1**

acids, to protect the two against harsh environment, thereby prolonging their sustainability. Lichenic acids include diverse metabolites including depsides, depsidones, dibenzofurans, diphenylether, xanthones, pulvinic acid derivatives etc [1]. To date, two lichenic acids usnic acid (dibenzofuran) and perlatolic acid (depside) have been reported from *C. portentosa* [2]. In present study, methanol extracts were prepared from *C. portentosa*, harvested from six Irish bogland ecosystems and their cytotoxicity and anti-inflammatory activity were measured using an in vitro model of immortalised bone marrow derived macrophages (iBMDM) cell line (▶ **Fig. 1**). Resazurin assay and ELISA techniques were used to investigate cell viability and to measure cytokine production including IFN-β, IL-6, TNF-α and Rantes, respectively. The extracts showed a clear inhibition of IFN-β and IL-6 while stimulation of Rantes production. HPLC, LC-ESI-MSn and column chromatography were used to separate and identify the active main components of the extracts. The metabolites, olivetolic acid, 4-O-Me-olivetolic acid and 2-O-Me-perlatolic acid were identified for the first time in *C. portentosa* along with previously reported usnic and perlatolic acids, and their structures were confirmed with 2D-NMR. These components may act as a precursor in biosynthesis of perlatolic acid. The cytotoxicity and anti-inflammatory screening of individual components of methanol extract is in progress.

References

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SL-E07 Short Lecture "Phytochemical, nutritional, and mineral content of four edible flowers"

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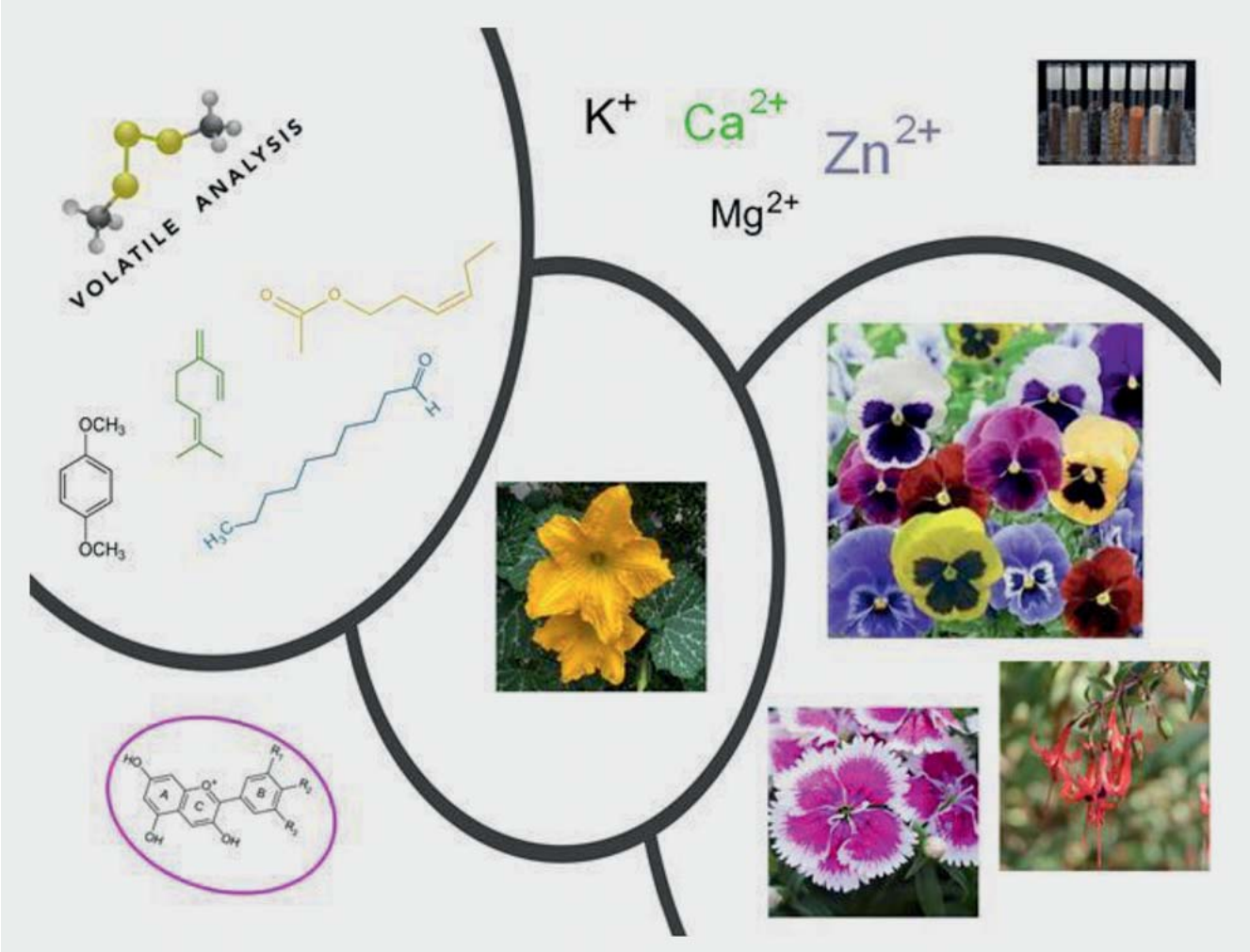
DOI 10.1055/s-0042-1758977

The consumption of flowers as food dates to ancient Greek and Rome and survives to the present day as part of the traditional cuisine of different countries [1]. The consumption of edible flowers (EFs) is significantly increased in the last few years, due to both their numerous healthy compounds and the heightened awareness of people to the food quality [1]. In this context four EFs were investigated to evaluate their aromatic profile and their nutritional value. The studied species were *Dianthus chinesis* L., *Viola cornuta* L., *Fucsia regia* (Vand. Ex Vell.) Munz and *Cucurbita moschata* Duchesne. p-Dimethoxybenzene (77.5%), cis-hexenyl acetate (31.2%) and decanal, non-terpene compounds, were the main constituents in volatile profile (by HS-SPME analysis) of *C. moschata* and *D. chinensis*, and *F. regia*, respectively. On the contrary *V. cornuta* was rich in terpenes mainly represented by myrcene (36.7%) and α-farnesene (34.5%). The extraction of the essential oil (EO) was carried out on *C. moschata, D. chinensis*, and *V. cornuta*. The EOs of the first two species maintained the prevalence of non-terpenes even though heneicosane (34.9%) and behenic alcohol (39.6%) becomes the principal ones, respective-



▶ **Fig. 1**

ly, while *Viola* EO was rich in (E)-palmitoleic acid (31.3%). From the nutritional point of view, *F. regia* have the most interesting flowers, with the highest content of polyphenols, anthocyanins, calcium, iron, and zinc. *D. chinensis* contains the higher soluble sugars, and common *C. moschata* flowers contain high quantities of crude proteins, phosphorous, potassium and magnesium and low content of secondary metabolites.

Reference

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SL-E08 Short Lecture "New strategies for the treatment of urinary tract infections: *Equisetum arvense* herb extract as inductor of Tamm-Horsfall Protein against E. coli"

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DOI 10.1055/s-0042-1758978

Tamm-Horsfall Protein (syn. THP, Uromodulin) is secreted in the epithelial cells of the thick ascending limb of the Henle loop. THP is capable of binding uropathogenic *E. coli* (UPEC) and therefore prevents interaction with eucariotic host cells [1,2]. Stimulation of THP secretion represents a new strategy for the prevention of urinary tract infections (UTI). Recently, Cranberry extracts have been shown to exert antiadhesive activity against UPEC by strong THP-stimulation after oral intake, correlated with significant antiadhesive effects of UPEC against bladder cells [3].

For identification of further potential THP stimulators, a biomedical study in 60 healthy volunteers (30 male, 30 female) was conducted. Morning midstream urine of volunteers (groups n = 10) after seven-day oral intake of six different herbal extracts was collected. Urine from volunteers treated with an aqueous extract of Equiseti herba (EqW) showed significant increase of [THP/Creatinine] (> 300%) and clinical parameters indicated additional diuretic effects. Urine samples also showed a decrease in the relative bacterial adhesion of UPEC to T-24 bladder cells. Antiadhesive effects correlate directly with the respective THP concentrations in the tested urine samples. LC-MS/UV analysis of EqW identified > 80% of all eluted peaks and ICP-OES was used for quantitation of the silicium content in EqW and in the urine samples.

The combined in vivo/ex vivo study proves antiadhesive and diuretic effects of horsetail extract after oral application, rationalizing the traditional use of *Equisetum* extracts in the prevention of UTI.

References

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SL-E09 Short Lecture "Comparison of anti-proliferative activity of β-damascenone and β-ionone"

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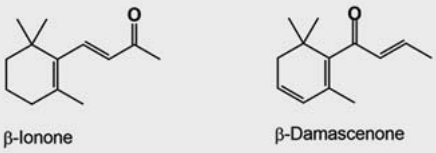
DOI 10.1055/s-0042-1758979

Damascones and ionones belong to a series of structurally related C13-norisoprenoids, commonly referred to as "rose ketons". Up to date, cancer research has mainly focused on β-ionone and its analogs, which have been described as promising anti-cancer agents. Especially β-ionone has shown its abilities in vivo and in vitro, although EC_{50} values were comparably high [1]. Recently, it was discussed that reduced COX-2 expression contributes to the anti-proliferative effect of β-ionone [2]. However, β-damascenone, which has already proven to inhibit COX-2 gene expression in vitro [3], has never been investigated in terms of anti-proliferative activity.



▶ **Fig. 1** Structures of *β*-ionone and *β*-damacenone.

To address the question, whether damascone-type C13-norisoprenoids are also potent inhibitors of cell proliferation, the leukemia cell line CCRF-CEM was treated with β-damascenone and β-ionone (▶ **Fig. 1**) in several concentrations. After 72 h, the amount of metabolic active cells was evaluated using XTT-assay. Interestingly, β-ionone was not able to inhibit proliferation of this cell line in concentrations up to 50 µM, whereas β-damascenone inhibited cell proliferation at concentrations between 50 and 5 µM (EC_{50} = 8.33 ± 0.6 µM). Similarly, β-damascenone was superior to β-ionone when tested on other malignant cell lines (WM-9, MDA-MB-231, HCT-116 and U-251). At the same time, β-damascenone showed only moderate cytotoxic effect on non-malignant MRC-5 lung fibroblasts, reducing cell viability to 72.48 ± 2.7% at 50 µM, indicating a selective anti-proliferative effect. Further investigations regarding the activity of other damascone-type C13-norisoprenoids are in progress.

References

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SL-E10 Short Lecture "Flavonoid sulfates in *Pelargonium sidoides* root extract EPs® 7630"

Authors Kulić Ž[1], Zeller M[1], Butterer A[1], Ahlert S[1], Jeschor R[2], Schneider H[1]
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DOI 10.1055/s-0042-1758980

EPs® 7630, a proprietary hydroethanolic extract of *Pelargonium sidoides* roots is the active principle in herbal medicinal products used for the treatment of respiratory tract infections such as acute bronchitis or common cold. Recently, in vitro antiviral effects against SARS-CoV-2 were reported for EPs® 7630 [1]. These findings confirmed the well-established broad antiviral properties of the extract. The constituents of the extract described in literature are mainly polymeric prodelphinidins, benzopyranone sulfates, purins and peptides/amino acids [2].

During our systematic phytochemical research, a new class of compounds could be detected, namely flavonoid sulfates. The two major compounds taxifolin-3-sulfate and epitaxifolin-3-sulfate were isolated and characterized by NMR spectroscopy. Quantification revealed the sum of 0.25% (m/m) of both epimers in the extract. Two other flavonoid sulfates could be detected by HPLC-UV-HRMS, which, according to the UV and mass spectra, may correspond to (2R,3R)-dihydroisorhamnetin sulfate and it's (2R,3S)-epimer.

While flavonoid glycosides (e.g., taxifolin-3-O-β-D-glucoside) and aglycones (e.g., quercetin) are already known constituents in *Pelargonium sidoides* aerial parts and roots, respectively [3], our findings are the first report of flavonoid sulfates in *Pelargonium sidoides* roots, to the best of our knowledge.

Planta Med 2022; 88: 1385–1588

Recently, taxifolin was identified in silico as a potential inhibitor of SARS-CoV-2 protease [4] and RNA-dependent RNA polymerase [5], respectively, which may further support the aforementioned antiviral effects of the extract.

References

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Wednesday, August 31 | Short Lectures F

Bioinformatics in natural products Drug Discovery

SL-F01 Short Lecture "Combination of high-throughput reversed docking and 13C NMR-based chemical profiling for new antimicrobial compounds and potential biological target identification"

Authors Darme P[1,3], Cordonnier J[1,3], Escotte-Binet S[3], Remy S[1], Borie N[1], Sayagh C[1], Hubert J[2], Aubert D[3], Villena I[3], Nuzillard J–M[1], Dauchez M[4], Baud S[4], Steffenel L-A[5], Voutquenne-Nazabadioko L[1], Renault J-H[1]
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DOI 10.1055/s-0042-1758981

The chemical space covered by natural products characterized by a high chemical diversity, remains a very interesting reservoir for the new drug discovery [1]. Nevertheless, the exploration of this chemical space for the search of biologically active compounds, either in its totality, or from physical substances such as extracts, or chemically simplified fractions obtained after a work of phytochemistry, requires the development of specific tools. Moreover, the search for antimicrobial compounds, quite easy to implement for the first biological evaluations, is often more complicated when it comes to highlighting the biological targets and the involved mechanisms of action. A software called AMIDE (for AutoMated Inverse Docking Engine) based on AutoDock-GPU and developed in 2014 to perform inverse docking on High Performance Computing, was optimized to allow high throughput screening of very large ligand datasets on large biological target libraries for an improved workflow leading to better performance and reliability [2]. As a first example, AMIDE combined with a ^{13}C NMR-based chemical profiling strategy [3] was used to highlight the anti-Toxoplasma gondii effect of lupane-type triterpenes, especially betulone, from the bark of black alder (Alnus glutinosa) and the identification of CDPK3, ENR and ROP8 proteins as potential targets [5]. The second case of study concerns the preliminary results obtained after the simultaneous in silico screening of two large databases (a natural product database named PNMRNP of 211 k natural products and the French national chemical library containing 70 k chemical compounds) on a dataset of 15 proteins of SARSCoV-2.

References

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SL-F03 Short Lecture "From in silico to in vivo: *Psychotria nemorosa* alkaloids counter protein toxicity in *Caenorhabditis elegans*"

Authors Kirchweger B[1], Klein-Junior LC[2,3], Pretsch D[1], Chen Y[1], Cretton S[4], Gasper AL[5], Vander Heyden Y[6], Christen P[4], Kirchmair J[1], Henriques AT[3], Rollinger JM[1]
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DOI 10.1055/s-0042-1758982

Usually, novel natural products are isolated in small amounts. This makes it difficult to explore their pharmacological targets in vitro and explore their effects in vivo. Hence, approaches to streamline these obstacles are pursued, two of which are addressed in this study: (1) In silico models to rationalize molecular target testing, (2) *Caenorhabditis elegans* as in vivo model to test compounds at the scale of an in vitro cellular assay.

We present a discovery pipeline for two azepine-indole alkaloids, nemorosine A (1) and fargesine (2), which have been identified as the main azepine-indole alkaloids of *Psychotria nemorosa* [1]. To explore their pharmacological profile, we applied an in silico molecular target fishing approach which is based on 3D similarity searches of the ChEMBL database [2]. Hereby, structurally related compounds that modulate the 5-HT2 receptor were identified. In vitro experiments confirmed an agonistic effect of 1 and 2 at the 5-HT2A receptor. This and the previously reported target profile of 1 and 2, which also includes BuChE and MAO-A inhibition [1], prompted the evaluation of these compounds in several *C. elegans* models linked to 5-HT signalling and proteotoxicity. Alkaloids 1 and 2 inhibited *C. elegans* motility and pharyngeal pumping. They alleviated amyloid beta proteotoxicity in transgenic strain CL4659 and reduced α-synuclein accumulation in transgenic strain NL5901.

These results add to the multi-target profiles of 1 and 2 and corroborate their potential in the treatment of neurodegeneration. They also highlight the capability of pipelines employing both in silico and nematode models [3].

References

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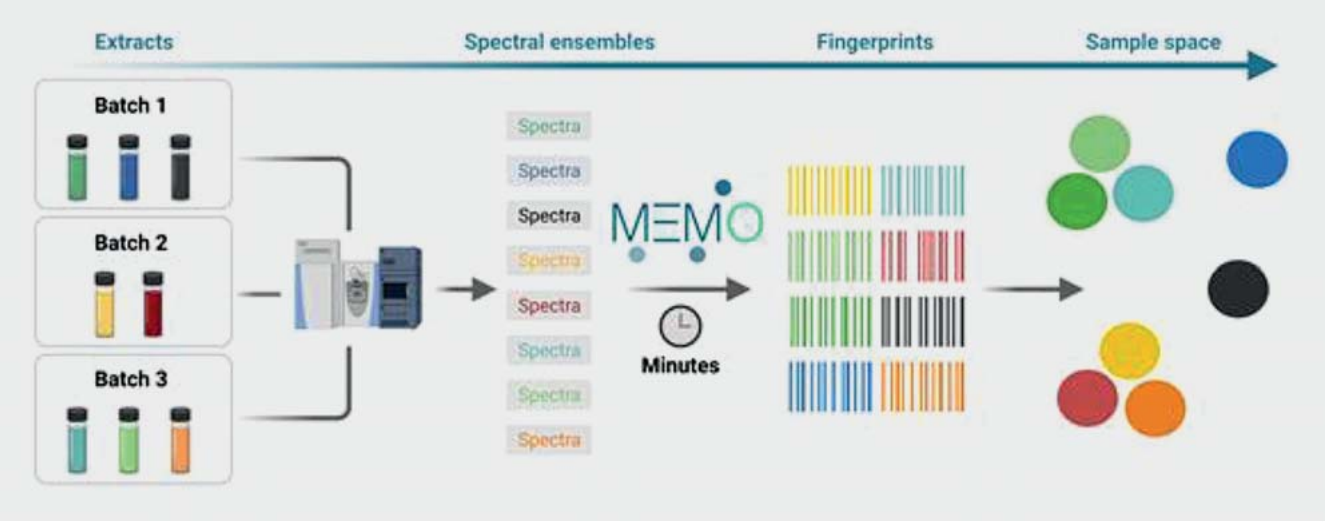

▶ **Fig. 1**

SL-F04 Short Lecture "Mass spectrometry-based sample vectorization for exploration of large chemodiverse datasets and efficient identification of new antiparasitic compounds"

Authors Gaudry A[1,2], Huber F[3], Flückiger J[1,2], Quirós L[1,2], Rutz A[1,2], Kaiser M[4,5], Grondin A[6], Marcourt L[1,2], Ferreira Queiroz E[1,2], Wolfender J-L[1,2], Allard P-M[1,2,7]

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In natural products research, UHPLC-HRMS/MS has become the reference method for the analysis of crude extracts. The profiling of hundreds to thousands of extracts results in large ensembles of data. This data is used in downstream analyses such as molecular networking, annotation processes to perform dereplication, or bioactive compounds prioritization [1–3]. Most of these analyses rely on so-called aligned datasets, where the occurrence of compounds among samples is compared usually via retention time and m/z comparison. The subsequent analysis of samples acquired in different batches or using different LC methods is thus complicated because of the inevitable shifts across the LC and MS dimensions. To compare large ensembles of chemodiverse extracts without relying on a prior alignment step, we developed a new method, called MEMO, which generates a unique fingerprint for each extract by aggregating its fragmentation data [4]. Using these fingerprints, similarities among extracts can then be spotted in a sample-space visualization (▶ **Fig. 1**). To benchmark the method, we applied it to a massive set of 1,600 chemodiverse plant extracts profiled in the frame of an antiparasitic screening. By combining MEMO with different annotation tools and the bioactivity results, we could rapidly identify that among the eight extracts active against *Trypanosoma cruzi*, six presented similar MEMO fingerprints and were found to be rich in rotenoids. The rotenoids' activity against *T. cruzi* was confirmed for deguelin and rotenone with an IC_{50} of 0.025 µM and below 0.005 µM respectively.

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SL-F05 Short Lecture "Application of NMR and LC-MS based chemometrics in an artificial extract as proof of PEGASUS concept"

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Conventional methods for detecting bioactive ingredients in plant extracts are time consuming, costly, and often result in the isolation of moderately active substances, or the detection of already known natural products. For this reason, chemometric methodologies have been developed for the detection and identification of metabolites in complex mixtures.

The aim of PEGASUS is to evaluate and optimize HetCa approach [1] to identify bioactive metabolites in plant extracts prior to isolation. Hence, a mixture of 59 standard substances (artificial extract) was prepared. The constituents of the artificial extract were selected to cover a wide range of polarity and different biological activities against the enzyme tyrosinase and the free radical DPPH, including strong and medium inhibitors as well as metabolites with no activity, thus, simulating a plant extract. FCPC was employed for the fractionation of the artificial extract using a step-gradient method with eight steps

to provide the concentration variance needed for the application of the statistical correlations. Subsequently, the inhibitory activity of all the FCPC fractions against tyrosinase and DPPH was evaluated, while their chemical profile was recorded using NMR spectroscopy and LC-MS spectrometry. Spectral information was processed with MATLAB for the implementation of HetCa approach (SHY and STOCSY algorithms [2,3]), to obtain pseudo-spectra (heterocovariance plots) that indicate the bioactive compounds.

PEGASUS incorporates chromatographic and spectroscopic techniques and bioactivity results along with advanced chemometric tools and could be established as a method of choice for the rapid and effective identification of bioactive natural products in plant extracts prior to isolation.

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SL-F06 Short Lecture "In-depth exploration of *Strychnos* alkaloids by molecular networking: Discovery of strychnine in new species"

Authors Bonnet O[1], Beniddir MA[2], Champy P[2], Kagisha V[3], Nyirimigabo A[3], Hamann C[1], Jgerenaia G[4], Ledoux A[1], Tiabou Tchinda A[5], Angenot L[1], Frédérich M[1]

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Due to their wide variety of traditional uses [1] and promising activities against *Plasmodium* parasites [2], plants of the *Strychnos* genus have been very well studied. Moreover, different attempts to draw an intrageneric taxonomy were made, based on morphological (Duvigneaud) and genetic (Setubal) characters. Moreover, in the Setubal et al. (2021) study, the results concluded that the classification established by Duvigneau is currently the most appropriate [3].

In this context, my research project consists in exploring the chemodiversity of *Strychnos* alkaloids in order to identify new bioactive metabolites against malaria and cancer, but also to study the chemotaxonomy of *Strychnos* genus. To achieve these objectives, 44 extracts, from 28 species of *Strychnos*, were studied by LC-MS/MS and molecular networking. Furthermore, by comparison with MS/MS spectra databases, known and unknown metabolites were annotated. Among the known ones, strychnine was surprisingly detected in seven *Strychnos* species for the first time, namely in *S. tricalysioides, S. camptoneura, S. congolana, S. boonei, S. densiflora, S. tchibangensis* and S. *usambarensis*. The TLC, HPLC, NMR and UPLC-MS/MS analyses allowed to detect the presence of this compound.

This novel identification of strychnine, allowed by the sensitivity of the technique used, offers new insights in the chemotaxonomy of the *Strychnos* genus. The perspectives are, on the one hand, further delineation of indole monoterpene alkaloids distribution in *Strychnos* spp., in regard to their taxonomic organization and, on the other hand, the identification of original bioactive compounds in this series.

The authors declare that they have no conflict of interest.

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Wednesday, August 31 | Short Lectures F

Animal Health care

SL-F07 Short Lecture "The effect of *Melissa officinalis* extract and chlorogenic acid on intestine motility of broiler chicken – ex vivo study"

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Melissa officinalis is a very common herb with high palatability. It also has a long history of being used in traditional medicine. This study aimed to develop knowledge about *Melissa* extract and its main active substances in regards to broilers intestine contractility – proximal and distal part of jejunum.

Materials and methods: The experiments were conducted on longitudinal jejunum samples collected from routinely slaughtered birds on two different parts of the jejunum: proximally and distally to Meckel diverticulum. The effect of standardized *Melissa officinalis* extract (Nor-Balm®) [1] and chlorogenic acid on spontaneous and ACh-induced activity was evaluated under isometric conditions [2].

Results: The results revealed dose-dependent potency of *Melissa* extract to increase the magnitude of acetylcholine-induced contraction. Besides, the extract enhanced spontaneous contractility in distal part of the jejunum but reduced this strength in proximal part. In case of chlorogenic acid the spontaneous motility in both parts was dose-dependently decreased. Chlorogenic acid inhibited remarkably the contraction induced by ACh in both parts.

Conclusions: The results of the performed study indicate that *Melissa officinalis* can be used to control gastrointestinal motility in chicken. Its ability to limit the size of ACh-induced contractility might be beneficial in hypermotility disorders in broilers. It seems that chlorogenic acid does not contribute to the final effect of the plant extract what proves more complex interaction and probable contribution of other phytoconstituents.

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SL-F08 Short Lectures "Effects of offering a combination of *Curcuma* and *Scutellaria* plant extracts on laying hen thermal tolerance under hot temperate conditions"

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DOI 10.1055/s-0042-1758987
Background: Heat stress challenges laying hen health and welfare, adversely affecting their productivity. Certain plant secondary metabolites may alleviate effects, due to their antioxidant and anti-inflammatory activities in the intestine and liver.
Aims: Herein, we investigated the effects of dietary supplementation with a phytonutrient solution (PHYTO) consisting of a plant extract combination of *Scutellaria baicalensis* and *Curcuma longa* on layers raised in summer Mediterranean conditions.
Materials and Methods: Four hundred, 24-week-old hens were allocated in 50 cages and were offered a diet either containing 2 g/kg of PHYTO or not, for 8 weeks. Egg production and feed intake were recorded weekly. At the end of the trial two hens per cage were blood sampled for assessment of blood markers, one of which was euthanized for histopathological evaluation of the liver and intestine and assessment of intestinal histomorphometry.
Results: PHYTO supplementation significantly increased egg production rate at weeks 26–27 and for the overall production period ($P < 0.01$), and feed: egg ratio at weeks 26–27 and 28–29 ($P < 0.05$). The degree of liver necrosis and microvascular thrombosis was lower ($P < 0.05$) whereas intestinal villosity was increased in duodenal and jejunal segments ($P < 0.05$) by PHYTO supplementation. Supplemented hens had reduced ($P < 0.05$) serum levels of corticosterone, TBARS and alanine aminotransferase activity. Levels of serum antioxidant enzymes were not affected, apart from catalase, which was reduced in supplemented birds.
Conclusion: Supplementation with PHYTO increased laying hen productivity, and improved laying hen thermotolerance.
Conflicts of interest: None

SL-F09 Short Lecture "Isolation of antibacterial compounds from *Searsia batophylla* and their activity against diarrhoeagenic *Escherichia coli*"

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DOI 10.1055/s-0042-1758988
Searsia species (Anacardiaceae) are used for different medicinal purposes in southern Africa, including gastrointestinal disorders, diarrhoea and gallsickness in cattle. The present study aimed to evaluate the bioactivity and cytotoxicity of fractions and isolated compounds from the leaves of *Searsia batophylla* (Codd) Moffett. The crude acetone extract was partitioned with water, ethyl acetate, hexane, chloroform, methanol and butanol. A two-fold serial dilution assay was used to determine the antibacterial activity of fractions and purified compounds against *Escherichia coli* 25 922, and an *E. coli* isolate from a clinical case of diarrhoea in cattle. Vero monkey kidney cells were used to determine cytotoxicity. Bioassay-guided fractionation of the chloroform fraction yielded three compounds. Structure elucidation was done using nuclear magnetic resonance (NMR) spectroscopic analysis, ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) and gas chromatography-mass spectrometry (GC-MS). Fractions had varying MIC values ranging from 0.04 to 2.50 mg/mL. Dodecanamide, 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (α, β-unsaturated ketone), and 3-oxo-olean-18-en-28-oic acid were isolated from *S. batophylla* leaves. Although the chloroform fraction had a minimum inhibitory concentration (MIC) value of 0.16 mg/ml, the compounds had MIC values ranging from 0.63 to 0.93 mg/ml. Compounds isolated from *S. batophylla* therefore most likely have synergistic antibacterial activity. All fractions and compounds had relatively little cytotoxicity. Further investigations are ongoing regarding the biofilm and quorum sensing inhibitory activities of the extract, fractions and compounds as well as activity against other bacterial clinical isolates.

Monday, August 29 | Poster Session I

- Ethnobiology – Ethnobotany – Biodiversity (P-001 – P-015)
- Natural compounds from marine organisms, fungi and microorganisms – Endophytes and microbes (incl. Microbiome) (P-016 – P-033)
- Analysis and authenticity – Quality control – Metabolomics (P-034 – P-081)
- Circular economy-Bioeconomy-Green technologies-Sustainable development of agricultural/industrial by-products (P-082 – P-097)
- Biotechnology-Bioengineering (P-098 – P-101)
- Chemistry and bioactivity of natural products (P-102, P-200, P-207)

P-001 How do medicinal plants contribute to the treatment of childhood diseases in rural areas of North-West Province?

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DOI 10.1055/s-0042-1758989
In South Africa, the majority of people still rely on traditional medicine for primary health care [1–3]. However, there is still a dearth of information on medicinal plants used to manage childhood diseases in many provinces including the North West Province. Hence, the current study documented medicinal plants used to manage childhood diseases in the North West Province [4]. An ethnobotanical survey was conducted among 101 participants using semi-structured interviews (face-to-face). Ethnobotanical indices such as the FC, UV and ICF were used for data analysis. We recorded 61 plant species from 34 families as herbal medicine used for managing 8 categories of diseases. Skin-related diseases were the most prevalent childhood diseases managed by the participants. Based on their FC values that ranged from approximately 0.9–75%, *A. elongatum* (75.2%) and *C. diffusa* (45.5%) were the most popular medicinal plants among the participants (▶ **Table 1**). Based on ICF, skin-related diseases had the highest ICF value (0.99). This category had 381 use-reports, comprising of 34 plant species (55% of total plants) used for childhood-related diseases with *E. elephantina* being the most cited plants in this category. Leaves (23%) were the most frequently used plant parts. Decoctions was the main methods of preparation, and the plant remedies were mainly administered orally (60%) and topically (34%). The current study revealed the continuous dependence on plant for primary health care relating to childhood diseases in the study area. However, the phytochemical profiles and the safety of these identified plants remain an essential aspect for future research.
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Planta Med 2022; 88: 1385–1588

▶ **Table 1** Ethno-botanical information on 10 top plants used for the treatment and management of childhood disease and well-being in Ngaka Modiri Molema and Bojanala districts of North West Province, South Africa.

Scientific name & Family [Voucher number]	Local name	Plant part & method of preparation	Childhood diseases/conditions	Administration and Dosage	[a] Plant form	[b]N	FC	Cs
Aptosinum elongatum Eng **Scrophulariaceae [TPN 016]**	Ditantanyane	Stem, Infusion	Umbilical cord, muscle fits, measles, bladder inflammation, weight and appetite	Orally (3 ×/day)	H	76	75.2	LC
Bulbine frutescens (L) Willd **Xanthorrhoeaceae [TPN 004]**	Makgabe-nyane	Rhizome/ bulb, roots Infusion, maceration	Sunken fontanelle, umbilical cord; body rash, sores, phlegm and urinary tract infection	Topical and orally (2 ×/day)	H	22	21.7	LC
Commelina diffusa Burm.f. **Commelinaceae [TPN 039]**	Kgopokgolo	Rhizome/ bulb, Decoction	Umbilical cord, purgative the child, preventing evil spirits and weak child	Orally, (2 ×/day)	H	45	44.5	LC
Elephantorrhiza elephantina (Burch) Skeels **Fabaceae [TPN 051]**	Mositsane	Roots, maceration or poultice	Infective eczema, diarrhoea, ulcer, burns and measles	Orally and topical (3 ×/day)	H	18	17.8	LC
Euphorbia prostrata Aiton **Asparagaceae [TPN 019]**	Letswetlane	Rhizome, enema or decoction	Constipation and phlegm	Orally, (As needed)	H	32	31.6	NE
Hypoxis hemerocallidea Fisch., C. A.Mey. & Ave-Lall. **Hypoxidaceae [TPN 058]**	Tshuka ya poo	Roots, decoction	Sunken fontanelle, bladder inflammation, kidney failure, urinary tract infection, bronchitis pneumonia, child cleanse influenza and ulcer, gastro-intestinal and appetite	Orally, Topical and orally (2 ×/day)	H	20	19.8	LC

The botanical names of the plants were verified using the World flora online (http://www.worldfloraonline.org/) and conservation status were verified using South African Red data list (http://redlist.sanbi.org/species) [a]Plant form: T = Tree, S = Shrub and H = Herb. [b]N = Number of participants. Ethnobotanical Index used, N = Frequency of Citation; Use-value = UV; Conservation status = CE = Critically Endangered; NE = Not Evaluated; LC = Least common; IA = Invasive alien species and EN = Endangered

P-002 Comparative study on chemical profile and antioxidant activity of *Artemisia pontica* L. during different vegetation stages in Lithuania

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In order to solve the problem of conservation and increase diversity of *Artemisia* (L.) genus plants it's necessary to determine their biologically active compounds and benefit on human health [1,3]. Different studies indicate positive effects of Artemisia extracts and essential oils for the treatment of malaria, cancer, inflammation and infections by fungi, bacteria and viruses such as SARS-CoV-2 [1,2].

The object of investigation was *Artemisia pontica* (L.) – a medicinal (aromatic) plant of Asteraceae (Bercht. & J. Presl) family, introduced in the Middle of Lithuania since 1973.

The aim of this study was to determine qualitative and quantitative phenolic content and antioxidant activity in *Artemisiae pontici* herbae samples were collected at different vegetation stages.

The methanolic *Artemisiae pontici* herbae extracts reported the highest total phenolic (271.93 ± 2.54 RE mg/g), FRAP (62.17 ± 0.32 µmol TE/g DW) and CUPRAC (152.94 ± 3.42 Mm TE/g) amount in massive flowering vegetation stage. Moreover, the highest value of total amount of flavonoids (8.56 ± 0.09 RE mg/g) and DPPH (87.26 ± 0.30 RE mg/g) were determined in the end of flowering, ABTS•+ (85.57 ± 0.89 µmol TE/g DW) in the beginning of flowering. The following phenolic acids and flavonoid glycosides were identified (caffeic, chlorogenic, neochlorogenic, 4,5-dicaffeoylquinic, 4-O-caffeoylquinic, 3,5-dicaffeoylquinic, 3,4-dicaffeoylquinic, isorhamnetin-3-rutinoside, luteolin-7-O-glucoside, luteolin-7-rutinoside, rutin. The significantly higher ($p < 0.05$) amount of chlorogenic (188.04 ± 0.81 mg/g) and 3,5-dicaffeoylquinic acid (122.16 ± 2.12 mg/g) were detected in the intensive growth vegetation stage.

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P-003 Medicinal plants in the treatment of skin diseases in Serbia during 19th and 20th century

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DOI 10.1055/s-0042-1758991

The purpose of this study was to collect and analyse plant-based treatments applied in Serbian folk medicine for treating skin disorders during the nineteenth and early to mid-twentieth century. We have consulted the most relevant ethnographic literature that was in any larger segment devoted to the folk medicine. This investigation has identified 164 plant species from 63 families and one mushroom species as being applied in the treatment of ailments categorized as skin diseases in the International Classification of Primary Care. Frequently used families were: Asteraceae (14 taxa), Lamiaceae (13 taxa), Solanaceae (10 taxa) and Rosaceae (8 taxa). Among plant parts leaves were the most utilized (21%) followed by aerial parts, roots, fruits and seeds. Mostly plant species were applied externally (60.98%), 9.15% of mentioned species were used both internally and externally and 4.27% were administered only externally. Herbs were processed and taken in different ways and forms, both fresh and dry. As fresh they were directly applied, or previously mashed and mixed with water, milk, vinegar, oil, honey, lard and butter. In case of *Arnica montana, Fumaria officinalis, Galium verum*, and *Conium maculatum* juice squeezed from fresh parts of the plants was used. Skin problems recorded in our investigation could be divided in three main categories: hair problems, bites and inflammatory skin diseases such as eczema, psoriasis etc. After comparison with contemporary ethnobotanical investigations conducted on the territory of Serbia, we observed that significantly lower number of plant species was recorded in modern studies.

P-004 Ethnopharmacological study of plants used against skin diseases in the region of Komotini

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The treatment of skin diseases is a global issue and despite scientific progress in the field of wound healing, it is often found that effective treatment is insufficient, especially when serious pathological conditions (e.g., diabetes) coexist. It is worth mentioning that numerous plants are still used today in the traditional medicine of many countries, not only for wound healing but also for the treatment of other skin diseases [1,2]. Given that Greek traditional medicine is a rich source of information their documentation, combined with scientific validation of their efficacy, could contribute to the discovery of new and remarkable therapeutic agents. As part of the European EthnoHERBS, an ethnobotanical study was carried out in the area of Komotini, Northeastern Greece, regarding the use of aromatic and medicinal plants for skin treatment. After processing the data of 50 questionnaires completed by residents of Komotini, 62 aromatic and medicinal plants were found to be used in skin diseases such as St. John's wort, calendula, chamomile, aloe, nettle, and rosemary, while tobacco was also mentioned due to its extensive production in the past. The most frequently mentioned families were Asteraceae, Lamiaceae, Rosaceae, Solanaceae and Violaceae. Comparing the scientific bibliographic data with the information obtained from our study, it was proved that the traditional use of the mentioned plants against skin diseases such as application of lemon balm in herpes [3], use of bergamot in acne [4] and elderberry in sunburns [5], were all confirmed in the literature thanks to their pharmacological properties.

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P-005 Validation of the traditional use of *Achillea moschata* Wulfen at gastric level: an ethnopharmacological approach

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Traditional plant knowledge in mountain areas has become an essential source of ethnomedicinal findings, basis for the selection of plants as potential source of new bioactive compounds [1]. In this context, an ethnobotanical survey, part of the European Interreg Italy-Switzerland B-ICE project, investigated the traditional uses of the autochthonous plant species in Valmalenco (Sondrio, Italy). From 2019 to 2022, a total of 401 informants were interviewed. The inflorescences of *Achillea moschata* Wulfen were 40%-mentioned for the use in digestive tract disorders, such as gastritis, an inflammatory-based disease often associated with *Helicobacter pylori*.

The present research aims at verifying the potential traditional properties of *Achillea moschata* Wulfen inflorescence extract at this level.

Infusion, decoction, and ethanol : water (50 : 50) extracts were prepared, in order to compare traditional preparations and different extraction solvents. The phytochemical profile of the extracts showed similar quali- and quantitative content of polyphenols, correlating with the activity at biological level. The extracts had similar anti-inflammatory activity when studied in an in vitro model of non-tumoral gastric epithelial cells (GES-1) stimulated with TNF-α or H. pylori-infection. The preparations inhibited the NF-κB driven transcription, IL-8, and IL-6 release, with IC_{50}s ranging from 30 to 100 µg/mL. The anti-bacterial effect was assessed on H. pylori by evaluating the minimum inhibitory concentration (MIC): the extracts showed activity starting from 100 µg/mL.

This is the first study demonstrating the anti-inflammatory and anti-bacterial properties of traditional preparations of *Achillea moschata* Wulfen inflorescences against *H. pylori*-related gastritis.

The authors have no conflict of interest to declare.

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P-006 A tale of two chamomiles: profiling the differences between *Tripleurospermum tempskyanum* (Freyn & Sint.) Hayek and *Matricaria recutita* L

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Matricaria and Tripleurospermum (Anthemideae, Asteraceae) are two closely related genera with a wide distribution across the Mediterranean basin. Until recently, the multitude of morphological similarities constituted a cause of taxonomic confusion among botanists, with several Tripleurospermum species being attributed to the Matricaria genus in nomenclature. Nevertheless, in recent years, studies around the genome of those plants assisted to the definite separation of the two genera [1]. In this context, the present work describes the phytochemical investigation of the aerial parts of the common chamomille, M. recutita (Mr) and T. temspkyanum (Tt), a rare species which has only been encountered in certain parts of Greece and Turkey.
On one hand, LC-HRMS analysis of the plants' polar extracts, revealed a similarity among the two species, with their profiles being characterized by a plethora of cinammic acid derivatives, flavonoids, fatty acids and certain coumarins. However, the main differences could be found in their major flavonoids, with Mr and Tt being abundant in apigenin and luteolin derivatives, respectively. On the other hand, GC-MS analysis of the plants' essential oils (eo) and non-polar extracts, showed significant variation among the two plants, especially for their main constituents. Finally, fractionation of Tt's non-polar extract, led to the isolation and unambiguous structure elucidation of its major volatile compounds. Overall, the current study provides novel insight on the phytochemical character of the two genera contributing to the elucidation of taxonomic contoversities.
The author is thankful to the Special Account for Research Grants and the NKUA for funding their participation.
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P-007 Hunting Luteolin in the Greek biodiversity for the treatment of periodontal diseases

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Periodontitis comprises an inflammatory disease of the periodontal tissues that is commonly initiated by bacterial microorganisms. If it is not properly treated, complications may occur involving gum recession, alveolar bone destruction and ultimately tooth loss [1]. During the early stages of periodontitis, conservative treatment techniques are preferably used, with the application of different pharmaceutical formulations being one of them; anti-bacterial and anti-inflammatory agents are the most frequently prescribed drugs [2]. However, these medications are often accompanied by side effects that can decrease patient adherence and compliance. For this reason, alternative treatments are mainly sought in the field of natural products.
Specifically, flavonoids have attracted a great amount of interest, due to their increased biological index and the fact that they can be abundantly found in many natural sources, such as fruits, vegetables, and seeds. Luteolin, belonging to flavonoids, is present in various plants of the Mediterranean region and especially the Greek biodiversity, and possesses significant anti-inflammatory properties [3]. In the present work several plants of the Greek flora (e.g., Origanum vulgare, Thymus vulgaris, Salvia officinalis, Laurus nobilis species) were grown and their extracts obtained by sonication were examined by HPLC-DAD method for their content in luteolin. Seasonal variation of luteolin in the above plants was also assessed.
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P-008 Use of Natural Products During the COVID-19 Pandemic: An Exploratory Cross-Sectional Study in Saudi Arabia

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During the COVID-19 pandemic, the use of Natural Products (NP) became an emerging treatment as well as prevention option among several communities. In Saudi Arabia, there is an increasing interest of the public in using NP for COVID-19. This study aimed to explore and document the types of NP and attitudes of NP' users among Saudi citizens. This was a cross-sectional study with data collected through an online survey distributed through social media on adult Saudi citizens between 18 September 2021 and 23 January 2022. Among the participants, Honey (80.1%), Lemon/Lime (*Citrus* sp.) (65.4%), Orange (*Citrus sinensis*) (54.1%), and Ginger (*Zingiber officinale*) (50.8%) were the most frequently used NP. Individuals infected with COVID-19 utilized NP to treat symptoms such as loss of tasting and smelling (12.6%), cough (12.2%), fatigue (12.2%), headache (11.9%), fever (11%), nasal congestion (9.1%), malaise (8.2%), shortness of breath (7.3%), and sneezing (4.5%). The most common source of information were friends (44.5%), internet (42.6%), social media (38.7%), doctors (18.7%), and pharmacists (12%). About half of the participants used NP in its basic form (54.8%). The most common reason for using NP was to induce the immune system (86.5%). In conclusion, the types and attitudes of NP used for COVID-19 among citizens in Saudi Arabia were documented. This trend of using NP for disease treatment and prevention should be highly considered in future medical research, as well as in the pharmaceutical sector in terms of treatments and products development.

P-009 Medicinal Plants of Guinea-Bissau's Bissorã and Dabatear geographic regions

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DOI 10.1055/s-0042-1758997
The use of medicinal plants in Guinea-Bissau is recognized and documented in different ethnobotanical surveys and published works. However, no data were available on some geographical regions. The present work intends to improve this knowledge by means of an ethnobotanical survey carried out in the Bissorã and Dabatear (Bula) regions of this country to identify the most used medicinal plants and to analyze and understand the different forms of preparation and use of each plant in these two regions.
An ethnobotanical mission was carried out in the communities of the Bissorã and Dabatear regions. For collecting the data, semi-structured interviewees, observation, and field walks were employed from January to February 2015. Corresponding plant material was collected by traditional medicine practitioners (TMPs) or by members of the team, to allow their subsequent botani-

cal identification and laboratory studies. All collected data were digitally recorded (sound and image). The survey was conducted in Creole and Mancanha languages. A total of 30 medicinal plants belonging to 18 species and 13 families are used in 15 traditional herbal recipes (THR) used by 11 TPMs. These medicinal plants and THR were used to the relieve symptoms or treatment of 22 of the most local common diseases, namely headache, yellow fever, allergies, inflammation, pain, wounds, fevers, anemia, psoriasis, skin diseases, vomiting, bladder inflammation, malaria, conjunctivitis, sexually gonorrhea, diarrhea, ringworm, kidney pain, and disease groin.
A special knowledge to the participating TMPs. All collected data will be returned in an adequate manner to the participating community.

P-010 Phytochemical screening of *Gentiana purpurea*, an important Norwegian medicinal plant

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Gentiana purpurea L. (Gentianaceae family) was one of the most important medicinal plants in Norway in the 19th and 20th centuries, both for veterinary and human use. Indications were all kind of stomach diseases, especially diarrhea, but also chest diseases such as bronchitis, and to treat coughing and the cold. The roots are known for the intense bitterness, which can be explained by the high content of secoirioids. Secoiridoids, such as gentiopicroside and amarogentin have previously been identified in the plant [1], however a detailed overview of the metabolites in the traditional preparations that were commonly used has not previously been described. The aim of the presented study was to perform a phytochemical screening of water and ethanol extracts of *G. purpurea*.
The crude extracts were applied to a Diaion HP-20 column and eluted with water and methanol. The methanol fractions were subjected to C18 flash chromatography, Sephadex LH-20 and preparative HPLC to obtain pure compounds. NMR and MS were used for structure elucidation. Isolated compounds included secoiridoid glucosides, flavonoids, a lignan, and other phenolic compounds, and represent both new natural products and new compounds for *G. purpurea*.
We have no conflict of interest.
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P-012 Giuseppe Raddi: The importance of your legacy for Biodiversity in the 3rd Millennium

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DOI 10.1055/s-0042-1759000
This project presents the discovery of the new plant world in Brazil through the work of 19th century botanist and ecologist Giuseppe Raddi. In October 2017, the University of Florence, Italy, celebrated 200 years since Raddi's travels to Brazil and his scientific expedition that included collecting and categorizing over 4000 plant species in 10 months. Raddi's collecting and research provided and subsequently defined the Brazilian ecosystem now known as the Atlantic Forest. Giuseppe Raddi's journey to Brazil began with Princess Leopoldina of the Habsburgs and other scientists and artists on the Journay from Italy to Rio de Janeiro. This project brings to life the journeys, research, discoveries of Giuseppe Raddi and the social conditions of the time in Brazil.
Goals: Presents the value of plants discovered in Brazil more than 200 years ago for contemporary medicinal and nutritional and ornamental uses. Brings to life the conditions on board during the ocean voyage to Rio de Janeiro and the social and political issues during his time in Brazil which are highlighted today through Giuseppe Raddi's diaries. Launch of the first Leandra's Brazilian Encyclopaedia.

▶ **Fig. 1**

Giuseppe Raddi's life in Italy, Brazil and Egypt – essays and excerpts from his diaries. The trajectory of his life, presented in a book containing Giuseppe Raddi's travel records and studies, the arrival of Princess Leopoldina and other important European scientists and naturalists on the journey to Brazil, as well as reports on his stay in Egypt at Champollion Expeditions and local conditions during that time.

P-013 Herbs and spices mentioned in first Latvian cookbooks

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The cookbook "The First Cookery Book Translated from German books" was published near Valmiera in 1795 by the German priest of Rubene Christoph Harder. It was the first cookbook in Latvian language with 414 food recipes. One year later, in 1796, "Latvian cookbook for manor chefs on teaching to cook and prepare all kinds of gentlemen's dishes" was published in Jelgava, also in Latvian, and contained 560 food recipes. In these two books, a variety of herbs and spices are mentioned such as the following: chervil, chives, endive, marjoram, parsley, parsnip, thyme, almonds, bay leaf, caraway, cardamom, capers, cinnamon, cloves, ginger, lemon peel, lovage, nutmeg as well as nutmeg flowers, pepper, vanilla etc. These ingredients can be found practically in all recipes' chapters – soups, meat and fish dishes, vegetables, pates, desserts – with the aim to enrich the flavour and taste of the dishes as well as to make their texture juicier. As salt was not used in big quantity, by additives of herbs and spices, the sweetness and the bitterness of served dishes was balanced.
This research is funded by the Latvian Council of Science, project "National Identity: Gastropoetic Aspect. Historical, International and Interdisciplinar Contexts" No. lzp-2019/1-0294.

P-015 Will the role of Indigenous Knowledge Systems help in sustaining utilization and conservation of indigenous medicinal plants?

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DOI 10.1055/s-0042-1759002

Several rural communities use indigenous knowledge systems (IKS) for decision making related to human and animal health, education, food security, and natural resources management. The utilisation of indigenous plants by local communities is not random and haphazard, rather, local communities have utilised their IKS to foster sustainable utilisation and conservation of indigenous plants. Only a small proportion of this knowledge has been documented, yet it remains a valuable repository that provides us with information on how numerous local communities have interacted with the flora and fauna of their changing environment. The IKS is culturally enshrined and comprised of behavioural corrective norms, capable of changing local peoples' perspectives towards biodiversity resources. Therefore, the current presentation considers the indigenous cultures and strategies that have shaped the sustainable utilization and conservation of medicinal plants. Indigenous practices are important for the sustainable utilisation and conservation of medicinal plants by rural communities which are enshrined in myths, taboos, values, folklore, traditional beliefs, rituals and traditional institutions. Myths and taboos have been used to conserve various indigenous resources for sustainability, based on prior knowledge of the importance of a particular genetic resource. Traditional leadership are the primary custodians of rural areas and play a significant role in protecting and preserving indigenous resources through igniting the cultural values and norms within the communities. It is important to understand the IKS before conserving the medicinal plants because about 80% of people worldwide depend on medicinal or traditional health care from medicinal plants.

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P-016 Discovery of Bioactive Secondary Metabolites from Fungal Endophytes Using Chemical Elicitation and Variation of Fermentation Media

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DOI 10.1055/s-0042-1759003

Endophytic fungi are an important source of bioactive secondary metabolites [1]. In this study, fungal endophytes obtained from A*STAR's Natural Product Library (NPL) and previously isolated from different habitats of Singapore were investigated for their diversity and biological activities. A total of 222 fungal strains were identified on the basis of sequence analysis of ITS region of the rDNA gene. The identified fungal strains belong to 59 genera distributed in 20 orders. Majority of the identified strains (99%; 219 strains) belongs to the Phylum Ascomycota, while two strains belonged to the phylum Basidiomycota and only one strain was from Mucoromycota phylum. The most dominant Genus was *Colletotrichum* accounting for 27% of all the identified strains. Chemical elicitation using 5-azacytidine and suberoylanilide hydroxamic acid (SAHA) and variation of fermentation media resulted in the discovery of more bioactive strains. Bioassay-guided isolation and structure elucidation of active constituents from three prioritized fungal strains; *Lophiotrema* sp. F6932, *Muyocopron laterale* F5912 and *Colletotrichum tropicicola* F10154 led to the isolation of a known compound; palmarumycin C_8 and five novel compounds; palmarumycin CP_{30}, muyocopronol A–C and tropicicolide. Tropicicolide displayed the strongest antifungal activity against *A. fumigatus* with an IC_{50} value of 1.8 µg/mL. Palmarumycin C_8 revealed the best antiproliferative activity with IC_{50} values of 1.1 and 2.1 µg/mL against MIA PaCa-2 and PANC-1 cells respectively [2]. The findings from this study demonstrates how diversification of growth media and use of selected chemical epigenetic modifiers can facilitate the discovery of new bioactive microbial natural products.

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P-017 Assessment of the antimicrobial and anti-virulent properties of the marine bromophenol methylrhodomelol against *Pseudomonas aeruginosa*

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Bromophenolic compounds from marine origin offer a plethora of structural features and known bioactivities, of which an antibacterial activity has been described for a fair amount of bromophenols [1]. For the red algal metabolite methylrhodomelol an antimicrobial activity has been suggested [2], however, it has never been confirmed experimentally. Therefore, aim of the current study was to assess potential antibacterial properties of this compound against the gram-negative pathogen *Pseudomonas aeruginosa*, including an investigation of its antivirulent potential.

Methylrhodomelol showed an antiproliferative activity against P. aeruginosa in Vogel-Bonner minimal medium (VB-MM) with a minimum inhibitory concentration (MIC) ranging from 25–50 µg/mL (positive control: gentamicin, MIC 0.3 µg/mL) for the laboratory strains ATCC27 853 and ATCC9027. Bacteriostatic effects were also observed in four different multiresistant clinical isolates of *P. aeruginosa* (MIC range: 50–100 µg/mL), whereas the compound did not show cytotoxicity in eucaryotic Vero cells (IC_{50} > 200 µM). In addition, antivirulent assays on bacterial motility, biofilm formation and proteolytic activity were performed in *P. aeruginosa* ATCC27 853, however methylrhodomelol did not show any significant activity regarding the tested virulence traits.

In summary, we report a moderate bacteriostatic activity of the marine bromophenol methylrhodomelol against *P. aeruginosa* in VB-MM, shedding some experimental light on its proposed antimicrobial potential.

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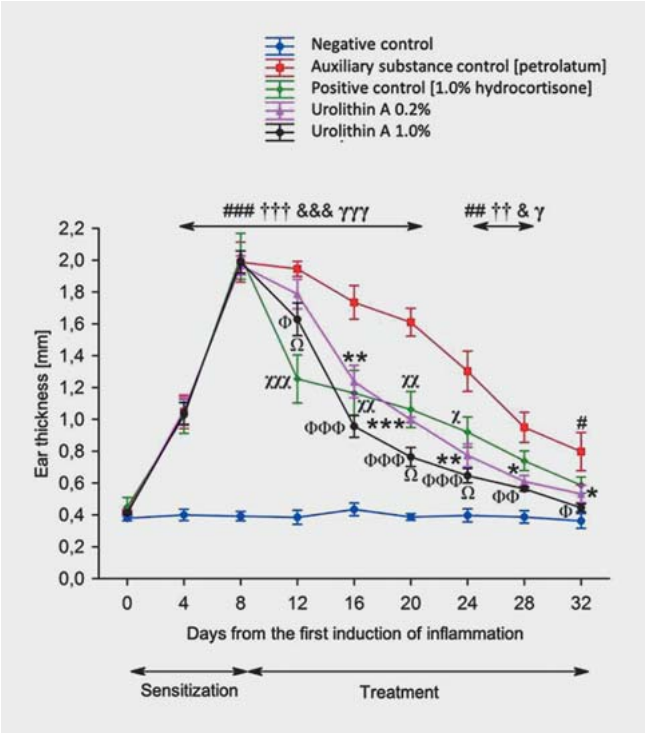

▶ Fig. 1

P-018 Application of urolithin A – a postbiotic metabolite produced by human gut microbiota, in topical treatment of atopic dermatitis

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DOI 10.1055/s-0042-1759005

Urolithin A (UA) is a postbiotic metabolite produced by human gut microbiota from ellagitannins. Unlike ellagitannins, UA has well-documented bioavailability, and its anti-inflammatory properties were proven in many studies. However, following absorption in the intestine, UA is conjugated with glucuronic acid and hence is present in the form of inactive phase II metabolites in tissues and bloodstream. Above limitations related to oral application of ellagitannins and UA have led to attempts to use its anti-inflammatory potential in a topical formulation applied to the skin.

Composition of an ointment was based on white petrolatum with 0.2% or 1.0% UA. The formulation containing 1.0% hydrocortisone was applied as a positive control. The induction of dermatitis on the ear skin of Wistar rats involved local infiltration of 2,4-dinitrochlorobenzene.

Ear edema was significantly reduced after the introduction of the topical treatment with both concentrations of UA and hydrocortisone. Behavioral assessment showed a reduction in scratching rate for both UA and hydrocortisone, however the effect was significantly more pronounced for UA. The observed anti-inflammatory activity of UA was associated with immune cells count decrease and attenuation of pathohistological changes in the examined skin lesions. In vitro experiments using human fibroblasts (NHDF) and keratinocytes (HaCaT) indicated attenuation of LTA-induced inflammatory response.

The obtained results indicate the potential of application a composition containing UA in topical therapy of skin inflammations, in treatment of which hydrocortisone or other steroids are currently used.

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P-019 Developing co-cultures of bacterial isolates from the bryozoan *Cristatella mucedo* for the discovery of novel secondary metabolites

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In nature, bacteria usually exist as part of diverse microbial communities where different types of interactions occur. The study of well-defined microbial interactions and their metabolic activities has led to the discovery of new bioactive secondary metabolites[1]. Advances in bacterial genomics, metagenomics, and the study of secondary metabolite biosynthesis pathways revealed the potential of certain species to produce compounds that could never be produced in the laboratory-based cultivation of single isolates. Genes that encode biosynthetic pathways for the synthesis of secondary metabolites (BGCs), are localized in specialized regions of the bacterial genomes. Most of these BGCs are "silent" under laboratory conditions and the environmental signals triggering their expression remain largely unknown. Recent studies demonstrate that co-cultivation of bacteria stimulates the production of novel secondary metabolites never before detected in monocultures[2]. In this study, we explore the biosynthetic potential of the microbiota from a fresh-water bryozoan *Cristatella mucedo*. In particular, we focus on isolation and co-cultivation of bacteria other than well-studied *Streptomyces* species. For this purpose, representatives of 28 bacteria genera have been isolated, taxonomically classified and genome sequenced. The selection of isolates with highest number of unique BGCs and hence capacity to produce secondary metabolites was performed using antiSMASH software[3]. Liquid co-cultivation with *Bacillus* sp. and *Rhodococcus* sp. isolates followed by HPLC, and LC-MS analyses revealed the induction and upregulation of several compounds, including a potential new natural product.

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P-022 New Mycosporin Amino Acid identified in a Trentino "Chlorella-like" Microalgae as source for ecologically friendly sunscreen

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DOI 10.1055/s-0042-1759008

Microalgae belonging to diverse evolutionary linages represent a novel and promising source of bioactive compounds to be involved in nutraceutical and cosmetic composition. These small organisms are characterized with a fast grow rate, low doubling time and low nutrient requirements providing excellent preconditions as cell biofactory. Certain microalgae are known to be able

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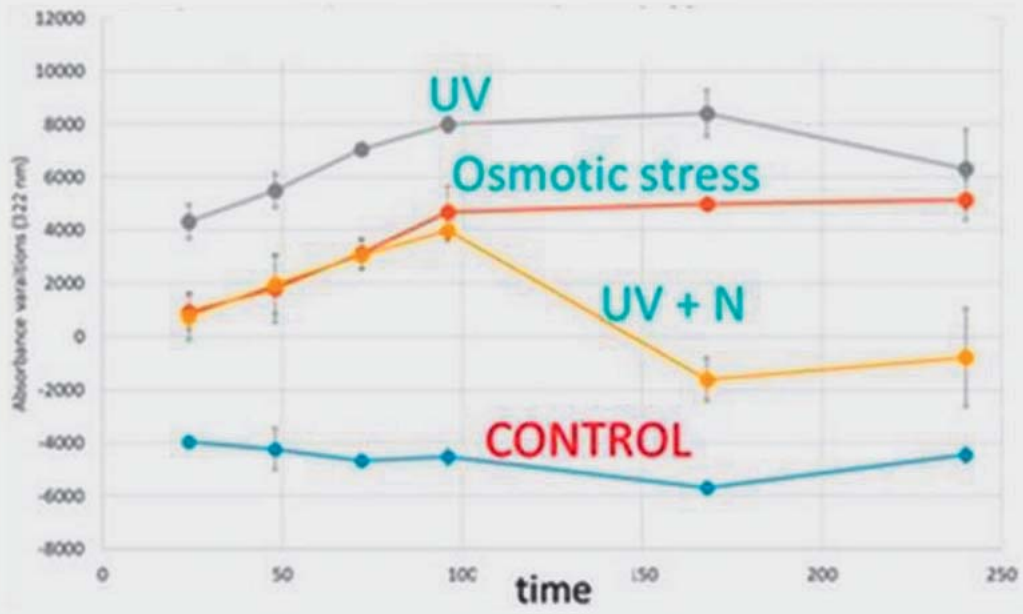

▶ **Fig. 1** MAA accumulation under stress conditions; UV, osmotic stress and UV plus nitrogen (N) stress.

to synthesize secondary metabolites under stress conditions. One of this class of metabolites are mycosporine-like amino acids (MAAs) isolated so far from several marine microorganisms. MAAs are highly promising compounds characterized by ultraviolet radiation (UV) absorbing capacities and are recognized as a potential source of ecologically friendly sunscreens [1]. MAAs absorb damaging UV radiation with maximum absorption in the range of 310–360 nm, including both UVA and UVB ranges. We characterized a microalgae strain from the genebank of FEM (NORCCA K-1801). The strain was tentatively assigned to the Scenedesmaceae family, whereas its classification within the *Chlorella* genus should be excluded. Further phylogenetic characterization is ongoing. Additionally, this strain was found to accumulate a yet unknown MAA which give raise to high potential of novel bioactivities. The MAA accumulation could be increased upon various stress, i.e., UV treatment, osmotic media conditions or combination of both (▶ **Fig. 1**). After upscaling in up to 500 l fermenter isolation of the pure MAA will enable the structural elucidation and functional characterization of its bioactivities. The final aim is to test various MAA containing extracts as supplement in new crème as sunscreen.

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P-023 Phytoene accumulation in the food grade fungus *Blakeslea trsipora* by interfering pathways of β-carotene and ergosterol synthesis

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Natural carotenoids are compounds of great interest due to their health-promoting actions. The colourless, UV radiation-absorbing phytoene, although largely neglected in the past, is attracting much attention recently [1]. In vitro plant tissue cultures, algae and microorganisms are efficient biotechnological tools to obtain phytoene [2]. The fungus *Blakeslea trispora* takes a prominent place among microbial producers of β-carotene as the exclusive product of the carotenoid pathway. In contrast to lycopene synthesis [3], little attention has been devoted to studies of phytoene accumulation in the fungal cells. As a gateway in the pathway, phytoene may be retained by inhibiting the metabolic flux.

The present study investigated the carotenoid pattern in *B. trispora* mated culture (ATCC14271, 14272) treated with multi-target inhibitors interfering with the β-carotene and ergosterol pathways that share common precursors, via phytoene desaturase, lycopene cyclase and squalene epoxidase inhibition. More specifically, our work presents the impact of the addition of the extrernal regulators, diphenylamine, 2-methyl imidazole, and terbinafine, and the fermentation time in stimulating phytoene accumulation.

A C30 RP-HPLC-DAD analysis method was applied for the quantitative analysis of phytoene, lycopene and β-carotene. Results showed that the type and level of inhibitor affected both qualitatively and quantitively the profile of the above carotenoids. Lycopene was the predominant one in 2-methyl imidazole treated cells. The level of phytoene exhibited fluctuations, but in all cases its concentration was comparable or even higher than in tomato products. The prospective of phytoene production by *B. trispora* should be considered a promising area of research.

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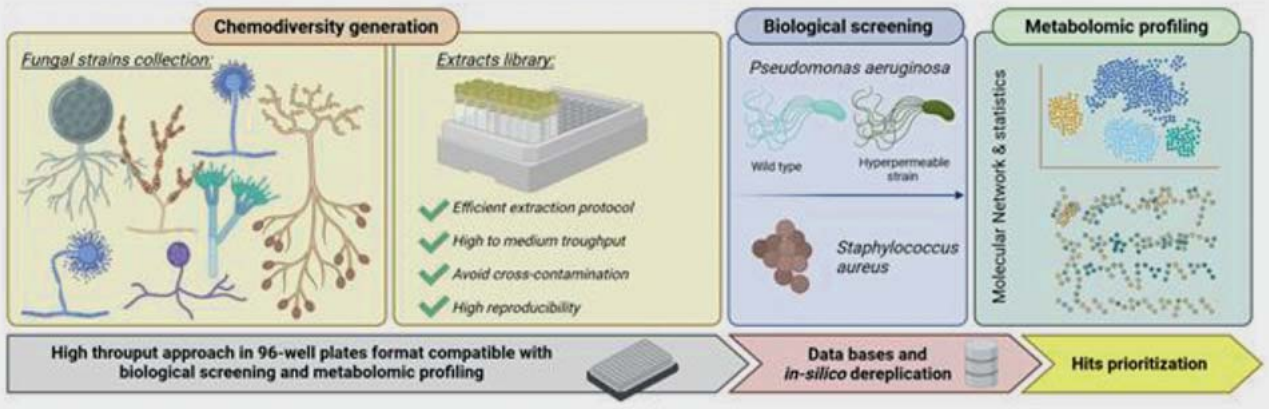

▶ **Fig. 1** Workflow overview.

P-024 Development of an efficient fungal micro-cultivation workflow integrating metabolomics and innovative bioassays for efficient antimicrobial hits prioritization

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This project aims at the development of a screening platform for efficient antimicrobial hits prioritization originating from fungal strains. Given the size of the collection (> 2500) and the resulting number of extracts, a miniaturized approach was developed (▶ **Fig. 1**).

Culture, extraction, bioassays and LC-MS/MS analysis presented here take advantage of the medium-throughput capacity and compatibility across the platforms confered by the 96-well plate format. All generated extracts were enriched by solid phase extraction (SPE) and systematically submitted to antimicrobial bioassays as well as metabolomic profiling. An hyper-permeable Pseudomonas aeruginosa strain, designed to maximize the detection of antibacterial hits, and a wild type Staphylococcus aureus strain were used as target organisms. The MS data of all extracts were incorporated, together with the bioactivity results, into massive and multi-informative molecular networks [1]. Such spectral organization, annotation and visualization tools facilitate chemical and biological exploration of the fungal collection at a molecular level. Furthermore, these data, in combination with dereplication tools based on mass spectrometry, were used to identify active molecules.

The evaluation of the methodology with state-of-the-art metabolomic workflow revealed sufficient metabolite production and reproducibility at this scale and great compatibility with both bioassays and metabolites profiling. This workflow led to the identification of antimicrobials from *Alternaria alternata* as a proof of concept. The methodology's efficiency, which allows the microculture, extraction and analysis of hundreds of fungal strains in parallel in only a few days, makes possible mutiplexing experiments to induce biological gene clusters. This tool will be used for efficient antimicrobial hit prioritization.

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P-025 A new iminol derivative from *Streptomyces cacaoi* in new fermentation conditions

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Marine-derived organisms have varied secondary metabolism due to their adaptation to extreme conditions of marine environments. This fact has made marine-derived Actinobacteria promising sources of new/novel compounds. In addition, the expression of secondary metabolite gene clusters is typically under the control of environmental conditions that cause many of the biosynthetic gene clusters to be silent under laboratory conditions. Thus, the determination of proper fermentation conditions becomes crucial for discovering new molecules [1].

Herein, we report an unusual new iminol derivative from marine-derived *Streptomyces cacaoi* fermentation that was carried out under optimized culture conditions. *S. cacaoi* was isolated from a sediment sample collected from the Mediterranean coast of Turkey. In previous studies using M6, one of the most preferred media, we obtained new polyether ionophores [2,3]. Later on, the metabolite diversity increased significantly with the optimization of the culture conditions, and we conducted new fermentation studies using the optimized medium. Using silica gel open-column chromatography, a colourless and slightly 254 nm UV active compound was isolated. Detailed inspection of 1D and 2D NMR spectra proposed a tautomeric amide form. Together with the HR-APCI-MS analysis, the structure was established as 5-hydroxy-1,6-diazacycloundec-5-en-2-one. This molecule was probably a cyclic dipeptide deriving from the deamination and decarboxylation of two amino acids, respectively, aspartic acid and lysine. In conclusion, this study signified the importance of manipulation of culture conditions to discover unusual new/novel natural products.

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Planta Med 2022; 88: 1385–1588

P-026 Metabolomic analysis of *Aspergillus tubingensis* (IBT23488)

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The black aspergilli (*Aspergillus* section Nigri) are an important group of species in food, medical mycology and biotechnology. In biotechnology, they are used in many processes such as for the production of biomass, organic acids, enzymes and isolation of secondary metabolites. *A. tubingensis* strains have been reported to be prolific producers for many classes of natural products like alkaloids, anthraquinones, terpenoids and γ-naphthopyrones. Moreover, some of the above-mentioned secondary metabolites shows significant biological activity and also represent novel structure, such as antiviral tubingensins. In present study, *A. tubingensis* IBT23488, forming sclerotia, was fermented in YES media and LC-MS analysis shows the presence of aurasperone C and E, malformin A1, tubingensin A and B, alfavinine as well as some new indole diterpenes. Till now, purification of the crude ethyl acetate extract of *A. tubingensis* led to the isolation of a known compound nigragilin. Isolation of new indole terpenoids is on-going. Furthermore, while co-culture of the fungus *A. tubingensis* in various media (OAT, PDA and CYA) with bacteria of genus *Streptomyces* indicate the suppression of the production of some secondary metabolites, it seems that amphotericin-producing *Streptomyces* induce the production of indole terpenes, the same way as amphotericin B in preliminary experiments.

P-027 Evaluation of β-carotene production from *Dunaliella* strains isolated from Greek solar saltworks

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The microalga *Dunaliella* sp. exhibits a cosmopolitan distribution, colonizing environments of high salinity and solar radiation, including the salt pans of Greece. *Dunaliella salina* is one of the richest natural sources of β-carotene, a natural pigment that presents a growing commercial demand at a global level owing to a wide range of applications in the food and cosmetic industry. In addition, β-carotene isolated from *Dunaliella* biomass is one of the few microalgae-based ingredients that has been authorized by the European Food Safety Authority as a food ingredient.
The research project "Pilot cultivation of locally isolated *Dunaliella* strains for the production of β-carotene" (PILOUS) aims to showcase the most promising Greek *Dunaliella* isolate(s) in terms of β-carotene productivity as well as the culture conditions that induce the intracellular β-carotene synthesis.
In this context, four *Dunaliella* strains isolated through the project have been included in a fractional factorial biomass experiment. Where the main effects and the interactions of three parameters (three levels for each parameter): light intensity (40, 100, and 500 µmol m-2 s-1), salinity (1, 2, and 2.5 M NaCl) and nitrogen availability (0, 0.2 and 1 g KNO3 L-1) have been studied in terms of biomass and β-carotene productivity. Under those treatments β-carotene production ranged between 7–18 mg g-1 dw). The stress factors applied did not yield the maximum β-carotene concentration potential stated in the literature. Further studies are needed to elucidate the β-carotene production in the first *Dunaliella* spp. strains isolated from Greece during PILOUS.
This research has been co-financed by the European Union (European Regional Development Fund) and Greek resources through the National Action "Special Actions" AQUACULTURE "-" INDUSTRIAL MATERIALS "and" OPENNESS "Innovation (EPANEK), GSRT, NSRF 2014–2020 (PILOUS project, MIS5 045 805. Project title: "Pilot cultivation of locally isolated Dunaliella strains for the production of β-carotene" (PILOUS)

P-028 A new promising astaxanthin producer: a Greek *Haematococcus pluvialis* isolate

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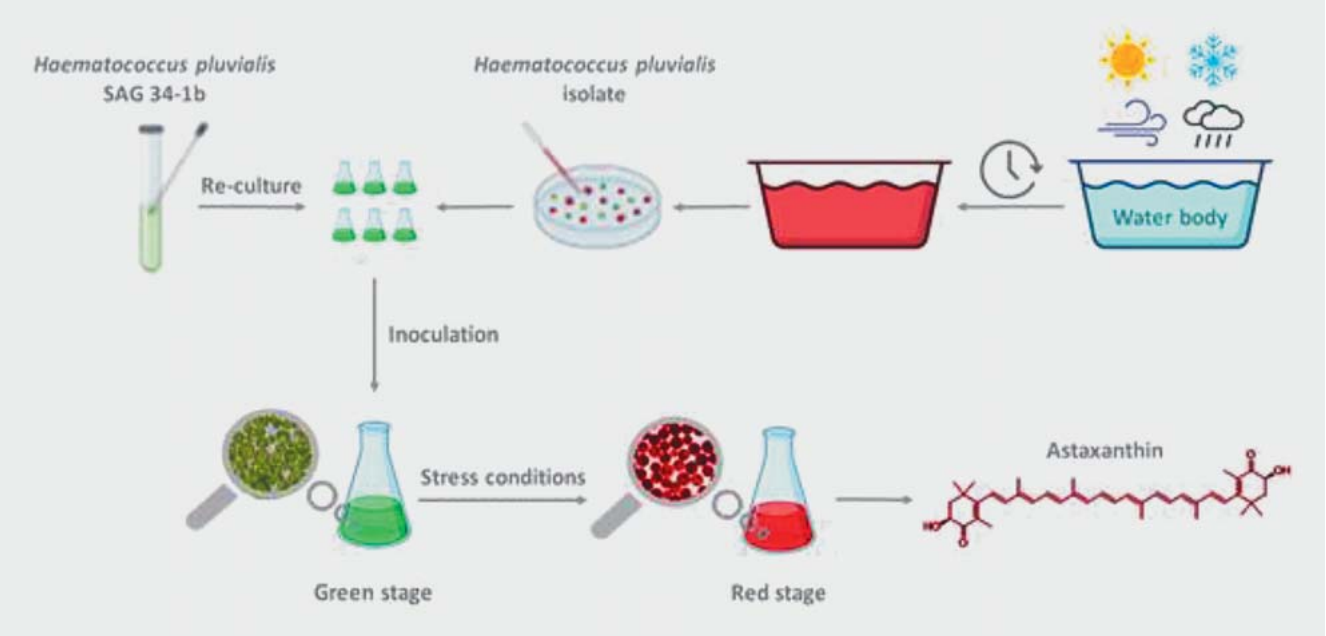
The ketocarotenoid astaxanthin constitutes a raw material with significant commercial potential for the pharmaceutical and cosmetic industry due to its antioxidant and anti-inflammatory properties [1]. The microalga *Haemato-*



▶ **Fig. 1**

coccus pluvialis is the principal natural producer of the blood-red pigment astaxanthin, the de-novo biosynthesis of which occurs under unfavourable growth conditions, such as continuous and high light intensities, high temperature, nutrient deprivation, increased salinity, etc. [2].
In this study, a new air-dispersed *H. pluvialis* strain isolated from artificial low-volume water containers after six weeks of exposition in the outdoor environmental conditions of Thessaloniki (Greece) in the late winter – early spring season of 2022, and the *H. pluvialis* SAG 34–1b strain are being evaluated for their astaxanthin production capacity. Firstly, the strains were cultivated under growth-favouring conditions (green-stage) until a sufficient inoculum was produced. Subsequently, new 500 mL Erlenmeyer flasks containing 250 mL of nitrogen-deprived BBM medium were inoculated with 500 mg/L dry biomass (DW) in duplicate for each strain and incubated for 12 days at 25 °C under a constant agitation rate of 140 rpm and continuous high-intensity illumination. The cultures were monitored every 48 h in terms of astaxanthin accumulation [3], chlorophylls and carotenoids concentration, while morphological and morphometric observations were performed daily.
Preliminary results indicated a preeminence of the new solate on astaxanthin accumulation performance compared to *H. pluvialis* SAG 34–1b strain, as well as faster conversion of the green-flagellated stages into large red-cysts. However, further studies on the two cultivation stages are needed to establish the maximum astaxanthin productivity for each strain.

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P-029 "AntiAging" Project: Exploitation of Greek microbial diversity for the development of innovative cosmeceuticals and food supplements

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Greek ecosystems are a fertile yet under-explored ground for the study of Actinobacteria–renowned producers of bioactive compounds. By exploring this under-investigated biodiversity and chemodiversity of Greek actinobacterial strains, the "AntiAging" project aims to uncover potentially novel natural compounds with anti-aging activity that can be formulated as cosmeceutical and nutraceutical products. In total, 1000 isolates belonging to the Athens University Bacterial & Archaea Culture Collection (ATHUBA)–some originating from unique environments (caverns, volcanoes, thermal springs, etc.)–were studied. An ultrasound-assisted extraction process was designed using solvents of increasing polarity to generate a customized in-house library of 2000 extracts. All extracts were investigated for their potential anti-aging properties and more specifically were assessed via enzymatic and cell-based assays for their anti-elastase and anti-tyrosinase activities. Based on this bioassay-guided approach, the metabolic screening and compound annotation of the most promising isolates is conducted via a high-throughput dereplication method (UPLC-HRMS). This leads to a targeted isolation and identification of compounds using state-of-the-art chromatographic and spectroscopic techniques (HPLC-DAD-ELSD, UPLC-HRMS, NMR). A smaller yet significant number of extracts demonstrated > 50% elastase inhibitory activity, whereas a considerably larger number exhibited > 50% tyrosinase inhibitory activity. Interestingly, in some cases, the bioactivity exceeded 80% in in vitro assays. The latter results show a hidden yet powerful potential of harnessing Greek microbial wealth in the context of the science of anti-aging.
"AntiAging" – GSRT – No. T2ΕΔΑ-01 410.

P-030 Study of *Micromonospora* sp. from the mesophotic zone of the Gulf of Thailand aimed at discovery of anti-aging natural products

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Marine actinobacteria have attracted the attention of the scientific community in recent years, as a novel source of a wide variety of bioactive natural compounds. In continuation of our work towards the investigation of this microbial group within the TASCMAR project framework, a strain identified as *Micromonospora* sp. (XLm-22-s1) was chosen for further study and bio-guided isolation of its secondary metabolites. After cultivation in both solid and liquid conditions, the extraction of the cultures was conducted using ethyl acetate and methanol. Four extracts (two ethyl-acetate and two methanolic extracts of solid and liquid cultures, respectively) were enzymatically evaluated via bioassays for their anti-elastase, anti-tyrosinase and anti-proteasome activities. The ethyl acetate extract of the solid culture demonstrated 57.51% proteasome inhibitory effect. The profiling and fractionation of the extracts were performed using various chromatographic methods (preparative TLC, semi-prep HPLC, CC). From the analysis of the obtained spectroscopic data (1D and 2D NMR, LC-HRMS), seventeen compounds were identified, of which six belonged to the diketopiperazine group, three were characterized as indolocarbazole derivatives, three were alkaloids, one was identified as a quinone, a single compound belonged to amino acids, and lastly, a nucleobase and a nucleoside were also identified. While sixteen of the aforesaid compounds are well described in the literature, one represents a novel natural product. All compounds were further evaluated for their anti-aging activity. In conclusion, the isolate of *Micromonospora* sp. has proved to be an important source of bioactive secondary metabolites. The Authors declare that there is no conflict of interest.

P-031 Secondary metabolites of fungus *Thyronectria* sp. and their antifungal and anticancer activities

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The genus *Thyronectria* belonging to the Nectriaceae family is predominantly characterized by perithecia covered with yellow scurf, whereas after drying its upper part, it usually becomes cupulate. The identified species of this genus (32 species) are rarely pathogenic, and are generally distributed in subtropical and temperate areas, growing on dead branches. So far there is no report on a chemical profile or biological activities of this genus [1]. This study aimed to isolate and identify secondary metabolites from a solid culture of *Thyronectaria* sp. on breakfast cereals, as well as to evaluate their antifungal and anticancer properties. Our bioprospecting using bioactivity guided isolation led to purification of a known halogenated heat shock protein 90 inhibitor radicicol (1) [2], two known cleistanthane type diterpenes (2 and 3), zythiostromic acid A and zythiostromic acid B, along with a new dimeric cleistanthane diterpene (4), for the first time from a solid culture of *Thyronectria* sp. Structures of

all isolates were established using 1&2D NMR and HRMS. The absolute configuration of 1 and 4 were deciphered using X-Ray crystallography. Furthermore, concentration-dependent investigations for their cytotoxic activity against a melanoma cancer cell line (A375), and antifungal activity against *Cryptococcus neoformans* and *Candida albicans* revealed compounds 4 and 1 to be responsible respectively for the bioactivities observed. Our results demonstrated that *Thyronectria* sp. investigated in this work is a potential source of bioactive compounds and requires further in-depth investigations.

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P-033 Chemodiversity and cosmetic potential of *Dendrobium fimbriatum* (Orchidaceae) fungal community

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Inhibition of tyrosinase, a key enzyme in melanin biosynthesis, is of great interest for the cosmetic industry, due to its skin-whitening properties and skin hyperpigmentation or age spots reduction effects [1].

Fungal melanin has important ecological and biochemical functions for persistence in the environment and within organisms [2]. Recently, fungal endophyte have shown anti-tyrosinase activities highlighting plant fungal community as an underexploited source of promising inhibitors [3].

The cultivable part of the fungal community of Dendrobium fimbriatum Hooke, 25 species belonging predominantly to *Fusarium, Trichoderma, Colletotrichum, Curvularia*, and *Phomopsis* genus, was evaluated as a source of cosmetic active ingredients.

The metabolite profiles of all strains were investigated by a dereplication strategy based on LC-HRMS/MS and molecular network (MN) representation based on fragmentation similarities. In parallel, extracts were screened on enzymatic bioautography methods for anti-tyrosinase and antioxidant activities. Active compounds were desorbed and analyzed by MS for annotation using MS/MS databases with taxonomical information [4,5].

The chemodiversity of the community was significant with terpenoids, amino acids and peptides, alkaloids, fatty acids, and polyketides compounds. MN representation revealed similitude/difference in metabolic pattern of the extracts that were used for making correlation with the bioassay.

All extracts had anti-tyrosinase and antioxidant actions with some similar and species or genus specific active compounds. The resulting MN highlighted various analogues active compounds.

The fungal community of *Dendrobium fimbriatum* appears as a rich source of cosmetic active ingredients. Indeed, the lack of annotation for several clusters indicate potential novel tyrosinase inhibitors.

The authors declare no conflict of interest.

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P-034 Analysis and quantitation of bioactive compounds in Norway spruce balm

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The balm produced by *Picea abies* (L.) H. Karst. (Pinaceae), a coniferous tree also known as Norway spruce, is traditionally used to treat acute, chronic and infected wounds. The bioactive compounds are lignans like pinoresinol and different diterpene resin acids (DRAs). In order to guarantee consistent pharmaceutical quality of Norway spruce balm and commercial products thereof, it is necessary to provide analytical methods for the quantitation of bioactive substances [1,2].

HPLC-UV/DAD allows the analysis and quantitation of lignans, hydroxylated resin acids and dehydroabietic acid. However, most of the DRAs of the abietane and the pimarane type show high structural similarity and cannot be separated sufficiently by HPLC-UV/DAD. This obstacle was eliminated by the development of a separation and quantitation protocol using ultra high-performance supercritical-fluid chromatography (UHPSFC) hyphenated to a quadrupole mass detector. Seven DRAs all with the same molecular weight of 302 g/mol (pimaric acid, sandaracopimaric acid, palustric acid, isopimaric acid, levopimaric acid, abietic acid and neoabietic acid) were separated using a Torus 2-Picolylamin column and CO_2 and ethanol as mobile phase. Both methods can be applied to examine the composition of the raw balms and available commercial products. Furthermore, samples drawn from Franz diffusion cells can be analysed in order to determine the quantity of bioactive compounds in an acceptor medium after permeation through a membrane made of pig skin.

The established methods represent an important tool for quality control of already existing and development of new Norway spruce balm products.

The authors declare no conflict of interest.

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P-035 New targets to combat bacterial infections: Confocal microscopy to investigate bacterial invasion?

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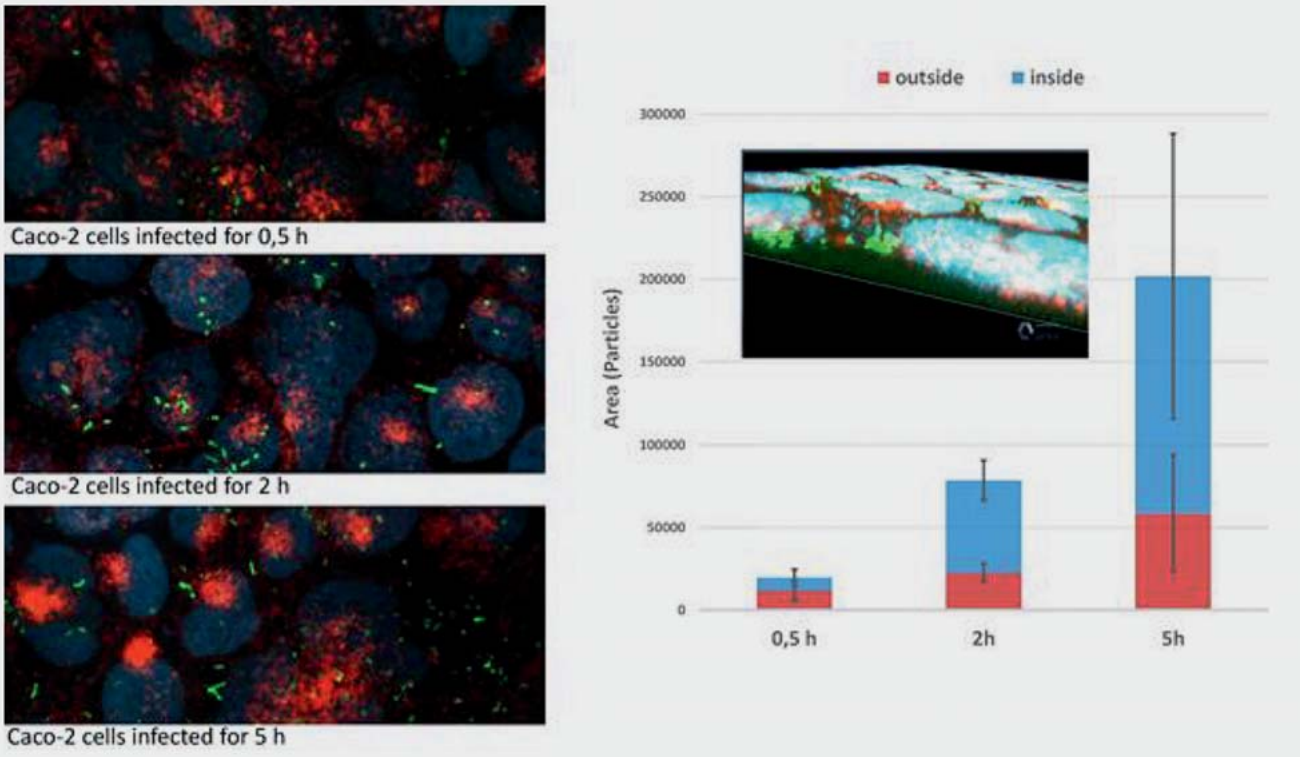
DOI 10.1055/s-0042-1759021

The search for innovative antibacterial natural products has pinpointed new targets. Inhibition of host-pathogen interaction has been shown to reduce the infection risk and the progression of the infection [1–4]. Also, invasion into host cell can be influenced by natural products. The standard method to investigate bacterial invasion is the gentamicin protection assay, which require great workload and material requirements. The statistical significance is highly dependent on the cultivability of the bacteria including whether VBNC forms are present.



▶ **Fig. 1** Time dependency of adhesion and invasion of bacteria.

More specific and effective can be the monitoring of bacterial invasion by use of confocal laser scanning microscopy (CLSM). It can be determined at which position of the cell the bacteria are located, thus providing a direct indication of both adhesion and invasion. Also, time-dependent and quantitative evaluations can be performed easily by CSM. Using *Campylobacter jejuni* as model organism and Caco-2 cells, an easy-to-use CSM-protocol has been developed. Host cells are stained fluorescently with DAPI for detection of intracellular compartments and Alexa-WGA-594, a lectin that binds to the cell membrane, to localize adherent bacteria. The bacteria themselves are fluorescently labelled with CFDA-SE. Invasion is performed as in the standard method. After the images are acquired, it can be determined to what degree the bacteria are adhered and/or invaded, and qualitatively, it can be assessed where they prefer to reside. Depending on laboratory equipment and experimental conditions, the adhesion and invasion of bacteria to and into the cell can be recorded as a time-lapse in a live cell imaging chamber and evaluated (▶ **Fig. 1**).

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P-036 Quest for New Lead Compounds Against Malaria Based on Natural Prodrugs Present in *Nauclea pobeguinii* and Their Metabolites

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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 was purified and tested for in vitro activity against malaria. Multivariate data analysis using OPLS-DA proposed alkaloids with a β-carboline moiety as active principles, suggesting that antiplasmodial activity of N. pobeguinii derives from an additive or synergistic effect of multiple minor alkaloids present in the bark extract and their metabolites.

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Planta Med 2022; 88: 1385–1588

P-037 Application of immunoassay method for determination of licochalcone A in licorice derived products and cosmetics

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Licochalcone A is a licorice chalcone that belongs to the flavonoid subclass. The pharmacological activities of licochalcone A are well known in anti-microbial and anti-inflammation properties. In clinical setting, it is used for reducing the inflammation-related symptoms such as redness and rash, as well as intended for strengthen skin. However, the current method for analysis of licochalcone A is high performance liquid chromatography (HPLC) which its sensitivity is not sufficient for licochalcone A analysis [1]. Especially, the cosmetic products, which consists of multi-ingredients and contained small amount of licochalcone A (normally less than 0.05%) [2]. The previous study developed the monoclonal antibody specific to licochalcone A [3]. Moreover, the ELISA method was developed and validated. In this study, we aim for applied ELISA method for determination of licorice products including herbal powders and cosmetic preparations. The result showed that the licochalcone A was found in herbal compound drugs in range 3.89–6.46 mg/g dry weight. In case of cosmetic product, it was found about 0.05–8.84 µg/g dry weight. The result indicated that ELISA method can be used for quantitative analysis of licochalcone A in all type of preparations (serum, lotion, gel, and cream) while HPLC has limitation due to the sensitivity. Therefore, the ELISA was a good alternative method for determination of licochalcone A in sample with complicate ingredients and contained small amount of licochalcone A.

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P-038 Gender-specific concentrations of Tamm-Horsfall protein (THP): biomedical study to explore THP differences during the search of THP-inductors by nature

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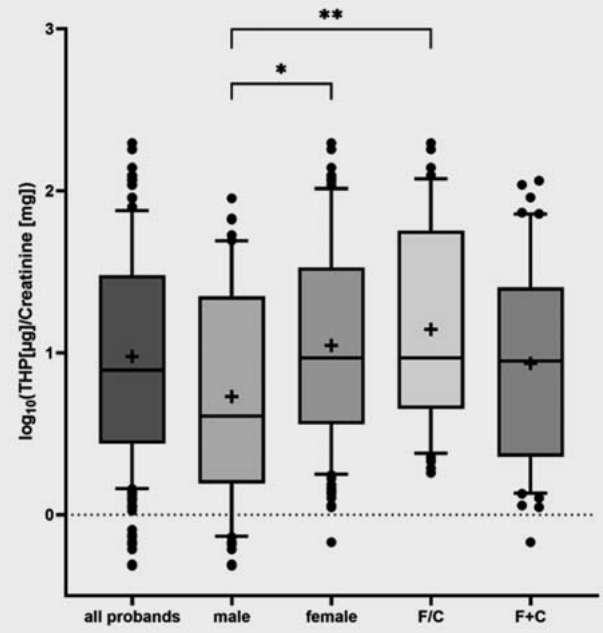
Tamm-Horsfall Protein (THP) is a highly mannosylated glycoprotein, expressed in the kidney. THP binds to the mannose sensitive adhesin FimH of uropathogenic *E. coli* (UPEC) and thus prevents FimH-mediated adhesion of UPEC to host cells. Natural substances as inducers of THP formation offer potential for innovative therapeutic strategies against urinary tract infections (UTI). Recently it has been shown that THP secretion is significantly stimulated by extracts from cranberry fruits (*Vaccinium macrocarpon*), resulting in increased anti adhesive properties against UPEC [1]. Surprisingly, this THP-stimulating effect turned out to be gender dependent, with men benefiting more than women regarding THP. For this, the following study aimed to quantitate basal THP levels without any intervention in human female and male urine samples to monitor gender-specific differences, this also within the fact, that the prevalence of UTI is significantly higher in women than in men [2].
For THP quantitation a specific ELISA was established and validated [3]. Within a biomedical study, urine was collected from 179 volunteers and THP was quantified. Subgroup analysis indicated significant lower average $\log_{10}$(THP/Crea) concentration in male urine compared to women (∅ 0.73 vs.1.05, $p < 0.05$). Female subjects without intake of hormonal contraceptives have a significantly higher THP secretion compared to men (∅ 1.15 vs. 0.73, $p < 0.01$). Urine from women, treated with hormonal contraceptives showed a tendency towards reduced log10(THP/Crea) ratios (∅ 0.94).
Further research (e.g., glycosylation pattern, efficiency of binding pathogen) is needed to establish a correlation between THP fine structure and the prevalence of UTI.



▶ **Fig. 1** $\log_{10}$ (THP [µg]/Crea [mg]) of study population (subgroups: male; female; F/C – female without contraceptives; F+C – female with additional intake of hormonal contraceptives) depicted in a box-plot chart (10–90 percentile). + represents mean value. Data were processed with nonparametric ANOVA (Kruskal-Wallis). Subsequently, post-hoc test was conducted with Dunn's multiple comparison. Statistical significance could thus be assessed (* $p < 0.05$; ** $p < 0.01$).

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P-039 Identification of biomarkers of *Lyme borreliosis* by non-targeted metabolomics

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Lyme borreliosis (LB) is the most notable vector-borne infectious disease caused by members of spirochete group *Borrelia burgdorferi* sensu lato and is

spread by ticks from Ixodes family. LB metabolomics have been studied before, but currently, there is no routine analytical method in use for identifying the early stages of the LB, before antibodies are formed after several weeks from the infection [1–3].

