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