The aim of this study is to develop a non-targeted metabolomics-based method for identifying small specific biomarkers and metabolic routes from human blood serum samples that are affected by the neurological form of LB, Lyme neuroborreliosis (LNB), by utilizing UHPLC-DAD-HESI-Orbitrap-MS platform together with cutting edge in silico tools. This metabolomics data workflow was applied to select a biosignature for classifying the healthy controls from LNB samples of two different time points: acute infection and treated infection (12 months after the infection). Proteins were precipitated from the sample matrix by using methanol before the high-resolution MS acquisition via negative ESI mode.

The used metabolomic UHPLC-MS/MS-data workflow approach pointed towards different biomarker groups from the sample matrix including different acylcarnitines, hydroxy-, nitro- and polyunsaturated fatty acids, diacylglycerols, peptides, triglycerides and sphingolipids. The results show promise for applying non-targeted LB metabolomics to create targeted methods in the future for early LB diagnostics.

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P-041 Identification of high content in carnosic acid Greek genotypes of *Rosmarinus officinalis* and *Salvia officinalis* by a fast HPLC-PDA-MS protocol

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In the framework of a national project aiming at exploring the biodiversity for the selection, development and sustainable use of Greek Medicinal and Aromatic plants, the species *Rosmarinus officinalis* and *Salvia officinalis* were selected. The selection was done based on their high content of carnosic acid which is a recognized antioxidant with wide applications in the food and cosmetic industry [1,2]. The project includes collection of a large number of genotypes, genetic analysis by use of microsatellites and genotyping by sequencing, chemical analyses by HPLC-PDA-MS for the monitoring of production of carnosic acid. A fast extraction protocol followed by a short HPLC-PDA-MS method based on a RP-C18 column was developed and optimized for the separation of carnosic acid from carnosol from other metabolites present in the extracts. The method was validated as required prior to metabolomic analysis and showed adequate precision (%RSD ranging from 0.20 to 2.86) and accuracy (less than 15% in three concentration levels, ranging from 0.16 to 2.75). 60 different genotypes were collected from each species and evaluated for their content of carnosic acid. Quantitative results showed a great variance of the chemical content of carnosic acid among different genotypes. *R. officinalis* genotypes from the area of Amaliada, Elis and *S. officinalis* genotypes from Mesovouni, Ioannina were evidenced as the richest in carnosic acid.

Acknowledgements

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P-042 Quality control of flowers of wild and cultivated *Primula veris* L. from Greece

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As massive harvests of *Primula veris* L. across the mountainous areas of Northern Greece threaten the survival of its wild-growing populations, a project between the Aristotle University of Thessaloniki, ELGO Agronomic Institute and the local Greek authorities of Epirus was undertaken. Scope of the project was the development of a sustainable exploitation strategy comprising the domestication of native *P. veris* and the investigation of its chemical content in order to assure the efficient supply of raw material of stable quality. In this framework, an HPLC protocol was developed for the quality assessment of both wild and cultivated *P. veris* flowers. Chemical analyses showed that *Primula* flowers were particularly rich in flavonol triglycosides, derivatives of quercetin, isorhamnetin and kaempferol. An HPLC-PDA method was developed for the determination of the flavonoids 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. RSD values ranged between 1.58 and 4.85 and the recovery ranged between 93.5% and 102.1% with RSD values < 5%, within the acceptable limits. 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]. Most notably, flower samples from cultivated plants had a similar profile compared to the native populations, characterized by the prevalence of isorhamnetin-3-O-triglucoside.

This study is funded by the Region of Epirus, Greece.

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P-043 Milk thistle product authentication using LC-MS and DNA metabarcoding

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Silybum marianum (L.) Gaertn. (milk thistle, Asteraceae) preparations are among the most common hepatoprotectants used in complementary and alternative medicine but are purported to have much broader health-promoting effects due to their rich source of bioactive pharmacologically active compounds [1–3]. However, these preparations are very often highly processed and sold as complex mixtures, making classical analytical methods for quality assurance limited in their ability to identify the target plant species, and to detect non-targeted species [4]. To overcome this limitation, various emerging technologies have been recommended to be used in order to ensure the quality and safety of such complex products [4,5]. In this study, LC-MS-based metabolomics and DNA metabarcoding were used for the authentication of eighteen products, containing *Silybum marianum* (L.) Gaertn. and/or silymarin according to the label, collected from different retailers and importers in Romania and Germany. Our results showed different degrees of variations between declared and detected ingredients. We conclude that LC-MS-based metabolomics and DNA metabarcoding can improve regulatory applications and can be effectively integrated into the toolbox of analytical methods for quality control of plant-based preparations.

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CNCS – UEFISCDI, project number PN-III-P1-1.1-PD-2019-0522, within PNCDI III, and by a grant of the Ministry of Research, Innovation and Digitization through Program 1 – Development of the National R&D System, Subprogram 1.2 – Institutional Performance – Projects for Excellence Financing in RDI, contract no. 2PFE/2021. The authors declare no conflicts of interest.

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P-044 Quality variation of Maidong (*Ophiopogon* and *Liriope* spp.) along the value chains- HPTLC fingerprint as an effective method

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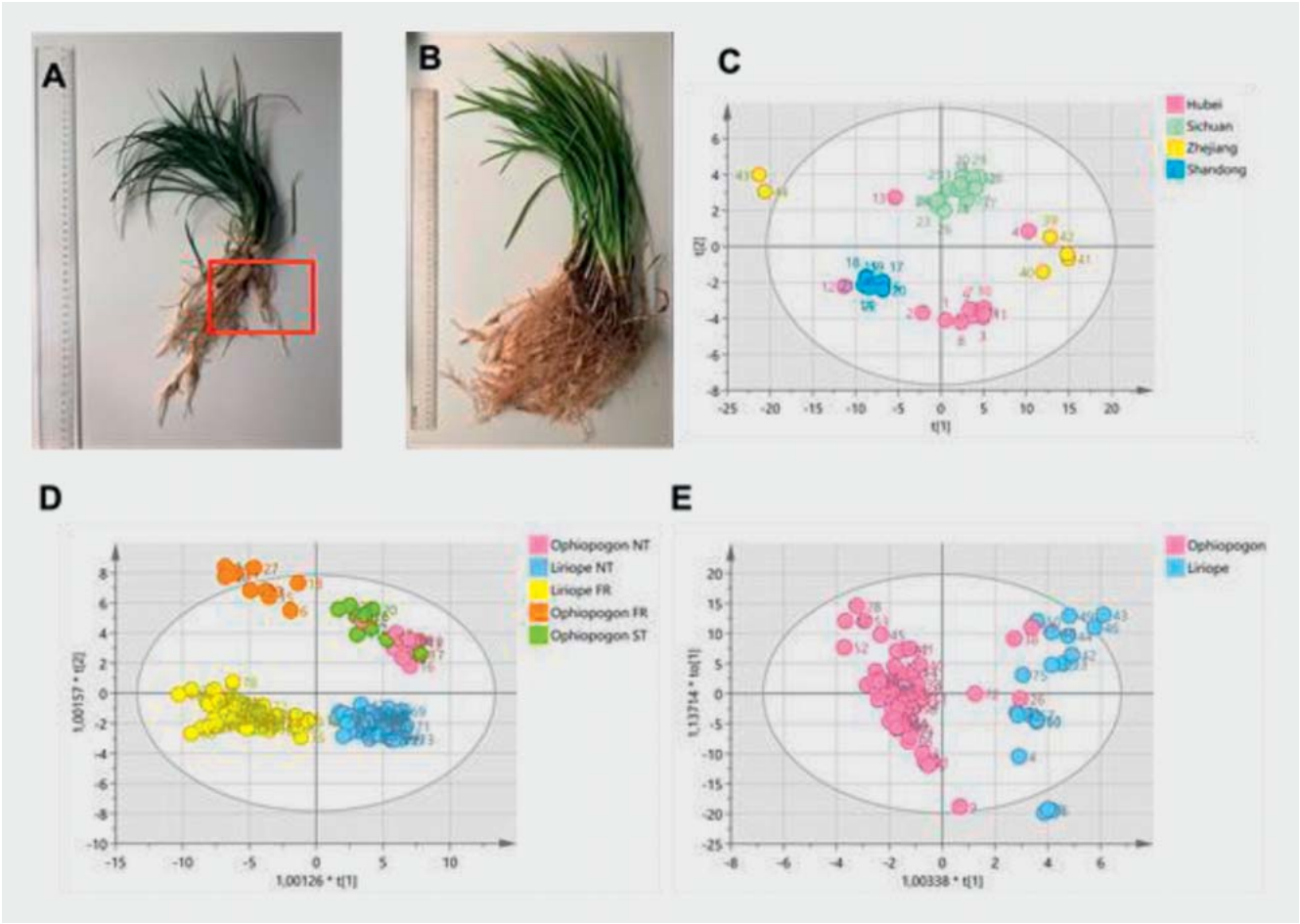
Maidong are the tuberous roots of *Ophiopogon japonicus* and, interchangeably, *Liriope spicata* and for over thousands of years have been used as medicines in China. Mislabeling exists on the market as- *O. japonicus* is 3–5 times more expensive than *L. spicata*. Moreover, the price of different types of tuberous roots differs. Therefore, it is necessary to understand the quality variation of Maidong along the value chains.

Maidong were sampled from various stages along the value chains, including samples from fields and markets. Specifically, Maidong are collected from four production regions in China; additionally, the underground part of the plants were separated and classified as stem tuberous roots, normal tuberus roots and fibrous roots (▶ **Fig. 1**A, B). Moreover, Maidong are purchased from markets in China and EU. HPTLC was applied to evaluate their fingerprints. Data analysis was accomplished by PCA and OPLS-DA.



▶ **Fig. 1** Plant part illustration and PCA results (A. *Ophiopogon japonicus*. Stem tuberous roots (ST) are the ones in the red triangular, and they are usually within 3 cm to the stem. Normal tuberous roots (NT) are the ones happen near the tip of fibrous roots. Fibrous roots (FR) are the slim roots; B. *Liriope spicata*; C. PCA loading score of samples from different geographical regions; D. Samples from different plant part (Ophiopogon means *O. japonicus*, Liriope means *L. spicata*); E. OPLS-DA loadings score of commercial samples.

HPTLC fingerprints effectively discriminate samples both at species and geographical level (▶ **Fig. 1**C, E). The different section of the tuberous roots of *O. japonicus* showed no significant differences in their profile. The fingerprints of the fibrous and tuberous roots of both species indicate high similarities in the type of metabolites but differ from concentration- the concentration tends to be higher in fibrous roots than in tuberous roots (▶ **Fig. 1**D), providing scientific metrics for further exploration of fibrous roots being used as medicines. Pharmacognostic assessment combined with the use of quantified markers can facilitate the quality control and, ultimately, sustainable development of Maidong.

The authors declare no conflict of interest.

P-045 Role of high-performance thin-layer chromatography method in separation and analysis of withanosides-withanolides with flavonoid glycoside in *Withania somnifera*

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Withania somnifera (L.) Dunal (WS), known as Ashwagandha, is an Indian herb classified as Rasayana in Ayurveda for rejuvenating and health-promoting activities. A sensitive and robust high–performance thin–layer chromatography (HPTLC) method for the estimation of withaferin A (WFA), withanoside IV (WSIV), withanoside V (WSV), and kaempferol based glucoside (KRG) was developed and validated in the roots and aerial parts of WS. This method reports the separation and simultaneous quantification of three diverse classes of WS with an analytical marker as KRG for effective detection and quantification of the aerial parts separating them from roots. The densitometric analysis was performed for KRG (at 254 nm), WFA, WSIV, and WSV (at 540 nm after derivatization) with characterization by HPTLC-MS/MS. The method was found linear ($R^2 > 0.99$), robust with excellent recoveries (80–100%). Furthermore, the class of pigment-based compounds in aerial parts at 366 nm in chemical fingerprinting in WS samples was separated with this method.

In this HPTLC-based assessment, WFA, WSIV, WSV, along with 12-deoxywithastramonolide, withanolide A, withanone, and withanolide B could be detected for quality control distinguishing them from other Withania species and other plant species reported earlier as adulterants. At the same time, the presence of flavonoid glycoside as an analytical marker with pigment zones can identify aerial parts, leaves, stems, and fruit with calyx and without calyx in the fingerprint analysis, separating them from root samples for authenticity. Thus, this HPLTC-based DS and MS/MS method were found rapid and economical for the estimation and surety of quality of WS samples.

P-046 Combination of two spectrophotometric methods for total quantification of steroidal and triterpenoid saponins contents in saponin plants mixtures

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Saponins are complex molecules constituted of a sapogenin associated with one or more osidic chains. Their properties are used in many industrial sectors such as food, cosmetics, agricultural and pharmaceutical. Depending on saponin plants, the sapogenin could be steroidal or triterpenoid leading to a large diversity of saponins. The control and quantification of total saponins is therefore a challenge due to this structural diversity. In a previous study, we developed a fast and accurate colorimetric method for the total quantification of steroidal and triterpenoid saponins [1]. In strong H_2SO_4 conditions (50% v/v) with p-anisadehyde, an identical chromophore is formed at 600 nm for all saponins allowing their quantification. Interestingly, utilization of soft H_2SO_4 conditions (12.5% v/v) with p-anisaldehyde form a chromophore at 425 nm only with the spirostan and furan structures present in steroidal saponins [2]. Coupling the spectrophotometric method at 425 nm with that of 600 nm could permit obtaining steroidal saponins content as well as the triterpenoid saponins content in a mixture of both saponins types. In this study, several blends of Camellia oleifera (triterpenoid saponins) and *Trigonella foenum-graecum* (steroidal saponins) extracts were assayed with spectrophotometric methods at 425 and 600 nm. Results showed that the values obtained experimentally were relatively close to those found by calculation with a bias which did not exceed 7.7%. This application could have great potential in the industry by example for the standardization of plant material constituted of two saponins types or to unravel plant adulteration issues.

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P-047 Quantification of *Camellia oleifera* extract in complete feed with UHPLC-MS/MS using a double "one-point" standard addition

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A specific and sensitive method for the quantification of *Camellia oleifera* in complete feed based on UHPLC-MS/MS has been developed and validated. The additive studied is a natural commercial feed additive (Cosap®, Nor-Feed, France) used in animal nutrition, consisting of tea seed extract in which camelliaside A has been identified and quantified. Camelliaside A was used as phytomarker for the quantification of the additive with a level of 3 µg/g of phytomarker in complete feed. To compensate for the loss of camelliaside A during the extraction step, a glycosylated flavanone is used as internal standard. Because of matrix effects that can occur in mass spectrometry, standard addition method was selected for the quantification. To decrease the workload of the standard addition method; a simplified approach based on the "one-point" addition was used for camelliaside A and internal standard 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 *Camellia oleifera* extract 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].

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P-048 Acute toxicity of *Stizophyllum perforatum* leaves crude extract and determination of verbascoside by a validated HPLC-DAD method

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Stizophyllum perforatum (Cham.) Miers (Bignoniaceae) crude extracts showed trypanocidal and leishmanicidal properties, additionally, verbascoside was the main active ingredient in the crude ethanol extract. We decided to quantify only verbascoside due to its biological properties and relative abundance in the crude extracts investigated during the study [1–3]. The aims of this study were to develop and validate a method for verbascoside determination in extracts of *S. perforatum* by HPLC-DAD using mangiferin as the internal standard, and to evaluate acute toxicity. The HPLC analysis was performed on a Luna ODS Phenomenex column, with a gradient mode, using water with acetic acid and acetonitrile. The method presented selectivity for verbascoside and mangiferin. The calibration curves were found to be linear in a concentration range of 0.8–60 µg/mL. In addition, this method provides recoveries between 102.82 and 111.04% and RSDs lower than 5.0%. In the acute toxicity test, the crude extract CE-2 did not cause deaths and significant behavioral changes in animals treated with a 2000 mg/kg dose. Thus, indicating a lethal dose of LD50 > 2000 mg/kg for CE-2. Additionally, macroscopic and histo-

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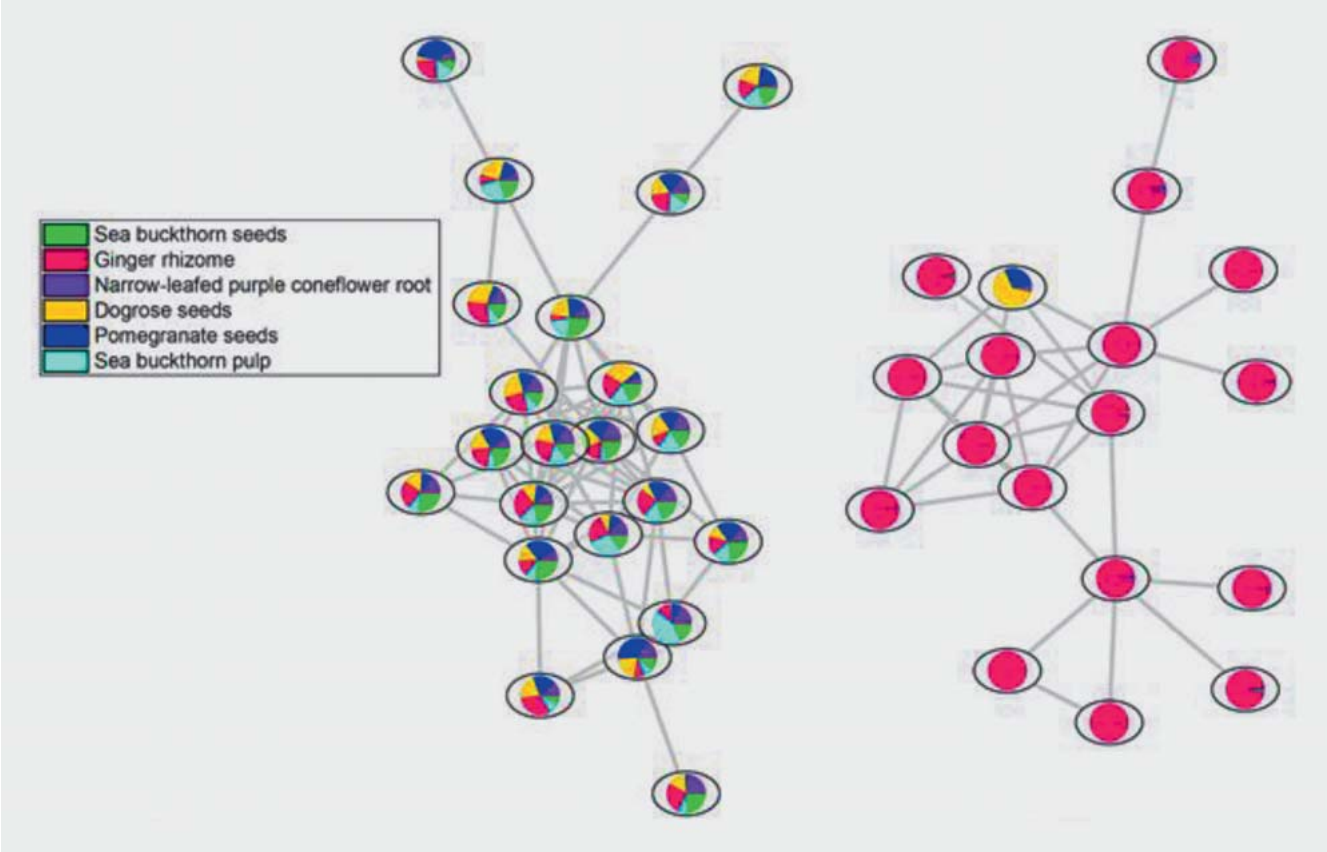

▶ **Fig. 1** Two clusters from the molecular network of plant extracts derivatized with TMSH, the left showing compound equally distributed over all samples and the right showing compounds specifically found in ginger.

pathological analyzes confirmed that CE-2 did not induce significant toxicity in liver and kidney tissues. In CE-2 verbascoside concentration was 3.78 ± 0.14 µg/mL, 0.63 % relatively to the dried extract. Thus, the developed method proved to be reliable and pertinent for quantification of verbascoside in *S. perforatum* samples, and regarding the acute toxicity study, the data suggested that CE-2 could be safe in a single administration.

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P-049 Optimization of a GC/MS-based Screening of Plant Extracts using GNPS

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An uncountable variety of plants and organisms inhabits an even bigger number of natural products [1]. These valuable compounds are still mostly unexplored, as research mainly focusses on thousands of molecules already identified and named.

Using GC/MS-analysis and GNPS [2,3] different extracts of five plants (sea buckthorn, ginger, narrow-leafed purple coneflower, dogrose and pomegranate) were investigated in an untargeted approach.

The extraction of the plant material was carried out successively with supercritical carbon dioxide (kindly provided by Flavex Naturextrakte GmbH) and methanol. The methanolic extract was further purified via liquid-liquid extraction and the ethyl acetate phase as well as the carbon dioxide extract was further investigated using GC/MS. Therefore, the derivatization step and the GC/MS method were optimized. Subsequently, the data were prepared for application using Global Natural Products Social Molecular Networking (GNPS) and different parameters during network creation and annotation were evaluated. The molecular networks visualized using Cytoscape show differences between different extracts as well as between different plants. Clusters were found which occurred in all samples as well as plant specific clusters (▶ **Fig. 1**).

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P-050 Mass spectrometric tools for species identification from leaf bud metabolites

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Previous studies have demonstrated that leaf surface metabolites can be used for distinguishing between birch species [1,2]. In this study, the aim was to develop a rapid LC-MS based fingerprinting method for the leaf bud metabolites other common Finnish trees and shrubs. The extracts were screened for marker candidates using two approaches: 1) UHPLC-ESI-Q-Orbitrap analysis combined with MZmine 2 data processing to obtain unique species-specific markers and 2) UHPLC-ESI-QqQ analysis and selection of main ions from the full scan spectra to obtain species-sensitive markers. Depending on the species, 1–6 marker candidates were chosen for further testing. Two competitive fingerprinting methods utilizing selected ion recording were created and tested for specificity and repeatability with 4–10 replicates per species.

The first approach was superior in creating selective fingerprints. However, the best repeatability and selectivity for three species were achieved by using the markers obtained with the second approach. The final tool included a rapid 10-min extraction of the metabolites, filtration and analysis with the 5-min LC-ESI-QqQ fingerprinting method and provided unique and repeatable chromatographic profiles for 13 species. The developed tool was suitable for the species identification based on the easily interpreted fingerprints and could be useful in the quality control of different products derived from leaf buds.

There are no conflicts of interest to declare.

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P-051 Profiling of pyrrolizidine alkaloids and quantitation of the retronecine-core in crude plant extracts: A Qual-Quan approach by UHPLC-TOF/MSe and UHPLC-TOF/MRM

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Pyrrolizidine alkaloids (PA) are secondary metabolites of high toxicological relevance. Their occurrence has triggered the development of several PA quantitative methodologies based on a limited number of certified standards, including time-consuming purification steps. Herein, we shed light on the variability of PAs by screening PA-containing extracts from Boraginaceae, Fabaceae and Compositae families. Additionally, we proposed a quantification methodology based on UHPLC-HRMS for the evaluation of the total PA content as retronecine-equivalents (RE) directly from crude matrices. In total, 105 PAs were identified using HRMSe experiments, specific MS/MS fragmentation patterns, a customized in-house library and literature data. Among them, 43 retronecine/heliotridine-type PAs, 13 platynecine-type derivatives, as well as their 45 corresponding N-oxides, were reported. Furthermore, three otonecine and one trachelanthamidine necine base were observed. Interestingly, 18 PAs were annotated as glycosylated derivatives, reported for the first time in the literature. The quantitative study recorded PA concentrations ranking from 8.64 ± 0.08 to 3096.28 ± 273.72 µg RE/g extract dry weight in shoots of *Alkanna graeca* and in leaves of *Crotalaria retusa*, respectively. The methodology showed LLOD and LLOQ values of 3.46 and 11.52 pg · mL^{-1}, respectively, offering a good precision (2.81–7.60% RSD) and accuracy (96.96–105.19%). Besides, a correction factor coupled to the detailed dereplication process was developed for each extract (ranging from 1.96 to 2.48) in relation to their unique PA content. The present methodology will facilitate PA quantification directly from crude extracts and avoid the underestimation of PA real content in botanical extracts.

P-052 The metabolic profile of *Anchusa officinalis* L. differs according to its associated arbuscular mycorrhizal fungi

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A recent study demonstrated the impact of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis* MUCL41 833 on the metabolome of *Anchusa officinalis* [1]. However, it is not known whether AMFs belonging to the same genus impact the primary (PM) and secondary (SM) metabolites of *A. officinalis* in a similar way. Therefore, we tested the hypothesis that different strains of AMF affect the plant metabolome differently. Four AMF species, belonging to the genus *Rhizophagus* (*R. irregularis* MUCL41 833, *R. intraradices* MUCL49 410, *R. clarus* MUCL46 238, *R. aggregatus* MUCL49 408), were considered and their effects on the PMs and SMs of A. officinalis evaluated under controlled semi-hydroponic cultivation conditions. An untargeted metabolomic analysis was performed using UHPLC-HRMS followed by a multivariate data analysis. Forty-two compounds were reported to be highly modulated in relation to the different AMF associations. Among them, six new SMs were tentatively identified including two acetyl- and four malonyl- phenylpropanoid and saponin derivatives, all presenting a common substitution at position C-6'' of the glycosidic moiety. In addition, an enhanced accumulation of PMs and SMs was observed for *R. irregularis* and *R. intraradices*, showing a stronger effect on *Anchusa officinalis* metabolome compared to *R. clarus* and *R. aggregatus*. Therefore, our data suggest that different AMF species belonging to the same genus specifically modulate the metabolome of *A. officinalis*. This observation can contribute to the selection of the most adequate AMF species for the production of targeted compounds.

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P-053 Ring B substitution pattern and glycosylation strongly affect the stability of flavonols towards in vitro digestion

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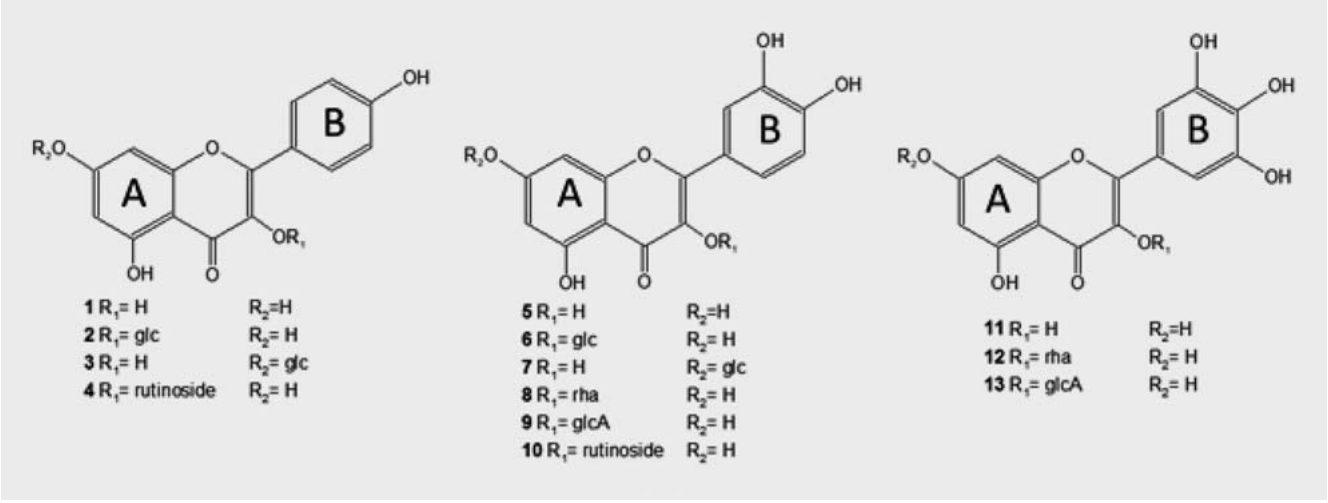
In vitro digestion models are frequently used to study the bioaccessibility and stability of plant constituents in food and medicinal plants [1]. Varying degrees of stability towards in vitro digestion have been reported for flavonols. Moreover, flavonols possessing catechol or pyrogallol substitution in ring B have been reported to be unstable in cell culture media or buffer solutions frequently used in pharmacological in vitro assays [2,3].

In this study, the stability of flavonols with phenol, catechol and pyrogallol substitution in ring B and different position and degree of glycosylation (▶ **Fig. 1**) should be assessed in an in vitro digestion protocol based on the INFOGEST consensus method [4]. Flavonoid levels in the different digestion phases were determined by HPLC-UV.



▶ **Fig. 1** Flavonols subjected to in vitro digestion. **1:** kaempferol; **2:** kaempferol-3-O-*β*-D-glucoside (astragalin); **3:** kaempferol-7-O-*β*-D-glucoside (populnin); **4:** kampferol-3-O-*β*-D-rutinoside (nicotiflorin); **5:** quercetin; **6:** quercetin-3-O-*β*-D-glucoside (isoquercitrin); **7:** quercetin-7-O-*β*-D-glucoside (quercimetrin); **8:** qercetin-3-O-*α*-L-rhamnoside (quercitrin); **9:** quercetin-3-O-*β*-D-glucuronide (miquelianin); **10:** quercetin-3- O-*β*-D-rutinoside (rutin); **11:** myricetin; **12:** myricetin-3 O-*α*-L-rhamnoside (myricitrin); **13:** myricetin-3-O-*β*-D-glucuronide.

While all flavonoids were found to be stable in the oral and gastric digestive phase, stability towards intestinal phase digestion was found to be strongly dependent on ring B substitution. While kaempferol and its glycosides remained unaffected, the levels of quercetin were reduced to 26.8% of a quercetin control solution prepared in ethanol. The observed reduction was obviously caused by enzymes or bile, since quercetin was stable when incubated in the intestinal phase buffer only. Myricetin was undetectable after incubation in intestinal phase buffer with and without enzymes and bile, indicating that its decline is due to instability in intestinal phase buffer. For the respective glycosides, lower degrees of reduction were observed, indicating that glycosylation generally enhanced the stability of quercetin and myricetin towards intestinal phase digestion.

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P-054 Integrating analytical methods for endocannabinoid system exploration: Determination of endogenous and plant cannabinoids in relevant matrices

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A growing body of evidence highlights the relevance of the endocannabinoid system (ECS) in various physiological and disease states [1]. Phytocannabinoids, notably Δ9-tetrahydrocannabinol (Δ9-THC) were firstly studied to investigate mechanisms of action associated with *Cannabis sativa* L., their prolific natural source. Such biological effects were attributed to the modulation of the ECS, which was found to be subject to pleiotropic signaling by endogenous ligands, referred to as endocannabinoids. The complex role of this system and related molecules in different biological substrates is yet to be elucidated. In view of this, reliable determination of both endo- and phytocannabinoids is of crucial importance, considering the various analytical challenges involved.

In this work, efficient chromatographic methods were developed and validated in physiologically relevant matrices, providing required performance characteristics in terms of sensitivity and cost-effectiveness. Liquid chromatography high-resolution mass spectrometry (LC-HRMS) was employed for identification and quantification of major endocannabinoids 1-AG, 2-AG and anandamide in biological samples. With respect to *C. sativa* biomass, major bioactive cannabinoids such as Δ9-THC, CBD and their biogenetic precursors THCA, and CBDA were determined using an optimized UPLC-PDA methodology. Both approaches effectively overcome the well-reported limitations [2] associated with separation and stability of the studied compounds, through optimized protocols for sample preparation and analysis. It is envisioned that the developed integrated approach will enable further exploration of the ECS and ultimately drive therapeutic interventions based on this multifaceted system.

The authors declare no conflict of interest; Funding: CANNABinAutism (ERDF & Greek National Funds, ID: T2EΔK-02075).

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P-055 Medicinal Cannabis Grown in Germany – First Experiences and Quality Standards

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The German Cannabis Agency was established in 2017. Following the new German legislation and the provisions of the "Single Convention on Narcotic

Drugs" (United Nations) [1], the German Cannabis Agency should control cultivation of *Cannabis* for medical use in Germany and its distribution, which is ensured by a wholesaler selected in a European tender. Three production facilities have been established by contract partners, following the legal standards for narcotics, medicinal products and in particular GACP and GMP. An important objective was to provide patients Medicinal *Cannabis* with a high level reproducible pharmaceutical quality. There are different types of Medicinal *Cannabis*, which are defined by a different content of THC and CBD. Appropriate specifications for each type have been established complying with standards defined for herbal medicinal products. Initially, stability was set to 8 months, based on data from literature and preliminary studies. Ongoing stability studies and supplementing studies on in-use-stability indicate that, after completion of the studies, there will be the option to increase the stability.
The first cannabis for medical use grown in Germany was released to the German market in July 2021 with BfArM/Cannabis Agency acting as wholesaler and pharmaceutical company. A total of about kg Medicinal *Cannabis* has been sold via 240 pharmacies to patients (as of May 2022). Only few complaints on pharmaceutical quality have been reported and evaluated. In forthcoming years, the full production quantity will be available and a total of about 2800 kg Medicinal *Cannabis* will be cultivated in Germany per year.

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P-056 LC-HRMS based metabolite profiling for quality control of Greek honey via bioinformatic tools

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DOI 10.1055/s-0042-1759041

Honey constitutes a high-value food commodity both nutritionally and commercially. Hence, it is often faced with adulteration adversities. The variance in its physicochemical properties along with its high-sugar content, make it a difficult matrix to be analyzed. Aside from conventional methods, metabolite profiling approaches enveloped in the field of foodomics, could offer a more extensive coverage of honey's secondary metabolites and provide solutions in authentication aspects. The aim of the present study was to apply LC-HRMS based metabolite profiling to map Greek honeys and set a standard in terms of quality control and authentication focusing on phenolics. Statistical tools were exploited to study parameters, such as botanical and geographical origin, and collection period of more than 250 samples and reveal potential biomarkers [1]. Briefly, acquired spectrometric data were processed prior to being subjected to multivariate analysis. The sensitivity of HRMS led to the identification of more than 150 secondary metabolites [1]. Statistical models showed low dispersion, high robustness, and good classification according to each studied parameter. Characteristic compounds were annotated in favour of different botanical and geographical origins, indicating their significance in the discrimination amongst classes. In conclusion, LC-HRMS based metabolite profiling proved to be an effective method in honey quality and authenticity evaluation. It is the first time that LC-HRMS is enlisted for the analysis of a large number of Greek honeys and the correlation with their origin under metabolomics approach.
The authors declare no conflict of interest; Honey Roads (Project Code: 2018ΣΕ01 300 000)

Reference

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P-057 Feature-based molecular networking approach for the search of unknown dihydrochalcone derivatives in the leaves of *Malus* species and hybrids

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Apple fruits and apple-derived products are very popular due to their nutritious and health promoting properties. Fruits but also leaves are known to accumulate various polyphenolic compounds with dihydrochalcones (DHCs) as characteristic secondary metabolites. There is strong evidence that DHCs greatly contribute to the health-beneficial activities of apple fruits itself, but also to those of apple derived products. Due to breeding and selection during the cultivation of apple trees, a huge diversity of different apple cultivars and hybrids was created. Therefore, we were interested in the possible differences the secondary plant metabolite composition of cultivars, hybrids, and wild species. Feature-based molecular networking (FBMN) utilizing a set of UHPLC-HRMS2 data of methanolic leave extracts of fifteen *Malus* ssp. revealed potentially unknown DHCs in two different clusters of the FBMN. The features of interest were found in higher amounts in a wild type apple species (*Malus micromalus* Makino) and were identified after isolation and NMR-based structure elucidation as 2′,4′,6′-trihydroxy-dihydrochalcone-4′-*O*-β-D-glucopyranoside [1] as well as two acylated DHC derivatives: 6′′-*O*-*p*-coumaroyl-trilobatin [2], and a yet unreported natural product 6′′-*O*-*p*-coumaroyl-sieboldin. With those findings, we were able to proof the value of FBMN as an efficient tool also for chemotaxonomic investigations and the identification of unknown natural products.

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P-058 Pharmacokinetics and metabolism of oleocanthal, a natural anti-inflammatory agent of olive oil

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Oleocanthal (Oleo) is a secoiridoid exclusively found in olive oil, which lately gained great scientific interest due to its strong anti-inflammatory activity [1]. Several biological properties have been ascribed to Oleo and its administration has been associated with the reduce of inflammation markers and the prevention of various human pathologies [2]. However, limited data exist for Oleo metabolic fate in in vivo systems, which could evidence the mechanisms behind its biological activities. Furthermore, its pharmacokinetic properties (PK) have never been described so far. Interestingly, Oleo has never been detected in biological fluids, possibly due to its sensitive and labile chemical structure consisted of two highly reactive aldehydes and an easily hydrolyzed ester bond. In the current study, a suitable mice model based protocol was set in a standard Oleo dose of 5 mg/Kg and plasma samples were collected in five different time points. For this purpose, an optimized extraction protocol was

developed for the recovery of Oleo from mice plasma and a special UPLC-Orbitrap-MS methodology was developed and applied for the detection of Oleo and its metabolic derivatives in mice plasma. The PK characteristics (Tmax and/or Cmax) of Oleo were studied and its metabolic derivatives were identified and determined in time along with their relative content. Biomarkers were proposed and associated for the first time with Oleo supplementation in vivo.

The authors declare no conflict of interest

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P-059 Volatilomics of Spanish style cv. Chalkidiki green table olives spontaneously fermented in Reduced NaCl Content Brine

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w>Table olives are important components of the Mediterranean diet, and their consumption is expanding worldwide due to their potent health benefits related to the presence of polyphenols, fatty acids and vitamins. Reducing NaCl content in table olive processing is important to establish the product as a functional food [1]. However, it is challenging as it influences the complex microbiota evolving in the fermentation process, which has an impact on the safety and quality of the final product. Volatilomic approaches have been used to detect specific molecules indicative of the metabolic activities of the microbial communities occurring on table olive fermentation and are associated with desirable flavors or defects [2]. In this context, in the present study, the volatile organic compounds of reduced NaCl brines (NaCl-KCl-CaCl2, 4–3%-1%) [3] were determined during spontaneous fermentation of Spanish style cv. Chalkidiki green table olives in pilot scale plastic vessels (220 L) for two processing periods (2020/21, 2021/22) using SPME/GC-MS. In control brine, NaCl was adjusted to 8%. A total of fifty-five metabolites comprising of acids, alcohols, aldehydes, esters and phenols were identified. In both treatments, acetic acid, propanoic acid, ethanol, 3-methyl-1-butanol, phenylethyl alcohol, ethyl acetate, 3-methyl-1-butanol acetate ethyl hexanoate and ethyl octanoate were the superior volatiles. Principal component analysis of the data helped to discriminate the tested samples according to treatment, processing period and fermentation time. This is the first systematic study on the volatile profile of table olives from cv. Chalkidiki processed under reduced salt.

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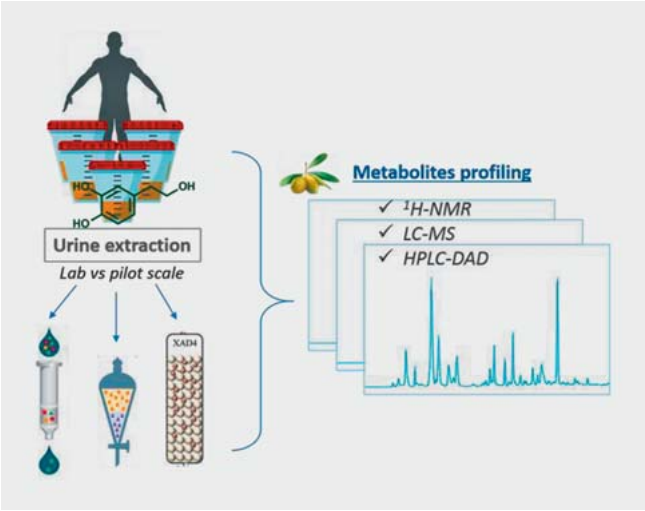

▶ **Fig. 1**

P-060 Integration of different extraction protocols for the metabolite profiling of urine after hydroxytyrosol supplementation in humans

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Urine has been demonstrated to be a valuable sample matrix across multiple omics field for monitoring information of human dietary exposure [1]. Usually interpretation of biomarkers/biochemical pathways are incorporated as analysis standpoints by means of LC-HRMS or (HR)NMR. However, the lack of appropriate standards, poses the risk of vague or imprecise metabolites identification in such approaches. Isolation of urine metabolites and their unambiguous structure elucidation could be the recourse in urine dereplication and biomarkers identification, after dietary interventions. The easy and non-invasively collection of urine could positively conduce in a large-scale approach for extraction and consequent metabolites isolation and unambiguous identification. Hence, the aim of the current study was the integration of different extraction protocols with pilot-scale potentials for urine extraction and metabolite profiling thereof, after hydroxytyrosol supplementation. Towards this purpose, hydroxytyrosol was supplemented as a soft capsule (15 mg/day) to a healthy volunteer for two weeks and 24-h samples were collected. Typical analytical protocols used for biological samples pre-treatment i.e., LLE, SPE [2] were applied together with enrichment methodologies such as macroporous adsorption resins (XAD4, XAD7, ion-exchange) which are employed in natural products research [3]. All derived extracts were analyzed via HPLC-DAD, UPLC-HRMS and NMR to investigate their profile and evaluated in terms of yield, richness and identified metabolites. The proposed methodology was efficiently applied for urine extraction in large volume offering the potential of metabolites isolation and accurate characterization of HT metabolites.

The authors declare no conflict of interest.

The authors thank Oliveheart program and Intermed S. A. for granting HT capsules.

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P-061 Human plasma metabolic changes induced by plant protein-enriched biscuits consumption via LC-triple-TOF employing DIA and LC-orbitrap using DDA

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Protein-enriched foods have been shown to exert appetite-regulating properties[1]. In this study, a wheat biscuit enriched with legume proteins was examined regarding its effects on weight loss and the metabolic profile of obese individuals. A 3-months, two-arm parallel dietary intervention of 80 overweight volunteers was conducted, in which half of the volunteers consumed daily a protein biscuit enriched, while the rest consumed a conventional wheat biscuit. Both groups were subjected to a weight loss diet.

The intervention's plasma samples were analyzed for 44 AA, incorporating a LC-triple-TOF system and the aTRAQ Kit. Moreover, the samples were subjected to metabolomic analysis with two LC-MS platforms, LC-triple-TOF employing SWATH acquisition mode (Data Independent Acquisition, DIA) and LC-Orbitrap using Data Depended Acquisition (DDA) mode, in order to compare the effectiveness of the instrumentations. In parallel, an investigation of the variations of the plasma metabolites before and after the intervention was carried out.

According to the study results, it was observed that the trial group presented a more considerable reduction in body mass index compared to the control group. Furthermore, changes were observed in the metabolic profile of the Individuals. For instance, obesity biomarkers' plasma concentrations, like aromatic AA, were reduced after dieting and protein supplementation.

Overall, functional foods supplemented with protein improve the metabolic responses related to appetite regulation. In contrast, both analytical platforms are proving to be powerful tools for metabolic profiling on complex samples and human intervention studies.

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P-063 Optimization of Extraction Conditions and Identification of Organic Volatile Compounds from Strawberry Genotypes

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Strawberry (*Fragaria × ananassa*) enjoys great popularity and has become the most consumed berry fruit worldwide, due to its unique aroma and high nutritional value. Numerous studies have been conducted over the last years on strawberry volatiles (VOCs) which are responsible for its intense fruity aroma. In this work, liquid-liquid extraction (LLE) and gas chromatography–mass spectrometry (GC-MS) were used to analyze and identify VOCs in the fruit of 20 strawberry genotypes cultivated at Berryplasma World Ltd. plantations (Varda, Ilia, Region of Western Greece). Considering the physiological pH of the strawberry fruit (~ 4–4.5), a variety of inorganic salt solutions ($SnCl_2$, $CaCl_2$, NH_4SO_4, NH_4Cl, and NaCl) were screened and incorporated into the fruit matrices aimed at maximizing the VOCs extraction. Finally, extraction was performed with ethyl acetate. The optimal chromatogram in terms of the number of identified VOCs was obtained using sodium chloride. The characteristic strawberry VOCs, found in most of the genotypes analyzed to date, consist of furanones, such as 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifuran) and 4-methoxy-2,5-dimethyl-3(2H)-furanone (furaneol) and 5-hexyldihydro-2(3H)-furanone (γ-decalactone); esters, including ethyl butanoate, ethyl hexanoate and methyl hexanoate; acids including trans-(E)-cinnamic acid, butanoic and hexanoic acid and terpenoids such as linalool, trans-(E)-nerolidol and bisabolol oxide B. Ongoing experiments in our laboratory are currently directed towards the extraction and determination of VOCs of all strawberries genotypes tested.

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P-064 Investigating the application of Raman spectroscopy on the discrimination of *Origanum* taxa and the quantification of their essential oils constituents

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The potential of Raman spectroscopy for the identification and quantification of essential oils constituents obtained from different *Origanum* taxa was investigated, particularly focusing on the reproducible analysis of volatile liquids in very small quantities. Compared to headspace Gas Chromatography Mass Spectrometry (HS-GC/MS), which is routinely applied for the analysis of essential oils [1], Raman spectroscopy offers the advantage of time efficiency, no sample preparation and non-destructiveness. It is also environmentally friendly and cost effective, as it does not require the use of chemical reagents [2]. A series of Raman measurements were conducted both on reference compounds present in essential oils and on samples of essential oils distilled from samples of *Origanum* taxa, either collected from nature or purchased from the market. To address issues such as the volatility and the volume requirement of the analytes, after testing different experimental arrangements, an in-house assembly based on the use of a capillary tube inserted in a 3D-printed adjustable base was selected as the most appropriate. The quantification method used for the essential oils' constituents was based on Multiple Linear Regression using the spectra of the reference compounds as input. The results were very encouraging, being in good agreement with the respective ones of GC/MS, with a deviation of less than 10%. Additionally, by collecting Raman spectra directly from the sessile glands of aromatic plants' leaves, it was possible to detect the characteristic substances and consequently facilitate the chemotaxonomy of *Origanum* taxa.

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P-065 LC-MS phenolic profile and quantification of rosmarinic acid in methanolic extracts from Bulgarian *Thymus* species

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Thymus species are well-known medicinal and aromatic plants widely used to heal various respiratory and gastrointestinal diseases due to their biological activities such as antibacterial, antifungal, spasmolytic and antioxidant properties. Bulgarian flora is represented by 21 species belonging to two sections – Hyphodromi and Serpyllum, as all they are poorly studied [1]. The phenolic profile of 15 *Thymus* species was analyzed by HPLC–DAD–MS, and a total of 22 individual compounds were identified – mainly phenolic acids, flavonoids and flavonoid glycosides, typical for the genus. In addition, the phenolic profile of the methanolic extracts displayed relatively constant qualitative composition in comparison to the data of their essential oils [2]. However, rosmarinic acid was found as the major compound, and common phenolic constituent, in all analyzed samples. Having in mind that rosmarinic acid is a valuable compound, widely used in food and cosmetic industries due to its proven antioxidant, anti-inflammatory, hepatoprotective and neuroprotective properties, its quantitation was further performed by HPLC–DAD. The results showed that the content of rosmarinic acid varies significantly (19.7 mg/g and 90.6 mg/g), with minimum and maximum content in *T. perinicus* and *T. pulegioides,* respectively. Although *Rosmarinus* species have been used as the main source of rosmarinic acid [3], our results show that Bulgarian *Thymus* species are valuable as a source of this substance and other biologically active phenols.

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P-066 Microflora-dependent conversion of secoiridoids from *Olea europaea* and their effect on bioavailability of hydroxytyrosol

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Hydroxytyrosol (HTyr), a dietary phenolic alcohol of olive oil, holds a plethora of bibliography and an EFSA health claim regarding its health promoting activities [1]. HTyr bioavailability has been mostly studied through ADMET assays and clinical trials, where it is supplemented directly as a pure compound, and indirectly as a structural moiety of oleuropein (Oleu), or as a constituent of the total polyphenolic fraction (TPF). However, the way HTyr is administered has been found to significantly affect its bioavailability, while its biotransformation in the colon by the human gut microbiota has also been highlighted as a very critical factor [2]. Since biotransformation studies of TPF and Oleu are limited, we aimed towards a detailed investigation of their microflora-dependent conversions, using the Gastro-Intestinal Dialysis Model with Colon (GIDM-Colon) [3] in order to investigate the availability of HTyr as individual component, as a part of Oleu or as a constituent of TPF. GIDM-Colon is a validated in vitro continuous flow dialysis system, simulating the physiological conditions of the human GI tract, including human gut microbiota. Throughout the in vitro model, biological samples were collected at crucial timepoints. Overall, in the case of TPF, it appears that the abundance of HTyr is relatively low throughout the 24 h metabolic line of gut microbial biotransformation. In contrast, Oleu seems to be an excellent reservoir that ensures increased bioavailability of HTyr over time, and it can be considered an effective prodrug.

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P-067 Development and validation of HPLC-ELSD method for *Pistacia lentiscus* L. characteristic triterpenic acids determination, in resin and food supplements

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Chios mastic gum (CMG) is the resinous secretion of the evergreen shrub *Pistacia lentiscus* var. Chia. CMG is a product with a long-standing ethnopharmacological history, officially recognized by the EMA as a traditional herbal medicinal product. Apart from its therapeutic value, CMG is also a unique PDO product of Greece, with a broad application in the food, cosmetics and food supplements industries. Due to its high market demand, extensive adulteration attempts with inferior quality gums and resins and packaging falsification is a pressing issue, since to this day no analytical method has successfully been applied for the crude resin' s quality control [1]. Therefore, in an effort to compensate for this evident gap in literature, we propose a simple, fast and cost-effective analytical solution to the quality control of CMG products, through the development of a robust, reliable and simple HPLC-ELSD methodology for the quantification of masticadienonic and isomasticadienonic acids, CMG's major and most characteristic triterpenes in mastic-containing samples. Validation of the developed method was performed according to the ICH guidelines, assessing parameters such as linearity, specificity, precision (repeatability and intermediate precision), accuracy, robustness and stability. The method was employed to the analysis of several substrates, including authentic and falsified crude resin samples and various food supplements with different formulations. The proposed method can be a useful tool for analytical laboratories working on the authentication and quality control of marketed CMG-based products.

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▶ **Table 1** Pesticide residues detected in herbal products analyzed in SFDA Laboratory.

Most repetitive compounds	Frequency (%)	Frequency (n)	Concentration Range (µg/kg)		LOQ (µg/kg)	LOD (µg/kg)
Carbendazim	23.1	3	25.290	161.210	25.000	8.000
Imidacloprid	30.8	4	44.980	251.730		
Propoxur	15.4	2	60.140	389.780		
Isoprocarb	15.4	2	106.280	244.260		
Metolcarb	7.7	1	72.160			
Cymoxanil	7.7	1	493.21			
Total	100.0	13				

P-068 Identification and quantification of pesticides residues in health and herbal products purchased from Saudi markets

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Background: Herbal products are consumed by approximately 80% of the world population owing to its beneficial effects to human's health.

Importantly, these products are from natural sources or have "Natural" in their labeling and so there are general presumptions of their safety and quality. However, there are literature data showing that herbal products could potentially be contaminated through many ways.

Objective: The study aimed to determine the pesticides residues in herbal products purchased from Saudi markets.

Materials and Methods: 41 products were extracted using QuEChERS, and then analysed using LC-MS/MS to determine 232 pesticide residues. Observed pesticides were compared to regulations of USP and SFDA.

Results: Results showed that pesticides residues were detected in 26.8% of the examined samples in concentrations ranging from 25.3 to 493.2 µg/kg. Moreover, 6 different pesticides residues were identified in the tested products as illustrated in ▶ **Table 1**.

Imidacloprid occurred most frequently in 4 samples, there are 2 samples has more than one pesticide residues while sample code 31 has the highest concentration of pesticide (493.2 µg/kg).

Finally, our findings revealed that all detected analytes were not in the "Herbal and Health Products" regulation in the SFDA and the (USP) which only determined 70 pesticides.

Conclusion: it is necessary to expand the SFDA's regulatory program for herbal products to cover as many possible pesticides as possible and to carry out frequent safety examinations of these herbal products, and demanding companies to conduct pesticide detection testing for finished herbal products.

P-069 Comprehensive characterization of hemp (*Cannabis sativa* L.) samples from Greece through combined NMR and LC-based metabolite profiling and biomarker identification

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Cannabis sativa L. (hemp) is an important source of bioactive compounds, such as cannabidiol (CBD) and cannabidiolic acid (CBDA). Other constituents, including minor cannabinoids, are increasingly gaining traction for informed plant utilization [1,2]. Phytochemical composition may be affected by variety, geographic origin, harvesting year or cultivation environment, inevitably resulting into different pharmacological effects [3]. In this regard, characterization and classification of *C. sativa* samples based on chemical profiles is important to promote standardization and consistency in products, practices, and therapeutic outcomes.

In this work, inflorescences belonging to different hemp varieties were collected from various regions of Greece during consecutive harvesting years. Following a simple and streamlined sample preparation protocol, the ethanol extracts were analyzed using a combination of NMR and LC-based techniques aided by chemometric processing. 1H-NMR proved to be a quick and informative tool, offering an overview of metabolite classes, including lipids and minor phenolics. Quantitative determination of target cannabinoids was performed by LC-PDA providing useful information, which was expanded by an LC-MS based untargeted workflow with focus on minor cannabinoids. Multivariate techniques, such as PCA and PLS-DA, were applied to reveal patterns and class structure within the samples. In pursuit of efficient biomarker identification, the class-determining metabolites as pointed out by each platform were explored and compared. Overall, the proposed approach allowed comprehensive characterization of *C. sativa* inflorescences and classification according to important parameters, being the first of its kind for Greek samples.

The authors declare no conflict of interest; Funding: CannabisMED (ERDF & Greek National Funds, ID: T1EDK-04301).

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P-070 Quantitative Profiling of the Chemical Diversity in the Plant Kingdom

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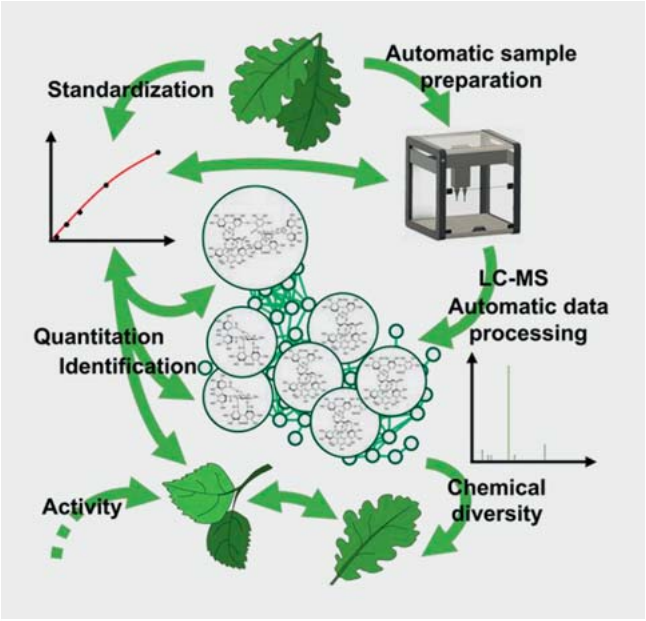
There are tens of thousands of specialized metabolites in the Plant Kingdom, such as polyphenols, alkaloids, glucosinolates, cyanogenic glycosides and terpenes. Even though it is shown that these metabolites have many types of relevant bioactivities, the actual chemical diversity of specialized metabolites in the Plant Kingdom is still uncharted. Our knowledge of the collection of the Botanical Garden of the University of Turku demonstrates this well; over half of the species are chemically completely unknown, let alone that the chemical compositions of individual species would be totally determined. Powerful tools are needed to be able to create a comprehensive picture of the chemical diversity in plants which is on the other hand constantly decreased by loss of biodiversity. We have created a fast compound-specific analytical platform that utilizes ultra-high performance liquid chromatography (Waters Acquity UHPLC)



▶ **Fig. 1** Single analytical platform with automatic data processing enables handling of thousands of plant samples. Quantitative information is combined with absolute quantitation data produced with compound-specific standards giving possibility to find chemical likages in the plant tree of life.

coupled with triple quadrupole (Waters Xevo TQ) and high-resolution mass spectrometry (Thermo Orbitrap QExactive) (▶ **Fig. 1**). A single quantitative platform with individual quantitation curves to all samples makes the whole data set fully comparable between all included samples. Produced data is processed utilizing automatic softwares such as MZmine2 and Global Natural Product Social Molecular Networking [1,2].

Approximately 4500 species from five continents will be analyzed with our platform. This will draw a wide quantitative picture of chemical diversity of plants. Most importantly, this data will give us unique opportunities to make conclusions on how different types of natural compounds are distributed in the Plant Kingdom and in the evolutionary plant tree of life [3].

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P-073 Pathogenic fungi influencing safety of raw material of oregano (*Origanum vulgare* L.) in Latvia

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DOI 10.1055/s-0042-1759055

Many fungal species infect oregano (*Origanum vulgare* L.) during cultivation, some of them produce substances of toxic character – mycotoxins, secondary metabolites [1]. In 2020, the research on diversity of fungi colonizing and damaging leaves of oregano was carried out. The samples of plant material were selected from an ex situ collection of aromatic and medicinal plants' genetic resources. Fungi, potential causing agents, were isolated from tissue of diseased leaves and were identified by sequencing nuclear ITS-rDNA region.

Quantitatively the most extensive group were represented by *Alternaria* sp. (36%), *Epicoccum* sp. (25%) and *Phoma* sp. (11%). Single isolates of *Ascomycota* sp., *Mucor* sp., *Botrytis* sp., *Stemphylium* sp. and *Chaetosphaeronema* sp. were also obtained from the leaves of oregano.

In scientific literature it is described *Alternaria* species produces more than 70 phytotoxins, they are accumulated in plant tissues [2,3]. Also is the genus *Phoma* are producer of mycotoxins [4]. *Boeremia exigua* var. *exigua* (syn. *Phoma exigua* var. *exigua*) we were observed in our study on oregano leaves.

The negative aspect of the occurrence of *Alternaria* and *Boeremia exigua* var. *exigua*) on oregano leaves, in addition to reducing the amount of 'Herba Origani' yield, is the ability to accumulate toxic substances, produced by the pathogens in the tissues. These species should be considered as particularly dangerous for oregano in Latvia.

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P-074 In-depth lipidomic profiling and classification of Greek bovine, ovine, buffalo, caprine and donkey milk by LC-MS/MS QTOF

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Milk lipidomics has recently been a topic of intense research interest. The notion is that milk lipids have specific roles in human diet and research has been indicative of the potential benefits of specific lipids. However, the entirety of the milk lipidome was relatively understudied, mainly due to equipment limitations. Recently, state of the art equipment and novel approaches on the lipidomic characterization of bovine milk [1,2], have helped to reveal the potential health benefits of specifics lipids and lipid classes [3]. Such developments suggest the need for a detailed lipidomic characterisation of milk produced by different farm animal species in Greece, that would benefit the dairy sector but also consumers.

Here, an in-depth lipidomic investigation is performed on five types of milk produced in Greece: bovine, ovine, buffalo, caprine and donkey, focusing mainly on the most abundant lipids in milk: triglycerides. Our results and statistical analysis demonstrate that the classification of milk is possible based solely on the lipidomic profiles of the analysed milk samples. Other potential applications of the extracted lipidomics profiles along with challenges faced for correct lipid identification are also discussed.

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P-075 Characterization of almond varieties from different regions of Greece during two harvesting years by HS-SPME/GC-MS and TD NMR Relaxometry analyses

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The almond (*Prunus dulcis*) is a nut largely consumed in the Mediterranean diet, considered one of the healthiest diets in the world. It has been approved that different types of almonds provide different nutritional characteristics [1]. The objective of the study was to evaluate the effect of geographical origin, harvesting year, and farming regime on the quality characteristics of Greek almonds. Time Domain Nuclear Magnetic Resonance (TD NMR) Relaxometry was used to determine the oil content of the different almond samples by using Hahn echo sequence (90 °-180 ° pulses). Oil content can be considered as an important quality parameter since it affects the shelf life and oxidative stability of the almonds [2].

Results showed a clear differentiation of the samples according to origin, farming regime and harvesting year. Chemometric characterization of almond samples by Principal Component analysis (PCA) revealed statistically significant volatile organic compound markers (aldehydes, ketones, alcohols, terpenes) for the differentiation of samples.

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P-076 Investigating quality characteristics of virgin olive oils from Crete. Integration of HS-SPME/GC-MS, 1H-NMR and FTIR-ATR analytical datasets

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Branded virgin olive oils from different regions of Crete, produced from olive drupes (Koroneiki and Tsounati cv) cultivated in both organic and conventional groves and for two consecutive years were examined with three different omic platforms. These platforms were HS-SPME/GC–MS, 1H-NMR, and FT-IR-ATR, which have used frequently in the field of olive oil analyses to address issues of quality and authenticity [1–3]. Toward this direction, the three techniques were examined for their capability to provide information on the influence of geographical origin, harvest year, and farming regime. The main objective of the study was the development of a "cross-metabolomics" approach, that is the integration of the three different datasets with the aid of multivariate statistical analysis to seek out correlations and examine whether the diagnostic potential in the sample analyses is improved. Such an approach, which is ongoing, is challenging as data analysis requires huge amount of work and high skill of data handling [4]. The presented work investigates the application of the cross-metabolomics approach for olive oil classification and further comments on its usefulness and future work required for further development.

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P-077 Development of a HS-SPME-GC-MS method for the characterization of Greek PDO and PGI wines and grape musts during alcoholic fermentation

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Greece is a country with a great production of quality wines, including Protected Geographical Indication (PGI) and Protected Designation of Origin (PDO) wines, produced of over 300 native cultivars. Several factors, such as cultivar, region, climate and vinification process, are considered to be important for the characterization of wine quality. In the present study, a HS-SPME-GC-MS method was developed after optimization of the extraction parameters. 70 samples of six monovarietal Greek PGI and PDO wine samples and 44 PDO Muscat of Alexandria grape must samples received several times during alcoholic fermentation were analyzed with the proposed method. Over one hundred metabolites were identified and relative quantified in all samples. Multivariate statistical analysis and biomarker assessment were per-

formed using the SIMCA package and the Metaboanalyst online platform to discriminate wine samples according colour and variety-region, to determine the authenticity of some floral Greek cultivars, such as Moschofilero, via typical compound classes and to monitor variations of the concentration of volatile metabolites of grape must during alcoholic fermentation.

Results of this work are shown that this method is suitable for the characterization of Greek PDO wines and grape must, while esters, alcohols and terpenes are the main responsible compound classes for the discrimination between cultivars. Moreover, other interesting results are that white Greek varieties are more floral due to their high concentration of terpenic compounds and there are several increases and decreases in the concentration of volatiles during fermentation according to compound group and the stage of fermentation.

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P-079 Development of HPTLC and UPLC methods for quality control of Hanshiyi formula

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The TCM formula Hanshiyi (HSYF) has been developed in China to treat patients with Covid-19. The formula contains *Arecae semen* (Jiao Binglang), *Armeniacae semen* (Kuxingren), *Atractylodis macrocephalae* rhizoma (Sheng Baizhu), *Atractylodis* rhizoma (Cangzhu), *Crataegi fructus* (Jiao Shanzha), *Cynanchi paniculati* radix et rhizoma (Xuchangqing), *Dryopteridis crassirhizomatis* rhizoma (Guanzhong), *Ephedrae* herba (Sheng Mahuang), *Eupatorii* herba (Peilan), *Gypsum fibrosum* (Shigao), *Hordei fructus* germinatus (Jiao Maiya), *Lepidii semen* (Tinglizi), *Massa medicata* fermenta (Shenqu), *Magnoliae* cortex (Houpo), *Notopterygii* rhizoma (Qianghuo), *Pheretima* (Dilong), *Pogostemonis* herba (Huoxiang), *Poria* (Fuling), *Tsaoko fructus* (Wei Caoguo) and *Zingiberis* rhizoma (Sheng Jiang). In official guidelines, the National Health Commission of China has recommended the use of HSYF based on a first retrospective cohort study, which showed a significant reduction of severe cases in the treated group (Tian et al. 2020).

Besides the identification of active principles and modes of action, one aim of this joint research project is to develop quality control systems for HSYF, which allow the identification of every ingredient based on marker compounds. Therefore, we have established HPTLC methods for the analysis of every individual component and finally also for the Hanshiyi mixture. In addition, 224 constituents in the mixture have been identified in the positive mode by using UPLC-HRMS (36 with reference substances), and 84 compounds in the negative mode (24 with reference substances).

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P-080 Evaluation of the new HPLC assay for *Hippocastani semen* PhEur

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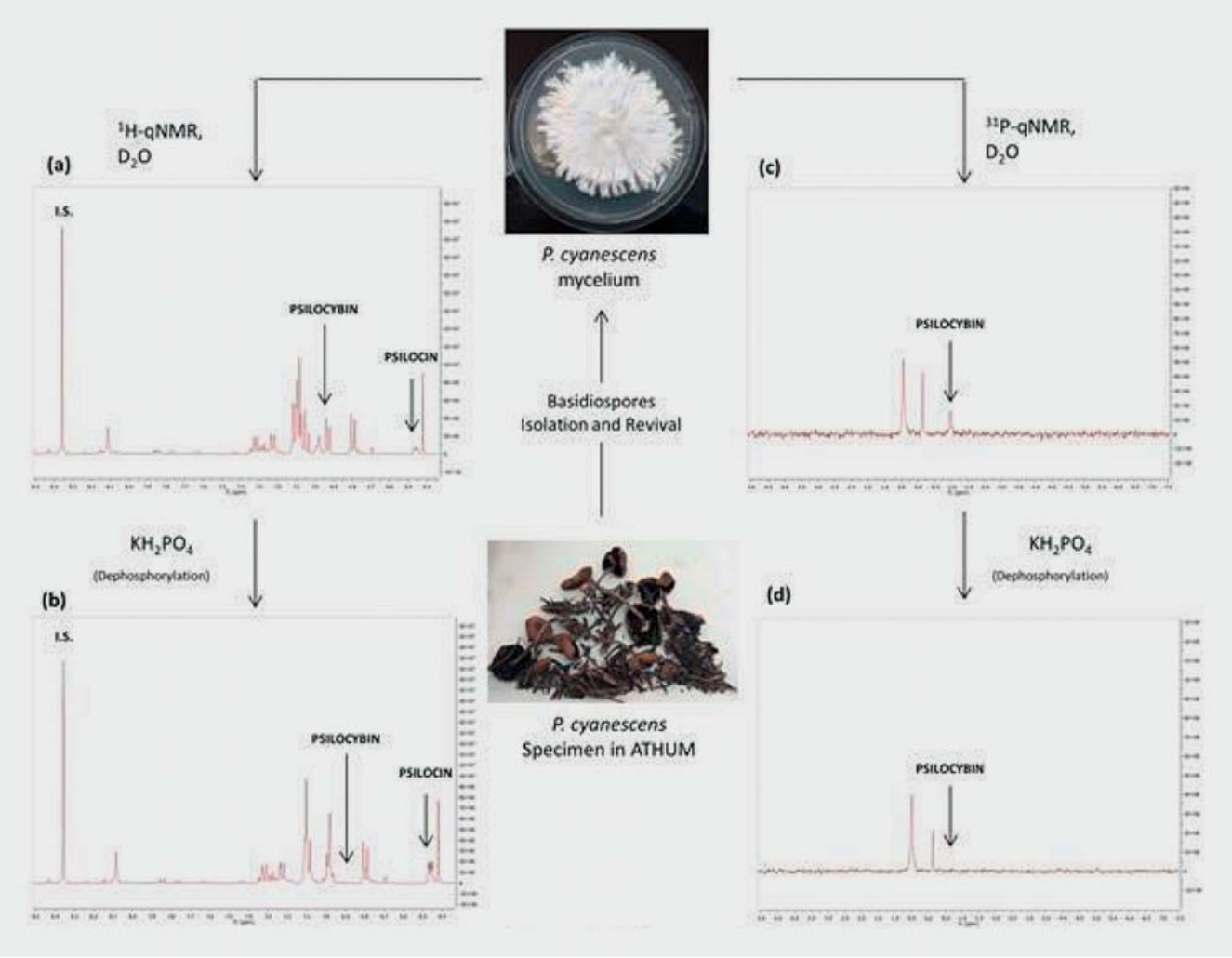
The assay of saponins in *Hippocastani semen* PhEur has to be performed by HPLC. While the assay in the German Pharmacopoeia (DAB) was based on photometric analysis of triterpene glycosides with a minimum limit of 3.0%, expressed as aescin, the assay according to the European Pharmacopoeia is using a HPLC separation of the saponins. The internal markers methyl salicylate and ibuprofen are used to indicate the range of peaks to be considered. Finally, a minimum content of 1.5% of triterpene glycosides, expressed as protoaescigenin is required. For the HPLC system, a system suitability test (SST) is mandatory, which has to be performed with aescin for LC assay HRS (supplied by EDQM) dissolved in the internal marker solution. Methylsalicylate has to elute in between 11.5 min to 15.5 min and has to have a signal-to-noise ratio of at least 10. Ibuprofen has to elute in between 34.0 min to 46.0 min, and aescin peaks A and B of the HRS have to have a minimum resolution of 2.0.

We have evaluated this method and found that for passing the SST is recommended to use the HPLC column recommended in the Knowledge Database of EDQM. Using a different column, which did not pass the SST led to wrong results with too low content. Using the column which passed SST, we compared the outcome of six batches of horse-chestnut seeds using the previous photometric assay (DAB) with the results of the HPLC assay (PhEur) and found that the results were quite similar (▶ **Table 1**).

▶ **Table 1** Comparison of results (% content of triterpene glycosides) usind photometric and HPLC assay.

	Results HPLC (PhEur)				Results Photometric Analysis (DAB)		
Batch	**Value 1**	**Value 2**	**Value 3**	**Mean**	**Value 1**	**Value 2**	**Mean**
1	4.6	4.8	4.7	4.7	4.82	4.57	4.69
2	6.3	6.3	5.5	6	6.17	5.85	6.01
3	4.2	3.8	3.8	3.9	4.95	4.69	4.82
4	6.4	6.1	5.8	6.1	6.46	6.13	6.29
5	5.4	4.9	5.3	5.2	6.14	5.82	5.98
6	5.6	6.3	6.1	6	5.7	5.4	5.55



▶ **Fig. 1** NMR spectra of *P. cyanescens*: (**a**) and (**b**) ^{1}H NMR before and after dephosphorylation, respectively; (**c**) and (**d**) ^{31}P NMR before and after dephosphorylation, respectively.

P-081 Direct Quantitation of Psilocybin and Psilocin by One-Dimensional 1H and 31P qNMR in a revived Greek specimen of *Psilocybe cyanescens*

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The genus *Psilocybe* of Basidiomycota includes more than two hundred species of mushroom-forming fungi, which are widely known for the production of the secondary metabolite psilocybin, a prodrug of its in vivo dephosphorylated active metabolite psilocin [1]. Psilocybin is being currently used in numerous clinical trials including the treatment of Major Depressive Disorder (MDD) [2], existential distress of terminally ill patients [3] and Alcohol Use Disorder (AUD) [4], creating the need of investigating and quantitating these substances.

An experimental study was carried out in order to test the viability of basidiospores coming from dried specimens. Among the various specimens of basidiomycetes kept at the Dried Specimen Collection of the Mycetotheca ATHUM (ATHens University Mycetotheca) was a specimen identified as *Psilocybe cyanescens* Wakef. [5]. Surprisingly, the spores of the specimen dried almost twenty-three years ago were germinated, producing mycelium which was subsequently studied for the production of the secondary metabolites of interest.

The lyophilized mycelium was extracted with methanol via ultrasounds. In order to verificate the presence of psilocybin, a dephosphorylation step was devised and the extract was subjected to GC/MS analysis, that revealed the presence of the dephosphorylated product psilocin. The initial and dephosphorylated extracts were analyzed with 1H and 31P NMR in D2O using calcium formate and dimethyl methylphosphonate as internal standards, respectively. To our knowledge, this is the first time a qNMR method is utilized for the analysis of psilocybin and psilocin containing fungi, without the need of separation and reference standards in less than five minutes.

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P-082 Ultrasound extraction of biologically active compounds from *Alhagi pseudalhagi* seeds

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The Leguminosae family has about 550 genera and 13 000 species distributed around the globe, being one of the largest families of the world's flora. The flora of Kazakhstan has 42 genera and 650 species [1]. Plants from the genus *Alhagi* possessed various biological activities [2]. We have isolated a complex of biologically active compounds from the seeds of *Alhagi pseudalhagi* by the method of ultrasound extraction [UE]. The chemical composition of the extract is studied by gas chromatography-mass spectrometry. UE was voiced using a Bandelin HD 2200 instrument, under the conditions: power 30 W, solvent – hexane, sound time 10 minutes, temperature 41 oC and the ratio of raw materials : extractant 1 : 10. Hexadecanoic (12.10 %), linoleic (12.05 %), oleic (10.18 %) acids and β-sitosterol (9.02 %) were found to be the main components of the extract. The yield of extractive compounds (EC) of *A. pseudalhagi* obtained by UE was compared with traditional maceration extraction. The output of EC using ultrasound is 0.86 %, then as for maceration this index is 0.74 %. These findings further illustrate that extraction of bioactive phytochemicals from plant materials using UE method consumes less extraction solvent and saves time.
The authors declare no conflict of interest.

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P-083 Valorization of bioactive compounds from *Matricaria recutita* essential oil distillation processes by-products

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The development of sustainable solutions for the management of by-products from industry is one of the main challenges of our society. The primary challenge for all kinds of industries in terms of green processes is the transition from a fossil resource-based economy to one based on renewable raw materials processing. The aim of this study was to obtain bioactive compounds for potential use in cosmetics with a characterized safety and efficacy profile from by-products of *Matricaria recutita* industrial processing by environmentally friendly supercritical fluid extraction technology. Extracts derived from chamomile industrial production by-products white flower petals were chemically characterized and revealed the presence of wide range polarity compounds such as tannins, flavonoids, carbohydrates, amino acids, vitamins, following by non-polar essential oils, carbohydrates, fatty acids, sterols and prenol lipids, depending on used extraction process type. Furthermore, the principal phenolic and flavonoid constituents such as coumarin, ferulic acid and apigenin derivatives were chromatographically characterized and quantified to investigate the relationship between their content and antioxidant activity. The elemental screening revealed the presence of iron, calcium, sodium, potassium, magnesium, manganese, zinc and copper. High antimicrobial activity of tested extracts was proved. Cytotoxicity assays demonstrated a high safety of obtained extracts and proof for cell-protecting activity was obtained using human skin cell cultures in vitro tests. Findings within this research suggest that byproducts of *Matricaria recutita* industrial processing are a potential source of valuable bioactive compounds, promoting their use in the field of cosmetics.

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P-084 Medicinal plant waste material as a potential source of sustainable agricultural pest repellents

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The development of new preparations that repel pests while maintaining the benefits that can help the transition to organic farming. Throughout history, medicinal plant extracts have been successfully exploited as insecticides and repellents. Natural pesticides are effective in controlling a wide range of pests, are inexpensive, biodegradable, have a variety of activities and have low toxicity [1]. The aim of this study was to develop raw water extract formulations from essential oil distillation by-products with agricultural pest repellent properties compatible with organic farming regulations. A hot water extraction was used to obtain the extract formulations of bioactive compounds after the atmospheric steam distillation. Phytochemical screening of total flavonoids, tannins, saponins, sugars and phenolic content as well as antioxidant activity using the DPPH assay, was carried out using high-throughput 96-well plate spectroscopic assay methods. Analyses of extract formulations by LC-qTOF-MS revealed bioactive constituents belonging to flavonoids, organic acids, coumarins, lignans, iridoids, alkaloids, aldehydes, ketones and fatty acids. Besides, allelopathic effects of extract formulations were tested using HS-GC-MS analyses of volatile organic compounds released from host plant *Cucumis sativus* leaves, as well as repellent activity on common agricultural insects such as green peach aphid, greenhouse whitefly and large cabbage butterfly were also tested. The study revealed potential of tested extract formulations significance in developing agricultural pest repellents as an alternative to synthetic insecticides

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P-085 Green Extractions of Bioactive Secondary Metabolites in By-Products of the Coffee Production Chain

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It is estimated that less than 1 % of all the medicinal plant species used worldwide are cultivated commercially [1]. On the other hand, only a small fraction of plants from millennial agricultural crops is traded, with the rest of the plants being transformed into low value-added products or discarded in the fields impacting the environment (greenhouse gas emissions, water and soil contamination with emerging contaminants, such as caffeine) [2,3]. This

work aimed to determine the performance of green solvents for the extraction of coffee by-products from farms to a factory. Water was the best solvent for caffeine and chlorogenic acid from defective green coffee, while all hydrophilic eutectic solvents (ES) surpassed water in the extraction of chlorogenic acid from coffee pulp. For by-products generated inside a coffee mill, at least one ES was as good or better at extracting these compounds compared to water. Choline chlorine with lactic acid or sorbitol-based ES stood out, leading to caffeine (6.4–20.5 mg. g-1) and chlorogenic acid (56.6–64.4 mg. g-1) rich extracts. Using these solvents were either equal or similar to the greenness of neat water according to Analytical Green Calculator. The array of hydrophobic solvents tested showed different selectivity, allowing the identification of 12 compounds that have not been reported in coffee by-products. These results demonstrate the ability to obtain a new and controllable range of extracts from highly available coffee by-products using green and functional solvents.

The authors declare no conflict of interest.

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P-086 Salt the root to make it good? Growth and metabolic profile of *Scutellaria baicalensis* under NaCl treatment

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Background: *Scutellaria baicalensis* Georgi is a valuable medicinal plant of the Lamiaceae family. The roots are used in Traditional Chinese Medicine (Huangqin) and included in the European Pharmacopoeia monograph (Baikal skullcap root). Major bioactive compounds, reaching 20% of dried mass are lipophilic flavones with unsubstituted B-ring – baicalein and wogonin and their respective glucuronides – baicalin and wogonoside. However, the content of these compounds is variable and the environmental factors behind this variation remain partially unknown.

Aims: The role of these compounds in stress response was postulated, so we investigated the effect of NaCl treatment on growth and metabolic profile – phenolic compounds and amino acids.

Results: Short-term exposure to salt stress resulted in marked (30–100%) increase of baicalein, baicalin, wogonin and wogonoside in the roots. The roots grown with addition of 50–150 mM NaCl were also thicker and had higher fresh mass. Conversely, in aerial parts, the content of individual major flavonoids (scutellarein and oroxylin A glucuronides) decreased by 10–50%. The soluble amino acid profile was also altered significantly – proline, isoleucine, leucine and tryptophan increased, whereas arginine, asparagine as well as glutathione levels decreased. In conclusion, a moderate salt stress exerted a beneficial effect on root mass and pharmaceutically relevant flavonoids content in *Scutellaria baicalensis*.

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P-087 Sustainable exploitation of *Castanea sativa* L. pruning by-products as a source of bioactive compounds with anti-inflammatory activity in intestinal cells

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Agronomic practices such as pruning of a chestnut tree (*Castanea sativa* Mill., [*C. sativa*]) produce a large amount of several by-products, such as wood and buds. These wastes are an interesting source of bioactive molecules, mainly phenolics compounds, mostly phenolic acids and ellagitannins.

Chestnut pericarp and episperm have been recently identified as a rich source of proanthocyanidins and showed a significant anti-inflammatory activity in gastric epithelial cells [1]. This research aimed therefore to investigate the potential anti-inflammatory and antioxidant activities of chestnut by-products in intestinal cells. Individual ellagitannins and total phenolic compounds were analyzed as well. Hydroalcoholic (H_2O: EtOH 50:50) and aqueous extracts of *C. sativa* woods and buds were prepared. To study the anti-inflammatory effect, the extracts were assayed in an in vitro model of undifferentiated human intestinal Caco-2 cells (colonocytes) stimulated with IL-1β-IFNγ. Antioxidant activity of the extracts was evaluated through DPPH and ORAC assays.

Hydroalcoholic extracts of C. sativa woods and buds inhibited the NF-kB driven transcription and the release of CXCL-10 in a concentration-dependent manner, both induced by IL-1β-IFNγ. The aqueous and hydroalcoholic extracts exploited antioxidant activity (30–50 mmol Trolox eq/g) and contained bioactive molecules such as phenolic compounds (290–430 mg gallic acid equivalent/g) and ellagitannins.

C. sativa pruning wastes are highly rich in bioactive compounds and performed both antioxidant and anti-inflammatory activities in intestinal cells. This preliminary work contributes to the valorization of chestnut by-products and their possible recycle.

The authors declare no conflict of interest.

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P-088 Optimization of the germination of edible Mediterranean wild halophytes

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Despite the growing interest on the commercial exploitation of salt tolerant plants (halophytes), either as food or sources of bioactive products, scientific efforts for its cultivation are scarce, and there is a need to develop production systems that are economically, socially and environmentally feasible [1–3]. This work aimed to optimize the germination of 8 edible halophytes, targeting treatments to break seed dormancy (gibberellic acid, chemical and mechanical scarification, water soaking and thermal shock), substrate (perlite, vermiculite, sand, coco peat, and combinations) and irrigation salinity (freshwater and 20.1 mS/cm). Germination percentages were in the range 11.1–95.6%; *Mesembryanthemum crystallinum, Salicornia ramosissima, Inula crithmoides* and *Portulaca oleracea* had germination rates above 50%. Mean germination times (MGT) ranged from 5–28 days, with *P. oleracea* displaying the best result (5–6.9 days) with freshwater irrigation. There was no relation between best germination rate and lower MGT. Seed treatments to break dormancy in *M. nodiflorum, Medicago marina* and *Ammophila arenaria* improved their germination in 7.8, 42.2 and 35.5% compared to the control. Notwithstanding that halophytes grow in saline environments, our results indicates that the germination of some species is compromised by salinity, and also that a treatment to break seed dormancy is required. The authors declares that there is no conflict of interest.

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P-089 Optimization of the cultivation of different ecotypes of *Sarcocornia perennis* L

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Sarcocornia A. J. Scott is a halophytic edible succulent with high economic value due to its nutritional, organoleptic and medicinal properties [1]. The goal of this work was to compare the agronomic and biochemical features of *S. perennis* ecotypes cultivated in a integrated multi-trophic aquaculture (IMTA) system. Ecotypes collected in Portugal (SP1, Tavira; SP2, Faro; and SP3, Portimão) was attempted in peat, coco peat and perlite (2:2:1). The effect of cold stratification, water salinity (tap water and 20.1 mS/cm) and photoperiod (darkness and 12:12 h light/dark cycle) was tested on germination, while the effect of the irrigation salinity (20.1, 35.3, 40.3 and 49 mS/cm) was evaluated on plant growth and biochemical properties. Methanol extracts were prepared from produced biomass and evaluated for pigments, total phenolic, flavonoids and condensed tannins contents, and in vitro antioxidant properties. Germination percentages were in the range of 5.7–50%. The germination of SP1 was enhanced with cold stratification. Maximum productivity, plant survival and chlorophyll levels were reached in medium salinities. SP2 and SP3 showed the highest total phenolic compound for medium salinities. Our results indicate that *S. perennis* can be cultivated in saline conditions while maintaining its productivity and biochemical properties, which are ecotype dependent. The authors declares that there is no conflict of interest.

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P-090 Effect of N fertilization on morphological, physiological, and essential oil content in rosemary

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Rosemary (*Rosmarinus officinalis* L.) is a perennial, evergreen aromatic plant with important commercial interest as it contains numerous essential oils (composed of terpenoid compounds) and phenolic constituents (natural antioxidant compounds). The interest in rosemary cultivation has increased in recent years because of its important effects on human and animal health. In addition, rosemary is a crop species which is well adapted to dry land conditions. In addition, nitrogen (N), one of the most essential nutrients for rosemary, is often applied to increase crop yield and improve product quality. The objective of this study was to evaluate the effect of N fertilization on morphological and physiological characteristics, and on the essential oil in Rosemary. The experiment was conducted in Thessaloniki, Greece, during the growing season 2021–2022 in a field with clay loam soil. Four treatments (control, 50 kg N ha-1, 100 kg N ha-1 and 150 kg N ha-1) were tested. Nitrogen fertilization affected the Leaf Area Index (LAI) and the Normalized Difference Vegetation Index (NDVI). Also, nitrogen fertilization had a significant effect on chlorophyll content, plant height, LAI and on essential oil content. This study provides some useful information about the effect of application of N fertilization on rosemary production, thus increasing our knowledge about the effect of N on crop production. This information can be used for better N management which can be used for cost effective application of fertilizers thereby leading to higher yield. However, the physiological basis of this effect remains unknown.

P-091 Effect of N fertilization on morphological, physiological, and essential oil content in oregano

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Oregano (*Origanum vulgare* spp. *hirtum*) is an important aromatic and medicinal plant that is used worldwide because of its antimicrobial, antifungal, insecticidal, and antioxidative effects on human and animal health. Oregano is a crop species which is well adapted to dry land conditions. In addition, nitrogen (N), one of the most essential nutrients for oregano, is often applied to increase crop yield and improve product quality. The objective of this study was to evaluate the effect of N fertilization in oregano cultivation. Four treatments were tested (control, 50 kg N ha-1, 100 kg N ha-1 and 150 kg N ha-1) in an experiment conducted in Thessaloniki, Greece, during the growing season 2021–2022 in a field with clay loam soil. Several different characteristics were used to evaluate the effect of N fertilization in oregano. Nitrogen fertilization affected the Leaf Area Index (LAI) and the Normalized Difference Vegetation Index (NDVI) index. There was an increase in chlorophyll content with N application and also an increase in plant height. These results show that N application can affect the growth and yield of oregano. This study provides some useful information about the effect of application of N fertilization on oregano production and secondary metabolites, thus increasing our knowledge about the effect of N on crop production. This information can be used for better N management which can be used for cost effective application of fertilizers, thereby leading to higher yield. However, the physiological basis of this effect remains unknown.

P-092 Effect of N fertilization on morphological, physiological, and essential oil content in sage

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Sage (*Salvia officinalis* L.) is a perennial, evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. The interest in sage cultivation has increased in recent years because of its antimicrobial, antifungal, insecticidal, and antioxidative effects on human and animal health. In addition, nitrogen (N), one of the most essential nutrients for sage, is often applied to increase crop yield and improve product quality. The objective of this study was to evaluate the effect of N fertilization on morphological and physiological characteristics, and on the essential oil in sage. Four treatments were tested (control, 50 kg N ha-1, 100 kg N ha-1 and 150 kg N ha-1). The experiment was conducted in Thessaloniki, Greece, during the growing season 2021–2022 in a field with clay loam soil. Several different characteristics were used to evaluate the effect of N fertilization on chlorophyll content, quantum yield efficiency, Normalized Difference Vegetation Index (NDVI), plant height, Leaf Area Index (LAI), and essential oil yield. Nitrogen fertilization affected LAI, plant height and NDVI index. There was an increase in chlorophyll content with N application and an increase also in plant height. These results show that N application can affect the growth and yield of sage. This study provides some useful information about the effect of application of N fertilization on sage production and essential oil content, therefore increasing our knowledge about the effect of N on crop production. However, the physiological basis of this effect needs further experimentation.

P-093 Grape leaves and canes as sources of high-value bioactive compounds: chemical characterization, antioxidant, and tyrosinase-inhibitory properties

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Grapes are one of the world's most important crops. Tons of liquid and solid waste are produced from winemaking and viticultural activities. Management of by-products is essential not only for achieving sustainability, but also for developing new ingredients for the food, pharmaceutical, and cosmetic industries. In the current study, we investigated the potential of grape cane (GCE) and leaf (GLE) ethanolic extracts as multifunctional cosmetic ingredients, obtained from 5 different varieties, both Greek and international, namely "Assyrtiko", "Rhoditis", "Muscat d'Hamburg", "Syrah" and "Merlot", cultivated in Thessaly, central Greece. Their chemical characterization was performed with LC-MS, whereas the quantification of the major ingredients with HPLC-DAD. A total of 18 and 15 compounds were identified and 8 and 5 of them were quantified in GCE and GLE, respectively. Stilbenoids were the most abundant phenolics in GCE, with trans-resveratrol and (E)-viniferin dominating, whereas hydroxycinnamic acids represented by caftaric acid, and flavonols, especially quercetin glycosides, were the most abundant in GLE. Antioxidant activity was assessed with the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging and the Ferric Reducing Antioxidant Power assays. All extracts showed rich antioxidant potential, yet the highest was exerted by Syrah GLE. The skin whitening capability of GCE and GLE was investigated using a mushroom tyrosinase inhibition assay and the results showed strong inhibitory activity. Overall, our findings demonstrate that grape canes and leaves could be valorized by generating bioactive extracts used as antioxidants and skin whitening raw materials in the cosmetic industry, satisfying the increasing interest for natural active ingredients among consumers.

P-094 Countercurrent Supercritical Fluid Extraction and Fractionation of High-Added-Value Compounds from Olive oil

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Olive oil (OO), widely known for its superior organoleptic properties, is at the center of research interest due to its high content in bioactives. Among them, hydroxytyrosol (HT), tyrosol (T) and the secoiridoids oleacein (OLEA) and oleocanthal (OLEO) are the major olive oil biophenols with established beneficial effects on human health, proven by a plethora of scientific data, with antioxidant, antitumor, anti-inflammatory, antimicrobial, and cardioprotective properties [1]. Since olive oil is one of the richest sources of bioactives, there are several studies available in literature regarding different methods and approaches for their isolation. However, based on our knowledge, there is no information available for the recovery of these constituents with the use of totally green and completely environmentally friendly methodologies. The aim of the current study was to develop an eco-friendly method for producing HT, T, OLEA and OLEO enriched extracts from olive oil, by applying Supercritical Fluid Extraction (SFE) technique and using as co-solvents distilled water, food grade i-PrOH and mixtures of these solvents thereof. The parameters that were tested were the pressure and temperature of the SFE-system, the flow rate of CO_2 and co-solvents, and as a result the optimal conditions for the production of HT, T, OLEA and OLEO-enriched extracts, was highlighted. Overall, this is the first time that a green SFE-based methodology was developed and applied, for olive oil biophenols-enriched extracts production.
The authors declare no conflict of interest.

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Reference
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P-095 A green methodology for extraction and isolation of squalene as value-added product from refinery by-products

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Olive Oil by-products are at the center of commercial interest due to their high content in bioactives [1]. Till today, most of the studies have focused on

olive leaves, olive mills by-products as well as by-products produced from the debittering process of edible olives while there is only few information available about the refinery residues produced during the olive oil refining process. Specifically, various types of by-products (soap faction obtained after the neutralization treatment, deodorizing by-products, solid waste resulting after demargarinization, acid oil from chemical refining, exhausted bleaching earths after filtration), are produced in Refinery with significant scientific interest due to their bioactive content. Among them, the deodorizing by-products are a valuable source of squalene, a triterpene characterized by an abundance of beneficial effects on human health such as antioxidant activity, tumor inhibitor and skin protective properties. The aim of the present study was the isolation of squalene from deodorizing by-products and the production of squalene-enriched extracts, by applying modern extraction and isolation methodologies such as Centrifugal Partition Chromatography (CPC) and Molecular Short Path Distillation (MSPD). Overall, in the context of the present study, a green-based methodology for squalene – enriched extracts production and high-purity squalene isolation was developed, utilizing the refinery by-products produced during the olive oil deodorization process thereof.
The authors declare no conflict of interest

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P-097 Optimization of environmentally friendly *Plantago major* L ultrasound-assisted extraction

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Plantago major L. is a species of flowering plant from the plantain family Plantaginaceae native to most of Europe and northern and central Asia, but widely naturalised worldwide [1]. In this work, green solvent-based ultrasound-assisted extraction of *P. major* bioactive phenolics was investigated in two steps. In the first step, 2-level factorial design was utilized for singling out the factors that impact the extraction efficiency of total phenols (TP), total phenolic acid (TPA), aucubin (Auc), and verbascoside (Ver), as dependant variables. Their content was determined by HPLC and spectrophotometric methods. The impact of the following independent variables was analyzed: glycerol content, 2-hydroxypropyl-β-cyclodextrin concentration, temperature, extraction duration, ultrasound power, and lactic acid content. The utilized 2-level factorial design indicated that glycerol content, temperature, ultrasound power, and lactic acid content significantly impacted extraction efficiency. In the second step, Box Behnken design was applied for the fine-tuning of the significant independent variables required to attain the maximum quantity of the target compounds. Four extracts, each containing the maximum of one target response, were prepared and the content of the target compounds determined. The prepared extracts were rich in phenolic compounds. The observed values for the responses were in good accordance with the predicted ones, the deviation being lower than 5%. The results have shown that the ultrasound-assisted extraction of P. major phenolics is an acceptable green alternative to traditional organic solvent-based extraction.

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P-098 In vitro cultures of antimicrobial and antibiofilm isoquinoline alkaloids containing ornamental plant *Corydalis cheilanthifolia* Hemsl

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Corydalis cheilanthifolia Hemsl. is an ornamental perennial of Papaveraceae originating from East Asia and commonly planted in hobby and public gardens. Our previous studies demonstrated high content of several isoquinoline alkaloids and polyphenolic compounds in various parts of cultivated *C. cheilanthifolia* [1].
In this study, we established in vitro culture of this species in order to provide a model for manipulation of alkaloid and polyphenol composition towards optimal antimicrobial properties and discovery of various factors influencing metabolic profile. Also, a method for fast clonal propagation may facilitate selection of superior quality plant material for pharmaceutical use.
In vitro cultures initiated from seedlings were established in form of proliferating microshoots, callus tissue and cell suspension. Out of several tested media, the MH3 composition [2] supplemented with benzyladenine and indoleacetic acid were optimal for growth and viability optimization experiments. The presence of targeted alkaloids as well as phenolic compounds was confirmed by chromatographic analysis. The extracts were found effective against human pathogens such as *S. aureus, P. aeruginosa, C. albicans* and multidrug resistant *Helicobacter pylori* [1,3]. Further investigations to reveal the relationships between the antimicrobial properties of individual constituents and the composition and quantities of alkaloids and polyphenols in cell and tissue cultures of this species. In conclusion, a significant antimicrobial potential of *C. cheilantifolia* and the possibility of obtaining the active compounds from in vitro cultures provide a feasible model for optimization of phytochemical composition targeting specific pathogens.

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P-099 Elucidating the interplay between Nrf2 and AMPK for optimal cellular stress resistance through natural products

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Nrf2 (Nuclear factor E2 p45-related factor 2) is a transcription factor and a master regulator of antioxidant response, facilitating cellular defense against oxidative and also xenobiotic stress. AMP- activated kinase (AMPK) is one of the master hubs ensuring cellular energy homeostasis. Previous studies indicated that numerous natural products with potential health benefit, such as sulforaphane or resveratrol, can activate Nrf2 and AMPK signaling. However, it is not completely understood whether both stress hubs act in parallel or in true cooperativity and interdependence to confer cellular stress resilience. Our previous data showed among others the existence of AMPK-dependent phospho-sites on Nrf2 whose mutations to alanine mainly resulted in altered expression amplitudes of selected Nrf2 target genes1. Here we show that in a KEAP1 (the canonical inhibitor of Nrf2)-deficient background, as often found in therapy-resistant lung cancer with overactive Nrf2 signaling, these phosphorylation sites lead to accelerated decay of Nrf2. Mutation of the serines to alanines enhanced stability of Nrf2 and impeded its interaction with the ubiquitin ligase βTRcP1. The effect of the phosphorylation sites was diminished under serum-free conditions. Furthermore, a direct interaction of AMPK with Nrf2 by employing Proximity Ligation Assay (PLA) could be demonstrated. Consequently, our data strongly suggest a functional crosstalk between Nrf2 and AMPK which, however, appears to be strongly context-dependent. Further investigations are warranted to fully understand and rationally exploit the potential of dual AMPK and Nrf2 activation by natural products for specific cellular environments.

The authors declare no conflicts of interest.

Reference

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P-100 Establishment and in vitro multiplication of the medicinal Iberian endemism *Limonium algarvense* Erben

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Coastal environments are greatly affected by climate change which is increasingly threatening its unique biodiversity, such as *Limonium algarvense* Erben, an endemic halophyte species of the Southwest area of the Iberian Peninsula known for its medicinal properties [1,2]. Therefore, this work aims at establishing and developing in vitro multiplication protocols, through culturing seedling shoot tips and axillary nodes from immature inflorescences as explants, and new shoots induction by supplementation of MS medium with different combinations of growth regulators (BA, NAA, KIN, IBA). Both explant types produced new shoots, and the combination of 2.5 mg/L KIN + 0.1 mg/L NAA induced the best shoot multiplication results (8 shoots/explant) with a normal appearance and easy to separate. The second multiplication cycle enhanced new shoots formation to 14 shoots/explant. Rooting experiments are ongoing, by testing different culture media, namely MS medium alone and in combination with IBA and activated charcoal. The establishment of an in vitro propagation procedure for the rare and endemic halophyte *L. algarvense* will enable the development of ex-situ and in-situ preservation programs for this species, as well as the sustainable biotechnological exploitation for medicinal purposes aiming at the creation of innovative commercial products.

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P-101 Sustainable Exploitation of Bio-Engineered Microorganisms for the Discovery and Development of Novel Biosurfactants and Siderophores with Industrial Applications

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SECRETed project aims to exploit the potential of Systems and Synthetic Biology toolboxes and their application within aquatic biotechnology to develop novel hybrid compounds for the agrochemical, pharmaceutical, cosmetic and chemistry sectors. Biosynthetic pathways of marine and extremophilic microorganisms will be reverse engineered to infer the individual roles of their constituent genes, which will be further combined for the production of non-natural biosurfactants and siderophores with tailor-made properties. Biosurfactants are compounds with surface-active nature tendency to adsorb at interfaces, while siderophores have the ability to chelate and transport Fe3+ ions. An iterative procedure following the Design-Build-Test-Learn methodological steps (▶ **Fig. 1**) will be used to produce new microbial strains that support the selected genetic elements and satisfy sustainable industrial processing solutions for the production of biosurfactants and siderophores. The amphiphilic nature of biosurfactants and marine siderophores provides an exciting opportunity to develop methods of biosynthesis that would enable the exchange of their hydrophobic and hydrophilic chemical parts enabling the development of new-to-nature compounds.

The development of such hybrid molecules would allow the exploration of new-to-nature compounds endowed with the combination of their respective properties, to address new applications. Machine Learning algorithms, inspection of databases, and new experimental and computational-based data will be employed to build a unique microbial amphiphilic compound chemical space to identify the desired genetic mechanisms. Detected genes will be reverse engineered to standardize and modularize associated metabolic elements, with a purpose to broaden their benefits by searching for Industrial-driven formulations based on suitable microbial hosts.

Acknowledgments

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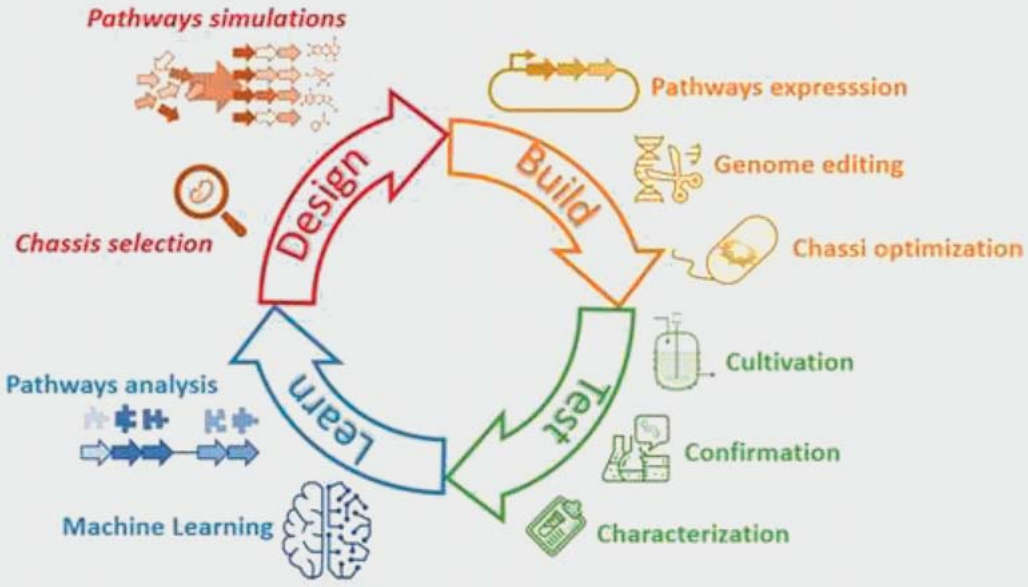

▶ **Fig. 1** The Design-Build-Test-Learn methodological approach that will be used to produce new microbial strains that satisfy sustainable industrial processing solutions for the production of new biosurfactants and siderophores.

P-102 Comparison of Catechic Tannins Contents of Some Species of *Juniperus, Prunus* and *Viburnum* Genus Growing in Turkey

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Tannins are polyphenolic, valuable plant secondary metabolites and water-soluble compounds found in many higher plants. They are divided into two groups as hydrolyzable tannins (gallotannins, ellagitannins) and condensed tannins (catechic tannins). Tannins are providing many benefits for human health. According to literature research, they have antioxidant, antimicrobial, cardioprotective, antidiabetic, antiobesity, antiinflammatory and wound healing activities [1,2]. The aim of our study is qualitative and quantitative comparison of catechin group secondary metabolite contents of the 70% methanolic extracts of *Juniperus communis, Juniperus oxycedrus* (Cupressaceae), *Prunus spinosa, Prunus divaricata* (Rosaceae), *Viburnum lantana* and *Viburnum opulus* (Caprifoliaceae) fruits grown naturally in Turkey. The analysis was performed on (+)-catechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate standard substances by using LC-MS/MS. Intersil-ODS-3 (5 µm, 4.0 mm × 250.0 mm) column was used. MeOH (100%) was as mobile phase (isocratic). Column temperature was 30 °C. Injection volume was 10 µl, flow rate was 0.8 mL/min and sample concentration was 10 mg/mL. The method was validated. While catechin was not found in extracts of *P. divaricata, P. spinosa* and *J. oxycedrus*; it was determined that these plants contain the most epigallocatechin compared to other substances. The plant containing the highest catechin and epigallocatechin was J. communis. It was found that the most abundant substance in *V. opulus* and *V. lantana* species compared to other compounds was catechin. All results are given at below (▶ **Table 1**). These species can be used as nutraceutical or functional food due to their tannins content.

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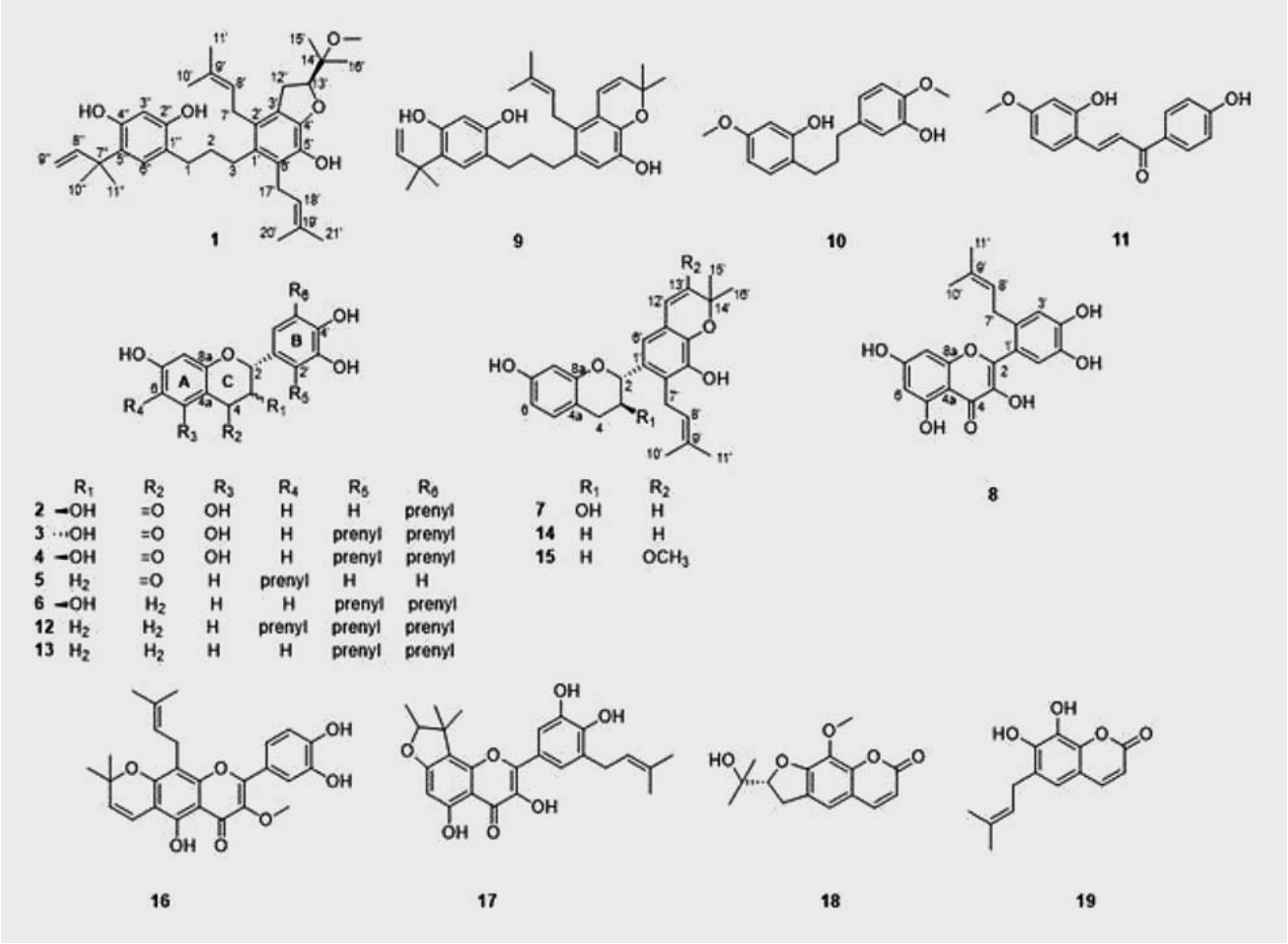
▶ **Table 1** The catechic tannins contents of extracts.

Extracts (70% MeOH) (10 mg/ml)	(+)-catechin (ng/ml)	(−)-epigallocatechin (ng/ml)	(−)-epigallocatechin gallate (ng/ml)	(−)-epicatechin gallate (ng/ml)
Prunus divaricata	–	17.6 ± 0.65	5.3 ± 1.02	10.2 ± 0.64
Prunus spinosa	–	15.1 ± 0.56	4.2 ± 0.19	9.6 ± 1.38
Viburnum opulus	293 ± 5.29	16.0 ± 2.31	19.8 ± 0.49	7.9 ± 0.22
Viburnum lantana	328.7 ± 0.58	128.3 ± 3.06	2.9 ± 0.07	8.5 ± 0.31
Juniperus oxycedrus	–	20.3 ± 2.01	3.6 ± 0.10	8.9 ± 0.25
Juniperus communis	1173.3 ± 5.77	208 ± 7.21	3 ± 0.14	9.2 ± 0.52



▶ **Fig. 1**

P-103 Antiosteoclastogenic active compounds from the roots of *Broussonetia kazonoki*

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DOI 10.1055/s-0042-1759083

To obtain antiosteoclastogenic active compounds from the natural sources, 19 compounds were isolated from the roots of *Broussonetia kazinoki* Siebold. We also report the isolation and structure determination of broussonol I from a natural source. The chemical structure of the isolated compounds was determined using conventional NMR and MASS data. Absolute configurations were assigned using time-dependent density functional theory calculations and Electronic Circular Dichroism (ECD) spectroscopy. The isolated 19 compounds (▶ **Fig. 1**) were screened for their effects on RANKL-induced osteoclast formation using RAW264.7 cells. Of them, broussonols F, G, and K showed dose-dependent antiosteoclastogenic activities with IC_{50} value of 6.33–14.13 μM against BMMs cells. Broussonol K exhibited the most potent inhibitory activity for osteoclast formation and possessed bone resorption suppressive activity.

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P-104 Main non-flavonoid constituent of birch leaves water extract – 3-hydroxy-1-(4-hydroxyphenyl)-propan-1-one 3-O-β-D-glucoside: isolation, permeability, and antiadhesive activity

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Birch leaves (*Betula pendula* Roth, *Betula pubescens* Ehrh.) is a plant material traditionally used in gout, rheumatism, arthritis, and urinary tract conditions. The main constituents of the birch leaf water infusion are flavonoids, phenolic acids, and glycosides. In previous reports, the diuretic activity of flavonoid-free birch leaves extract was confirmed [1]. The most abundant non-flavonoid constituent of the extract is 3-hydroxy-1-(4-hydroxyphenyl)-propan-1-one 3-O-β-D-glucoside (3,4′-DHPPG) [2]. The aim of the research was to isolate this compound from birch leaves extract and assess its permeability and antiadhesive activity.

The preparative HPLC was used for direct isolation of 3,4′-DHPPG from raw water infusion (yield 1.8% of dry weight). The obtained compound had 95.67% purity (HPLC). The antiadhesive activity of the compound was assessed by incubating the compound, FITC-labelled uropathogenic *E. coli* (NU14), and T24-bladder cells forwarded with flow-cytometry analysis. The antiadhesive activity was observed for 100 μM of 3,4′-DHPPG. The transport

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experiments were performed using Caco-2 monolayers with both raw extract and isolated compound solutions. In both modes, the significant permeability of 3,4′-DHPPG was observed.

The highest described isolation yield of 3,4′-DHPPG from a natural source was obtained [3]. Its antiadhesive activity can contribute to birch leaves beneficial properties considering the treatment of urinary tract infections. Based on the permeability experiments, it might be concluded that 3,4′-DHPPG is the most permeable constituent of the raw extract.

The authors declare no conflict of interest. The presented research was financially supported by the NCN research grant OPUS15 No. 2018/29/B/NZ7/01873 and the MUW Scholarship No. 06/F/MBM/21.

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P-105 Rationalized development of antiadhesive natural products against *Campylobacter jejuni*: molecular structure and antiadhesive activity of chitosans

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DOI 10.1055/s-0042-1759085

Infections caused by the food-borne bacteria *Campylobacter* are main causes of acute bacterial gastroenteritis worldwide [1]. Campylobacteriosis can range from mild symptoms to fatal illness. Multifactorial prevention strategies should be implemented to reduce prevalence of *Campylobacter* in the food chain. Especially antiadhesive strategies, inhibiting early host-pathogen interaction, are an innovative concept for reducing Campylobacter bacterial load in feedstock production [1].

During in vitro screening of natural compounds for antiadhesive activity against *C. jejuni* by flow cytometry of fluorescence labelled *C. jejuni* (DSM27585) on CaCo-2 epithelial cells, significant anti-adhesive effects were found for chitosans (poly-β-1,4-glucosamine, partially acetylated). Detailed structure-activity investigations using chitosans with varying degree of polymerization (DP) and different degrees of acetylation (DA) were performed (see ▶ **Fig. 1**). Data indicate that a high DP and a medium-range DA can be correlated with a strong anti-adhesive activity at a concentration of about 100 µg/mL. Antiproliferative activity of the chitosans tested against *C. jejuni* was observed only at higher concentrations (> 1 mg/mL). Vitality of the host cells is not influenced negatively by the selected chitosans at the concentrations tested. The reduced bacterial adhesion was also verified by advanced fluorescence and confocal microscopy. For identification of the potential molecular targets of the antiadhesive chitosan, *C. jejuni* whole protein lysates of chitosan-treated bacteria were investigated by 2D-gels, followed by MS-based proteome analysis.

The data described here provide a rationalized basis for further development of optimized chitosan as antiadhesive polysaccharide against *C. jejuni* and use in food technology and food processing.

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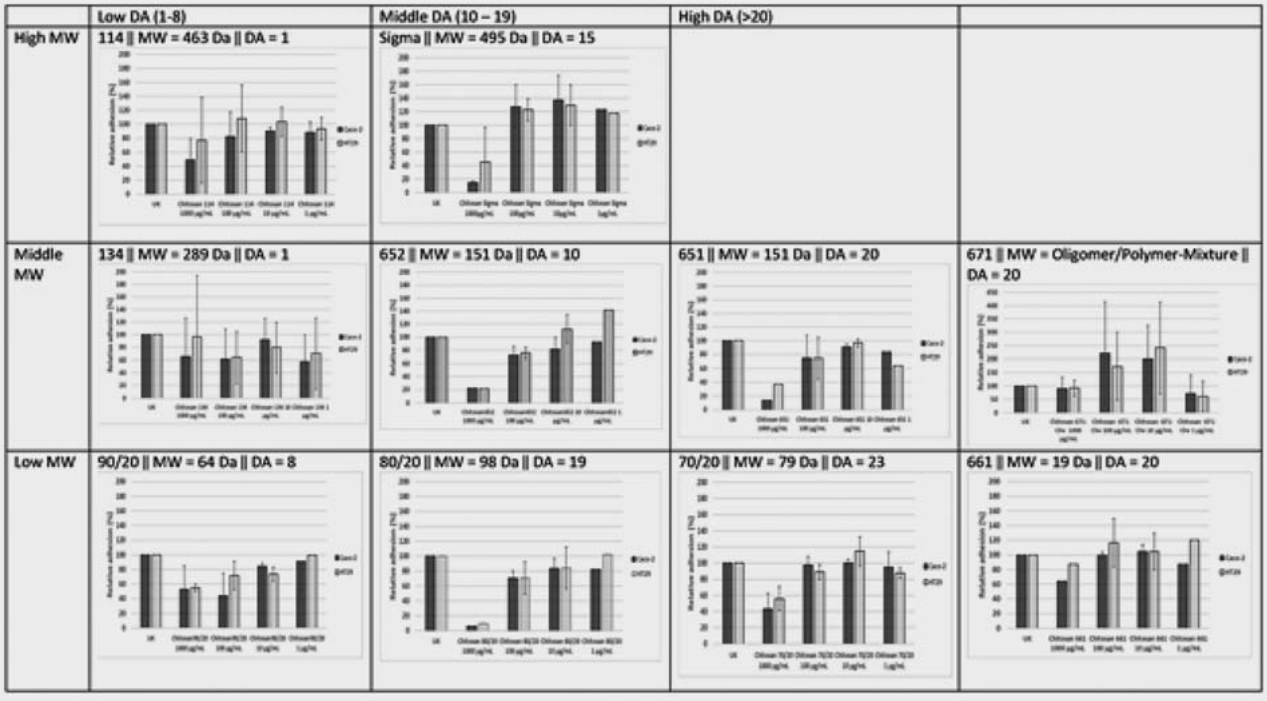

▶ **Fig. 1** Antiadhesive activity of different chitosans against *C. jejuni* on Coco-2 and HT-29 cells in flow cytometric assay.

P-106 Evaluation the enzyme inhibitory properties of Commandaria grape pomace extracts associated with hyperglycemia

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DOI 10.1055/s-0042-1759086

The grape pomace contains plethora of polyphenols as phenolic acids, flavanoids, anthocyanins, tannins and stilbenes, which are linked with diverse health effects. Many studies manifest that grape phenolics exert potent antihyperglycemic effects. The α-amylase and α-glucosidase inhibitory effects are considered as a possible mechanism of their action. Commandaria is a dessert wine, which is produced by wine-making sun-dried grapes. Thus, its pomace comprises high concentration of polyphenols due to dehydration. The objective of this work was to evaluate grape pomace phenolics as potential enzyme inhibitors related with hyperglycemia. Therefore, the effect of extraction systems (methanol 100%, methanol-water 80–20%, methanol-water 60–40%, ethanol 100%, ethanol-water 80–20%, ethanol-water 60–40%, acetone, acetone-water 80–20%, and acetone-water 60–40%) on the bioactive composition and enzyme inhibitory properties was studied.

Results demonstrated that ethanolic and hydroethanolic grape pomace extracts had the highest phenolic and hydroxycinnamate contents followed by methanolic and hydromethanolic extracts. Acetone and its aqueous mixtures also recover higher amounts of flavanols compared to alcohols. Furthermore, results showed the α-amylase and α-glucosidase inhibitory activities of Commandaria grape pomace extracts were strongly affected by extraction system. The a-amylase and a-glucosidase inhibitory effects (IC_{50}) ranged from 19.1 ± 0.8 to 27.6 ± 1.4 μg · mL^{-1} and from 70.4 ± 4.9 to 88.9 ± 6.7 μg · mL^{-1}, respectively. The findings suggest the use of pure alcohols and methanol-water (80–20%) to prepare extracts with potent enzyme inhibitory activities.

Overall, the current study highlights the Commandaria grape pomace as inhibitors of α-glucosidase and α-amylase and provides a knowledge-base for the selection of the most appropriate solvents to produce antihyperglycemic extracts.

P-107 Antioxidant activity and GC-MS analysis of the components of aqueous leaf extract of *Spathodea campanulata*

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DOI 10.1055/s-0042-1759087

The antioxidant capacity helps reduce the pro-oxidative state inflicted by reactive oxygen species (ROS) produced either from natural cell metabolisms or from external sources and increased in different chronic and degenerative diseases. Antioxidant compounds have received attention in recent years, particularly from medicinal plants. Several studies reported the bioactive compounds present in plants called phytochemicals produced in the secondary plant metabolism. Aim: To evaluate the antioxidant capacity and evaluate the main components in the aqueous extract. Material and Methods. Aqueous extract of leaves of *Spathodea campanulata* (AQESCL). The antioxidant activity was evaluated using the 1-1-dyphenyl-2-picrylhydrazyl (DPPH) method (Bonet and Brand-Williams, 1995). To identify the components present a gas chromatography-mass spectrometry on Agilent 7000C Triple Quad w/7890B GC/MS/MS system with an Agilent J&W GC Column was performed. Results. The AQELSC presented an EC_{50} of 70.86 ± 9.67 g/Kg DPPH, with a Specific activity of 7.84 X 10−3 representing high antiradical efficiency. GC-MS analysis revealed the presence of 36 constituents. The major components present in the aqueous extracts were: 9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol: 14.97%, Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene-: 11.13%, 2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3-ol, 14.19%, 1,5-Hexadien-3-ol, 3-methyl-6-(methylthio)-1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E,E)-: 6.77, and 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one 6.59, giving 53.95% of the components found. They have been related to different functions, including reducing oxidative stress. Conclusion. The AQESCL present high antioxidant activity due to the secondary metabolites present.

Acknowledgements
CIC-UMSNH-2022 for the partial support.
Keywords: Antioxidant activity, aqueous extract, GC-MS Analysis, Spathodea campanulata.

P-108 Nrf2 and VCAM-1 regulation may play a role in the anti-inflammatory mechanism of xylopic acid

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DOI 10.1055/s-0042-1759088

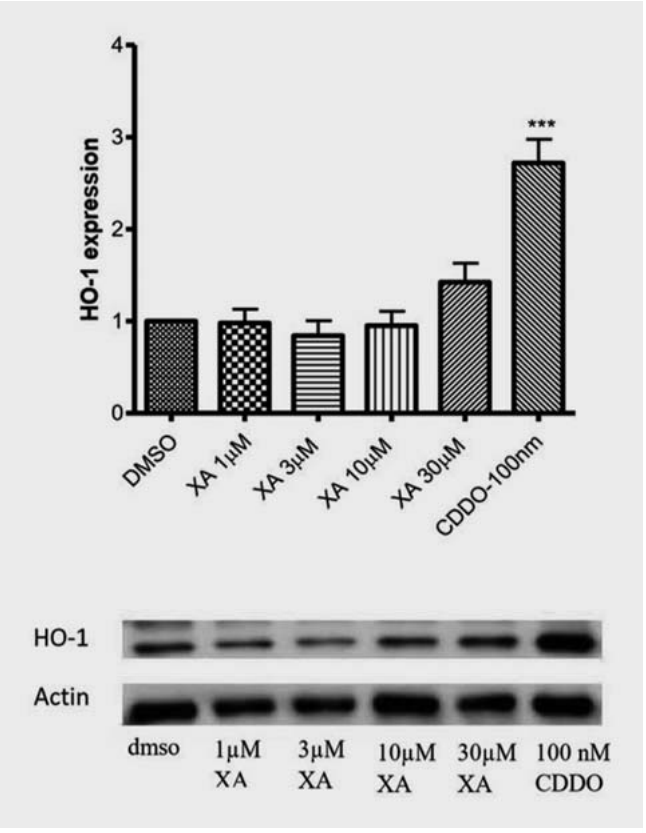
Background: Xylopic acid (XA) is a kaurene diterpene which naturally exists in African plants such as *Xylopia aethiopica* [1]. It has been documented to exhibit acute and chronic anti-inflammatory activities [2].

Aim: The current work sets out to identify the potential molecular inflammation target(s) of xylopic acid.

Methods: Selection of targets was based on the proven bioactivity of XA as potential anti-inflammatory agent using in silico prediction tool named SPiDER [3]. Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling and protein expression of Nrf2 target gene, heme oxygenase-1 (HO-1), were investigated using luciferase reporter gene assay and human umbilical vein endothelial cells (HUVEC) respectively, while effect on vascular cell adhesion molecule-1 (VCAM-1) expression was studied using HUVEC-tert cell line.

Results: XA showed enhanced activation of Nrf2 in a concentration dependent manner and slightly increased HO-1 protein expression levels. Expression of VCAM-1 was reduced to 70% in XA-treated cells.

Conclusion: XA exhibits its anti-inflammatory action via regulation of VCAM-1 and Nrf2 expression.



▶ **Fig. 1** Effect of xylopic acid on HO-1 protein expression levels in HUVEC cells. Values are mean ± SEM, n = 3. *** $p < 0.001$ compared to the control.

Planta Med 2022; 88: 1385–1588

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P-109 Hypoglycemic activity of leaves and flowers of *Spathodea campanulata* in diabetic type 2 rat model

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DOI 10.1055/s-0042-1759089
Plants synthesize a large variety of secondary metabolites, many with biological activities such as hypoglycemic activity, so they have been used as an alternative and coadjutants in the treatments against Diabetes mellitus. The objective of this work was to evaluate the hypoglycemic effect of Spathodea campanulata leaves and flowers aqueous extracts in a rat type 2 diabetes mellitus model. Material and methods: Aqueous extract of *S. campanulata* leaves (AQESCL) and flowers (AQESCF) (5 to 5000 mg/Kg). Acute evaluation of the hypoglycemic capacity of aqueous extract using glucose tolerance curves in type 2 diabetic male Wistar rats (from 100 mg) randomly distributed in the following groups (n = 6): Healthy controls, Diabetics with saline solution, Diabetics with AQESCL or AQESCF and Diabetics with Metformin; the induction of diabetes mellitus type 2 was performed by oral administration of 60% fructose. Results. The hypoglycemic activity for AQESCL and AQESCF respectively for the different doses analyzed where as follows: 5 mg/kg: 40.09 and 41.03%; 25 mg/kg: 29.74% and 63.03; 50 mg/kg: 30.72 and 83.78%, 150 mg/kg of 53.23 and 70.05%, 300 mg/kg of 86% and 10.91%, 2000 mg/kg of 23.36% and 130.15% 5000 mg/kg of 24.32 and 193%, in addition, as controls, the rats treated with saline showed a reduction of 0% (negative control) and those treated with metformin as positive control (100%). Conclusion. The optimal hypoglycemic dose of the aqueous extract for AQESCL was 300 mg/kg and for AQESCF was 50 mg/kg.

P-110 Flavonoids and xanthones from *Maclura cochinchinensis* (Lour.) Corner. and their antibacterial activity

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DOI 10.1055/s-0042-1759090
Maclura cochinchinensis (Lour.) Corner. has been used widely in traditional medicine, especially for the treatment of rheumatism, hepatitis, and neuralgia [1]. Previous phytochemical investigations of *M. cochinchinensis* resulted in the identification of a number of isoflavones, flavanones, and xanthones; many of these compounds exhibited interesting biological activities [1,2]. As part of an ongoing investigation for bioactive compounds from Thai medicinal plants, the fruit and leaf extracts of *M. cochinchinensis* were examined and these showed good antimicrobial activities with minimum inhibitory concentration (MIC) values in the range of 10–160 µg/mL. This prompted us to further investigate their phytochemicals and antimicrobial activities. The leaf and fruit extracts of *M. cochinchinensis* (Lour.) Corner were separately subjected to silica gel column chromatography to afford four new isoflavones, one new flavone, and 24 known compounds [3]. The structures of the new compounds were characterized by spectroscopic methods and mass spectrometry. All new isoflavones were 5,7,4′-oxygenated isoflavones containing a modified isoprenyl unit at C-8 except for macluracochinone A has a modified isoprenyl unit at C-6. A new flavone was a 2,5,7, 4′-oxygenated flavone, which contained an oxyisoprenyl unit at C-7 and an isoprenyl unit C-5'. All known compounds were eighteen isoflavones, one flavone, one flavanone, and four xanthones. Most of these compounds had one or two isoprenyl units. The antimicrobial activities of 12 of these compounds were evaluated. Of these, gancaonin M, lupiwighteone, lupalbigenin, warangalone, auriculatin, and millexatin F displayed good antibacterial activities against Gram-positive bacteria with MIC values in the range of 1–8 µg/mL [3].
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P-111 Asiatic acid inhibits pro-oxidant mediators-induced oxidative stress in human aortic endothelial cells

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Oxidative stress, characterized by an imbalance between the synthesis of pro-oxidant molecules and antioxidant mechanisms in cells, is a hallmark of early atherosclerosis. Hence, inhibition of oxidative stress could be a promising approach in prevention of atherosclerosis and the associated cardiovascular diseases. Asiatic acid is a major terpenoid isolated from *Centella asiatica* L. Urban and exhibits pharmacological activities including anti-hypertensive, cardioprotective and anti-hyperlipidemic activities [1]. However, the knowledge gap on how asiatic acid acts on oxidative stress that occurs in the endothelium remains unaddressed. The objective of this study was to assess the effect of asiatic acid on tumor necrosis factor alpha (TNF-α)- or hydrogen peroxide (H_2O_2)-stimulated oxidative stress using human aortic endothelial cells. Intracellular reactive oxygen species (ROS) levels and the activity of catalase (CAT) were evaluated. The protein expression of p47phox, which is a subunit of NADPH oxidases (No X) that generates ROS, was also assessed using Western blot analysis. The results showed that 40 µM of asiatic acid inhibited TNF-α-induced increased ROS release. Asiatic acid, at 10–40 µM, was found to prevent reduced CAT activity elicited by H_2O_2. Our optimization data also demonstrated that 10 ng/mL of TNF-α upregulated p47phox expression maximally at 1 h. Yet, asiatic acid did not suppress the increased p47phox expression. These findings indicate that asiatic acid alleviates endothelial oxidative stress by decreasing ROS production and enhancing CAT activity. But the exact Nox subunit which attributes to antioxidant effect of asiatic acid warrants further investigations. The authors declared no conflict of interest.
Funding
This work was funded under Fundamental Research Grant Scheme (FRGS/1/2018/SKK06/UTAR/02/7) by the Ministry of Higher Education, Malaysia
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▶ **Table 1** Cytotoxicity evaluation and antileishmanial activity against *Leishmania* spp. promastigotes.

Compound	CC_{50} (µg/ml) ± SD	IC_{50} (µg/ml) ± SD *L. infantum*			IC_{50} (µg/ml) ± SD *L. major*		
	J774A.1	promastigotes	amastigotes	SI	promastigotes	amastigotes	SI
TPF	270.2 ± 8.1	1186.5 ± 45.8	207 ± 6.6	> 1	976 ± 21.6	142.3 ± 28.2	> 1
Miltefosine	28.5 ± 3.7	2.5 ± 0.2	1.6 ± 0.8	> 1	3.4·± 0.3	2.4 ± 0.2	> 1

P-112 Antileishmanial potential of a total phenolic fraction rich in hydroxytyrosol and tyrosol and its additive interaction with miltefosine against *Leishmania*

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Leishmaniasis is a major public health problem, caused by protozoa of the genus Leishmania, with a wide spectrum of clinical manifestations. Chemotherapies include old drugs with drawbacks such as toxicity, high cost and emerging resistance [1]. Thus, the development of new therapeutics is focused on combination therapies and new formulations. Previous studies demonstrated that total phenolic fraction (TPF) derived from extra virgin olive oil, exhibits antileishmanial activity. Present study aims to evaluate the leishmanicidal activity of a TPF extract, especially rich in hydroxytyrosol (HT) and tyrosol (Tyr) biophenols, in combination with miltefosine, the standard antileishmanial drug, against L. infantum and L. major extracellular promastigotes and intracellular amastigotes. Its qualitative and quantitative analysis revealed a content of 7 and 42 mg/g of extract for HT and Tyr, respectively. TPF caused a concentration-dependent inhibition on promastigote and amastigote viability, at 72 h after treatment, for both strains. The half maximal inhibitory and cytotoxic concentrations (IC_{50} and CC50 values) of TPF and miltefosine were estimated using the resazurin cell viability assay (▶ **Table 1**). TPF (IC_{50}) inhibited parasite proliferation and triggered a significant enhancement of intracellular ROS levels in L. infantum and L. major promastigotes after incubation for 72 h, as it was demonstrated by increased fluorescence intensity compared to untreated parasites. Its antileishmanial effect in combination with miltefosine, was determined on promastigotes of both strains and isobolograms revealed additive effects. Our overall results strongly suggest that TPF rich in HT and Tyr active molecules, is a powerful inhibitory factor against viscerotropic and dermotropic *Leishmania* strains.

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P-113 Study of selected Kazakhstani sage extracts

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Plants of sage genus (*Salvia* L.) have been extensively used in folk medicine for the relief of pain, protecting the body against oxidative stress, free radical damages, angiogenesis, inflammation, bacterial and virus infection [1]. Polyphenolic compounds, essential oils and polysaccharides are traditionally considered responsible for the pharmacological action of sage [2,3]. In Kazakhstan, two species, namely desert and clary sage are the most notable, however, scarely studied.

As a result of our scientific-research work (under the project AP09563397, MES RK, 2021), the quality of plant material, optimal technologies for obtaining substances using conventional (maceration) and ultrasound-assisted extraction, component analysis together with storage regime were jointly established for the first time. Tests for extract with higher immunomodulatory activity for acute toxicity, phagocytic activity of macrophages, analysis using flow cytometry, determination of levels of cytokines and antibodies, histomorphological analysis have been carried out. The hydrophobic fraction, fractions of condensed tannins and polysaccharides were isolated, and their composition was determined.

It was established in particular that the maximum yield of a complex of biologically active compounds (extract) from clary sage by maceration was achieved when grinded (3 mm) plant material was extracted with 50% ethyl alcohol at a ratio of the selected extragent to the raw material equal to 1 : 10 within 24 hours. Based on the data obtained in vitro studies, it was shown that even at higher concentrations, sage extracts do not cause a significant toxic effect on human fibroblasts. Two of the extracts are suitable for preclinical tests on inflammation.

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P-114 Study of enzymatic changes in model object *Brachypodium distachyon* as part of its non-specific resistance to leaf rust

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According to CIMMYT, wheat provides 20% of the daily protein for 4.5 billion people. By 2050 the demand for wheat is expected to increase by 60%, which requires about 1.6% of annual wheat yield increase. At the same time, about 40% of food crops are annually lost due to plant pests and diseases. One of the key pathogens affecting wheat in Kazakhstan is leaf rust. With severe damage to plants, in the ears, fewer grains are formed; they are of low quality, lightweight, which is the main cause of the crop shortage (up to 40–60% in the years of epiphytothies).

In our research we used inbred lines of the wild wheat relative *Brachypodium distachyon*: Bd21, Bd 3–1, Bd 1–1 for a comparative study of non-specific resistance of the model plant to leaf rust. In particular our studies have shown that on the 3rd day after infection of plants of the Bd21 line, peroxidase (one of the most important catalysts among biotic factors protecting plants from phytopathogens) activity increases by 50%, Bd3-1 by 35%, Bd1-1 by 16.8% relative to the control, which is possibly associated with an increase in the content of H2O2 in the cells and non-specific response of plants to infection. Another interesting finding was related to inhibition of XDH activity possibly related to

increased resistance of Bd21. Some anatomical changes have also been observed, including increased indices of internal structures associated with a protective response to the pathogen damage. Research was funded by AP05 134 104 project (MES RK).

P-115 Comparative in vitro study of immunomodulatory activity of two extracts from the aerial part of *Limonium gmelinii*

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Limonium (L.) *gmelinii* (Willd.) Kuntze is a valuable industrially significant Kazakhstani plant. Its extracts and isolated compounds were proven to display antioxidant, hepatoprotective, antimicrobial, antimutagenic, antitumor, antiviral [1,2]; antitrypanosomal [3] and neuroprotective effects [4], which may indicate of potential immunomodulatory activity. Extracts obtained by conventional (maceration; CE) and ultrasound-assisted extraction (UAE) with 50% aqueous ethanol from the aerial part of *L. gmelinii* were compared by their yield, total phenolic and flavonoid content and in vitro immunomodulatory activity. Extract 1 (obtained by UAE) and Extract 2 (obtained by CE) differed slightly by their yield, total phenolic and flavonoid content: 30.25 ± 1.6% and 33.51 ± 2.4%; 378.1 ± 4.5 and 382.2 ± 3.3 mg GAE/g DW; 90.22 ± 2.8 and 94.61 ± 1.9 mg QE/g DW, respectively. ELISA showed that both extracts stimulated cytokines production in LPS-activated and inhibited them in non-activated murine macrophages and lymphocytes. Maximum stimulatory and inhibitory effect on macrophages was at a concentration of 10 µg/mL and 1 mg/mL, and 100 µg/mL for both extracts, respectively. The maximum stimulatory effect on IL-6 and TNF-α production by non-activated lymphocytes was found at 5 µg/mL and 1 µg/mL, respectively. Suppression of TNF-α was observed at all dosages; of IL-6 at 100 µg/mL. The proliferation assay demonstrated stronger suppressive effect of Extract 2; both preparations had a stimulatory effect on the inactivated lymphocytes. *L. gmelinii* extracts obtained by UAE and CE had similar immunomodulatory activity. The data obtained gives basis for further research into the mechanisms of this activity and active compounds responsible for it.

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P-116 Phytochemical profiles and Bioactivity of *Nepeta phyllochlamys* P. H. Davis

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There are about 275 taxa of *Nepeta* L. in the world [1]. In Turkey, *Nepeta* includes 45 taxa, 18 of which are endemic [2]. *Nepeta* species are widely used in folk medicine because of their antispasmodic, diuretic, antiseptic, antitussive, antiasthmatic, and febrifuge activities [3]. *Nepeta phyllochlamys* P. H. Davis is a local endemic in Antalya province, Turkey. This study aimed to in vitro antioxidant and antimicrobial activity of extracts prepared with different solvents (n-hexane, ethyl alcohol 70%, and ethyl acetate) of *N. phyllochlamys*. The antioxidant activities of the extracts of N. *phyllochlamys* were determined by the DPPH• scavenging activity assay. Furthermore, the composition of hydrodistilled essential oil (yield 0.7%.) of *N. phyllochlamys* was determined by GC/GC-MS analysis. 70% ethanol extract of *N. phyllochlamys* showed higher antioxidant activity (IC_{50}: 0.32 ± 0.025 mg/ml). However, all extracts of the *N. phyllochlamys* demonstrated weaker antioxidant activity than the standard gallic acid.
According to the GC-MS analysis, the major components were determined as caryophyllene oxide (%18.2), β-pinene (%15.6) and linalool (%11.0).
N. phyllochlamys extracts were evaluated against four bacterial and five candidal reference strains. The extracts showed weak activity against all bacterial strains in the MIC range of 2000–8000 µg/mL. It was determined that the lowest MIC value (125 µg/mL) and the highest anticandidal effect were in n-hexane extract against *C. tropicalis* (ATCC750).

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P-117 Role of calcium in the Eugenol-mediated reduction of Tobramycin resistance in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa infections are difficult to treat because the pathogen is resistant against a multitude of antibiotics including tobramycin. Tobramycin resistance is increased by Ca2+ induced transcription of efflux transporters [1] but can be abrogated by eugenol [2]. We investigated the influence of eugenol and Ca2+ on tobramycin resistance of P. aeruginosa. Minimum inhibitory concentrations (MIC) of tobramycin and eugenol against resistant *P. aeruginosa* isolates (including synergistic concentrations) were investigated by checkerboard microdilution. Eugenol reduced tobramycin resistance of *P. aeruginosa*. The resistance-modification was specifically reversed by calcium. The inhibition of eugenol-mediated resistance modification by Ca2+ provides evidence that eugenol disrupts Ca2+ homeostasis, which is essential for tobramycin efflux, as shown earlier [3]. The role of Ca2+ in the resistance-modifying activity of eugenol was further confirmed by complexing with EDTA. Results indicate that calcium plays a critical role in the reduction of *P. aeruginosa* tobramycin resistance by eugenol.

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P-118 Marshmallow and its action on inflamed 3D skin model mimicking atopic dermatitis

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The root of marshmallow (*Althaea officinalis* L.) is commonly known for its anti-inflammatory action and used traditionally in different inflammatory conditions like sore throat. Furthermore, there are some hints of its traditional, topical usage on inflamed skin conditions [1,2]. The aim of this study was to investigate the influence of marshmallow root extract on the gene expression of proinflammatory cytokines and further markers on active AD lesions by using a 3D skin model for atopic dermatitis (AD) [3].

The AD-like 3D skin model was obtained by systematic treatment of a reconstructed human epidermis (RHE) with a proinflammatory cytokine mix (IL-22, TNFa, IL-4, and IL-13). This proinflammatory cytokine treatment led to an upregulation of cytokines related to AD (CXCL1, IL-8. CCL-5, CCL-27), and increased the gene expression of AD related antimicrobial peptides such as human β-defensin-2 and S100A2. This inflamed, AD-like skin model was then used to study the anti-inflammatory effect of marshmallow root extract. The topical treatment of the inflamed RHE with the marshmallow extract reduced dose dependently the expression of the cytokines CXCL1, IL-8. CCL-5 and CCL-27 in comparison to the control. The upregulation of human β-defensin-2 and S100A2 in the inflamed condition was also reduced after the topical treatment with marshmallow.

These data support the traditional use of marshmallow in skin health, demonstrating its anti-inflammatory action after topical application.

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P-119 Identification and structural elucidation of anti-inflammatory stilbene oligomers and flavonoids from the subaerial parts of *Scirpoides holoschoenus*

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The plant *Scirpoides holoschoenus* (Linnaeus) Soják (syn. *Scirpus holoschoenus*) (Cyperaceae) is native to Mediterranean and Asian countries and spreads to the western Himalaya. The subaerial plant material is used at the shores of the Danube delta as a traditional remedy to treat liver disorders [1,2]. Up to now, the phytochemistry and bioactivity of *S. holoschoenus* has been poorly investigated [3,4]. Phytochemical investigations of dichloromethane and methanol extracts of roots and rhizomes of *S. holoschoenus* afforded 19 stilbenes, six flavonoids, six ferulic acid derivatives and four diterpenes, which have not been reported as constituents of this species. Among these constituents, ten derivatives represent previously unreported natural products. Structure elucidation was performed by HRESI-MS, NMR, GC-MS, and ECD data evaluation. The stilbene dimer trans-scirpusin B and trimer cyperusphenol B showed promising inhibitory activities of NO production in LPS-stimulated J774A.1 murine macrophages in a concentration-dependent manner (IC_{50} < 15 µM). Time-course experiments (3–20 h) in LPS-stimulated RAW 264.7 and THP-1 macrophages identified diprenylated flavonoids (euchestraflavanone A, gancaonin E and 2R-macarangaflavanone B) as highly active anti-inflammatory constituents. They attenuated the transcription levels of pro-inflammatory cytokines IL-1β and IL-6, measured by RT-qPCR, ELISA and Western blot analysis at concentrations of 0.1–5 µg/ml. The data obtained are the first results confirming the anti-inflammatory potential of S. holoschoenus and rationalize the traditional use of this plant to treat inflammation related disorders in the Danube delta.

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P-120 Development of a fluorogenic matrix metalloproteinase 9 screening assays (MMP-9)

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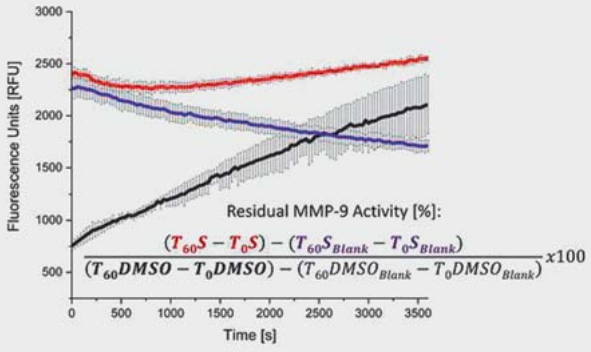
Matrix metalloproteinase 9, is a member of the MMP family of transmembrane zinc-dependent endopeptidase enzymes responsible for both physiological and pathophysiological tissue remodeling. In pathophysiological conditions, MMP-9 is upregulated and plays a key role in promoting the progres-



▶ **Fig. 1** Calculation of the fluorescence increase. The fluorescence pattern of the thest compound without enzyme and substrate must be considered in the assay (purple curve). The fluorescence profile of the DMSO blank without enzyme and substrate is almost constant during the assay.

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sion of various disease pathologies, including disorders during wound healing and inflammatory processes, such as arthritis, diabetes, and cancer. Subsequently, MMP-9 is an important target for inhibitor screening and led to an increased research interest in natural MMP-9 inhibitors, with a frequent focus on polyphenols [1,2]. A broad range of different substrate and enzyme types and concentrations is described in the literature, using a fluorescence detection mode. Fluorescence measurement by assessing its increase after preincubation with a test compound is basically trivial and a successful technique, yet our recent findings show that this detection mode may have some pitfalls. Natural products containing high intrinsic fluorescence may lead to unreliable inhibition constants of polyphenols such as caffeates and stilbene monomers, which can be classified as false positive inhibitors. In this study, we investigated the inhibitory potential of 32 Vietnames extracts and 37 natural products, including compounds previously reported as MMP-9 inhibitors, at a test concentration of 30 µg/ml and 25 µM, respectively. IC_{50} values of cyperusphenol B (15.8 µM), cyperusphenol D (18.3 µM) and porphyra-334 (28.0 µM) were determined by concentration-response curves and their inhibitory effects were confirmed by molecular docking experiments. These results will aid in the search for new natural MMP-9 inhibitors and contribute to a better understanding of the screening assay.

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P-121 Recycling Antibiotics with Natural Substances as Adjuvants using *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as Examples

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The development of resistance to antibiotics in bacterial pathogens is a threatening circumstance worldwide. The resulting need for effective therapeutics cannot be met by antibiotics alone [1]. One strategy to counter this threat is to combine antibiotics to which resistance already exists with natural products that enhance the antibiotic activity through resistance-modifying properties.

The purpose of this study was to increase the efficacy of the antibiotics by combining them with natural substances, so that even resistant bacteria react sensitively again. The resistance-decreasing effects of clove and cinnamon oil as well as lysozyme with carbapenem antibiotics (imipenem) and aminoglycosides (gentamicin) against the bacterial pathogens *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were investigated using checkerboard microdilution methods.

The essential oil of either plant species combined with lysozyme significantly increased the antibiotic effect. The minimum inhibitory concentrations (MICs) of gentamicin and imipenem against multidrug-resistant clinical isolates of the two Gram-negative bacterial species were reduced. For example, the MIC of gentamicin in a *P. aeruginosa* isolate was reduced from 13 107 (± 4487) mg/L to 32 mg/L by adding cinnamon oil and lysozyme (FICI 0.27).

The results show that the phenylpropanoid derivatives in both essential oils, in combination with lysozyme, are suitable for the development of new resistance-modifying substances against bacterial infections. Further work on resistance modification by these agents focuses on the analysis of as many clinical bacterial isolates as possible. In addition, the resistance-modifying properties could be further improved through chemical modification.

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P-122 Estrogenic activity of isoflavones derived from *Derris scandens* using MCF-7 cell

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Derris scandens (Hog Creeper Vine), commonly known as "Thao Wan Priang" in Thai, is a woody climbing vine in the Fabaceae family. This herb possesses analgesic and anti-inflammatory effects [1]. Isoflavonoids and their prenylated derivatives are the major compounds found in D. scandens. The chemical markers for this plant are genistein-7-O- [-rhamnopyranosyl-(1 to 6)-glucopyranoside] (GTG) and genistein, according to the Thai Herbal Pharmacopoeia (THP). Isoflavonoids are phytoestrogens present in fabaceous plants that can act as estrogen replacements [2]. Therefore, this study aimed to evaluate the estrogenic activity of GTG and lupalbigenin derived from D. scandens. In the present study, we used an E-screening proliferation assay in the MCF-7 cell line [3] and RT-PCR to investigate gene expression. The results showed that 1 µM GTG induced 71.15% proliferation compared to 0.1 nM 17β-estradiol (set up to 100% relative proliferative effect) while 1 µM lupalbigenin reduced proliferation to 18.72%. Furthermore, when 1 µM lupalbigenin was co-treated with 0.1 nM 17β-estradiol, cell proliferation was altered to 80.38%. The results suggest that lupalbigenin has anti-estrogen effect in vitro. The effect of GTG and lupalbigenin on gene expression levels were analyzed. GTG increased estrogen receptor gene expression (2.5-fold), while androgen receptor and TMPRSS2 gene expression were suppressed. Lupalbigenin significantly inhibited the androgen receptor on transcriptional level. Both compounds might contribute to the estrogenic effect of *D. scandens* extracts. However, further studies should be performed to confirm estrogenic activity of plant extracts.

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P-123 Effects of caffeoylquinic-acid-rich fractions of silver wormwood (*Artemisia ludoviciana* Nutt.) extract on rat kidney mitochondria functions

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Caffeoylquinic acids are specialized bioactive metabolites possess antioxidant activity [1]. Currently, increasing interest is shown towards mitochondria-targeted antioxidants. The aim of this study was to fractionate caffeoylquinic acids from cultivated silver wormwood herb acetone extract and evaluate their antioxidant activity, effects on kidney mitochondrial functions, and cytochrome-c-reducing properties. Caffeoylquinic acids were fractionated by column chromatography, aqueous monocaffeoylquinic acids (WS1) and methanolic dicaffeoylquinic acids (WS2) fractions were obtained [2]. Quantitative and qualitative analysis was performed by HPLC-PDA method. Preparation of isolated kidney mitochondria of rats described in [2,3]. The mitochondrial functions were measured using an Oxygraph-2k high-resolution respirometry system. The reduction of cytochrome c, and antioxidant activity in vitro were recorded spectrophotometrically [2,3]. The fractions were riched in caffeoylquinic acids. We identified chlorogenic acid (143.3 ± 2.8 mg/g DW), neochlorogenic acid (6.7 ± 0.1 mg/g DW), 4-O-caffeoylquinic acid (7.8 ± 0.2 mg/g DW) in WS1 fraction and 4,5-dicaffeoylquinic acid (101.1 ± 0.6 mg/g DW), 3,4-dicaffeoylquinic acid (175.9 ± 0.5 mg/g DW), 3,5-dicaffeoylquinic acid (378.8 ± 0.5 mg/g DW) in WS2 fraction by HPLC-PDA method. The highest radical scavenging and antiradical activities were observed in the WS2 fraction from silver wormwood herb acetone extract. The WS1 fraction showed lower antioxidant activity in vitro. WS1 at both concentrations (0.008 µg/mL and 0.8 µg/mL) had no effect on routine (V0) respiration rate. 0.8 µg/mL concentration of WS1 15% reduced state 3 respiration rates (VADP) as compared to the control group. WS2 also had no effect on V0. WS2 fraction at both concentrations diminished VADP in a dose-dependent manner. The higher concentration (0.8 µg/mL) of the WS2 23% reduced VADP as compared to the control group. The highest cytochrome c reducing capacity was observed in the WS2. WS1 less reduced cytochrome c. The results of our experiments revealed up-and-coming mitochondrial-function-modulating, antioxidant and cytochrome-c-reducing properties of caffeoylquinic-acid-rich fractions from cultivated silver wormwood herb extract based on a new mechanism of action.

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P-124 Bioaccesibility, uptake and transport mechanisms of monotropein and monotropein esters from *Gaultheria* berries

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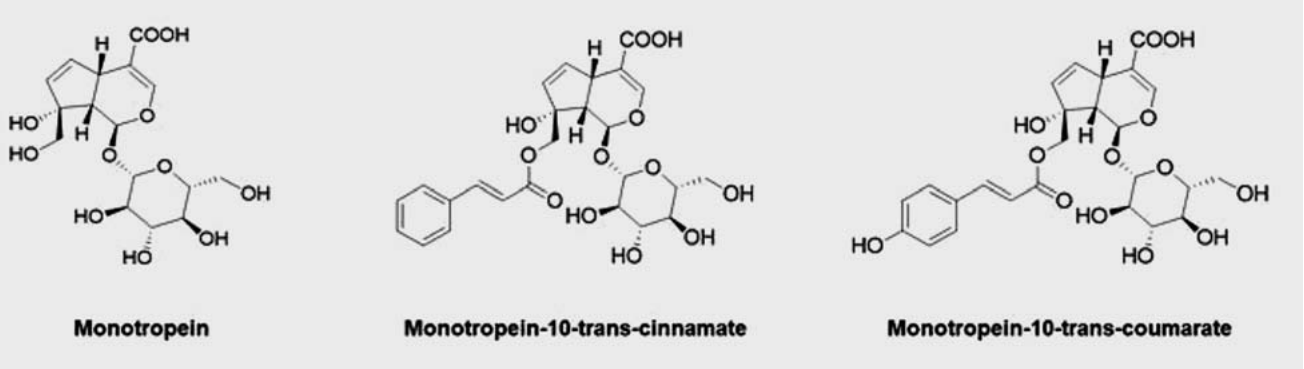
Iridoids are a group of secondary metabolites that can be found in berries from *Gaultheria* genus [1]. Monotropein (MT) has demonstrated health promoting properties in vitro and in vivo [2]. Scarce information about the bioavailability of MT can be found in literature. The aim of this study was to determine the uptake and transport mechanism of MT and MT esters, namely: cinnamate (MT-Cin) and coumarate (MT-Cou) (▶ **Fig. 1**), which are secondary plant metabolites present in *G. phillyreifolia* and *G. poeppigii* berries [3]. Berries, as well as the isolated esters and the commercial standard of MT were submitted to a simulated digestion model. After digestion, micelles were isolated, and the uptake and transport of the parent compound and esters was studied using differentiated Caco-2 cells. Under our assay conditions, the food matrix stabilized the compounds during digestion, but interfered with the micellization, resulting in a lower percentage of bioaccesible compounds. MT-Cin presented the highest uptake and transport, with a Cmax of 0.46 nmol/mg protein at a Tmax of 180 min, and an apparent permeability (Papp) of 0.050x10–6 cm/s. The presence of bromosulphalein, a known OATPB2B1 inhibitor, decreased the uptake of MT-Cin by 74.3%, while phlorizin, a known SGLT inhibitor, delayed the Tmax to 240 min. In the same line, both inhibitors induced significant changes in the Papp of both esters. In conclusion, the esters of MT have better physicochemical properties that lead to higher uptake rates and Papp compared to MT. However, the mechanism of transport through membranes remains to be clarified.



▶ **Fig. 1**

Planta Med 2022; 88: 1385–1588

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P-125 Comparison of antioxidant activity of *Trifolium pratense* L. extracts prepared using β- or γ-cyclodextrin-assisted extractions

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Red clover is valued for its positive influence of isoflavones on health and its potential use in preventing and treating chronic diseases [1,2]. The right excipients, such as β- or γ cyclodextrin, can increase valuable phenolic compounds in extraction media thus obtaining antioxidant-rich extracts, which can be used in the pharmaceutical industry.
This study aimed to investigate and compare the total phenolic content and antioxidant activity of red clover aerial parts ethanolic extracts prepared using traditional and cyclodextrins-assisted extraction methods.
All the samples were prepared using three different methods: reflux (1); reflux combined with ultrasound processing for 10 min (2); reflux combined with ultrasound processing for 30 min (3). Compared with samples prepared using traditional methods without cyclodextrins (control samples C1-3), β-cyclodextrin (B1-3) increased total phenolic content by 11.77% and γ-cyclodextrin (G1-3) by 28.66% on average. The highest antioxidant activity (ABTS and DPPH) was detected in the samples G1: 433.122 ± 2.61 µg TE/g (ABTS) and G2: 12.55 ± 0.59 µg TE/g (DPPH). Using the post-column ABTS method, the highest effect was found in sample G1: 30.16 ± 1.21 mg TE/g. However, the highest reducing power activity was determined in the B3 sample prepared using β-cyclodextrin (191.92 ± 0.73 mg FE(II)/g).
Both cyclodextrin-assisted extraction methods are useful tools for obtaining antioxidant-rich extracts for the production of nutraceuticals. Nevertheless, γ-cyclodextrin-assisted method showed greater results compared to β-cyclodextrin.
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P-126 Antiprotozoal activity of compounds isolated from *Psychotria leiocarpa*

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With an estimated amount of 241 million cases of malaria and 627,000 deaths from it in 2021, malaria remains one of the most important infectious parasitic diseases worldwide [1]. Due to increasing resistance against the drugs currently used to treat this illness, there is an urgent need to find new and more effective antimalarial therapies [2]. Over the years, medicinal plants have been an inspiring source for antimalarial compounds, such as quinine and artemisinin. Our approach to discover new drugs was to explore South American plants used in traditional medicine. The genus *Psychotria* is one of the most important within the Rubiaceae family and includes about 1200 species. This genus is reported as a source of alkaloids and iridoids, which exhibited psychotropic, anti-inflammatory, antioxidant, antimutagenic, antimicrobial and antiprotozoal activities [3]. The present study focused on the isolation and identification of bioactive compounds from the methanolic extract of the leaves of *Psychotria leiocarpa* (SisGen Brazil A9C0F2F), and the evaluation of their antiprotozoal activity towards Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani, and Plasmodium falciparum, as well as of their cytotoxicity against rat skeletal myoblast L6 cells. The extract demonstrated an activity against the NF54 strain of P. falciparum (IC_{50} value of 8.8 µg/mL). Fractionation and isolation combined to LC-HRMS/MS-based dereplication provided 10 compounds including alkaloids, iridoids and phenolic acids. The isolated compounds were tested for their antiprotozoal activity and cytotoxicity to determine their selectivity index and potential for future studies.
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P-128 A new alkaloid from *Scadoxus multiflorus*

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Scadoxus multiflorus (Martyn) Raf. (Amaryllidaceae) or blood lily is an ornamental plant native to tropical and southern Africa. It is traditionally used in the treatment of many respiratory problems (bronchitis, asthma, pneumonia, etc.), scabies, dropsy, and wound healing [1]. However, the alkaloidal constituents of *S. multiflorus* have rarely been explored and only a few are known [2,3]. In the course of our study of Amaryllidaceae alkaloids, a full investigation of the alkaloidal profile of bulbs of *S. multiflorus* was carried out using a combination of chromatographic and spectroscopic techniques, as well as computational calculations. As a result, a previously undescribed alkaloid (1), together with 14 known ones (lycorine, 2-O-methylpseudolycorine, narciclasine, lycoricidine, ungiminorine, ungiminorine N-oxide, narcissidine, sanguinine, montanine, 2-O-acetyl-chlidanthine, 2-hydroxy-O,N-dimethylnorbelladine, O-demethylmaritidine, 8-O-demethyloxomaritidine, 9-de-O-methyl-11β-hydroxygalanthamine), were isolated and characterized.
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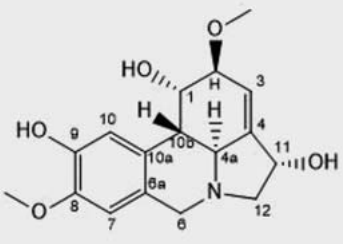

▶ **Fig. 1**

P-129 Rational search for natural inhibitors of the trypanosomatid pteridine metabolism

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DOI 10.1055/s-0042-1759108

Tropical and subtropical regions of the world are severely affected by millions of disease cases caused by trypanosomatid parasites. For example, human African trypanosomiasis (HAT) and cutaneous leishmaniasis (CL) are caused by *Trypanosoma brucei* (Tb) and *Leishmania major* (Lm) [1]. The identification of new drugs is urgently needed since current treatment options cause severe side effects and are frequently hampered by resistance developments of the parasites. Trypanosomatids possess a unique pteridine metabolism with an enzyme system consisting of the bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS) and the pteridine reductase 1 (PTR1) which represents a highly interesting drug target [2]. In continuation of our previous work [3,4], we aim for the identification of new lead structures with a dual inhibitory effect against the respective T. brucei (TbDHFR, TbPTR1) and L. major (LmDHFR, LmPTR1) enzymes. To this end, we used in silico methods (pharmacophore-based virtual screening of approx. 5000 natual products followed by multi-step docking procedures) to select candidates with potential inhibitory activity towards the target enzymes. Promising compounds were subsequently tested in vitro through spectrophotometric inhibition assays against recombinant DHFR and PTR1 of both investigated parasites. Out of 50 tested compounds, five were identified as dual inhibitors against the Tb enzymes so far ($0.2\,\mu M < IC_{50} < 83.6\,\mu M$). Against the corresponding enzymes of Lm, seven out of 35 tested natural products inhibited both of the enzymes in the micromolar range ($4.2\,\mu M < IC_{50} < 84.5\,\mu M$). The elucidation of further dual inhibitors as well as their kinetic mechanism(s) of inhibition are the focus of current studies.

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P-130 Characterization of proanthocyanidins from the bark of *Bassia longifolia*

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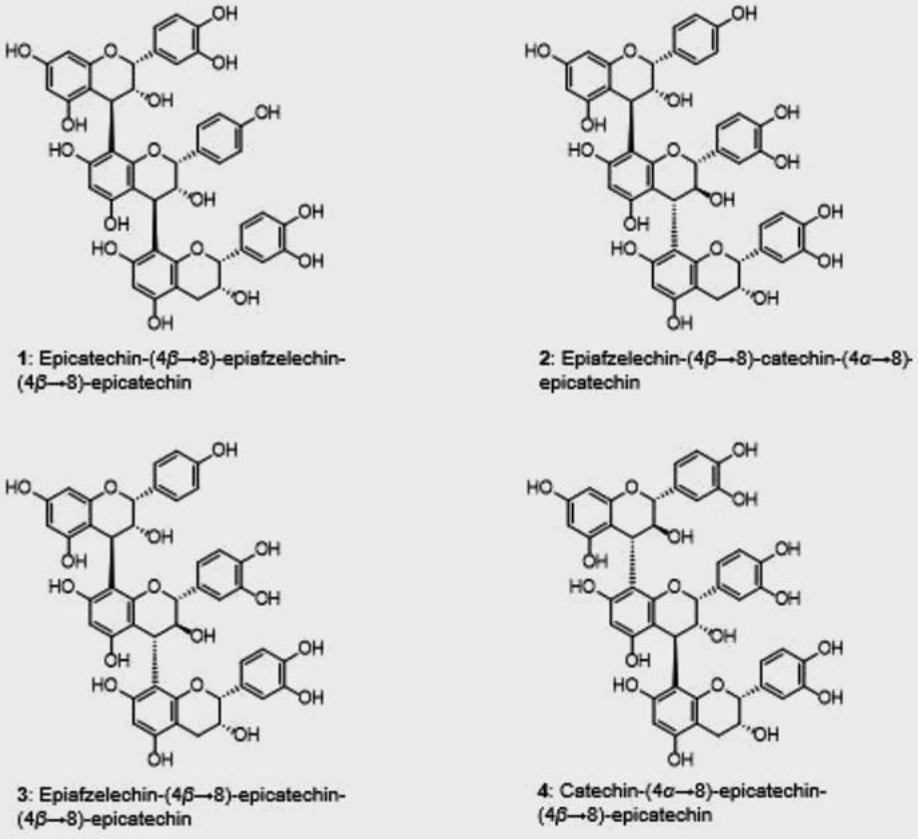
DOI 10.1055/s-0042-1759109

Bassia longifolia KOENIG (= Madhuca longifolia KOENIG, Sapotaceae) is one of more than 1600 different plant species that are used for traditional phytomedicine in Nepal [1]. It is an evergreen tree which grows about 20 m in height. Flowers and bark are used for both, food and medicine [2]. The bark



▶ **Fig. 1** Compounds **1** and **2** are isolated for the first time. Freephenolic NMR – data of compounds **3** and **4** are described for the first time.

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is used for cough, colds and bronchitis. The bark paste can be applied externally on cuts and wounds to stop bleeding [3].
Until then, analytical results and data are only available for the seeds [4,5] but data regarding the flowers and bark are not sufficiently available. Therefore, a characterization of the proanthocyanidin pattern was performed for the first time. From a methanolic extract obtained from the bark of *B. longifolia*, 12 compounds were identified. One tetrameric, six trimeric and five dimeric proanthocyanidins consisting of mono- and dihydroxylated flavan-3-ol-units were isolated by a combination of different chromatographic techniques. Their structures were elucidated by ^{1}H- and ^{13}C-NMR-spectroscopy including COSY, HSQC, HMBC and ROESY methods. Optical characterization was performed by polarimetry and circular dichroism. Two trimers with epiafzelechin-units were isolated for the first time (▶ **Fig. 1**). In addition, for another two trimers, NMR and optical data of the free phenolic compounds are shown for the first time. Future studies will show if these proanthocyanidins contribute to the pharmacological effect of *B. longifolia*.

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P-131 Phytochemical study of *Ulmus minor* subsp. minor fruits, a rich source of anti-inflammatory constituents

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Ulmus minor Mill. subsp. minor is a deciduous medium-sized tree native to Europe, extending to Central Asia and Northwest Africa [1]. The plant is used in popular medicine for the treatment of many ailments: the bark decoction is taken as an astringent for intestinal disorders or in local applications against skin diseases, while bark ointment is topically applied against rheumatism; the leaves are used boiled in vinegar as a pesticide to treat scabies [2,3]. Moreover, plant immature samaras are eaten raw in the Italian phytoalimurgical tradition as snacks, to flavor salads and thicken soups [4] but till now no deep chemical study on their secondary metabolites have been provided so far. Therefore, in this work the phytochemical investigation of plant species samaras EtOAc and n-BuOH extracts is reported for the first time, resulting in the isolation and characterization of twenty compounds including a new flavan-3-ol and a new trihydroxy fatty acid. The extracts and some compounds, selected for their isolation amount and not common distribution in the plant kingdom, were tested for their inhibitory effect on some mediators of inflammation in J774A.1 cells stimulated with lipopolysaccharide of Escherichia coli (LPS). NO release and iNOS and COX-2 expression were evaluated, and both the extracts and compounds significantly inhibited NO release as well as iNOS and COX-2 expression in macrophages. In particular, 8-(2-pyrrolidinone-5-yl)-catechin, oxylipin, and catechin 7-O-α-L-rhamnopyranoside (20–5 μM) seems to be the more promising in inhibiting the inflammatory response in macrophages ($P < 0.001$ vs. LPS control treated cells).

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P-132 Clinical validation of the utility of triterpene saponins from *Sapindus saponaria* and chroman hydrazone for topical treatment of cutaneous leishmaniasis

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DOI 10.1055/s-0042-1759111

Cutaneous leishmaniasis (CL) is an endemic infection in several countries worldwide. Because of variable response to therapy and frequency of relapses, more effective, safer, and inexpensive treatments are needed. The hederagenin glucoside saponins (SS) and chromane hydrazone (TC2) combined in a 1 : 1 ratio have high potential in antileishmanial therapy since both compounds alter the survival of *Leishmania* and the ability to infect adjacent macrophage [1]. In this work, we developed an ointment formulation containing 2% TC2 and 2% SS (w/w) and determined the skin permeation and the absorption but also the acute dermal toxicity by in vitro and in vivo assays. Last, the effectiveness and safety of the topical therapy to treat non-complicated CL was evaluated in an observational study in human and canine patients from endemic areas of Colombia. Both TC2 and SS diffused through pig ear skin and traces of TC2 but not SS were detected in the stratum corneum of mice at 6–24 hours. Neither TC2 nor SS were detected in plasma. The acute dermal toxicity was negative. Treatment with 2% TC2 – 2% SS ointment produced a complete long-term clinical cure in 10 patients (4 women and 6 men) and 56 dogs (24 females and 32 males) without adverse effects. All human and canine patients have remained disease-free for the last 24 months. In conclusion, these results support the use of topical therapy as a safer and new first-line local treatment of CL that could be further validated by controlled clinical trials.

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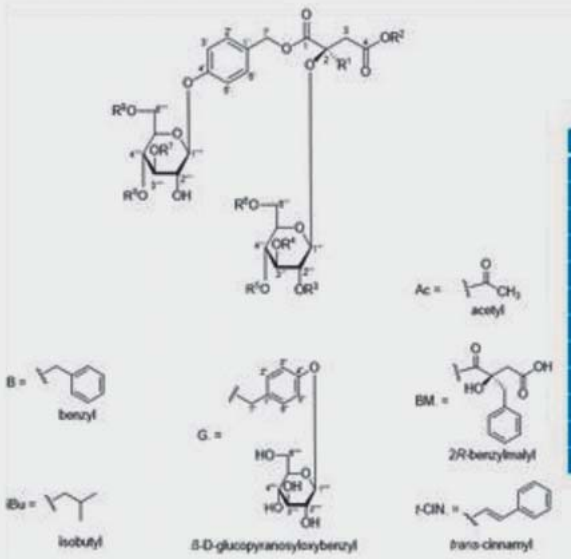

Compounds	R1	R2	R3	R4	R5	R6	R7	R8	R9
Arundinoside I (1)	B	H	Ac	Ac	H	Ac	H	H	Ac
Arundinoside II (2)	B	H	Ac	H	H	H	t-CIN	H	H
Arundinoside III (3)	B	G	Ac	Ac	Ac	BM	H	H	Ac
Arundinoside IV (4)	B	G	Ac	H	H	H	t-CIN	H	Ac
Arundinoside V (5)	B	G	Ac	H	H	Ac	t-CIN	H	Ac
Arundinoside VI (6)	B	G	Ac	Ac	H	H	t-CIN	H	H
Arundinoside VII (7)	B	G	Ac	H	H	H	H	t-CIN	Ac
Arundinoside VIII (8)	B	G	Ac	H	H	Ac	H	t-CIN	Ac
Arundinoside IX (9)	B	G	H	H	H	H	t-CIN	t-CIN	Ac
Arundinoside A (12)	B	G	Ac	H	H	H	t-CIN	t-CIN	Ac
Graminifoloside A (10)	iBu	G	Ac	H	H	H	H	t-CIN	Ac
Graminifoloside B (11)	iBu	G	Ac	H	H	H	t-CIN	t-CIN	Ac

▶ **Fig. 1**

P-133 Arundinosides I–IX and graminifolosides A–B: 2R-benzylmalate and 2R-isobutylmalates derivatives from *Arundina graminifolia*

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Glucosyloxybenzyl 2R-benzylmalate and glucosyloxybenzyl 2R-isobutylmalate derivatives are structurally unique group of plant metabolites that occurs primarily in the family Orchidaceae. There are very limited studies reporting this class of compounds due to their rare occurrence in nature. *Arundina graminifolia* D.Don Ochr. popularly known as bamboo orchid is widely distributed in subtropical Asia, particularly in Southeast Asian countries [1]. The roots/rhizomes and leaves are used for treating food poisoning, blood stasis, bacterial infections and rheumatism [1,2]. *A. graminifolia* is a rich source of bibenzyls phenanthrenes, phenolics and glucosyloxybenzyl 2R-benzylmalate derivatives [1,3–5]. As a furtherance to the studies on the bioactive constituents from *A. graminifolia* led to the isolation of eleven new compounds. Dried undergrounds parts of *A. graminifolia* were extracted with ethanol and the concentrated extract was partitioned into CH_2Cl_2 and EtOAc and H_2O fractions. The EtOAc fraction was subjected to centrifugal partition chromatography, sephadex LH-20 and semi preparative RP-HPLC to afford nine new glucosyloxybenzyl 2R-benzylmalate, arundinosides I–IX (1–9) and two new glucosyloxybenzyl 2R-isobutylmalates, graminifolosides A–B (10–11) (▶ **Fig. 1**). The structures were deduced using spectroscopic techniques including NMR and HRMS as well as comparing with previous literatures. Compounds 3–8 showed potent antioxidant activities in the ABTS radical scavenging, DPPH radical scavenging and FRAP activities. Furthermore, the compounds exerted minimal cytotoxic effects against RAW 264.7 cells and cytoprotective effects against hydrogen peroxide induced cell toxicity.

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P-135 Bioassay-guided isolation of cytotoxic retrochalcones from *Glycyrrhiza echinata* L. roots and elucidation of their cell death mechanisms

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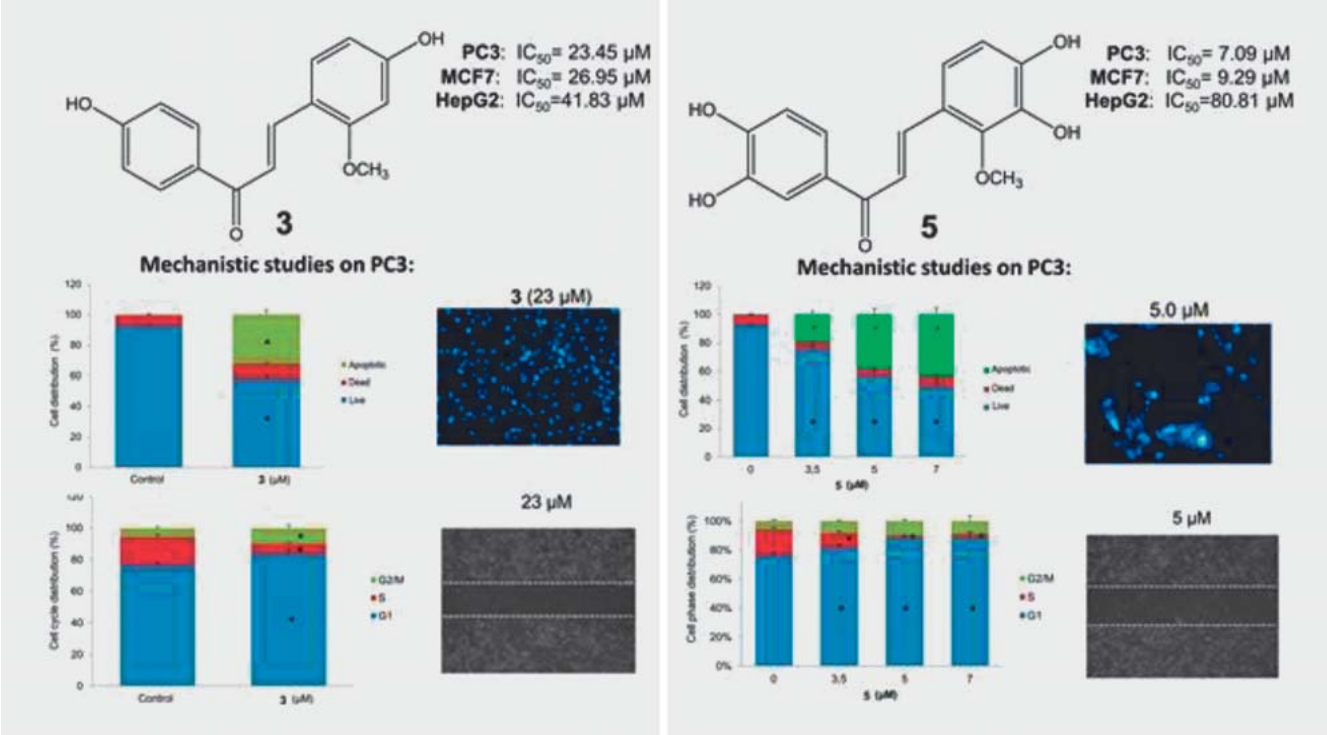
DOI 10.1055/s-0042-1759113

Extracts and some phenolic compounds of *Glycyrrhiza* L. have demonstrated evidential cytotoxic and antitumor activities through different mechanisms [1,2]. As a continuation of our studies on *Glycyrrhiza* sp. from flora of Turkey [3,4], this study aimed to isolate the cytotoxic metabolites from *G. echinata* L. roots by bioassay-guided fractionation and to elucidate their cell death mechanisms. For this purpose, in vitro cytotoxic activity of total MeOH extract as well as its subextracts were evaluated against PC3, MCF7 and HepG2 cells by MTT assay. Totally seven secondary metabolites were yielded from DCM (IC_{50} = 4.26 µg/mL-123.90 µg/mL) and EtOAc (IC_{50} = 23.41–146.90 µg/mL) subextracts through sequential chromatographic techniques. The isolates were identified as 4-hydroxybenzoic acid (1), isoliquiritigenin (2), echinatin (3), licochalcone B (4), tetrahydroxymethoxychalcone (5), vestitol (6) and medicarpin (7) on the basis of extensive NMR and MS analyses. According to in vitro cytotoxic effects of isolates, unusual chalcones characteristic for the genus *Glycyrrhiza*, namely retrochalcones (3–5) were found as the cytotoxically most effective compounds (▶ **Fig. 1**). Further mechanistic studies on

Planta Med 2022; 88: 1385–1588



▶ **Fig. 1** Cytotoxic activity mechanisms of **3** and **5**.

3 (23 µM) and 5 (5 and 7 µM) including Hoechst staining and wound healing assays along with apoptosis rate and cell cycle analyses indicated that both compounds induced apoptotic cell death, caused significant cell cycle suppression in G1 and G2/M phases and exhibited antimigratory property on PC3 cells while 5 showed selectivity to cancer cells (SI = 5.195). To conclude, tested retrochalcones most particularly 5, established remarkable anticancer effects via several mechanisms against androgen independent prostate cancer (PC3) cells, could be potential anticancer drug candidates.

Acknowledgements

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P-137 Computational Examination of Urolithins as Potential Scavengers of Chemical Carcinogens

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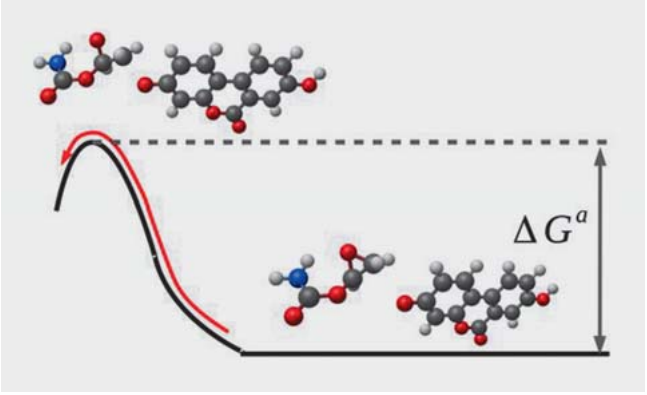
Tannins represent a rather diverse group of natural compounds that are of great technological importance while they might also exhibit some health beneficial properties [1]. Ellagic acid is a hydrolysis product of ellagitannins and has been subjected to several studies [2]. In our previous study, we demonstrated that ellagic acid forms a promising candidate for carcinogen scavenging [3]. However, its activity in the organism is limited by its rather low bioavailability. Because of this, it may act as a carcinogen scavenger in the digestive tract, while its activity in the cells is limited by pharmacokinetics. However, ellagic acid is by bacteria in the digestive tract metabolized into several urolithins, which can later be observed in various body fluids [4].

In this study, the chemical scavenging capacity of several urolithins (e.g., Urolithin B and A, Isourolithin A, and Urolithin AR) against nine ultimate carcinogens of the epoxy type at the Hartree-Fock and B3LYP levels of theory in conjunction with flexible basis sets and implicit solvation models were examined. The reactivities of ultimate carcinogens were additionally compared to the reactivity of ellagic acid and guanine, the most reactive nucleobase of DNA, against the same set of carcinogens [3,5].



▶ **Fig. 1** Schematic representation of a reaction between vinyl carbamate and Urolithin A.

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P-138 Isolation and structural elucidation of sesquiterpene lactones from *Achillea millefolium* L. and pharmacological investigation in an ICAM-1 in vitro model

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DOI 10.1055/s-0042-1759116

Achillea millefolium is one of the most widely used medicinal plants in the world and has been used in folk medicine for millennia [1]. Its broad spectrum of pharmacological activity is mainly due to secondary metabolites such as flavonoids and sesquiterpenes, in particular sesquiterpene lactones (SL) [2]. Due to the promising potential of SLs for medical applications, in course of this work SLs were isolated from *A. millefolium* and their in vitro pharmacological potential was investigated. The structural elucidation was performed by means of one- and two-dimensional NMR spectroscopy and HRESIMS. The isolated compounds were four SLs of the germacranolide-type and eleven SLs of the guaianolide-type as well as three unsaturated fatty acids and one flavonol. Two isolated compounds have not yet been identified for the species *A. millefolium* and six SLs are described for the first time (▶ **Fig. 1**). Furthermore, the biological activity of ten of these compounds was investigated using a human endothelial cell line (HMEC-1) as an ICAM-1 inflammation model [3]. For eight of the ten investigated compounds, a significant reduction in ICAM-1 production was detected which has not yet been described for any of these compounds. None of the investigated substances exhibited an exocyclic methylene structure. The reduced transcription of proinflammatory genes in literature is attributed in particular to this structure due to its interaction with the transcription factor NF-κB [4,5]. However, these results verify the ability of other structural motifs to initiate an anti-inflammatory effect in vitro.

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P-139 Secondary metabolites from *Gentiana cruciata* L

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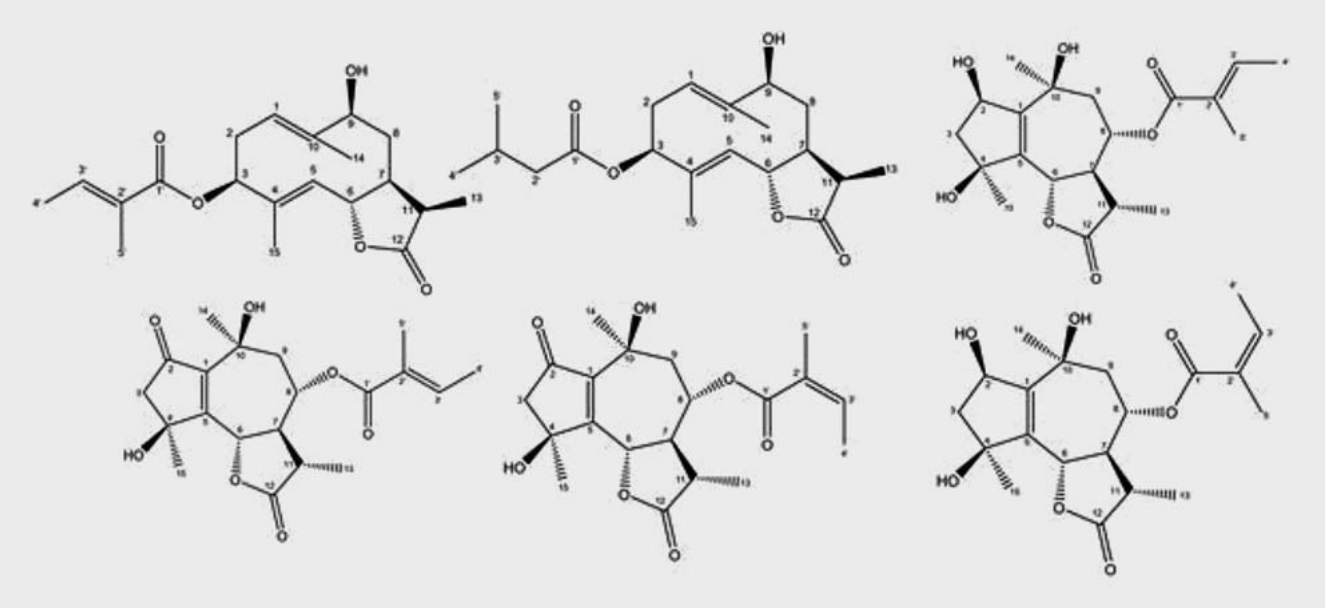
The genus *Gentiana* (Gentianaceae) is a large genus containing around 400 species worldwide [1]. Some of these species have long been used in different folk medicines for the treatment various disorders particularly against digestive problems. Among these species, *G. cruciata* is used to reduce blood cholesterol level, as an antidiabetic agent as well as to improve digestion and appetite in Serbia [2]. The extracts of this species were reported to possess in vivo hepatoprotective activity [3]. The genus *Gentiana* is represented by 12 species in the flora of Turkey including *G. cruciata* [4]. Few previous phyto-



▶ **Fig. 1** Six sesquiterpene lactones isolated and described for the first time in this work.

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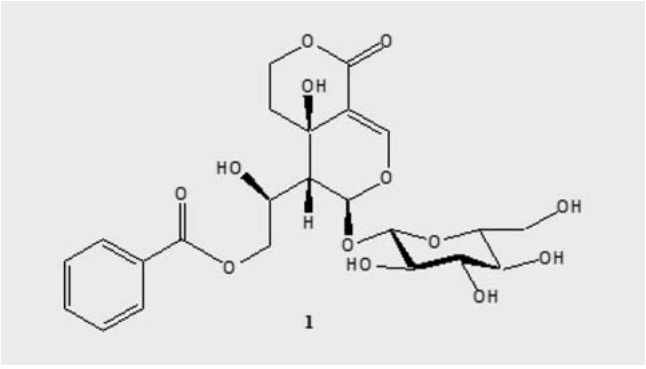

▶ **Fig. 1**

chemical studies revealed the presence of secoiridoids and flavon-C-glycosides from this species [1]. However, there is no detailed study on the isolation of its secondary metabolites. As a part of our ongoing studies on the Turkish *Gentiana* species [5], we aimed to isolate the secondary metabolites from the aerial parts of *G. cruciata* collected from Turkey. Phytochemical studies resulted in the isolation of a new secoirioid glycoside, cruciatoside (1) along with three known secoiridoid glycosides, eustomoside, gentiopicroside and 6′-O-β-D-glucopyranosyl gentiopicroside, one iridoid, loganic acid, three flavonoids, isoorientin, isoorientin 2′′-(E)-ferulate, isovitexin, one xanthone derivative, mangiferin, and one cyclitol, methyl-inositol. The chemical structures of the isolates were determined based on extensive 1D and 2D NMR experiments as well as HRMS analysis. This study also represents the first record of the occurrence of isoorientin 2′′-(E)-ferulate, 6′-O-β-D-glucopyranosyl gentiopicroside, eustomoside and methyl-inositol in *G. cruciata*. The in vitro anti-inflammatory and analgesic activities of the isolates are underway in our laboratory.

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P-140 Antioxidative activity of commercial liquorice samples and their phytochemical constituents in vitro on cellular level

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DOI 10.1055/s-0042-1759118

In previous studies we found a remarkable antioxidative activity of liquorice extracts in cell-based in vitro assays. When piglet intestinal epithelial cells (IPEC-J2) were pre-incubated with liquorice extracts and challenged by hydrogen peroxide, formation of ROS as measured by a fluorescein probe (DCFH-DA) was inhibited. Moreover, glycyrrhizin was not responsible for the observed activity. In order to identify the active principle, 10 commercial extracts of *Glycyrrhiza glabra* and one plant powder were compared analytically (LC-QTOF-MS) and tested for antioxidative effects in the same cell-based assay. Correlations between bioactivity and the amounts of phytochemicals and phytochemical classes were calculated. The more abundant phytochemicals were tested as pure compounds in the cell-based assay at 0.1–10.0 µg/mL. The liquorice samples reduced the oxidative stress signals by 31–83% when tested at 30–60 µg/mL. Of all tested phytochemicals, only glabridin significantly reduced the oxidative stress signal in cells by 33% at 10 µg/mL ($p < 0.05$). Also, glabridin abundance in liquorice samples was correlated with bioactivity ($r = 0.58$). Antioxidative effects of commercial liquorice samples seemed to be related to their flavonoids. Additive or synergistic effects are very likely, since none of the tested single phytochemicals showed an activity sufficiently high to explain the activity of extracts. Glabridin had the strongest antioxidative activity among the tested substances and might contribute most to the observed activity of the liquorice samples. The authors declare no confilct of interest.

P-141 Effects of selected *Cirsium palustre* extracts on intestinal motility – an ex vivo study

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DOI 10.1055/s-0042-1759119

Cirsium palustre (L.) Scop. (Asteraceae) is a species commonly found in Europe. In addition to the proven use of plants in traditional medicine, scientific studies indicated numerous pharmacological activities of their extracts and isolated compounds. This experiment is a continuation of the study aimed to clarify the utility of further *C. palustre* extracts and fractions to control colon dysmotility. The experiments were performed on the model of swine colon specimens, which can be used in translational medicine. Colon specimens were incubated in modified Krebs-Henseleit solution under isometric conditions [1]. The effect of aqueous extract (CP3) and diethyl ether fraction (CP4) of *C. palustre* on the spontaneous and acetylcholine (ACh)-induced contractility of colon was verified. An analytical approach based on LC-ESI-MS analysis to obtain a phytochemical profile was applied. The metabolite profile revealed that samples contained quinic acid and flavone derivatives as major constituents. The results indicated that CP3 and CP4 are potent modifiers of colon motility. The spontaneous activity of circular and longitudinal smooth muscle was significantly enhanced in the presence of both studied preparations. In the case of ACh-induced contractility, the effect was less spectacular and variable. The CP3 extract showed a tendency to increase the force of ACh-evoked reaction in both colon specimens whereas CP4 fraction slightly intensified and weakened longitudinal and circular colon smooth muscle, respectively. Bearing in mind the complexity of colon dysmotility in the course of functional intestine diseases, the preparations of C. palustre could be an interesting option for symptomatic therapy, particularly for adynamic ileus.

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P-142 Prenylated bicyclic acylphloroglucinols from *Hypericum tetrapterum* with antibacterial activity

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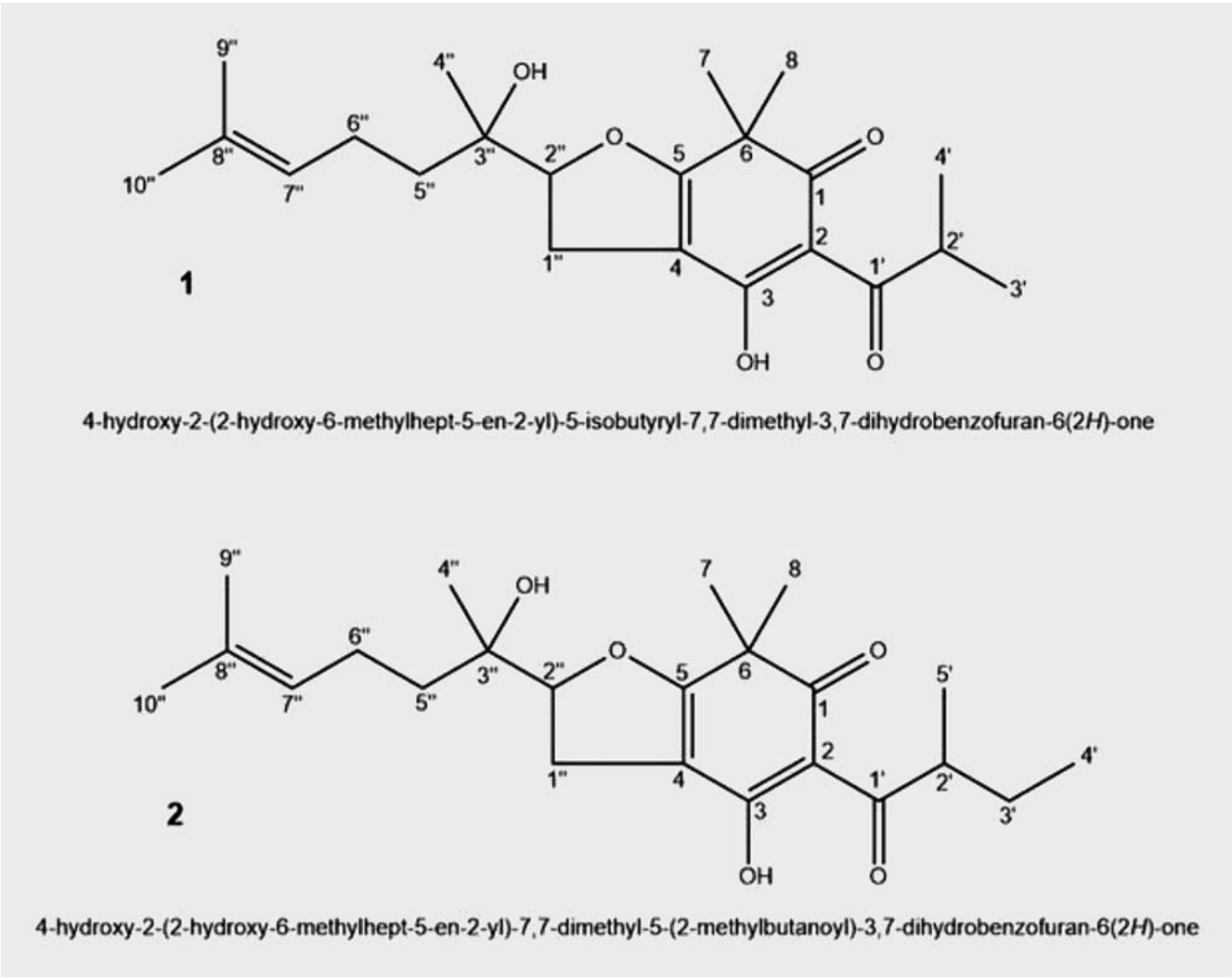
DOI 10.1055/s-0042-1759120

The genus *Hypericum* L. contains about 500 species, and it is almost spread all over the world avoiding only zones of extreme aridity, temperature and/or salinity [1]. Besides flavonoids, procyanidins and naphthodianthrones, the



▶ **Fig. 1** Structures of prenylated bicyclic acylphloroglucinols from *Hypericum tetrapterum* Fr.

acylphloroglucinols are an abundant class of secondary metabolites with hyperforin as the most prominent representative. Hyperforin occurring in *H. perforatum* L. contribute to the anti-depressive, but also to the antibacterial effect of its extracts [2]. *Hypericum tetrapterum* Fr. (*Hypericum*, Hypericaceae) is a perennial herb with a four-winged stem and is native to Europe, Western Asia and North Africa [3]. To date, the chemical composition of the plant, especially the profile of prenylated acylphloroglucinols, is mostly unknown. For the isolation of acylphloroglucinols a petroleum ether extract was prepared from the aerial parts of *H. tetrapterum* and 1H NMR-guided fractionation was performed. Structure elucidation based on 1H-, 13C- and 2D NMR (HSQC, HMBC, NOESY, COSY) spectroscopy as well as on data derived from mass spectrometry. Optical characterization was performed by polarimetry and circular dichroism. The obtained structures 1 and 2 are two prenylated bicyclic acylphloroglucinols with furanone structure (▶ **Fig. 1**) which were described here for the first time. The compounds were evaluated for their antibacterial activity against the gram-positive test germ Staphylococcus aureus with a modified dilution method as published previously [4] and showed antibacterial activity. Isolation and structure elucidation of further compounds with similar skeleton are in progress.

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P-143 Use of *Andrographis paniculata* in the management of acute bronchitis in primary care: protocol of a pragmatic randomized controlled trial

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DOI 10.1055/s-0042-1759121

Although the majority of respiratory tract infections, including acute bronchitis, are caused by viruses, 53% of diagnosed patients receive antibiotics (ABs)

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in Europe, which contributes largely to resistance to ABs [1]. *Andrographis paniculata* known for its antiviral, anti-inflammatory and immune system stimulating properties, is traditionally used in China, India and Thailand. This study aims to assess its efficacy in the management of acute bronchitis with or without COVID-19 and the feasibility to prescribe it in primary care.

This pragmatic randomized controlled trial will be carried out in primary care physicians' (PCPs) practices based in Switzerland. A total of 280 patients diagnosed with acute bronchitis will be included and randomized either in the usual care group or in the intervention group. At inclusion, symptoms will be assessed by PCPs in order to quantify the severity of five symptoms related to acute bronchitis, using the ABSS score [2]. A diary will be completed daily by the patient to report his/her symptoms. The primary outcome is determined by treatment efficacy by comparing the number of days needed to achieve a 50 % reduction in the ABSS score after peak of symptoms in each group. Secondary outcomes will measure the proportion of patients receiving antibiotics for the same illness episode as well as the proportion of PCPs agreeing to participate in the study and adhere to it.

This study will contribute to search ways to reduce antibiotics overuse as well as to understand better opportunities and barriers to prescribe herbal medicine in primary care.

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P-144 Antimicrobial and phytochemical analyses of European Larch resins and essential oils

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Malignant fungating wounds (MFW) affect 5 to 14 % of oncology patients and to date there is no standard therapy to manage these wounds [1,2]. Pinaceae resins have been investigated as promising wound healing agents [3] – *Larix decidua* resin has already been described for MFW treatment [4]. This study aimed to compare the non-volatile chemical composition of nine different Larch resins as well as their essential oil composition, using GC-MS, GC-FID and HPTLC as analytical tools. The antimicrobial potential was evaluated by agar diffusion test, using wound and skin bacterial strains. HPTLC results showed a similar qualitative profile for all resins. Using three different mobile phases, several diterpenes could be separated. The GC-MS confirmed the resins' similarity, and 4 compounds were identified using authentic standards: larixyl acetate, isopimaric acid, abietic acid, and dehydroabietic acid. GC-FID analysis of the resins' essential oils obtained by hydrodistillation revealed a consistent profile for all samples, allowing for the identification of alpha-pinene, beta-pinene and 3-carene, being alpha-pinene the major compound (825.3–896.7 µL/mL) in all oils. However, although the chemical profile was related in all samples, their antibacterial activity differed substantially. Results were promising for one essential oil (inhibition zones for all tested strains ~ 25 mm). Smaller inhibition zones were also seen for four other oils. In conclusion, our results showed that Larch resins from different manufacturers presented similar chemical profiles by HPTLC, GC-MS and GC-FID. Further investigation is ongoing to evaluate the biological activities and wound healing properties of *Larix decidua* from different harvest collections.

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P-145 Isolation, detection and pharmacological activity of the major paraconic acids from *Cetraria islandica*

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DOI 10.1055/s-0042-1759123

Paraconic acids are a group of secondary metabolites mainly found in lichens and selected fungi. Within this compound class, lichesterinic- and protolichesterinic acid have previously shown inhibitory activity on 5- and 12-lipoxygenase (LOX) [1,2].

This compound class show weak UV-light absorption, resulting in a low sensitivity in (LC-)UV detection [3]. Moreover, their very similar structure makes their separation rather challenging. The present project describes a two-step isolation protocol to obtain the major paraconic acids from commercially available *Cetraria islandica*. First, the crude extract is separated by size exclusion chromatography to obtain fractions composed of isobaric compounds. Subsequently, lichesterinic- and protolichesterinic acid were separated by fast centrifugal partition chromatography, while roccelaric- and nephromopsinic acid were isolated by column chromatography. We also developed analytical methods to detect these compounds by thin layer chromatography (TLC), LC-MS, and LC-ELSD (▶ **Fig. 1**).

The effects of the paraconic acids on lipid mediator biosynthesis in human peripheral blood mononuclear cells (PBMC) were investigated by UPLC-MS/MS-based lipidomics. We found that paraconic acids diminished the mobilization of polyunsaturated fatty acids and thus lipid mediator biosynthesis (at 3 µM), potently interfere with 5-LOX product formation (at 3 µM) and elevate the levels of cytochrome P450 monooxygenase-derived epoxyeicosatrienoic acids (EETs) (at 30 µM).

In conclusion, we managed the isolation of lichesterinic-, protolichesterinic-, roccellaric-, and nephromopsinic acid from *Cetraria islandica* with purities above 90 %, which favorably shift the lipid mediator profile of activated innate immune cells from pro-inflammatory to anti-inflammatory products, while generally dampening lipid mediator production.

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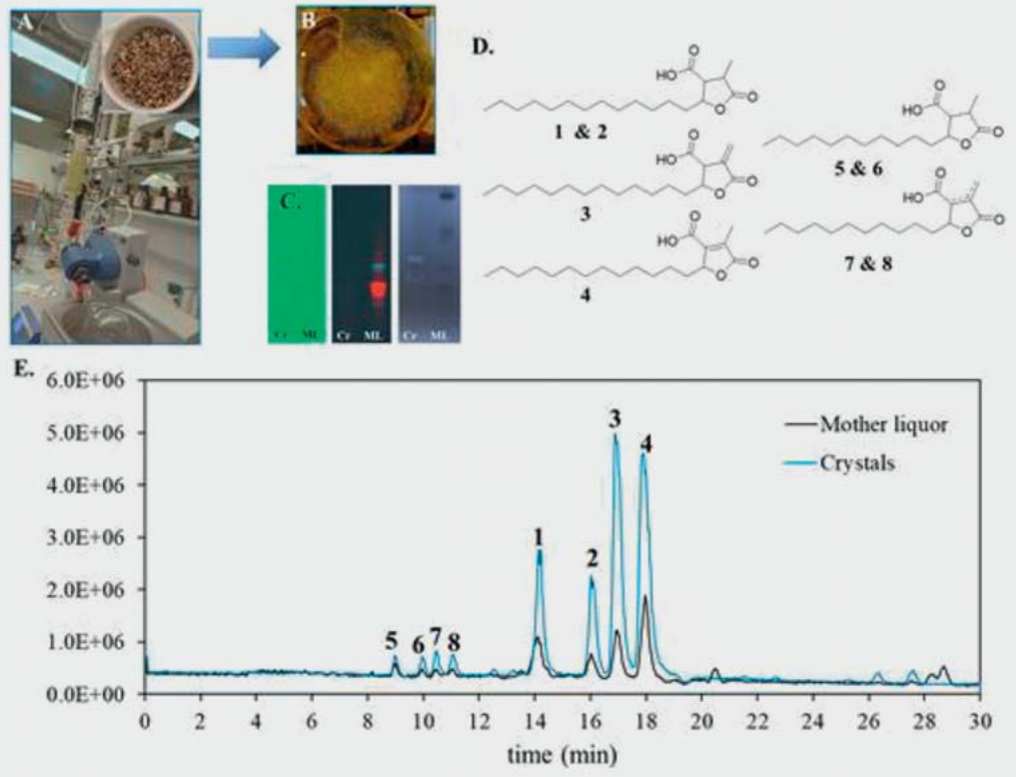

▶ **Fig. 1** **A** Soxhlet extraction of *C. islandica* with petroleum ether. **B** Crystallization of paraconic acids after Soxhlet extraction. **C** TLC analysis. **D** Chemical structure of compounds contained in crystal mixture: nephromopsinic acid, 1; roccellaric 2; protolichesterinic acid, 3; lichesterinic acid, 4; nephrosteranic acid and an isomer, 5 and 6; nephrosterinic acid and an isomer, (7 and 8). **E** LC-MS analysis of petroleum ether extract of *C. islandica* after crystallization.

P-146 Bioactivity of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* on Neonatal Group B Streptococcus

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Group B *Streptococcus* (GBS) is a harmless commensal bacterium in healthy adults, but it causes sepsis in neonates resulting in a high rate of mortality. This study was carried out to investigate the antibacterial activity of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* bioactive fractions on 35 multidrug resistant GBS strains implicated on neonatal sepsis as well as identifying the antibiotic resistant genes present. Minimum Inhibitory Concentration (MIC) and Minimum Bacteria Concentration (MBC) of the different plant biofractions was determined. The presence of erythromycin (ermB) and tetracycline (tetO) resistant genes was identified using duplex PCR techniques. Statistical analysis was done. The results obtained show that the plants have dose dependent activity against GBS. Ethanol biofraction of *R. vomitoria* had the highest activity with an MIC value of 12.5 mg/ml and MBC, 25 mg/ml followed by methanol biofraction of *B. pinnatum* with an MIC value of 50 mg/ml on 32 out of the 35 strains investigated, then MBC values at 50 mg/ml while N-hexane and aqueous biofractions had the least activity. Also, the presence of ermB and tetO resistant genes were present in all the ten representative GBS strains tested. The high rate of activity shown by the methanol and ethanol biofractions of both plants suggests that the plants can serve as a potential alternative for the treatment of neonatal sepsis. However, a further study on their in vivo activity is important in order to evaluate the efficiency, safety and potential adverse effects and drug herb interactions of the plants. No conflicts of interest.

P-148 Anti-inflammatory potential of phenolic compounds isolated from *Entada africana* Guill. & Perr. used in the Republic of Benin

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Entada africana is a West African tree with numerous ethnomedicinal uses. The bark and root of E. africana have been investigated for their phytochemical compositions. However, knowledge on phenolic composition of the leaves, which are predominantly used in the Republic of Benin for the treatment of wounds, fractures and sprains, and their bioactivity is still scarce. This study aims at elucidating phenolic compounds of a hydroalcoholic leaf extract of *E. africana* and assessing their bioactivity. Structural elucidation was performed using HRESI-MS and NMR methods. Bioactivity of *E. africana* crude extract, fractions thereof, and isolated compounds was evaluated using TNF-α

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stimulated human keratinocytes (HaCaT) as an in vitro model of skin inflammation. As a result, 11 phenolic compounds were identified in the hydroalcoholic leaf extract of E. africana, which moderately inhibited IL-8 release to 48.1 ± 3.5 % with an IC_{50} = 59.2 µg/mL. Among the 11 phenolic compounds, 3′,4′,7-trihydroxyflavone showed a significant inhibitory effect on IL-6 interleukin with a maximum inhibition of 74.4 ± 2 % and IC_{50} = 17.8 µg/mL and moderately reduced IL-8 interleukin secretion to 40.2 ± 5.1 % with IC_{50} = 126.2 µg/mL. This study provides for the first time an overview of the phenolic compounds present in the hydroethanolic leaf extract of *E. africana* and their anti-inflammatory potential, which support its traditional medicinal use in the treatment of wounds, fractures and sprains.

The authors declare that there is no conflict of interest.

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P-149 Myrrh and chamomile flower extract inhibit the release of the mast cell mediators β-hexosaminidase and histamine in IgE-stimulated RBL-2H3 cells

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Recent clinical evidence suggests efficacy of a traditional medicinal product containing the combination of myrrh (*Commiphora myrrha* (Nees) Engl.), coffee charcoal (*Coffea arabica* L.) and chamomile flower dry extract (*Matricaria chamomilla* L.) for the treatment of diarrhea and irritable bowel syndrome (IBS) [1]. Mast cells seem to play a key role in IBS symptom generation as recent studies show an increased activation and degranulation rate of mucosal mast cells in the intestinal tissue of IBS patients.

To evaluate the application of the herbal combination in the treatment of IBS, the effects of the herbal extracts on the release of mast cell mediators from RBL-2H3 cells were investigated. Therefore, the release of histamine (by ELISA) and β-hexosaminidase (by colorimetric enzyme substrate reaction) was quantified in cell culture supernatant after preincubation (18 h) with IgE (500 ng/mL) and the plant extracts followed by cross-linking with human serum albumin (HSA, 1 µg/mL) for 30 min.

Myrrh (MY) and chamomile flower (CH) reduced IgE/HSA-stimulated β-hexosaminidase release, with myrrh exerting a more distinct effect (MY: 35,7 % ± 3,6 % inhibition, IC_{50} = 10,34 µg/mL; CH: 20,0 % ± 5,0 % inhibition). In addition, chamomile flower extract (100 µg/mL) was able to reduce IgE/HSA-stimulated histamine release (38,1 % ± 3,1 % inhibition).

Thus, these results indicate a mechanistic basis for the use of the herbal combination of myrrh, coffee charcoal and chamomile flower extract for the treatment of diarrhea symptoms in IBS patients.

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P-150 Phenolics and polysaccharides isolated from the roots of *Aconitum septentrionale*, a Norwegian medicinal plant

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Aconitum septentrionale Koelle (Ranunculaceae family) is a medicinal plant widely distributed in Norway, Sweden and Russia, and well known for its toxicity. Alkaloids, such as lappaconitine, lappaconine and septontrionin [1–3], as

▶ **Fig. 1**

well as lipids and organic acids [4,5] have previously been identified in the plant. The high content of alkaloids is responsible for the poisonous effects of the plant, which also limits its utilization. However, other types of bioactive natural products like phenolics and polysaccharides that are present in most *Aconitum* plants, have not been studied in *A. septentrionale* yet. The aim of this study was to explore the content of phenolics and polysaccharides in a water extract of this plant, and to study their immunomodulating effects.

The water extract was applied to a Diaion HP-20 absorbent column and eluted with water and methanol. The water fraction was purified by ion exchange chromatography and gel filtration to isolate the polysaccharides, while the 25–100% methanol fractions were subjected to C18 flash chromatography, Sephadex LH-20 and preparative HPLC. Several phenolic compounds and three types of polysaccharides were isolated from water extracts of *A. septentrionale* roots for the first time. Further structure analysis and bioactivity testing of the polysaccharides and phenolics are now under investigation and will be presented.

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P-151 Phytochemical study of *Commiphora myrrha* (NEES) ENGL. reveals various sesquiterpene scaffolds

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DOI 10.1055/s-0042-1759128

Myrrh is the oleo-gum resin of *Commiphora myrrha* (NEES) ENGL. (Burseraceae) and has traditionally been used for the treatment of different, among other inflammatory diseases [1,2]. Searching for its activity determining ingredients, 20 sesquiterpenes of nine different structural types and one sesquiterpene dimer were isolated from an ethanolic extract by chromatographic steps and identified by NMR and CD spectroscopy and HRESIMS. Thereof, nine molecules were found for the first time as natural products (4, 8, 9, 14–18, 21), nine compounds the first time for the species *Commiphora* and three substances (1, 2, 19) are known as myrrh ingredients (▶ **Fig. 1**). Sesquiterpenes of the eremophilane- and nor-eudesmane-type have not been published for myrrh so far. Selected compounds (1, 6, 7, 16, 18 and 19) were tested on their biological activity in an ICAM-1 in vitro model in which no considerable effect was detected. The anti-inflammatory properties of myrrh [3,4] are therefore either based on its other ingredients or mediated via another pathway; the efficacy of a herbal preparation containing myrrh in the treatment of ulcerative colitis [5] could besides be promoted by synergistic and additive effects.

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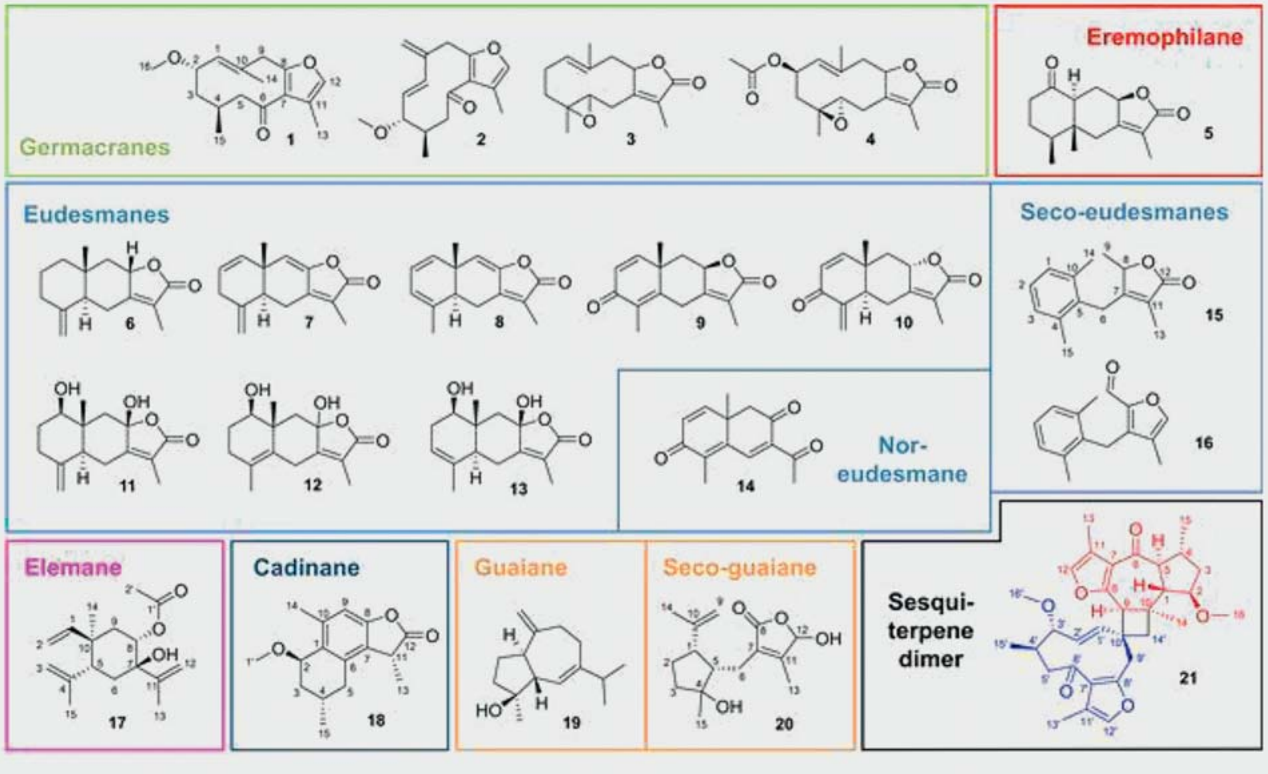

▶ **Fig. 1** Compilation of the 21 sesquiterpene derivates isolated from myrrh sorted by their structural skeleton types.

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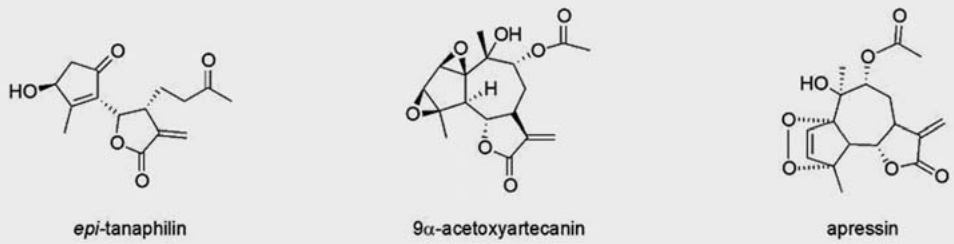

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P-152 Further constituents from *Ammoides atlantica* (Coss. & Durieu) H. Wolff and their cytotoxic activity evaluation

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Ammoides altantica (Coss. & Durieu) H.Wolff (Apiaceae) is a herbaceous plant endemic to Algeria, where it is consumed as a spice or used as an ethnobotanic remedy against headache, fever, and diarrhea [1]. Few chemical studies on the plant aerial parts are available in the literature, reporting the chemical composition of essential oil [2] and polar extracts, rich in flavonoids and phenolic acids [3]. Antibacterial, antioxidant, and anti-inflammatory properties were attributed to the investigated plant extracts [4]. The aim of the present study was the investigation of non-polar constituents of *A. altantica* aerial parts along with their cytotoxicity evaluation. The dried plant material was defatted with n-hexane and extracted with $CHCl_3$ to obtain a raw extract successively fractionated by silica gel column chromatography and RP-HPLC for the isolation of pure compounds. The separation process was assisted by a quali-quantitative analytical investigation by UHPLC coupled to an Orbitrap-based HR-MS. Seven new terpenoids, together with eight known sesquiterpenoids were finally isolated and characterized by 1D and 2D NMR, as well as HR-MS experiments. All compounds were assayed in human tumor cell lines. The known sesquiterpenes epi-tanaphilin, 9α-acetoxyartecanin, and apressin (▶ **Fig. 1**) showed a significant dose-dependent reduction in cell viability on most of the cell lines, especially in A549, A375, and Jurkat. The two most abundant and active compounds, epi-tanaphilin and 9α-acetoxyartecanin, were investigated for their effect on apoptosis and cell cycle. Results showed that both compounds induced a significant ($p < 0.001$) increase of apoptotic response in a dose-dependent manner.

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P-153 Bioactive boron compounds inspired from Nature

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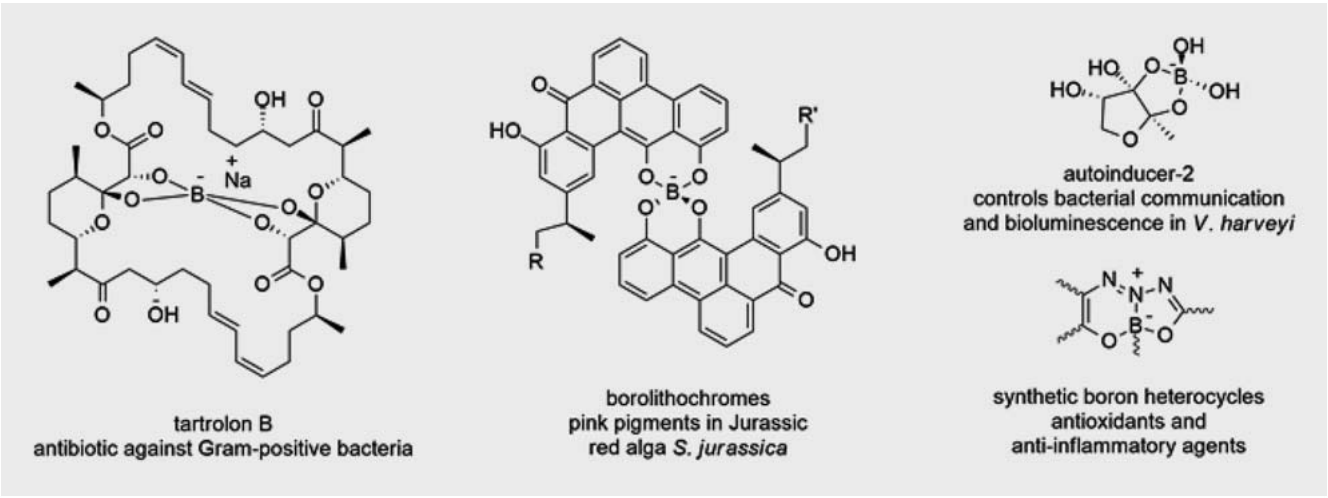
Boron can be primarily found in a wide variety of natural sources such as fruits, vegetables, and hazelnuts. It is an essential nutrient useful or necessary for the development of organisms. It plays a significant role in plants' structural integrity and metabolism, whereas in mammals it is essential for bone health and proper function of vitamin D [1]. Furthermore, boron exists in natural antibiotics, such as Boromycin, Asplasmomycin, Borophycin, and Tartrolons, possessing antibacterial, anticancer, and antiviral properties [2].
A variety of boron-containing bioactive compounds exhibit antibacterial, antifungal, antiparasitic, antiviral and anti-inflammatory activities [3]; some of these compounds have been recently approved by FDA as commercially available drugs. Velcade® and Ninlaro® are proteasome inhibitors for the treatment of multiple myeloma. Kerydin® is a benzoxaborole to treat fungal onychomycosis. Eucrisa® combats mild to moderate atopic dermatitis. Vabomere® is a combination drug, including Vaborbactam (a boronic acid component), a β-lactamase inhibitor, and Meropenem which impedes the synthesis of bacterial cell-wall, thus treating bacterial infections [1]. Calcium fructoborate (CFB) is a nature-identical mimetic of a molecule naturally present in fruits and is commercially produced as a dietary boron supplement for combating inflammatory and cardiovascular diseases and killing cancer cells [2].
Based on the above and our interest in boron derivatives, we designed the synthesis of bioactive boron-containing heterocycles and proceeded to the evaluation of their antioxidant and anti-inflammatory properties with the aim to develop new drug entities [4].



▶ **Fig. 1** Boron-containing heterocycles.

The authors are grateful to Alan and Linde Katritzky Foundation, ARKAT USA, for financial support of the research.

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P-154 N-Methyldihydrobenzo[c]phenanthridines Bearing a β-Aminoester Moiety as Potent Antibacterial Agents Against Antibiotic-Resistant Bacteria

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The emergence of antibiotic-resistant bacteria is considered a worldwide public health problem for which new antibiotics are needed [1]. Some benzo[c]phenanthridine-type alkaloids have attracted much interest as potential antibacterial agents that target FtsZ and antibiotic efflux proteins [2]. Herein, two natural dihydrobenzo[c]phenanthridines were isolated from the seeds of *Bocconia latisepala* and then subjected to copper-catalyzed benzylic functionalization to incorporate nitroester, cyanoester, and dialkylmalonic moieties. The in vitro evaluation of 15 semisynthetic compounds against clinical isolates of antibiotic-resistant gram positive (methicillin-resistant *Staphylococcus aureus*, linezolid-resistant *S. epidermidis*, vancomycin-resistant *Enterococcus faecium*, and multidrug-resistant *Mycobacterium tuberculosis*) and gram negative bacteria (carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*, and OXA-48 and NDM-1-producing *K. pneumoniae*) allowed us to discover promising antibacterial compounds with minimum inhibitory concentration values ranging from 1.56 to 50 µg/mL. Among bioactives, eight compounds selectively inhibited the growth of the antibiotic-resistant gram positive bacteria at MIC values (1.56–6.25 µg/mL) lower than the standard drug levofloxacin (12.5 µg/mL), whereas four derivatives resulted four times less active than levofloxacin against CR *A. baumannii.*

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P-155 Antimycobacterial Alkaloids from the Aerial Parts of *Bocconia latisepala* L

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The quest for novel antibacterial agents against antibiotic-resistant *Mycobacterium tuberculosis* has been focused to new scaffolds or pharmacophores with different targets or mechanisms of action [1]. *Bocconia* plants contain benzo[c]phenanthridine-type alkaloids that fulfill these features [2–3]. Herein we report the isolation and structural characterization of fourteen known and three new natural benzo[c]phenanthridines along with one new aporphine alkaloid from the aerial parts of *Bocconia latisepala*. The isolates were tested against sensitive (H37Rv) and multidrug-resistant (G122 and G133) *Mycobacterium tuberculosis* strains. Seven alkaloids exhibited moderate to potent activity against sensitive and resistant *M. tuberculosis* strains with minimum inhibitory concentration (MIC) values ranging from 6.25 to 50 µg/mL. Among bioactives, three alkaloids resulted four times less active than ethambutol (MIC = 3.125 µg/mL) against the H37Rv strain, while chelerythrine, (−)-6,12-dimethoxydihydrochelerythrine, and 12-methoxychelerythrine resulted equally active or two times less active than ethambutol (MIC = 6.25 µg/mL) against the G122 strain. To investigate the safety profile of the bioactives, cytotoxicity on human normal cell lines was determined. 12-Methoxychelerythrine resulted less toxic to mammalian cells than chelerythrine at 50 µM, thus becoming a promising antitubercular agent.

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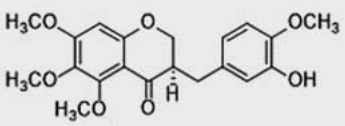

▶ **Fig. 1** Synthetic homoisoflavonoid GI50 (HREC) 0.035 µM.

P-156 Natural and Synthetic Homoisoflavonoids and Related Compounds for the Treatment of Macular Degeneration

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DOI 10.1055/s-0042-1759133
The inhibition of angiogenesis is a drug target with widespread application. Macular conditions affect around 1.5 million people in the UK, with most of them suffering from age-related macular degeneration. AMD is the biggest cause of sight loss in the UK, affecting about 600 000 people [1]. Wet age-related macular degeneration it is characterized by extensive neovascularisation of the retina. Current treatments include the use of anti-VEGF agents such as Bevacizumab (Avastin) and Ranibizumab (Lucentis). Due to the cost of these treatments and the variable outcomes, the possibility of a small molecule treatment is very attractive. Homoisoflavonoids are C-16 natural products isolated primarily from the Asparagaceae family (▶ **Fig. 1**). These compounds have shown promising activity in vitro and in some initial in vivo analyses. A wide range of homoisoflavonoids, both extracted from natural sources as well as of synthetic origin have been screened for selectivity towards human retinal microvascular endothelial (HREC) cells, as well as being assessed for their ability to inhibit tube formation using a Matrigel assay. The work has highlighted some key structural features that are important for activity and selectivity. Synthetic methodology has been developed and optimised to allow for the production of a range of structurally diverse homoisoflavonoids showing good activity and promise for further development as therapeutic agents.

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P-157 Phytochemical profile and antigenotoxic potential of *Bergenia crassifolia* (L.) Fritsch methanolic extracts

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Bergenia crassifolia (L.) Fritsch (Saxifragaceae) is in use for over a century as a medicinal plant in the treatment of various ailments, such as gastritis, enterocolitis, cold, fever, headache, and diarrhea. The root, rhizome, and dried leaves of *B. crassifolia* are most frequently used for medicinal purposes as they showed significant antioxidant, anti-inflammatory, and anticancer properties, with adaptogenic effects as well [1,2]. *B. crassifolia* has some characteristic constituents: bergenin, arbutin, and various tannins [1,3]. The aim of the current study was to evaluate the chemical composition of methanolic extracts of *B. crassifolia* flowers (BCF), leaves (BCL), and roots (BCR) and to show their antigenotoxic effect towards free radical-induced DNA disruption in vitro. The extracts were obtained using accelerated solvent extraction (ASE). Their composition was analyzed using LC-HRMS analysis. Bergenin and arbutin were detected in all three extracts. Other phenolic compounds, such as quinic acid, ellagic acid, quercitrin, and (epi)catechin gallate were also detected in all Bergenia extracts. Besides, there were tentatively annotated some compounds from classes like mono- and sesquiterpene glycosides, phenolamides, and glycolipids that have never been detected in *Bergenia* species before. *B. crassifolia* extracts, particularly BCF and BCL, efficiently protected DNA against oxidative damage caused by hydroxyl and peroxyl radicals in all applied concentrations (50, 100, and 200 µg/mL). The obtained data showed significant antioxidant and genoprotective potential of *B. crassifolia* that will be studied in detail, as well as the presence of some new compounds that have never been reported in *Bergenia* species so far.

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P-158 Anticancer Diterpenoids from African *Croton* Species

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We report the chemistry and anticancer activities of three African *Croton* species, *C. mubango* Mull. Arg., *C. haumanianus* J. Leonard and *C. dictyophlebodes* Radcl. -Sm. Forty-five previously undescribed diterpenoids and thirty known compounds were isolated from these species. NMR, MS, ECD, and DP4+ methods were used to determine the structures. Thirty diterpenoids were submitted to the NCI's Development Therapeutics Programme for testing in the NCI 60 anticancer cell line screening programme. Compounds 1–5 (▶ **Fig. 1**) exhibited 100% lethality against colon (HCT-116), melanoma (M14), and renal (786–0) cancer cell lines whereas compound 6 gave 99%, 89% and 82% cell lethality against melanoma (MALME-3M), renal (UO-31) and ovarian (IGROV1) cancer cell lines respectively at a concentration of 10–5 M [1–3].

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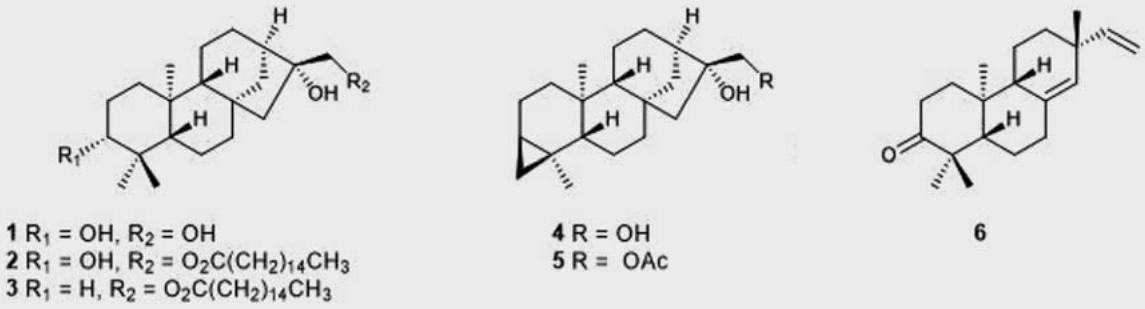

▶ **Fig. 1** Anticancer diterpenoids.

P-159 Interactions of selected flavone derivatives with model lipid membranes

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The widespread presence of flavonoids in the diet, low toxicity and beneficial effects on health make flavonoids interesting to study [1–2]. Explanation of interactions with membranes may contribute to understanding the molecular mechanisms of their actions important for the treatment of diseases [1,3–5]. The aim of the study was to characterize the interactions, location and influence of rare flavone derivatives on the dynamic and structural properties of model lipid membranes prepared from dipalmitoylphosphatidylcholine (DPPC) and egg yolk phosphatidyl-choline (EYPC). Analyses performed with the application of Fourier transform infrared spectroscopy (FTIR) for DPPC membranes at 25, 37 and 45 °C showed that all tested compounds become incorporated into the region of the polar heads of phospholipids. In the region of lipid chains, they cause an increase in the fluidity of membranes. At 25 °C, minor effects were observed in liposomes, and the tested compounds were not deeply embedded in the membranes. Similarly, for EYPC liposomes, all tested compounds interacted with the region of the choline heads of lipids but had little effect on carbonyl ester groups. The NMR technique confirmed the location of all tested compounds in the polar head zone and small effects on the hydrophobic region. All tested compounds have absorption maxima in the range of 250–350 nm. Here, we disclose the mechanism of previously unknown flavonoid action on membranes in terms of their incorporation and changes in the biophysical properties of the membranes, which are important from a medical point of view.

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P-160 Bio-elicitation stimulated isoquinoline alkaloids production in *Chelidonium majus* cells cultured on bio-nano-cellulose

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Enhanced biosynthesis of plant specialized metabolites that can be used for the medical purposes is one of the main goals of the natural product research. Microorganisms can provide an efficient tool for the production of valuable plant pharmacologically active substances. Among the most desirable are isoquinoline alkaloids due to their strong bioactivity. *C. majus* cells were used as a model system for the production of protoberberine, protopine and benzophenanthridine derivatives. The plant cells were cultured on bacterial nanocellulose (BNC) carriers [1]. To enhance biosynthesis, three strains of microorganisms, i.e., *Candida albicans, Staphylococcus aureus* and *Pseudomonas aeruginosa* were inoculated at the top or the bottom of BNC discs. The number of live plant cells was estimated after 2 and 4 weeks of culture (1.7×10^7/mL, and 4.1×10^7/mL, respectively).

3-day old cellulose matrices were most efficient. LC-MS/MS analysis showed different proportions of phenolic compounds and isoquinoline alkaloids among tested samples. After 4-weeks of culture, MALDI MSI chemical maps showed higher content of coptisine, sanguinarine, berberine, chelerythrine, chelidonine, allocryptopine in BNC containing S. aureus, than with *C. albicans* and *P. aeruginosa* or elicitor-free BNC. Hence, *S. aureus* can serve as effective

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elicitor in terms of isoquinoline alkaloid production in *C. majus* cells in combination with biologically inert BNC carriers.

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P-161 Polyphenolic profile in hairy root cultures of *Agastache rugosa* (Fisch. & C. A.Mey.) Kuntze

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Agastache rugosa (Lamiaceae) is one of the 50 most important herbs of Traditional Chinese Medicine. It is a rich source of phenolic acids and flavonoids [1]. Since, the biotechnology approaches can serve as a great tool for the plant metabolites production enhancement. A. rugosa hairy root cultures were established through the infection of *Agrobacterium rhizogenes* strain (A4). Transgenic status of the obtained plant material was confirmed by PCR using rolB and rolC specific primers. Transformed roots were cultured on MS and ½ MS liquid media for their biomass production using three bioreactor systems: a nutrient sprinkle bioreactor (NSB), Plantform and RITA. Fresh and dry weights (g/L) were recorded after 17 days of culture. NSB was found as the most efficient bioreactor system in terms of the biomass and the polyphenolic compounds production. Over 20 compounds were detected in the methanolic extracts of cultured roots. Rosmarinic acid was the most abundant. Its content varied between nearly 4 to over 9 mg/g of dry weight. The polyphenolic profile was dependent on the bioreactor system. Nevertheless, caffeic and caffeoylquinic acids derivatives as well as flavonoids such as apigenin derivatives were present in most samples. The infection of *A. rugosa* leaves with *A. rhizogenes* was an efficient method to obtain polyphenolic rich transformed plant material.

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P-162 Light and temperature influence on phytochemical profile of *Salvia yangi* shoots in vitro

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Salvia yangii BT Drew is a medicinal plant native to Central-West Asia. It grows under harsh environmental conditions in terms of intense solar irradiation and large temperature fluctuations. Phytochemicals that confer medicinal properties include a number of volatile terpenoids, oxidized abietane diterpenoids and rosmarinic acid in the aerial parts and tanshinones in the roots. In this study, we used an in vitro grown shoot model to observe changes in metabolic profile under three light intensities: low (70), moderate (130), and high (220 µmol m − 2 s − 1) and regular (25 °C) and elevated (30 °C) temperatures [1]. Chromatographic analysis (GC-MS and UHPLC) showed significant differences in the proportions of sesquiterpenes (alloaromadendrene, β-caryophyllene, α-humulene), as well as in carnosic (CA) and rosmarinic acid (RA) content. The relative contribution analysis of morhogenetic and metabolic response was performed to show the influence of introduced factors. CA was affected by culture duration, light and temperature treatment, whereas RA by light and temperature or light only. The CA content was higher at 30 °C, and further increased with the length of the culture.

Based on these results, a possible role of CA in a multi-level protective reactions against cell damage, caused by lipid degradation, is suggested. Hence, CA can be considered as a metabolic marker the concentration of which is increased in response to long- lasting intense light and increased temperature stimulation of *S. yangii*.

The study was funded by the Wroclaw Medical University grant no SUBK. D030.22.008 ‘Influence of illumination spectra on metabolic profile in *Salvia yangii* BT Drew’.

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P-163 Effect of fireweed (*Chamerion angustifolium* L.) leaf extract on mitochondrial functions in human colorectal Caco-2 cancer cells

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Extracts from various species of *Epilobium* display numerous therapeutic effects, including anti-inflammatory, antiproliferative, antimicrobial, antioxidant and others. Giving the importance of mitochondrial energy metabolism in cancer, we investigated the effect of fireweed (*Chamerion angustifolium* L.) leaf aqueous extract on the cell viability and mitochondrial bioenergetics in human colorectal adenocarcinoma cancer cell line Caco-2.

Caco-2 cells were incubated for 24 h in RPMI 1640 medium supplemented with 10 % of fetal bovine serum and 1 % penicillin/streptomycin at 37 °C in 5 % CO_2 humidity. Then cells were treated by fireweed leaf extract (IC_{50} = 0.8085 mg/ml) for 48 hours. Mitochondrial respiration rate was measured using an Oxygraph-2k at 37 °C with glutamate/malate and succinate as substrates [2].

In fireweed extract four phenolic acids (p-coumaric, elagic, chlorogenic, neochlorogenic), flavonoids (hyperoside and isoquercitrin) and oenothein B were determined by HPLC. Treatment of Caco-2 cells with fireweed extract dose-dependently suppressed (by 53–91 %) the viability. The mitochondrial function in Caco-2 cells exposed to fireweed extract decreased by 50 % (glutamate/malate) and by 35 % (succinate) and the leak respiration rate increased by 73 %, $p < 0.05$, showing the damage of mitochondrial function. Mitochondrial respiratory control index in Caco-2 cells after pre-treatment decreased by 55 % and 52 %. Moreover, the cytochrome c effect was slightly increased after pre-treatment of cells with fireweed extract showing the damage of mitochondrial outer membrane in Caco − 2 cells.

In conclusion, fireweed leaf aqueous extract diminished significantly the viability of Caco-2 cells and mitochondrial function in Caco-2 cells and could be promising therapeutic agent in cancer cells.

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P-164 Phytochemical Studies on Mastic Gum of *Pistacia lentiscus* var. chia Collected from Karaburun Peninsula and Neuroprotective Activities of the Isolates

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Pistacia lentiscus L., called mastic tree, grows extensively in coastal areas of the Mediterranean. It has been traditionally exploited as a dietary or cosmetic agent and to treat gastrointestinal disorders. While the chemical constituents of Chios mastic gum comprise natural polymers, essential oils, and triterpenic compounds, further studies have suggested that triterpenes are of great importance in biological activities such as anti-inflammatory, antimicrobial, antioxidant, and chemopreventive [1]. Since the abiotic factors affect the secondary metabolite profile [2], our group has decided to undertake a phytochemical study on *Pistacia lentiscus* var. chia growing in Karaburun Peninsula, İzmir-Turkey.

Isolation and purification studies were performed using chromatographic methods; subsequently, structural elucidation was done by spectroscopic methods (1D NMR, 2D NMR, MS and X-RAY). The detailed inspections of spectra helped us establish structures of twelve molecules possessing triterpenic skeletons (▶ **Fig. 1**), and two of them turned out to be undescribed compounds. Since oxidative stress leads to the progression of neurodegenerative disorders [3] and antioxidant activities of some Pistacia species are documented [4], the neuroprotective effect of the isolates was examined against H_2O_2-induced oxidative stress on SH-SY5Y cells. As differentiation agents were reported to affect potency of compounds [5], both differentiated and undifferentiated SH-SY5Y cells was used in this study. Two compounds (oleanolic acid and 17-β-hydroxy-28-norolean-12-ene-3-one) showed neuroprotective effects, while others demonstrated little or no effect. Interestingly, 17-β-hydroxy-28-norolean-12-ene-3-one exhibited protective activity in both differentiated and undifferentiated SH-SY5Y cells whereas oleanolic acid had effects only on undifferentiated cells.

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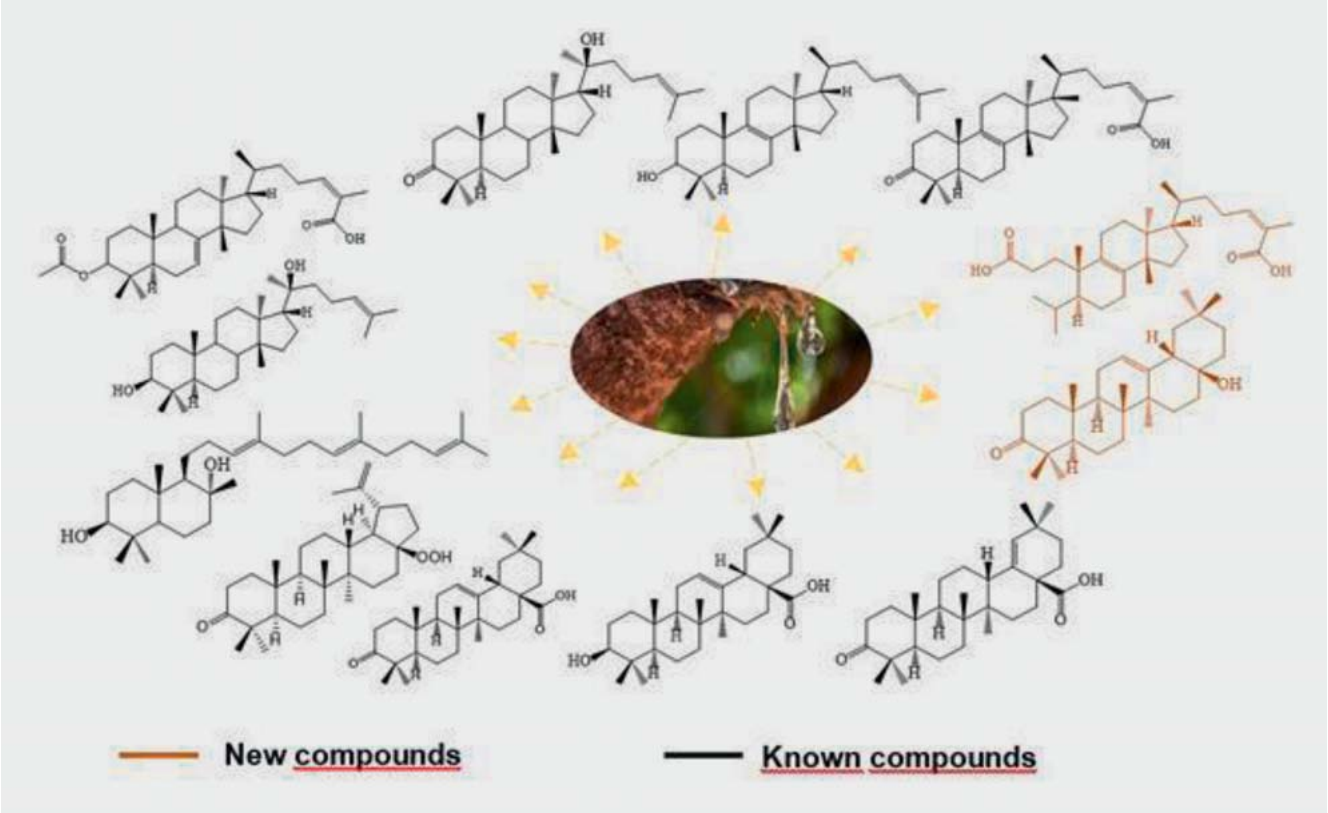

▶ **Fig. 1** Isolated triterpenoids from *Pistacia lentiscus* var *chia*.

P-165 Phytochemical and biological investigation of enzymatic wood extract of *Rosa hybrida* cv. 'Jardin de Granville'

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The potential of *Rosa hybrida* cultivar 'Jardin de Granville', a delicate clear pink flower was investigated with an innovative angle. Fresh woods were extracted with advanced enzymatic techniques, followed by a modern phytochemical method combining CPC fractionation and NMR [1]. 12 fractions were obtained using CPC fractionation. The acquisition of 13C NMR spectra of these 12 fractions was achieved which allowed to identify 15 compounds including catechin, epicatechin, β-hydroxypropiovanillone, 3-methoxygallic acid, galloylquinic acid. Transcriptomic analysis of normal human keratinocytes treated with this extract revealed interesting biological activities particularly on genes involved in antioxidant pathways (SELX, GPX4, CAT), skin barrier function (TGM1, KALK7, SPRRb, AQP9 ...), innate immunity (DEFB4 and PI3) or anti-inflammatory activity (IL-1A, SOCS1, PTGER4). This extract also displayed wound healing properties on a scratch test model using keratinocytes or fibroblasts.

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P-166 The novel therapeutic effect of *Acer palmatum* thumb. leaf extract in a rat retinal ischemic injury model induced by MCAO

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In this study, we investigated that the administration of *Acer palmatum* thumb. leaf extract (KIOM-2015E) protects against the degeneration of rat retinal ganglion cells after ischemia/reperfusion (I/R) induced by midbrain cerebral artery occlusion (MCAO). Sprague–Dawley rats were subjected to 90 min of MCAO produces transient ischemia in both the retina and brain due to the use of an intraluminal filament that blocks the ophthalmic and middle cerebral arteries, which followed by reperfusion under anesthesia with isoflurane. The day after surgery, the eyes were treated three times (eye drop) or one time (oral administration) daily with KIOM-2015E for five days. Retinal histology was assessed in flat mounts and vertical sections to determine the effect of KIOM-2015E on I/R injury. A significant fluorescence loss of brain-specific homeobox/POU domain protein 3A (Brn3a) and neuron-specific class III beta-tubulin (Tuj-1) were observed after five days in the PBS-treated MCAO group compared to the sham-operated control group. The glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS) expression were markedly increased under the same conditions. However, KIOM-2015E treatment reduced (1) MCAO-induced upregulation of GFAP and GS, (2) retinal ganglion cell loss, (3) nerve fiber degeneration, and (4) the number of TUNEL-positive cells. KIOM-2015E application also increased staining for parvalbumin and recoverin in rats subjected to MCAO-induced retinal damage. In conclusions, KIOM-2015E treatment exerted protective effects against retinal damage following MCAO injury, which KIOM-2015E may aid in the development of novel therapeutic strategies for retinal diseases, such as glaucoma and age-related macular disease.

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P-167 The effect of fertilizers on physiological growth, chemical, bioactive components and secondary metabolites in *Vigna unguiculate*

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The current study assessed the effect of different levels of poultry manure fertilizer (10, 30, 60 and 90 kg F/ha) and nitrogen fertilizer (30, 45, 60 and 90 kg N/ha) on the growth, yield, mineral composition, bioactive compounds and secondary metabolites in the legume crop *Vigna unguiculate*. At the end of 15 weeks, 90 kg F/ha and 45 kg N/ha enhanced the growth, yield, mineral composition, production of ascorbic acid, total phenolic and FRAP assay, this informed our decision for the selection of this treatments (45kgN/ha, 90kgF/ha and 0 kg/ha control) for further analyses of secondary metabolites. Poultry manure and nitrogen fertilizer improved the mineral compositions of cowpea leaves, and different accumulation trends were noted depending on different application levels, with poultry manure responding well. Thus, the application of organic poultry manure at 90 kg F/ha for cowpea cultivation should potentially be recommended in the Mpumalanga Province.

P-168 Bee Products in Cosmetic Industry: Propolis Extract a Potent "microbiome friendly" Active Ingredient

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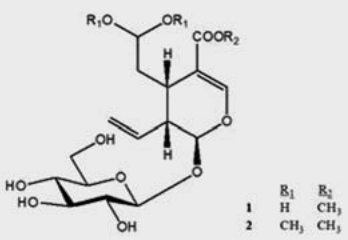
Propolis is a multifunctional material used by bees in the construction and maintenance of their hives. The main chemical classes present in propolis are flavonoids, phenolics and other various aromatic compounds. While use of products containing propolis have a long history, recently there has been renewed interest in it, and it is now increasingly being used in cosmetic industry with a promising role in future. The main objective of this study is to determine the chemical profile of several propolis extracts through HPTLC, in relationship with known markers such as pinocembrin, pinobaskin and other phenolic compounds. The results indicated that the extract of propolis diluted in 1,3-Propanediol has the richest profile and selected for further in vitro investigation. The in vitro study consists of testing a product solution within a sterile dilution medium and challenging this preparation with a prescribed inoculum of suitable micro-organisms. Based on the evaluation of a score algorithm, the tested product was characterized as microbiome friendly. Moreover, evaluation of the product's influence on the pathogenic microbiota indicated a selective action against *Propionibacterium acnes*, *Corynebacterium tuberculostearicum*, *Malassezia globosa*, *Candida glabrata* and *Streptococcus viridans*. Finally, the findings of this study suggest that the specific propolis extract can play an important role as an active ingredient in cosmetic products intended for the scalp skin, armpit, and intimate area. The authors declare no conflict of interest.



▶ **Fig. 1** Chemical structures of secoiridoids (**1** and **2**) from *S. atropurpurea*.

P-169 Secoiridoids, flavonoids and caffeoylquinic acid derivatives from the aerial parts of *Scabiosa atropurpurea* L

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The genus *Scabiosa* (Caprifoliaceae) comprises approximately 100 species worldwide, mainly distributed in the Mediterranean region. Some of these species have long been used in different folk medicines for the treatment of various disorders, particularly against bacterial and viral infections [1]. *S. atropurpurea* is used to treat measles and furuncles in Catalonia while its infusion is indicated as an anti-acne agent in the Iberian Peninsula [1,2]. The extracts of this species were reported to possess in vitro antimicrobial and antioxidant activities [2]. The genus Scabiosa is represented by 36 taxa in the flora of Turkey [3]. Previous phytochemical studies showed the presence of iridoid glycosides and flavonoids in *S. atropurpurea* [1]. The aim of this study is to isolate and identify the secondary metabolites from the aerial parts of *S. atropurpurea* growing wild in Turkey. The plant material was extracted with MeOH. Successive chromatographic studies on the MeOH extract afforded 10 secondary metabolites including two secoiridoid glycosides, lonicejaposide I (1) and secologanin dimethyl acetal (2), six flavonoids, isoorientin (3) hesperidin (4), rhoifolin (5), luteolin 7-O-β-D-glucopyranoside (6), kaempferol 3-O-(3′′,6′′-di-(E)-p-coumaroyl)-β-D-glucopyranoside (7), and kaempferol 3-O-(3′′-O-Acetyl, 6′′-O-(E)-p-coumaroyl)-β-D-glycopyranoside (8), two caffeoylquinic acid derivatives, chlorogenic acid (9) and 3,5-dicaffeoylquinic acid (10). The chemical structures of the isolates were determined based on extensive 1D and 2D NMR experiments as well as ESI-MS analysis. Compounds 1, 2, 4 and 7 are being reported for the first time from the genus *Scabiosa*.

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P-170 Isolation and biological activity of alkaloids from *Vinca minor* L. related to Alzheimer's disease

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Natural products, especially alkaloids, are still a substantial resource for the drug development [1]. Based on the preliminary screening of alkaloidal extracts of various plants against Alzheimer's disease, *Vinca minor* L. (Apocynaceae) have been chosen for a detailed phytochemical and biological study. *V. minor* L. is an evergreen trailing subshrub common in Europe with rich content of monoterpene indole alkaloids [2]. In this study, we have isolated and identified active alkaloidal compounds and assessed their potential to inhibit hAChE, hBuChE, POP, and GSK-3β – enzymes that play a key role in the pathophysiology of Alzheimer's disease. Using chromatographic methods, we have isolated 23 alkaloids; 11 of them have been reported in this species for the first time. One alkaloidal structure was undescribed and was named as vincaminorudeine. The most active compound was (−)-2-ethyl-3[2-(3-ethylpiperidinyl)-ethyl]-1H-indole with IC_{50} = 0.65 μM for the inhibition of hBuChE, and with IC_{50} = 58 μM for the inhibition of POP. Other alkaloids that exhibited significant inhibition against hBuChE (IC_{50} < 30 μM) were vincaminoreine, minovine, 16-methoxyminovine, vincorine, and tubotaiwine. None of the isolated alkaloids was active against hAChE. The (−)-2-ethyl-3[2-(3-ethylpiperidinyl)-ethyl]-1H-indole was also further studied for its pharmacokinetic, revealing a reversible competitive type of inhibition for hBuChE with Ki = 55 nM. This compound can also penetrate the blood-brain barrier by passive diffusion, as was assessed by the PAMPA study. Additionally, the alkaloid on the panel of ten cell lines showed non-cytotoxicity. These compelling results open a possibility for further research on this indole alkaloid.

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P-171 *Viscum album* ethanolic extract promotes MDA-MB-231 cell death by glycolytic enzymes inhibition

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Viscum album L. (Santalaceae) is a semi-parasitic plant which cytotoxic and immunomodulatory properties of its extracts have been described in oncological patients. Despite the well-known anticancer activity of these aqueous extracts, the alcoholic ones have also shown a great anticancer potential in in vitro studies, and these mechanisms deserve to be deep investigated. In this sense, this work evaluated the biological effects of *Viscum album* ethanolic ex-

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tracts (VAE) using two-dimensional (2D) and three-dimensional (3D) cell cultures in vitro. VAE were prepared by maceration of fresh plants from: *V. album* subsp. *album* growing on *Quercus* sp. and *V. album* subsp. *abietis* from *Abies alba*. The anticancer activity was evaluated in 2D and 3D models (MDA-MB-231 breast cancer cells) by MTT and glycolytic enzymes pathway analysis. The summer VAE at 0.5% v/v induced higher cytotoxic damage than winter preparations, and VAE from *Abies alba* and *Quercus* sp. promoted 35% and 68% tumor viability reduction in 3D model (72 h incubation), respectively. MDA-MB-231 glycolytic pathway in 2D model showed a decrease in the glucose consumption. Also, HK (Hexokinase), PFK (6-phosphofructo-1-kinase) and PK (Pyruvate kinase) activities were inhibited by VAE summer extracts after 48 and 72 h of incubation. The transmission electron microscopy of 3D spheroids showed chromatin condensation and accumulation of cytoplasmic vacuoles, after 4 h of VAE treatment. The 3D models are potentially approach in biological cancer research, and further experiments are ongoing to better understand the cellular targets triggered by VAE in in vitro models.

P-172 *Viscum album* hydrogel: physico-chemical evaluation and antiproliferative assay

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Viscum album L. is a plant species, which clinical importance has been registered either as monotherapy or adjunct therapy for patients with cancer [1,2]. It's well known the use of different extracts in the *V. album* preparations that influence the wide diversity of metabolites detected in these pharmaceutical preparations [3–5]. The objective of this study was to evaluate the physico-chemical and antitumoral in vitro effect of a hydrogel containing ethanolic dry extract of *V. album* (host tree Abies alba). For this, dynamic light scattering (DLS), transmission and scanning electron microscopy (TEM, SEM), and Ultraviolet Spectrometry (UV) were applied. The antiproliferative and cellular features were evaluated by MTT and Giemsa stain, respectively, using non-tumor (L929) and tumor (SCC-25) cells. DLS showed particles with an average size of 256 ± 7 nm (0 day) and 258 ± 1 nm, after 30 days storage. The TEM and SEM analyses revealed well-dispersed spherical droplets, with regular borders and nanometer sizes (300–500 nm). Flavonoids content in equivalents of rutin was 13.22 ± 0.02 mg/g. MTT showed IC_{50} of 333.40 µg/mL and 1433 µg/mL for SCC-25 and L929, respectively, with 4.3 of selectivity index for the tumor in relation to the non-tumor cell. Giemsa stain showed intensive damage in tumoral cells, such as: swelling and lysis, intensive vacuolization, chromatin condensation and fragmentation. In conclusion, the *Viscum album* hydrogel spontaneously formed stable nanoparticles and the formulation has a promising antitumoral activity highlighted by the tumor selective index and cellular damage.

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P-173 In vitro study of antioxidant and anti-inflammatory effects of *Dialium cochinchinense*

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Dialium cochinchinense Pierre. is an indigenous medicinal plant in Vietnam. Many parts of this plant have been used in folk medicine for the treatment of diarrhea, parasitemia, and urticaria. However, there is lack of reports about its pharmacological effects. Hence, the present study aimed to investigate the antioxidant and anti-inflammatory effects of 4 different parts of *D. cochinchinense*.

The stem barks (DCB), leaves (DCL), fruit coats (DCC), and seeds (DCS) of *D. cochinchinense* were collected in Khanh Hoa, Vietnam. Dried materials were extracted with 95% ethanol to yield the crude extract. All the obtained extracts were evaluated antioxidant and anti-inflammatory activities via DPPH assay and model LPS-induced inflammation in RAW 264.7 cells.

As the results, DCB and DCS extracts expressed significant DPPH scavenging activity (IC_{50} 9.35 ± 0.64, 8.40 ± 0.56 ppm, respectively), stronger than positive control (ascorbic acid, IC_{50} 13.9 ± 0.40 ppm). In addition, all of DC extracts treatment markedly decreased the production of NO, IL-1β, IL-6, and TNF-α induced by LPS in a dose-dependent manner by ELISA assay. The strongest anti-inflammatory effect was found in DCC and DCS extracts.

Overall, *D. cochinchinense* is a potential source of natural antioxidants with a beneficial effect against inflammation for pharmaceutical applications.

P-174 Secondary metabolites from endophytic fungus *Penicilium roseopurpureum* and investigation of their cytotoxic activities

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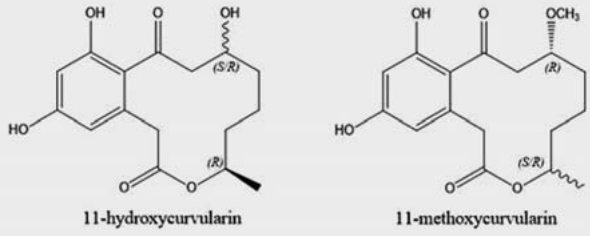
Endophytes are microorganisms, which colonizes in the internal and distinct tissues of the host plants. They attracted attention as a new source for drug discovery and development due to their rich metabolic profiles consisting of novel and bioactive compounds [1,2]. In our preliminary study, the chemical diversity and cytotoxic activity of an endophytic fungus, namely *Penicilium roseopurpureum* 1E4BS1 isolated from *Astragalus angustifolius*, have been demonstrated. The aim of this study was to obtain secondary metabolites of *P. roseopurpureum* and to determine their cytotoxic activity. The ethyl acetate extract of the fermentation broth afforded nine metabolites via isolation studies, and the structures were elucidated by spectral methods (1D-, 2D-NMR, and HR-ESI-MS). Five of the metabolites were found to be new anthraqui-



▶ **Fig. 1** Structure of 11-hydroxycurvularin and 11-methoxycurvularin.

none-type compounds. Additionally, the known compounds were identified as 11-methoxycurvularin (epimeric mixture), carviolin, 1-O-methylemodin and 11-hydroxycurvularin (diastereoisomeric mixture). Then cytotoxic activities of the metabolites were determined against three cancer (DU145, LnCaP, and PC3) and a non-cancerous (RPWE-1) prostate cell lines. 11-Methoxycurvularin (▶ **Fig. 1**) exhibited cytotoxic activity in a dose-dependent manner, whereas 11-hydroxycurvularin (▶ **Fig. 1**) had cytostatic properties. These results were further evidenced by 7-AAD/Annexin V staining, which showed that 11-methoxycurvularin induced cellular death while 11-hydroxycurvularin did not increase dead cell content compared to control. Lastly, cell cycle analysis demonstrated that compounds exhibited different cell cycle arrest patterns.

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P-175 New polymethoxyflavones from *Hottonia palustris* and their effects in an oral squamous carcinoma (SCC-25) cell line

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More than 90 % of types of oral cancers originate from the squamous cells that are inside of the oral cavity. It is estimated that in 2020, nearly 10 million deaths from cancer occurred worldwide, including approximately 180,000 deaths from oral cancer [1]. Our study involved the isolation and identification of flavonoid compounds from the aerial parts of *Hottonia palustris* L. (Primulaceae) and their cytotoxic effects on human tongue squamous cell carcinoma line SCC-25 and human oral keratinocyte. In particular, the current study documented the presence of previously isolated dibenzoylmethane (1) [2] and two flavones, 5,6′-dihydroxy-2′-methoxyflavone (2) and 5,6′-dihydroxy-2′,3′-dimethoxyflavone (3). Their structures were determined by spectral (NMR, MS) analyses. To our knowledge, metabolites 2 and 3 are new natural products to be reported in the plant kingdom. Compounds (1–3), zapotin (4) [3] and selected extracts (methanol – HP1, petroleum ether – HP8) were evaluated for cytotoxic activity by MTT assays [4]. The highest cytotoxic activity (IC_{50} after 48 h) was found for HP8 – 14.90 ± 0.74 µg/mL and for compounds (4) 20.33 ± 1.02, (2) 24.20 ± 1.21, and (1) 29.10 ± 1.45 µM/mL. Our data suggest that *H. palustris* is a source of polyphenols with chemopreventive and chemotherapeutic activities and potential to be developed as candidates for oral cancer treatment.

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P-176 *Pisolithus arhizus* (Scop.) Rauschert: chemical composition and biological activity

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Pisolithus arhizus (Scop.) Rauschert (Sclerodermataceae) is an ectomycorrhizal mushroom, known for its role in forest ecology due to its association with different tree genera such as Quercus and Eucalyptus [1]. In Africa and in Southern Italy, *P. arhizus* is traditionally used for wound healing and hemorragic disorders [2]. Previous phytochemical investigations reported the presence, as main components, of pulvinic acid derivatives, that give the characteristic red brown colour, benzoic acid derivatives, such as pisolithin A and B and triterpenes. [1–3]. Antioxidant and antifungal activity of spores, methanolic and ethanolic extracts of *P. arhizus* were reported [3–4]. This research aims to investigate the chemical composition and the biological activity of *P. arhizus* collected from hills of Benevento, Italy. In details, dried sphorophores were extracted using increasing polarity solvent, obtaining n-hexane, chloroformic and methanolic residues. The study of methanolic extracts led to the isolation of two pigments, norbadione A and bisnorbadioquinone A and phenolic acids derivatives. From the chloroformic residue, after silica gel column chromatography and RP-HPLC, twenty undescribed lanostane-type triterpenoids were purified. Among them, new tetracyclic triterpenoids with an unusual spiro scaffold were also characterized. The structures were elucidated by 1D-and 2D-NMR spectroscopy and confirmed by high resolution mass spectrometry. The absolute configuration at C-22 of 3,22-dihydroxy-24-methylenelanost-9-en-7-one was determined by the modified Mosher's method and its structure was confirmed by X-ray analysis. The antitumoral activity of extracts and pure compounds was also investigated on human T lymphocyte (Jurkat), human glioblastoma (U-87 MG), human leukemia monocyte lymphoma (U937), and cervical cancer (HeLa) cell lines.

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P-177 Polyprenylated aromatic acylphloroglucinols from *Hypericum cordifolium* Choisy

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Hypericum represents the largest genus of Hypericaceae and species are distributed almost all over the world. *Hypericum perforatum* L. is the medicinally most used species in Europe, and its extracts showed anti-depressant, anti-inflammatory and anti-bacterial effects (Barnes et al. 2001). Nevertheless, various *Hypericum* species are used in traditional medicine of other cultures with further indications (Zhang et al. 2020) and thus the investigation of secondary metabolite profiles and therapeutic potential of other unexplored Hypericum species is still important.

As part of our research a dichloromethane extract of the aerial parts from *Hypericum cordifolium* CHOISY a species native to Nepal was phytochemically examined. *H. cordifolium* is traditionally used for the treatment of menstrual

disorder, backache, dislocation of bone, fever, and diarrhea (Basyal und Bhandari 2020). The extract was fractionated by open CC, CPC and semi-preparative HPLC techniques to gain 4 known acylphloroglucinols (Uralione D, Clusiachromene C, Clusiacitran A, Clusiacitran B) previously isolated from *Clusia multifolia* and *Hypericum uralum* (Gonzalez et al. 1995; Fun et al. 2006; Zhou et al. 2016), and 4 hitherto unknown polyprenylated phloroglucinols (1–4, Fig. 1) all showing aromatic acyl substitution. The structures were elucidated with 1D and 2D NMR spectroscopy together with high-resolution electrospray ionization mass spectrometry. All compounds will be tested for antibacterial activity.

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P-178 The study of choleretic activity of *Artemisia* L. herb extracts

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Artemisia L. species are used in medicine for the appetite stimulation and as choleretic, antimalarial, antitumor, anti-inflammatory and antimicrobial agents. These pharmacological properties of *Artemisia* L. herb are due to its diverse chemical composition [1–3].

The aim of the research was to study choleretic properties of *Artemisia absinthium* and *Artemisia vulgaris* standardized extracts [4].

To investigate the dose-dependence of the choleretic effect we used the extracts in doses: 25, 50 and 100 mg/kg body weight of rats. The strongest choleretic effect was observed for Artemisia absinthium extract at a dose of 50 mg/kg; bile secretion increased by 64.6%, which is 1.64 times higher than in the control group of animals,and exceeded the comparison drug "Flamin" by 19.2%.

By decreasing the percentage increase in the average rate of bile secretion, the studied drugs are arranged as follows: *Artemisia absinthium* extract at a dose of 50 mg/kg (64.6%) → "Flamin" at a dose of 50 mg/kg (45.4%) → extract *Artemisia absinthium* at a dose of 100 mg/kg (35.4%) → *Artemisia absinthium* extract at a dose of 25 mg/kg (27.3%).

Administration of *Artemisia vulgaris* extracts at doses of 25, 50 and 100 mg/kg body weight contributed to a moderate stimulation of bile secretion in experimental animals, but the excretion intensity was lower than comparison drug "Flamin".

Thus, pharmacological studies prove the viability of *Artemisia absinthium* herb extract introduction for the treatment of the hepatobiliary system diseases.

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P-179 Hepatoprotective activity of liquid extract of *Gentiana asclepiadea* roots

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Gentiana root preparations are used to stimulate appetite, secretion of digestive glands, bile secretion; they have anti-inflammatory, hepatoprotective and antiseptic properties [1,2].

The aim of the research was to study the hepatoprotective activity of *Gentiana asclepiadea* roots liquid extract using acute carbon tetrachloride hepatitis model in rats in comparison with Silymarin [3,4].

Liver damage was caused by subcutaneous administration of 50% carbon tetrachloride oil solution (0.8 ml per 100 g of body weight of the animal) for two days with 24 hours interval. The extract and Silymarin (25 mg/kg) were administered intragastrically 1 h before and 2 h after it. 24 h after the last administration of carbon tetrachloride, biochemical and functional parameters of the liver and serum, liver mass ratio were determined.

With the introduction of *Gentiana asclepiadea* liquid extract and Silymarin, the enzymes activity in experimental animals serum decreased relative to the control group values: alanine aminotransferase – 2.08 and 1.92 times, aspartate aminotransferase – 1.92 and 1.8 times, alkaline phosphatase – 1.37 and 1.32 times, respectively; the level of TBA reactants in the serum decreased by 1.58 and 1.52 times, and in the liver homogenate – by 1.78 and 1.68 times, respectively.

When using *Gentiana asclepiadea* roots liquid extract, the decrease in liver mass was 22.8% compared with the group of control pathology.

In conditions of acute toxic hepatitis caused by carbon tetrachloride, *Gentiana asclepiadea* root extract shows a pronounced hepatoprotective activity, which indicates the possibility of expanding the diversity of hepatoprotectors.

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P-181 Wound healing potential of extract and fractions of elderberry (*Sambucus nigra* L.) leaves

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Sambucus nigra leaves have been used in traditional folk medicine to treat wounds, burns, ulcers, and inflammation of the skin and eyes. However, its therapeutic properties on human skin cell models have not been investigated so far.

The aim of the research was to investigate the effect of 70% ethanolic extract and its fractions (dichloromethane, diethyl ether, ethyl acetate, n-butanol and water residue) on the inflammatory response of cells directly involved in the wound healing process (neutrophils, keratinocytes and fibroblasts). Physical

(UV radiation) and biological (bacterial derived products, TNF-α and interferon γ (IFN-γ)) factors were used to induce the inflammatory response. The potential effect on the regeneration of epidermal damage was determined using the in vitro scratch assay. Additionally, chemical composition of extracts and fractions was characterized.

The tested samples strongly inhibit the secretion of TNF-α and reactive oxygen species by human neutrophils. In addition, they reduce the secretion of chemokine 8 (IL8) by keratinocytes exposed to UV radiation and TNF-α/IFN-γ stimulation, and at the same time they increase the secretion of interleukin 6 (IL6) responsible for their migration and proliferation. The scratch assay showed that the samples increasing IL6 secretion by keratinocytes simultaneously improved their ability to migrate to the site of injury, whereas urolithin A (positive control), which reduces the secretion of IL6, lowers their migration. The inflammation induced on the fibroblasts was reduced by both the urolithin A and the extract and fractions.

Elderberry leaves may have a potentially beneficial effect on wound healing and skin inflammation.

P-182 The effects of novel telomerase activators on human adipose-derived mesenchymal stem cell (hAD-MSC) proliferation and osteogenic differentiation

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Recently, telomerase activator small molecules from natural sources have attracted attention owing to their great potential as therapeutic tools for healthy aging and preventing degenerative diseases. Also, these compounds have great potential in stem cell research since MSCs exert low/absent telomerase activity [1], which might be the overriding reason for the main drawback of their in vitro long-term manipulations. Our previous studies utilizing fungal biotransformation on *Astragalus* cycloartane-type sapogenins (e.g., cycloastragenol, astragenol) have afforded novel molecules with potent telomerase activation [2]. Some of these compounds (E-CG-01, E-AG-01, E-AG-02) were selected for further studies to evaluate their potential in stem cell research.

Our preliminary investigations on hAD-MSCs have demonstrated that these molecules increase proliferation by about 25–30% between the 2 to 300 nM concentration range. At the same time, cycloastragenol (CG; 2 nM), a well-known telomerase activator [3], and EGF (1.7 nM) resulted in a 20% increase. Additionally, the effects of the compounds on osteoblastic differentiation were undertaken. The results showed that CG derivatives E-CG-01, E-AG-01, and E-AG-02 ameliorated osteoblastic differentiation with increased calcification due to increased alkaline phosphatase (ALPase) activity. Higher ALPase activities were observed for E-AG-01 and E-AG-02 at lower concentrations (0.1 to 300 nM), whereas CG and E-CG-01 provided the same effects at higher concentrations (30 to 1000 nM).

Further studies are warranted to understand the positive effect of our compounds on MSC differentiation, whether it originates from only telomerase activation or regulation of other pathways.

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P-183 In vitro gastrointestinal biotransformation of a Devil's claw (*Harpagophytum procumbens*) extract

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Roots of the Devil's claw (*Harpagophytum procumbens* D. C., Pedaliaceae) have traditionally been used to treat arthritis and rheumatic diseases and their anti-inflammatory activity was attributed to the presence of iridoid glycosides, of which harpagoside (Fig. 1) is the main representative [1]. However, while hydrolysis of the glycosidic bonds of the iridoid glycosides is required for displaying anti-inflammatory activity [2], the final active compounds are unknown. Therefore, we aimed to determine the metabolic fate of a Devil's claw extract by means of an in vitro gastrointestinal model (GIM) [3]. 300 mg of H. procumbens extract was submitted to this model, which is comprised of a stomach, small intestine and colon phase. Samples were collected at different time points and experiments were performed in triplo. All samples were analyzed by UPLC-HRMS. An automated data analysis workflow allowed monitoring of the relative abundances of individual compounds over time.

The iridoid glycosides harpagoside and harpagide were identified in the 80% methanolic extract and were still present after passage through the GIM. However, a clear reduction of the tentatively identified iridoids pagoside and pagide could be observed after 48 h of colon fermentation. Also, various other iridoids were tentatively identified in the crude extract, as well as after the GIM experiment, including 8-O-(p-coumaroyl)-harpagide, procumbide and 6′-O-(p-coumaroyl)-procumbide. Levels of these compounds seem to reduce in particular during the small intestine and colon phases. Further data-processing is ongoing in order to derive more detailed information on the gastrointestinal biotransformation of the Devil's claw constituents.

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P-184 Phytochemical evaluation of *Meum athamanticum* Jacq, a traditional aromatic and liqueur plant of European mountains

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Background: *Meum athamanticum* Jacq is an alpine aromatic plant of the Apiaceae. The roots (Bärwurz) are used in German-speaking mountain population in Europe to make herbal liqueurs. Due to the previously confirmed high content of phthalides, it may possess beneficial pharmacological properties, largely unstudied. However, the content of these compounds is variable, and the geographic diversity is poorly known.

Aims: In this study, we analyzed the GC-MS phytochemical profiles of plants from a wild population on its north-eastern border of distribution (Sudeten Mts. in SW Poland) and attempted to isolate the phthalide compounds for pharmacological evaluation.

Results: The roots contained Z-ligustilide (> 80% of relative content in the extracts) is a predominant compound, accompanied by other minor phthalides and a fatty alcohol – falcarinol (10% relative content in the non-polar ex-

tracts). A simple silica gel column chromatography allowed for selective enrichment in phthalides to > 92% (87% Z-ligustilide, 2.7% E-ligustilide, 2.6% Z-butylidenephthalide) and subsequent isolation of the major constituent. Aerial parts contained less phthalides (up to 10% relative content) and were dominated by sesquiterpenoids such as spathulenol and α-farnesene.
In conclusion, the high content of Z-ligustilide was confirmed in all parts of the plant and proved feasible to simple isolation procedures from solvent extracts. A pharmacological application of this herb should be considered beyond its current popularity as alcohol beverage ingredient.
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P-185 A new tool to investigate the structural characteristics of sesterterpenoids in marine and terrestrial organisms

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The design of a relational database, based on highly controlled taxonomies and ontologies [1], that will form the core of a site open to interdisciplinary collaboration is presented [2,3]. In the logical scheme (▶ **Fig. 1**) the centrality of the organism concept in relation to the compound whose chemical classes, activities and extraction mechanisms are stored is highlighted. Bibliographic information covers the years from 1918 to 2022. The sesterterpenoids are classified based on their structural complexity, from linear pentaprenyl, to carbocyclic and heterocyclic ring-containing scaffolds. Marine and terrestrial organisms, including higher plants and insects are reported. Phylum Porifera is the richest source, yet the original producers are often believed to be their associated microbes, even if scarcely proved. Certain spongivore molluscs bioaccumulate or biotransform sponge-derived sesterterpenoids for their own defense, while a few molluscs and cnidarians produce them de novo. In environmental and human pathogenic microorganisms, the production of sesterterpenoids changes in response to stressors and dictates fluctuation in complex microbial communities. Bioactivities as antimicrobial, ichthyo- and phyto-toxic, nematocidal, anticancer, anti-inflammatory, and modulation of neurodegenerative processes are reported, as well as activity in the treatment of metabolic diseases such as type-II diabetes, hypercholesterolemia and obesity and as immunosuppressive molecules. For each compound information about the extraction procedure is provided, including pretreatment of the sample, solvent(s) of extraction and partitioning, and analytical technique. The database is built and tested with data provided by all co-authors. At present, over 350 living organisms (from 4 kingdoms) producing more than 1300 compounds have been catalogued.

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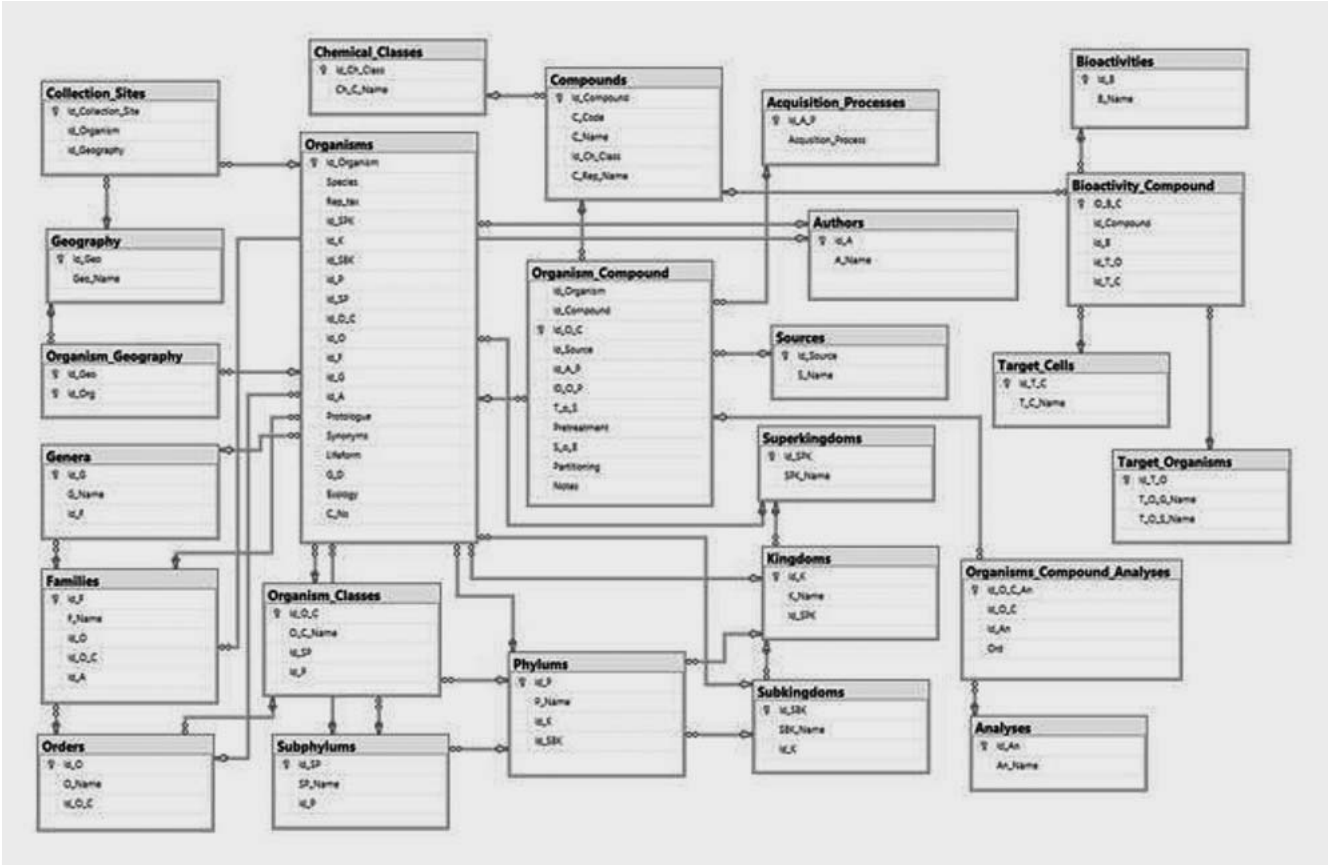

▶ **Fig. 1** Logic scheme of the proposed database.

P-186 Phytochemical Screening and In Vitro Micropropagation of sea-holly (*Eryngium maritimum*)

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Sea-holly (*Eryngium maritimum*) is listed in the Red Data Book of the Baltic Region and commercial collection of herbs in the wild is therefore prohibited. However, the plant has potential applications for herbal treatment, food consumption, and in cosmetics, which is the most important precondition for their commercial cultivation. Seeds are difficult to germinate, therefore, in vitro methods of micro-propagation are needed as an alternative to seed propagation. The species are poorly studied in the Baltic state's region and are not commercially grown. Therefore, the aim of the study was to develop methods for species propagation in vitro and subsequent adaptation ex vitro and cultivation in field conditions as well as to determine the phytochemical composition of the plant's aboveground and belowground parts. Experimental variants for the micropropagation included both different combinations of various cytokinins and auxins at various concentrations as well as various nitrogen source concentrations of the media. The composition of the medium significantly influenced the number of shoots. Shoots cultured on 0.5 mg/L meta-topolin and 0.1 mg/L Indole-3-acetic acid had the highest propagation rate. Various concentrations of Indole-3-acetic acid and nitrogen source were tested for rooting. Addition of auxin slightly increased the number of roots. *E. maritimum* revealed the presence of triterpenoid saponins, phenolic compounds, chlorogenic acid, rosmarinic acid and essential oil. The headspace gas chromatography mass spectrometry method was found to be the best choice as a rapid screening method to obtain results from fresh micropropagation plant samples, without complicated sample preparation. Research is funded by ERDF 1.1.1.1/19/A/083.

P-187 Wound healing activity of medicinal plants and their application in photodynamic therapy (PDT)

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Infected chronic wounds are difficult to treat, and often lead to heavy economic burden on health care systems. Bacterial resistance due to misuse/overuse of antibiotics is a challenge for infection treatment.

Photodynamic therapy works by light activation of a locally applied photosensitizing agent that triggers the release of reactive oxygen species and singlet oxygen, causing oxidative damage on cells and bacteria. Plants are sustainable sources of accessible treatment, especially in low-to-middle income countries. The first photosensitizers were natural compounds, but only few taxa have been explored for PDT application.

Therefore, this project aims to investigate the possible use of medicinal plants in the application of PDT for the treatment of infected wounds. The UV absorbance of crude extracts was assessed firstly. Anti-microbial test proved 4 medicinal plants, *Polygalae japonica* Houtt., *Morinda citrifolia* L., *Semiaquilegiae adoxoides* (DC.) Makino, and *Turpiniae arguta* Seem, having limited activity against two pathogenic microbes (▶ **Table 1**). In the ultraviolet-visible light (UV/VIS) spectrometry determination, only acetone extracts of *M. citrifolia* (AEMC) absorbed visible light. Dark cytotoxicity of AEMC was detected at a concentration of 10% on 4T1 and MRC-5 cells. With light irradiation, the cytotoxicity of AEMC increased on MRC-5 cells (5%). For the first time our study revealed light-activated cytotoxicity of the AEMC on 4T1 and MRC5 cell lines. Further exploration on the light cytotoxicity to bacteria is essential to study if the light enhances antimicrobial effect of *M. citrifolia* and its potential in treating infected wounds.

We have no conflicts to disclose.

▶ **Table 1** The MICs value of 4 medicinal plants on *S. aureus* and *S. pyogenes*.

Plant species	Minimum inhibitory concentration (mg/ml)	
	Staphylococcus aureus (ATCC25923)	Streptococcus pyogenes (ATCC19615)
Polygalae japonica Houtt	> 1024	512
Morinda citrifolia L.	1024	512
Semiaquilegiae adoxoides (DC.) Makino	1024	512
Turpiniae arguta Seem	> 1024	1024

P-188 Volatiles from all *Juniperus* trees growing wild in Greece

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Juniperus (Cupressaceae) comprise 8 species in Greece. The Lake Prespa region is one of the least areas with well-preserved forest habitats of Greek juniper (*J. excelsa*) in the Balkan Peninsula [1]

The seed cones, mostly of *J. communis*, are medicinally used as diuretic and for the relief of digestive disorders, while essential oils are widely applied in food and cosmetic industries.

We report in this study the volatiles (GC-MS) from leaves and cones of wild trees of *Juniperus oxycedrus* L. subsp. *deltoides, J. excelsa, J. foetidissima, J. communis* subsp. *communis* from Lake Prespa region (W Macedonia), together with *J. macrocarpa* and *J. phoenicea* from the island of Lesvos (N Aegean).

The results showed that in most of the studied *Juniperus*, oxygenated monoterpenes followed by sesquiterpenes appeared as the most abundant metabolites, except of *J. macrocarpa* which contained mainly sesquiterpenes followed by their oxygenated derivatives.

All studied essential oils belonged to α-pinene chemotype with amounts of α-cedrol, sabinene, limonene, myrcene, as the most abundant metabolites. Several differences in yields and chemical composition were detected comparing the studied samples. Furthermore, the obtained results in comparison with literature from *Juniperus* of different geographic origin showed qualitative and quantitative differences, which could be attributed to the unique micro-climate conditions of Prespa region and the volcanic island of Lesvos.

All samples have been evaluated for their total phenolic content, antiradical and antimicrobial activity showing that the leaves have higher phenolic content and antioxidant activity compared to the seeds and they will be potential further exploited.

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P-189 Phytochemical analysis and biological properties of *Heliotropium procumbens* from Panama

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Heliotropium (Boraginaceae) is a plant genus widespread, used in folk medicine for its healing properties (against inflammation, rheumatism, skin disorders etc), known source of pyrrolizidine alkaloids (PAs) and their N-oxides (PANOs), and phenolic metabolites [1].
In the framework of our phytochemical studies on Boraginaceae plants [2], we report herein the phytochemical analysis of *H. procumbens* aerial parts growing in Panama.
The LC-MS analysis revealed the presence of PANOs, among which three new natural products: helifoline, 9-angleloylheliotridine and hydroxy-platynecine as well as the known 7-angeloylheliotridine, senecionine, europine, lycopsamine and Intermedine. Additionally, the PAs riddelin, europine, lycopsamine, hydroxyplatynecine together with N, N-diferuylo-spermidine and several amino-alcohols were identified.
Moreover, 18 phenolic metabolites were identified (LC-MS), comprising of 5 flavonoids, 3 caffeic acid derivatives and 10 benzoic and cinnamic acid derivatives, among which luteolin-7-glucoside, rosmarinic acid and lithospermic acid have been isolated and structurally determined (NMR).
Antioxidant properties of methanol (ME) and water (WE) extracts were assessed via eight different assays (TPC, TFC, DPPH, ABTS, CUPRAC, FRAP, Metal chelating and Phospholybdenum). The extracts displayed considerable DPPH and ABTS scavenging activity and noticeable reducing power in CUPRAC and FRAP.
The inhibitory activity of the extracts was determined [2] against cholinesterases (AChE, BChE), agents with important role in the function of the nervous system, against α-amylase and α-glucosidase, a useful strategy for the management of obesity and diabetes, as well as against tyrosinase, a skin whitening factor.
H. procubens could be considered potential sources of bioactive metabolites exploited for further research or potential applications.
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P-190 Evaluation of honeybees' products from lake Prespa region-Greece

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Due to consumers' growing interest for natural and healthy foods, in connection with our continuous studies on bee-keeping products [1,2], we present in this study the pollen profile and chemical analyses of 11 honey samples, 9 propolis and 3 royal jellies (RJ) from the lake Prespa region, further evaluated for their bioactivities.
The lake Prespa (NW Greece) is the highest tectonic lake in the Balkan with a unique collection of flora including over 1,800 plant taxa. The pollinic spectra of all samples, showed an interesting and unusual combination mainly from Rosaceae, Fagaceae Fabaceae and Boraginaceae families, which could develop a specific labelling, as Country of Origin Labelling or Certificate of Specific Character for these products.
Among the volatile substances (GC-MS) of honeys, a high percentage of methyl- and propyl-benzene derivatives were the most characteristic constituents, some of them found before in Greek pine and thyme honey as well as in Rosaceae plants (*Prunus*).
The analyses of propolis samples, led to the classification of propolis of European type (rich in phenolic acids, flavonoids) while only one sample was rich in diterpenes, probably due to the endemic unique forest of Greek juniper in the Prespa's watershed. From this sample the diterpenes 7-oxodehydroabietinol, was isolated for the first time in propolis samples worldwide while has been previously identified in *Juniperus oxycedrus*. The studied RJs samples showed the typical profile of C10-acids, characteristic in all RJ according to literature [3].
All samples have been evaluated for their antioxidant (DPPH) and antimicrobial activity showing interesting results.
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P-191 Extension of Prenyl Side Chain of Flavanones as Tool to Prevent Cell Death due to Oxidative Stress

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Recently, a unique class of flavonoids, characterized by the presence of prenylated side chains, called prenylated flavanones, gain more and more attention in research. Since this group defines the second most abundant class of flavonoids, they may have a huge potential as therapeutic agents against multiple diseases [1]. Possibly prenylation of those substances could be related to an improved neuro-regenerative potential [2]. Anti-oxidative activity is a major issue related to neuroprotection. Therefore, the influence of the substitution of a prenyl, geranyl, or farnesyl group at position C-8 of the flavanone naringenin concerning cytotoxic and neuroprotective effects against oxidative stress in neuronal SH-SY5Y cells was investigated. Extension of the prenyl side chain revealed an increasing cytotoxic effect against SH-SY5Y cells. However, a neuroprotective effect was determined, when cells were preconditioned with those flavanones four hours before oxidative stress was induced by NO, released from sodium nitroprusside (SNP). Since neither pretreatment with naringenin nor 8-prenylnaringenin, but treatment with 10 µM 8-geranylnaringenin and 5 µM 8-farnesylnaringenin could prevent cell death after oxidative insult, the anti-oxidative effect of prenylated Flavanones may be related to the length of prenyl side chain. Consequently, the extension of the prenyl side chain of prenylated Flavanones is supposed to have major effects on the biological activity, which could be explained by changes in the substances lipophilicity, membrane attachment and transmembrane transport capability [3,4].
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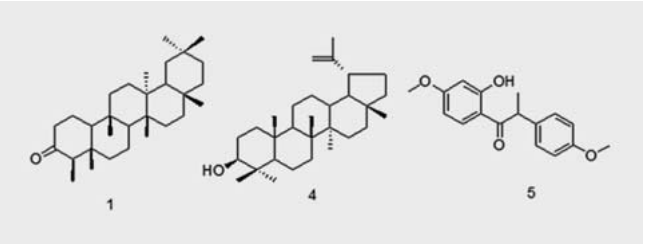

▶ **Fig. 1**

P-192 Phytochemical investigation of wild and cultivated *Pterocarpus angolensis*

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Pterocarpus angolensis (Family: Fabaceae) is an indigenous medicinal plant found in east and southern Africa [1]. *P. angolensis* is used for the treatment of stomach troubles, diarrhoea, fevers, mouth ulcers, nose bleeding, sore eyes, and skin problems [2]. The phenolic compounds of the *Pterocarpus* genus were found to exhibit antimicrobial, anti-inflammatory, and antioxidant activities [3]. Limited phytochemistry of *P. angolensis* was carried out; hence, further investigation is needed. In this study, the main focus was to isolate natural compounds from the stem bark of *P. angolensis* and determine their in vitro anticancer activities. The wild and cultivated powdered stem bark of *P. angolensis* was extracted sequentially with increasing polarity from hexane, dichloromethane, ethyl acetate, and methanol. The DCM crude extracts were loaded on column chromatography for fractionation. Fractions were collected based on the TLC profile and Rf values and further purified using the preparative TLC method. The stem bark of *Pterocarpus* afforded seven compounds namely, Friedelin (1), 3α-Hydroxy-2-oxofriedelane (2), 3-Hydroxy-2-oxofriedel-3-ene (3), lupeol (4), 4-O-methylangolensin (5) Tridecyl ferulate (6) and stigmasterol (7). The structures of the isolated pure compounds were elucidated and identified using spectroscopic methods such IR, MS, 1D and 2D NMR and by comparison of literature values. The crude extracts and isolated compounds were screened against breast cancer cell lines (MCF-7) and normal cells (Vero). Compound 5, wild CDM extract, cultivated DCM extract and wild hexane extract showed selective anticancer activities with LC50 values of 48.11, 44.30, 13.50 and 206.97 µg/mL, respectively.

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P-193 *Paeonia clusii* subsp. *clusii* seeds: phytochemical profile (LC-MS), q-NMR determination of paeoniflorin and biological activities

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Paeonia is the single genus in Paeoniaceae family, while *P. clusii* subsp. *clusii* is endemic on the islands of Crete and Karpathos in Greece [1]. The roots of many *Paeonia* species are highly valuable crude drugs in TCM [2]. In recent decades, a large number of publications have reported diverse biological activities including anti-inflammatory, analgesic, antivirus, CNS- and cardiovascular- protective effects [2].

The black fertile seeds of this species have been phytochemically studied for the first time. UHPLC-HRMS methodology led to the identification of 68 metabolites, including 24 monoterpene glycosides (with a common pinane skeleton), 11 oligostilbenes (resveratrol oligomers), 19 flavonoid derivatives and several phenolic compounds (gallic acid derivatives), iridoid glycosides, diterpenoids and triterpenoids. Furthermore, trans-resveratrol, trans-resveratroloside, viniferin, gnetin H, together with luteolin and luteolin-3'-glucoside, luteolin-3',4'-di-glucoside apigenin, hispidulin as well as the taxonomic marker of the genus paeoniflorin have been isolated and structurally determined by NMR.

Moreover, q-NMR methodology has been used to measure peoniflorin content in the examined extract and was found to be the most abundant metabolite (34.05%). Paeoniflorin is considered to be the most characteristic chemotaxonomic metabolite among peonies and has exhibited various pharmaceutical properties [2].

Furthermore, the total phenolic content has been evaluated (Folin-Ciocalteu method) showing high values (116.04 ± 2.6 mg GAE/g), which can be attributed to the rich phenolic profile, as well as the antioxidative capacity using the free radical inhibition assay (DPPH 43.47 ± 1.04 mg GA/g extract in concentration 100 µg/ml) indicating a potent antioxidant activity for this plant.

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P-194 Hydrolysable Tannin–Protein Interactions: The Devil Is in the Structural Details

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Tannins and their various bioactivities have been studied for decades because of the multiple bioactivities they express. Due to the high number of studies carried out with various methods, varying tannin and protein sources as well as variable tannin purities, it is difficult to combine these results and fashion them into a meaningful whole. Moreover, regarding bioactivity, the devil is in the details and small changes in tannin structure may drastically alter the obtained bioactivity.

Our ultimate goal is to be able to predict the bioactivities of not yet studied compounds directly from their structural features. In the present work, we chose altogether 32 tannins from multiple branches of the biosynthetic pathway of hydrolysable tannins to make conclusions on their protein precipitation capacity and the compositions of the formed complexes. For this, we utilised a turbidimetric plate reader method and ultrahigh performance liquid chromatography with diode array detection.

Our results indicated clear relationships between the structural features of hydrolysable tannins and their ability to form insoluble complexes with the model protein. In addition, the compositions of the formed complexes depended on the exact tannin structure. Altogether, our results highlighted the importance of the structural details of tannins to better understand their various bioactivities and possible use in different applications.

Authors declare no conflicts of interest.

P-195 Anti-inflammatory and Anti-diabetic Potential of *Echinoderm* Species from Mindanao, Philippines

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The island of Mindanao in the Philippines is a haven of marine resources. The abundance of echinoderm species serves as the motivation of the present study to discover new anti-inflammatory and antidiabetic natural sources

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from the sea. Methanol and hexane extracts of seven echinoderm species, namely, *Culcita novaeguineae* (Cn), *Marthasterias glacialis* (Mg), *Strongylocentrotus droebachiensis* (Sd), *Bohadschia argus* (Ba), *Acanthaster planci* (Apl), *Actinopyga palauensis* (Apa), and *Choriaster granulatus* (Cg) were subjected to bovine serum albumin (BSA) denaturation and α-amylase inhibition assays to evaluate their anti-inflammatory and antidiabetic potentials, respectively. At 25 µg/mL, all hexane extracts exhibited anti-inflammatory activity comparable to the positive control Naproxen (% inhibition of BSA denaturation = 90.75 ± 1.72) with Apl (% inhibition = 93.07 ± 1.35) leading. Among the methanol extracts, Ba exhibited the highest anti-inflammatory activity (% inhibition = 93.01 ± 2.29) and only Apa registered no inhibition. For antidiabetic activity at 25 µg/mL, the hexane extract of Sd (% α-amylase inhibition = 95.75 ± 0.32) exhibited the highest activity while those of Apl, Apa, and Mg showed no inhibition. All the methanol extracts demonstrated varying α-amylase inhibition with Apa registering the highest (% inhibition = 91.70 ± 0.32). Moreover, all α-amylase inhibitions were comparable to the positive control acarbose (Glucobay, % α-amylase inhibition = 73.01 ± 4.59.) The results are promising, but further studies need to be done to confirm these results prior to purification and isolation of bioactive compounds from these marine species.

The authors have no conflicts of interest in the conduct of this study.

P-196 Matrix free LDI-HRMS combined with MixONat assisted 13C-NMR dereplication: A chemometric approach to identify bioactive natural products from crude extracts

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DOI 10.1055/s-0042-1759172

Natural Products (NPs) are known for a wide range of interesting biological effects [1]. Avoiding the repetitive isolation of previously described NPs and time-consuming bioassay guided fractionation strategies, the early-on identification of active metabolites from complex mixture has become a key element of NPs research. Merging chemical profiling with biological data, chemometrics [2] represent a cornerstone of this strategy. In the process, Ultra High-Performance Chromatography (UPLC) coupled with High Resolution Mass Spectrometry (HRMS) is considered as method of first choice for data acquisition. Despite its indubitable assets, UPLC-HRMS may nevertheless encounter certain limitations linked to solvent limitation, ionizability of analytes and the differentiation of (stereo)isomers. Addressing these issues, matrix free laser desorption ionization-HRMS (LDI-HRMS) [3] assisted by 13C NMR dereplication (MixONat) [4] may provide alternative methodological approaches. As a working example, a holistic chemometric approach was applied using UPLC-qTOF versus LDI-HRMS assisted by 13C NMR for the identification of anti-AGEs NPs (ability to prevent the formation of advanced glycation end products [5]) from *Garcinia parvifolia* bark extracts.

The chemometric analyses permitted to highlight several markers as potentially active. While both, UPLC and LDI-MS, facilitated the detection of the same active markers into the PLS (loadings plot), MixONat confirmed the presence of rubraxanthone (1), isocowanol (2) and parvixanthone G (3). Subsequently, the anti-AGEs assay revealed a very good activity for these three compounds. Overall, our results showed that a joint LDI-HRMS and 13C NMR approach (▶ **Fig. 1**) provides a simple and efficient strategy for the chemical characterization of major active constituents in extracts.

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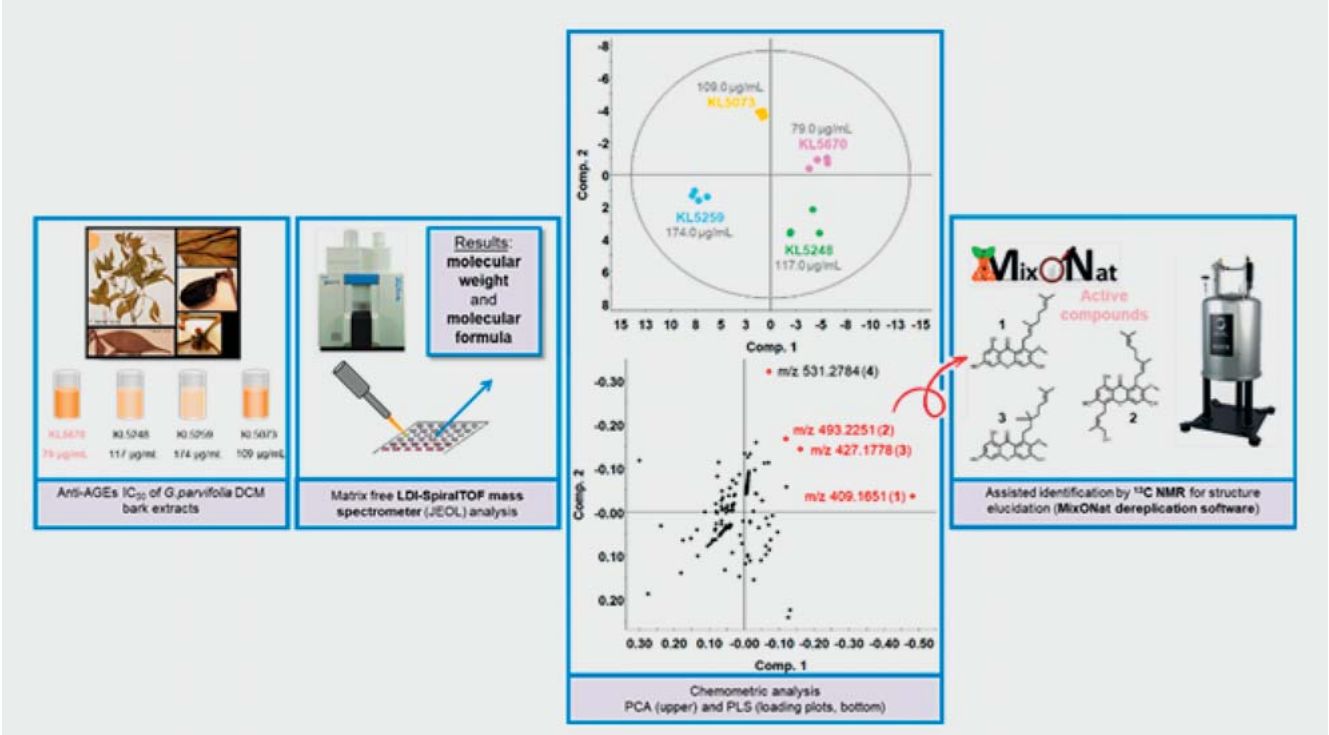

▶ **Fig. 1**

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P-197 Traditional Chinese Medicine: A path worth exploring in the fight against COVID-19?

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DOI 10.1055/s-0042-1759173

By the end of December 2019, a new coronavirus SARS-CoV-2 capable of causing pneumonia and respiratory failure emerged in Wuhan, China. The disease resulting from the virus was named COVID-19. [1,2] Despite the efforts to contain the spread of the virus, by March 2020, the WHO declared the world is facing a full-scale pandemic. TCM has been practiced for more than 5000 years and has gathered knowledge throughout the millennia in the fight against epidemic and pandemic threats. [3,4] In this study, 69 TCM entities, as well as eight formulas, were tested in vitro to observe any possible antiviral activity against SARS-CoV-2 using Vero E6 cells as host cells for the virus. In conclusion, four entities and a formula showed remarkable antiviral activities against SARS-CoV-2: *Moutan cortex, Glycyrrhizae* radix et rhizoma, *Armeniacae semen* amarum, *Cinnamomum ramulus*, and Qingfei Paidu Decoction. The Qingfei Paidu Decoction contains twenty-one herbs and not only such as: *Ephedrae herba, Glycyrrhizae radix, Armeniacae semen, Gypsum fibrosum, Cinnamomi ramulus, Alismatis* rhizoma, *Polyporus, Atractylodis macrocephalae* rhizoma, *Poria, Bupleuri* radix, *Scutellariae* radix, *Pinellinae* rhizoma, *Zingiberis* rhizoma, *Asteris* radix, *Farfarae* flos, *Belamcandae* rhizoma, *Asari* radix et rhizoma, *Dioscoreae* rhizoma, *Aurantii rructus* immaturus, *Citri reticulatae* pericarpium and *Pogostemonis* herba. These results support the idea of using TCM as an adjuvant treatment to the western ones in the fight against COVID-19 disease.

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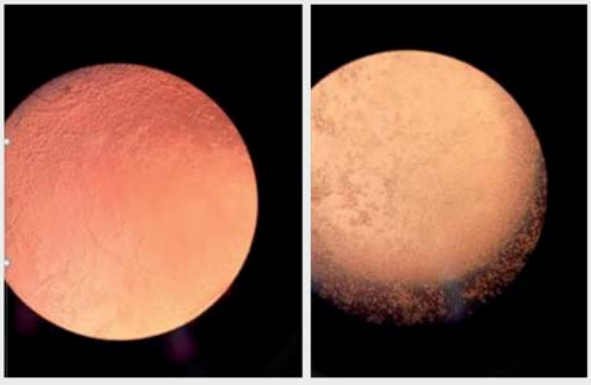

▶ **Fig. 1**

P-198 Bioaccessibility of *Salvia pratensis* L. phenolic compounds during in vitro gastrointestinal digestion

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This study aimed to determine the phenolic profile of the aerial part (SPA) and root (SPR) methanol extracts of *Salvia pratensis* L. and changes in the concentration of some identified compounds in these extracts during in vitro digestion process. A standardized static in vitro digestion method [1] was applied to simulate the oral, gastric, and intestinal phases of digestion of extracts followed by LC/MC analysis [2] in each phase of digestion. Using the UHPLC–MS4 Orbitrap analysis, 67 phenolic compounds were detected in SPA and SPR extracts. Among them, a total of 20 phenolic compounds were quantified in SPA, while 18 were quantified in SPR by UHPLC-DAD/(−)HESI-MS/MS analysis. The most dominant components in both extracts were rosmarinic and caffeic acids, whereas salvianolic acids A and B were found in high concentrations in the SPR extract. During in vitro gastrointestinal digestion of extracts, the content of total phenolics and flavonoids, as well as the antioxidant activity of extracts have not significantly changed compared with values determined initially. The results showed reduced bioaccessibility of rosmarinic acid in SPA and SPR, as well as salvianolic acid A in SPR during the simulated digestion process. However, digestion conditions did not affect the bioaccessibility of protocatechuic and p-hydroxybenzoic acid, while the concentration of caffeic acid increased during the intestinal phase of digestion. Considering the high diversity of phenolic compounds identified in extracts and high concentrations of some of the identified phenolic acids, *S. pratensis* may be used as a good alternative to the common sage.

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P-199 *Lysimachia vulgaris* L. aerial part and root methanol extracts as potential α-amylase and α-glucosidase inhibitors

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DOI 10.1055/s-0042-1759175

Anti-diabetic properties of many plants are documented so far, predominantly due to some polyphenolic compounds that can inhibit enzymes involved in the digestion of carbohydrates and significantly reduce the postprandial increase of blood glucose [1]. *Lysimachia vulgaris* L., commonly known as yellow loosestrife, is a medicinal plant traditionally used to treat ulcers, fever, inflammation, and diarrhea. Its antifungal, antibacterial, anticancer, and antioxidant properties have also been verified. The aim of this investigation was to evaluate the possible inhibition activity of α-amylase and α-glucosidase using aerial part (LVA) and root (LVR) methanolic extracts of *L. vulgaris*. The results showed that LVR has a significantly higher ability to inhibit both enzymes (IC_{50} = 146.34 µg/mL for α-amylase: IC_{50} < 31.25 µg/mL for α-glucosidase) in comparison with LVA extract. Furthermore, when compared to acarbose (IC_{50} = 340.13 µg/mL), a drug used as medicine for many years to treat diabetes mellitus type 2, LVR had approximately ten times higher inhibition activity of α-glucosidase. However, acarbose was significantly more effective in the inhibition of α-amylase compared with the tested extracts.

The obtained results are in correlation with our previous research which confirmed that LVR is richer in the phenolic compounds content in comparison with LVA. Due to the strong inhibition potential of LVR extract, further research may be on the identification and quantification of compounds in extracts responsible for the inhibition of these enzymes, as well as in vivo animal experiments for possible treatment of diabetes mellitus type 2 using *L. vulgaris*.

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P-200 Chemical characterization and leishmanicidal activity of *Araucaria* sp Brazilian brown propolis

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DOI 10.1055/s-0042-1759176

Brazilian brown propolis from *Araucaria* sp is composed predominantly by a mixture of acid diterpenes and displays a wide spectrum of biological activities, including antiparasitary [1,2]. Thus, the hydroalcoholic extract of Brazilian brown propolis (EBBP) was tested against promastigote forms of *Leishmania amazonensis*. The result revealed that EBBP is a rich source of potent leishmanicidal compounds since it displayed an $IC_{50} = 8.32 \pm 0.61\ \mu g \cdot mL^{-1}$ after 24 hours of incubation. Then, ten diterpenes were isolated from EBBP, identified and also biologically investigated but none of them showed promising leishmanicidal effect (IC_{50} values higher than 50 µM). Due to these results, only EBBP was evaluated at its IC_{50} concentration on intracellular amastigotes. After 48 hours of treatment EBBP reduced the internalization of parasites in about 58%. Morphological analysis of promastigotes by scanning electron microscopy treated with EBBP at $8.32\ \mu g \cdot mL^{-1}$ (IC_{50} concentration) revealed noticeable differences between the treated parasites and the control group, such as the losing of their fusiform morphology, and a significant damage in the cell membranes. In general, the results described herein pointed out EBBP as a promising natural source for further in vivo leishmanicidal investigation and bring news perspectives about synergistic studies between the main isolated diterpenes.

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P-207 13C NMR and MixONat software: Useful tools to help elucidate the composition of propolis samples collected in Benin and Congo

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DOI 10.1055/s-0042-1759177

Bees use propolis to seal and smooth out the internal walls of hives and as a protective barrier against fungal and bacterial infections. Propolis is a resinous natural substance collected by honeybees from buds and exudates of various plants, mixed with beewax and salivary enzymes. Therefore, its chemical composition is geographically dependent. Propolis are generally classified as "poplar-type" in temperate zones and "green Brazilian- ", "Clusia-", "Macaranga- " or either Mediterranean-type in tropical zones [1]. While flavonoids and phenolic acids are the major classes of compounds in propolis from temperate areas, tropical propolis, especially those from Africa, are often less well known. Though previous studies on West-African propolis exhibited anti-trypanosomal polyprenylated stilbenes (Ghana, [2]) or other bioactive prenylated isoflavonoids (Nigeria, [3]) there was no report on chemical composition of Beninese propolis.

The aim of this study was to determine the composition of propolis samples collected in different zones of Benin and in Congo and to evaluate their antioxidant and/or anti-AGEs activities.

The phytochemical composition of EtOH extracts from eight batches was studied using coupled chromatographic methods (GC/MS, HPLC/MS). The association of a 13C-NMR dereplication process using MixONat software and adapted databases allowed to characterize straightfully products in mixtures [4,5].

In addition to triterpenoids, one Beninese propolis sample exhibited an original composition with antioxidant stilbenoids/phenanthrenoids when another contained anti-AGEs prenylated and geranylated flavanones. Finally, resorcinols and phenols derivatives as well as triterpenes described in *Mangifera indica* were identified in the Congolese sample.

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Wednesday, August 31 | Poster Session II

- Chemistry and bioactivity of natural products (P-201 – P-206, P-208 – P-300B)
- Bioinformatics in natural products Drug Discovery (P-301 – P-309)
- Botanical products (Herbal Medicinal Products – Food supplements – Cosmetics – Botanical Medical Devices) (P-310 – P-361B)
- Natural products against respiratory infections (P-362 – P-371)
- Animal Health care (P-372 – P-385)
- Formulation – Pharmaceutical technology – Drug delivery systems (P-386 – P-400)

P-201 *Cardiospermum halicacabum* extract does not display glucocorticoid-like activity

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Cardiospermum halicacabum L. (Sapindaceae) is a medicinal plant used in European phytotherapy and in Ayurveda for the treatment of a variety of inflammatory diseases [1]. A recently published paper described a glucocorticoid-like activity of *C. halicacabum* extract similar to dexamethasone [2]. The aim of this study was to verify this effect. An ELISA assay was employed to assess

adrenocorticotropic hormone (ACTH) secretion in murine ACTH-secreting corticotroph cells AtT-20/D16v-F2, treated with *C. halicacabum* extract (CE). Dexamethasone was used as a reference glucocorticoid. Cell proliferation was assessed by colorimetric measurement of XTT. Proliferation of AtT-20/D16v-F2 cells was not suppressed by CE in a time-dependent manner (up to 96 h exposure). Treatment with dexamethasone (100 nM) reduced ACTH release (− 29 % as compared to control untreated cells), whereas treatment with CE (50 µg/ml) did not affect ACTH levels. Corticotropin-releasing factor (CRH; 100 nM) significantly stimulated ACTH secretion (+ 55 % vs. control untreated cells). Dexamethasone (100 nM) significantly reduced CRH-induced ACTH secretion (− 41 % vs. CRH treated cells), while CE (50 µg/ml) did not affect the ACTH release. Finally, ACTH secretion was impaired more markedly by dexamethasone after 48 h incubation than after 24 h, whereas it remained the same upon treatment with CE. Consequently, in our study CE up to a concentration of 50 µg/ml did not exhibit any significant glucocorticoid-like effect.

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

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P-202 Unfolding the chemical features and biological properties of honey: an integrated and comparative study

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Honey is a unique product that can be used directly from nature without any processing. Chemically, it is a saturated aqueous sugars solution enriched with a wide range of other constituents belonging mainly to phenolics, amino acids and proteins, vitamins, lipids, minerals, organic acids [1]. Nevertheless, honey is highly diverse and complex material of unexpected nature since its composition depends on several factors, such as botanical and geographical origin, environmental conditions but also bee species [2]. Furthermore, the medicinal importance of honey has been documented in the world's oldest medical literatures, and since ancient times, it has been known to possess among others, antioxidant, wound-healing, antibacterial and antiviral properties [3]. The aim of the current effort was the comparative study of honeys from Ikaria (Greece), Åland (Finland), Corse and Ouessant (France) using in parallel, different analytical methods i.e., HPLC-DAD, HPTLC, LC-HRMS, GC-MS and NMR. Another objective was the integration of obtained data towards the targeted isolation and identification of marker compounds specific for each honey as well as the evaluation of the tyrosinase, collagenase, hyaluronidase and elastase inhibitory and antioxidant properties of the individual phenolic extracts (non-sugar part). Furthermore, an attempt to correlate honey samples with honey plants (e.g., *Rubus fruticosis, Erica cinerea* & *Calluna vulgaris*), was carried out concerning both polar and non-polar constituents employing GC- and LC-MS.

The authors declare no conflict of interest.

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P-204 Pumpkin pulp extracts from a Serbian *Cucurbita maxima* breeding collection: profile of phenolics and in vitro bioactivity

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Pumpkins and pumpkin-based products are considered as functional food due to the presence of various bioactive compounds such as polyphenols, triterpenoids and carotenoids. Their consumption has been shown to confer multiple benefits on human health, such as anticancer and anti-inflammatory activity. Considering this fact, the phenolic profile of methanolic extracts of *Cucurbita maxima* Duchesne four accessions (Max 113, Max 118–1, Max 117, Max 1) and their cytotoxic properties were evaluated in a trophoblast cell line (HTR-8/SVneo) and three human cancer cell lines (JEG-3, JAR, and HeLa).

The phenolic compounds have been determined using LC/MS-MS technique [1]. The obtained results indicated that quinic acid was present in the highest amount in sample Max 113 (71 ng/mg), while amentoflavone was the most abundant phenolic constituent in the sample Max 1 (100 ng/mg). Furthermore, the results of the MTT assay showed that (▶ **Fig. 1**) extracts at 10 and 100 µg/mL significantly increased the viability of HTR-8/SVneo, JEG-3, and JAR cells, with most pronounced increase in the treatment with Max 113. Even at the highest concentration (1000 µg/mL), none of the extracts had a cytotoxic effect on any of these cell lines. However, in HeLa cells, the extracts did not exhibit significant influence on cell viability at concentrations of 10 and 100 µg/mL but showed modest cytotoxic effects at 1000 µg/mL. These findings indicate that selected pumpkin varieties could be suitable candidates in national breeding programs and thus contribute to the improvement of human diet and health.

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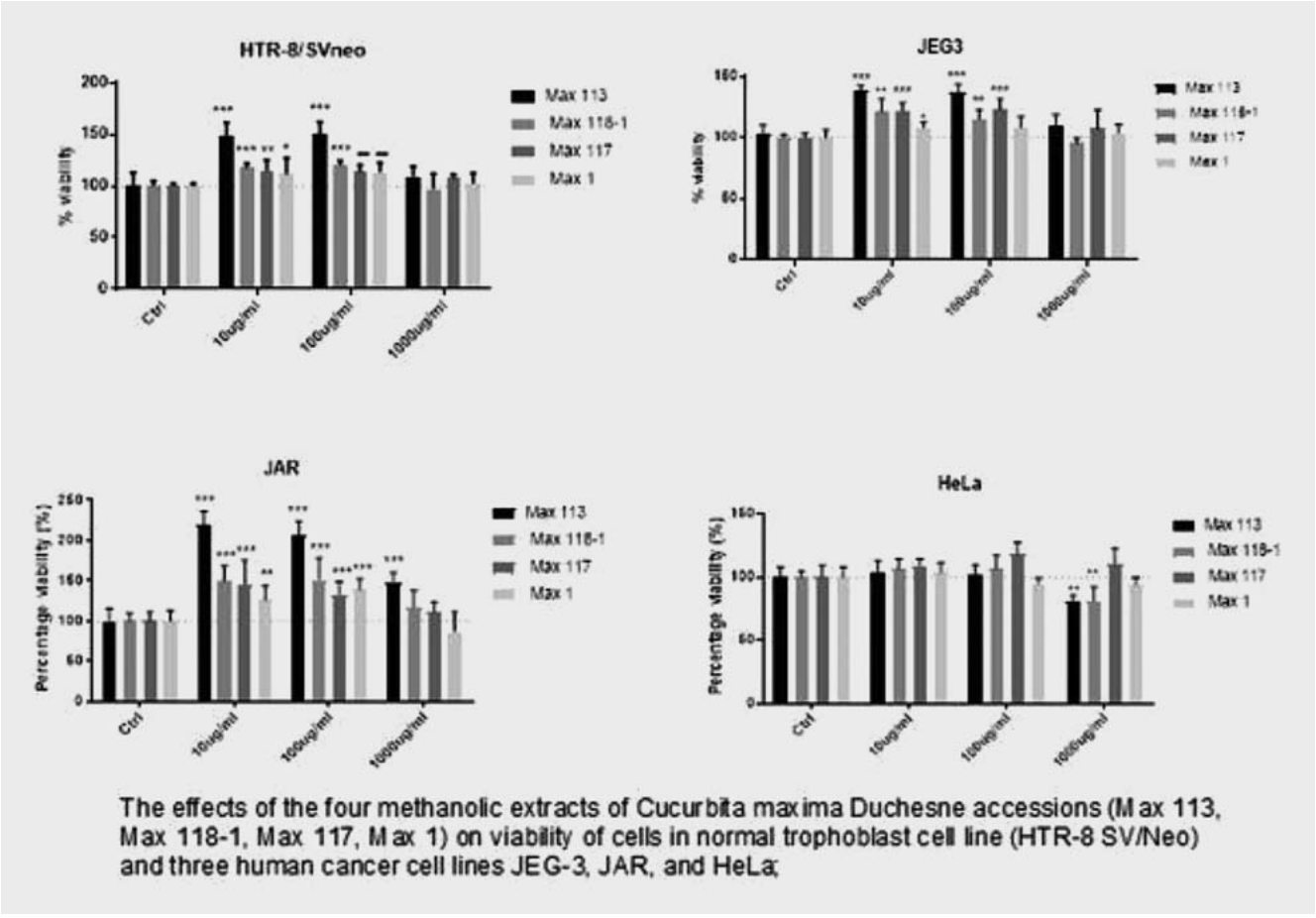
[1] Orčić D, Francišković M, Bekvalac K et al. Quantitative determination of plant phenolics in Urtica dioica extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. Food Chem 2014; 143: 48–53



▶ **Fig. 1** The effects of the four methanolic extracts of *Cucurbita maxima* Duchesne accessions (Max 113, Max 118–1, Max 117, Max 1) on viability of cells in normal trophoblast cell line (HTR-8 SV/Neo) and three human cancer cell lines JEG-3, JAR and HeLa.

P-205 Mycochemical profile and biological activity of edible mushroom species *Cyclocybe aegerita* (V. Brig.) Vizzini 2014

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In this study, phenolic profile and bioactivity of four extracts of edible *Cyclocybe aegerita* species originating from Novi Sad, Serbia, were evaluated. Bioactivity was assessed by a set of in vitro antioxidant assays and one antidiabetic assay. The antioxidant potential of chloroform ($CHCl_3$), methanol (80% MeOH), ethanol (70% EtOH) and water (H_2O) extracts were determined using several assays: DPPH and ABTS as well as Ferric Reducing Antioxidant Power (FRAP) [1], while α-amylase [2] was used in order to determine potential application of tested mushrooms in treatment of diabetes mellitus. The LC–MS/MS analysis [3] confirmed the presence of six phenolic compounds in examined extracts, among which quinic acid followed by hydroxybenzoic acids p-hydroxybenzoic acid and cinnamic acid were the most abundant. The highest amount of selected phenolic compounds was quantified in MeOH extracts, with the exception of quinic acid which was found in the highest amount in the water extract (485.89 ng/mg dry weight).

The MeOH extract showed the highest antioxidant potential, while the water extract stood out as the most promising sample with antidiabetic activity (891.52 ± 112.27 mg AKA eq/g dry weigh) what is in correlation with quantified phenolic compounds.

The results obtained in this study indicate that the *C. aegerita* extracts are an interesting source of bioactive compounds and possess remarkable bioactivity. Therefore, further research should be conducted in order to confirm their application in the food and pharmaceutical industry.

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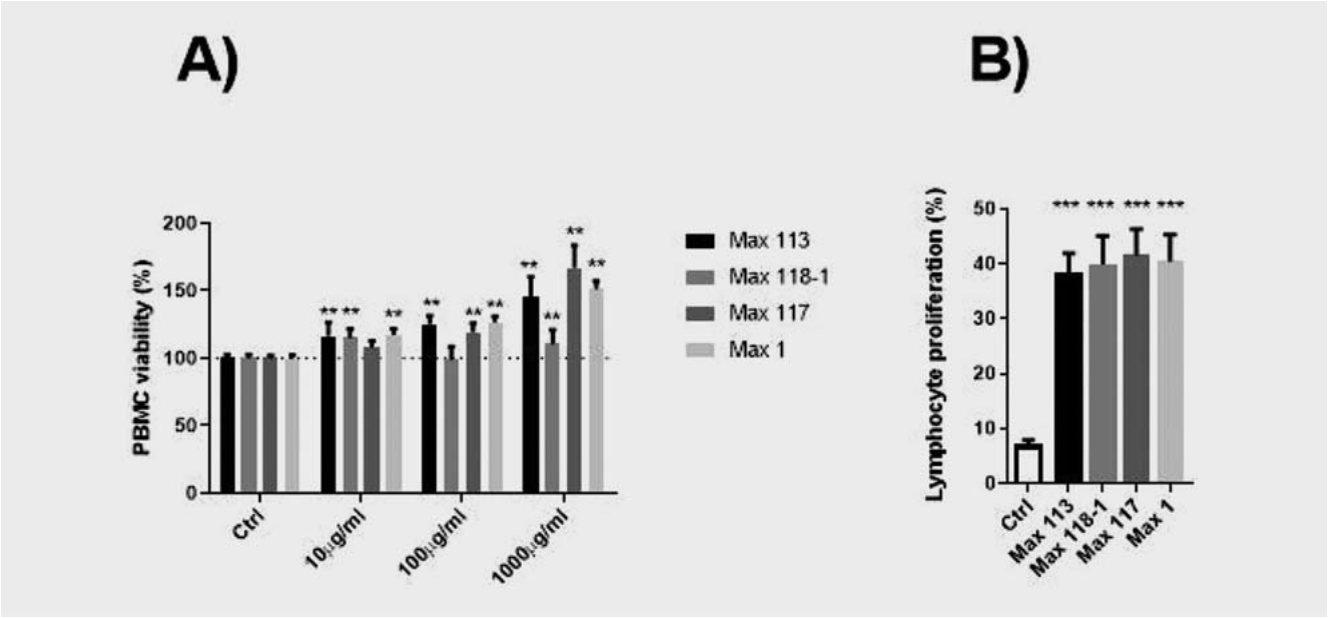

▶ **Fig. 1** The effects of the four methanolic extracts of *Cucurbita maxima* Duchesne accessions (Max 113, Max 118–1, Max 117, Max 1) on **A** human peripheral blood mononuclear cells (PBMC) viability and **B** lymphocyte proliferation.

P-206 The effects of methanolic pumpkin pulp extracts from a Serbian *Cucurbita maxima* breeding collection on human lymphocyte proliferation

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DOI 10.1055/s-0042-1759182

Although pumpkin extracts are associated with health-promoting properties, the effects of pumpkin pulp extracts have only barely been investigated. In this study, the effects of methanolic pulp extracts from four *Cucurbita maxima* Duchesne accessions (Max 113, Max 118–1, Max 117, Max 1) were evaluated for cytotoxic and anti-proliferative properties in human peripheral blood mononuclear cells (PBMCs) (▶ **Fig. 1**). PBMCs were isolated from 6 healthy donors and cultured with different concentrations of the pulp extracts (10,100 and 1000 µg/mL) for 24 hours, and an MTT assay was performed to evaluate cytotoxicity. For analysing lymphocyte proliferation, a carboxyfluorescein succinimidyl ester (CFSE) dilution-based proliferation assay was used after 72 h of cultivation with the extracts. The MTT results showed that all four extracts caused a significant increase in PBMC viability compared to control, and the increase was concentration dependent. The most profound increase was observed with 1000 µg/mL concentrations for all extracts. Moreover, the MTT results confirmed that even at high concentration none of the extracts had cytotoxic effect in PBMC. Furthermore, the results of the CFSE assay showed a highly stimulatory effect ($p < 0.001$) on lymphocyte proliferation and the magnitude of this effect was comparable between the four extracts. In conclusion, the findings reveal that the treatment with the four pulp extracts of Cucurbita maxima could increase viability in a dose-dependent manner and induce proliferation in human PBMCs, without exhibiting cytotoxicity at examined concentrations. These results indicate the safety of pumpkins pulp extracts and improve the overall current understanding of their potential health benefits.

P-208 Optimization of lavender distillation process by studying the essential oil's chemical composition and antioxidant activity

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DOI 10.1055/s-0042-1759183

Lavandula angustifolia (common name lavender) is a flowering plant of the Lamiaceae family and among the most valuable aromatic and medicinal plants for perfumery, cosmetics, pharmaceutical, agricultural and food industry [1].

Aim of the present study was to find the appropriate pilot scale distillation conditions that optimize the yield and pleasant aroma of the essential oil (EO) of lavender cultivated in northern Greece. Specifically, all steam distillations were conducted at the industrial distillery "Vessel Essential Oils" and process parameters were: time, temperature and pressure, implementing a Face-Centered Composite experimental design.

EOs' composition was determined by Headspace GC-MS analysis. Prior to the final analyses, pretreatment, sampling, chromatography and MS conditions were optimized. The in vitro antioxidant activity of EOs from all distillation conditions was evaluated via the inhibition of linoleic acid peroxidation assay, as well as through the soybean lipoxygenase inhibition study to investigate the anti-inflammatory activity [2,3].

With the applied GC-MS method, more than 35 volatile compounds were identified in the lavender's EOs, and their relative percentages were recorded. The desired space was determined by applying a three-dimensional response surface analysis of the independent and dependent variables, selecting as optimization criteria the EO's yield, along with the relative abundance of linalool, which is the primary compound responsible for lavender's pleasant fragrance.

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The authors declare that they have no conflict of interest.

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P-209 Optimization of distillation process parameters of oregano by determining bioactive aroma constituents and in vitro antioxidant activity

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Origanum vulgare (commonly known as oregano) is a widely used aromatic herb of the Lamiaceae family that has been growing in the Mediterranean region since antiquity. Oregano plants exhibit antioxidant and antimicrobial properties and have great economic value for perfumery, agricultural and food industry [1,2].

Aim of the present work was to optimize the conditions of the distillation process that maximize the yield of specific bioactive compounds of the essential oil (EO) of oregano cultivated in Greece. Steam distillation was conducted at a pilot scale at the industrial distillery "Vessel Essential Oils" and the selected parameters of the implemented Face-Centered Composite experimental design were: time, temperature and pressure.

EOs' composition was determined by Headspace GC-MS analysis. The in vitro antioxidant activity of EOs from all samples was measured in terms of the interaction with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) after 20 and 60 min [3,4]. Most prominent EOs were also tested as inhibitors of lipid peroxidation.

With the applied GC-MS method, more than 20 volatile compounds were identified in the oregano's EOs. Data processing showed that the factor that mainly affects yield, is distillation time. The desired space was determined by conducting three-dimensional response surface analysis of the independent and dependent variables, choosing yields of thymol and carvacrol as optimization criteria.

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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P-210 A comparative study of the antioxidant and free radical scavenging activity of *Prunella vulgaris* herbal substances

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The common selfheal, *Prunella vulgaris* L. [PV] is a pharmacopoeial medicinal plant [1], with a cosmopolitan distribution, diverse and contradictory ethnobotanical data on the used morphological parts and their application as a folk remedy.

The aim of the study is to evaluate the antioxidant capacity of herbal substances *Prunellae* herba [PVh] and *Prunellae* spica [PVs] from Bulgarian populations.

Plant material from 14 bulgarian localities of PV, representatives of the main of habitat types, was studied. The total phenol content (Folin-Ciocalteu reagent), the radical scavenging (DPPH•) and antioxidant ($O_2^{\bullet-}$) properties of water and ethanol extracts of PVh and PVs were determined.

The obtained data for the water extracts show higher radical activity and antioxidant activity for PVh compared to PVs, corresponding to the established total phenolic content (mean 44.54: 13.71 mg PG/g dw sample). Ethanol extracts show higher activity compared to water and higher antioxidant activity for PVh (40% and 60% ethanol extract).

The obtained results show three main types of localities have been identified: highland, typical and anthropogenic. In connection with an analysis of the Bulgarian localities, 6 model populations of Prunella vulgaris were selected. A population from the Pirin floristic region (1740 m above sea level) with valuable qualities (antioxidant activity) has been determined – a potential source of biologically active substances and plant material for research and cultivation.

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The authors declare that they have no conflict of interest.

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P-211 Chemical and biological evaluation of plants from the Greek flora towards their use as cosmetics

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Greece is known for its unique biodiversity, consisting of approximately 6.000 species of higher plants. Of these, ~ 15% of the plant taxa are endemic making Greece an area of high conservation priority [1,2]. Due to its wealth, there is an increasing interest in the study of the Greek flora, since a great variety of bioactive compounds can be obtained [3]. The aim of this study was to evaluate the chemical content and the biological properties of various plants of the Greek flora for their potential use in cosmeceuticals. 52 plant materials from different areas were selected, collected and extracted using tow techniques i.e., Supercritical Fluid Extraction (SFE) & Accelerated Solvent Extraction (ASE). The chemical profiling of the extracts was performed with LC-HRMS and HPTLC. Dereplication based on high resolution mass spectra gave infor-

mation about the composition and possible bioactive compounds of the extracts. Furthermore, total phenolic content, antioxidant activity, cytotoxicity and UV-protective activity of the extracts on human skin fibroblast cultures were analyzed. Most extracts (59%) were not cytotoxic for human skin cells, while the rest were used in the photoprotection assay at the highest non-cytotoxic concentration. Until now 10 extracts have been identified combining antioxidant with photoprotective activity. In conclusion, based on the chemical content and in correlation with the biological profile of these extracts, they could serve as an excellent base for the development of novel and high-quality cosmetics.

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P-212 Plants of the Greek Flora as photoprotective cosmeceuticals

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Exposure to solar ultraviolet (UV) radiation is the main environmental factor in the development of hyperpigmentation, photoaging, photosensitivity and skin cancers [1,2]. The use of medicinal plants and their extracts as an efficient nature-based strategy to protect against skin pathologies is growing. Many of these extracts have phenolic compounds, such as flavonoids, with well-established photoprotective or/and anti-photocarcinogenic activity against UV-induced damages [3]. The great diversity of Medicinal and Aromatic Plants (MAPs) growing in Greece offers vast possibilities for the discovery of novel photoprotective compounds of natural origin [4]. In view of the above, in this work, a variety of extracts from plants collected from the wild or cultivated in Greece, were biologically evaluated for UV-protection and antiaging activity against a panel of healthy human epidermal cell lines. Results so far indicate that certain extracts from mountain tea and olive leaves have suitable properties to be used as cosmeceuticals, as they have low to non-existent toxicity and strong antioxidant activity. Most importantly, they exhibit significant photoprotective effects against UVA-induced cell damage with >40% cell viability recovery of irradiated fibroblasts. The assessment of the photoprotective effects of the extracts against UVB-induced cell damage is currently in progress. Overall, the experimental results clearly indicate the great potential of the under-study extracts to act as sun photoprotective agents for cosmetic applications.

The authors declare no conflict of interest.

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P-213 Evaluation of *Sideritis* species as agents for food supplements

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According to the World Health Organization, 80% of the general population in developed countries use supplements to support specific physiological functions. Natural products are a rich source of molecules that can be effectively used in dietary supplements, as a means of promoting health, relieve and prevent [1,2]. The current study aims to investigate the chemical content of three *Sideritis* water extracts, specifically *S. scardica, S. perfoliata* and *S. raeseri* and evaluate their potential to be used in food supplements. The samples were treated according to predefined conditions. The plant materials were dried, pulverized and water extracts were prepared. HPLC-DAD, HPTLC, and LC-HRMS were used for analysis and chemical markers were identified. The extracts showed many similarities concerning their chemical profile and most differences were quantitative. Phenolic acids, like 5-caffeoylquinic acid and p-coumaric acid 4-O-glucoside and phenylethanoid glycosides, like forsythoside B and verbascoside were detected. The major metabolites of the extracts were acetylated flavonoids glycosides, such as isoscutellarein 7-O-[6‴-O-acetyl]-allosyl(1 → 2)glucoside, 4′-O-methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl (1 → 2)glucoside and isoscutellarein 7-O-[6‴-O-acetyl]-allosyl(1 → 2)-[6″-O-acetyl]-glucoside. The extracts were fractionated and purified with preparative column chromatography and prep-HPLC and the structures of the isolated compounds were identified with NMR. The extracts were evaluated for their antioxidant activity, showing IC_{50} values in the DPPH assay less than 50 µg/ml. In conclusion, *Sideritis* species provide water extracts rich in bioactive constituents that can be used in the production of food supplements.

The authors declare no conflict of interest.

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P-214 Unlocking Nature's Pharmacy: Effects of processing on chemical composition and biological activity of *Echinacea purpurea*

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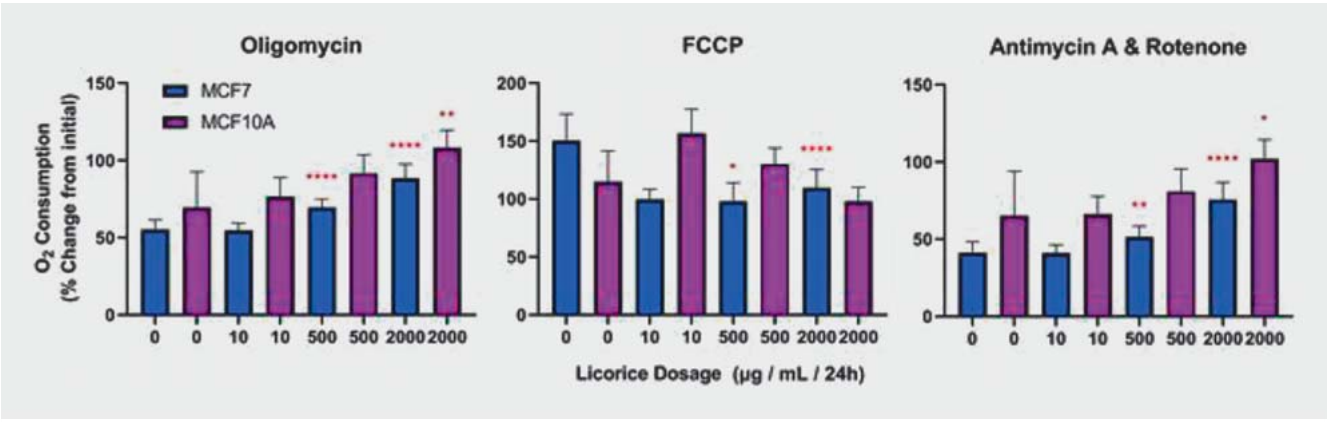
Quality, safety, and efficacy of traditional herbal medicinal products (THMPs) are essential requirements for their registration as THMPs in the EU under Directive 2004/24/EC. Many factors affect quality, including biotic and abiotic. In addition, factors associated with post-harvest storage and processing can also affect metabolomic fingerprints, and hence biological activity of THMPs. In the current study we have investigated the effects of age and processing on the metabolomic fingerprints of tinctures of *Echinacea purpurea*, one of the top selling herbal medicines used to treat common cold symptoms, sore throats and infections of the upper respiratory tract. Tinctures of *E. purpurea*, were prepared in our laboratories with plant material grown in the midlands of Ireland. Two-, and three-year-old plants were collected. Fresh and air-dried plant material was investigated. Plant material was chopped or shredded, and all



▶ **Fig. 1** Changes in O_2 consumption after administration of mitochondria modulating drugs in liquorice pre-treated cells.

samples were prepared as tinctures, which were evaluated after 6 weeks and again after 18 weeks. Samples were analysed by HPLC and ^{1}H NMR. Distinct qualitative and quantitative differences were found between fresh and dried tinctured samples and between tinctures that were prepared from chopped and shredded material. Two-year-old *E. purpurea* contained a higher concentration of chicoric acid than three- year-old plant material. *E. purpurea* that had been chopped contained a higher concentration of chicoric acid in contrast to the shredded plant material. All the tinctures were non-toxic to and inhibited the production of IFN-β in vitro in the iBMDM (immortalised bone marrow derived macrophage) cell line. IL-6, TNF-α and Rantes, were not affected.

P-215 Licorice Root Ameliorates Drug Induced Mitochondrial Stress in MCF7 and MCF10A Cells

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Liquorice (*Glycyrrhiza* spp.) is used in Chinese medicine to reduce the potential toxicity of other herbal medicines [1], but its mechanism is not fully understood. We investigated these effects on mitochondrial function in vitro.

MCF7 (cancer) and MCF10A (control) cells were treated for 24 hours with liquorice tea extract (Pukka Herbs; UK) at low (10 µg/mL), medium (500 µg/mL) and high (2000 µg/mL) dosages. A SeaHorse MitoStress test [2] measured O_2 consumption while four mitochondria modulating drugs were injected at specific intervals:

- Oligomycin: ATP Synthase inhibitor
- FCCP: Uncoupling agent
- Antimycin A & Rotenone: Complex III and I inhibitors

Liquorice reduced the influence of all four drugs in both cell lines (▶ **Fig. 1**). MCF7 cells showed a concentration dependent effect. Cell viability, reactive oxygen species and membrane potential showed no significant changes, except an increase in membrane potential in MCF7 cells.

Previous studies reported liquorice protected cells from antimycin A [3] and rotenone [4,5]. We confirm this and also show that liquorice can protect mitochondria from Oligomycin and FCCP, suggesting that this tea extract can maintain mitochondrial function in the presence of a broad spectrum of cytotoxins. This may have implications for the use of liquorice in drug regimens that affect mitochondrial function, potentially increasing tolerance or reducing efficacy of the drug. Further assessment of liquorice treated cells exposed to drugs could help to elucidate this mechanism and effects on patient populations.

The author (SW) receives funding from Pukka Herbs. The researchers declare no conflict of interest.

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P-216 Exploring the biotechnological value of *Corema album* leaves

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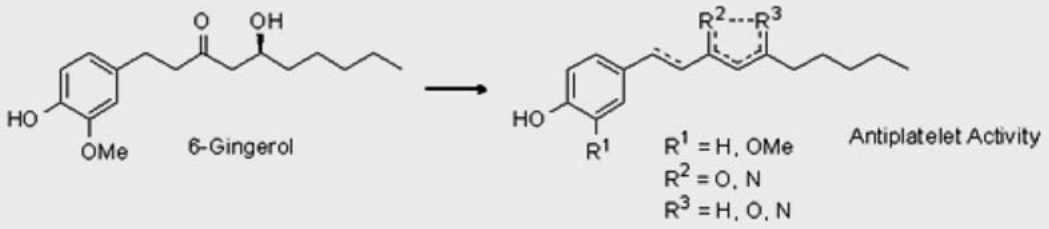
Corema album is an halophytic perennial shrub commonly known as "Camarinha", with edible fruits traditionally used in popular medicine as an antipyretic. Recent studies evaluated biological activities of natural extracts from *C. album* and confirmed their medicinal properties, namely in terms of protection against oxidative stress related diseases [1,2]. Aiming at the biotechnological valorization of this species, this work prepared ethanol and acetone extracts from its leaves, and evaluated them for total content of phenolic compounds, radical scavenging activity through DPPH and ABTS methods and for inhibitory capacity towards tyrosinase, an enzyme related with hyperpigmentation and food oxidation. The total content of phenolic compounds was higher in the ethanol extract (536.4 mg GAE/g) than in the acetone one (260.5 mg EAG/g). Ethanol extract showed higher antioxidant capacity by neutralizing DPPH (IC_{50} = 0.116 mg/mL) and ABTS (IC_{50} = 0.168 mg/mL) radicals than the acetone (DPPH, IC_{50} = 0.280 mg/mL; ABTS, IC_{50} = 0.347 mg/mL). The ethanol extract also exhibited the higher tyrosinase inhibition (IC_{50} = 2.844 mg/mL). Our results suggest that the ethanol extract from *C. album* leaves could be further explored as a source of polyphenolic compounds with antioxidant and tyrosinase inhibition properties, with possible uses in the food and pharmaceutical industries.

The authors declares that there is no conflict of interest.



▶ **Fig. 1** 6-Gingerol and its synthesized derivatives.

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P-217 *Cakile maritima* seeds as a source of bioactive compounds

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DOI 10.1055/s-0042-1759192

Cakile maritima Scop. (sea rocket), is an edible halophyte plant with several medicinal uses, such as antiscorbutic, digestive, and diuretic. Sea rocket contains several nutrients, including vitamins, fatty acids, and minerals, and displays relevant functional properties, such as in vitro antioxidant and neuroprotective [1,2]. Its seeds are rich in phosphatidyl- choline, a potential pharmaceutical ingredient especially in the treatment of neurological and liver diseases [2]. Having in mind the biotechnological valorization of sea rocket seeds, this work evaluated ethanol and acetone extracts for total phenolic content, in vitro antioxidant capacity (radical scavenging on DPPH and ABTS), neuroprotection (inhibition of acetylcholinesterase, AChE) and depigmentating/food preservative properties (tyrosinase inhibition). The ultrasound ethanol extract showed the highest AChE activity (75.84%) and total phenolic content (389.76 GAE/g DW). The overnight ethanol extract had the highest radical scavenging activity on DPPH (IC_{50} = 3.66 mg/mL) and ABTS (IC_{50} = 2.8 mg/mL). No relevant tyrosinase inhibition was detected. Our results suggest that sea rocket seeds could be further explored as a source of polyphenolic enriched extracts with antioxidant properties and AChE inhibition.

The authors declare that there is no conflict of interest.

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P-218 6-Gingerol Derivatives as Promising Antiplatelet Leads

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Ginger (*Zingiber officinale*, Roscoe) is a widely used spice since ancient times, and there are many reports about its roots' constituents, mainly gingerols and shogaols, for their beneficial bioactivities in health and disease [1,2]. In this context, gingerols were reported to have promising antiplatelet activity [3]. In this work, 6-gingerol's structure was used as a core for the synthesis of derivatives (▶ **Fig. 1**) that were tested for their inhibitory activity against AA-induced platelet aggregation and COX-1, and for their antioxidant activity. In silico ADME and docking studies were also performed. Ligand lipophilic efficiency (LLE) was used as a scoring function towards the best lead. The natural compound 3 showed the most promising antiplatelet activity with an IC_{50} of 2.1 µM, while a semisynthetic new compound 17 had an IC_{50} value of 3.1 µM. These results were supported by COX-1 IC_{50} values of 4.36 and 5.84 µM for compounds 3 and 17, respectively. Further, the lowest in silico binding affinity, i.e., − 9.5 kcal/mol was calculated for compound 17. The LLE results, however, pointed out compound 11 as our best lead for further development.

The authors declare no conflict of interest.

Acknowledgements

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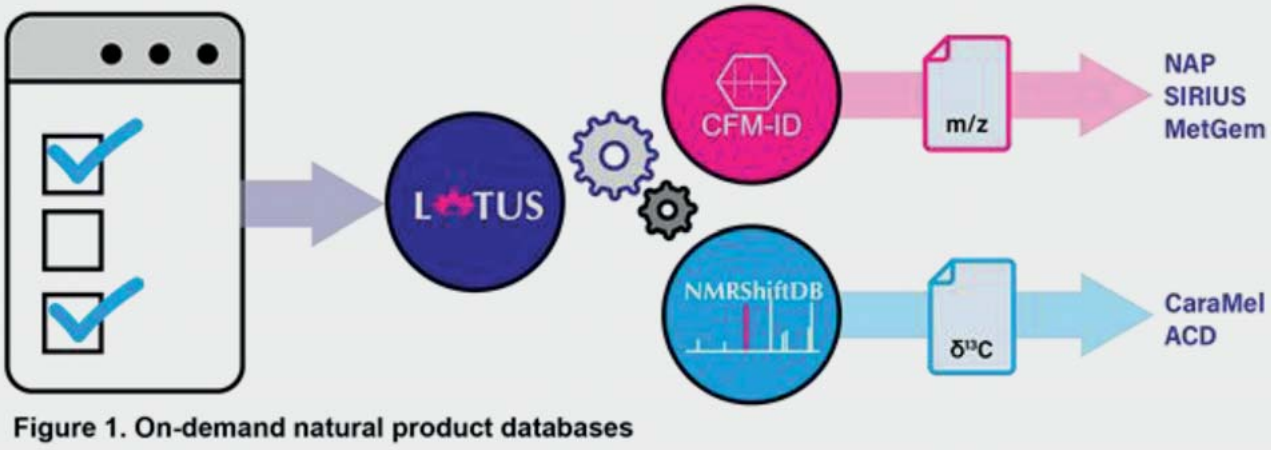

▶ **Fig. 1** On-demand natural product databases.

P-219 Assisting 13C NMR and MS/MS joint data annotation through on-demand databases

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Compound identification in complex mixtures by NMR and MS is best achieved through experimental databases (DB) mining. Experimental DB frequently show limitations regarding their completeness, availability or data quality, thus making predicted database (e.g., ISDB, PNMRNP) of increasing common use [1]. Querying large databases may lead to select unlikely structure candidates. Two approaches to dereplication are thus possible: taxonomical filtering (either biological or chemical) of the DB before search or taxonomical scoring of the results after a large-scale search [2]. The present work relies on the former approach. The corresponding dereplication tool involves the selection of the structure set of interest from the largest available structural DB and the prediction of the associated NMR and MS spectral data (▶ **Fig. 1**).

As far as we know, NMRshiftDB2 is the only open-source 13C NMR chemical shift predictor that can be freely operated in batch mode [3]. CFM-ID 4.0 is one of the best-performing open-source tools for ESI-MS/MS spectra prediction [4]. LOTUS is a freely usable and comprehensive collection of secondary metabolites [5]. It can select compounds according to substructure, chemical class, or taxonomical source. Integrating the open-source database and software LOTUS, CFM-ID, and NMRShiftDB2 in a dereplication workflow requires presently programming skills, owing to the diversity of data encoding and processing procedures. A graphical user interface that integrates seamlessly database building and spectral data prediction still does not exist, to the best of our knowledge.

The present work proposes a coherent software tool that assists secondary metabolites specialists to identify mixture components in a simple way.

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P-221 Unlocking Nature's Pharmacy: An Electrophysiological Assessment of The Impact of Cannabis-Derived Terpenes on Acute Seizure Activity In Vitro

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Herbal medicines have been used to treat epilepsy for centuries. *Cannabis* derived cannabanoids have received attention for their anti-epileptic activity. This study investigates the acute anti-epileptic potential of terpenes found in the Cannabaceae family of plants.

Extracellular local field potential (LFP) recordings, elicited using the proconvulsant 4-aminopyridine, were employed to measure seizure like events (SLEs). Terpenes under examination were added to the circulating artificial cerebrospinal fluid. And the effects of each terpene on seizure duration (SD), first spike amplitude (FSA) and spectral power density (SPD) were analysed.

Linalool, myrcene, and β-caryophyllene produced significant alterations in the seizure parameters measured. Linalool (300 µM) significantly reduced both SD ($n = 7$, $P < 0.01$) and SPD ($n = 7$, $P < 0.05$). β-caryophyllene (100 µM) also produced a significant reduction in SD ($n = 10$, $P < 0.05$), SPD ($n = 10$, $P < 0.05$) and FSA ($n = 10$, $P < 0.01$). Finally, myrcene was observed to significantly reduce SD at both concentrations tested (10 µM $n = 7$, $P < 0.05$; 30 µM $n = 7$, $P < 0.05$). D-limonene, α-humulene and α-pinene all failed to produce a significant change in any of the parameters measured.

These studies demonstrate that the terpenes linalool, α-caryophyllene and myrcene commonly found in cannabis 'cultivars' are capable of acutely reducing seizure like activity in an in vitro model of seizure generation. This anticonvulsant activity may be attributable to modulation of glutamate neurotransmission, voltage-gated sodium channels or cannabinoid type-2 (CB2) receptors. Future work will aim to reveal the mechanistic nature of the effect of the terpenes observed in this current study.

P-222 Exploration of phytochemical content of cultivated *Stachys iva* Griseb

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Genus *Stachys* L. consists of 300 species as annual or perennial herbs or small shrubs. In terms of phytochemistry, the plants of the genus are rich in constituents with therapeutic and economic applications. In continuation of our research on this genus [1,2], we report herein, for the first time, the phytochemical investigation of the essential oil (EO) and the dichloromethane-methanol extract of cultivated *Stachys iva* Griseb., by the means of different analytical techniques. 1D and 2D NMR experiments were used for the identification and characterization of the chemical components of the extract. The EO was obtained by hydrodistillation and was analysed by GC-MS technique. Overall, thirteen compounds were revealed from the studied extract, including two iridoids, two phenylethanoid glycosides, and nine flavonoids. Moreover, a comparative study between the dichloromethane-methanol extract and the previously studied polar extract [2] was conducted. Regarding the chemical content of the EO, the main constituents were geranyl-α-terpinene, caryophyllene oxide and epi-α-bisabolol. In conclusion, the dichloromethane-methanol extract of the cultivated *S. iva* contains a variety of natural products, with pharmacological interest. As far as we know, this study is the first report on the chemical composition of the EO of the specific species.
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P-223 In vitro cercaricidal activity and phytochemical profile of *Vernonia britteniana* root

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DOI 10.1055/s-0042-1759197
Schistosomiasis is a neglected disease in tropical and subtropical countries. Angola, despite the significant improvement of some of its global health indicators, still has a high incidence of neglected diseases like schistosomiasis. In some regions, medicinal plants are the most available or even the only treatment to this disease in rural communities. *Vernonia britteniana* Hiern (Asteraceae), root is one of the medicinal plants identified by us as used in Angolan traditional medicine to treat schistosomiasis. Our study aimed to evaluate the in vitro cercaricidal of two extracts (water, WE) and hydroethanolic 70%, HE70) made with this herbal medicine. Additionally, the phytochemical profile of these extracts was also accessed. The cercaricidal activity was evaluated against *Schistosoma mansoni* cercariae, exposed to different concentrations of each extract (500, 438, and 125 µg/ml) and observed at 30, 60, 90, 120, and 150 min. Praziquantel (10 µg/ml) was used as positive control and 1% of DMSO+water and/or deionized water as the negative control. The chromatographic profile was assessed by LC-UV-MS. Both extracts showed cercaricidal activity, with 100% cercariae mortality at 500 µg/ml after 30 min, and an LC50 of 438 µg/ml, after 120 min ($p < 0.05$). Chlorogenic acid, caffeic acid, 3,5-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquic acid) and vernosides A, B and D were identified for the first time in this medicinal plant and they are the main compounds of both extracts. Our results reinforce the therapeutic usefulness potential of the flora of the studied region.

P-224 Graphical polyphenol analysis: a tool to visualize chemotaxonomic and other patterns

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Polyphenols and other phenolic compounds are under an increasing interest because of their health benefits and medicinal applications. In plants they have an important role in defence chemistry against herbivores, pathogens, UV radiation and other threats. New bioactive compounds are found on new plant species, and there is a growing need to screen increasing amounts of species to reveal their polyphenol content. As analytical methods become more efficient, the data handling becomes considerably more challenging. New methods to analyze and visualize large data sets are needed.
We have created a tool to present differences in polyphenol content and bioactivities between plant samples, species, genera and families. Our tool creates contrast for every studied bioactivity and polyphenol group at one chart, which allows us to distinguish unique patterns for every taxon at one glance.
To test our tool, we studied the variability of chemically diverse plant species. The variation level was measured within 31 populations and seasonal variation over three growing seasons. Eight polyphenol groups were measured using the group-specific UPLC-DAD-MS/MS method [1,2] and two most important bioactivities were tested with the oxidative activity assay [3] and the radial diffusion assay [4].
Our tool helps to visualize, how the combination of qualitative and quantitative data of the polyphenol groups and related bioactivities can effectively discriminate species. For some of the species, their variation caused some noise in the visual tool, but that was overcome by careful selection of the parameters to be visualized.
We have no conflicts of interest to disclose.
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P-225 Effects of *Vitis vinifera* leaf extracts on inflammation and vascular permeability

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Wine production leads to large amounts of by-products, such as leaves, which are rich in flavonoids that are effective in venous insufficiency [1,2]. Venous insufficiency results from venous hypertension and leads to edema, skin lesions and eventually venous ulceration. It is characterized by increased vascular leakage, inflammation factors (IL-6, TGF-1), adhesion molecules (sICAM, sVCAM) and metalloproteases (MMP2, MMP9). Here, we investigated the effect of *Vitis vinifera* leaf extracts on inflammation and vascular permeability. Human endothelial (EA. hy926) and fibroblast (DLF) cell lines were pretreated

with six leaf extracts (1 µg/ml). Inflammation was induced by TNA-α (100 ng/ml). Twenty-four hours following TNF-α stimulation, the levels of secreted IL-6, TGF-β1, sVCAM, sICAM and MMP9 levels were quantified with ELISA. The two most effective extracts were tested in the carrageenan (2 % w/v)-induced paw edema model and the VEGF (50 ng)-induced vascular leakage model in mice, using the EVANS blue method. The aqueous extract E13 and the enriched phenolic fraction E15 were the most effective in reducing TNF-α induced IL-6, TGF-β1, sICAM, sVCAM and MMP9 in both EA. hy925 and DLF cells. Also, E13 (30 mg/Kg) and E15 (30 mg/Kg) reduced carrageenan-induced edema and E13 (30 mg/Kg and 100 mg/Kg) significantly reduced vascular leakage induced by VEGF. In summary, the aqueous extract E13, effectively reduced inflammation and vascular leakage in vitro and in vivo suggesting it could be further evaluated in humans suffering from venous insufficiency.
The authors declare no conflict of interest.

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P-226 Phytochemical screening, in vitro antioxidant and antimicrobial activity of *Periploca chevalieri*, an endemic medicinal plant from Cabo Verde

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Periploca chevalieri Browicz (Apocynaceae) is an endemic species of Cabo Verde. The aerial part of this medicinal plant is used to treat cough, diabetes symptoms and as cardiotonic. This study focused on the phytochemical screening, antioxidant activity and antimicrobial activity of *Periploca chevalieri*.
The phytochemical screening of aqueous and hydroethanolic (70 %) extracts was made through TLC, and major class of compounds (total phenols, flavonoids, and condensed tannins) were estimated colorimetrically. The antioxidant activity was determined through the assessment of the reduction capacity (CUPRAC and FRAP) and radical scavenging capacity (DPPH assay). The antimicrobial activity was determined by the broth microdilution method (range of concentrations between 156.25 µg/mL and 2500 µg/mL), against both Gram-positive and Gram-negative bacteria.
The hydroethanolic extract presented the highest content in phenolic compounds (415.19 ± 1.14 mg equiv. gallic acid/g dry extract), flavonoids (0.794 ± 0.011 mM equiv. catechin/g dry extract) and condensed tannins (35.7 ± 0.03 mg equiv. cyanidin chloride/g dry extract). It also displayed great antioxidant activity by the DPPH assay with an IC_{50} of 23.9 ± 0.5 µg/mL. Regarding the antimicrobial activity both extracts presented in vitro antibacterial activity against the Gram-positive bacteria being the *Bacillus cereus* (MIC = 312.5 µg/mL) and *Staphylococcus aureus* MRSA (MIC = 625 µg/mL) the most sensitive ones to the hydroethanolic extract.
P. chevalieri extracts exhibited antioxidant and antimicrobial activity suggesting its potential as a source of phytochemicals with useful pharmacological activity.

P-227 Phytochemical, pre-clinical safety and efficacy evaluation of two Portuguese *Asphodelus* leaf extracts

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Asphodelus bento-rainhae subsp. *bento-rainhae* (Ab), an endemic species with relevant interest due to conservation concerns and *Asphodelus macrocarpus* subsp. *macrocarpus* (Am), are commonly known by the Portuguese name "abrótea" and their leaf (AbL, AmL), has been traditionally used for the treatment of ulcers, urinary and inflammatory disorders.
In this study, hydroethanolic extracts (70 %) of dried leaf of both species were prepared and the main classes of secondary metabolites, including, total phenolic, flavonoid, anthraquinone, condensed and hydrolysable tannin and terpenoid contents were detected and quantified by spectrophotometric methods. Liquid-liquid (L-L) partition of crude extracts were obtained using ethyl ether (AbL-1, AmL-1), ethyl acetate (AbL-2, AmL-2) and water (AbL-3, AmL-3). Phytochemical screenings of all extracts were conducted using LC/ESI/MS and LC/UV-DAD co-chromatographic techniques. Moreover, in vitro determination of antioxidant activity by FRAP and DPPH assays and preliminary genotoxicity/carcinogenicity by Ames test were performed.
Twelve compounds, namely, neochlorogenic acid, chlorogenic acid, caffeic acid, ρ-coumaric acid, ferulic acid, isoorientin, isovitexin, luteolin, diosmetin, aloe-emodin, chrysophanol and β-sitosterol were identified as major constituents in both species. Among the species, AbL fractions showed stronger antioxidant activity in comparison to AmL fractions and among the fractions, AbL-2 (IC_{50}: 0.8 mg/mL) and AmL-2 (IC_{50}: 1.2 mg/mL) exhibited the highest activity when compared to all the other fractions. No genotoxicity/mutagenicity potential of crude extracts (up to 5 mg/plate, with/without metabolomic activation) was observed in both species.
The obtained results will contribute to quality evaluation and validation of the studied *Asphodelus* species and their future use as herbal medicines.

P-228 Evaluation of in vitro hypoglycemic activity of *Periploca chevalieri*, an endemic medicinal plant from Cabo Verde

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Natural sources of α-glucosidase and α-amylase enzyme inhibitors present an interesting therapeutic approach in the treatment of postprandial hyperglycemia by decreasing the carbohydrate digestion rate and have the potential to prevent the development of diabetes mellitus type 2. The aerial part of *Periploca chevalieri* Browicz (Apocynaceae), an endemic species of the Cabo Verde archipelago, is used in traditional medicine for the treatment of diabetes symptoms. The aim of this study was to evaluate the pre-clinical antidiabetic potential of aqueous and hydroethanolic (70 %) extracts prepared with this medicinal plant and to characterize the main secondary metabolites that could be involved in this activity.
The chemical characterization of both extracts was made through LC/UV-DAD. The α-amylase and α-glucosidase inhibitory potential of these extracts were investigated through colorimetric methods.

Based on the obtained LC/UV-DAD data and using co-chromatographic techniques with correspondent reference standards, 3-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, quercetin-3-O-galactoside and catechin were identified as the major secondary metabolites present on both aqueous and hydroethanolic extracts of *P. chevalieri*. All the extracts inhibited α-glucosidase and α-amylase enzymatic activities in a dose-dependent manner. For α-glucosidase, the detected inhibitory activity (IC_{50} value 0.093 ± 0.002 mg/mL) was significantly higher than that of acarbose (4.20 ± 0.18 mg/mL).
Obtained results revealed that both extracts have the potential to decrease postprandial hyperglycemia, corroborating the traditional use of *P. chevalieri* in the management of diabetes mellitus type 2.

P-229 Brazilian propolis: a multifaceted natural product that modulates HIF-1 pathway – new perspectives for cutaneous wound healing and regeneration

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The ultimate outcome of skin wound healing is repair, a form of incomplete regeneration characterised by scars proportional to the duration and severity of inflammation. However, recent evidence suggests that the pharmacological stabilisation of the transcription factor HIF-1α can evoke a regenerative phenotype [1]. Brazilian propolis elicits multifaceted activities on the wound microenvironment, counteracting the drawbacks of inflammation while modulating HIF-1 pathway, thus being a candidate to trigger the cellular regenerative program.
The aim of this study was a comprehensive in vitro evaluation of green and red Brazilian propolis biological activities on human keratinocytes (HaCaT) and dermal fibroblasts (HDF).
Following an initial phytochemical characterisation, the ability of propolis to impair NF-κB-driven transcription was evaluated upon TNF-α or IL-1β stimulation, showing a greater activity of red propolis (IC_{50} < 10 µg/mL in HaCaT, IC_{50} = 23.6 µg/mL in HDF), prominent also on TNF-α-induced IL-8 secretion (IC_{50} = 11.89 µg/mL in HaCaT, IC_{50}=5.89 µg/mL in HDF). The investigation of HIF-1α stabilisation and nuclear translocation suggested the activity of both propolis on HaCaT, while only red propolis was active on HDF. These results have been confirmed through immunofluorescence (▶ **Fig. 1**), western blot, and qPCR of HIF-1 target genes. Finally, a bioguided fractionation has been performed to better clarify the chemical species responsible for the biological activities.
The results of this study contribute to elucidate the molecular mechanisms behind the promising activity of Brazilian propolis in wound healing, shedding light on the possible exploitation of these natural products in skin regeneration.
The authors have no conflicts of interest to declare.

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P-230 Potent Anti-staphylococcal Metabolites in *Salvia miltiorrhiza*

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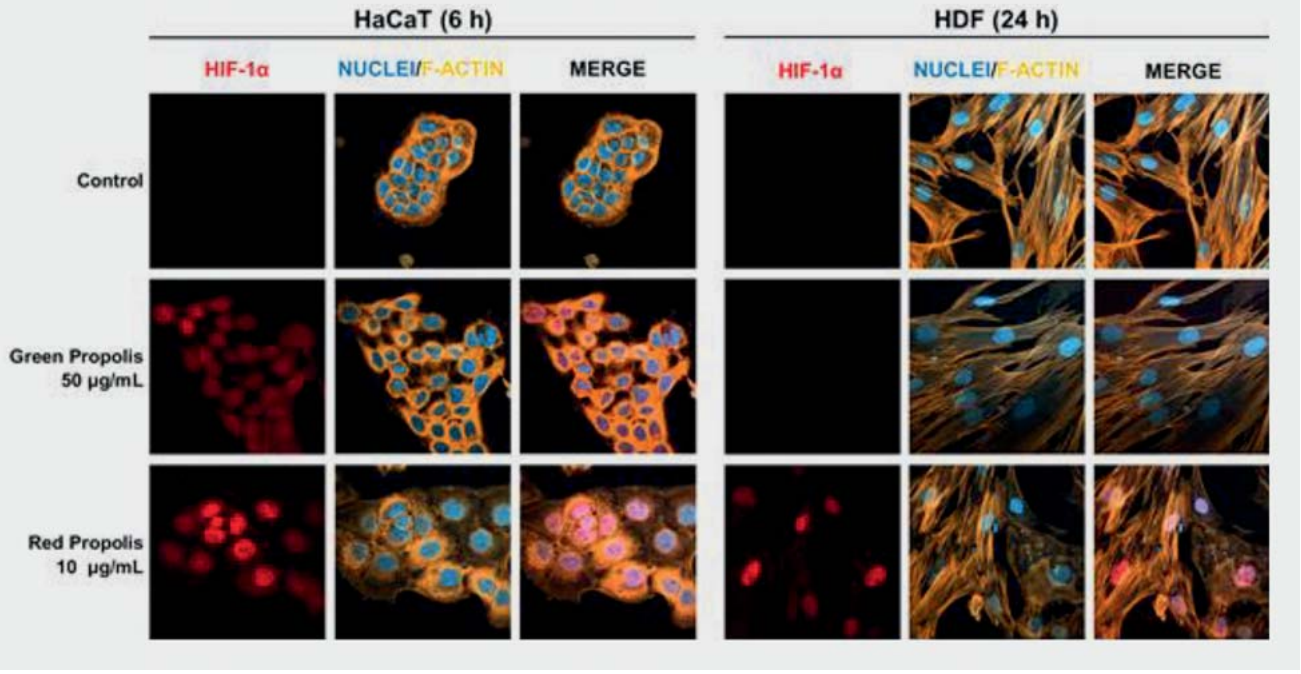
Development of novel agents to treat infections caused by multidrug-resistant bacteria is an urgent priority. Medicinal plants are a recognised source of diverse bioactive compounds with capacities to tackle such organisms [1]. The root of *Salvia miltiorrhiza* Bunge (red sage or Danshen, Lamiaceae) has been used traditionally to treat coronary heart diseases, although *S.miltiorrhiza* extracts have also been shown to possess antimicrobial activities against a range of pathogenic organisms [2,3].
The aim of this study was to investigate metabolites from *S.miltiorrhiza* previously uncharacterised in terms of antimicrobial activities and potential to negate efflux-mediated resistance in Staphylococcus aureus. These metabolites were tanshinone I, tanshinone IIA, cryptotanshinone, dihydrotanshinone, salvianolic acid A, Miltirone, protocatechuicaldhyde, rosmarinic acid, caffeic



▶ **Fig. 1** Representative confocal micrographs of immunofluorescent staining with anti-HIF-1α, DAPI (nuclei), and phalloidin (F-actin).

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acid and danshensu. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBC) assays were determined against *S. aureus* ATCC25 923, SA-1199B+NorA (expressing the NorA efflux pump) and XU212 +TetK (expressing the TetK efflux pump). Miltirone and dihydrotanshinone had the lowest MIC values; 0.5–1 µg/mL. The MBC values of Miltirone for *S. aureus* ATCC25 923 was 2 µg/mL, for SA-1199B+NorA and XU212+TetK was 4 µg/mL. However, the MBC value for dihydrotanshinone was > 128 µg/mL for all *S. aureus* strains, indicating bacteriostatic activities. At sub-inhibitory concentrations, both metabolites 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. The MIC of the other metabolites ranged between 512–64 µg/mL.

Overall, evaluation of these compounds indicates that they could be drug leads in managing staphylococcal infections.

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P-231 Enhancing natural products annotation in dual ^{13}C-NMR and LC-HRMS^2 based complex mixtures chemical profiling through custom in silico databases

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The chemical profiling of plant extract usually involves a dereplication step commonly based on LC-HRMS^2 or NMR. The high sensitivity of MS provides numerous but sometimes incorrect candidates whereas the low sensitivity and the high universality of NMR lead to less but more accurate annotations. Despite their complementarity, both analytical techniques are rarely used in combination. This study focuses on the chemical profiling of the bark of *Larix decidua* through both LC-HRMS^2 and NMR data analysis (▶ **Fig. 1**). In a first time AcOEt crude bark extract was fractionated by Centrifugal Partition Chromatography (CPC). In a second time the 12 fractions of decreasing polarity were analyzed both by LC-HRMS^2 and by ^{13}C NMR, in order to benefit from advantage of both techniques (sensibility, universality resp.). Data were analyzed in parallel workflows. On one hand, pre-treated LC-HRMS^2 data (MZmine 3) [1], were submitted to the Ion Identity Molecular Network workflow (including NAP and MolNetEnhancer) [2,3] and additionally annotated via SIRIUS4 [4]. On the other hand, ^{13}C NMR data was subjected to the CaraMel workflow [5]. The whole annotation process was realized using in silico spectral database restricted to compound reported in Pinaceae plant family. Databases were generated via an in-house graphical interface based on LOTUS NMRShiftDB and CFM-ID. Thus, this work shows how the combination of analytical techniques, and the use of custom database can support chemical profiling of complex mixtures and increase the annotation confidence.

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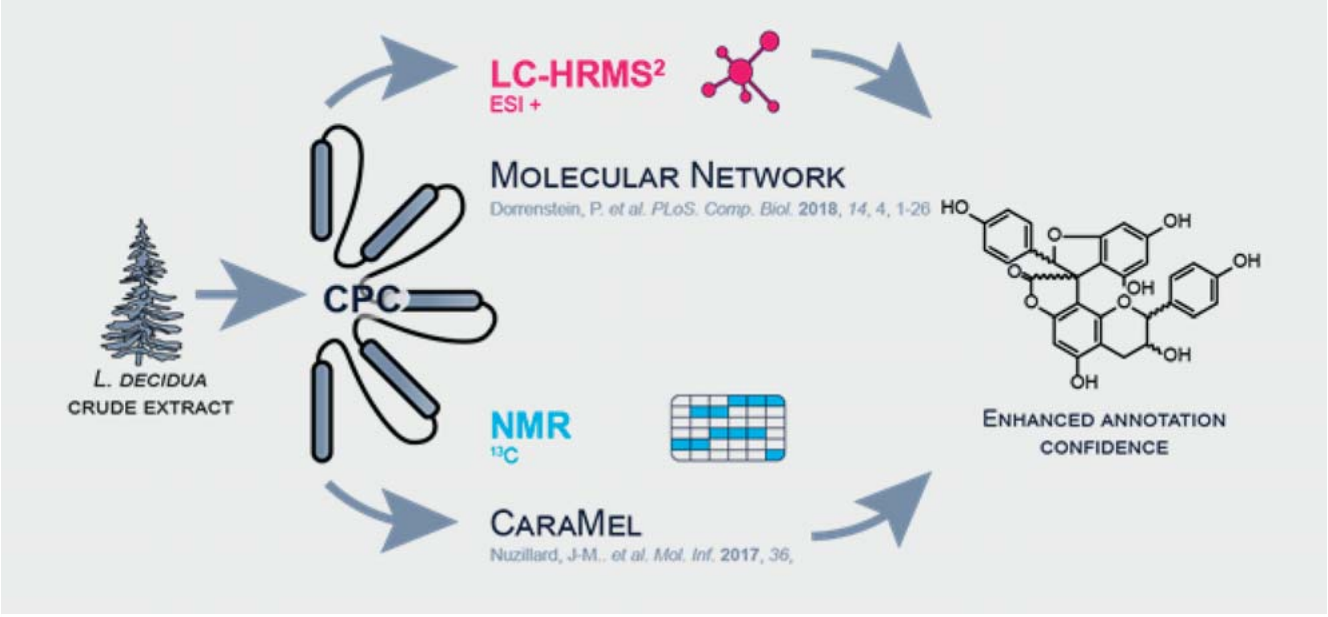

▶ **Fig. 1** *L. decidua* crude extract chemical profiling workflow.

P-232 Volatiles of *Capparis cartilaginea* Decne. from Saudi Arabia

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Capparis cartilaginea (CC), a medicinal plant of the family Capparaceae grows abundantly in wild arid regions of Saudi Arabia. The plant is a xerophytic perennial plant with a remarkable adaptability to harsh environments typical for the Arabian Peninsula. It has been used in traditional Arabian medicine for inflammation, earache, headache, bruises, snakebite, and childbirth [1]. The aim of this study was the analysis (using GC and GC/MS) of the chemical composition of the essential oil hydrodistilled from the leaves of *Capparis cartilaginea* and the comparison of the composition of the oils from a number of *Capparis* taxa (present study and literature data by using multivariate statistical analyses (MVA), viz., agglomerative hierarchical cluster analysis (AHC) and principal component analysis (PCA).

The analysis of the essential oil obtained from the leaves of Saudi Arabian *Capparis cartilaginea* Decne enabled the identification of 41 constituents, comprising 99.99% of the total oil composition. The major compounds identified were isopropyl isothiocyanate (31.0%), 2-methyl-2-butenenitrile (21.4%), Isobutyronitrile (15.4%), and 3-methylbutanenitrile (8.2%). Other classes of compounds identified include monoterpenes (1.9%) and esters (0.6%). The chemical composition of the derived oil and 12 additional oils obtained from selected Capparis taxa were compared using Multivariate Statistical Analyses (Principal Component Analysis and Agglomerative Hierarchical Cluster Analysis). The results of the statistical analyses distinctly separate CC from other members of its genus on the basis of its components and their relative amounts. Moreover, environmental, and geographical stressors may be implicated in the essential oil profile of plants found within the genus Capparis.

P-233 In vitro growth-inhibitory effects of phytochemicals and their synthetic analogs against intestinal bacteria associated with colorectal cancer

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Colorectal cancer (CRC), defined as an adenocarcinoma of large intestine, is the second most deadly cancer that caused 0.9 million deaths in 2020 worldwide [1]. China and the United States have the highest estimated number of new cases [2]. Gut dysbiosis is one of the factors associated with an increased risk of developing intestinal cancer. Various phytochemicals and their synthetic analogs (e.g., quinoline derivates) have been found to inhibit gut pathogenic microorganisms [3], however, their effect on CRC associated microorganisms has not been determined yet. Therefore, the aim of this study was to test in vitro growth-inhibitory effects of ten substances (berberine, bismuth subsalicylate, ferron, 8-hydroxyquinoline, chloroxine, nitroxoline, salicylic acid, sanguinarine, tannic acid, and zinc pyrithione), together with six conventional antibiotics (ceftriaxone, ciprofloxacin, chloramphenicol, metronidazole, tetracycline, and vancomycin) against CRC-causing pathogens (*Clostridium septicum, Escherichia coli, Fusobacterium necrophorum, Fusobacterium nucleatum, Peptostretococcus anaerobius* and *Streptococcus bovis*) using broth-microdilution method assessing minimum inhibitory concentrations (MIC) [4,5]. Nitroxoline (MICs = 8–16 µg/ml), zinc pyrithione (MICs = 4–32 µg/ml) and chloroxine (MICs = 4–64 µg/ml) have been found to be the most active substances. *E. coli* and *S. bovis* were the most susceptible bacteria with MICs ≥ 4 µg/ml. These findings indicate that 8-hydroxyquinoline alkaloids and coordination complexes of zinc are chemical structures with potential to inhibit growth of pathogenic gut microorganisms associated with CRC development. We declare no conflict of interest.

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P-234 Characterization of the type of interaction between terpenoids and cannabinoids compounds of hemp plant against MDA-MB-231 cancer-cells by isobologram analyses

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Scientific interest in phytocannabinoids research is currently experiencing a significant increase with the numerous evidences that support their therapeutic potential. Beside the psychoactivity of tetrahydrocannabinol (THC), other cannabinoids such as Cannabidiol (CBD) or Cannabigerol (CBG) display interesting effects on cancer progression via interactions with the endocannabinoid system [1]. Additionally, hemp contains other compounds such as mono/sesqui-terpenes (β-Caryophyllene) that have also been shown to exert anticancer actions [2]. The aim of this study is to characterize the type (antagonistic, synergistic and additive) of effects between CBD/CBG/terpenes on the triple negative breast cancer cell line (MDA-MB-231). The method of isobologram analysis was used for appraising the type of interactions between hemp compounds [3]. In case of additivity, the addition of drug A while drug B dose is lower than its IC_{50} allows to produce the same efficacy. When the combination of the drug A and B is more effective than the single drug, these two drugs are considered to have a synergistic interaction.

The potential of cannabinoids is no longer to be proven, and our study demonstrated an IC_{50} of 5,57 ± 0,54 µg/mL for the CBD and 8,43 ± 0,30 for the CBG on MDA-MB-231 cancer cells. The isobologram curves suggest an additivity effect between CBD and CBG but also seem to exhibit a synergistic effect when a slight dose of CBD is added to CBG. Taking together, these results contribute to the understanding of the "entourage effect" interactions describing in the *Cannabis* plant.

The authors declare no conflict of interest.

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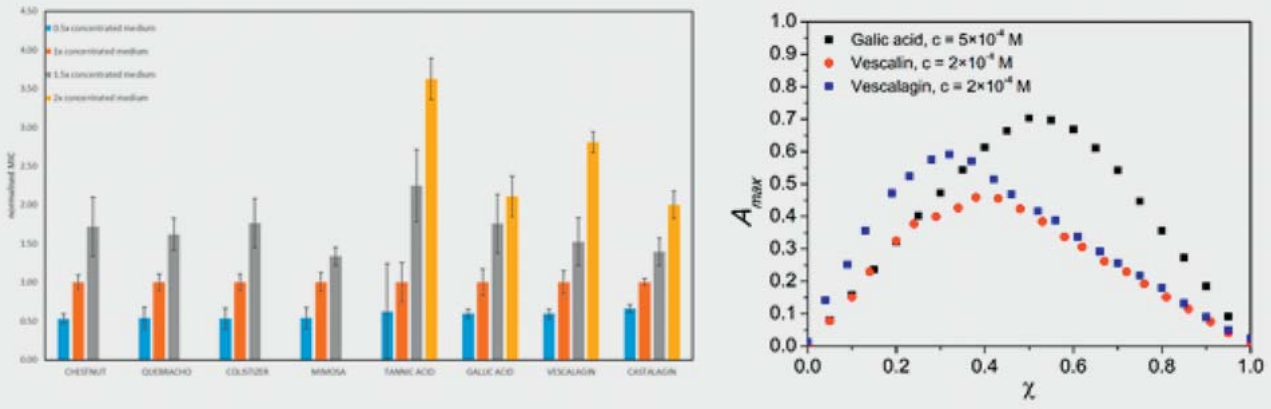

▶ **Fig. 1** Left – MIC values of samples at differently concentrated media, right – Job plots of coordination compounds.

P-235 Investigation of the mechanism of tannin antimicrobial action

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Plant extracts have been shown to possess a range of biological activities, including antimicrobial effects [1–3]. Tannins influence bacterial growth through several mechanisms. One of these is the formation of coordination compounds with essential metal ions. The complex composition of the extracts makes it difficult to establish composition-to-activity and structure-to-activity relationships. The aim of our study was to isolate individual tannin components (vescalin, vescalagin, castalin, castalagin) from chestnut extracts and to analyse the influence of commercial extracts and pure tannins on the growth of Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. Antibacterial activity was followed by monitoring the effect of tannins on the minimum inhibitory concentration (MIC), as well as on the duration of the lag phase. In the case of *Escherichia coli*, MIC values were found to increase proportionally to the concentration of the medium, while for tannin concentrations lower than the MIC, the duration of the lag phase increased exponentially with the increasing tannin concentration [4]. For *Staphylococcus aureus* dependencies were not so pronounced. Pure compounds with MIC values lower than those of the extracts best inhibit bacterial growth and thus contribute significantly to the antibacterial activity of tannin extracts. Therefore, the formation of coordination compounds between gallic acid, vescalin, castalin, vescalagin, and castalagin with Fe (II) ions was studied using UV-Vis spectroscopy and Job's method (▶ **Fig. 1**). It was determined that vescalin and castalin bind two iron (II) ions, while vescalagin and castalagin bind three iron (II) ions, which corresponds well to their MIC values [5].

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P-236 Unlocking Nature's Pharmacy: Composition and bioactivity of essential oil of bog-myrtle (*Myrica gale*) grown on Irish boglands

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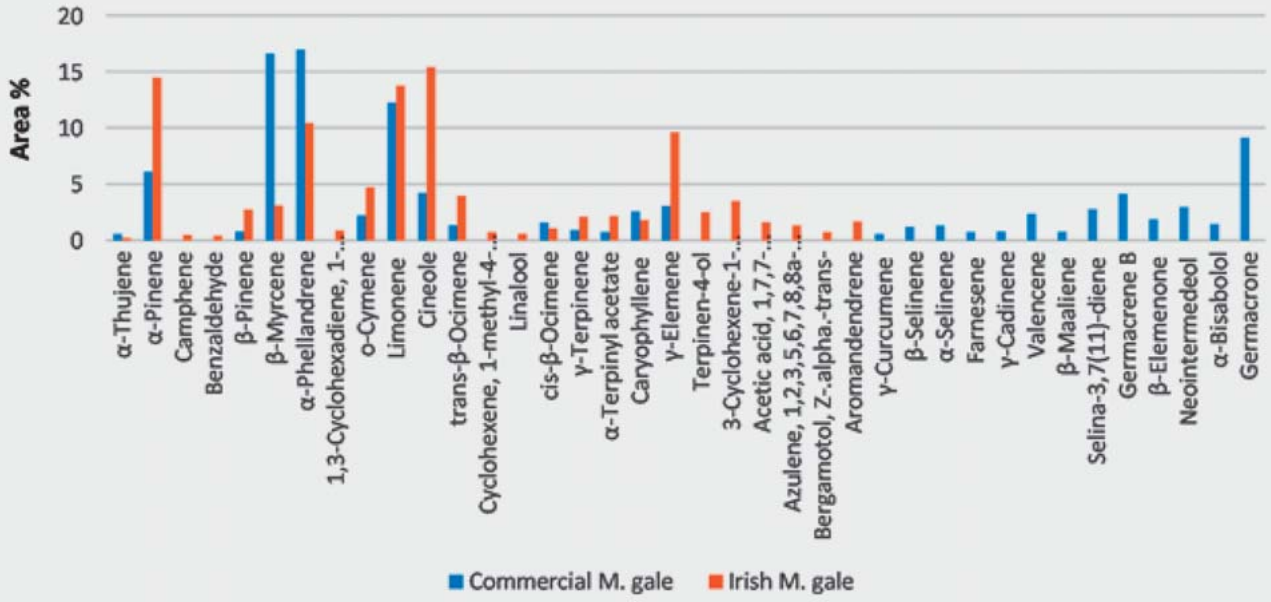
DOI 10.1055/s-0042-1759210

Myrica gale is an acidic soil loving aromatic shrub that grows throughout parts of the Northern Hemisphere in bogs, fens and heaths and at lake edges. Its catkins and leaves secrete an aromatic oil known historically to repel insects. Commonly known as bog-myrtle, its branches were placed in bedroom cupboards and stuffed into mattresses to repel moths and bed bugs and a twig of bog-myrtle worn in a shirt buttonhole was thought to keep biting midges at bay. Many insecticides, from both natural and synthetic sources, exert their action through modulation of cholinesterases. This work examined the anticholinesterase activity of essential oil from Irish *Myrica gale* leaves and a Canadian commercial preparation by TLC-bioautography with detection by both a diazotization method and the Ellman method with determination of IC_{50}s by colorimetric assay. The chemical composition of the essential oils was examined by GCMS and compared. The essential oils of Irish and Canadian *M. gale* were partially similar in qualitative profile with α-pinene, β-myrcene, α-phellandrene, o-cymene, limonene, cineole and γ-elemene as major compounds in common. Qualitative as well as quantitative differences between the oils were observed (▶ **Fig. 1**). Irish *M. gale* essential oil and the Canadian oil were both found to be low potency inhibitors of acetylcholinesterase with IC_{50}s of 0.36 mg/mL and 0.55 mg/mL respectively. Assessment of the inhibitory activity of individual oil components is in progress.



▶ **Fig. 1** GCMS composition profile of Irish *Myrica gale* essential oil and a Canadian commercial preparation.

P-237 Homoisoflavonoids from *Eucomis bicolour*, *Eucomis autumnalis* and *Scilla peruviana* (Asparagaceae)

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DOI 10.1055/s-0042-1759211

Natural products chemistry involves the extraction of potentially active compounds from plants. A class of these active compounds is called homoisoflavonoids and they are frequently extracted from the Asparagaceae family. They are regarded as hopeful pharmacological candidates due to their anti-inflammatory, anti-bacterial and antioxidant effects. Due to their anti-bacterial and anti-inflammatory activity, these plants are often used in traditional medicine, particularly in their native home of South Africa [1]. In addition, homoisoflavonoids have been shown to exhibit antiangiogenic activity, reducing the excessive formation of blood vessels. Several homoisoflavonoids have been investigated as prospective treatments for various major causes of blindness: proliferative diabetic retinopathy, retinopathy of prematurity and wet age-related macular degeneration. These are all characterised by abnormal blood vessel growth at the back of the eye.

Various homoisoflavonoids have been investigated previously for their antiangiogenic activity [2] and it has been determined that the stereochemistry of the chiral centre (C-3) in the homoisoflavonoids affects the activity.

Three plants from the Asparagaceae family were investigated: *Eucomis bicolour*, *Eucomis autumnalis* and *Scilla peruvian*a. The bulbs of each of the plants were extracted using dichloromethane and methanol. These extracts were separated using gravity column and medium pressure flash chromatography to obtain pure compounds. Nuclear magnetic resonance spectroscopy was used in order to elucidate the structure of the compounds [3].

Homoisoflavonoids were isolated from each of the plant species and identified. These homoisoflavonoid will be screened for their anti-inflammatory and antiangiogenic activity.

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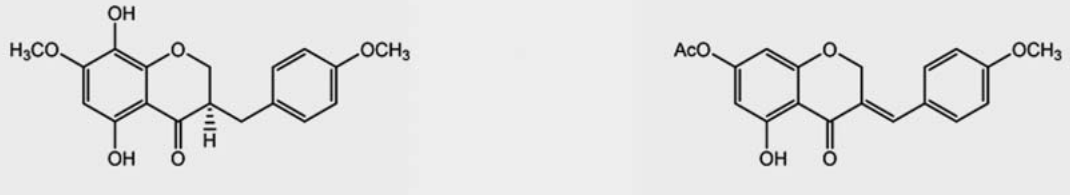

▶ **Fig. 1** Two homoisoflavonoids from *Scilla peruviana* (left) and *Eucomis autumnalis* (right).

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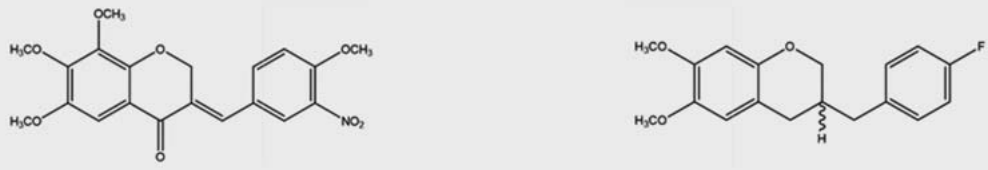

▶ **Fig. 1** Examples of synthetic homoisoflavonoids.

P-238 Synthesis of Derivatised Homoisoflavonoids to Target Ocular Angiogenesis

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Homoisoflavonoids, a class of naturally occurring compounds long used in traditional medicine, have been shown previously to possess antiangiogenic activities against human retinal endothelial cells [1]. This provides an exciting opportunity for the treatment of ocular disease associated with excessive retinal blood vessel formation, such as proliferative diabetic retinopathy, wet age-related macular degeneration, neovascular glaucoma, and retinopathy of prematurity [2]. Existing therapies for such conditions consist of large molecules which require intravitreal injections by medical professionals. In addition, drug resistance and undesirable side effects make treatment of diseases associated with retinal angiogenesis difficult for many. [1] Thus, development of a small molecule biologic, such as a homoisoflavonoid, may provide an alternative and less invasive route of drug administration, as well as tackle resistance issues. Though potentially active homoisoflavonoid compounds may be found in nature from plant families such as Asparagaceae, [3] here, we describe the synthesis of and derivatised (E)-3-benzylidene-4-chromanones and 3-benzylchromanes via cyclisation of 3-phenoxypropanenitrile intermediates [4,5] which will be evaluated for anti-proliferative activity.

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P-239 Phytochemical and antioxidant evaluation of the ex-situ cultivated species *Petromarula pinnata* (L.) A. DC. and *Campanula cretica* (A.DC.) Dietr. (Campanulaceae), from Crete (Greece)

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The family Campanulaceae contains almost three thousand perennial, biennial and annual herbaceous species occurring on all continents except Antarctica, organized in 84 genera [1]. The genus *Petromarula* includes only one known vulnerable species, *P. pinnata*, endemic to the Greek island of Crete in the Mediterranean. *C. cretica* is a rare and vulnerable Greek native perennial species that is also protected by the Greek Presidential Decree 67/1981 [1,2]. The aim of this study is to evaluate the antioxidant and anti-inflammatory activity and the total amount of phenolic compounds of the hexane, dichloromethane, methanol and hydromethanolic extracts of fresh and air-dried leaves of the two plants in two different growth stages. DPPH free-radical scavenging, inhibition of linoleic acid lipid peroxidation, inhibition of soybean lipoxygenase and Folin-Ciocalteu in vitro methods have been used in this experiment. *P. pinnata*'s methanol extracts showed the highest % interaction with DPPH (86.81–97.80%), the hydromethanolic extracts showed the strongest inhibition of lipid peroxidation (up to 91.32%) and none of the extracts inhibited the soybean lipoxygenase. The hexane extracts had the highest total phenolic content (up to 275.54 mg/L of gallic acid). *C.cretica*'s methanol and hydromethanolic extracts exhibit the highest interaction with DPPH (up to 95.05%), all of them inhibited lipid peroxidation (39.35–96.21%), while the hexane and dichloromethane extracts showed strong inhibition of the soybean lipoxygenase (up to 82.09%). The dichloromethane and methanol extracts had the highest total phenolic content (up to 142.801 mg/L of gallic acid). In conclusion, *P. pinnata and C. cretica* have high antioxidant activity, but only the latter showed anti-inflammatory activity.

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P-240 Total phenolic content, antioxidant activity and trace elements in the leaves of conventionally vs. organically cultivated *Sambucus nigra* L

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The fruits and flowers of *Sambucus nigra* (black elderberry) are used traditionally in culinary preparations while the leaves, flowers and fruits have been used in traditional medicine for the treatment of common cold, fever, coughing, constipation, inflammatory conditions, as well as a diuretic and diaphoretic agent [1]. Numerous pharmacological studies confirm the immunomodulatory, antioxidant, anti-inflammatory, anti-cancer and antimicrobial activities of S. nigra extracts. Polyphenols, such as phenolic acids, flavonoids and anthocyanins are perhaps the most important bioactive compounds of black elderberry [2]. In our study, we examined the total phenolic content (using the Folin-Ciocalteu method) and the in vitro antioxidant activity (by the DPPH assay) of hydromethanolic extracts from the leaves of organically and conventionally cultivated Greek *S. nigra* plants. The leaf samples were also assessed by means of ICP-OES analysis for their content in trace elements. The total phenolic content of the leaves' extracts ranged from 84.984 ± 1.984 mg GAE/L to 226.724 ± 2.755 mg GAE/L, while their antioxidant activity, calculated as % Radical Scavenging Activity (% RSA) was particularly high, from 78.065 ± 1.837 % to 100.00 ± 0.884 %. Finally, the ICP-OES analysis showed that K was the most abundant macroelement, with concentrations fluctuating between 5.972 ± 0.057 mg/g and 10.053 ± 0.240 mg/g, followed by Ca (3.062 ± 0.061–6.799 ± 0.036 mg/g), whereas Al (71.943 ± 2.928–363.647 ± 1.457 mg/g) and Fe (66.801 ± 4.392–342.641 ± 3.642 mg/g) were the micro-elements with the highest content.

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P-241 Effect of the vacuum-drying process of saffron on its pigment and volatile content and profile

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Dehydration of the flower red stigmas of the Crocus sativus L. plant is necessary to yield "saffron" that is highly valuable in the global market both as a coloring spice and as a medicinal herbal product [1]. In the case of the Greek PDO "Krokos Kozanis", traditional drying involves long exposure (12–24 h) of the fresh stigmas to mild, controllable temperature (25–30 oC). Such methods favour enzymatic degradation of crocetin sugar esters and picrocrocin that are responsible for saffron colour and taste respectively [2], leading to the formation of safranal and isophorone-related compounds as the major flavour constituents [3]. In the present study, we investigated the fate of those secondary metabolites (profile and concentration) after employing two vacuum drying methods to produce saffron at low to mild temperatures. Freshly collected stigmas from the Kozani region were exposed to different time, temperature, and tray load conditions until complete dehydration in the absence of oxygen. A sample dried according to the traditional method was used as a reference. The samples were examined macroscopically, via tristimulus colorimetry, FTIR spectroscopy, UV-Vis spectrophotometry, as well as liquid and gas-chromatographic techniques (HPLC-DAD, HS-SPME-GC-MS). The results highlight differences in the pigment and volatile profiles that help to understand the chemical transformation pathways. The antioxidant performance of the sample extracts in a lecithin-liposome oxidation model was also evaluated to highlight possible effects of the compositional changes upon drying. The results could be of interest to the local saffron producers and the relevant food/pharmaceutical industries.

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P-242 Metabolite fingerprinting of *Sideritis* taxa infusions

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Sideritis L. genus finds great ethnomedical importance in the regions of the Mediterranean basin and Balkan peninsula [1,2]. In folk medicine, herbal preparations of *Sideritis* plants are widely used for various diseases in Greece and Cyprus. Specifically, their infusions are consumed as diaphoretic, diuretic, tonic agents, as well as to treat inflammation of the respiratory tract, stomach disorders, and common cold [1,2]. Previous studies reported the rich phytochemical profiles of *Sideritis* taxa [2]. In continuation of our studies on the genus *Sideritis* [3,4], we focused on the metabolite fingerprinting of the infusions of different *Sideritis* taxa. The samples originated from wild or/and cultivated populations from Cyprus (*Sideritis cypria* Post. and *S. perfoliata* L. subsp. perfoliata) and different areas of Greece (*S. euboea* Heldr., *S. scardica* Griseb., *S. clandestina* (Bory & Chaub.) Hayek subsp. *clandestina*, *S. raeseri* subsp. *attica* (Heldr.) Pap. et Kok., S. *raeseri* Boiss. & Heldr. subsp. *raeseri* and *S. sipylea* Boiss.). The chemical fingerprints of the samples were explored by GC-MS and LC-UV and MS/MS techniques. For GC-MS analysis of the infusions silylated derivatives were produced by using derivatisation reagent. The present study revealed differences in the chemical profiles of the infusions based on different geographical origins and environmental conditions.

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P-243 Cold water extract of Tiger Milk Mushroom and its fractions protect against UVB-induced toxicity in nematode Caenorhabditis elegans

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Tiger Milk Mushroom or *Lignosus rhinocerus* (Cooke) Ryvarden (LR) found in Southeast Asia including Malaysia and Thailand is an edible mushroom known and studied for its various ethnobotanical and pharmacological properties. Cold water extract of *L. rhinocerus* (CLR) was found to be rich in antioxidants as well as to enhance stress resistance and extend lifespan in *Caenorhabditis elegans* in a recent study [1], though its effect on Ultraviolet-B (UVB) induced oxidative stress and photoaging have not yet been explored. UVB exposure on normal human skin induces oxidative stress and DNA damage, leading to several complications such as erythema, sunburn, photoaging and skin cancer. Hence, it is imperative to prevent or reduce the effect of UVB exposure. The present study focuses on the protective effects of CLR and its high, medium, and low molecular weight (HLR, MLR, and LLR, respectively) fractions against UVB (60 J/M²)-induced toxicity in vivo using C. elegans. After pre-treatment with 50 µg/mL of CLR extract, lifespan of C. elegans was found to be significantly increased compared to untreated group. The effect corresponded with the increase in the expression of antioxidant genes and decrease in apoptosis-related genes. Interestingly, only HLR and MLR (50 and 100 µg/mL, respectively) showed protection against UVB. These results suggests that the cold-water extract of LR could protect *C. elegans* against UVB induced damage. Furthermore, the anti-inflammatory and anti-photoaging properties shown by LR are worth investigating for developing innovative skin care products to protect the skin against deleterious effect of UV exposure.

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P-244 Composition of the essential oils of ten *Salvia* taxa from Greece

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The genus *Salvia* L. (Lamiaceae family) includes over 900 species [1]. In Greece, 26 *Salvia* taxa are found in different geographical areas [2]. The aim of this study was to investigate the volatile compounds of wild-growing and/or ex-situ cultivated *Salvia* taxa (species and subspecies), to reveal the chemical variabilities of the essential oil (EO) content due to different geographical and environmental conditions. Therefore, dried aerial parts of 10 Salvia taxa, namely S. *aethiopis, S. amplexicaulis, S. argentea, S. candidissima, S. pratensis* subsp. *pratensis, S. ringens, S. sclarea, S. teddii, S. verticillata,* and *S. virgata* were collected from wild and/or ex-situ cultivated populations from 18 different regions of Greece. In total, 24 EOs were obtained by hydrodistillation from the above taxa and were analysed by GC-MS analyses in triplicates. The major constituents of the EOs were: 1,8-cineole in *S. ringens* and cultivated *S. amplexicaulis*; germacrene D in *S. amplexicaulis, S. argentea,* and *S. candidissima*; caryophyllene E in *S. aethiopis, S. pratensis* subsp. *pratensis* and *S. teddii*; linalool acetate in *S. sclarea*; nerolidol in *S. verticillata*; sabinene in *S. virgata*. Their concentrations varied greatly between the samples examined. Furthermore, high variation was observed in the percentages of the volatile compounds among the 20 wild-growing and the 4 cultivated samples. This study is the first report on the chemical variability of the EOs of Greek *Salvia* species, which originated from wild-growing populations and/or ex-situ cultivated samples. The composition of *S. teddii* EO, an endemic Greek species, is reported herein for the first time.

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P-245 Targeted liquid chromatography-quadrupole Orbitrap mass spectrometry method for the quantification of phenanthrenes in *Juncus compressus* Jacq

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Phenanthrenes are a promising group of natural small molecules, possessing noteworthy pharmacological (e.g., antiproliferative, antibacterial, anti-inflammatory and sedative) activities.

The primary goal of our work was to develop an ultra-high performance liquid chromatography (UPLC) combined with an Orbitrap mass spectrometer (UPLC–Orbitrap–MS/MS) analytical method for the quantification of phenanthrenes in Juncus species. At first, methanol extracts were prepared from *Juncus compressus* Jacq. samples collected from different places in Hungary. After evaporation, the extracts were dissolved in 50% of aqueous methanol and solvent-solvent partition was performed with dichloromethane as phenanthrenes are generally enrich in this fraction. The presence of seven compounds, namely compressin A, effusol, effususol, juncusol, 7-hydroxy-1-methyl-2-methoxy-5-vinyl-9,10-dihydrophenanthrene, effususin A, and dehydroeffusol, isolated previously from J. compressus were investigated in the methanol and dichloromethane extracts [1]. In order to obtain a fast and reliable analytical method, several chromatographic and mass spectrometric parameters were investigated. Chromatographic separation, carried out on a C30 column using gradient elution, and mass spectrometer operating in negative parallel reaction monitoring mode was proved to be the best experimental conditions for determination of phenanthrenes. For the external calibration procedure, a semisynthetic derivative of juncuenin B was selected as an internal standard [2]. Dichloromethane extracts are generally more enriched than methanol extracts in phenanthrenes except for compressin A and 7-hydroxy-1-methyl-2-methoxy-5-vinyl-9,10-dihydrophenanthrene. Effusol was the most abundant phenanthrene in the investigated *J. compressus* extracts. Overall, the developed targeted analytical method can be suitable for quantifying phenanthrenes in other Juncaceae samples.

The authors declare no conflict of interest.

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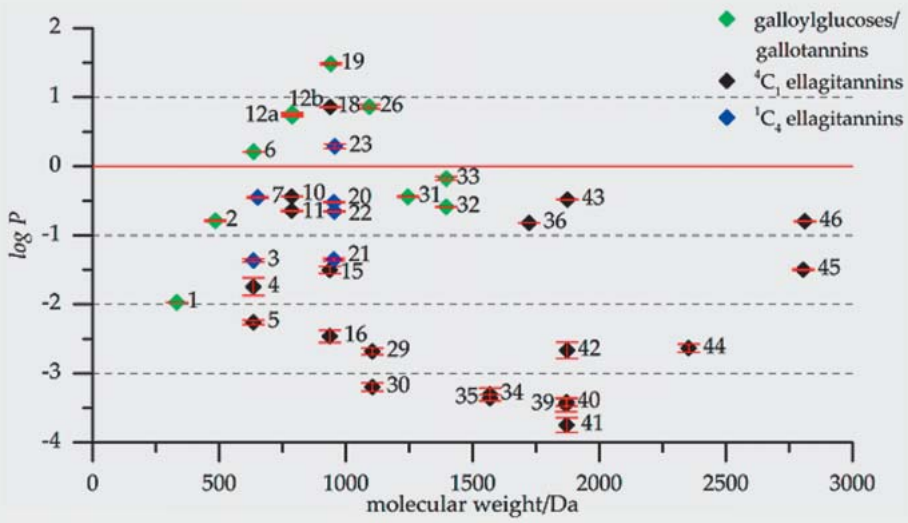

▶ **Fig. 1** LogP values of 47 hydrolysable tannins plotted against their molecular weight measured with UPLC showing galloylglucoses/gallotannins, $^4C_1$ glucose core ellagitannins and $^1C_4$ glucose core ellagitannins in different series. The numbers refer to Figures 1 and 2 and Table A1 in [1]. The figure has been previously published in [1] and is reused under open access Creative Commons CC BY 4.0 license.

P-246 Lipid Interactions and Hydrophobic Properties of Hydrolysable Tannins

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Hydrolysable tannins (HTs) are plant specialized metabolites, which have, for example, nutritional and pharmacological properties. Hydrophobicity is a fundamental physico-chemical property used to estimate the potential activities and interactions of different compounds with macromolecules like lipid vesicles. HTs have abundant structural variability and their hydrophobicity likewise varies notably with respect to different structures and functional groups therein.

The partition coefficients of 47 characterized HTs (▶ **Fig. 1**) were measured and structural features affecting the hydrophobicity of the HT structure found [1]. Notably the number of free galloyl groups, conformation of the polyol glucose, substitution of the anomeric position of glucose, molecular weight and the flexibility of the structure had the largest effect on the observed hydrophobicities.

On the basis of these results, the interactions of 13 HTs with biomimetic lipid vesicles from *Escherichia coli* (*E. coli*) were studied with high resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy [2]. HT structures that could penetrate into the lipid bilayer were determined by observing the changes in the lipids 1H chemical shifts and calculating the cross-relaxation rates from nuclear Overhauser effect spectroscopy measurements between the lipid protons and aromatic protons of HTs. Additionally, the thermodynamics of the interactions between a wider subset of the 47 HTs and biomimetic lipid vesicles from *E. coli* were studied with isothermal titration calorimetry (ITC) [3]. Based on both of these studies the prominence of free galloyl groups, flexibility and increased molecular weight was observed.

Authors declare no conflicts of interest.

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P-247 Antiproliferative, anti-inflammatory and antioxidant activity of *Ptaeroxylon obliquum* leaf extracts and fractions

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Ptaeroxylon obliquum (Thunb.) Radlk. (Rutaceae) is traditionally used in South Africa to treat many ailments including inflammation-related diseases. Approximately 20 % of cancers are induced by chronic inflammation or other infections. In this study, in vitro antiproliferative, anti-inflammatory and antioxidant activity of *P. obliquum* acetone and aqueous leaf extracts and fractions prepared using column chromatography were determined. Antiproliferative activity was evaluated against Vero cells, human breast cancer (MCF-7), hepatocarcinoma (HepG2), lung adenocarcinoma (A549) and human cervical cancer cells (Hela) using a colorimetric tetrazolium bromide assay. Soybean 15-lipoxygenase (15-LOX) inhibitory assays were used to evaluate the anti-inflammatory activity. Radical scavenging activity was tested using 2, 2-diphenyl-1-pircrylhydrazyl (DPPH) and 2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Water extracts scavenged ABTS radicals with IC_{50} values as low as 29 µg/ml. Acetone extracts and fractions had good activity against 15-LOX with IC_{50} values of 6–10 µg/ml and 22–23 µg/ml respectively. Most acetone extracts were toxic to HepG2 cells with LC50 values from 2.2–10 µg/ml and were less toxic to other cell lines including non-cancerous Vero cells, with promising selectivity index values ranging from 5 to 22. Aqueous extracts and fractions were non-toxic at the concentrations tested against all the cell lines. Morphological analysis of HepG2 and Hela cells using light microscopy showed that acetone extracts changed the morphology of the cells, and further investigation is ongoing. The acetone extract had selective antiproliferative and anti-inflammatory activity, supporting the use of *P. obliquum* in traditional medicine against inflammatory-related diseases including cancer.

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▶ **Table 1** Antiplasmodial activity and cytotoxicity of *Catatia cordata* and *Symphonia eugenioides* crude extracts.

Species	Extracts	Antiplasmodial activity	Cytotoxicity		Selectivity index	
		W2	A2058	MDA-MB-231	A2058/W2	MDA-MB-231/W2
		IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)		
C. cordata	Dichloromethane	9.23 ± 1.77	> 50	> 50	> 5	> 5
	Methanol	63.39 ± 23.17	> 50	> 50	> 0.78	> 0.78
S. eugenioides	Dichloromethane	7.70 ± 0.62	17.17 ± 3.33	14.48 ± 6.48	2.23	1.88
	Methanol	21.81 ± 7.40	> 50	> 50	> 2	> 2
Artemisinin		3.29 ± 0.87*				

* ng/mL

P-249 Antiplasmodial and cytotoxic activities of *Catatia cordata* Humbert (Compositae) and *Symphonia eugenioides* Baker (Clusiaceae), two endemic plants of Madagascar

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Madagascar has an important diversity of endemic plants. However, this resource is little explored. *Symphonia eugenioides* and *Catatia cordata* are two endemic species of Madagascar which haven't yet been subjected to any biological nor chemical studies. The present research aimed to evaluate the antiplasmodial activity of these species. The purpose is to find novel antimalarial compound within these species. The antiplasmodial activity of dichloromethane and methanol extracts of *S. eugenioides* (bark) and *C. cordata* (leaves) were evaluated against the chloroquine-resistant *Plasmodium falciparum* strain (W2). The cytotoxicity was also tested on human melanoma A2058 and human breast cancer MDA-MB-231 cell lines (▶ **Table 1**). The dichloromethane extracts of *S. eugenioides* and *C. cordata* showed the best antiplasmodial activity, with IC_{50} values of 7.70 ± 0.62 and 9.23 ± 1.77 µg/mL, respectively. The dichloromethane extract of *S. eugenioides* had also a significant cytotoxic activity against A2058 and MDA-MB-231cell lines with IC_{50} values of 7.17 ± 3.33 and 14.48 ± 6.48 µg/mL, respectively. The different chromatographic analysis (LC-DAD and TLC) indicated the presence of ursolic acid and oleanolic acid in dichloromethane extract of *S. eugenioides* while chlorogenic acid was present in methanolic extract of *C. cordata*. This is the first report on antiplasmodial, cytotoxic activities and chemical content for *S. eugenioides* and the first findings on species belonging *Catatia* genus. The results have indicated that these two plants can potentially be used to treat malaria. *S. eugenioides* may also contain some potent active compounds for developing anticancer agent.

The authors declare no conflict of interest.

P-250 *Fraxinus ornus* bark as a rich source of bioactive agents

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Fraxinus ornus (Oleaceae) bark contains a plethora of bioactive natural products and is used in traditional medicine for the treatment of inflammation, arthritis, and dysentery [1]. As the few existing research works are old and fragmented, there is a need to update the scientific data, especially regarding the phytochemistry and bioactivity. Thus, the goal of this work was the use of synchronous analytical techniques for the in-depth analysis of bark' chemical content, the isolation of its main secondary metabolites using "green" chromatography and the assessment of their antioxidant and enzymatic activity. The dry pulverized bark was initially defatted with n-Hept and extracted successively using EtOAc and EtOH/H_2O. Both extracts were subjected to UPLC-HRMS/MS resulting in the identification of 56 secondary metabolites, which mainly belong to coumarins, secoiridoids, and phenylethanoids. The EtOAc extract was further analyzed by centrifugal partition chromatography (CPC) in order to isolate its main components. 5 g extract were fractionated in a preparative CPC column, using gradient elusion-extrusion method [2], with a series of 4 biphasic systems composed of n-Hex/EtOAc/EtOH/H_2O. The direct recovery of 752 mg of Ligstroside and 745 mg of Esculin in high purity was achieved while the further purification of selected CPC fractions led to the isolation of 10 more secondary metabolites. Both extracts and isolated compounds were evaluated for antioxidant, anti-tyrosinase, anti-elastase and anti-collagenase activity and showed promising results for their use as active agents.

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P-251 Anti-SARS-CoV-2 activity of polar extracts from different plant parts of *Echinacea purpurea* and *Pelargonium sidoides*

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In the context of the COVID-19 pandemic, two plants were selected to be studied for their in vitro antiviral potential against the SARS-CoV-2 coronavirus: *Echinacea purpurea* and *Pelargonium sidoides*. Both these plants already showed an antiviral activity against other coronaviruses in the litterature and are traditionally used to prevent or treat common cold. The plants were cultivated indoor, in the framework of the "Tropical Plant Factory" project, funded by FEDER and DGO6-Walloon Region.

The anti-SARS-CoV-2 activity of aqueous decoctions and ethanolic extracts, prepared from different plants parts, was evaluated through the observation of the inhibition of the cytopathogenic effect caused by the SARS-CoV-2 on Vero E6 cells. SARS-CoV-2 infected cells were treated (triplicate, n = 2 independent assays) with 6 two-fold dilutions of each extract (final concentrations = 3.125–50 µg/ml) and the minimal inhibitory concentration was determined as the lowest concentration inhibiting the cytopathogenic effect caused by SARS-CoV-2 infection, without causing any cytotoxicity, compared to control cells not infected by the virus.

Both plants showed significant activity against SARS-CoV-2 infection with some differences according to the plant part and the type of extract. The best results were obtained with *Pelargonium* flowers EtOH extract (MIC = 6.25 µg/ml), followed by *Echinacea* roots (ethanol extract) and flowers (both extracts) with a MIC = 12.5 µg/ml.

Further phytochemical studies are in progress to identify the antiviral compounds in these 2 plants. The activity of the pure compounds detected in the active extracts, as cichoric and caftaric acids in *Echinacea*, will also be evaluated.

P-252 Compositions of polyphenols and pharmacological study of residue by-products developed from the American basil and wild bergamot post-distillation wastes

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The yield of essential oils isolated from plants is not very high and the hydrodistilled residue by-products rich in polyphenols could be used to increase the profitability of such plant raw materials [1–4]. The aim of the study was to evaluate the compositions and contents of phenolic compounds in the dry extracts obtained from the by-product of American basil (*Ocimum americanum* L.) and wild bergamot (*Monarda fistulosa* L.) as well as to investigate their safety and pharmacological activities. The conducted chromatographic analyses of polyphenols revealed the domination of rosmarinic acid in both obtained dry extracts. Its amount analyzed by high-performance liquid chromatography method was 91.23 ± 1.62 mg/g in the *Monarda fistulosa* dry extract (ME) and 78.70 ± 1.13 mg/g in the *Ocimum americanu*m dry extract (OE). Luteolin-7-O-glucoside was the second predominant polyphenol of both extracts, but its content differed significantly (76.30 ± 1.50 mg/g and 17.22 ± 0.49 mg/g, respectively). Caffeic acid (21.62 ± 0.17 mg/g) followed by apigenin (15.12 ± 0.15 mg/g) were the other major compounds in the ME, whilst rutin (11.20 ± 0.26 mg/g) and ferulic acid (8.21 ± 0.09 mg/g) predominated in OE. The free radical scavenging activity against DPPH of ME and OE were IC_{50} = 0.285 mg/mL and IC_{50} = 0.298 mg/mL, respectively. Both tested extracts dose-dependently decreased the paw oedema in rats suggesting their anti-inflammatory properties. The administration of extracts at the doses of 500–5000 mg/kg to rats did not reveal any toxic reactions that indicates their safety. Consequently, the studied by-products are promising sources of bioactive compounds with antioxidant and anti-inflammatory effects.

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P-254 Exploring seeds of *Tamarix africana* Poir as a source of bioactive natural products

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Tamarix africana Poir is an invasive salt tolerant plant (halophyte) with medicinal uses towards gastric and duodenal diseases. The commercial use of invasive species is one of the possible strategies for their control, as already applied to some macroalgae species. In this context, and having in mind existing information regarding *Tamarix* species, we evaluated the antioxidant activity of a decoction, infusion and tincture of seeds of *T. africana* (radical scavenging of DPPH and ABTS, chelating activity of iron: ICA, and copper: CCA, and iron reducing antioxidant power: FRAP), antidiabetic potential through inhibition of α-glucosidase and α-amylase enzymes, and for total phenolic and flavonoid contents. Tincture was the most active sample with the following IC_{50} values: DPPH = 0.167 mg/mL, ABTS = 0.241 mg/mL, FRAP = 0.080 mg/mL; CCA: 0.255 mg/mL; α-glucosidase: 0.125 mg/mL. Tincture had the highest phenolic content (349 mg GAE/g extract), while the infusion showed the highest flavonoid level (52.7 mg QE/g extract). Our results suggest that natural products from *T. africana* seeds may hold potential in the pharmaceutical, food and cosmetic industries. Future studies are in progress targeting the ex vivo evaluation of relevant properties and chemical characterization of the active samples.

The authors declares that there is no conflict of interest.

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P-257 Unlocking nature's Pharmacy from Bogland Species: Root and aerial extracts of Tormentil exhibit antimicrobial and antibiofilm effects against *Acinetobacter baumannii*

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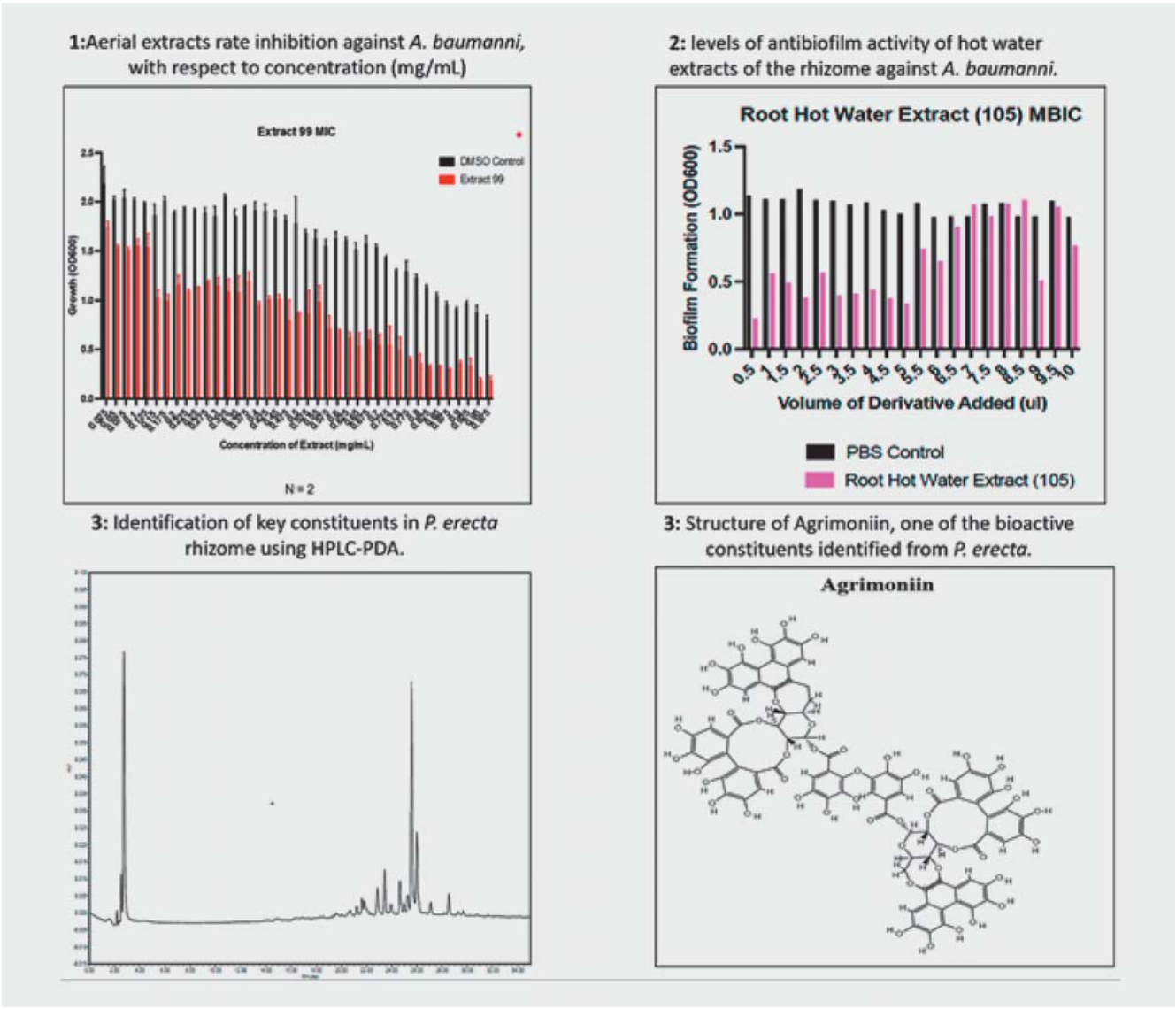
Potentilla erecta L. (Rosacea), "Tormentil", is found on peat soil associated with Irish boglands and is widespread across Europe. The species has been used traditionally with mentions of *P. erecta* in ancient herbal texts to treat oral cavity ulcerations, along with certain contagious diseases [1]. Research into *P. erecta* has revealed anti-viral and antibacterial properties, with several publications attributing this to the tannin levels present in the rhizome. *P. erecta* has proven effective against biofilm-forming strains of *Streptococcus* mutans, as well as strains that exhibit antibiotic resistance. Contemporary research into the antibacterial properties have attributed this to the total tannin content, but there still capacity for a more profound correlation between the bioactivity and the phytochemical composition of the species. *Acinetobacter baumannii* is currently at the top of the World Health Organisation's list for pathogens that are in urgent need for novel therapeutics [2]. Following preliminary testing, we have found tormentil root and plant extracts to have an antimicrobial and antibiofilm effect against a multidrug resistant strain of *A. baumannii* (▶ **Fig. 1**). Both antimicrobial and antibiofilm effects appear to be dose dependant. This plant contains up to 20% tannins and 5% ellagitannins and one of the most abundant phytochemicals in this plant is the hydrolysable tannin agrimoniin [3]. In this study we found that agrimoniin displayed an antimicrobial and antibiofilm against *A. baumannii* suggesting that this may be the primary phytochemical responsible for the antimicrobial activity of Tormentil.



▶ **Fig. 1** Phytochemical characterisation and biological analysis of *Potentilla erecta* L.

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P-258 *Hyperacanthus* genus (Rubiaceae): an underexplored source of bioactive compounds

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The *Hyperacanthus* genus (Rubiaceae) contains species endemic to southern Africa and mainly to Madagascar [1–5] that remains chemically and biologically underexplored. This study aimed to describe chemical constituents and

investigate biological activities of three endemic species of Madagascar belonging to this genus: *H. thouvenotii, H. poivrei* and *H. mangoroensis*. Twelve compounds were isolated, and two other compounds such as oleanolic acid and chlorogenic acid were detected for the first time from *Hyperacanthus* genus. The antiplasmodial and cytotoxic activities of the crude extracts and compounds were performed on *Plasmodium falciparum* 3D7 strain, on human melanoma A2058 and human breast cancer MDA-MB-231 cell lines, respectively. The dichloromethane extracts of these species showed a promising to moderate antiplasmodial activity with an IC_{50} ranging from 13.40 ± 1.61 to 19.71 ± 1.68 µg/mL and a selectivity index (SI) up to 3.87. The in vitro antioxidant activity was assessed by DPPH method and expressed by its IC_{50} and antioxidant activity index (AAI). All of the methanol extracts and infusions of the three species have a very strong antioxidant activity with IC_{50} and AAI values ranging from 9.36 ± 0.85 to 11.18 ± 1.31 µg/mL and 4.59 ± 0.40 to 3.86 ± 0.45, respectively. This is the first isolation of moretenol, 24-hydroxy α-amyrin and 24-hydroxy β-amyrin from plants belonging to the Rubiaceae family. 2,5 dioxoimidazolidine-4-carboxylic acid was isolated for the first time from the natural source. These results suggest that *Hyperacanthus* species have a potential for exploitation as a source of antimalarial and antioxidant agents.

The authors declare no conflicts of interest.

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P-259 Semi-synthetic derivatives of Amaryllidaceae alkaloid ambelline as potential lead structures for drug development

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Amaryllidaceae alkaloid ambelline, belonging to the crinane-type subgroup, lacks any significant biological activity. However, its analogs prepared by the C-11 hydroxyl group's derivatization possess various pharmacological properties.

Within the current study, thirty-two derivatives were developed and tested for inhibitory activity of cholinesterases and in vitro cytotoxicity to screen their biological activity.

Seven aromatic derivatives with different substitutions on the attached aromatic ring showed inhibitory potency against hBuChE (IC_{50} < 5 µM), of which 11-O-(1-naphtoyl) ambelline (26) was the most promising, with an IC_{50} value of 0.10 ± 0.01 µM.

The cytotoxic potential of all derivatives was determined on a panel of nine human cancer cell lines and one noncancerous cell line. 11-O-(4-chloro-3-nitrobenzoyl) ambelline (32) had the most satisfactory cytotoxic potency among the ambelline derivatives, with IC_{50} ranging from 0.6 ± 0.1 µM (MCF-7) to 9.9 ± 0.2 µM (PANC-1). Derivative 32 was active even against resistant tumor cell lines, such as HT-29 and PANC-1. The most active selective inhibitors of hBuChE are not cytotoxic and could be used as lead structures for a new series of ambelline derivatives, hence the need for further research.

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P-260 Phytochemical study of *Campanula pelviformis*, an edible species of eastern Crete

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Campanula pelviformis is a plant species that belongs in the Campanulaceae family. That plant is narrowly endemic, it grows in Sitia, Eastern Crete. In this particular study, *C. pelviformis* was phytochemically analysed.

The methanolic extract of its aerial parts was fractionated with liquid-liquid extraction (distribution) with four different solvents of increasing polarity: petroleum ether, ethyl acetate, 1-butanol and water. The petroleum ether, ethyl acetate and butanol extracts were further studied for their chemical composition. So far ten secondary metabolites of the plant were isolated: a polyacetylenes: lobetyolin (1), an alcohol: gentiobioside of 1-octen-ol (2), two phenylpropanoids: demethylsyrrigin (3) and wahlenoside A (4), two chlorogenic acid ester, the chlorogenic acid methyl ester (5) an the chlorogenic acid boutyl ester (6), two flavonoids, nicotiflorin (7) and rutin (8), and two megastigmane glucosides, corchoionoside A (9) and glucoside of 6-hydroxy-4-megastigmen-3,6-dione (10). This is the first research that concerns the phytochemical composition of this endemic Greek plant. All these compounds (1–10) were isolated from this particular species for the first time. Moreover, this is the first time that megastigmanes are reported in the genus *Camlanula*. The chemical structures of the isolated compounds were established by 1D and 2D NMR analysis (1H, 13C, gDQCOSY, gHSQCAD, gHMBCAD), and through comparison with the literature.

P-261 Antibacterial activity of *Calamintha mentifolia* Host. essential oils

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Background: *Calamintha menthifolia* Host grows very rarely in natural conditions in the Right-Bank Forest-Steppe (right bank of the Dnieper River) but introduced in many botanical gardens and collections. The aim of the study was to investigate the antibacterial activity of the essential oil (EO) of *Calamintha menthifolia* Host. EOs obtained from crude and dry shoots of *C. mentifolia* (flowering stage) grown and harvested from experimental plots of Institute of rice NAAS of Ukraine, and subjected to hydrodistillation in a Clevenger apparatus, where the final yield was 1.8 and 1.7%. To determine the antibacterial activity used the generally accepted method of diffusion into agar in the modification of wells. One-day cultures of microorganisms were used as a test culture: *Escherichia coli, Bacillus subtilis, Pseudomonas fluorescens, Proteus vulgaris, Micrococcus luteus, Staphylococcus albus*. 0.06 ml of oil was added to the well. The diameter of the growth retardation zones around the test cultures was measured in mm after one days. Ciprofloxacin, and EO of *Lavandula angustifolia* Mill. were used as controls. The result was from 40.2 ± 0.2 to 45.2 ± 0.2 mm in diameter of the zone of growth retardation of the studied strains. The most sensitive were *S. albus, E. coli, and P. fluorescens. P. vulgaris* was the least sensitive. Ciprofloxacin had a 25% higher activity against all investigated strains. The activity of *Lavandula angustifolia* EO was almost twice less effective compared to investigated EOs of *C. mentifolia* Host. Conclusion: *C. menthifolia* EO is promising as a therapeutic due to its high antibacterial properties.

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P-262 Toward the Design and Synthesis of Novel Oleocanthal – Based compounds as Potential Anticancer Agents

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DOI 10.1055/s-0042-1759235
Extra virgin olive oil (EVOO), a well-known source of polyphenols, has attracted considerable scientific attention in recent years because of their biological activities and health protective effects. Among them hydroxytyrosol, tyrosol and their corresponding EDA esters, oleacein and oleocanthal respectively, acknowledged as the key ingredients responsible for the health benefits of EVOO, are the subject of intense scientific study due to their important biological properties. The significant interest in these high-value natural compounds and the difficulty to isolate them in high amounts in pure form, has triggered the development of various synthetic approaches involving multistep total synthesis with low overall total yields.
In this regard herein we describe the development of a concise and scalable procedure, for the synthesis of various oleocanthal and iso-oleocanthal analogues. The synthesis is performed by a straightforward biomimetic and stereocontrolled approach, starting from oleuropein, an oleacein precursor highly abundant in olive leaves. Several oleocanthal analogs with improved cytotoxic activity against various cancer cell lines have been synthesized. The results so far indicate that oleuropein could serve as an excellent alternative starting material for promising oleocanthal active derivatives.
The authors declare no conflict of interest.
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P-263 Isolation and Phytochemical characterization of the Secondary metabolites from Flowers of the cultivated orchid *Cymbidium* sp. from Samos Island

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Orchidaceae family, commercialized either as loose flowers or as potted plants in floriculture worldwide. The non-marketable parts (unsuitable flowers, leaves, pseudobulbs, roots) generate an enormous quantity of by-products with potential for utilization in the production of bioactive substances and dermocosmetic products. Following our previous work regarding the phytochemical analysis and dermo-cosmetic evaluation of cultivation by-products [1], we proceeded with the phytochemical analysis of the discarded flowers of *Cymbidium* sp.
The study started with the extraction of dry materials with dichloromethane, ethyl acetate and methanol. The following qualitative analysis (TLC, HPLC-UV-DAD, UPLC-HRMS) revealed that EtOAc extract was the richest in secondary metabolites and the most promising for further treatment. The phytochemical analysis of this extract was achieved following an orthogonal chromatographic process including the initial fractionation by centrifugal partition chromatography (CPC) and subsequently a purification step using LPLC and prep-HPLC. 5 g of the extract were efficiently fractionated in a preparative CPC column using the biphasic system n-Hexane/ACN/Isopropanol 1.6/1.6/0.2 (v/v/v) in elution-extrusion mode. The following analysis of selected fractions lead to the isolation of 13 natural compounds, of which 7 belongs to sterols and triterpenes, 3 fatty esters of triterpenes and 3 phenolic acids. It is important to note that the isolation of 6′-fatty ester of daucosterol is described herein for the first time. The structure of the isolated compounds was identified by NMR and MS spectroscopy.
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P-265 Exploring the dynamics of developmental stages of micropropagated medical cannabis (*Cannabis sativa* L.) through chemical characterization and optimal plant regeneration

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The usage of medical cannabis (*Cannabis sativa* L.) for therapeutic purposes has been rising in many countries due to increasing evidence for medicinal benefit and favorable regulatory policies [1]. Cannabinoids, particularly Δ9-tetrahydrocannabinol (THC) and its acidic counterpart tetrahydrocannabinolic acid (THCA) are considered the main bioactive constituents of the plant, though C. sativa is known to produce a wide array of phytochemicals [2]. In line with urgent clinical and research demands, large amounts of plant biomass are required for consistent production of high-quality extracts and isolation of cannabinoids.
Biotechnological approaches, such as in vitro propagation, enable intensified plant multiplication without compromising chemical consistency for end uses [3,4]. However, during developmental stages of growth various changes occur in metabolite profiles, affecting cannabinoids among others. Chemical characterization of the respective samples is thus of critical importance to provide deeper insight into the dynamics of tissue culture. In this work, an efficient protocol was developed and optimized using stem explants for indirect organogenesis. Appropriate additive and plant growth regulator (PGR) regimens were established, and acclimatized plantlets were well developed. In parallel, metabolomic analyses were conducted based on suitable techniques, such as Nuclear Magnetic Resonance (NMR) spectroscopy, to acquire rapidly relevant data and monitor systematic chemical differences among the tissue culture samples. Overall, the implemented strategy allowed to gain further knowledge into the biochemical mechanisms underlying growth and developmental processes in the course of medical cannabis tissue culture.
The authors declare no conflict of interest; Funding; Stavros Niarchos Foundation (grant number KA 14320).
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P-266 Chemical characterization and biological activity of *Melissa officinalis* extracts from dried plant and solid waste from essential oil industry

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Essential oil industry generates great amount of waste biomass rich in bioactive compounds and potential source of phenols, flavanoids, etc. Lemon balm (*Melissa officinalis* L.) is a plant of the Mint family (Lamiaceae) and its largely used for production of essential oil. For this plant strong antioxidant and anticholinesterase activities were reported due to its high content of important biologically active substances such as rosmarinic, caffeic, ferulic, protocatechuic, vanillic, sinapinic acids, rutin, etc. [1,2]. Hence, the waste biomass from lemon balm essential oil production could be a valuable source of bioactive compounds.

Herein, we present the comparison of the chemical composition and biological activity of *Melissa officinalis* extracts obtained from dried plant material and solid waste from essential oil distillation. Additionally, the extracts were enriched of rosmarinic acid (RA) by acid-base extraction. An HPLC method was developed for the quantification of rosmarinic acid in the extracts. The chemical profiling was performed by HPLC-DAD, LC-MS and HPTLC.

Finally, the biological activity of the extracts was evaluated by antimicrobial activity against gram-positive (*S. aureus, B. subtilis, L. monocytogenes*) and negative (*E. coli, P. aeruginosa, S. enteric*) bacteria. On the other hand, was studied cytotoxicity and cytopathological effect of RA on BJ cell line.

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P-267 Stability study of the active pharmaceutical substance shikonin

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Shikonin and its esters have been used as active pharmaceutical ingredients in approved medicines (HELIXDERM®) for their tissue regenerative, strong wound healing and anti-inflammatory activity. Thus, their physicochemical stability during process and storage is crucial. The chiral pair alkannin and shikonin is susceptible to photo-degradation, thermal degradation and polymerization [1,2].

The aim of this study was to examine the stability of shikonin with time: a) in solutions, with different solvents (methanol, acetone, chloroform and n-hexane) under heating and light (natural sunlight and artificial irradiation) and b) in powder form, under heating and laser irradiation at 532 nm. Shikonin in various solvents was proved unstable under sunlight exposure, storage at ambient temperature, and heating, as shown by HPLC-DAD, UHPLC-MS and NMR analyses. The main by-products formed under several storage conditions and heating were characterized. The concentration of shikonin in all solutions exposed to sunlight decreased within two weeks, exhibiting a different degradation rate depending on the solvent used, but resulting to the same main by-product. Shikonin in methanol heated over 60 °C led to the formation of shikonin dimers, whereas in n-hexane was stable under heating up to 70 °C. When shikonin in powder form was heated at 150 °C, beyond its melting point, its dimeric and oligomeric moieties were formed with time. By irradiating the powder up to 60 minutes, with low enough laser intensity to avoid melting, no significant indications of by-products are evident, as shown by HPLC-DAD.

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P-268 An UHPLC-MS based metabolomic approach to explore effects of bacterial endophytes on *Alkanna tinctoria* (L.) Tausch cell suspension metabolome

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The plant endo-microbiome might influence the production of secondary metabolites (SMs). In this work, the interaction between *Alkanna tinctoria* (L.) Tausch cell suspension and eight of its bacteria endophytes was investigated through an Ultra-High-Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS) untargeted metabolomics approach (▶ **Fig. 1**). Principal component analysis and hierarchical clustering heat map were applied to the data analysis to visualize the potential modification of the metabolome. The bacteria endophyte-induced metabolites were then putative classified and identified using Molecular Networking. UHPLC-MS-based hierarchical clustering analysis, principal component analysis together with MS/MS molecular networking highlighted significant modifications in SMs production caused by bacteria endophytes.

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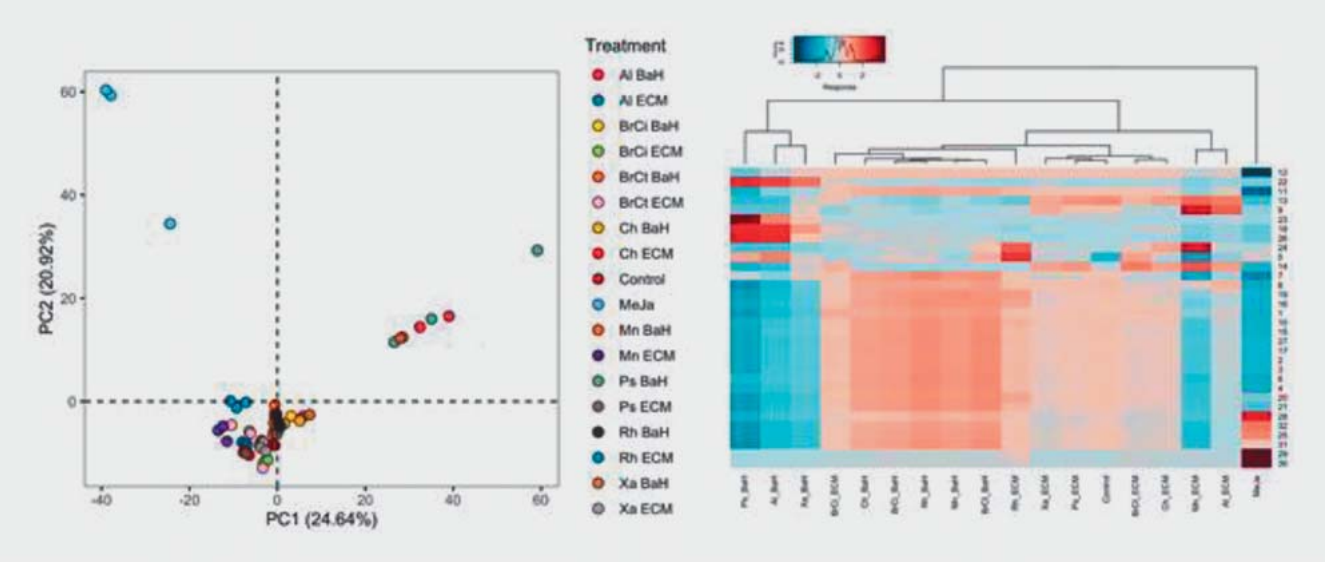

▶ **Fig. 1** Influence of bacterial endophytes on *Alkanna tinctoria*.

P-269 Antiproliferative sesquiterpene lactones from *Ambrosia artemisiifolia* L

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Ambrosia artemisiifolia is a quickly spreading invasive species in Europe. This species has not been used extensively in folk medicine although it contains sesquiterpene lactones, which are characterized with remarkable bioactivities. The aim of our work was to isolate and identify sesquiterpene lactones from *A. artemisiifolia* and to study their potential antiproliferative effects.

We isolated a new seco-psilostachyinolide derivative, 1,10-dihydro-1′-noraltamisin, and seven known compounds from the plant by different chromatographic methods. The structures of the compounds were elucidated by 1D and 2D NMR, HR-MS spectroscopy. The cytotoxic and antiproliferative effects of the isolated compounds were assayed on human colonic adenocarcinoma cell lines and human embryonal lung fibroblast cell line using MTT assay. The selectivity of the effect was calculated toward the normal cell line. Drug interactions with doxorubicin was studied on multidrug resistant Colo 320 cells.

Acetoxydihydrodamsin was the most cytotoxic on sensitive (Colo205) cells. 1′-noraltamisin and psilostachyin exerted significant antiproliferative effects on the multidrug resistant Colo320 cell line and had moderate selectivity against human embryonal lung fibroblast cell line. However, none of the isolated compounds showed inhibitory activity on ABCB1 efflux pump (P-glycoprotein), nor on the bacterial efflux pumps.

P-270 Anthelmintic activities of procyanidins targeting the cuticle of the nematode Caenorhabditis elegans – microscopic observations and the impact of interflavan-linkages

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Due to the dramatic emergence of resistances to standard anthelmintics, condensed tannins (syn. proanthocyanidins) have been proposed as a sustainable control of nematode infestations in livestock [1,2]. Proanthocyanidins have therefore been extensively investigated previously for their anthelmintic properties in vivo and in vitro. However, despite the large amount of studies, a detailed understanding of the mode of action is still lacking.

Our study focused on effects in *Caenorhabditis elegans*, a common model organism for nematodes. Extracts enriched in procyanidins (PC) showed nematicidal activity in young adult worms [3,4]. Moreover, inhibition of molting was observed in all larval stages L1 to L4. Damages to the cuticle and the underlying muscle fibers were revealed by atomic force, differential interference contrast and fluorescence microscopy, whereas internal tissues such as the intestine appeared unaffected. Interestingly, the nematicidal activity of PCs was not only determined by their molecular size, but to an unexpected extent also by the type of interflavan linkage with LC50 values of different isolated tetrameric PCs ranging from 224 µM to 1172 µM (72 h of incubation; positive control: levamisole-HCl 40 mM, negative control: DMSO 1 %).

In summary, the current findings underpin the nematodes' collagenous cuticle as a major target for anthelmintic proanthocyanidins, resulting in worms impaired in molting and locomotion. While this effect is generally shared by the entire substance class, significant differences in the efficacy among pure PCs suggest that a detailed phytochemical knowledge is beneficial for an optimized application of tanniferous plant extracts and preparations for anthelmintic use.

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P-271 Cytotoxic activities of 15-hydroxy-angustilobine A, a major indole alkaloid from the leaves of *Alstonia boonei*

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Alstonia boonei De Wild. is a medicinal plant traditionally used in West Africa for treatment of various ailments. The composition of the bark has been frequently investigated due to its popular use against malaria [1], however, not much is known about the phytochemistry of the leaves [2], although they are prepared as decoctions to treat different types of cancer in Ghana [3].

The cytotoxic activity of a hydroethanolic leaf extract was therefore assessed in a panel of ten cell lines (A549, MCF-7, MDA-MB- 231, A431, LNCap, RD-ES, CADO-ES-1, REH, HL-60 and Vero) by MTT assay including doxorubicin as positive control (IC_{50} 0.02–13.4 µM). The cell viability was reduced in all cell lines (IC_{50} 22.8–73.1 µg/mL) and a subsequent bioassay-guided fractionation revealed 15-hydroxyangustilobine A (15-HA) as the only compound significantly affecting cell viability (IC_{50} 15.5–72.9 µM; MCF-7, RD-ES, CADO-ES-1, REH, HL-60, CCRF-CEM and Vero). Additionally, 15-HA led to apoptosis and cell cycle arrest at G2/M phase, determined by flow cytometry. Fluorescence microscopy and assessment of topoisomerase I activity indicated a mode of action distinct from other natural product derived anticancer drugs, e.g. vincristine, camptothecin or paclitaxel. Further indole alkaloids representing major phytochemical constituents of the extract, namely alstrostines C–E, 12-methoxyechitamidine, 19-oxo-12-methoxyechitamidine, 6,7-seco-angustilobine B and 6,7-seco-19,20-α-epoxyangustilobine B, were isolated, but did not contribute to the bioactivity.

In summary, the current study identified the major indole alkaloids that were previously unreported in *A. boonei* leaves and revealed 15-HA as the active component causing moderate cytotoxicity in selected cell lines.

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P-272 Phytochemical study of a chemotype of *Cannabis sativa* L

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Cannabis sativa L. (Cannabaceae) was one of the first plant ever domesticated by humans, spreading its cultivation worldwide over the past 10,000 years. For its importance in alimentary, medicinal, and industrial fields, this plant could be considerate as a multi-purpose crop characterized by the presence of phytocannabinoids. However, economic sustainability of *C. sativa* cultivation should encompass the possibility of recovering non-narcotic secondary metabolites from its by-products [1]. In this study the phytochemical profile of monoecious chemotype (Ermo, V) of *C. sativa* and *C. sativa* exemplifying distinct chemotypes of hemp, both grown in Northern Italy, were compared, by investigating the accumulation of the phytocannabinoids and of the major flavonoids. The *C. sativa* extracts were characterized by liquid chromatography coupled to high-resolution mass spectrometry LTQ-orbitrap and NMR. The extract of plant obtained from the *C. sativa* chemotype V showed a phytochemical profile different from that typical of *C. sativa*; indeed, in chemotype V phytocannabinoids were not detected. Conversely, the LCHR-MS analysis of this extract highlighted the presence of compounds belonging to different classes of non-cannabinoid phenols. Flavonoids, fatty acids, terpenes, and ionone derivatives were also detected [2,3]. Both extracts were then tested on several gram positive and gram-negative bacteria strains using the broth dilution methods [4], in order to determine their minimal inhibitory concentration (MIC). The extract of *C. sativa* rich in phytocannabinoids showed a significant antimicrobial activity against *Listeria monocytogenes*, with a MIC value of 20 µg/mL, while the one from chemotype V was inactive towards all the testes species.

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P-273 Antitrypanosomal activity of semisyntetic enone-type derivatives

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The protozoan *Trypanosoma cruzi* (*T. cruzi*) causes Chagas disease, a neglected tropical disease, which aggravates between 6 and 7 million patients' life in global. Mostly it is spreaded via vectoral infection of Triatominae bugs [1].

In our previous study, 52 ecdysteroids' antitrypanosomal activity was tested on *T. cruzi* epimastigotes. Cinnamic ester derivatives of 20-hydroxyecdysone (20-E) and E and Z 6-tert-butyl-oxime ethers of 20E 2,3 : 20,22-diacetonide possessed promising selective antiparasitic activity at 5 µM [2,3].

The aim of our current work was to prepare new antitrypanosomal compounds from further natural enones. Our first target compound group was ecdysteroids derivatives that contain both previously identified pharmacophores, i.e., cinnamic ester and t-butyl-oxime ether moieties. The esterification of the two 6-t-butyl-oxime ether isomers were carried out applying cinnamic acid, EDAC, DMAP in dry dichloromethane. RP-HPLC methods were used to isolate the compounds. Four new ecdysteroid derivatives were obtained, and they were identified by NMR as the 2-, the 2,22-, and the 2,3,22-cinnamic ester of the E isomer and the 2-cinnamic ester of the Z isomer. Secondly, a set of new protoflavonoid derivatives were prepared, including ethers, oximes, semicarbazides, thiosemicarbazides.

The testing of altogether 16 compounds against *T. cruzi* is currently ongoing.

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P-274 *Platismatia glauca* and its secondary metabolite caperatic acid exhibit potential to treat central nervous system diseases

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The lichen *Platismatia glauca* is a source of chemical compounds with valuable biological properties. Thus, the aim of this study was to explore the neurobiological activity of dichloromethane, acetone, methanol, methanol-water, and water extracts from P. glauca as well as its secondary metabolite, caperatic acid. To confirm the presence of caperatic acid in *P. glauca* extracts FT-IR study was used. For more accurate identification the second derivative infrared spectra, received by the Savitzky–Golay polynomial fitting method, were analyzed. The composition of the tested extracts was also assessed using GC-MS chromatography. Our research proved the in vitro inhibition of acetylocholinesterase and butylocholinesterse by lipophilic extracts of *P. glauca* and by caperatic acid. The putative binding sites of the molecule for the enzyme were revealed using molecular docking methods. Moreover, *P. glauca* extracts showed a high chelating capacity for Fe2+ and Cu2+ ions, important for protecting against β-amyloid precipitation and alleviating Alzheimer's disease progression. The anti-inflammatory activity of the examined substances was proved by hyaluronidase inhibition capacity. Moreover, our research also suggested the anti-glioblastoma activity of the studied extracts, especially the lipophilic ones. Dose-dependent cytotoxicity against T98G and U-138 MG glioblastoma cell lines was shown using MTT analysis. In conclusion, *P. glauca* extracts and caperatic acid can be regarded as the source of compounds with valuable central nervous system therapeutic potential.

P-275 Phytochemical analysis of the decoction of *Capparis cartilaginea* leaves by high-speed counter-current chromatography (HSCCC)

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DOI 10.1055/s-0042-1759248
Capparis cartilaginea (CC) Decne (Capparaceae) is a plant found in Saudi Arabia and is recognized by the local population as a traditional herb used as decoctions to treat pain and inflammatory conditions such as rheumatism, arthritis, and gout [1]. Compared to other members of the family, there are limited studies on *C. cartilaginea* phytochemistry and biological activities, which shows the importance of having this species validated via its chemical and pharmacological studies. A simple and efficient HSCCC method has been developed for the preparative separation of six flavonoids, one phenolic acid and a nucleoside from the extract obtained from CC tea. A solvent system composed of ethyl acetate–butanol–water (2 : 3 : 5, v/v/v) was optimized for the separation. The upper phase was used as the stationary phase, and the lower phase was used as the mobile phase. Under the optimized conditions, kaempferol-3-O-rutinoside (nicotiflorin) (17.0 mg), quercetin-3-O-rutinoside (rutin) (108.6 mg), kaempferol 3- neohesperidoside (21.7 mg), kaempferol 3-(2 G-rhamnosylrutinoside) (Clitorin) (78.5 mg), quercetin 3-(2 G-rhamnosylrutinoside) (60.0 mg), isorhamnetin-3-o-rutinoside (Narcissin) (10.0 mg), gallic acid (12.6 mg), and adenosine (10.4 mg) were separated from CC tea extract. The structures of the isolates were identified by ESI-MS, ^{1}H- and ^{13}C-NMR analyses and their purities were determined using HPLC. These compounds have been isolated from this species for the first time, except for nicotiflorin, rutin and gallic acid, previously isolated from *C. cartilaginea* leaves.

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P-276 In vitro growth-inhibitory effect of essential oils against plant pathogenic bacteria and fungi in vapour phase

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Diseases caused by plant pathogens lead to significant losses to food crops in agricultural storages and greenhouses. In closed environment, plant diseases are currently controlled with fungicides, however, reliance on this single strategy leads to problems such as harmful residues and health issues of workers. Therefore, there is an increasing pressure to find more natural methods of disease control in special environment. One of the prospective methods could be the use of essential oil (EO) vapours [1]. EOs have previously demonstrated antimicrobial activity against number of plant pathogens. Despite the great antimicrobial potential of EOs, growth-inhibitory effects of their vapours have poorly been investigated against microorganisms causing plant diseases in closed environment [1]. In this study we determined chemical composition and antimicrobial activity of EOs and their vapours against plant pathogens such as *Aspergillus niger, Fusarium oxysporum* and *Pectobacterium carotovorum*. EOs of *Allium sativum, Cinnamomum zeylanicum, Citrus sinensis, Syzygium aromaticum* and *Thymus vulgaris* were obtained using hydrodistilation. Subsequently, minimum inhibitory concentrations (MICs) were identified using broth microdilution volatilization method [2,3]. The chemical composition of the most effective EOs have been determined using GC/MS. The results showed that the *C. zeylanicum, T. vulgaris* and *S. aromaticum* EOs produced the highest antimicrobial activity in vapor or liquid phase (MICs 256–1024 µg/mL). The major components were E-cinnamaldehyde (cinnamon), thymol (thyme) and eugenol (clove). In conclusion, above-mentioned EOs could be used for the development of new products for control of pathogenic microorganisms causing spoilage of agricultural products or plant diseases e.g., controlled atmosphere.
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P-277 Prenylated acylphloroglucinols from aerial parts of *Hypericum scabrum*

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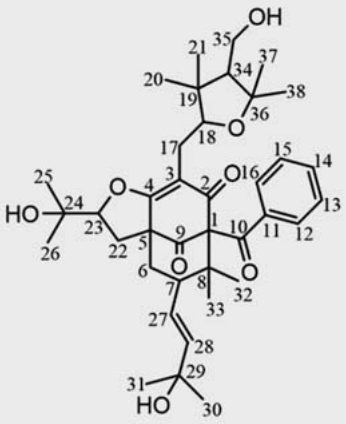
Hypericum is one of the biggest genus of family Hypericasease, with multifarious applications in traditional medicine around the world. *H. scabrum*,



▶ **Fig. 1** Structure of acylphloroglucinol **1**.

one of the species of *Hypericum*, growing in Iran, has been used in Iranian folk medicine as an antiseptic, a sedative, an analgesic, and for the treatment of headaches [1]. Several classes of compounds were reported from *Hypericum* genus, and polyprenylated acylphloroglucinols (PPAPs) are among those well-known secondary metabolites isolated from this genus to date. The structural diversity of above-mentioned compounds leads to broad ranges of bioactivity observed for them including anti-depressant, anti-HIV, anti-neurodegenerative, and anti-inflammatory [2]. MS-based analysis of the fractions obtained from chromatographical separation of the methanolic extract of *H. scabrum* displayed the presence of new phloroglucinol derivatives. Therefore, this work aimed for i) isolation of new phloroglucinols from the aerial parts of *H. scabrum*, ii) investigation of their inhibitory activity against 5-lipoxygenase (5-LOX), and iii) deciphering their absolute configuration using chiroptical and computational approaches. Five new prenylated acylphloroglucinols along with two known ones are reported (example scaffold below), the structures of which established using 1&2D NMR and HRMS, and their absolute configuration determined using electronic circular dichroism (ECD) and quantum chemical calculations. Investigation of their 5-LOX inhibitory activity at a single concentration of 10 µM revealed that only compounds 3 and 4 have moderate activity in cell free assays; however, no activity was observed on human neutrophils.

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P-278 Chemical characterization, antioxidant and anticancer activity evaluation of methanolic and alkaloid extracts of two *Retama* species

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Retama dasycarpa Coss. and *Retama sphaerocarpa* (L.) Boiss. are two Moroccan medicinal plants, belonging to the Fabaceae family. These plants have been used traditionally for the treatment of different diseases such as purgative, anthelmintic, and abortive. Moreover, studies have been reported on several pharmacological activities, including hypoglycemic, cytotoxic, antiviral and anti-inflammatory [1].

In the frame of a project oriented to study the phytochemical composition and the bioactivity of Moroccan medicinal plants, an investigation of methanolic and alkaloidic extracts from the aerial parts of *Retama dasycrpa* and *Retama sphaerocarpa* was carried out. Then the antioxidant and antiproliferative activity against melanoma cell lines (A375) and adenocarcinomic human alveolar basal epithelial cell lines (A549) by MTT assay were studied [2]. The characterization of the metabolites was performed by HRMS/MS. The analysis of the methanolic extract revealed the presence of different flavonoids and particularly isoflavones, while the alkaloid extract showed the presence of quinolizidine alkaloids [3].

The results of the antiproliferative activity showed that alkaloidic extracts from both species were able to inhibit cell growth in a dose-dependent manner after 48 h of treatment, in the (A549) cell lines. The IC_{50} values for (A549) were 10.20 ± 1.48 and 18.12 ± 0.72 µg/ml, respectively, while no activity was observed for (A375) cell lines. Furthermore, the antioxidant assays of the methanolic extracts showed that *R. sphaerocarpa* exhibited the highest DPPH free radical scavenging activity with EC_{50} = 222±4.2 µg/ml compared to 314 ± 7 µg/ml for *R. dasycarpa*.

Both species could be considered as potential sources of bioactive compounds that can be useful for pharmaceutical treatment.

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P-279 The effect of *Pinus halepensis* resin on the aromatic profile of Greek retsina

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DOI 10.1055/s-0042-1759252

Retsina is a Greek white or rosé wine which has been made for at least 2000 years. It is produced by the traditional method of adding pine resin (species *Pinus halepensis*) into the must. It is protected by the Greek and European legislation as "Traditional Appellation" which is used exclusively for wines produced according to traditional methods of a specific area or country [1].

The present research focuses on the effect of some parameters related to the resin and the vinification process on the aromatic profile of the wine during the vinification and aging process. The examined parameters are as follows:

- The collection time period of the resin
- The geographical origin of the resin
- The contact duration of the resin with the fermented must

The analytical technique of Headspace SPME Gas Chromatography-Mass Spectrometry (GC-MS) was used to study the volatile aromatic compounds extracted from the resin to the wine.

Sensory analysis took place in parallel to examine its correlation with chemical analysis' results.

The results show that the geographical origin of the resin has no clear effect on the aromatic profile of the wines, while both the collection period and contact duration have a significant impact.

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The authors declare that they have no conflict of interest.

Reference

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P-280 GC-MS, HPLC analysis and antioxidant estimation of n-hexane and methanol leaf extracts of *Spondias mombin* growing in Bayelsa State, Nigeria

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DOI 10.1055/s-0042-1759253

Medicinal plants are considered as a rich source of phytocompounds which have therapeutic values and so can be used in drug development and synthesis [1]. Natural antioxidants such as flavonoids have shown to play a key role in protecting human body from the damaging effects of free radicals [2]. The leaves of *Spondias mombin* are commonly used in Bayelsa State, Nigeria for ethnomedicinal purposes to treat diabetes, diarrhea, dysentery, cold, and gonorrhea. This study was carried out to evaluate the antioxidants properties and phytochemical composition of n-hexane and methanol leaf extracts of *Spondias mombin* plant using GC-MS and HPLC. The antioxidants evaluation of the plant extracts showed that the total phenol, total flavonoid content and total antioxidant capacity were found to be in the range of 9.99 ± 2.26 (mgGAE/g), 9.99–23.40 ± 0. 11 (mgQE/g) and 7.00–10.02 ± 5.04 (mgAAE/g) dry extract respectively. GC-MS analysis showed the presence of many bioactive components which include longifolene, stigmasterol ester, retinoic acid methyl ester, phytol, ergost-25-ene − 3,5,6,12-tetrol phthalic acid ester, octadecadienoic acid methyl eater, limonene pivalate, n-tetracosanol and bisnorhopane. Also, the HPLC analysis of the methanol extract (▶ **Fig. 1**) gave the following major flavonoids – (+) catechin, apigenin, naringenin, luteolin, kaemferol, quercetin, isorhamnetin and myricetin. The present study provides evidence that the leaf extracts of *Spondias mombin* are a potential source of natural antioxidants which may act as a chemo-preventative agent.

No conflict of interest.

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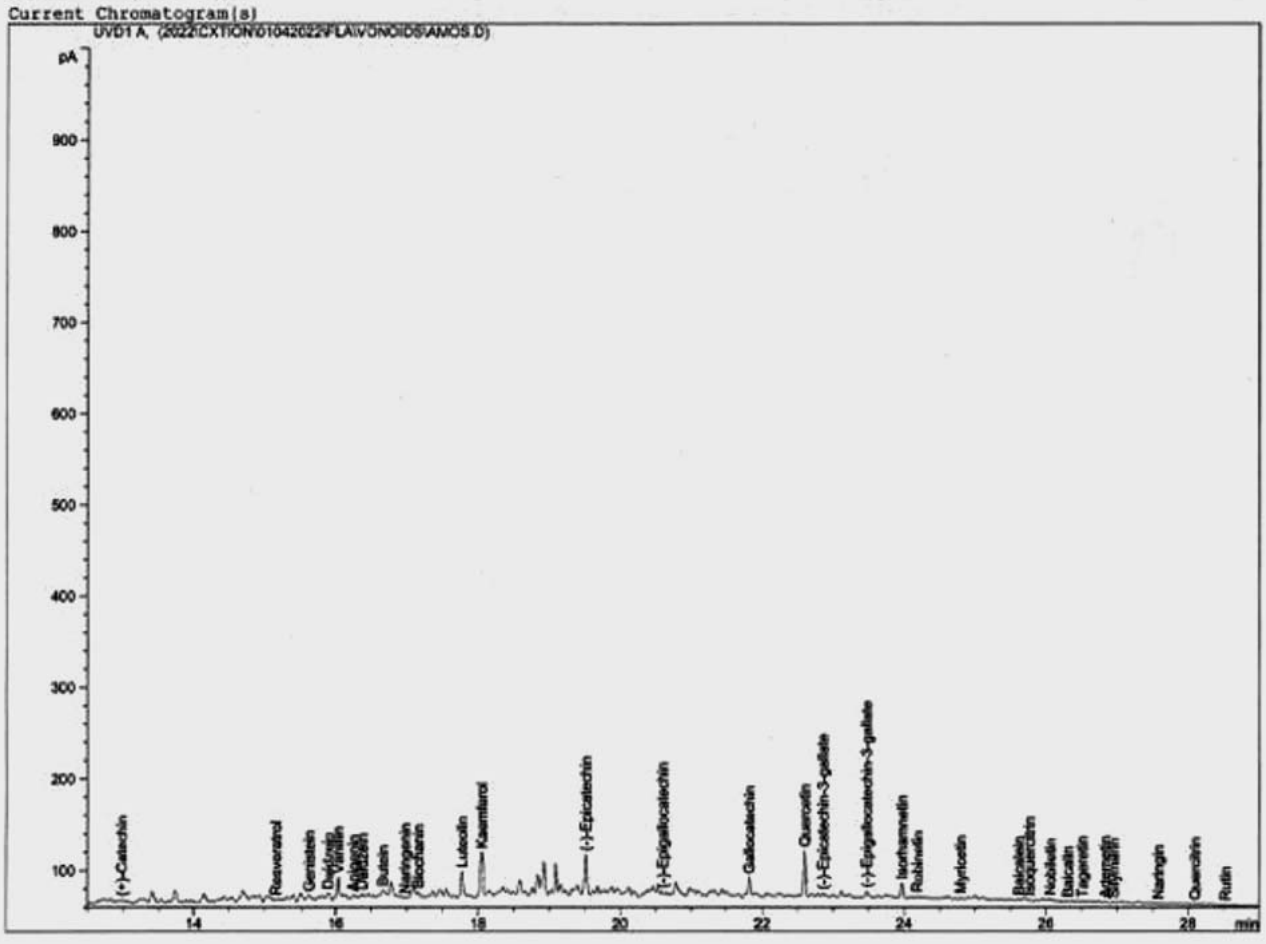

▶ **Fig. 1**

P-281 Vasculoprotective and Neuroprotective Effects of Various Parts of Pomegranate: In Vitro, In Vivo, and Preclinical studies

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DOI 10.1055/s-0042-1759254

Fruits rich in polyphenols have been shown to have health benefits associated with antioxidant and anti-inflammatory properties. Ellagitannins (ET) and ellagic acid (EA) are polyphenols found in fruits and seeds. Pomegranate (*Punica granatum* L.) is a rich source of ET and is considered as a new therapeutic agent. A small number of clinical trials in humans have highlighted the positive effects of pomegranate juice and extract consumption on cardiovascular health. It could be used as a future therapeutic agent towards hypertension, heart disease, Alzheimer.

The present study was performed to evaluate the antioxidant/free-radical scavenging properties of the juice, peel and seed extracts of pomegranate from mainland Greece. The peel homogenates from both pomegranate cultivars Central Macedonia (B) and Thrace (C) showed higher DPPH activity than that of the aril juices and seeds extracts. For both pomegranate cultivars B and C, peel homogenates and aril juices showed higher reducing activity than the seeds extracts. C peel homogenate was a strong reducing agent. Peel homogenates showed higher total phenol content than the aril juices. Total flavonoids (TF) and hydrolysable tannins (HT) results showed that peel homogenates from both pomegranate cultivars contained significantly higher contents than juices. Peel homogenates from both cultivars showed higher EA content than juices. B cultivar in its peel homogenates showed higher free radical scavenging activity, total phenol, total flavonoid, hydrolysable tannins and ellagic acid contents than C cultivar. Results of this study are very promising and suggest that pomegranate peels could be a rich source of beneficial phytochemicals.

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P-283 Biotransformation of diterpenes from Brazilian Brown Propolis by *Cunninghamella echinulata*

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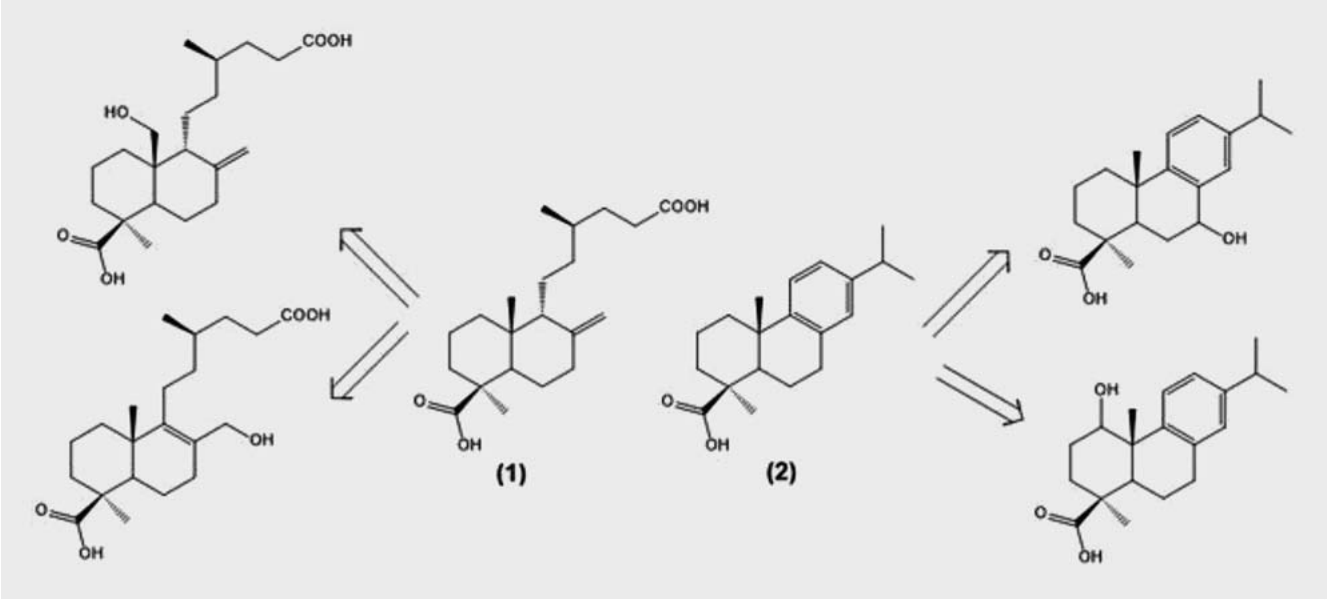
DOI 10.1055/s-0042-1759256

Propolis has attracted great scientific interest due to its biological properties, nutrition uses, and worldwide recognized traditional applications. Brazilian brown propolis (BBP) produced in Paraná state has *Araucaria* as the primary botanical source, its exudates a resin composed predominantly of a mixture of acid diterpenes. Diterpenes have been identified as active compounds in several medicinal plants showing remarkable biological activities [1,2]. Biotransformation processes are interesting tools for the structural modification of natural products with complex chemical structures, which are difficult to achieve using chemical reactions. In this way, our objective was obtaining chemical derivatives through fungal transformation from diterpenes isolated from BBP. For that, samples of BBP from *Apis mellifera* were collected in União da Vitoria, Paraná and were extracted with hydroalcoholic solution by maceration. From extract, six diterpenes were isolated, the two major compounds, diidroagatic acid and desidroabietic acid, were submitted to the biotransformation process (▶ **Fig. 1**). The inoculums were grown in pre-fermentation medium and transferred to a fermentation medium where they were incubated with the compounds [3]. The biotransformation process was monitored through aliquots analyzed by HPLC. Two products not reported in the literature were obtained from diidroagatic acid. For dehydroabietic acid, there



▶ **Fig. 1** Chemical structures of compounds diidroagatic acid (**1**) and desidroabietic acid (**2**) and their derivatives obtained by fungal transformation using *Cunninghamella echinulata*.

Planta Med 2022; 88: 1385–1588

were three known products. From the different transformations catalyzed by enzymatic systems, the selective hydroxylation at non-activated carbons is considered the main reaction promoted by fungus in natural compounds. In agreement with this information, it was also found that *C. echinulata* were able to efficiently perform the introduction of hydroxyl groups in the chemical structures.

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P-284 In vitro α-glucosidase inhibitory activity and phytochemical characterization of *Anisotes trisulcus* leaf extract

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DOI 10.1055/s-0042-1759257

Diabetes Mellitus type 2 (DM2) is a life-threatening disease manifested by hyperglycaemia. Lowering postprandial hyperglycaemia by inhibiting carbohydrate digestive enzymes, including α-glucosidase, is one of the current clinical approaches to control blood glucose levels. *Anisotes trisulcus* is a medicinal plant species native to Saudi Arabia and was used traditionally to treat diabetes [1,2]. This work investigates the α-glucosidase inhibitory activity of *A. trisulcus* leaf extract and characterises its bioactive metabolites. The methanol extract of the leaf (200 µg/ml) possessed high inhibitory activity (%65.87 ± 2.01, IC_{50} 111.60 ± 2.81 µg/ml) compared to acarbose (%50.17 ± 1.58, IC_{50} 214.3 ± 6.23 µg/ml). Bio-guided fractionation was conducted on the methanol extract utilising different separating techniques. Twenty-two compounds, mainly flavonoids and quinazoline alkaloids, were tentatively characterized in the methanolic extract using a dereplication technique. Several detected metabolites of fractions demonstrating potent inhibition remain unidentified. An aggregation assay utilising 0.01% Triton-X was conducted to assess the specificity of inhibition. The detergent did not affect the inhibitory activity of the methanol extract and analysed fractions. The observed inhibitory activity could be part of the possible mechanism by which *A. trisulcus* extract could possess antidiabetic activity. Further studies are warranted to isolate and characterize compounds contributing to the inhibitory activity and assess their potential for managing DM2. We declare no conflicts of interest.

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P-286 Viscosity-enhanced spectroscopY combined with dynamic NMR for atropisomers analysis of synthetic (−)-cannabidiol derivatives

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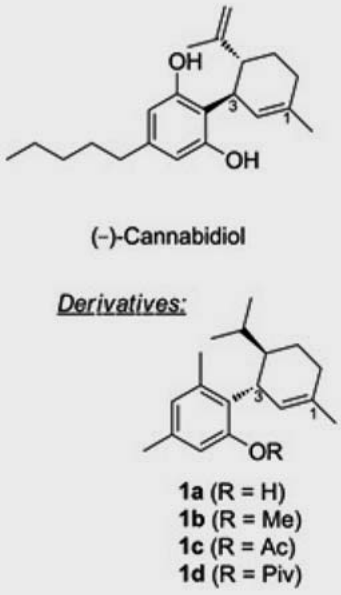
DOI 10.1055/s-0042-1759259

Mixture analysis by NMR is a topic that is nearly as old as NMR itself and for which only a few solutions have been proposed, such as LC-NMR hyphenation, diffusion-ordered spectroscopy (DOSY), assisted or not by matrix effect, multiquantum spectroscopy combined (or not) with broadband homonuclear decoupling, sparse sampling, ultrafast data acquisition, multiplet selective excitation, or tensor decomposition methods.



▶ **Fig. 1** Structure of (−)-Cannabidiol and their synthetic derivatives.

The recent use of viscous solvents has provided an exciting approach called ViscY (Viscosity-enhanced spectroscopY) for studying mixtures by lowering the molecular tumbling rate in solution [1–4]. As a result, the molecules display a negative nOe regime, and their resonances can be sorted according to their ability to exchange magnetisation through intramolecular spin diffusion. The 2D 1H-1H NOESY spectrum of a mixture reveals correlations between all 1H resonances of each analyte when recorded in spin diffusion conditions, thus giving access to individual 1H NMR spectra of the mixture components.

Dynamic NMR is suitable to characterise atropisomerism, associated principally with single bonds that join a pair of hindered planar groups. The kinetic/activation parameters of the chemical exchange process resulting from barrier to rotation are accessible [5]. To date, conformational control about C(sp2)-C(sp3) single bonds remains unexplored because restricted rotation around such bonds is extremely rare.

We have first combined the spin diffusion phenomenon with dynamic NMR to reach the same time, the structure elucidation, and the determination of kinetic/activation parameters of the restriction rotation about Aryl-C(sp3) bond of synthesised (−)-cannabidiol derivatives (see ▶ **Fig. 1**), using the viscous binary solvent DMSO-d6/water.

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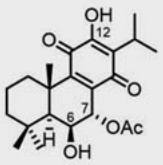

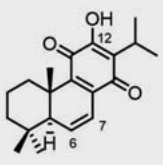

▶ **Fig. 1**

P-287 Natural and semi-synthetic royleanone diterpenoids from *Plectranthus* spp. as potential anti-tumoral agents

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DOI 10.1055/s-0042-1759260

Natural products are an important source of lead compounds for drug discovery. *Plectranthus* (Lamiaceae family) is an Old-World genus widely used in traditional medicine, whose species are rich in pharmacologically active compounds, specifically diterpenes. Two important lead molecules reported in *Plectranthus* spp. are the diterpenoids 7α-acetoxy-6β-hydroxyroyleanone (Roy, ▶ **Fig. 1**) and 6,7-dehydroroyleanone (DeRoy, ▶ **Fig. 1**) [1]. Previous studies reported in vitro activity of Roy and DeRoy against several breast cancer cell lines [1,2]. Furthermore, in silico studies suggested promising interactions of these natural royleanones with protein kinase C (PKC) isoforms [2].

The key point of this work was to prepare new functionalized derivatives of Roy and DeRoy and evaluate their effect on two cancer targets, PKC isoforms and the efflux pump, P-glycoprotein (P-gp). New royleanone derivatives were obtained by hemi-synthesis, starting from Roy and DeRoy. Some of these compounds were evaluated as PKC (α, βI, δ, ε and ζ) activators. One benzoylated analogue showed the ability to selectively activate PKC-δ, while DeRoy displayed improved PKC activity, compared with the positive control, in all tested isoforms. Additionally, P-gp inhibitory potential was evaluated in human non-small cell lung carcinoma NCI-H460 and its MDR counterpart NCI-H460/R. Natural royleanones Roy and DeRoy showed similar cytotoxic activity against both NCI-H460 and MDR cancer cell lines. Interestingly, the benzoylated derivatives displayed the most promising results, showing an increased P-gp inhibitory activity and suggesting a relevant role of this moiety for the cytotoxic activity. Several other derivatives are currently under investigation as potential chemotherapeutic agents.

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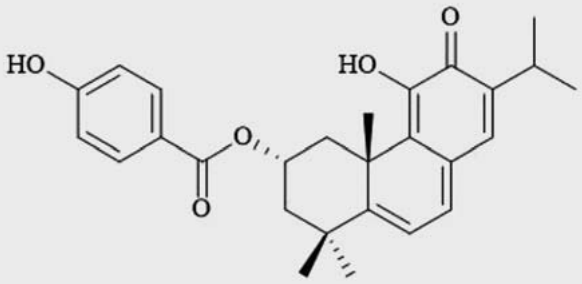

▶ **Fig. 1** Parviflorone D.

P-288 Phytochemical study and bioactivity assessment of extracts from *Plectranthus ecklonii* Benth

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Plectranthus genus plants belong to Lamiaceae family, commonly known for their use as aromatic and medicinal properties. Furthermore, *Plectranthus* species are particularly rich in phenolic compounds and abietane-type diterpenes, such as royleanones, able to justify their use in traditional medicine against a wide range of diseases, including skin disorders and cancer [1,2].

In order to study the phytochemical composition and the biological activity of *P. ecklonii* Benth., ultrasound-assisted extractions were carried out using methanol and acetone as extraction solvents.

The phytochemical analysis revealed the predominant presence of phenolic compounds in the methanolic extracts, while abietanes were identified as the most occurring secondary metabolites in the acetone ones.

Methanolic extracts were screened to assay their potential bioactivity as antioxidant, antimicrobial and on skin-related enzymes, as well as their general toxicity. The results showed a very promising antioxidant activity, but only a moderate effect against bacteria; however, no relevant general toxicity was highlighted. Good tyrosinase inhibition was observed, together with an excellent inhibitory activity on collagenase, making the methanolic extract a promising raw material to be used for the development of dermocosmetic formulations, especially those with anti-ageing activity.

Considering safety issues, only diterpene Parviflorone D (▶ **Fig. 1**) has been isolated and characterized from acetone extracts. Its cytotoxic activity has been evaluated against several cancer cell lines, always showing an IC_{50} in the low micromolar range.

Further studies are currently ongoing on both the extracts to investigate about other relevant biological activities and ascertain their safety, for internal and topical uses.

* Both authors contributed equally to this work.

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Planta Med 2022; 88: 1385–1588

P-289 Lipidic extracts from *Hermetia illucens* larvae as potential ingredients for dermocosmetic applications

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There is a growing trend for novel cosmetic products based on natural ingredients to improve the biocompatibility of formulations. The *Hermetia illucens* – Black soldier fly (BSF) – larvae biomass has promising applications as a source of value-added products to be used in health and cosmetic products due to its high content in mono- and polyunsaturated fatty acids (mainly lauric acid) [1].

In our previous work, some extracts were performed based on literature search and the fatty acid profile was evaluated. Lauric acid was establish as the major component in the organic extracts (41 % to 62 %) prepared. Furthermore, significant amounts of palmitic, oleic and linoleic acids were also observed in organic extracts. The aqueous extraction also provided considerable concentrations of lauric acid, and polyunsaturated fatty acids (PUFA) which have great potential in skincare cosmetics [2].

The present work has the goal to optimize the complete extraction process. Based on this, we studied the high extraction yield technique and the corresponding extraction solvent. Two different extraction techniques were performed (decoction and microwave-based method) using two organic solvents (acetone and n-hexane). Higher extraction yields were obtained compared to the previously reported values [2].

Moreover, the chemical composition and bioactivities of the extracts obtained are currently under evaluation, mainly the inhibition of enzyme related to skin conditions. Other studies are ongoing to ascertain the safety and efficacy of the larvae extracts for their dermocosmetics uses.

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P-290 Qualitative evaluation of phenolic compounds and free amino acids in *Cirsium vulgare* plant raw materials

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DOI 10.1055/s-0042-1759263

Cirsium vulgare (Savi) Ten. is also known as a bull thistle. It is a species of the Asteraceae, genus Cirsium. This plant is known as biennial native in most of Europe, Western Asia, Northwestern Africa [1]. Studies show that the main active compounds in Cirsium genus plants are flavonoids. It is known that *Cirsium vulgare* contains secondary metabolites such as sterols and triterpenes, aliphatic aldehydes, phenolic acids [2]. Also, it is suspected that this plant accumulates amino acids like other plants of this genus. Due to this, our main research aim was to identify phenolic compounds and amino acids in *Cirsium vulgare* plant leaves, roots, flowers raw materials. For these plant parts qualitative evaluation HPLC-PDA (for phenolic compounds) and GC-MS (amino acids) methods were used. Results showed that main phenolic compounds accumulated in *Cirsium vulgare* raw materials water/ethanol extracts were chlorogenic acid, neochlorogen, p-cumaric acid, hyperoside, isoquercitrine, apigenin, apigenin-7-O-glucoside and luteolin. Amino acids accumulated in plant were L-Alanine, Glycine, L-Valine, L-Leucine, L-Isoleucine, L-Proline, L-Serine, L-Threonine, L-Phenylalanine, L-Aspartic acid, L-Glutamic acid, L-Lysine and L-Tyrosine. A relation between detection of active compounds and the phenological stage and/or part of the plant raw materials were found. The greatest variety of amino acids and phenolic compounds was found during the mass flowering of plants, in the flowers and leaves, respectively.

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P-291 Antioxidant and cosmeceutical activity of *Plantago major* extracts

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Plantago major L. (Plantaginaceae), known as great platain, is a widespread used medicinal plant. The aerial parts contain numerous active compounds including flavonoids, polysaccharides, terpenoids, lipids, iridoid glycosides, and caffeic acid derivates [1]. *P. major* contains verbascoside, a derivate from phenolic acid, and aucubin, an iridoid with numerous beneficial skin-related properties such as wound healing and anti-inflammatory effects [2]. In this work, P. *major* extracts were prepared with biocompatible solvents, such as water, glycerol, and lactic acid by using ultrasound bath extraction for 20 minutes. According to the results of the previously performed Box-Behnken design and subsequent optimization, four extracts were prepared: the extract with the highest concentration of polyphenol (OPT-TP), phenolic acid (OPT-TPA), aucubin (OPT-AUC), and verbascoside (OPT-VER) were used to determinate the antioxidant effect by using DPPH radical scavenging activity and reducing power. The activity related to inhibitory effects of skin-related enzymes elastase, hyaluronidase, and lipoxygenase, were tested. All the prepared extracts displayed strong radical scavenging and reducing properties. OPT-AUC showed the best anti-elastase (69.34 ± 4.29 µg/mL) and lipoxygenase (50.43 ± 1.85 µg/mL) activity. On the other hand, OPT-TP showed the best anti-hyaluronidase (16.96 ± 0.51 µg/mL) activity. In addition to excellent antioxidant and enzyme-inhibiting properties, the extracts have the additional advantage of being prepared using skin-friendly solvents which can make them ideal ingredients for cosmetic products.

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P-292 New methods of isolation of olive secoiridoids and systematic study of their anti-proliferative/ cytotoxic effect on multiple cancer cell lines

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Olive oil phenols (OOPs: oleocanthal, oleacein, oleuropein aglycone and ligstroside aglycone), have been associated with the prevention of many types

of human cancers. Previous studies have demonstrated that certain of these compounds inhibit cell proliferation and induce apoptosis. However, there is no systematic study of all the compounds under the same conditions due to the difficulties related to their isolation in pure form. We have developed new methods for large-scale selective extraction of these compounds [1,2], making them affordable for detailed investigation. One step extraction of olive leaves using cold water and EtOAc permitted isolation of oleacein in 95 % purity without chromatography. Similarly, extraction of intact olive leaves with dichloromethane permitted isolation of pure oleomissional without chromatography. Alkaline treatment of oleomissional led quantitatively to oleuropein aglycon. Finally, water extraction of olive oil led to pure oleocanthal that was further oxidized to oleocanthalic acid. All the pure compounds as well as their combinations were assessed for their cytotoxic action on sixteen human cancer cell lines. EC_{50} values were calculated for each isolated compound and their cytotoxic/anti-proliferative effect were estimated when different OOPs were combined. Finally, olive oil extracts of determined OOPs' content were tested. It was demonstrated that all OOPs impair cancer cell viability in a dose and time dependent manner in all cancer cells, while oleocanthal (EC_{50} = 9 µM) was the most effective in all cancer cell lines. Most combinations of OOPs showed strong synergistic effect, whereas OOPs' extracts strongly impaired tumour cell viability even in the most resistant cancer cell lines.

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P-293 Preliminary Phytochemical Analysis and Antimicrobial Activity Study of *Thymus transcaucasicus* from Turkey

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DOI 10.1055/s-0042-1759266

Medical equipment is responsible for the majority of hospital-acquired illnesses. Bacteria on joint prostheses and dental implants can cause loosening and failure. The combined activity of enzymes produced by Streptococcus mutans and adhesive enhancing substances secreted by *Candida albicans* forms biofilms on tooth surfaces [1–3]. Pseudomonas and Enterobacteriaceae species (including *Klebsiella* species and *E. coli*) are placed in the most critical priority category, whereas numerous resistant bacteria (including *Enterococcus faecium, Staphylococcus aureus,* and *Helicobacter pylori*) are categorized in the high priority group. Bacteria adapt in response to antibiotic therapy and acquire antibiotic resistance, often as a result of abuse or misuse [4].

The present study concentrated on the preliminary and chromatographical qualitative phytochemical analysis of *Thymus transcaucasicus* aerial and subterranean parts, and the antimicrobial activities of the various extracts (petroleum ether, chloroform and ethanol extracts; infusion and decoction) and essential oil from its aerial parts. While the essential oil was the most active samples among all samples, the hexane extract exhibited the highest antimicrobial activity among all extracts. *Staphylococcus aureus* was found more sensitive against the extracts than other microorganisms. The chloroform and acetone extracts showed the highest activity against this bacteria. In light of all data, it can be estimated that, *T. transcaucasicus* has a therapeutical potential to the treatment of antimicrobial disorders. This goal will be pursued in our studies on this species.

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P-295 Screening of the antioxidant capacity of Cyperaceae species and isolation of stilbenoids and other phenolic compounds from *Carex* praecox

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DOI 10.1055/s-0042-1759267

Plant derived natural products have always been important in drug development [1]. Plants of Cyperaceae family occur worldwide and accumulate a wide variety of secondary metabolites with noteworthy biological activities (e.g., flavonoids, lignans, stilbenes). *Carex* with approx. 2000 species is the largest genus of the family [2].

The aim of our work is to investigate Cyperaceae species native to the Carpathian basin. Previously, an antibacterial screening study of 39 species was performed. Different extracts (hexane, chloroform, ethyl acetate) of the plants were tested for their antibacterial activity on eight strains; among them the EtOAc-soluble extracts showed remarkable antibacterial effect on several strains. Currently, we are evaluating the xanthine-oxidase inhibitory and the antioxidant activity of the above-mentioned plant extracts by using DPPH and ORAC assays.

Based on the notable antibacterial activity, *Carex praecox* was chosen for further preparative work. We have already reported the investigation of the chloroform fraction of the plant [3]. From this extract, 14 compounds, including two new flavonoids and two new lignans were isolated. Now, we present the phytochemical investigation of the EtOAc-soluble extract of *C. praecox*. Altogether 11 compounds, including vanillic acid, two flavonoids (tricin, quercetin), a chalcone (cilicinone B), six stilbenoids (resveratrol, cis- and trans-ε-viniferin, cis-miyabenol C, kobophenol A, carexinol A) and a new lignan derivative were identified so far from the EtOAc extract using multistep chromatographic methods.

The authors declare no conflict of interest.

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P-296 PTP1B and α-glucosidase inhibitory activities of *Hedera rhombea* compounds

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To obtain antidiabetic active compounds from the natural sources, we led to the isolation of 32 compounds from the fruits of *Hedera rhombes*. Their structures and absolute configurations were elucidated by extensive analysis of NMR spectroscopic data, HRMS, and ECD calculations [1]. Among the isolated compounds (▶ **Fig. 1**), falcarindiol and caffeoyltryptophan showed significant PTP1B inhibition with IC_{50} values of 7.32 and 16.99 µM, respectively, compared to those of the positive controls [sodium orthovanadate (IC_{50} = 17.96 µM) and ursolic acid (IC_{50} = 4.53 µM)]. These two compounds along with several other compounds displayed significant α-glucosidase inhibitions

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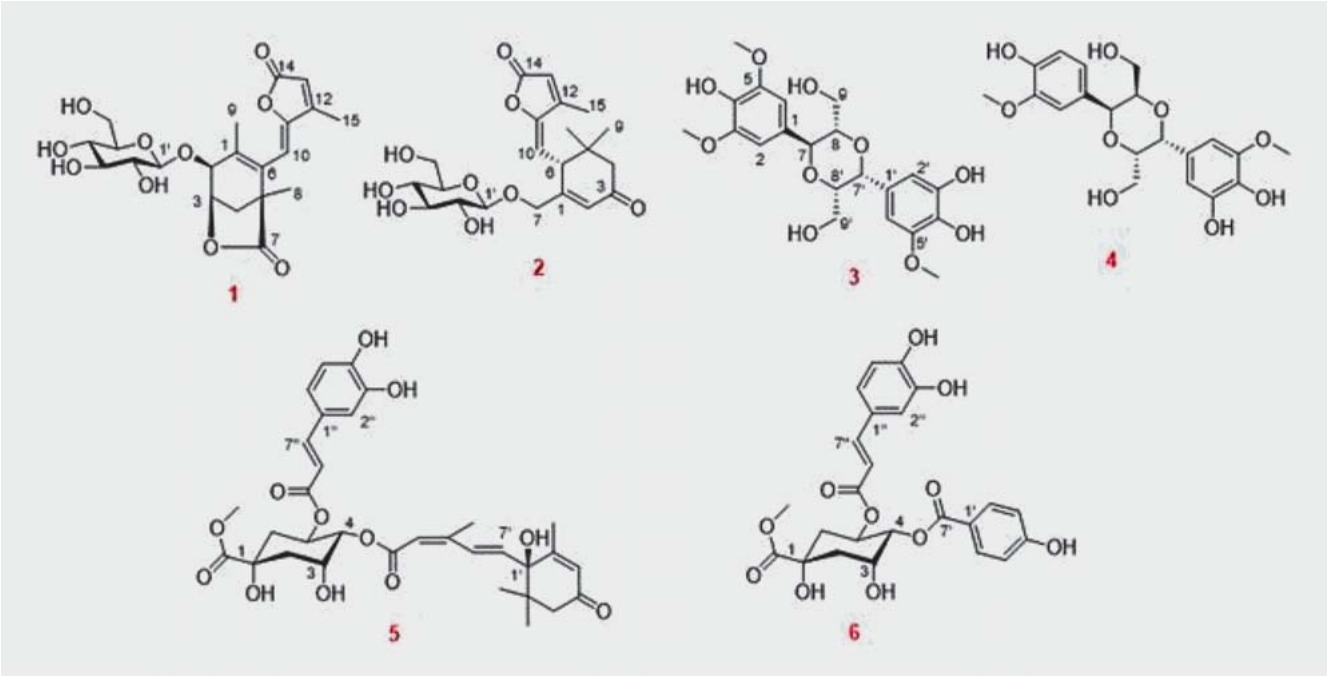

▶ **Fig. 1**

with IC_{50} values ranging from 12.88 to 91.89 μM, stronger than that of the positive control (acarbose, IC_{50} = 298.07 μM) [2].

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P-298 Diarylheptanoids isolated from *Alpinia officinarum* as novel influenza neuraminidase inhibitors

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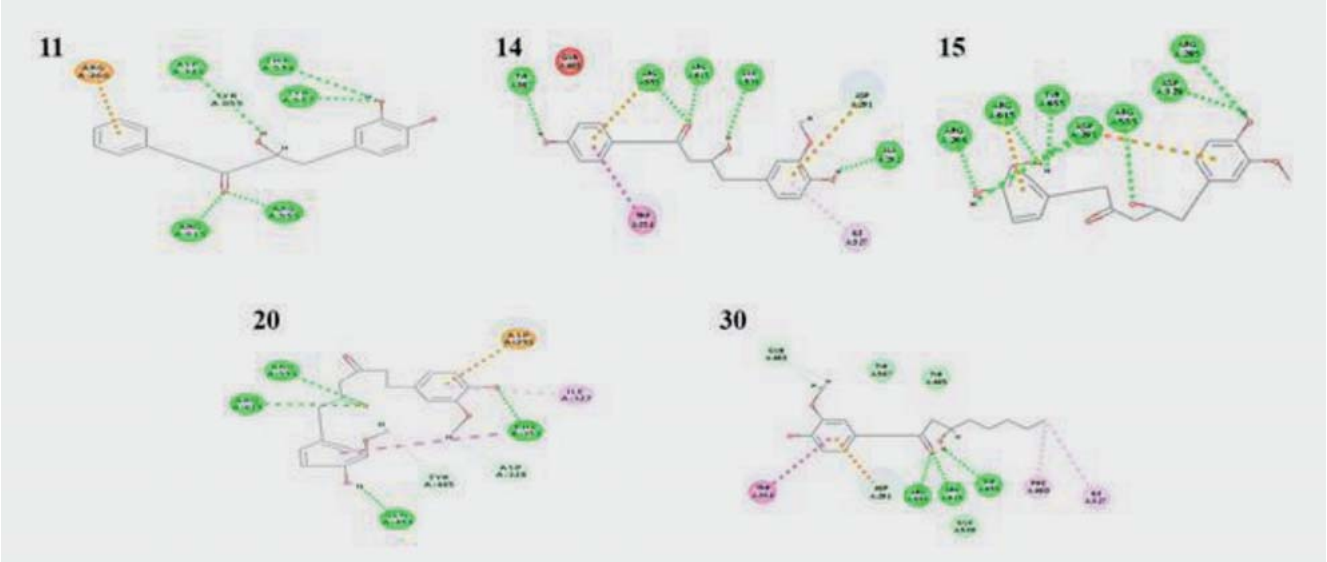
DOI 10.1055/s-0042-1759270

Neuraminidase is an important surface glycoprotein of the influenza viruses [1]. The main role of neuraminidase is to remove the sialic acid groups from glycoproteins at the surfaces of host cells, resulting in the release of virion progeny from infected cells [2]. Diarylheptanoids, known to be abundant in



▶ **Fig. 1** The receptor-ligand interacting modes of 5 hits in the active site of neuraminidase.

A. officinarum, have been reported to have neuraminidase inhibitory activity. In this study, *A. officinarum* was selected as a natural resource to investigate the correlation between neuraminidase and diarylheptanoid. Four new diarylheptanoids along with 26 known diarylheptanoids were isolated from the *A. officinarum* extract. The molecular docking studies were performed to discover putative active binding site and corresponding binding conformation of isolated diarylheptanoids. Among the isolated diarylheptanoids, 10 compounds showed relatively stable binding energy levels in neuraminidase. Interestingly, five (11, 14, 15, 20 and 30) of these 10 compounds also showed strong inhibitory activity in neuraminidase enzyme analysis (▶ **Fig. 1**).

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P-299 Bark extracts of *Holoptelea integrifolia* and their bioactive components on canine demodicosis through anti-inflammation on COX-2 and iNOS inhibition

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Holoptelea integrifolia (Roxb.) Planch or Indian elm is medicinal plants used in many countries including Thailand. The fresh stem bark has been traditionally treated on canine demodectic mange (CDM) that caused by parasitic mites which inflammation is one main symptom [1,2]. However, intensive research in molecular level of *H. integrifolia* bark extracts on CDM has not been reported. The aim of this study was to establish the efficacy of *H. integrifolia* fresh stem bark extracts on CDM treatment through anti-inflammation in cell culture. The fresh stem bark was extracted with various solvents such as hexane, dichloromethane, ethyl acetate, ethanol, methanol and water. The crude extracts were analyzed for major active components by thin layer chromatography (TLC) using toluene: ethyl acetate: ethanol (8 : 6 : 1) as a mobile phase. The anti-inflammatory activity was investigated in RAW 264.7 murine macrophage cell line. The inflammatory genes, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions were evaluated by reverse transcription quantitative polymerase chain reaction (RT-qPCR). The triterpenoids, oleanolic acid, lupeol and friedelin were spotted as the phytochemical component in the crude extracts. The hexane extract showed the most effectiveness. COX-2 and iNOS gene expressions were potently suppressed by hexane extract at 5 µg/µL and all phytochemical compounds at 10 µg/µL. Among them, lupeol exhibited the most effectiveness at the same concentration (▶ **Fig. 1**). This study supports the ability of using *H. integrifolia* fresh bark extracts and its phytochemical compounds on CDM healing through an anti-inflammatory effect.

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P-300 High Resolution Structures of Natural Products in Solution Based on DFT Calculations of NMR Chemical Shifts

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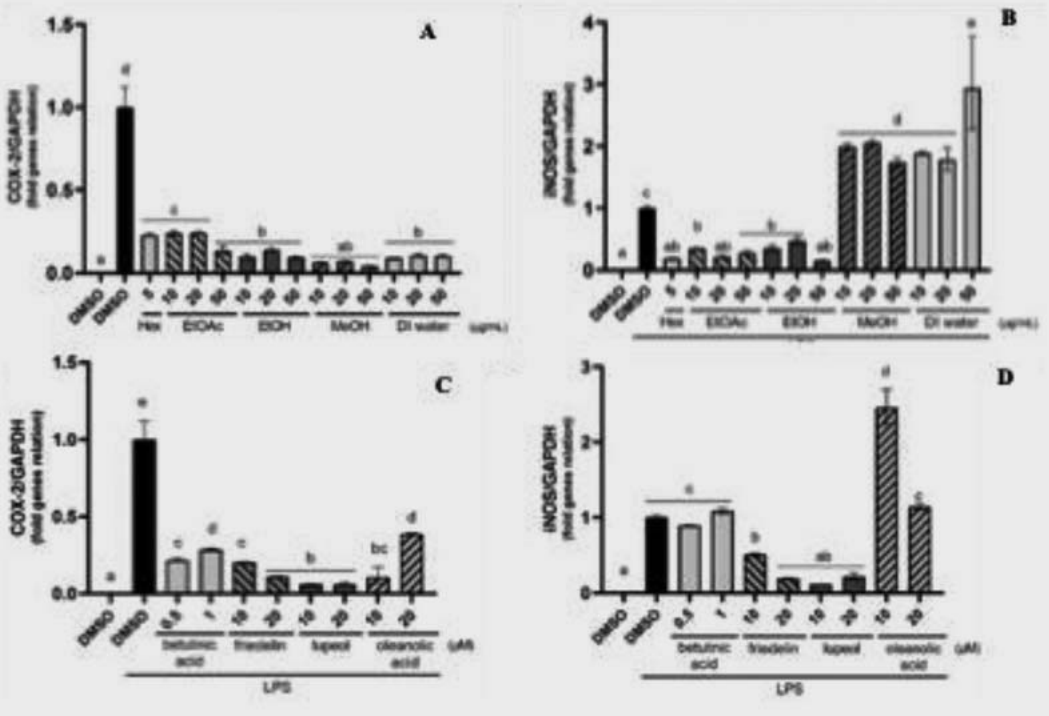
X-ray and neutron diffraction methods have emerged as the most widely used tools for structural analysis in the solid state. X-ray and neutron diffraction



▶ **Fig. 1** Effects of *H. integrifolia* extracts (**A, B**) and their bioactive compounds (**C, D**) on pro-inflammatory genes transcription in LPS-stimulated RAW 264.7 cells. Data were plot as mean ± error. The alphabet indicated the different groups.

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structures, however, may be different with respect to those in solution state due to different solute-solvent hydrogen bonding and stacking interactions. Extrapolation, therefore, of the molecular conformations in the crystal to possible conformations in solution is, in several cases, problematic. We present in this lecture the use of DFT calculations of NMR chemical shifts as a promising approach in obtaining high resolution structures in solution [1–5]. Emphasis will be given in structure elucidation of diastereomers due to the presence of multiple chiral centers. It is demonstrated that the difference between experimental and DFT calculated chemical shifts can provide an excellent method for obtaining high resolution structural and conformational information in solution beyond the current limits of the single crystal X-ray, the crystalline sponge and neutron diffraction methods.

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The author declares no conflicts of interest.

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P-302 INVENTA: a workflow for discovering chemical novelty in natural products extracts libraries

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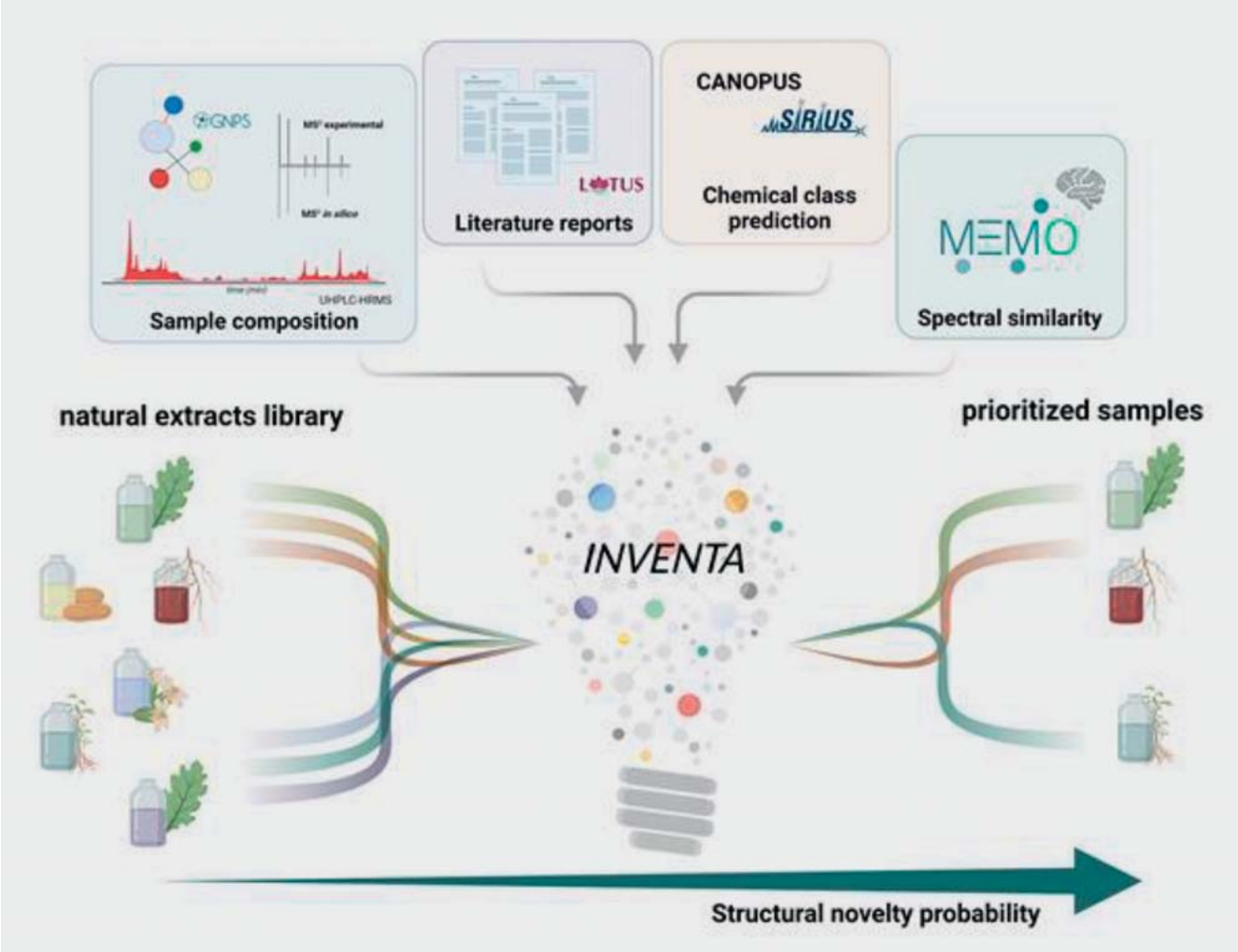
In Natural Product (NP) research the efficient prioritization of samples in extract libraries has become a key element for the discovery of original active



▶ **Fig. 1**

specialized metabolites [1]. INVENTA is an automated untargeted mass spectrometry (MS) structure-based prioritization workflow that allows extract selection, based on the possibility of structural novelty of their metabolites, from libraries of biological samples analyzed by in-depth untargeted UHPLC-MS/MS metabolite profiling. To achieve this, spectral organization is performed through molecular networking and MS/MS spectra are annotated by a combination of advanced computational methods that yield molecular formula and chemical classes assessment as well as best candidate structure ranking. INVENTA integrates previous literature reports on the taxon through automated search in an online resource for NP structure occurrences in their source organisms (LOTUS) [2]. Furthermore, INVENTA uses the chemical distances between samples to assess the chemical diversity of a given sample within a library of profiled extracts [3]. Based on such data INVENTA provides combined scores that allow extracts prioritization based on chemical novelty and results can be complemented with bioactivity screening data for the identification of novel bioactive NPs. As a proof of concept, INVENTA was applied to a collection of taxonomically related samples of the Celastraceae family. The ethyl acetate extract of *Pristemira indica* roots was highlighted as a potential source of original metabolites. The phytochemical study resulted in the characterization of 13 new dihydro-β-agarofuran sesquiterpenes and illustrated how Inventa can speed up the discovery of novel natural products.

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P-303 Triterpenes in a plant extract increase their beneficial transcriptional effect on inflammation and adipogenesis

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DOI 10.1055/s-0042-1759274

Previous studies have shown that an extract of *Eucalyptus tereticornis* (OBE100), with ursolic acid (UA), oleanolic acid (OA), and ursolic acid lactone (UAL) as the main molecules mixed with unknown minor metabolites, provided superior anti-inflammatory and hypolipidemic effects than reconstituted triterpenoid mixtures in mouse models [1,2]. The present work aims to analyze the effect of OBE100 and triterpenes on the gene expression in human U937 macrophage cell line and primary cultured human adipocytes.

Cells were treated with OBE100, UA, OA, UAL or M1, a triterpene mixture. RNA was sequenced using the DNBseq platform. In U937 macrophage, we found 39 differential expressed genes (DEGs) between OBE100 treatment vs. activated control (AC), 11 DEGs between M1 vs. AC, 5 DEGs between UA vs. AC, 2 DEGs between OA vs. AC and 3 DEGs between UAL vs. AC. Different inflammatory genes were only downregulated in OBE100 treated cells (CXCR5, DNM3, EGR3, IL2RA, KIR3DX1, MASP2, PTGIR). In primary cultured human adipocytes, we found 34 DEGs between OBE100 vs. differentiated control (DC), 13 DEGs between M1 vs. DC, 14 DEGs between UA vs. DC, 32 DEGs between OA vs. DC, and 36 DEGs between UAL vs. DC. OBE100 inhibited the expression of inflammation-related genes (CXCL10, CCL5) and upregulated the expression of genes related to insulin sensitivity, glucose homeostasis, preadipocyte state and inhibition of adipogenesis (NFATC2, FOSB, KLF2, ITGA11, DACT1, GREM2).

These results suggest that the transcriptional effect of OBE100 treatment is higher, inhibiting the expression of genes involved in inflammation and adipogenesis.

P-304 *Garcinia mangostana*, anti-acne and anti-inflammatory potential: An in silico study

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DOI 10.1055/s-0042-1759275

Acne vulgaris is a common skin disorder characterized by the formation of lesions resulting from the obstruction and inflammation of pilosebaceous units and the overgrowth of the skin bacterium *Cutibacterium acnes* [1]. A number of potential drug targets have been identified for the treatment of acne. This includes the KAS III and exo-β-1,4-mannosidase enzymes that are involved in *C. acnes* fatty acid biosynthesis and the degradation of the host's N-glycans to provide *C. acnes* with nutrients and possibly contribute to its virulence, respectively [2,3].

Formulations containing *Garcinia mangostana* (mangosteen), traditionally used in Thai medicine in the treatment of skin diseases, have shown efficacy in treating acne, though the mechanisms of action are unknown [4]. In this study, an in-silico molecular docking approach using AutoDock Vina, was used to predict the binding affinity and ligand efficiency of 104 phytochemicals isolated from mangosteen fruit/pericarp towards KAS III, exo-β-1,4-mannosidase, as well as human c-Jun N-terminal kinase (JNK1) involved in inflammation [5].

Of the phytochemicals displaying strongest ligand efficiencies (LE), illustrated in ▶ **Fig. 1**, gentisein and 2,4,6,3′,5′-pentahydroxybenzophenone showed the best LE toward KAS III (LE-values = 3.7 and 3.6, respectively), greater than that of phloretin (LE-value = 3.2). Mangostanaxanthone VI showed the best LE towards exo-β-1,4-mannosidase (LE-value = 4.5). Garcinoxanthone T showed the best LE towards JNK1 (LE-value = 4.4), greater than that of quercetagetin (LE-value = 4.3), a known inhibitor of JNK1.

Further studies exploring the biological effects of these xanthones, and benzophenones in vitro are warranted.

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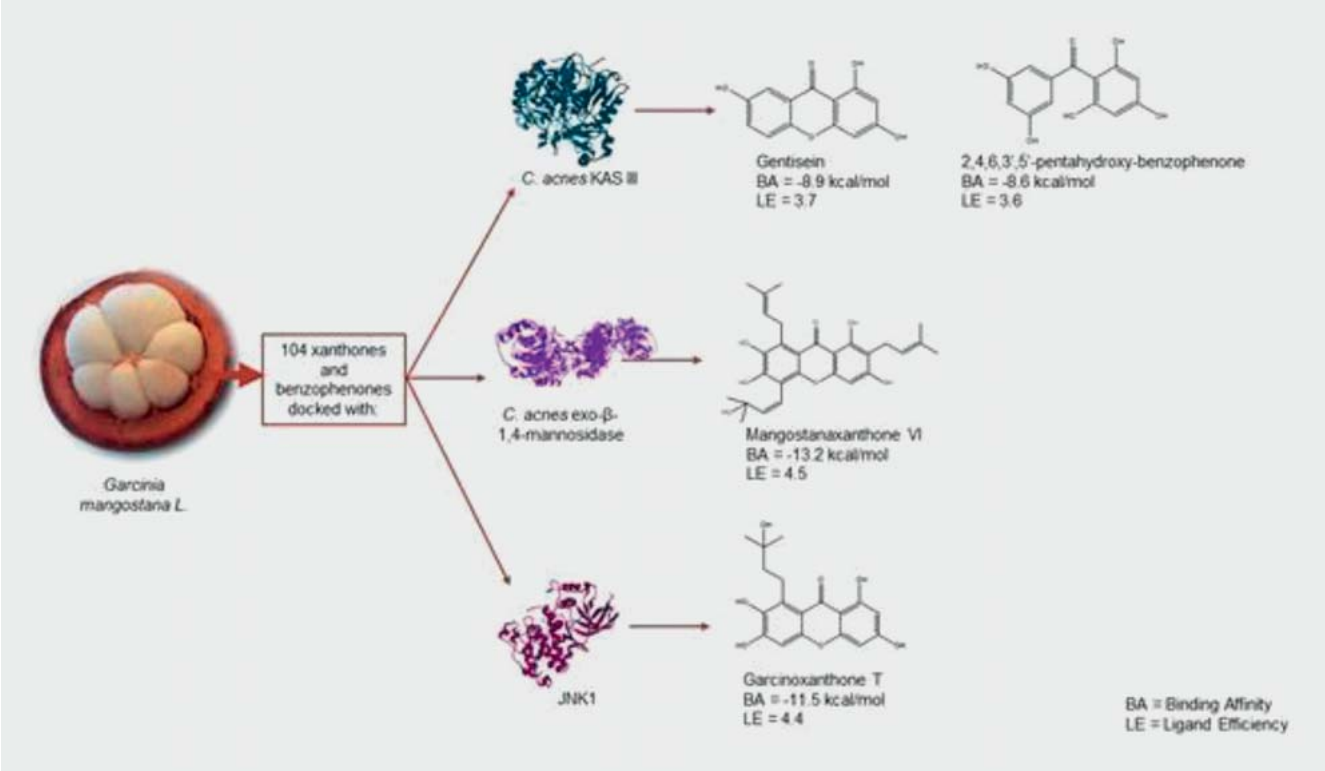

▶ **Fig. 1** Overview of study design and resulting predicted binding affinities (BA) and ligand efficiencies (LE) for the highest scoring *Garcinia mangostana* phytochemicals against *C. acnes* KAS III, *C. acnes* exo-β-1,4-mannosidase and human JNK1 enzymes.

P-305 *Carissa spinarum* L.: a study using ethnomedicine-guided systems pharmacology in identifying a mechanism of action of a medicinal plant

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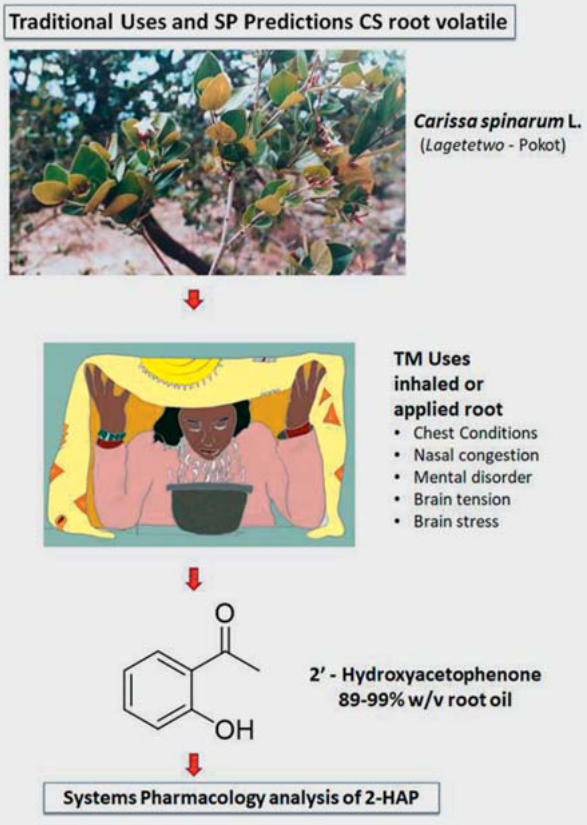
The literature of Pokot ethnomedicine (Kenya) shows that several species used by the Pokot are widely used throughout Kenya [1]. One of these species, *Carissa spinarum* L. (CS) (Apocynaceae), was selected for further ethnomedical [2] and phytochemical investigation. The use of the inhaled root in chest congestion led to the analysis of steam distillate of CS root, finding a major metabolite, 2′-hydroxyacetophenone (2-HAP), with little known bioactivity [3]. This study aims to investigate the mechanism of action (MOA) of 2-HAP relative to traditional usage of root volatile using systems pharmacology (SP). General SP analysis found 2-HAP to be a druggable molecule with predicted overlaps with ethnomedical use of volatilised root in respiratory, central nervous system and inflammatory conditions. As a folk treatment of respiratory disease, further research is required to elucidate its MOAs and explore its therapeutic potential for treating lung inflammatory disorders. Literature and SP studies revealed core targets for molecular docking analysis. Good results for PTGS2, IL18, NOS3, ACE2, PTGS1, CCNA2 and ALB were confirmed by molecular computational analyses indicating a potential MOA of 2-HAP in pulmonary inflammatory and immune responses. This study confirmed the therapeutic effect of CS, a tree widely used in African and Asian countries to treat different diseases. The potential mechanisms of 2-HAP were revealed by network pharmacology and molecular computational analyses. Our results offer a different perspective using modern pharmacological mechanisms which may assist in the global fight against the COVID-19 pandemic. Further cell and animal models are necessary to verify the relevant pathways and targets.



▶ **Fig. 1**

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P-307 STW5-II addresses Colon Targets of the Irritable Bowel Syndrome with predominant Constipation as well as with predominant Diarrhoea in vitro

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STW5-II is a plant combination of six plants (*Iberis amara* L, *Mentha piperita* L, *Matricaria chamomilla* L, *Glycyrrhiza glabra* L, *Carum carvi* L, *Melissa officinalis* L) indicated for the treatment of irritable bowel syndrome (IBS) in Germany. IBS is a heterogenous globally prevalent disorder categorized into constipation, diarrhoea and mixed.

In the present study we compared the gene expression (GE-) profiles obtained from colon cells (NCM460) treated with STW5-II and its combination partners with GE- profiles obtained from colon biopsies from IBS- patients with predominant symptoms of constipation (IBS-C) or diarrhoea (IBS-D) available from the GEO-data base.

Two clinical studies were identified with samples obtained from the rectal colon of IBS-patients. GE-profiles of colon samples were compared to GE-profiles of the colon cells cellline (NCM460) treated with STW5-II and significantly differing from the controls. In the first study of 4049 genes significantly differing between healthy and diseased donors (IBS-C), 292 were modulated also by STW5-II. In the second study of 1987 genes significantly regulated in samples from IBS-C (n = 18) compared to healthy donors and of 2353 genes in IBS-D (n = 27), 105 and 95 common genes for the subgroups IBD-C and IBD-D respectively were identified by comparison with the GE-profiles of colon cells after treatment with STW5-II. Common genes include heat shock protein 90 alpha (HSP90A), which has been previously validated by us (RT-PCR) and is thought to play a role in gastric apoptosis and inflammation.

STW5-II addresses subset-specific mechanisms underlying IBS related to constipation and to diarrhoeae in vitro.

P-308 Natural product analogues from the dihydrochalcone series as multi-target inhibitors of cancer cell growth

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DOI 10.1055/s-0042-1759278

Chalcones are a well-known family of natural flavonoids, renowned for a variety of pharmacological activities such as their anti-inflammatory, anti-bacterial and also anti-cancer properties. We investigated synthetic and semi-synthetic derivatives of MF-15, a benzylated dihydrochalcone isolated from *Melodorum fruticosum* leaves, in an in silico profiling approach and discovered several distinct bioactivities on individual protein targets. One or a combination of these activities lead to in vitro anti-proliferative activity on different cancer cell lines, previously published in [1] and [2].

The most potent compound in the series so far, BnDHC51, was shown to inhibit 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD5) (91 % at 10 µM) as well as the androgen receptor (AR). BnDHC51 also displayed a concentration-dependent inhibition of liver cancer cell proliferation (IC_{50} = 0.07 µM for Hep3B, IC_{50} = 0.55 µM for HUH7) showing a more than 10-fold higher activity than the parent compound MF-15 (IC_{50} = 6.4 µM for Hep3B, IC_{50} = 8.6 µM for HUH7). HEK293T cells were inhibited by BnDHC51 only with an IC_{50} of 12.8 µM.

In silico docking studies were used to propose a binding mode for the benzylated dihydrochalcone scaffold to 17β-HSD5 as well as the AR DNA binding site. The scaffold's impressive polypharmacological profile with the dual anti-androgenic effect, in combination with its previously shown anti-inflammatory properties (unpublished results) render it an extraordinary natural product derived lead structure.

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P-309 Cheminformatics identification of modulators of key carbohydrate metabolizing enzymes from *Crescentia cujete* towards interventive T2DM therapy

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DOI 10.1055/s-0042-1759279

The therapeutic use of oral hypoglycaemic agents in the effective management of type-2 diabetes mellitus are without adverse effects; thus, calls for alternative and novel candidates from natural products in medicinal plant. The study explored computational approach (molecular docking and molecular dynamics simulation) in the determination of antidiabetic effect of probable candidates from the *Crescentia cujete* secondary metabolites. The molecular docking results identified the best 5 compounds for each target of diabetes enzymes (alpha-glucosidase, dipeptidyl peptidase-IV, aldose reductase and protein tyrosine phosphatase-1B) where all these compounds (except against PTP-1B) depicted higher docking scores greater than the respective standards (acarbose, Diprotin A, ranirestat) and were further subject to molecular dynamics simulation (MDS) over a period of 100 ns. The results similarly revealed some of the compounds such as benzoic acid and phytol (− 48.414, − 45.112 kcal/mol respectively) as well as chlorogenic acid (− 42.978 kcal/mol) and naringenin (− 31.292 kcal/mol) produced higher binding energy affinities better the standards [acarbose (− 28.248 kcal/mol), ranirestat (− 21.042 kcal/mol)] against alpha-glucosidase and aldose reductase respectively (and vice versa for DPP-IV and PTP-1B) though, these results were inconsistent with findings from the post-dynamics simulation metrics. While isoflavone (alpha-glucosidase), xycaine (DPP-IV), luteolin (aldose reductase) and chlorogenic acid (PTP-1B) were affirmed as best inhibitors of respective enzyme targets, luteolin and chlorogenic acid may be suggested and proposed as probable candidates against type-2 diabetes mellitus and related retinopathy complication based on their affinity for 3 (DPP-IV, aldose reductase and PTP-1B) out of the 4 targets. Further studies are warranted in vitro and in vivo on the antihyperglycaemic effects of these candidates.

P-310 Impact of microwave-assisted, subcritical water, and HVED extraction on the content of bioactive components of quince leaves (*Cydonia oblonga*) extracts

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Native to the regions of west Asia quince (*Cydonia oblonga*) is a small tree or a shrub which is now spread throughout the world [1]. Application in traditional

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medicine proved that quince leaves possess significant medicinal properties. Modern medicine states that quince leaves extracts have cardiovascular, antibacterial, antifungal, antidiabetic, and antioxidant activity [2]. In order to enhance the bioactivity of quince leaves extracts, three green extraction techniques were applied. The applied techniques were microwave-assisted extraction (MAE), subcritical water extraction (SWE), and high voltage electric discharge (HVED) extraction. The efficiency of the extraction techniques was determined by analyzing the content of total flavonoids and antioxidative activity of the extracts. MAE was conducted at five different temperatures (40, 60, 80, 100, 120 °C) and two different extraction time (5 and 10 min) using 50% ethanolic solution as solvent. The HVED was conducted at three different frequencies (40, 70, 100 Hz) and three different extraction times (1, 5, 15 min) while five different temperatures were applied for SWE (100, 125, 150, 175, 200 °C). The content of total flavonoids was ranging between 62.67 and 640.71 mg CAT/g DE, while the IC_{50} value for the DPPH test was in the range from 7.65 to 0.907 µg/mL. The highest activity was achieved by applying HVED extraction at the conditions of 40 Hz and 15 min. By applying these extraction techniques, it was proved that extracts rich in bioactive components with high antioxidative activity can be obtained with the reduced negative impact on the environment.

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P-311 Phytochemical analysis, metabolism, anti-adhesive and anti-inflammatory activity of infusion from silver birch leaves (*Betula pendula* Roth)

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DOI 10.1055/s-0042-1759281

Silver birch leaves (*Betula pendula* Roth/*Betula pubescens* Ehrh.) is a plant material used in the treatment of urinary tract disorders as a diurectic agent [1]. It is administered orally as infusion.

The aim of the research was to establish the phytochemical composition of infusion, evaluate its metabolism by human gut microbiota as well as the anti-adhesive and anti-inflammatory potential of raw extract and mixtures obtained after metabolism.

The infusion was analysed using UHPLC-DAD-MS. It was shown that it contains 4-hydroxyphenyl-propan-1-one-3-O-β-D-glucoside, salidroside, caffeoylquinic acid derivatives and flavonoids. The incubation of extract with gut microbiota for 24 h showed the degradation of major natural products present at the starting point. Some small molecular metabolites were detected. The anti-adhesive assays using infusion shown that it inhibits the adhesion of *E. coli* to blader epithelial cells (T24) in the concentration range 62.5–250 µg/mL. The anti-inflammatory potential of the extract and mixtures of gut microbiota metabolites was assessed using human neuthophils after the stimulation with LPS. The production of IL-8, IL-1β and TNF-α was checked by ELISA. The infusion in the concentration range 6.25–50 µg/ml influenced the production of cytokines. The best anti-inflammatory activity was observed for raw extract at 12.5 µg/ml. The evaluation of the anti-adhesive and anti-inflammatory potential of mixtures of metabolites didn't show activity. Conclusively, silver birtch's infusion, rich in phenolics which are metabolized by gut microbiota. The raw extract exerted both anti-inalfmmatory and anti-adhesive activity, however, the metabolism eliminates the observed bioactivity.

The research was funded by Polish National Science Centre OPUS_15 No. 2018/29/B/NZ7/01873.

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P-312 Seven-day oral intake of herbal preparation as potential stimulators for Tamm-Horsfall Protein – An ex vivo Study

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DOI 10.1055/s-0042-1759282

Urinary tract infections (UTI) are one of the most common infectious diseases [1]. In Europe, traditional herbal materials and extracts from various plants are widely used for the prevention and therapy of UTI. In most cases, the respective effects of the extracts are attributed to the increase in urinary flow [HMPC]. The aim of this study is to investigate whether selected herbal preparations stimulate the secretion of Tamm-Horsfall protein (THP) in urine over seven days of oral consumption. The following preparations were investigated: *Levistici radix* infusion, *Juniperi galbulus* infusion, *Mate folium* extract, *Taraxaci* herba cum radice extract, *Urticae folium* extract and Equiseti herba extract (5 male and 5 female for each group). Dosing was performed according to the respective recommendation of HMPC of EMA. THP in morning urine (day 1, 3, 6 and 8) was quantified by use of a validated in-house ELISA. Day 1 urine serves as untreated control urine.

THP and THP normalised to creatinine [µg/mL and µg/mg] levels of pooled urine are elevated prominently in the Equiseti herba group (> 300%). In this group, a diuretic effect and alteration of electrolyte/creatinine ratio is also present. All other groups did not show any diuretic effect nor a stimulation of THP secretion. Equiseti herba is recommended for the treatment of uncomplicated urinary tract infections, as it not only increases the volume of urine but stimulates THP secretion. Thus, pathogens such as uropathogenic *Escherichia coli* can be excreted more effectively [2].

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P-313 The impact of Excipients and Extraction Methods on the Yield of Naringin and Naringenin

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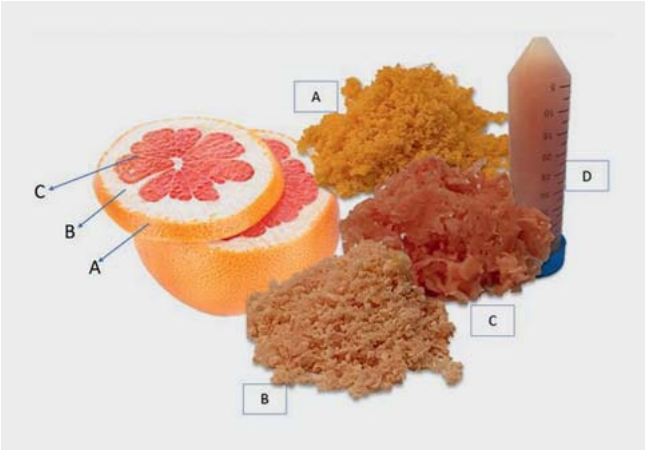
While flavanones exist in a variety of chemical forms, their favourable health effects are most prominent in their free form–aglycones. Their concentrations in grapefruit (*Citrus x paradisi* L) extracts vary according to the extraction and hydrolysis methods used. The primary aim of the work was to maximize the yields of naringin and naringenin from fresh grapefruit fruits of various parts (flavedo, albedo, and segmental) using different extraction and hydrolysis methods. In addition, evaluate the excipient material–magnesium aluminometasilicate– and determine its influence on the qualitative composition of grapefruit extracts.

The grapefruit fruits were collected from the local market in Mastaičiai, Kaunas district, Lithuania. The fruit was separated into the albedo, and segmental parts, then chopped with a food processor and frozen in a freezer (– 18 ± 0.9 °C) until extraction. Extracts were obtained by heat-reflux extraction (HRE), ultrasound-assisted extraction with an ultrasonic homogenizer (UAE*), and ultrasound-assisted extraction with a bath (UAE). UAE using a bath was modulated using thermal hydrolysis, adding additional excipient.

The highest yield of naringin 17.45 ± 0.872 mg/g was obtained from an albedo sample under optimal conditions using ultrasound-assisted extraction; a high yield of naringenin 35.80 ± 1.79 µg/g was produced using the heat reflux method from the segmental part. Meanwhile, UAE with thermal hydrolysis significantly increased from the albedo and segmental parts: naringin (from 17.45 ± 0.872 mg/g to 25.05 ± 1.25 mg/g), and naringenin (from 0 mg/g to 4.21 ± 0.55 µg/g). Additionally, magnesium aluminometasilicate revealed significant increases in naringenin from all treated grapefruit parts.



▶ **Fig. 1**

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P-314 Extraction of chokeberry anthocyanins using natural deep eutectic solvents

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Fruits of chokeberry (*Aronia melanocarpa* (Michk) Elliot) contain high levels of phenolic compounds including anthocyanins which show strong antioxidant activities and reduce the risks of metabolic syndrome and age-related disease. Beyond internal use, chokeberry extracts can be incorporated into pharmaceutical formulations for topical application. The challenge in anthocyanin extraction is their limited chemical stability. In this study, an innovative eco-friendly method for the extraction of chokeberry anthocyanins was investigated. Anthocyanin extractions were performed using natural deep eutectic solvents (NaDES) coupled with ultrasound-assisted extraction. For this purpose, nine different NaDES composed of choline chloride as a hydrogen bond acceptor and organic acids, sugars, polyols, and an amide as hydrogen bond donors were screened. NaDES composed of choline chloride and malic acid was selected and used further for optimization of extraction conditions (sonication time, temperature, content of water in NaDES) by response surface methodology. Highly accurate predictive models for cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, and total anthocyanins were developed. Optimal conditions for simultaneously maximizing the anthocyanins extraction yield were 42.7 °C, 90 min, and 40% (w/w) water in NaDES. In the next stage of this study, the possibility of improving anthocyanins extraction yields at high extraction temperature by incorporating different concentrations of hydroxypropyl-β-cyclodextrin (HP-β-CD) into selected NaDES was investigated. The extraction yield was improved at HP-β-CD concentrations up to 3% (w/w). Developed methods can be useful for anthocyanins extraction from chokeberry fruit aimed to obtain standardized extracts for the application in pharmaceutical, cosmetic, and food industries.

P-315 Real-world evidence: evaluating a herbal combination treatment (valerian, passionflower, hawthorn and black horehound, BAY-987204) for mental stress

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Many people experience chronic detrimental stress at some point in their lives. Typical symptoms like nervousness, irritability and sleep problems negatively impact quality of life. Some people turn to prescription or OTC sedatives, which often have unwanted side effects without actually solving the problem. Well-tolerated herbal alternatives might be more helpful.

The aim of the study was to generate further evidence for the purported benefits of the traditional herbal combination product BAY-987204 in relieving mild symptoms of stress and aiding sleep by collecting retrospective information from consumers in France.

Users of BAY-987204 were recruited from a consumer panel comprising people who indicated that they had taken/were taking the product for one of the indications listed on the product label (at time of survey: minor anxiety states, mental stress, sleep disorders).

520 respondents completed the survey: 89% took the product to improve sleep, 78% to reduce stress. BAY-987204 improved both sleep- and stress-related symptoms. Results did not differ when stratified by age, sex or employment status. Ca. 60% agreed or strongly agreed that quality of sleep had improved, they felt more rested, relaxed and less irritable during the day and could cope with stress and problems more calmly. Most respondents (68%) reported feeling better within seven days.

In this real-world survey, BAY-987204 was found to aid sleep and relieve mild symptoms of stress, reducing irritability and increasing relaxation. The use of BAY 987 204 was associated with a rapid perceived onset of effect and a high level of consumer satisfaction.

P-316 Evaluating the effects of a herbal combination preparation (BAY-987204) on symptoms of mental stress and sleep in a real-world survey

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Chronic stress is seen as the number one health risk of our time. In addition to the serious long-term implications, typical symptoms like nervousness, irritability and sleep problems also negatively impact quality of life. The treatment of choice would be to reduce stress, however, this is not always possible. Some medicinal plants with a long-standing tradition for use in this indication might support in coping with stressful times.

A combination of four of these herbs (valerian, passionflower, hawthorn and black horehound, BAY-987204) has been used in France for decades to relieve symptoms of mild anxiety, mental stress and sleep disorders. The current post-marketing survey assessed the self-reported effects of the herbal preparation, now also available in Germany (Calmalaif®), in a real-world setting during product use. Consumers were recruited while buying BAY-987204 and were asked to fill an online questionnaire at least 2 weeks after buying the product.

At the time of this first assessment, 533 respondents completed the survey. The herbal combination improved both stress and sleep related symptoms. 82% agreed that they can handle daily stress better and more than 80%

agreed that they felt less nervous, tense, irritable and unsettled. 76% or more agreed that it improved their sleep. General well-being improved in 87% of users.
In the current survey, the majority of users agreed that BAY-987204 relieved mild symptoms of stress and improved sleep. The use of BAY-987204 was associated with high level of consumer satisfaction. 92% of users stated that they would recommend the product.

P-317 MAMA Decoction, an herbal antimalarial preparation, alters the disposition of amodiaquine in humans

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MAMA decoction (MD), a preparation from the leaves of *Mangifera indica, Alstonia boonei, Morinda lucida,* and *Azadirachta indica*, was co-administered with amodiaquine, and resulted in the synergistic clearance of malaria parasites in a previous report [1]. The pharmacokinetic basis for this observation, significant increases in the exposure and half-life of desethylamodiaquine, the major metabolite of amodiaquine, was reported in mice [2]. Here, a further evaluation of these previously identified herb-drug interactions was carried out in healthy human volunteers.
Single oral doses of amodiaquine (10 mg/kg) with/without MD (0.25 L, 0.04% w/v gedunin) were co-administered to 16 healthy volunteers in a three-period crossover design. Blood samples were collected between 0 h and 48 h for each study period and analysed for amodiaquine and desethylamodiaquine contents. The effect of MD on amodiaquine disposition across study periods was investigated using a non-linear mixed-effect pharmacokinetic model which estimated population parameters with the stochastic approximation expectation maximization (SAEM) algorithm implemented in Monolix 2020R1.
The disposition of amodiaquine and desethylamodiaquine were each described, adequately, by two- and one-compartment structural models respectively, and a first-order oral absorption rate. The co-administration of amodiaquine with MD resulted in about 41% decrease in the apparent volume of distribution of amodiaquine (VAQ/F). Chronic administration of MD prior to the dosing of amodiaquine led to a 22% decrease in VAQ/F.
MD appeared to decrease the tissue partitioning of amodiaquine in humans. The consequence of this for effective parasite clearance in humans is, however, unknown.
The authors declare no conflict of interest.
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P-318 Pharmacological interactions of a herbal combination of myrrh, chamomile flowers and coffee charcoal for the treatment of gastrointestinal disorders

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Synergistic interactions play a major role in phytomedicine, as they often justify the therapeutic superiority of plant combinations over single compounds. A herbal combination consisting of myrrh (*Commiphora myrrha* (Nees) Engl.), coffee charcoal (*Coffea arabica* L.) and chamomile flower dry extract (*Matricaria chamomilla* L.) for which anti-inflammatory, spasmolytic and barrier-stabilizing activity have been demonstrated [1], is traditionally used for the treatment of gastrointestinal diseases.
Aim of the present study is to provide an overview on the pharmacological interactions of the herbal components. Thus, spasmolytic activity was determined by isometric contraction measurements using isolated rat small intestinal preparations. In a cell model of the inflamed intestinal mucosa consisting of LPS-activated THP-1 macrophages and an epithelial monolayer (Caco-2/HT-29-MTX cells), influences on pro-inflammatory cell-cell signaling (IL6, IL8, TNF, PGE2) by ELISA and a resulting barrier impairment of the cell monolayer were investigated by transepithelial resistance measurements. The characterization of the pharmacological interaction was based on the combination index (CI) according to Chou 2006 [2].
Additive and synergistic effects were observed, especially for the inhibition of pro-inflammatory cell-cell signaling of IL-6 (CI = 0.1), TNF (CI = 0.4) and PGE2 (CI = 1.1) from activated macrophages and IL-8 (CI = 0.7), PGE2 (CI = 0.6) from intestinal epithelial cells as well as barrier stabilization (CI = 0.8). The spasmolytic activity of chamomile flowers and myrrh complemented each other additively (CI = 1.0).
In conclusion, cooperative effects between herbal ingredients, in combination with a multi-target approach, may reinforce the use of herbal drugs combinations.
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P-319 Effect of *Tormentillae tinctura* on the barrier function of Caco-2 cells monolayer

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Tormentillae rhizoma is a traditionally used pharmacopeial plant material with various applications related to gastrointestinal tract ailments. The plant material is taken orally, the most popular preparation is a tincture (*Tormentillae tinctura*, TT). Due to its tannin-reach composition, it can offer an opportunity for novel approaches in the therapy of Leaky Gut Syndrome [1,2].
The research was aimed to establish the effect of TT on the gut barrier integrity with transepithelial permeability assessment of its constituents.
The cytotoxicity of the TT towards Caco-2 cells was investigated using the MTT test. The barrier functions of Caco-2 monolayer were examined by 24 h incubation with TT accompanied by real-time monitoring of the transepithelial electrical resistance (TEER). Analysis of substances permeating through the monolayer was conducted using UHPLC-DAD-MS.
It was shown that TT is not cytotoxic towards Caco-2 in a concentrations 0,0625–5 mg/ml. The addition of TT did not affect the condition of Caco-2 monolayer based on TEER monitoring. Significant differences for TT constituents with regard to their migration through monolayers were observed. Compounds with the best permeability were saponin aglycones, mainly tormentic acid derivatives, which were detected in the acceptor medium together with some triterpene glycosides. Instead, highly abundant in TT oligomeric procyanidins were not able to cross the monolayer.
The research has indicated, for the first time, which natural products contained in *Tormentillae tincura* can cross the intestinal barrier in vitro and potentially express their bioactivity beyond the gut lumen.
Project financial support: Polish National Science Centre research grant Sonatina 5 2021/40/C/NZ7/00 231.
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P-320 Prospects for expanding *Solidago herba* base among the representatives of the genus from the flora of Ukraine

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More than 100 species of the genus *Solidago* L. (Asteraceae family) are known in the world flora, 8 species grow in Ukraine, the most widespread are *S. virgaurea* L., *S. canadensis* L. and *S. caucasica* L. Goldenrod medicines have diuretic, choleretic, antibacterial, anti-inflammatory and astringent activities.

According to the State Pharmacopoeia of Ukraine, the raw material is *Solidago* herba, whole or cut, dried flowering aboveground parts of Solidago gigantean or *Solidago canadensis*, their varieties or hybrids (Content of flavonoids: not less than 2.5%, in terms of hyperoside and dry raw materials).

The aim of the research is a comparative phytochemical study of the most widespread species in Ukraine: *S. virgaurea, S. canadensis* and *S. caucasica* to expand raw material base.

The objects of the research were S. *virgaurea, S. canadensis* (Ivano-Frankivsk) and *S. caucasica* (Kyiv) herbs. TLC were used to identify BAS in the objects, for assay – pharmacopoeial methods of spectrophotometry and titrimetry.

As a result of the phytochemical analysis in the Goldenrod herbs were found flavonoids, tannins, aminoacids, polysaccharides, saponins, coumarins and traces of alkaloids.

The content of tannins, flavonoids, ascorbic acid and organic acids were determined. The content of flavonoids in the raw materials almost didn't differ. The highest content of tannins (4.34 ± 0.02%) and flavonoids (2.58 ± 0.02%) are in *S. virgaurea*.

Thus, it is advisable for *Solidago* herba to harvest all three species *S. virgaurea, S. canadensis* and *S. caucasica*, taking into the account their prevalence and possibility of cultivation in Ukraine.

P-321 New Hops-Products for Neuroprotection and Regeneration: Consumer Insights and Product Expectations

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Innovative Natural Health Products (NHP) – including herbal medicinal products as well as natural food supplements – show growing demand in Germany. In recent years researchers scrutineered by-products of the hops processing industry and found secondary plant compounds (prenyl flavonoids) having the potential to stimulate the formation of neurons [1–3]. These herbal extracts have the potential to extend the use of hops (*Humulus lupulus* L.) as a medicinal plant from sleep aids to modern therapeutic approaches in the fields of neurodegenerative diseases (e.g., dementia, depression) and neuroprotection (e.g., cognition enhancers).

To address the potential target group of these new application fields a deeper understanding of consumer demand and the reasons, leading to the choice of new NHPs, is essential. Therefore, a national representative online survey with 1.707 participants was carried out in Germany in April 2022. The survey queried the general use of NHPs in Germany and the interest for an innovative cognition enhancer based on hops. Product attributes that attract potential consumers to such a product, was determined with a choice experiment. All these analyses will contribute to assess the therapeutic demand and to define a target group for new neuroactive hop-NHPs.

There are no conflicts of interest.

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P-322 Pharmacogenetics and herbal medicines. Considerations about interactions herb-drug

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Background: The science of pharmacogenomics has advanced significantly in the last five years, but it is still in infancy and is mostly used on research basis. Pharmacogenomics may involve the pharmacokinetic or pharmacodynamic pathways to affect herb-drug interaction. Due to the fast growing in the consumption of phytomedicines, it is necessary the investigation of mechanism of actions of these products with more rigor and is important to know the implications of the interaction with drugs based on pharmacogenetic.

Aims: To present an updated report about this novel topic pharmacogenetic and its relation with herbal medicines focus to interaction herb-drug.

Results: The herbal medicines like synthetic drugs have been showed their bioactivation through cytochrome P-450, the main enzyme involved in the metabolism of xenobiotics. The main enzymes involved in the metabolism of phytomedicines, the advantages and disadvantages of bioactivation to metabolites less or more toxics are described as well as the pharmacodynamic interactions involving herbs. Moreover, the herbs which affect the P-glycoprotein activity in vitro will be showed. These studies strengthen and optimize the safety of herbal medicines. Some examples will be shown like: St. John's Wort, Baicalin, Gingko, etc.

Conclusion: The hope for the future is that through personalized medicine, doctors and patients will be able to make better-informed choices about treatment. This treatment will avoid the adverse drug reaction to the medication and will improve the diagnosis diseases as well as the prevention and treatment of diseases.

Conflict of interest

The author declares no conflict of interest.

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P-323 Isolation of Phenolic Compounds from *Alchemilla phegophila* Juz

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Medicinal plant raw materials contain groups of biologically active substances that have various effects on the human body, it is used in folk and scientific medicine for both prevention and treatment of many diseases. The scientific interest was caused by the *Alchemilla phegophila* Juz. plant, which is an independent botanical type of *Alchemilla* L. It is a perennial herbaceous plant of

grey green colour with an upright densely pubescent stem, 35–50 cm high, which has experience in folk medicine [1,2].
The purpose of the work was to select phenolic compounds from the herb *Alchemilla phegophila* Juz.
With the help of qualitative reactions and chromatographic analysis in the herb *Alchemilla phegophila* Juz. the presence of flavonoids, hydroxycoric acids and coumarins was established.
For the separation of phenolic compounds *Alchemilla phegophila* Juz. the method of adsorption chromatography on polyamide columns was used, followed by repeated chromatography, fractional crystallization, and preparative chromatography on paper. Chromatographic separation was subjected to water and ethyl acetate fractions [3].
As a result of a study of the herb *Alchemilla phegophila* Juz. the following phenolic compounds were isolated: 4 hydroxycinnamic acids (n-coumaric, caffeic, ferulic, chlorogenic), 3 coumarins (umbelliferone, esculetin, scopoletin) and 6 flavonoids (luteolin, kaempferol, astragalin, quercetin, hyperoside, routine).
The obtained data indicate the prospect of using the herb *Alchemilla phegophila* Juz. as sources of biologically active substances of phenolic nature.

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P-324 Antiproliferative and pro-apoptotic effect against HaCaT human keratinocytes of hydrogels containing polymeric micelles as delivery systems for oregano essential oil

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Background: Polymeric micelles-based hydrogel formulation was developed using a mixture of two poloxamers, as solubilizers for – Origanum vulgare L. essential oil (OEO), but also as gelling agents. This topical product can represent an alternative to the current invasive approach for the management of skin tags, which include keratinocytes among the main constituents.
Aim: To evaluate in vitro the antiproliferative and pro-apoptotic potential and mechanism of action of standardized OEO (thymol and carvacrol as main components) as such and incorporated 5% into the developed hydrogel vehicle containing 20% Pluronic® F-127, 1% Pluronic® L-31 and water on HaCaT human keratinocytes
Results: Six different concentrations of the samples were tested, namely 10, 25, 50, 100, 150 and 200 µg/mL (24, 48 and 72 h). MTT and "scratch assay" were used to determine the antiproliferative potential of the samples, LDH assay for the cytotoxic effect, DAPI staining and caspase-3 to detect the pro-apoptotic potential. OEO 5% formulation and OEO alone produced a dose-dependent decrease in cells viability especially at 72 h post-stimulation. At the same interval, LDH release and caspase-3 were detected and following DAPI staining the cells showed that chromatin condensation is increasing dose-dependent and signs of nuclear membrane blebbing were observed.
Conclusion: Tested samples induced in a dose dependent manner antiproliferative, pro-apoptotic and cytotoxic effect on HaCaT human keratinocytes. The hydrogel did not influenced the biological activity. The results are promising for furtherer in vivo tests on skin papilloma
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P-325 Vegetable butters and oils: scientific evidence for cosmetic and therapeutic use

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Vegetable butters and oils have been used for centuries in the care and treatment of skin disorders. However, scientific evidence about their mechanisms of action and effectiveness after dermal application is still limited [1].
Vegetable butters and oils are composed of triglycerides and unsaponifiable compounds. Triglycerides are mainly responsible for a non-specific emollient effect, which results in decreased transepidermal water loss and thus improved skin hydration. The specific action includes antimicrobial, anti-inflammatory and antioxidative effects, which are expressed by unsaponifiable compounds and free fatty acids derived from triglycerides. In contrast to beneficial dermal effects, lipid barrier disruption has been observed in vegetable oils with the predominant oleic acid [2,3]. Recent evidence shows that in inflammation-affected skin, oils high in oleic acid, together with the lack of or low linoleic acid, cause additional stratum corneum damage, while oils high in linoleic acid and saturated fatty acids express positive effects. Non-affected skin is generally resistant to the damaging potential of oils high in oleic acid [4].
In conclusion, dermal use of vegetable butters and oils is supported by evidence, as they are affordable, easily accessible, generally linked to good skin compatibility, have fewer side effects than specific conventional treatments, and function as effective pharmaceutical ingredients in dermal treatments and as active cosmetic ingredients.

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P-326 *Arnica* planta tota is superior to *Arnica* flos in terms of anti-inflammatory properties in vitro and in vivo

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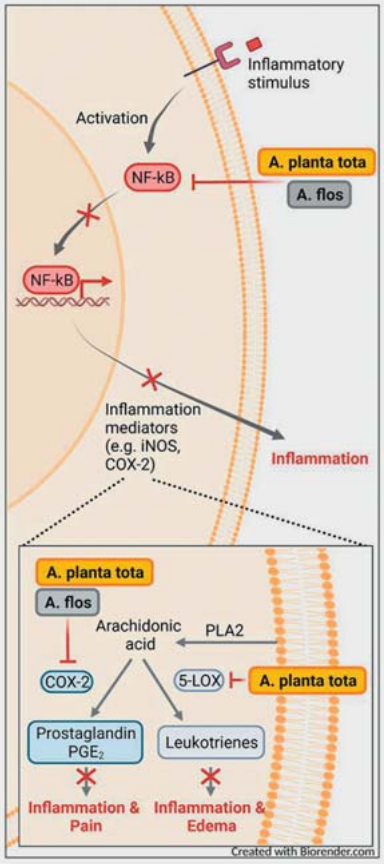
Traditionally *Arnica montana* L. is indicated for treatment of blunt injuries like strains and bruises that are physiologically accompanied by local inflammation, including activation of the NF-κB pathway and release of pro-inflammatory leukotrienes and prostaglandins.
Here we aimed to evaluate the anti-inflammatory efficacy of ethanolic extracts from A. planta tota and A. flos.
Dry extracts from *Arnica montana* L. were prepared using liquid extracts from *A.* planta tota, fresh plant or *A.* flos, fresh flowers (DER 1 : 1,1, 30% ethanol (m/m)). NF-κB activation was analysed in a reporter assay with the human Jurkat cell line. Stimulated human PMNLs or monocytes were used for analysis of 5-lipoxygenase product formation and PGE_2 release. In vivo anti-inflammatory properties were analysed in a carrageenan-induced paw edema mouse model.
A. planta tota and *A.* flos concentration-dependently inhibited NF-κB activation (IC_{50}: 15.4 µg/ml and 52.5 µg/ml), and PGE_2 release from monocytes (IC_{50}: 26.7 µg/ml and 105.4 µg/ml). 5-lipoxygenase product formation in PMNLs was inhibited by A. planta tota but not A. flos (IC_{50}: 59.3 µg/ml and > 300 µg/ml). Topical application of *A.* planta tota or *A.* flos inhibited significantly the carrageenan-induced paw swelling.



▶ **Fig. 1** Anti-inflammatory properties of A. planta tota and A. flos [rerif].

Thus, the Weleda *Arnica* planta tota extract demonstrated superior anti-inflammatory activity in vitro and in vivo.
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 (▶ **Fig. 1**). However, further studies are required to further characterise potential therapeutic advantages of *A.* planta tota preparations.

P-327 A multi-component herbal preparation induces colon relaxation via inhibition of M3 muscarinic receptor

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The herbal multi-component preparation MCP (contained in Weleda Digestodoron®) is traditionally used for treatment of gastrointestinal dysmotility. Patients present heterogeneous syndromes such as motility disturbances leading to abdominal pain and disordered bowel habits. Our study aimed to investigate the mode of action of the MCP single herbal extracts (*Dryopteris filix-mas, Phyllitis scolopendrium, Polypodium vulgare* (PV) and *Salix folium*) on intestinal motility. Dry extracts were prepared from the liquid extract from fresh plants.
We studied the in vitro effect of the extracts on G-protein-coupled-receptors known to play a role in the etiology of gastrointestinal disorders: muscarinic M3, opioid δ and μ and serotonin 5-HT4 receptors. Only PV inhibited 3H-4-DAMP binding to M3 receptors (IC_{50}: 259 μg/mL) and its activity shown by inhibition of Ca2+ release (IC_{50}: 79 μg/mL). Opioid and 5-HT4 receptors' activities were not affected.
To study the PV anti-cholinergic activity, two ex vivo approaches were performed in circular muscle strips from guinea-pig colon. First approach evaluated the relaxing effect of PV (5 mg/mL) [1]. Following a contraction induced by muscarinic agonist Carbachol (Cch), ethanol-free PV was added and produced a relaxation of the contraction (76% relaxation). Second approach investigated the PV antagonist activity on Cch-induced concentration-response curves (CRC). When compared to the Cch-CRC before PV addition (EC_{50}: 0.12 μM), the presence of PV produced a significant parallel rightward shift of the second Cch-CRC (EC_{50}: 1.53 μM).
These data suggest that the MCP contained in Weleda Digestodoron® induces relaxation on colon Cch-induced contraction via the anti-cholinergic activity of PV (▶ **Fig. 1**).

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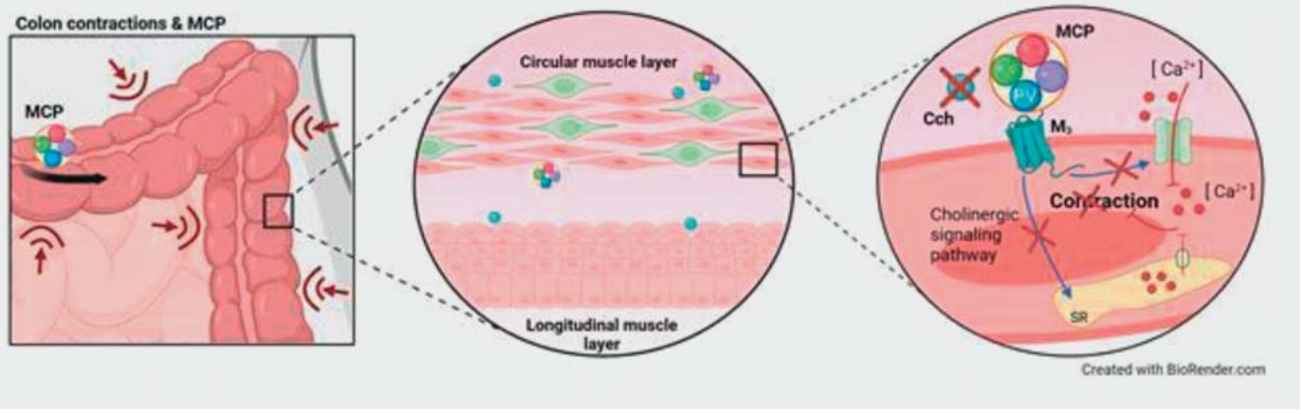

▶ **Fig. 1** The herbal multi-component preparation (MCP) induces a relaxation effect on colon Carbachol (Cch)-induced contraction via the anti-cholinergic activity of *P. vulgare* [rerif].

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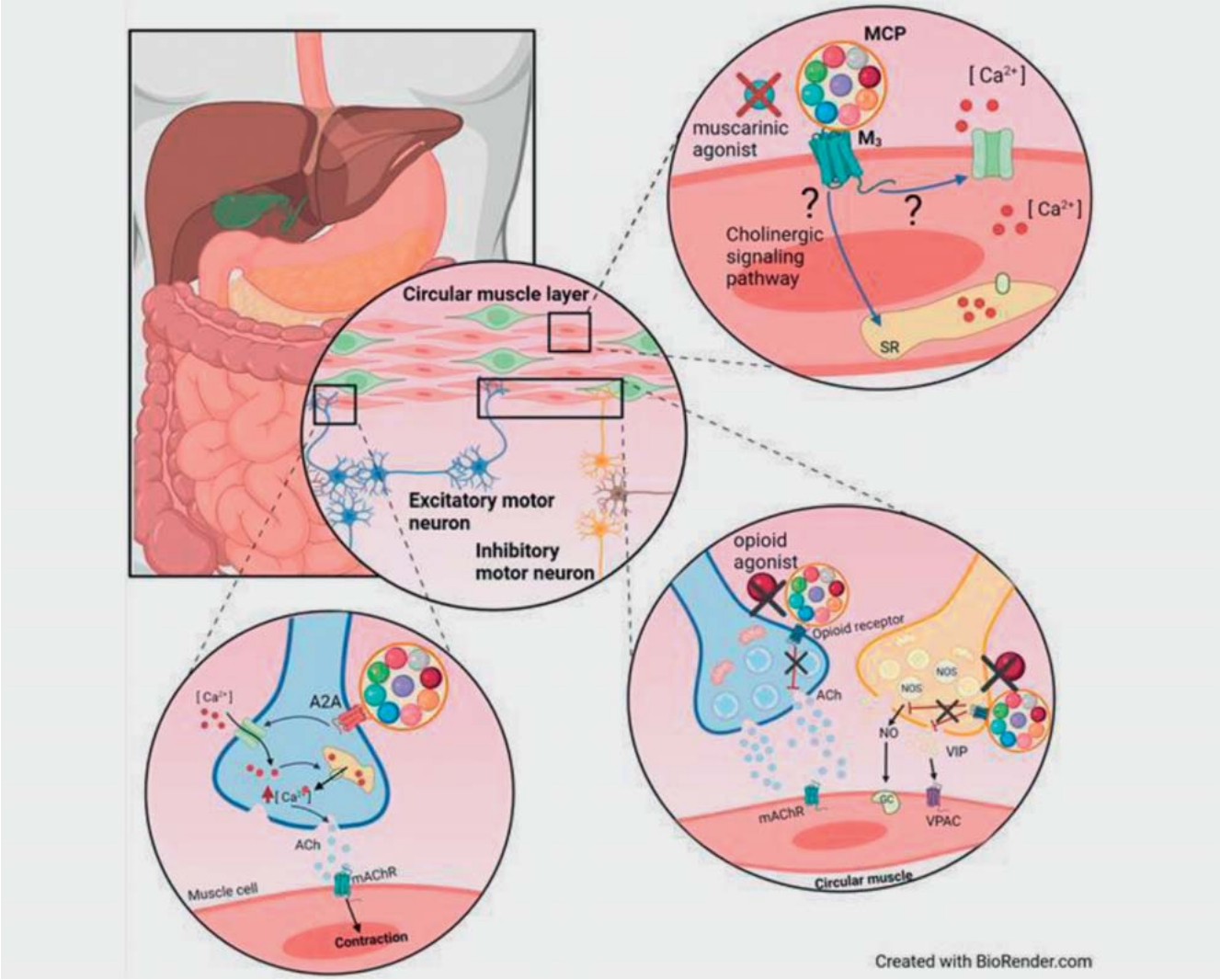

▶ **Fig. 1** The herbal multi-component preparation (MCP) affects the activity of different receptors involved in gastrointestinal motility disorders [rerif].

P-328 Target identification of a multi-component herbal preparation in gastrointestinal dysmotility disorders

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Weleda Amara oral drops (Amara-Tropfen) is traditionally used for treatment of gastrointestinal dysmotility disorders and contains a herbal multi-component preparation (MCP) of nine bitter hydroethanolic herbal extracts (*Achillea millefolium, Artemisia absinthium, Centaurium erythraea, Cichorium intybus, Gentiana lutea, Juniperus communis, Peucedanum ostruthium, Salvia officinalis* and *Taraxacum officinale*). Patients suffering from motility disorders present heterogenous syndromes such as epigastric pain, vomiting and inflammation. However, the origin of these symptoms remains poorly understood. Several studies have shown the efficacy of phytotherapeutic combinations in improving dysmotility symptoms. Therefore, here we aimed to identify disease-modifying targets of MCP in vitro.

We investigated different G-protein-coupled-receptors known to play a role in the pathogenesis of gastrointestinal disorders: muscarinic M3, adenosine A2A, serotonin 5-HT4, opioid δ and μ receptors. MCP was studied for binding affinities to M3 and A2A receptors and for functional activity on 5-HT4, opioid δ and μ and A2A receptors. The MCP dry extract was prepared from the commercial medicinal product Weleda Amara oral drops.

MCP inhibited the binding of 3H-4-DAMP to M3 (IC_{50}: 380 μg/mL) and A2A receptors (IC_{50}: 91.3 μg/mL). In addition, MCP potentially impaired the activity of opioid δ and μ receptors, shown by inhibition of intracellular cAMP release (IC_{50}: 304 & 256 μg/mL, respectively) and increases Ca2+ intracellular levels via activation of A2A receptor (IC_{50}: 318 μg/mL). However, 5-HT4 receptor activity was not affected.

These data suggest that the herbal extract combination MCP contained in Weleda Amara oral drops affects receptors involved in gastrointestinal motility disorders (▶ **Fig. 1**).

P-329 Mechanisms of action of a multi-component herbal preparation in inflammatory bowel disease: anti-inflammatory activity

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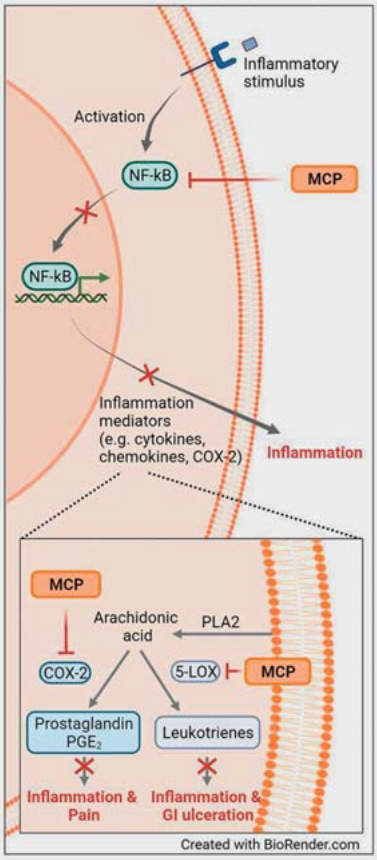
Weleda Amara oral drops is traditionally used for treatment of gastrointestinal dysmotility disorders and contains an herbal multi-component preparation



▶ **Fig. 1** The herbal multi-component preparation (MCP) efficiently inhibits pain and inflammation mediators such as 5-LOX and COX-2 enzymes, cytokines and the NF-κB pathway [rerif].

(MCP), i.e., nine bitter hydroethanolic herbal extracts (*Achillea millefolium, Artemisia absinthium, Centaurium erythraea, Cichorium intybus, Gentiana lutea, Juniperus communis, Peucedanum ostruthium, Salvia officinalis* and *Taraxacum*). Patients suffering from inflammatory bowel disease (IBD) have been characterized by impaired intestinal motility associated with mucosal inflammation. In this study, we investigated the anti-inflammatory properties of MCP and its individual extracts. The MCP dry extract was prepared from the commercial medicinal product Weleda Amara oral drops (Amara-Tropfen).

Inflammation is typically characterized by release of pro-inflammatory leukotrienes, prostaglandins and cytokines and activation of NF-κB pathway. Analysis of inhibition of pro-inflammatory and pain-related enzymes 5-LOX and COX-2 was performed using recombinant human enzymes. Proinflammatory cytokine release was studied using human peripheral blood mononuclear cells (PBMCs) and NF-κB activation was analyzed in a reporter assay using human Jurkat cell line. MCP (IC_{50}: 3 µg/mL) and its single herbal extracts inhibited COX-2. MCP (IC_{50}: 3.34 µg/mL) and the single herbal extracts *Achillea millefolium, Juniperus communis, Taraxacum officinale, Salvia officinalis and Peucedanum ostruthium* strongly inhibited 5-LOX enzymes in a concentration-dependent manner. In addition, MCP concentration-dependently inhibited NF-κB activation (IC_{50}: 56 µg/mL) as well as the release of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, MIP-1α and TNF-α).

These data suggest that the herbal extract combination MCP contained in Weleda Amara oral drops is an efficient treatment option for patients suffering from IBD based on its potent anti-inflammatory and pain-related efficacy (▶ **Fig. 1**).

P-330 Combination of *Primula veris, Onopordum acanthium* and *Hyascyamus niger* extracts and their effects on the activity of cardiac ion channels

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Cardiovascular diseases are common chronic diseases which are often associated with regulatory dysfunction such as hypertension, chronic heart failure and cardiac arrhythmias. In this context, ion channels are important therapeutic targets for all aspects of cardiac function. Here we show in vitro cardiac ion channel activity investigations of *Primula veris, Onopordum acanthium* and *Hyascyamus niger* extracts that are components of the herbal medicinal product Cardiodoron®, traditionally used in the treatment of cardiovascular problems.

Dry extracts of *P. veris, O. acanthium* and *H. niger* were prepared according to V.2a HAB and applied to stably transfected CHO and HEK293 cells to measure currents of human sodium, potassium and calcium ion channels with the patch clamp technique.

P. veris and *H. niger* significantly inhibited late current of NaV1.5 ion channels (35% relative remaining current (RRC)) to a similar extent as the positive reference Ranolazine (32% RRC), that is associated to a diastolic relaxation of the heart muscle for improved blood supply and alleviation of angina pectoris symptoms. Another finding was the selective inhibition of HCN4 channels by *P. veris* and *H. niger* (22% RRC) compared to the inhibition by Ivabradine (16% RRC), which is associated to a reduction of heart rate in tachycardia. Furthermore, we could show the inhibition of calcium ion channels CaV1.2 (18% RRC) and CaV3.2 (22% RRC) by cowslip and henbane. In conclusion, these findings may indicate antiarrhytmic and anti-hypertensive effects of the herbal extracts. However, functional studies are needed to determine the potential therapeutic benefits of the proposed combination.

P-331 *Euphrasia* planta tota – an anti-inflammatory multi-target application

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Euphrasia officinalis is a traditional medicinal plant commonly used to treat irritated eyes in allergic or non-infectious conjunctivitis and catarrhal inflammation by relieving symptoms such as redness, swelling, pain and increased lacrimation. These issues are usually associated with activation of the nuclear factor-kappa B (NF-κB) signalling pathway, increased oxidative stress and the release of pro-inflammatory mediators such as leukotrienes, prostaglandins and metalloproteinases (MMPs).

However, the characterisation of the pharmaceutical mode of action of *Euphrasia* is rather scarce. Here we present several pharmacological targets effectively treated by *Euphrasia* planta tota (EPT), demonstrating its anti-inflammatory properties in vitro.

Dry extracts of EPT were prepared according to V.3c HAB. NF-κB activation was analysed in a reporter assay using the human Jurkat T cell line, showing concentration-dependent inhibition of NF-κB translocation by EPT (IC_{50}: 50.7 µg/ml). We also detected the reduction of oxidative stress with EPT in the DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay (IC_{50}: 133 µg/ml). Furthermore, we revealed the effective enzyme inhibition of the inflammatory mediators cyclooxygenase-2 (IC_{50}: 7.6 µg/ml) and 5-lipoxygenase (IC_{50}: 27.9 µg/ml), which play a major role in pain and edema formation. In addition, we demonstrated the reduced activity of several matrix metalloproteinases by EPT, showing the ability to prevent tissue damage, a common driver of the inflammatory cascade in eye irritation.

These mechanisms targeted by EPT contribute to an overall anti-inflammatory response, which may ultimately promote the symptom relief in irritated eyes. However, further studies are needed to more precisely determine the potential therapeutic benefits of EPT.

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P-332 Inhibition of key inflammatory targets by *Malva sylvestris* L. flos in the context of dry eye disease

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Dry eye disease (DED) is an ocular disorder usually associated with inflammation of the corneal surface due to hyperosmolarity and loss of tear fluid. In this context, inflammation is characterised by activation of the nuclear factor-kappa B (NF-κB) signalling pathway and release of inflammatory mediators such as matrix metalloproteinases (MMPs). In particular, increased MMP-9 levels have been found to accelerate the process of tissue degradation in the apical corneal epithelium, contributing to its desquamation and barrier disruption, and also playing an important role as a biomarker in DED [1,2].

Here, we report the beneficial anti-inflammatory effects of *Malva sylvestris* L. flos (MS) dry extracts, which may have a positive impact on the treatment of DED.

The dry extracts were prepared from the ethanolic extracts of the dried flowers of *Malva sylvestris* L. flos (∅ = D1 V.20, Eth. 43 % m/m flos).

We have demonstrated concentration-dependent inhibition of NF-κB translocation by MS in a reporter assay using the human Jurkat cell line (IC_{50}: 34 µg/ml) indicating its down-regulation of immune responses and inflammation. In addition, we report reduced activity of several MMPs under MS treatment in enzyme inhibition assays, particularly for inhibition of MMP-9, which plays a key role in the inflammatory cascade in eye irritation by degrading inflamed tissue (IC_{50}: 30.8 µg/ml).

Overall, these mechanisms targeted by MS contribute to an overall anti-inflammatory response and may ultimately lead to a beneficial symptom relief in irritated eyes. However, further studies are needed to more precisely determine the potential therapeutic benefits of MS.

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P-333 Interaction of the extract from marigold flowers and comfrey root with human skin microbiota

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Marigold flowers (*Calendula officinalis*) and comfrey root (*Symphytum officinale*) are plant materials traditionally used as an anti-inflammatory and wound healing topical remedies for skin diseases. Also, they are the components of many popular ointments, creams, and tinctures applied directly to the skin [1,2].

The aim of the research was to establish the phytochemical composition of 70 % ethanolic extracts, the verification of its influence on the biodiversity of the skin microbiome and evaluate its metabolism by human skin microbiota.

The extracts were analyzed using HPLC-DAD-MS. It was shown that *Calendula officinalis* mostly contains phenolics, flavonoids, and saponins, and Symphytum officinale is rich in alkaloids, lignans, and phenolic acid derivatives.

16 s rDNA sequencing showed that tested extracts (at 2 mg/ml) have no influence on the biodiversity of the skin microbiota after 24 h incubation. However, some qualitative changes in microbiota composition were observed. Changes in extracts composition after incubation with skin microbiota for 24, 48, and 72 h were also assessed. Tinderesting application and data-transformation script in R [3] were used to screen for potential metabolites and microbiota-enhanced biodegradation.

The results showed extracts alter the composition of skin microbiota. That may play a role in extracts' activity in the treatment of skin diseases. Microbiota-enhanced biodegradation of extracts was detected using a machine learning approach. Some metabolites were detected in samples with comfrey root.

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P-334 First evidence of the compatibility of a specific herbal multi-component preparation with intestinal bacteria

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The herbal medicine Weleda Amara oral drops contains a multi-component preparation (MCP) consisting of nine different hydroethanolic herbal extracts (*Artemisia absinthium, Centaurium erythraea, Cichorium intybus, Gentiana lutea, Juniperus communis, Milefolii herba, Peucedanum ostruthium, Salvia officinalis* and *Taraxacum*). It is indicated for the treatment of moderate digestive disorders like fullness, heartburn, nausea and disturbed gastrointestinal motility. Optimal digestive function is inevitably linked to a healthy intestinal microbial composition. Thus, medication should not interfere with the intestinal microbiota.

Here we aimed to evaluate the antibacterial impact of the MCP towards distinct bacterial strains using an in vitro approach.

The MCP dry extract was prepared from the final medicinal product Amara oral drops (Amara-Tropfen). Cloves, a known antibacterial spice, was used as herbal reference compound. Bacterial strains were incubated in liquid culture under constant shaking at 37 °C for up to 7 h together with test samples or controls.

The MCP did not reveal antibacterial effects towards the intestinal commensal bacterial strains *Escherichia coli* or *Enterobacter cloacae* up to a concentration of 10 mg/ml. Interestingly, the growth of *E. cloacae* was significantly increased by the MCP. The growth of *Bacillus subtilis* was not affected at low concentrations but inhibited at high concentrations. The reference extract from cloves significantly inhibited the growth of all tested bacterial strains.

These in vitro experiments indicate that the MCP is compatible with the intestinal bacterial strains *E. coli* and *E. cloacae.*

However, further studies are required to characterise the compatibility of the Amara oral drops with the intestinal microbiota.

P-335 Traditional Asian medicinal plants as components in food supplements released on the Bulgarian market

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Food supplements (FS) can include constituents such as herbal substances or preparations, which can exert a pharmacological, immunological, or metabolic action [1]. Patients show increased interest in FS (desire for greater quality of life, wrongfully equating natural with safe and decreased faith in allopathic medicine) and pharmacists are expected to advise their use with limited available information [2].

The constituents of FS released on the Bulgarian market were analysed as registered with the Bulgarian Food Safety Agency. Taxa used in traditional Asian medicine and constituents of FS were identified. Safety information available on the European Medicines Agency's was analysed.

Out of 2 610 analysed FS – 467 (21,6%) have such constituents. 118 taxa were identified. Most often included were *Camellia sinensis* (70 times cited), *Ginkgo biloba* (40), *Withania somnifera* (25), *Panax ginseng* and *Eleutherococcus senticosus* (24 times each). The family represented by the highest amount of taxa is Fabaceae (11 taxa), followed by Lamiaceae (9 taxa) and Apiaceae (6 taxa). Safety information was available for 12 of the 118 taxa. Of the determined taxa, 15 are with an unknown genecenter or with an areal of distribution too wide, outstretching the confines of Asian territories.

More research on the risks associated with exposure to FS needs to be conducted before their use can be properly monitored and advised.

Acknowledgements

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The authors declare that they have no conflict of interest.

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P-336 Analyzing field horsetail products on the market – identifying marsh horsetail impurities

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Equisetum arvense L. (field horsetail) is a plant with variety of properties and uses mentioned in ethnomedicinal sources – diuretic, wound healing, hemostatic, antigout. The species' range of distribution in Bulgaria is throughout the country. *Equisetum palustre* L. (marsh horsetail) has similar macroscopic diagnostic characteristics and habitat. These similarities pose a threat of contamination of *E. arvense* products on the market and therefore to human health since *E. palustre* contains poisonous alkaloids – palustrine, palustridiene [1,2].

The contamination with *E. palustre* in 19 products from the market and pharmacies in Bulgaria has been investigated. 15 of them (78,9%) are mono component, while the other 4 (21,1%) are herbal tea mixtures. Their identity and purity have been determined in three steps – 1. Macroscopic determination; 2. Microscopic determination; 3. Thin layer chromatography [3]. 4 (21,1%), 6 (31,6%) and 6 (31,6%) contaminated products have been identified by the respective analyses. Correlation has been found between the three analyses – same samples have been identified as contaminated by all the analyses. Combining the three methods has been proved beneficial in identifying impurities in the products.

The obtained results show the lack of strict enough control over gathering and identification of plant material used in the analyzed products on the market. Product safety and effectiveness may be decreased and a threat to the population that uses them may be present.

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The authors declare that they have no conflict of interest.

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P-337 Chemical composition, antioxidant, anti-diabetic, and cytotoxic properties of the halophyte *Frankenia laevis* L

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Frankenia species are used in Asian traditional medicine, but just a few studies have reported the in vitro biological properties of *Frankenia laevis* L. [1–3]. Thus, this work explored this species as a potential source of bioactive compounds. Methanol and dichloromethane extracts were prepared from aerial organs (flowers, leaves and stems) and assessed for their chemical composition by HPLC-ESI-MS/MS. The extracts were evaluated for in vitro antioxidant capacity (DPPH and ABTS radical-scavenging, iron reducing power, copper and iron chelation), inhibitory effects on enzymes related with neurodegeneration (AChE and BuChE), Type-2 diabetes (α-glucosidase and α-amylase), hyperpigmentation/food oxidation (tyrosinase), and cytotoxicity towards human hepatocarcinoma (HepG2) cells. Fifty-one molecules were identified in the extracts, including derivatives of phenolic acids, lignans and flavonoids, monoterpenes, and hydroxylated derivatives of linoleic acid. The methanol extract was effective in DPPH and ABTS radical-scavenging (EC_{50} = 0.25 and 0.65 mg/mL, respectively), copper chelation (EC_{50} = 0.78 mg/mL), and iron reduction (EC_{50} = 0.51 mg/mL) activities, whereas the dichloromethane extract had high iron chelating ability (EC_{50} = 0.76 mg/mL). Both extracts inhibited α-glucosidase, especially the dichloromethane (EC_{50} = 0.52 mg/mL). This extract also exerted a significant selective cytotoxicity towards HepG2 cells (EC_{50} = 52.1 µg/mL, SI > 1.9). Generally, extracts from *F. laevis* aerial parts showed to be a promising source of natural compounds for pharmaceutical and/or food additives applications due to their high antioxidant, anti-diabetic, and cytotoxic properties.

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P-338 Identification of biologically active substances of *Ajuga reptans* L

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Phytochemical analysis of raw materials is a necessary step in the analysis of plant raw materials.

We consider *Ajuga reptans* of Lamiaceae family to be a very prospective object for research. Chemical composition of *Ajuga* plants was studied by Turkish and European scientists, but any information on the study of these plants in Ukraine has not been found. *Ajuga reptans* contains a variety of BAC that exhibit anti-inflammatory, hepatoprotective, wound healing and antimicrobial activity [1–3].

The aim of the work was to identify groups of BAC of *Ajuga reptans* raw materials.

Phytochemical study of leaves, flowers and herb of Ajuga reptans was carried out to identify the main groups of BAC. Raw materials were harvested during the mass flowering period. To identify phenolic compounds a sample of 10.0 g of *Ajuga reptans* raw materials was extracted on a water heater under reflux condenser for 1 hour at the boiling point of the extractant. The extracts were evaporated to 1/10 of their volume, applied to Filtrak FN-1 chromatographic paper and studied by two-dimensional chromatography in solvent systems of 15% glacial acetic acid solution and n-butanol – glacial acetic acid – purified water (4 : 1 : 5). The dried chromatograms were studied in UV-light before and after the treatment with chromogenic reagents.

Taking into account the mobility of substances and the fluorescence in UV-light before and after the treatment with reagents 6 flavonoids, 2 hydroxycinnamic acids and 1 coumarin were identified on chromatograms.

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P-339 Oregano in European food market: Botanical identification and quality control in the light of *Codex Alimentarius* standard

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Oregano is a very popular herb widely used worldwide for food flavoring in a number of recipes and dishes. European consumers usually buy dried oregano in various packages with crushed of rubbed leaves and flowering tops. The aim of this study was threefold: (i) to find out the botanical identity of the plant material used in the oregano packages sold in the European food market (ii) to measure the density (number/mm^2) and size (diameter) of leaf sessile glands, and (iii) finally, to look for any relation between glands' size and density and the total essential oil (EO) content. Commercial samples of oregano were purchased from supermarkets and local food stores of southern, central and northern European countries. The botanical identification of plant material was based to the key-characters (calyx-form, bracts, size and density of leaf sessile glands) [1–4]. Three taxa of the genus *Origanum* were intentified to sold as oregano, viz. *O. onites, O. vulgare* subsp. *hirtum* and *O. vulgare* subsp. *vulgare*. A wide range of the glands' density and size as well as of the total EO content was found. On the basis of their EO content (mL/100 g D.W.) the oregano samples are grouped in three quality-categories, as given in *Codex Alimentarius* [5]: Extra: oil content > 2.5%, Class I: oil content 2.4–1.6, and Class II: oil content < 1.5. Finally, the correlation found between the glands' size with the EO content may lead to a rapid and economical assessment of the commercial oregano quality.

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P-340 *Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw. from Holy Mount, Chersonisos Athos (GR1230003): An oregano or a thyme plant?

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Origanum vulgare L. subsp. *hirtum* (Link) Ietsw. (known as Greek oregano) is grown in Balkan Peninsula and Turkey. Besides its popularity as spice in everyday cooking, the essential oils (EOs) of Greek oregano have been extensively studied for their antiviral, antimicrobial and antifungal properties [1]. Athos, commonly named as Holy Mount (Άγιο Όρος in Greek), is a NATURA 2000 site (CHERSONISOS ATHOS: GR1230007) [2] and the locus classicus of *Origanum hirtum* Link wherefrom the type specimen was collected by P. Sintenis and J. Bornmüller in 1891 [3]. Results published to date suggest that the EOs of Greek oregano grown wild in Athos have a high thymol content (up to 66.4% in the summer) [4–5]. The aim of this study was to compare the morphology and EOs constituents of *O. vulgare* subsp. *hirtum* plants collected from two new locations in the central-western part of Athos: (i) near the Monastery of St. Gregory and (ii) near the small settlement Dafni. Morphological micro-characters from calyces, bracts and leaves were counted and presented. Plants from both localities were exceptionally rich in EO content, 6.0 and 7.1 mL/100 g D.W., respectively. Noticeable differences have been found in their EOs composition-studied by Headspace GC/MS- and particularly in the ratio of the two isomeric phenols, namely carvacrol (C) and thymol (T). Carvacrol, that gives plants the so-called "oregano" smell, was the main EO component in St. Gregory's sample (C/T = 46), whereas thymol, which is responsible for what is called "thyme" smell, dominated the EO's flavour from Dafni (C/T = 0.048).

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P-341 *Echinacea* and Propolis phytoconstituents: Development of a novel skin care formulation with in vitro Sun Protection Factor (SPF) boosting properties

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The design of sunscreen formulations with increased Sun Protection Factor (SPF) by combining relatively low concentration of synthetic sunscreens and natural ingredients that boost SPF, is of great interest for the personal care products industry. The study concerned the synthesis of a novel formulation based on *Echinacea* extract and Propolis oil and the evaluation of its potential in developing a broad-spectrum sunscreen formulation.

Echinacea extract and/or Propolis oil were incorporated into a novel sunscreen formulation containing, Ethylhexyl Triazone (EHT), Octocrylene (OCR), Diethylamino Hydroxybenzoyl Hexyl Benzoate (DHHB) and Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (MBBT). The SPF in vitro of the novel formulation was measured based on Diffey–Robson's method. The comparative study between the combination of EHT, OCR, DHHB, MBBT with or without EHMC vs. the combination of EHT, OCR, DHHB, MBBT and phytoconstituents separately or mixed showed that the SPF was boosted by 29%.

We were able to develop a formulation including *Echinacea* and Propolis phytoconstituents very efficient in boosting SPF measured in vitro by > 29%, in comparison with organic sunscreens. These results make *Echinacea* and Propolis phytoconstituents a promising additive for the design of broad-spectrum photoprotective products.

The authors have no conflicts of interest

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P-342 Proteolytic Enzymes Papain and Chymotrypsin Combined with Laser Techniques for the Management of Facial Hirsutism

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Background. Papain is a proteolytic enzyme, derived from the latex of *Carica papaya*. It is used for depilatory preparations and has been proposed as a safe treatment for hirsutism. Photo-epilation is the treatment of choice for hirsutism, but it does not offer complete and persistent results. Meanwhile, iontophoresis of proteolytic enzymes papain and chymotrypsin to skin of experimental animals, has shown long-lasting depilatory effects. Therefore, we think it would be of interest to combine photo-epilation with application of these enzymes, as a more natural approach, to enhance treatment efficacy. Methods. In this randomized controlled blinded clinical study, 30 adult Caucasian women with facial hirsutism were divided into two groups: Group I, treated with combination of laser Alexandrite 755 nm and iontophoresis of aqueous solutions papain 0.48% and chymotrypsin 0.29% and Group II, treated with laser Alexandrite 755 nm alone. Evaluations were based on digital hair counts. Comparison between groups of percentage change from baseline to 6 months was performed using the independent samples t-test and the statistical package SPSS vr 21.00 (IBM Corporation, Somers, NY, USA). The study has been approved by the Research Ethics Committee of the University of West Attica. Results. The decrease of percentage change from baseline to 6 months of the 'Total Number of Hairs' was statistically significantly higher in Group I compared to Group II ($p = 0.017$). Conclusions. Iontophoretic delivery of papain and chymotrypsin provides a more natural adjuvant treatment, with a potential to increase laser-induced hair reduction in hirsute patients.

▶ **Table 1** Analysis of total number of hairs variable between Groups I and II.

Groups	Baseline	After 6 months of treatment	% Decrease
I (Laser + Enzymes)	73.53 ± 13.93	37.60 ± 11.55	− 48.70%±13.77
II (Laser)	77.60 ± 24.47	56.40 ± 27.0	− 25.60%±32.39
p-value			0.017

All values are presented as mean ± SD.

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P-343 St John's wort: Flavonoids favourably influence the gut microbiota

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The gut microbiome has a major impact on human health. Dysbiosis can contribute to various mental disturbances via the gut-brain axis [1], such as depression, for which St. John's wort is effective. St. John's wort (*Hypericum perforatum* L.) contains 2–4% quercetin glycosides [2]. These are metabolized to quercetin in the gut. [3]. The aim of the study was to determine whether quercetin owes its beneficial effect by affecting the microbiome. A literature survey was therefore conducted according to PRISMA for 'quercetin AND gut microbiome'.

The search resulted in finding 4 in-vitro and 7 in-vivo studies with quercetin in animal models. The studies showed that quercetin can prevent the growth of potential pathogenic bacteria, such as *E. coli*, and promote the growth of beneficial short-fatty-acid-producing bacteria, such as Bifidobacterium. [4,5]. Some SCFAs-producing bacteria and SCFAs are decreased in people with depression [6]. The available studies show that quercetin can increase the levels of these bacteria, thereby increasing the levels of SCFAs, which may have a positive effect on depression via the gut-brain axis.

In conclusion one can confirm that quercetin is not only an important constituent of St. John's wort, but its effect on the microbiome might help to understand the mechanisms of action leading to the beneficial use of St. John's wort in depression.

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P-344 Phytotherapeutics in children: Clinical trials vs. Real World Data-which is the better approach?

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Introduction: The number of phytotherapeutics available for the use in children has been decreasing in recent decades, particularly in EU member states, as many products previously used without concerns have no longer been approved for the pediatric age group due to “lack of scientific data.”
Aim: To address this, the review of clinical trials vs. the generation of Real-World Data should be explored.
Methods: Firstly, a systematic review of clinical trials worldwide was conducted according to the PRISMA statement [1,2]. Secondly, the PhytoVIS data base, a pharmacoepidemiological data base documenting the use of phytomedicines in 20,870 patients in Germany according to EnCePP [3], was evaluated [4].
Results: The review included 133 clinical trials, of which 43 were double-blind, most with small group sizes and using partly poorly defined products. From the PhytoVIS data base 2063 data sets from children of all age groups in a wide range of indications were evaluated. Neither tolerability nor the perceived therapeutic benefit were age dependent, and data also showed no influence of the use within age limits compared with the small number of cases below the approved age limits, physicians and pharmacists being the main source of recommendation.
Conclusions: While available clinical trials on phytotherapeutics mostly have limited added value for the documentation of a safe use in children, Real World Data can provide information on a wide range of herbal medicinal products. Accordingly, the large-scale generation of Real-World Data should be encouraged, and regulatory acceptance activated.
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P-345 Phytotherapy for pain: data from the PhytoVIS study, a NIS in 20,870 users of herbal medicinal products

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DOI 10.1055/s-0042-1759315
Introduction: Herbal medicinal products (HMPs) are widely used by patients suffering from pain. However, clinical studies are only available for some of the products, meaning that pharmacoepidemiological data can be an important source of information.
Aims: Therefore, data from the PhytoVIS study, probably the world’s largest pharmacoepidemiological study on the use of HMPs [1], were evaluated.
Methods: The PhytoVIS data were collected in medical practices and pharmacies in accordance with the specifications of the ENCePP code [2] and screened based on indication groups.
Results: A total of 1,350 of a total of 24,056 datasets document the use of HPMs for pain treatment. Patients of all ages used HMPs. Formulations for external use dominated with 30% to 50%, especially in children and elderly patients, followed by solid and liquid oral dosage forms. CGI-E self-reported effect assessment was best for e.g. neck pain, bruises, back pain and headaches, and still good for e.g., migraine and arthralgia. More than 90% of the patients in each age group rated the tolerability as “good” and no intolerances of concern were reported. About 25% of HMPs were recommended by pharmacists and 15% by medical doctors.
Discussion and conclusions: As the patients reported, the phytotherapy for pain was well tolerated and effective. The data be utilized as a basis for therapy decisions.
Acknowledgments
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P-346 Studies of herbal drug preparations containing anthraquinone derivatives in the Ames test: No hints on mutagenicity

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Introduction: Herbal medicinal products play an important role in the treatment of gastrointestinal ailments, including constipation. Clinical studies and many years of experience have proven the effectiveness of the extracts of a number of herbal drugs that have a group of ingredients in common, the anthraquinone derivatives. The genotoxic safety of these drugs has been repeatedly discussed over the last years [1,2].
Objective: Additional data on the genotoxic potential are therefore desirable, for allowing assessments of the therapeutic safety of these drugs, using the

Ames test according to the genotoxicity guideline of the Herbal Medicinal Product Committee HMPC of the Europen regulatory agency EMA [3].
Methods: Extracts from *Aloes* folii succus siccatus, *Rhamni pushianae* cortex, *Frangulae* cortex, *Rhei* radix, *Sennae fructus* and *Sennae folium* drugs, which had been prepared and characterized analytically according to Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP), were tested in the Ames test, following the OECD [4] and HMPC guidances [3].
Results: The extracts did not show a mutagenic effect, even not in the highest concentrations according to the OECD guidance, and irrespective of their contents of anthraquinone aglyca.
Discussion: The data add to the evidence from the published literature, which was evaluated systematically according to the PRISMA statement [5] and support the therapeutic safety of the extracts when used according to the instructions in the resp. HMPC monographs [3].

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P-347 Species of the genus *Melampyrum* as a source of flavonoids

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Among the main tasks of modern pharmaceutical science is the search for plants that can be a source of diverse chemical composition and action of biologically active substances, which have a therapeutic effect and at the same time cause minimal side effects. Such medicinal plant raw materials are used to develop effective medicines.
This applies to plants that have a long history of use in folk medicine, which include species of the genus *Melampyrum* in the family Scrophulariaceae. These are annual herbaceous plants that are widespread in Ukraine. The therapeutic properties of interception are explained by the composition of BAS, among which a significant number are flavonoids [1].
E. E. Galishevskaya, V. M. Petrychenko, E. N. Skryabina conducted research that showed that the grass meadow contains 17 flavonoids. The content of flavonoids in meadow grass is 0.39%, in flowers – 3.17% [1].
Melampyrum pratense is used in traditional Austrian medicine in the treatment of various conditions associated with inflammation. *Melampyrum pratense* extracts stimulate peroxisome receptors α- and γ-, which are well known for their anti-inflammatory activity. Fractionation revealed several active flavonoids.
The flavonoids apigenin and luteolin have been identified as the main components responsible for anti-inflammatory properties. They effectively inhibited tumor necrosis factor α [2].
Thus, the above data show the prospects for the study of species of the genus *Perestrich* for the content of flavonoids with the subsequent development of drugs.

P-348 Self-perceived knowledge concerning herbal medicine: A consumer segmentation study in Germany

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Herbal medicine (HM) has often been described as one of the most popular forms of complementary and alternative medicines [1]. In the German market, preparations for self-medication dominate [2]. Additionally, many consumers using HM in self-medication do not inform their physicians about this [3]. As HM also can have side or interaction effects with other remedies [4] consumers' knowledge is decisive to guarantee save usage.
The aim of this study was to segment German consumers based on their knowledge concerning different topics of HM, and to characterize the resulting segments by sociodemographic characteristics and consumers' wish for further information on HM. In 2018, data of n = 2.520 HM users were collected using a cross-sectional, nationwide online survey and a standardized questionnaire [5]. Respondents had to evaluate their level of knowledge concerning different aspects of HM (e.g., medical or potential side effects). Using two-step cluster analysis, four consumer segments were identified: Individuals of cluster 4 (25.9%) perceived their level of knowledge as good, while respondents of cluster 1 (10.6%) perceived it as very poor. The latter showed the highest share of men, young people, and the lowest wish for further information on HM. In contrast, Cluster 2 (28.8%) and 3 (34.6%), both with a low-medium or a medium level of self-perceived knowledge, had the highest wish for further information. Results of the study can be used to address the information need concerning HM of different consumer groups and thus can help to ensure a safer usage of HM.

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P-349 Effect of isoquinoline alkaloids on trout performance antioxidant status and filet fatty acid profile

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Main targets in the aquaculture industry are to maintain fish health and to improve performance. We studied the effect of plant-based isoquinoline alkaloids (IQs) on growth performance parameters, hematological parameters,

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oxidative status and filet traits of rainbow trout (Oncorhynchus mykiss) under conventional farming. The experiments were carried out at the fish trout farm of Ministry of Agriculture in Louros River, Epirus. A total of 300 sub-adult trouts (63.6 g ± 5.1), were randomly allocated into 3 different treatments with four replicates. The control group (T1) was fed a basal diet, while the other two groups were fed diets supplemented with IQs (Sangrovit®) 500 (T2) and 1000 (T3) g/t of feed, respectively. Results related to body weight, feed intake, feed efficiency and mortality show that the T3 group exhibited substantial increased body weight and SGR compared with the other two groups. IT can be deduced that IQs beneficially affected fish growth rate. Results for fatty acid profiles for the experimental groups record an increased amount of healthy UFA in groups T2 and T3 compared with T1. In terms of feed oxidative status T1 displayed significantly higher TBARS values compared to the IQ groups, which also exhibited higher levels of glutathione peroxidase (GSH-PX) activity in serum. This result indicates that plant-based IQs beneficially altered the filet oxidative status, in agreement with the levels of GSH-PX in the examined serum samples. Plant-based isoquinoline alkaloids (IQs) can be regarded as efficient natural growth promoters in trout.
All authors declare no conflict of interest.

P-350 Effect of dietary herbal extracts on broiler growth performance and intestinal microbiota

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This trial examined the effects of dietary use of an oregano, garlic, camelina and crithmum extract either encapsulated in cyclodextrin or in an aqueous form, on growth performance and on intestinal microflora ecosystem. Control Group A (CL) was fed basal diets based on maize and soybean meal with a trial duration of 35 days. Bacterial community diversity and structure in the ileum and caecum samples after slaughter was investigated through 16S rRNA gene high-throughput amplicon sequencing on the V3-V4 hypervariable region, generating over 30,000 reads/sample. Fimicutes, Proteobacteria and Bacteroidota were the most representative phyla in all diets in both caecum and ileum samples. Intestinal microflora was also dominated by Actinobacteriota, whereas this phylum was less representative in caecum. Alpha-diversity indices highlighted higher species richness and diversity in caecum versus ileum samples, as well as in treatments with the aqueous extract of herbal mixture but only in caecum. Taking into consideration that ileum and caecum samples appeared to consist of heterogenous bacterial assemblages, the beta diversity of the bacterial communities was higher between groups rather than within groups for both tissues. As for LefSe analysis, the most abundant bacterial taxa serving as potential biomarkers in the aqua-herbal diet belong to the class of Campylobacteria (ileum) or Clostridia (caecum), whereas in the cyclodextrin-herbal diet, to the families of Lactobacillaceae (ileum) and Methanobacteriaceae (caecum). In conclusion, dietary mixtures of herbal extracts improved protein and lipid oxidation in meat and increased beneficial lactic acid bacteria in caecum.
All authors declare no conflict of interest.

P-351 *Cannabis* edibles: a new Russian roulette?

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The use of cannabis and cannabinoids are mainly reserved for medical application; however, the regulation in some countries has decriminalised the recreational use of these. Furthermore, the *Cannabis* and CBD can be found in edibles, as well. These edibles are available in dedicated food stores, and more accessible online. Although, some parts of hemp can be used as food ingredients with limitation on THC content (< 0.2–0.3 %), Cannabis extract and CBD have not been used traditionally.
Aim of this work was to screen online available edibles, focusing on sweets (i.e., brownie, muffin, cookie, lollipop, and candy). Seven products were purchased on http://emag.hu in November 2021 and follow-up screening was repeated in May 2022. All products were coming from the same manufacturer. The labels of products were not informative enough to learn the exact content of sweets regarding Cannabis and cannabinoids. For cannabinoid content analysis HPLC-DAD was applied, focusing on CBD and THC. No cannabinoid could be detected in lollipops and candies. Interestingly, the CBD and THC content in cookies, brownies and muffins showed high fluctuation. Even the cookies within the same batch had CBD and THC in range of barely detectable amount to > 0.2 %. These results might suggest that cookies, brownies and muffins were containing *Cannabis*. The inhomogeneous cannabinoid-content might suggest food technology failure during the production. Consumption of these products is just like playing Russian roulette.

P-352 Valorization of Olive Leaves By-Products: Mapping of Greek olive leaves based on biophenols content

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By-products produced during olive tree cultivation and particularly olive leaves (OLs) resulting from the pruning of olive trees are considered agricultural residues, without being further exploited. Nevertheless, they are a source of valuable bioactive ingredients. Among them simple phenols and phenolic acids, secoiridoids, flavonoids and terpenoids are some of the main chemical classes of bioactives, identified in OLs with proved biological properties such as antioxidant, antitumor, anti-inflammatory, antimicrobial and cardioprotective activity.
In the present work a systematic study towards the investigation of the phytochemical content of olive (Olea europaea L.) leaves was performed. A high number of samples of different olive tree varieties, cultivation practices and geographical regions of Greece were investigated, using HPLC-DAD and LC-HRMS & HRMS/MS methodologies. The aim was to highlight the crucial parameters that affect the content and composition of OLs biophenols. Based on our knowledge it is the first time that so many olive leaf samples were studied, with the results of this work showing significant variations in the contents of Oleuropein, Hydroxytyrosol, Luteoline-7-glucoside, Apigenin-7-glucoside and Verbascoside while their levels are directly correlated on olive tree varieties, cultivation practices and Greek geographical regions
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P-353 Improvement of gastrointestinal discomfort and inflammatory status with a proprietary Wild Thyme extract

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According to the results of a nationally representative survey of over 71,000 Americans, 61% reported having had ≥ 1 gastrointestinal symptom (GIs) in the past week. The most commonly reported symptoms were heartburn/reflux (30.9%), abdominal pain (24.8%), bloating (20.6%), diarrhea (20.2%), and constipation (19.7%). Less common symptoms were nausea/vomiting, dysphagia and bowel incontinence [1]. Moreover, in a large-scale multinational study, they found that more than 40% of persons worldwide have GIs [2].

Considering these, Finzelberg has started a new product-concept: a science backed aqueous extract for gut health. The proprietary aqueous extract of Wild Thyme herba (*Thymus serpyllum*) (DEV native 4–8 : 1) has demonstrated interesting effects to reduce symptoms related to gut health conditions and displays an anti-inflammatory effect [3]. The proprietary extract is hydrophilic, can easily be dissolved in water and comprises different flavonoids like rosmarinic acid.

Finzelberg's aim was to investigate the effects of the proprietary *T. serpylli* herba extract on GI discomfort bowel movements in healthy human subjects in comparison to a placebo product. The placebo controlled clinical study revealed the possible interactions from Wild Thyme herba extract in the gut mechanism: The intake of 600 mg helped potentially to alleviate digestive discomfort. Furthermore, Wild Thyme extract benefit of targeting the gut microbiota to create improvements in well-being, providing a natural and safe way to improve and adjust the gut microbiota, which can help to maintain and/or improve the digestive health.

The authors declare no conflicts of interest.

Keywords: Gastrointestinal discomfort, Wild Thyme, Thymus serpyllum, Lamiacea, gut health/microbiota, Bloating

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P-354 Using *Astragalus membranaceus* (Fisch) Bge. to Treat Skin Diseases: Comparison of Traditional Uses and Research Results

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Astragalus membranaceus (Fisch) Bge. (AM), a member of restoratives for invigoration qi, has been used to treat patients with skin diseases in the framework of traditional medicine. The major efficacies of AM related to skin diseases are tonifying defensive qi and securing the exterior, expelling toxins and pus, and promoting tissue regeneration and wound healing. We investigated the traditional usages of AM described in the textbook and encyclopedia, and we also investigated scientific research using Pubmed and national digital library of Korea. In our opinion, tonifying defensive qi and securing the exterior effect of AM is related to the photo-protective, anti-aging and protecting effects on normal skin tissue. Expelling toxins and pus and promoting tissue regeneration and wound healing effects are closely related to the anti-inflammatory effects and promoting healing of wounds or ulcers on the body surface respectively. In addition, astragaloside IV, formononetin, calycosin, cycloastragenol (TA-65) and gamma aminobutyric acid were the major components related to therapeutic effects of AM on skin diseases. The therapeutic effects of AM on skin diseases were divided into three categories according to the theory of traditional medicine, and its effects in each category can be explained by scientific experiments.

P-355 Fine characterisation of a commercially available *Citrus*-based product used in animal nutrition reveals it's standardization

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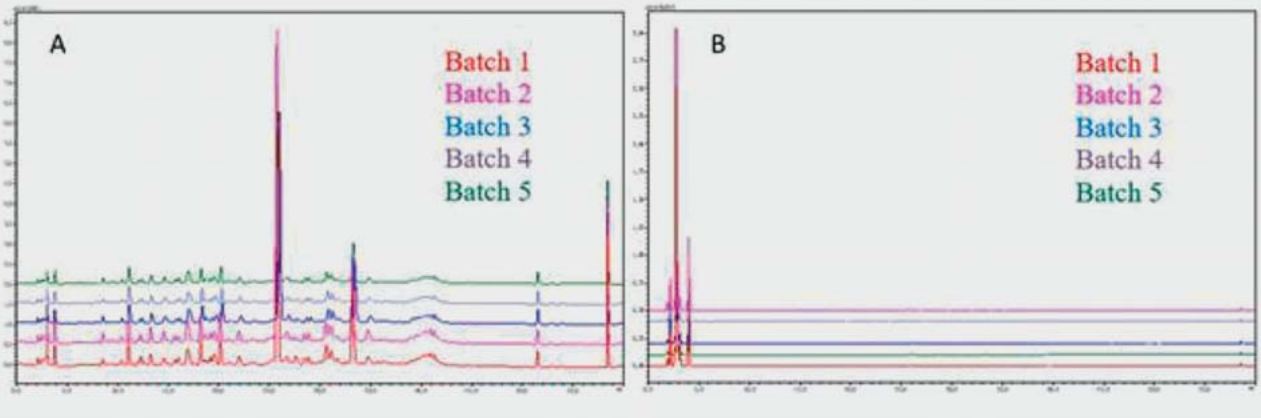
Citrus-based products (CBP) have more and more interest in animal nutrition, as growth performances enhancer. However, when added to the feed, their effects can be different, depending on the used product. In fact, some CBP has shown positive effect on animal performances [1] whereas some others didn't show any effect [2,3]. A possible explanation of these differences may be the composition. The lack of composition data make the understanding of their mechanisms of action unclear. This study aimed to assess the composition of a commercially available CBP (CA-CBP) which has already shown a beneficial effect on animal performances.

Based on the obtained HPLC profiles (▶ **Fig. 1**), no variation has been observed in the composition and the concentration of active compounds of



▶ **Fig. 1** HPLC profile of 5 SCE batches manufactured over 2 years. **A** $UV_{280\,nm}$ profile. **B** ELSD profile.

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5 CA-CBP batches. These 5 batches have been manufactured for over 2 years. The MS/MS analysis performed on CA-CBP allowed to identify pectic oligosaccharides as major compounds as well as 30 secondary metabolites, including hesperidin, naringinin, and eriocitrin in the product. The characterisation performed on CA-CBP allowed to highlight its standardization and stability. These two elements may explain the reproducible effects observed on animal performances. Moreover, the characterization data allowed to set up a list of hypotheses regarding the mode of action of the product. Indeed, the identified compounds are well known for their beneficial effect on animals, according to the scientific literature.

This study evidence the importance of CBP characterization in order to better understand the mechanism of action and provides tools for formulas improvement in the future.

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P-356 Screening anti-DPP-4 activity of Medicinal Plants in Traditional Recipes and Candidate Anti-diabetic Compounds from *Hydnophytum formicarum* Jack. Tubers

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Diabetes Mellitus (DM) is a health problem in the world. The number of patients is increasing every year. One of the mechanisms of DM treatment is anti-dipeptidyl peptidase-4 (DPP-4) activity, which has become an attractive oral anti-hyperglycemic agent. The Traditional Thai medicine recipe, the Wang-Nam-Yen hospital recipe, has been traditionally used to treat Diabetes Mellitus for a long time in the hospital, but its beneficial mechanisms have not been described. This study aimed to investigate the antidiabetic effects of 26 medicinal plants from the Wang-Nam-Yen recipe. To achieve DPP-4 inhibitory activity and isolate the pure compound from the medicinal plant, which showed the best bioactivity, were investigated. The result shows that *Hydnophytum formicarum, Urceola minutiflora,* and *Lagerstroemia speciosa* inhibited DPP-4 with more than 70% inhibition at 50 µg/mL (82.8% ± 0.8, 71.9% ± 0.3, and 71.1% ± 0.1, respectively compared with standard Diprotin A at 50 µg/mL, 90.1% ± 0.4). The tubers of *H. formicarum* extract were identified as the most bioactivity for anti-DPP-4 activity (IC_{50} = 33.87 ± 0.02 µg/mL) and were thus isolated to obtain 1 pure compound and 1 mixture compound, Palmitic acid (1) exhibited DPP-4 inhibitory activity at IC_{50} value of 73.82 ± 2.64 µg/mL, and a mixture of stigmasterol and β-sitosterol (2) at 78.58 ± 0.92 µg/mL. Many herbs in the Wang-Nam-Yen preparation possessed properties predictive of antidiabetic treatment. The results also suggest the possibility of further use of *H. formicarum* and its isolated compounds as an alternative DM treatment in the future.

P-357 The potential of biotechnological studies on chia (*Salvia hispanica* L.) in vitro cultures

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Salvia hispanica L. (Lamiaceae), is a species providing chia seeds, which, thanks to their rich chemical composition and valuable biological activities, are classified as so-called "healthy food" [1,2].

The biotechnological experiments involved agitated and Plantform-bioteactor grown *S. hispanica* microshoot cultures maintained in Murashige-Skoog medium [3] with 2 mg/L6-benzyladenine. The stimulatory effect of culture media supplementation with precursor compound – L-phenylalanine (Phe), and the effect of elicitation with methyl jasmonate (MeJa), on the production of phenolic compounds was proved.

Eight compounds: quercetin, chlorogenic acid, cinnamic acid, caffeic acid, neochlorogenic acid, protocatechuic acid, syringic acid and rosmarinic acid (quantitatively dominant in all samples) were determined with an HPLC-DAD method [4] in the extracts from experimental cultures.

The study of the dynamic of phenolic compounds accumulation showed, that the total content of estimated compounds was dependent on the duration of culture growth period and ranged from 107.14 to 748.61 mg/100 g s. m.

The highest content of rosmarinic acid (4050.38 mg/100 g s. m.; 60 times higher than in the control), was found in the extracts of agitated cultures supplemented with 1 mmol/L Phe on 7th day of 13 days growth period.

Studies on Plantform grown microshoots also proved high production of rosmarinic acid (3261.08 mg/100 g DW) which was obtained after media supplementation with 3 mmol/l Phe on the 17th day of 25 days growth period.

S. hispanica in vitro cultures can be proposed as a source of phenolic compounds, especially rosmarinic acid, a compound with valuable antioxidant properties. I declare no conflict of interest.

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P-358 Green extraction of bioactive compounds from the traditional Balkan flora using cyclodextrins

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In the Balkan area, several plant species have been used in folk medicine over many centuries. In the present study, four plant species that are known from some historical texts as well as modern-day investigations and data bases were examined for their therapeutic action in various skin diseases and conditions. Fumaria officinalis L. is used for eczema, scabies, *Satureja montana* L. for skin infections, for the treatment of insect bites, bee and wasp stings, *Verbascum thapsus* L. for wound healing, eczema, acne, and *Veronica officinalis* L. for the treatment of wounds, or snake bites.

The purpose of this work is to study the extracts of the four species that were produced using two different types of green extraction – with and without beta-hydroxypropyl cyclodextrins. For both the hydro-glycerol extracts pro-

duced with rapid extractors type TIMATIC (Technolab) and the extracts that were encapsulated with cyclodextrins, antioxidant activity and total phenolic content were assessed. Furthermore, the physicochemical and the microbiological stability of these extracts were also evaluated. Given their traditional uses as wound healing and anti-inflammatory agents, the extracts were also studied for their effects on cellular proliferation and the transcriptional regulation of genes implicated in relevant regulatory pathways.

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P-359 Determination of antioxidant and cosmeceutical activity of eco-friendly *Helichrysum italicum* extracts

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Immortelle (*Helichrysum italicum* (Roth) G.Don, Asteraceae) is a widespread Mediterranean plant [1]. It is widely used in traditional medicine, but also in anti-aging natural cosmetics. In this work, the cosmeceutical activity of two eco-friendly extracts of *H. italicum* was investigated. The extracts were prepared using the extraction protocol previously developed through a response surface methodology procedure using 2-hydroxypropy-β-cyclodextrin encapsulation with lactic acid as co-solubilizer. Antioxidant and cosmeceutical activity of the extracts was determined by the enzymatic and UV/VIS spectrophotometry methods. Free-radical-scavenging activity (RSA) of the extracts was assessed with the 2,2-diphenyl-1-pycrylhydrazyl (DPPH) method. Cosmeceutical activity was investigated by using anti-elastase, anti-collagenase, and anti-hyaluronidase assays [2]. First extract, OPT1, was rich in phenolic acids (2.11 ± 0.07 mg/mL) while the second one had high content of total phenolic compounds (up to 4.38 ± 0.04 mg/mL). Both extracts showed significantly higher antioxidant activity compared to positive control (butylated hydroxyanisole (BHA). They displayed excellent anti-elastase (IC_{50} for OPT1 and OPT2 were 22.36 ± 0.12 µg/mL and 20.07 ± 0.97 µg/mL, respectively), anti-collagenase (IC_{50} for OPT1 and OPT2 were 12.04 ± 1.03 µg/mL and 14.39 ± 0.71 µg/mL, respectively), and anti-hyaluronidase (IC_{50} for OPT1 and OPT2 were 14.31 ± 0.29 µg/mL and 19.82 ± 1.53 µg/mL, respectively) activity. As the extracts were prepared using the ingredients that can be incorporated into skin products, the formulation of such products may be achieved using less time and energy while still maintaining excellent cosmeceutical activity.

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P-360 *Juniperus communis* cell culture derived extracts for skin protection and regeneration

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Juniper is a valuable raw material for manufacturing of food, pharmaceuticals and cosmetics. Demand for juniper derived ingredients is growing and creates challenges for sustainable use of this natural resource. One of the most efficient ways to respond to industry needs is the use of biotechnological manufacturing methods. Interest in biotechnologically produced botanical compounds increases, with cosmetic industry being the leader in the application of such ingredients. Aim of our study was to evaluate the potential of Juniperus communis cell cultures as sources of skin protecting and regenerating cosmetic active ingredients. Various cultivation conditions were tested, and extraction solvents compared. HPLC analyses confirmed the high flavonoid content in the cell biomass extracts, with procyanidines and prodelphinidines being the dominating compounds. High levels of beta-thujaplicin were also detected. Antioxidative activity was confirmed by DPPH and nitric oxide scavenging assays. Antimicrobial activity against Gram positive bacteria was shown. Flow cytometry was used to quantify reactive oxygen species (ROS) in human keratinocytes, and it was found that extracts reduced accumulation of ROS by up to 50%, thus protecting cells from oxidative stress. Quantification of senescence markers and collagen I gene expression analyses were performed to evaluate the skin regenerative potential. Extract reduced the accumulation of senescence marker by more than 30% and induced expression collagen I in dermal fibroblasts. Chemical composition and biological activities of J. communis cell culture extracts confirm the high potential of it as efficient cosmetic active ingredient. The work has been supported by ERDF project No. 1.1.1.1/19/A/075.

P-361 Identification of phenolic compounds of *Ajuga reptans* L

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Phytochemical analysis of raw materials is a necessary step in the analysis of plant raw materials.

We consider *Ajuga reptans* of Lamiaceae family to be a very prospective object for research. Chemical composition of Ajuga plants was studied by Turkish and European scientists, but any data on the study of these plants in Ukraine have not been found. *Ajuga reptans* contains a variety of BAC (biologically active compounds) that exhibit anti-inflammatory, hepatoprotective, wound healing and antimicrobial activity [1–3].

The aim of the work was to identify groups of phenolic compounds of *Ajuga reptans* raw materials.

Phytochemical study of leaves, flowers and herb of *Ajuga reptans* was carried out to identify the main groups of phenolic compounds. Raw materials were harvested during the mass flowering period. To identify phenolic compounds a sample of 10 g of *Ajuga reptans* raw materials was extracted on a water heater under reflux condenser for 1 hour at the boiling point of the extractant. The extracts were evaporated to 1/10 of their volume, applied to Filtrak FN-1 chromatographic paper and studied by two-dimensional chromatography in solvent systems of 15% glacial acetic acid solution and n-butanol – glacial acetic acid – purified water (4 : 1 : 5). The dried chromatograms were studied in UV-light before and after the treatment with chromogenic reagents.

Taking into account the mobility of substances and the fluorescence in UV-light before and after the treatment with reagents 6 flavonoids, 2 hydroxycinnamic acids and 1 coumarin were identified on chromatograms.

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P-361B Effect of hyssop syrup on syndrome-differentiated mild to moderate asthma: A pilot clinical trial

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Asthma is one of the most common diseases of respiratory system. *Hyssopus officinalis* L. is a medicinal herb that have shown anti-asthmatic effects. In this study, efficacy of hyssop in mild to moderate asthma was investigated considering the phenotype of the patients. It was a randomized triple-blind placebo-controlled trial on 60 mild-to moderate asthmatic patients. Participants were randomized to receive either hyssop syrup (5 ml twice daily containing 6 g *Hyssopus officinalis* L. extract) or plain sugar syrup (5 ml twice daily) for 4 weeks as adjuvant to routine treatment. Asthma Control Test (ACT), pulmonary function tests, Expert Panel Report 3 (EPR3) and wheezing severity were considered as outcome measures. A significant improvement was observed in forced expiratory volume in 1 second (FEV1), ACT (at 4th week), peak expiratory flow (PEF), maximal expiratory flow rate 25–75 (MEF25-75%), and wheezing severity in the patients with productive cough in hyssop group. However, those with dry cough got worse regarding these indices. Results of this trial revealed the importance of syndrome differentiation in asthmatic patients according to their specific phenotype in order to increase the accuracy of treatment and response rate. Future trials are guaranteed to approve this method of case selection in asthma treatment
Keywords: Asthma; Hyssopus officinalis; Persian Medicine; Randomized Controlled Trial; Respiratory System; Syndrom Differentiation

P-362 Immunomodulatory properties of selected plant extracts in inflammatory respiratory diseases

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Virus-induced respiratory diseases are very common. Herbal preparations are often used for treatment, but the mechanisms of action on the cellular level are largely unknown. The goals of the project are to identify new candidates and to analyze underlying mechanisms of action.
Human A549 lung epithelial cells were treated with synthetic RNA analogs loxoribine and polyU to stimulate a TLR7/8 mediated immune response, mimicking viral infection. From a panel of twelve plant extracts, extracts of *Cistus incanus* and *Rosmarinus officinalis* in non-toxic concentrations could be shown to decrease secretion of cytokines IL-6, IL-8, IFNλ1, IFNλ2/3, and IFN-β compared to the positive control. Based on that, conclusions could be drawn on the underlying immunomodulatory signaling pathways, as said cytokines are particularly associated with the JAK-STAT, MAPK, and PI3K-AKT pathways. Furthermore, an Air-Liquid-Interface model was established, in which air-exposed epithelial lung cells are treated with nebulized plant extracts in a highly standardized manner. In the Air-Liquid-Interface model the anti-inflammatory effect of glycyrrhizin on LPS treated A549 cells could be shown.
Our data suggest multiple underlying signaling pathways, eventually leading to the immune-modulating effects of *Cistus incanus* and *Rosmarinus officinalis* extracts in human lung epithelial cells. The Air-Liquid-Interface model will allow examination of plant extracts regarding their inhalative effect.

P-363 Anti-avian influenza virus H5N3 activity of ethanol extract of *Psoralea drupacea* Bge. in chicken embryos

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Currently, no means of specific prevention and treatment of avian influenza are known. Lack of specific prevention measures, in other words the impossibility of vaccine development in the traditional way on chicken embryos due to the pathogenicity of the avian influenza virus (A/H5N1, A/H5N3) for chickens and embryos makes the search for active substances against influenza A viruses topical [1]. Herbal medications are deemed as alternative drugs. Typically, they are believed to be safer than chemical drugs, and less likely to collide with the stable viruses due to their multivalent functions [2]. The present work studies the anti-avian influenza virus H5N3 activity of ethanol extract of *Psoralea drupacea* Bge.root in chicken embryos. The ethanol extract has a high antiviral activity against this strain of influenza in comparison with commercial drugs such as tamiflu and remantadin. The plant extracts in doses of 0.63–10 mg/chicken embryo (CE) completely suppress 100 infectious doses of avian influenza virus strain A/tern/South Africa/1/61 (H5N3). Whereas Tamiflu suppresses the reproduction of the avian influenza A/H5N3 virus by 40% only at the maximum dose (10 mg/CE), and remantadin has no inhibitory activity against this virus. Studies have shown the possibility of using the extract as a virulide for the prevention and treatment of avian influenza in veterinary medicine.
The authors declare no conflict of interest.
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P-364 Antibacterial potential of essential oils from South-East Asian aromatic plants against respiratory pathogens

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Respiratory infections belong to the leading causes of morbidity and mortality in developing countries, including regions in South-East Asia [1]. Inhalation therapy is possible way of treatment when the active agents are delivered directly to the site of infection in respiratory system [2]. The plant essential oils (EOs) are of great potential for inhalation due to their volatility. Recently, several studies investigating antimicrobial effects of EOs derived from South-East Asian plant species have been summarized [3], however the evaluation of their vapors against bacterial respiratory pathogens are in lack until now. Therefore, in this study, antibacterial activity of EOs from seven less known Cambodian and Philippine aromatic plants namely *Alpinia cumingii, Alpinia elegans, Amomum pierreanum, Cinnamomum bonii, C. dimporphandrum, C. inners,* and *Xanthostemon verdugonianus* was tested using broth microdilution volatilization method [4] in liquid and vapor phase against *Haemophilus influenzae, Staphylococcus aureus,* and *Streptococcus pneumoniae*. As a result, all EOs tested have shown a certain degree of antibacterial effect with minimum inhibitory concentrations (MICs) ranging from ≥ 128 and ≥ 1,024 µg/mL in liquid and vapor phase, respectively. The EO of *A. elegans* seed possessed the highest antimicrobial activity against *S. aureus* in liquid phase (MIC = 128 µg/mL). In summary of this study, the results suggest potent growth-inhibitory effects of EOs obtained from different parts of Cambodian and Philippine aromatic plants that could be used for the treatment of respiratory infections. However, further research focused on chemical analysis, toxicity and in vivo evaluation is necessary to be carried out.

Conflicts of interest
The authors declare that they have no conflict of interest
Acknowledgments
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P-365 Antibacterial activity of essential oil-bearing herbs in vapour phase against respiratory pathogens and headspace analysis optimization of *Thymus vulgaris* sample

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While aerosolized antibiotics remain the recommended treatment for lower respiratory infections, difficulties to implement nebulization techniques have limited their widespread adoption. The volatile constituents of essential oils (EOs) appear as a promising alternative in the development of novel inhaled antibiotic therapy. In this study we first determined the in vitro antibacterial activity of five EO-bearing herbs vapour phase against respiratory pathogens including *Staphylococcus aureus, Streptococcus pyogenes* and *Haemophilus influenzae* using broth-microdilution volatilization (BMV) method. Then, with the aim of optimizing a protocol for the characterization of EO vapours, the chemical profile of the most active EO (*Thymus vulgaris*) was determined using two headspace sampling techniques coupled with GC/MS. While *Pimpinella anisum, Mentha × piperita, Foeniculum vulgare* and *Eucalyptus globulus* vapours didn't exhibit any antibacterial activity at MIC1024 µg/mL, MICs of *Thymus vulgaris* EO samples ranged from 512 to 1024 µg/mL. Moreover, GC/MS analysis results showed a different distribution of compounds in the headspace: thymol peak percentage area was unusually low – 5.27% (HS-SPME) and 0.60% (HS-GTS) – compared to the EOs (max. 48.65%). Multiple factors could explain these results such as parameters related to the use of a fibre coating assembly but also the experimental conditions like the matrix used. Overall, this study suggests that the procedure examined could be further exploited to better assess the benefits of EO volatile compounds for inhalation therapy against respiratory infections; however, further toxicological evaluation will be necessary to verify its potential practical use. We declare no conflict of interest.

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P-366 Growth-inhibitory effect of volatile compounds and essential oils from Indian medicinal plants against respiratory pathogens using broth macrodilution volatilization method

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Recently nebulised antibiotics have been preferred over systemic therapies for treating respiratory infections (e.g., cystic fibrosis) [1]. However, the delivery of aerosolised antimicrobial particles in the lower respiratory tract can be problematic due to the drug particle size. In this context, plant-derived products, especially essential oils (EOs), maybe interesting alternatives. The unique physicochemical feature of high volatility allows easy inhalation and uniform distribution of EOs' active substances [2]. Therefore, the current study aims to evaluate the antibacterial potential of 3 essential oils [*Cymbopogon citratus* (DC.) Stapf, *Cyperus scariosus* R.Br., and *Trachyspermum ammi* (L.) Sprague] obtained from Indian medicinal plants and 3 plant-derived volatile compounds (β-thujaplicin, thymohydroquinone, and thymoquinone) against bacteria associated with respiratory infections (*Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae,* and *Streptococcus pyogenes*) using recently developed broth macrodilution volatilization method. This assay combines the principles of broth microdilution volatilization and standard broth macrodilution methods and enables rapid, simple, cost- and labour-effective screening of volatile agents [3]. Among tested compounds, thymohydroquinone and thymoquinone showed highest antibacterial activity against H. influenzae, with minimum inhibitory concentrations (MICs) of 4 and 8 µg/mL in the liquid and vapour phases, respectively. *T. ammi* EO exhibited the most significant activity against *H. influenzae* with MICs of 128 and 256 µg/mL in the broth and agar. This study suggests the potential of *T. ammi* EO, thymohydroquinone, and thymoquinone for practical use in inhalation therapy. However, further experiments on their safety and in vivo efficacy are required to verify the practical applicability. We declare no conflict of interest.

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P-367 Antiviral effect of Lotus (*Nelumbo nucifera* Gaertn.) Leaf Water Extract against Influenza A Virus

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Lotus (*Nelumbo nucifera* Gaertn.) is a perennial aquatic plant containing many kinds of bioactive ingredients, including alkaloids and flavonoids. In this study,

we first demonstrated that the water extract of lotus leaf (WLL) has a strong inhibitory effect on influenza A virus (IAV) infection by inhibiting neuraminidase (NA) and hemagglutinin (HA). We investigated the effect of WLL on viral infection using fluorescent microscopy and fluorescence-activated cell sorting (FACS) analysis with green fluorescent protein-tagged Influenza A/PR/8/34 virus. Plaque inhibition assay and cytopathic effect detection assay were used to confirm the antiviral effect of WLL. WLL significantly inhibited influenza viral infection, in a dose-dependent manner. Immunofluorescence (IF) analysis confirmed that WLL reduces influenza HA, NA, M2, and NP protein expression. WLL strongly reduced both HA and NA activity of IAV. Among six components in WLL, isoquercitrin exerted a potent antiviral effect. Additionally, WLL strongly suppressed the cytopathic effect by H1N1 or H3N2 IAV infection. In conclusion, our results suggest that WLL could be used as a natural viral inhibitor for H1N1 and H3N2 influenza viral infection.

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P-368 Molecular networks for the exploration of large libraries of plant extracts and the targeted isolation of new anti-infective natural products

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Tuberculosis has proven to be a particular threat to humanity, claiming about 1.5 million human lives each year. While treatments for tuberculosis exist, there is still an urgent need for new molecules to overcome the challenges of antibiotic resistance. Plants offer a great source of chemical diversity to generate new drug candidates, provided one can easily map the chemical content of such complex matrices. In this context, a library of about 1600 plant extracts was analysed through untargeted fragmentation experiments in high-resolution mass spectrometry (HRMS/MS), to generate a Molecular Network (MN) compiling the entire chemical content of the sample library. Theoretical spectral database and taxonomically informed metabolite annotation were used to dereplicate structures based on HRMS/MS data [1]. This study led to the creation of a virtual library of 37 304 compounds. The first operation consisted in exploring this virtual chemical library to spot structural analogues of benz[g]isoquinoline-5,10-dione, an active azaanthraquinone derivative found in a previous screen with an infection model system using the amoeba *D. discoideum* as a host and *Mycobacterium marinum* [2], which is closely related to *M. tuberculosis*, as the pathogen. The extract of *Cananga latifolia* (Annonaceae) was prioritised and efficient targeted isolation with high-resolution chromatography [3], yielded 12 compounds, 5 of which are closely related structural derivatives of the active azaathraquinone structure. The small amounts of pure compounds generated (100 µg) were screened on our model infection system to reveal their bioactive potential, with compounds such as "onychin" standing out and confirming the initial hypothesis.

P-369 *Rhodiola rosea*: Anti-influenza virus aspects of a popular adaptogen

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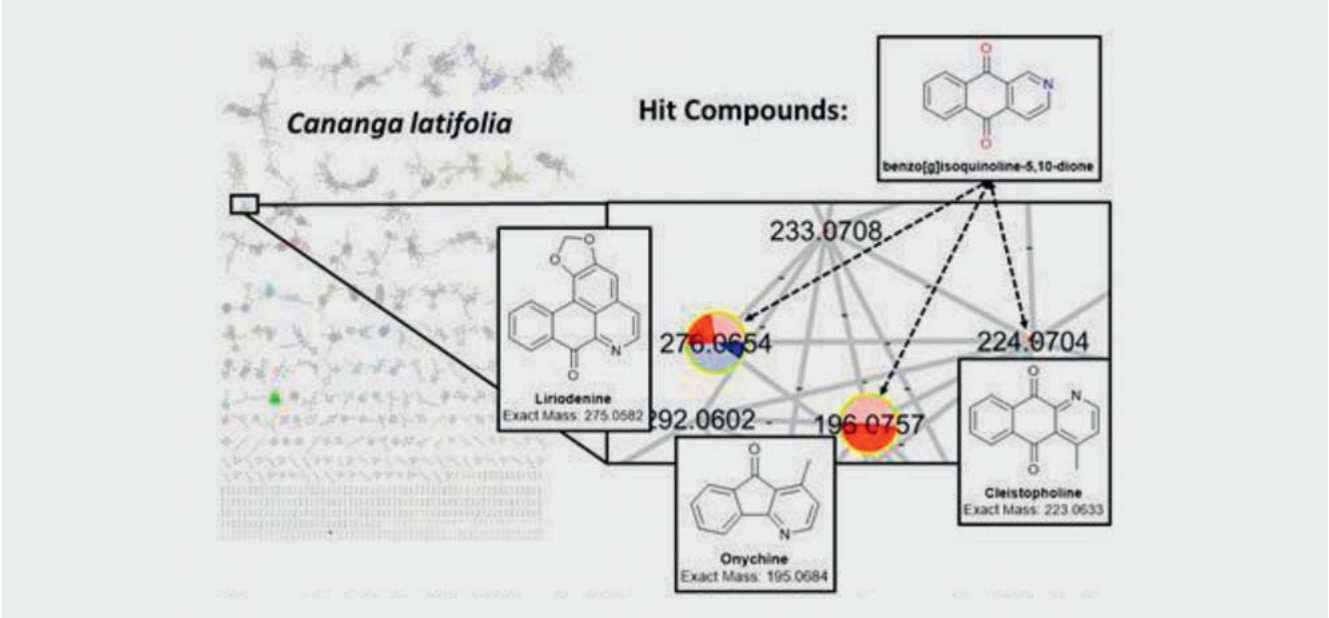
Preparations of *Rhodiola rosea* are popular as adaptogen to treat stress, fatigue, and weakness. Marker compounds such as rosin, rosiridin, rosarin, rosavin, and salidroside [1] were quantitatively analysed in herbal drugs as well as commercial preparations using a supercritical fluid workflow [2]. However, *R. rosea* also exhibits anti-influenza virus activities which could not be explained by the effect of known adaptogenic compounds.



▶ **Fig. 1** On the left: Molecular Network of extracts from *Cananga latifolia* (bark and subterranean parts). On the right: cluster of interest with analogues of a hit compound dereplicated and then isolated.

Hence, the aim was to investigate the antiviral effect regarding i) combinatorial effects of isolated constituents, ii) the influence of tannins, iii) the mode of action, and iv) resistance development.
Interestingly, a tannin fraction (TE) composed of prodelphinidin gallate oligomers was detected as main contributor to the antiviral activity. TE inhibited the plaque-production of influenza virus A(H1N1) pdm09, A(H3N2), and B isolates with IC_{50}s between 0.12–0.53 µg/mL. Mechanistic studies proved a virucidal activity, inhibition of viral adsorption, viral neuraminidase activity, and virus spread. No resistance development was observed in vitro [3].
These anti-influenza insights support a yet unknown aspect of adaptogenic properties of *R. rosea*.
The authors declare no conflict of interest.

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P-370 *Echinacea* for Prevention/Therapy of Respiratory Tract Infections during Covid-19 Pandemic: Insights from recent in vivo and in vitro Investigation

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Background: Continuous mutation of SARS-CoV-2 and novel variants of concerns (VOCs) pose a serious threat to containment by immunization. The quest for additional broad-spectrum antivirals is therefore still ongoing.
Aims: A randomized, open, controlled clinical study in 120 adults investigated a freshly harvested *Echinacea purpurea* extract [Echinaforce®/EF] for the prevention and treatment of respiratory tract infections (RTIs) during Covid-19 pandemic (Nov2020-May2021). At the same time, in vitro studies shed light on the modes-of-action of the observed broad antiviral effects.
Results: During 5 months of prevention [2/2/1 mt], 21 and 29 nasopharyngeal/blood samples tested positive for any virus in the EF and in the non-treatment control group, of which 5 and 14 samples tested SARS-CoV-2 positive (RR = 0.37, p = 0.03). EF acute treatment significantly reduced the overall virus load in swab samples of patients by at least 2.12 log10 or approx. 99% ($p < 0.05$), the time to virus clearance by 8.0 days for all viruses (p = 0.02) and by 4.8 days for SARS-CoV-2 ($p > 0.05$) in comparison to control. Two hospitalizations occurred with control and none during EF prevention.
EF broadly inhibited SARS-CoV-2 VOCs in vivo (alpha/beta/gamma/delta/eta/omicron) at physiological concentrations ($EC_{50} \leq 12$ug/ml) due to interaction of alkylamides with receptor binding domain (RBD) of spike protein (virucidal) as well as inhibition of TMPRSS-2 serine protease on host cell (cell-protective).
Conclusion: EF extract shows broad antiviral effects in vitro and in vivo and reducing the risk of viral RTIs, including SARS-CoV-2. By substantially suppressing virus loads in infected individuals, EF offers a supportive addition to existing mandated treatments such as vaccinations.

P-371 Reduction of viral load in patients with acute sore throats: Results from an observational clinical trial with *Echinacea*/*Salvia* lozenges

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Introduction: Acute tonsillopharyngitis or sore throat is an initial and frequent sign of respiratory tract infections "RTIs" and represents an optimal occasion for early and effective antiviral treatment.
Aim: This study investigated safety and efficacy of *Echinacea purpurea*/*Salvia off*. lozenges on acute sore throat symptoms and the viral load in infected patients.
Method: 74 patients (13–69 years) with acute sore throat symptoms (< 48 h) were treated with five lozenges per day (each containing: 800 mg Echinaforce® extract and 379 mg Salvia officinalis fresh herb extract [A.Vogel AG, Switzerland]) for 4 days. Symptom intensities were recorded in a diary and oropharyngeal swab samples collected for virus detection and quantification via RT-qPCR.
Results: 98.6% of patents rated the tolerability as (very-) good, no RTI developed, and no antibiotic treatment was required (0%). Taking a single lozenge reduced throat pain by 48% ($p < 0.001$) and tonsillopharyngitis symptoms by 34% ($p < 0.001$). 24.3% of patients tested virus positive. Viral loads were reduced by 62% ($p < 0.03$) after taking a single lozenge and by 96% ($p < 0.02$) after 4-days of treatment compared to pretreatment.
Conclusion: *Echinacea*/*Salvia* lozenges represent a valuable and safe option for the early treatment of acute sore throats, effective in reducing symptoms and viral loads in the throat.

P-372 The effect of the bakuchiol-enriched fraction of *Psoraleae drupacea* Bge. hexane extract on *Brucella melitensis* in vitro

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Brucellosis is a particularly dangerous and socially significant infection, causing significant economic damage and causing a high level of disability of patients. Brucellosis is a systemic zoonotic infection transmitted from animals to humans through the consumption of infected products, direct contact with infected animals or inhalation with aerosols. It is caused by bacteria of the genus *Brucella* [1]. Fruits of *P. drupacea* Bge. exhaustively extracted with hexane by maceration. Next, the hexane extract was separated by silica gel column chromatography and a fraction was obtained, according to GC/MS, containing 96.69% of bakuchiol. The present experimental in vitro study was carried out to evaluate the anti-brucella activities of the bakuchiol-enriched fraction. The reference *Brucella* 16 M strain was studied using the serial dilution method. It was revealed that the studied *Brucella* 16 M strain was sensitive to this fraction even at low concentration (the ratio of solvent-extract is 1 : 0.039). The current findings have clearly demonstrated that the bakuchiol-enriched fraction of *P. drupacea* Bge. fruits extract possess antibrucellosis activity.
Studies have shown the possibility of using the bakuchiol-enriched fraction of *P. drupacea* Bge. fruits extract as a bioadditive for the prevention and treatment of brucellosis in veterinary medicine.
The authors declare no conflict of interest.

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P-373 The eubiotic potential of *Lythrum salicaria* L. in piglets nutrition

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Lythrum salicaria herb (LSH) was applied in therapy of diarrhea since ancient times. Taking into consideration the historical use of LSH in treatment of diar-

rhea in farm animals, the aim of the study was to examine its influence on processes associated with maintaining intestinal epithelium integrity, enteropathogenic Escherichia coli (EPEC) growth and adhesion as well as impact on gut microbiota homeostasis in piglets.
LSH was not only inhibiting EPEC growth but also its adhesion to IPEC-J2 intestinal epithelial cell monolayers. Inhibitory activity towards EPEC growth was additionally confirmed ex vivo in distal colon samples of piglets. LSH and its dominating C-glycosylic ellagitannins were stimulating IPEC-J2 monolayer formation by enhancing claudin 4 production. Despite the determined anti-ETEC properties, LSH did not negatively affect alpha diversity and metabolism of intestinal microbiota of post-weaning piglets ex vivo. Changes in microbial taxa abundances were induced, some of which correlated with the formation of postbiotic metabolites, namely urolithins, which proven anti-inflammatory properties can beneficially contribute to gut health of piglets during the weaning period.
The conducted studies support the purported anti-diarrheal properties of LSH revealing its eubiotic effects, that not only respect the ecological context of preserving the homeostasis of intestinal microbiota but also can prevent infection and support the intestinal epithelium development in post-weaning piglets. The obtained results serve as an initial point for further studies on development of novel, sustainable feed additives dedicated to farm animals being scientifically based alternatives to antibiotics.
Supported by Alexander von Humboldt Foundation Research Fellowship for Postdoctoral Researchers.

P-374 In vitro growth-inhibitory activity of plant extracts and phytochemicals against diarrhoea-associated and probiotic bacteria of veterinary importance

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Diarrhoea is a common cause of morbidity and mortality of farm animals, especially during the pre-and post-weaning periods of youngs. Among others, bacterial infections are the leading causes of neonatal diarrhoea. Though antibiotics effectively prevent casualties, they often induce gut dysbiosis [1,2]. Thus, it warrants searching for new alternatives that can inhibit the growth of pathogens without affecting beneficial gut bacteria. Therefore, this study aimed to evaluate the growth-inhibitory effects of 18 ethanolic plant extracts and 6 phytochemicals against 12 diarrhoea-associated bacteria, 6 bifidobacteria and 6 lactobacilli by the broth microdilution method following protocols of the CLSI [3] and Hecht (1999) [4] for aerobes and anaerobes, respectively. Extract of *Embelia ribes* fruits showed strong growth inhibitory activity against *Bacillus cereus* and *Listeria monocytogenes* with minimum inhibitory concentrations (MICs) of 64 and 128 µg/mL, respectively. It was only moderately toxic to 10 out of 12 probiotic bacteria (MICs ≥ 256 µg/mL). Besides, 8-hydroxyquinoline exhibited potent activity against *Streptococcus bovis* (MIC8 µg/mL) and 8 other infectious agents (MICs 32 µg/mL); and was weakly toxic to all the probiotic strains (MICs ≥ 128 µg/mL). Sanguinarine displayed strong effects in 3 pathogens (MICs 32 µg/mL). Results of 8-hydroxyquinoline and sanguinarine were in accordance with our previous research [5]. The study suggests that *E. ribes* extracts inhibited pathogens and had lesser toxic effects on probiotic bacteria. Thus, it may be of future veterinary importance to treat diarrhoea in farm animals, although animal trials are needed. We declare no conflict of interest.
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P-375 *Hottonia palustris* L. extracts as *Apis mellifera* boosters in the fight against nosemosis

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The honeybee plays a key role in plant pollination and food production. These insects are thus ecologically and economically invaluable. Alarmingly, the numbers of bee colonies have declined in recent years. This may be due to pesticide application, weather conditions and the availability of food resources but also to the spread of new diseases and parasites [1]. One of the main causes of honeybee death is nosemosis. Nosemosis is caused by species of microsporidia of the genus Nosema. These microsporidia are intracellular parasites that attack the epithelial cells of the honeybee midgut, where these pathogens multiply and mature. Honeybee digestion is disturbed, malnourishment occurs, and physiological and anatomical changes to the midgut cells ensue. These processes weaken the bees and ultimately kill them, which can lead to colony collapse. The present study aimed to investigate *Hottonia palustris* L. (Primulaceae) extracts as therapeutics for nosemosis infection in honeybees. Water extract (HP3) and ethyl acetate (HP5) or butanol (HP6) fractions administered orally to artificially infected honeybees significantly lowered the level of nosemosis, up to 98%, compared to control honeybees. An analytical approach based on LC-PDA-MS/TOF was also applied to obtain phytochemical profiles of all analyzed samples. In the control group of an infected honeybee not treated with preparations, the level of nosemosis was 87.6 mln spores/bee, and in the group treated with the most effective HP6 preparation (1 mg/mL), the infection level was lowered to 1.6 mln spores/bee. Therefore, *H. palustris* extracts can be considered promising agents in combating nosemosis in honeybees.
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P-376 *Scleranthus perennis* extracts prolonged the honeybee lifespan

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Honeybees are very important pollinators, and their role in ecosystems cannot be overestimated. *Apis mellifera* workers pollinate approximately 80 percent of flowering plants; only in the United States are efforts estimated up to $15 billion worth of crops annually performed. Moreover, honeybees produce honey, wax, pollen, royal jelly, and propolis, products eagerly used by many people. Unfortunately, honeybees suffer from the effects of pesticides, immu-

nodeficiencies, beekeeping practices in which antibiotics are used, malnutrition, and disease. These factors can drastically reduce the honeybee lifespan [1]. The aim of the study was to determine whether *Scleranthus perennis* L. (Caryophyllaceae) extracts can prolong the honeybee lifespan, especially after infection with pathogenic fungi from the genus Nosema. The aerial parts of *S. perennis* were collected in the Białystok area of Poland. Extraction and fractionation were performed as previously described [2]. The plant material was powdered and extracted using an ultrasonication bath. All extractions were performed using solvents such as water (SP3), ethyl acetate (SP5), and n-butanol (SP6). The most effective extract, SP3 (1 mg/mL sugar syrup), prolonged the honeybee lifespan by approximately 30% in both Nosema-infected and healthy (not infected) honeybees. Nosema-infected honeybees in the control group lived for 20 days, and after administration of the extract, their lifespan was prolonged to 26 days. The lifespan of healthy honeybees from the control group was 26 days, and after administration of the extract, it was prolonged to 34 days. *S. perennis* extracts have potential as honeybee lifespan enhancers.

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P-378 Chemical analyses and in vivo properties, of a combination of Olive-Oregano oils of Greek origin, for competition horses

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The history of veterinary herbal remedy has followed parallel route alongside the evolution of human medicine. The “Hippiatrika” are famous documents related to Ancient Greek/Roman practitioner’s, so called “Hippiatros”, studies on horses.

Extra virgin olive oil (EVOO) is liquid fat from olives (*Olea europaea*) consisted of approx. 80% on oleic acid, polyphenols and vitamins (E, K), used as food supplement for horses with positive impact on their coat shine, supporting gastrointestinal functions. Oregano essential oil (OEO) is widely used in veterinary medicine, mostly due to its antimicrobial properties [1].

The chemical profiles of EVOO (Kalamata, Peloponnesus) and OEO *Origanum vulgare* sp *hirtum* (Kozani, W Macedonia), were analysed, showing low (0.5%) free acidity and high carvacrol (76.97%) content, for EVOO and OEO respectively. The effects of a combination EVOO+OEO were tested, after their use as a daily supplement in seven competition horses. The overall athletic performance and health of all five horses improved during test period as indicated by clinical examination (increase of PCV, significantly decrease of SGOT, AST, GGT, SAA). One control horse (not fed with EVOO+OEO) did not show improvement, while another one receiving only EVOO exhibited stable values. No horses exhibited lameness or signs of colic during trial. The use of EVOO +OEO as a nutritional supplement showed positive effects in the appearance and athletic performance of all animals, with safety, as the calculated maximum safe concentration of the OEO in complete feed is reported recently at 88 mg/kg for horses [2]. Further in vivo studies are ongoing.

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P-379 Testing properties and biological activities of several saponin plants in laboratory for animal nutrition

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Saponins are secondary metabolites widely distributed in the vegetal kingdom, which are characterized by their structure containing a steroid or triterpenoid sapogenin attached to one or more sugar chains [1]. The market trend for natural products coupled with their properties and mounting evidence on their biological activity (surfactant, membrane-permeabilization, ammonia-binding, lysis of protozans) as led to the emergence of saponins as commercially significant compounds with expanding applications in animal nutrition: reducing ammonia and methane emissions, limiting coccidiosis and other pathogens [2]. Several sources of saponin plants can be used: *Yucca schidigera, Trigonella foenum-graecum, Quillaja saponaria, Chenopodium quinoa, Camellia oleifera* etc. However, the structural diversity existing within each saponin plant as well as their content can lead to different properties and biological activities that could affect animals in a host of different ways both positive and negative. In this study, several saponins plants were evaluated in laboratory with standardized methods: foam index, heamolytic capacity, ammonia binding capacity and saponins content. The results showed a great diversity in the properties tested and provided an overall picture of each saponin plant. These data can be useful in formulating new products for a specific application in animal nutrition.

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P-380 Disclosing the use of salt tolerant plants as sources of veterinary products for the treatment of gastrointestinal nematodes (GIN) infections

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Using plants and their metabolites is a valuable strategy to tackle parasitic infections by gastrointestinal nematodes (GIN) in integrated control strategies aiming at reducing dependence on synthetic drugs. Several salt tolerant plants (halophytes) have ethnoveterinary uses as antiparasitic, and are rich in bioactive molecules (e.g., tannins), and are therefore potential sources of anthelminthic phytotherapeutic products. This work explored the tannins content and the in vitro athlelminthic properties towards eggs and larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* of acetone extracts made from aerial organs of halophytes common in the Mediterranean, namely *Pistacia lentiscus* L., *Cladium mariscus* (L.) Pohl, *Inula crithmoides* L., *Helichrysum italicum* (Roth) G. Don subsp. *picardi* (Boiss. & Reut.) Franco, *Calystegia soldanella* (L.) R. Br., *Medicago marina* L., *Plantago coronopus* L., *Limoniastrum monopeta-*

lum (L.) Boiss. and *Crucianella maritima* L. *Cladium mariscus* was one of the active in both GIN and life stages, it inhibited larvae exsheathment (IC_{50} ranging from 77.8–88.9 µg/mL), without differences between both parasite species, and was more effective towards eggs *H. contortus* (IC_{50}= 496.6 µg/mL) than *T. colubriformis* (IC_{50}=2575.5 µg/mL^{-1}). The main compounds identified in the *C. mariscus* extract, by HPLC-ESI-MSn analysis, were flavan-3-ols (epigallocatechin, catechin), proanthocyanidins, luteolin, C-glycosyl luteolin, a kaempferol glucoside, and an apigenin flavone, the majority with recognized anthelmintic effects.

The authors declares that there is no conflict of interest.

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P-381 The effect of grape extract protection on the stability of polyphenols in the rumen: an in vitro study

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Polyphenols of natural grape extract (NGE) have beneficial effects on antioxidant defense and immunity in ruminants [1,2]. However, polyphenols in NGE can be rapidly degraded in the rumen due to floral fermentation affecting their antioxidant capacity [3]. Therefore, a rumen-by-pass formula to protect these active molecules in the rumen is crucial to maintain their biological activities. This study presents the stability of a protected NGE (PNGE) with natural waxes compared to an unprotected NGE (UNGE) in rumen fluid in-vitro. First, the content of polyphenols in both formulas was standardized. 0.4 g of PNGE and 0.3 g of UNGE were enveloped in filter paper in triplicate, then incubated in 20 mL of rumen fluid for 1 h (T1) and 6 h (T6), in water bath at 39 °C (T0) with stirring. At the end of incubation periods, 2 ml formic acid were added to stop the fermentation and 20 ml of hexane were added. Anthocyanins are recovered in the aqueous phase and analyzed by HPLC-UV at 520 nm. Results showed that at T0 when products have not undergone the fermentation, the same anthocyanin profile was found for both groups. After 1 h of incubation, anthocyanins content dropped by 49%±7 in UNGE group and only 17% ± 2 in PNGE group compared to T0. Similarly, at T6, there was a dramatic reduction in anthocyanin compounds in UNGE samples, up to 92% ± 3, while it was only 53% in the case of PNGE ones. The obtained results demonstrated the strong sensibility of NGE and the efficiency of PNGE formula in protecting NGE's active molecules in rumen.

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P-382 Effect of dietary herbal extracts on broiler meat oxidation and activation of stress-induced proteins

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This trial examined the effects of dietary use of an extract including oregano, garlic, camelina and rock samphire either encapsulated in cyclodextrin or in an aqueous form on meat oxidative stability and stress-induced protein activation of breast and thigh meat samples. Control Group A (CL) was fed basal diets based on maize and soybean meal. The duration of the trial was 35 days. Mixed broiler chicks (Ross-308, 120 individuals, one-day-old) were randomly allocated to three groups with four replicates. The results showed that breast chemical composition was similar among the three groups. Lipid oxidation and protein carbonyls were reduced in both groups that received the herbal extracts either in encapsulated or aqueous form. Furthermore, to address cellular stress and signaling responses, expression patterns of heat shock proteins (HSP60, HSP70, HSP90) and MAPKs members (p38, p44/42) were investigated in breast and thigh tissues using Western Blot analysis. According to the protein expression patterns, the formulated diets elicited tissue-specific cellular response. Compared to the control, the encapsulated diet resulted in significant HSPs induction and MAPKs activation, while the aqueous diet decreased or maintained most of the examined proteins at constant levels. In conclusion, dietary mixtures of herbal extracts improved protein and lipid oxidation in meat and decreased significantly proteins associated with stress signaling.

All authors declare no conflict of interest.

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P-383 Diploma of herbal medicine in the veterinary schools of France: current state and prospects

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In recent years, the popularity and the enthusiasm for human and veterinary phytotherapy have continued to increase in France and in the world, especially because of the distrust towards pharmaceutical products. Until 2018, veterinary schools in France did not offer training in herbal medicine, and those provided in continuing education by the French faculties of pharmacy and medicine are not fully adapted to veterinary practice. It is in this context that the representatives of Pharmacy and Pharmacology, and Food/Nutrition and

Botanical Units of the 4 French Vet schools wanted to take the lead to teach veterinary herbal medicine by creating an inter-school professional diploma, as a recognition for the Veterinarian and a way to establish the rules of use of the plant for animal health.
The Diploma of phytotherapy is a 1-year (4-modules) program that has been accredited in 2019 by the the National Council of the Order of Veterinarians. More than 120 Veterinarians have been trained since 2018. The training provides the Veterinary Practitioner both traditional and scientific-based knowledges of herbal medicine with the objective to allow him to prescribe a treatment and/or a phytotherapeutic strategy adapted to the animal disease.
In this presentation, the teaching program, the pedagogical objectives, the methods of learning, the evaluations of the teaching by the trainees since 4 years and the prospects of the training were presented and discussed.

P-384 Effect of Brewers' Yeast Beta-Glucan Supplementation on Immune Parameters in Horses

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Beta – glucans are well-known for their positive immune effects, which have been successful proven in various human and animal studies. But there are less informations about using beta – glucans in horses. The purpose of this study was to identify the influence of purified brewers' yeast beta-glucan on different immune parameters in horses.
Material & Methods: Polish sport horses (500 kg) were fed for a period of 21 days different dosages of β- glucans (2 g/day, 5 g/day and 10 g per day). Serum blood was taken before administering the preparation (day 1) and on day 7, day 14, day 21 and 7 days after last application (day 28). The kinetics of changes in the immune parameters like lysozyme, total protein and gamma-globulin were measured in the serum blood. The study was done acc. to the ethical committee.
Results: Supplementing of purified brewers'yeast beta-glucans increased already 7 days after feeding lysozyme activity, total protein, gamma-globulin and ceruloplasmin, in all dosages. The highest increase was found for lysozyme, total protein and gamma-globulin on day 14 and 21, with 5 and 10 g per day. 7 days after feeding beta-glucans a significantly high activity of lysozyme and gammaglobulin were found compared to the baseline values before feeding, for 5 and 10 g per day.
Conclusion: The obtained results suggest that brewers'yeast beta glucans have a stimulating effect esp. on non-specific cellular and humoral defence mechanisms on the immune system of horses. The highest levels of activity were observed at the dose of 5 and 10 g/day.

P-386 Maslinic Acid Derivative Nanoemulsion: Physicochemical Characterization, Antimicrobial Activity and Three-Dimensional (3D) Reconstructed Human Epidermal Model Screening

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Background: Maslinic acid belongs to the class of pentacyclic triterpene and has been reported to possess various therapeutic effects, including anti-inflammatory, antimicrobial and antitumor properties. Furthermore, derivatives of maslinic acid have been indicated to elicit improved biological activity compared to the parent compound.
Aim: The present study focuses on the formulation and characterization of a maslinic acid derivative (EM2) nanoemulsion followed by evaluation of the antimicrobial activity and the possible skin irritation effect induced on the three-dimensional (3D) reconstructed human epidermal tissues.
Results: EM2 nanoemulsion was characterized by different techniques: Zeta Potential, Dynamic Light Scattering and Transmission Electron Microscopy. The formulation was tested for its antimicrobial activity (disk diffusion method and the dilution method) against 5 bacterial strains and 2 fungi. Levofloxacin and fluconazole were used as positive control. EM2 nanoemulsion (1 mg/mL) elicited an antifungal effect on *Candida albicans* and *C. parapsilosis* (inhibition areas were 29.6 and 30 mm, respectively). Similar effects were obtained for EM2 alone. The experiments performed on the 3D reconstructed human epidermal tissues were made in standard conditions that complied with OECD Test Guideline 439 and have ECVAM validation. The tissue models viability was not affected following samples application.
Conclusion: EM2 nanoemulsion produced an antifungal effect and did not display a significant skin irritative effect on the 3D-tissue model, thus the sample can serve as a promising formulation in skin care treatment
Conflicts of interest
The authors declare no conflict of interest.
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P-387 Encapsulation of two distinct *Cistus* essential oils in β- and γ-cyclodextrins

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Cistus shrubs are aromatic with terpene-rich antimicrobial essential oils that are used as food and cosmetic ingredients. *Cistus creticus* L. is native to Greece while *C. ladanifer* L. grows in the Western Mediterranean. Essential oil encapsulation protects the volatiles from degradation and reduces their volatility. Cyclodextrins increase their water solubility, as well. The aim of this study was the investigation of the chemical composition of commercially available essential oils of *C. creticus* and *C. ladanider*, and their encapsulation efficiency in β- and γ-cyclodextrins. GC-MS analysis showed that the oil of *C. creticus* comprised mainly of sesquiterpenes (48%) and diterpenes (> 18%), whereas monoterpenes were only 10%. *C. ladanifer* essential oil contained mostly monoterpenes (70%); sesquiterpenes constituted 22% and diterpenes less than 1%. The main ingredient in *C. creticus* oil was manoyl oxide, while in *C. ladanifer*, α-pinene. The percentages of the *C. creticus* and *C. ladanifer* oils encapsulated in γ-cyclodextrin (89 and 87%, respectively) were higher than those in β-cyclodextrin (73 and 79%). In addition, the determination of surface oils revealed that the monoterpene-rich oil was entirely trapped in cyclodextrins' cavity, whereas a significant percentage (about 50%) of the *C. creticus* volatiles were adsorbed only on the surface of β-cyclodextrin; γ-cyclodextrin larger cavity accommodated more *C. creticus* volatiles and only 10% were adsorbed on its surface. The ongoing GC-MS analysis will shed light on these mechanisms. This study illustrates the species-specific differences in *Cistus* essential oils and the impact of those differences on the encapsulation in cyclodextrins of different size.

P-388 Functionalized nanoformulation based on enriched-triterpenes fraction target adipose tissue, as a potential treatment to obesity and type-2 diabetes: preliminary data

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Previous studies have shown that the intraperitoneal administration of enriched triterpenes extracts (OBE100), obtained from *Eucalyptus tereticornis*, reduces metabolic alterations in a diet-induced obese (DIO) mouse model [1]. The present work aims to develop a phytotherapeutic prototype of triterpenes encapsulated in functionalized nanoparticles (FNP) to target abdominal

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adipose tissue in a DIO model. Encapsulating therapeutic principles in functional nanocarriers allows the site, dose, and release time control.
The PLGA-b-PEG-Maleimide polymer was conjugated with the peptide P3 (CKGGRAKDC) labelled with cyanine 7 (CY7). Peptide P3 binds to prohibitin, a marker of adipose tissue [2]. The PLGA-b-PEG, PLGA-b-PEG-Peptide and OBE 100 were used to form the FNP using the emulsion-solvent evaporation technique. The FNP was purified, and the particle size, Z-potential, encapsulation efficiency (EE) and loading capacity (LC) were determined. The size was checked by scanning electron microscopy (SEM), and the CY7 fluorochrome in the assembled particle was verified. The FNP was administered intravenously in the DIO mouse model, and its body distribution was evaluated ex vivo.
We developed a FPN with size of 278 nm, + 3,1 ZP, 0,16 PDI, 80 % EE, 1–4 % LC. SEM confirmed the size and showed a homogeneous distribution. The functionalization of the nanoparticles was verified by the Cy7 fluorescence marker anchored to the peptide. The affinity of the FNP to adipose tissue after 1.5H of intravenous administration of 80 mg OBE100/kg animal weight was demonstrated.
A formulation of FNP was developed based on a natural extract with potential antidiabetic activity to be selectively directed towards abdominal adipose tissue.

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P-389 Construction of thermodynamic phase diagrams for luteolin – povidone binary solid mixtures

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Luteolin (LUT) is a bioflavonoid that possesses several formulation-related problems due to its poor aqueous solubility [1]. This limitation may be resolved by formulating the API in its amorphous form. In this context, the aim of the present study is the thermophysical characterization of amorphous LUT and its binary mixtures with povidone (PVP), in order to determine the critical formulation parameters (e.g., recrystallization and/or phase transition regions) for the preparation of LUT amorphous drug formulations. In this vein, phase transition diagrams (i.e., temperature vs. concentration plots) of the active substance with PVP were constructed using differential scanning calorimetry (DSC) and the Flory-Huggins (FH) lattice theory, through which the dispersion of the pharmaceutical substance in the polymer behaves exactly like a polymer solution, with the difference that the API itself is in the place of the solvent. Fitting of the FH equation in the experimentally (DSC) determined drug melting temperatures at various LUT/PVP ratios, revealed a negative FH interaction parameter (χ) indicating that the API is thermodynamically miscible with the said polymer. Additionally, it was found that at 25 °C, which is a common storage temperature for pharmaceutical products, the "metastable" zone of the mixture (i.e., the zone between liquidus and spinodal) extends at concentrations below 10 % w/w of the active substance, indicating that in ASDs having higher API quantities drug-polymer amorphous-amorphous phase separation is favored.

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P-390 Selection of polymeric matrix/carrier(s) for the preparation of Siderol solid dispersions

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Despite the increased interest in natural products, APIs that occur from nature do not possess ideal properties to be formulated properly. Amongst them, Siderol (SDR), an active ent-kaurane diterpenoid substance having antimicrobial, antioxidant and antiviral activities [1,2], shows extremely low aqueous solubility, leading to poor in vivo bioavailability. Therefore, the aim of the present study was to identify the most promising polymeric matrix/carrier(s) for the preparation of binary drug-polymer solid dispersions in an attempt enhance drug' water solubility. Four widely used polymers and copolymers, namely polyvinylpyrrolidone (PVP) hydroxypropyl cellulose (HPC-SL), copovidone (coPVP) and Soluplus® (SOL) were evaluated in terms of drug's recrystallization inhibition and crystal growth rate. In regard to SDR's recrystallization, results showed that HPC-SL and SOL were able to stabilize the amorphous API, as compared to PVP and coPVP where a rapid and extended recrystallization was observed immediately after the preparation of the binary solid dispersions. In terms of API's crystal growth, results showed that all solid dispersions exhibited significantly lower growth rates as compared to the neat API (i.e., 0.94, 0.08, 0.38, 0.25 and 0.05 mm/sec for the neat SDR, and SDR- PVP, SDR-HPC-SL, SDR-coPVP and SDR-SOL solid dispersions, respectively), with SOL showing the most promising drug crystal growth rate inhibition. Therefore, based on the findings of the present study, SOL and HPC-SL can be considered as the most promising matrix/carrier candidates for the preparation of SDR solid dispersions.

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P-391 Molecular dynamics simulations of amorphous siderol

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Despite the several pharmacological uses of siderol (SRD) its successful formulation is limited by its extremely low aqueous solubility [1]. One way to overcome this, is to administrate the drug in a highly disordered – amorphous – state. However, in order to prepare a stable amorphous drug product, it is crucial to gain an insight into the several molecular phenomena occurring within this thermodynamically unstable state. Nowadays, this in-depth understanding can be expedited by the utilization of molecular simulations, and therefore, the aim of the present study was to apply molecular dynamics (MD) simulations in order to evaluate the several interactions occurring within the amorphous structure of the API. In this vein, SDR's initial molecular structure (obtained from cultivated *Sideritis scardica* [2]) was used (after energy minimization) to construct an amorphous cell consisting of 30 drug mole-

cules. This drug amorphous assembly was validated by comparing the MD simulated glass transition temperature (Tg) with experimental results obtained from DSC. Results showed that the theoretical and the experimental Tgs were in good agreement, indicating that the prepared amorphous assembly can be considered reliable. Following this validation process, the amorphous SDR structure was subjected into a long MD simulation run for 10 ns. The analysis of the obtained trajectory revealed the formation of hydrogen bonds within the amorphous API structure. The nature and strength of these intermolecular H-bonds was analyzed from the obtained simulation results, and their interplay with API's amorphous stability during storage was evaluated.

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P-392 Selection of amorphous solid dispersion matrix/carriers for Luteolin

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Luteolin (LUT), a bioflavonoid found in many plants, shows significant formulation problems due to its poor aqueous solubility and water stability [1]. One way to overcome these limitations is to formulate the drug in suitable amorphous solid dispersions (ASDs) [2]. However, the selection of a proper ASD matrix/carrier is a nontrivial task. Therefore, the aim of the present study is to evaluate several polymeric matrix/carriers and select the most promising for the preparation of a stable LUT ASD system. Initially, the glass forming ability of LUT was evaluated using a DSC-based method [3]. Results showed that the API is a good glass former since no thermal events were observed during drug's quench-cooling and reheating. Then, six commonly used polymeric ASD matrix/carriers (namely povidone, PVP, coPovidone, coPVP, hydroxypropyl cellulose, HPC-SL, hydroxypropyl methyl cellulose acetate succinate, HPMC-AS, Eudragit® RS, Eud-RS, and Soluplus®, SOL) were tested as to whether they can inhibit successfully LUT's recrystallization. In this vein, binary ASDs were prepared using the film-casting method. Then, the ASD casts were placed in accelerating storage conditions (40 ± 2 oC/75 ± 5% RH) and the formation of LUT crystals was evaluated via polarized light microscopy. In the case of the pure API, and ASDs using Eud-RS, HPC-SL, HPMC-AS and SOL, high drug's recrystallization was recorded starting from day one. On the contrary, binary LUT ASDs using PVP and coPVP showed good amorphous stability, up to 21 days of storage, attributed to API-polymer strong intermolecular interactions (revealed via ATR-FTIR spectroscopy).

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P-393 Luteolin – polyvinylpyrrolidone amorphous formulations: analysing intermolecular interactions via molecular dynamics simulations

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Luteolin (LUT) is a bioflavonoid that possesses several formulation-related problems due to its poor aqueous solubility [1]. This limitation may be resolved by formulating the API in its amorphous form. In this context, the aim of the present study is the thermophysical characterization of amorphous LUT and its binary mixtures with povidone (PVP), in order to determine the critical formulation parameters (e.g., recrystallization and/or phase transition regions) for the preparation of LUT amorphous drug formulations. In this vein, phase transition diagrams (i.e., temperature vs. concentration plots) of the active substance with PVP were constructed using differential scanning calorimetry (DSC) and the Flory-Huggins (FH) lattice theory, through which the dispersion of the pharmaceutical substance in the polymer behaves exactly like a polymer solution, with the difference that the API itself is in the place of the solvent. Fitting of the FH equation in the experimentally (DSC) determined drug melting temperatures at various LUT/PVP ratios (R^2 of fitting was 0.988), revealed a negative FH interaction parameter (χ) indicating that the API is thermodynamically miscible with the said polymer. Additionally, it was found that at 25 °C, which is a common storage temperature for pharmaceutical products, the "metastable" zone of the mixture (i.e., the zone between liquidus and spinodal) extends at concentrations below 10% w/w of the active substance, indicating that in ASDs having higher API quantities drug-polymer amorphous-amorphous phase separation is favored.

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P-394 In depth analysis of pilocarpine-carbomer molecular interactions via molecular dynamics simulations

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Pilocarpine (PIL) is a cholinergic agonist of natural origin used in the treatment of glaucoma and dry mouth. A major formulation-related drawback for this drug is its high chemical degradation in the presence of water (due to hydrolysis and epimerization). One way to overcome this limitation is to prepare hydrogel complexes of the API with carbomer (CRB) [1]. In this case, the formation of strong molecular interactions between the API and CBR are responsible for keeping the latter stable when exposed to an aqueous environment. Hence, in order to gain a further insight into PIL-CRB stabilization process, the present study attempts to unravel the nature of these interactions with the aid of molecular dynamics (MD) simulations. In this direction, a PIL-CRB amorphous assembly was initially prepared containing 10% w/w of the API.

After equilibration, the well relaxed assembly was subjected to long run (10 ns) MD simulations. A similar procedure was also followed for the neat API, containing 20 molecules of PIL, and the neat copolymer, containing a 30-monomer chain. The final 3.0 ns of the trajectory were used for computing the cohesive energy (Ecoh) and solubility parameter (δ) for each compound, and for calculating the respective radial distribution functions. Results confirmed, on a theoretical basis, the formation of significant intermolecular interactions between the drug and the copolymer, which can be considered as responsible for API's stabilization in the presence of aqueous environments. These results were in good agreement with experimental data (using ATR-FTIR).

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P-395 A phenolic-rich extract from *Ugni molinae* berries reduces abnormal protein aggregation and improve motor behavior in models of huntington's disease

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Huntington's disease (HD) is an autosomal–dominant inherited neurological disorder caused by an unstable trinucleotide CAG repeat expansion at the N-terminus of gene encoding the huntingtin protein (Htt) [1]. The mutation results in Htt proteins with an abnormal length of polyglutamine (polyQ) repeats [2]. This abnormal aggregation of mutant Htt (mHtt) promotes neuronal dysfunction and death of medium spiny neurons in the striatum, resulting in altered motor control and cognitive function [2]. Effective treatments for HD are still pending. Previously, our group identified the presence of polyphenols in leaves from the Chilean-native berry *Ugni molinae* [3,4], whose extracts showed a potent anti-aggregation activity in models of Alzheimer's disease [4]. However, beneficial effects of murtilla berry extracts were not investigated. Thus, we evaluated the efficacy of fruit extracts from different genotypes of *U. molinae* on reducing protein aggregation using cellular models of HD [5]. One extract (ETE 19–1) significantly reduced polyglutamine aggregation levels.

Aims: To determine the beneficial effect of ETE 19–1 extract in HD preclinicals models

Materials & Methods: R6/2 HD mouse model was treated with ETE-19-1 by Gavage daily for one month. We evaluated motor capacity by Rotarod test and protein aggregation in the brain tissue by western blot.

Results: HD preclinical models treated with ETE 19–1 shows that improves motor function and reduces protein aggregates in striatum.

Conclusion: Bioactive components in extracts from *U. molinae* berries have positive effects on HD. This demonstrates the potential effect of native polyphenols to treat neurodegenerative diseases associated with protein aggregates.

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P-396 Bioactive 3D printed scaffolds for the treatment of periodontal diseases

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Periodontitis is a bacteria-driven inflammatory disease of the periodontium, which can progressively cause tissue destruction and loss of alveolar bone and periodontal ligament, leading eventually to teeth loss [1]. Periodontitis has a high prevalence affecting millions of people worldwide; thus, there is an increasing need for effective treatments. Today the clinically available options for treating periodontitis are classified into non-surgical and surgical techniques. The first ones aim at controlling inflammation and preventing further progression of the periodontal disease, whereas the latter are usually applied in cases of extensive damage. Modern techniques also involve the use of grafts or biological agents aiming to augment the regenerative potential of the periodontal tissue. However, no treatment has succeeded in delivering a complete regeneration of the damaged tissue. In this regard, new techniques have emerged as promising therapeutic solutions, with three-dimensional (3D) printed scaffolds being one of them, offering a plethora of advantages. In this frame, various active pharmaceutical ingredients (APIs) with anti-inflammatory and anti-bacterial properties can be incorporated in 3D scaffolds and delivered in the area of interest, achieving an enhanced therapeutic effect. Curcumin is a natural product that alleviates periodontitis symptoms and impedes bacterial infections [2]. In the present work we fabricated and characterized curcumin- and ibuprofen-incorporated 3D printed biocompatible polymer scaffolds for the treatment of damaged periodontal tissues. Both a fused deposition modeling printer and a bioprinter were used and different methods (co-extrusion, co-dilution, impregnation) for the incorporation of APIs into several polymers were applied.

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P-397 Formulation strategies to stabilize pilocarpine hydrochloride solutions

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Xerostomia (or dry mouth) is defined as a subjective complaint of dry mouth which commonly exists as a consequence of reduced salivary flow (hyposalivation). Amongst the several treatments of xerostomia, the use of systemati-

cally administered sialagogues seems to be the most promising approach. Pilocarpine (PIL) is a naturally derived compound isolated from the leaves of *Pilocarpus microphyllus* and *Pilocarpus jaborandi*, which is capable of inducing the secretion of natural saliva from the undamaged part of the salivary glands through its action on muscarinic receptors. It is a parasympathomimetic agent that acts primarily as a non-specific muscarinic acetylcholine receptor agonist with mild beta-adrenergic activity. Despite its significant efficacy, a drawback regarding PIL's administration in the form of a solution (such as mouthwashes targeting xerostomia) is its chemical degradation due to the hydrolysis of the γ-lactone moiety and the epimerization of the α-carbon of the lactone ring. In order to overcome this limitation, commercially available products are formulated at low pH values (between 4 and 5) where the drug is almost fully ionized, leading, hence, to poor bioavailability. Therefore, the aim of the present study was to identify new strategies in order to improve PIL solution stability, and, consequently, improve its bioavailability. In this direction, the present study reveals that the chemical stability of PIL in non-aqueous solvents (i.e., glycerol and PEG-400) with or without the presence of suitable polymeric stabilizers can be considered as a promising strategy to enhance PIL solution stability.

P-398 Nanovesicles as smart drug delivery systems to load resins and essential oils or their combinations

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In the framework of the European Project EthnoHERBS, four plant species have been selected for this study, namely *Origanum dictamnus* L. (Lamiaceae, Cretan Dittany), *Salvia fruticosa* L. Mill. (Lamiaceae, Greek Sage), *Pistacia lentiscus* L. (Anacardiaceae, Mastic Tree), and *Cistus creticus* L. subsp. *creticus* (Cistaceae, Pink Rockrose/Ladano). The essential oils (EO) were obtained by steam distillation from Cretan dittany and Greek Sage, while the resins were collected from Pink Rockrose and Mastic Tree. GC and HPLC analyses were done to obtain the fingerprints of EOs and resins. Due to the high volatility of the constituents and possibly loss of activity, nanovesicles were developed containing a combination of EO and resins.

Nanovesicles made of phosphatidylcholine and cholesterol in a 60:1 gravimetric ratio according to our previous studies were produced [1,2] and tween 80 (3% v/v) was added to the hydration medium as a stabiliser of the vesicle's bilayer vesicles obtaining sizes of ca. 100 nm with polydispersity of about 0.2. Cretan Dittany and Pink Rockrose (1%) were loaded in the vesicles with a very high encapsulation efficiency (EE, > 95%). Other nanovesicles were prepared using two different gravimetric ratios, namely 3:1 and 6:1 (phosphatidylcholine and Mastic Tree) and loaded with 1% Greek Sage and EE was > 95%. Sizes of all developed nanovesicles were always lower than 130 nm and polydispersity less than 0.2. Preliminary stability testing (one month) evidenced a chemical and physical stability of the formulations, while release studies are ongoing.

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P-399 Encapsulating silybin in PCL-based electrospun nanofibers

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Silybin is the most active component of milk thistle (*Silybum marianum*) extract (silymarin). It is a polyphenolic flavolignane presenting a therapeutic effect on many diseases [1]. In this study, silybin was encapsulated in polycaprolactone (PCL) fibrous membranes, prepared using the electrospinning technique [2]. The morphological characteristics of the composite fibrous structures were examined using Scanning Electron Microscopy. In all cases, fibrous structures with a minor number of beads were obtained, as shown in ▶ **Fig. 1**. Thermal analysis using differential scanning calorimetry revealed that no shift of the PCL's melting point occurs in the composite scaffolds. Thermogravimetric analysis showed that, while PCL practically exhibits one decomposition stage initiated at around 310 °C, the composite membranes exhibit two decomposition stages, one at around 280 °C (temperature that pure silybin shows maximum decomposition rate) and one in the temperature range of 320–350 °C. Since only the PCL's onset decomposition temperature is shifted, it is concluded that no extensive interaction between PCL and silybin exists and, thus, no alteration of the bioactivity of silybin is expected. To evaluate the amount of loaded silybin, the prepared nanofibers were initially dissolved and the amount of released silybin was measured using ultraviolet–visible (UV-Vis) spectrophotometry. The potential cytotoxic activity of the composite scaffolds was assessed in human embryonic kidney HEK-293 [3] and in human hepatocarcinoma HepG2 cells [4], applying the MTT assay. A conspicuous inhibition effect of silybin-encapsulated nanofibers against the carcinoma cell line was observed, underlying the antitumor potential that the electrospun polymer composites exhibit.

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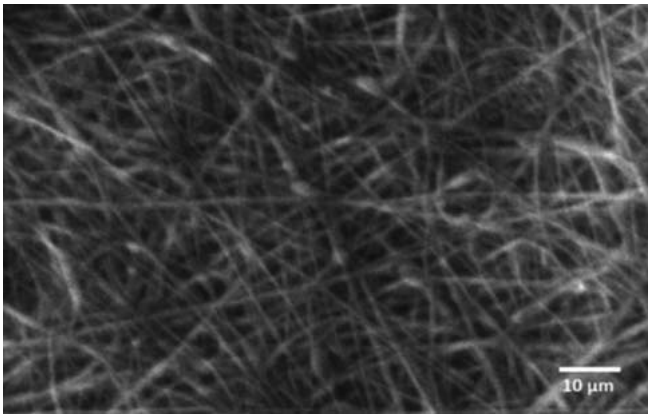

▶ **Fig. 1** PCL fibrous structure containing 5% wt. of Silybin.

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P-400 Bis-MPA hyperbranched dendritic nanocarriers of a structurally characterized flavonoid morin-Zn (II) complex with antioxidant and anticancer potential

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Flavonoids are plant secondary metabolites with several beneficial biological activities [1]. Morin is a flavonol with remarkable anticancer properties, though with limited solubility and stability in physiological media [2]. Metal ion complexation and/or encapsulation in nanocarriers is known to improve the bio-utilization of flavonoids. In this work, in view of the biological importance of Zn (II) ion, the synthesis, characterization and encapsulation of a Zn (II)-morin complex into bis-MPA dendrimeric scaffolds was effected. Both the Zn (II)-morin complex and the generated nano-formulations were characterized with physico-chemical and microscopy techniques and biologically evaluated for their antioxidant and anticancer activity against human A549 and H520 lung cancer cells and healthy human MRC-5 lung fibroblasts. The produced nano-formulations, empty and loaded, consisted of monodisperse nanoparticles with spherical morphology and neutral surface charge and sizes ranging between 80–180 nm. The entrapment efficiency and loading capacity of the loaded samples were 96.10% and 24.12%, respectively, whereas the release of the encapsulated complex was almost complete (98.7%). In vitro cytotoxicity studies against A549 and H520 lung cancer cells showed that morin exhibited cytotoxicity with IC_{50} values of 93.09 ± 3.58 and 88.19 ± 2.31 μM. The Zn (II)-complex exhibited greater anticancer activity than plain morin with IC_{50} values of 48.22 ± 4.33 and 50.71 ± 3.71 μM, whereas the loaded nano-formulations induced approximately 50% higher cytotoxicity than the Zn (II)-complex with IC_{50} values of 25.12 ± 3.93 and 22.51 ± 3.34 μM. The toxicity of the loaded and empty samples was over 180 μM, demonstrating the lack of significant cytotoxicity against healthy MRC-5 fibroblasts.

No conflict of interest was declared by the authors.

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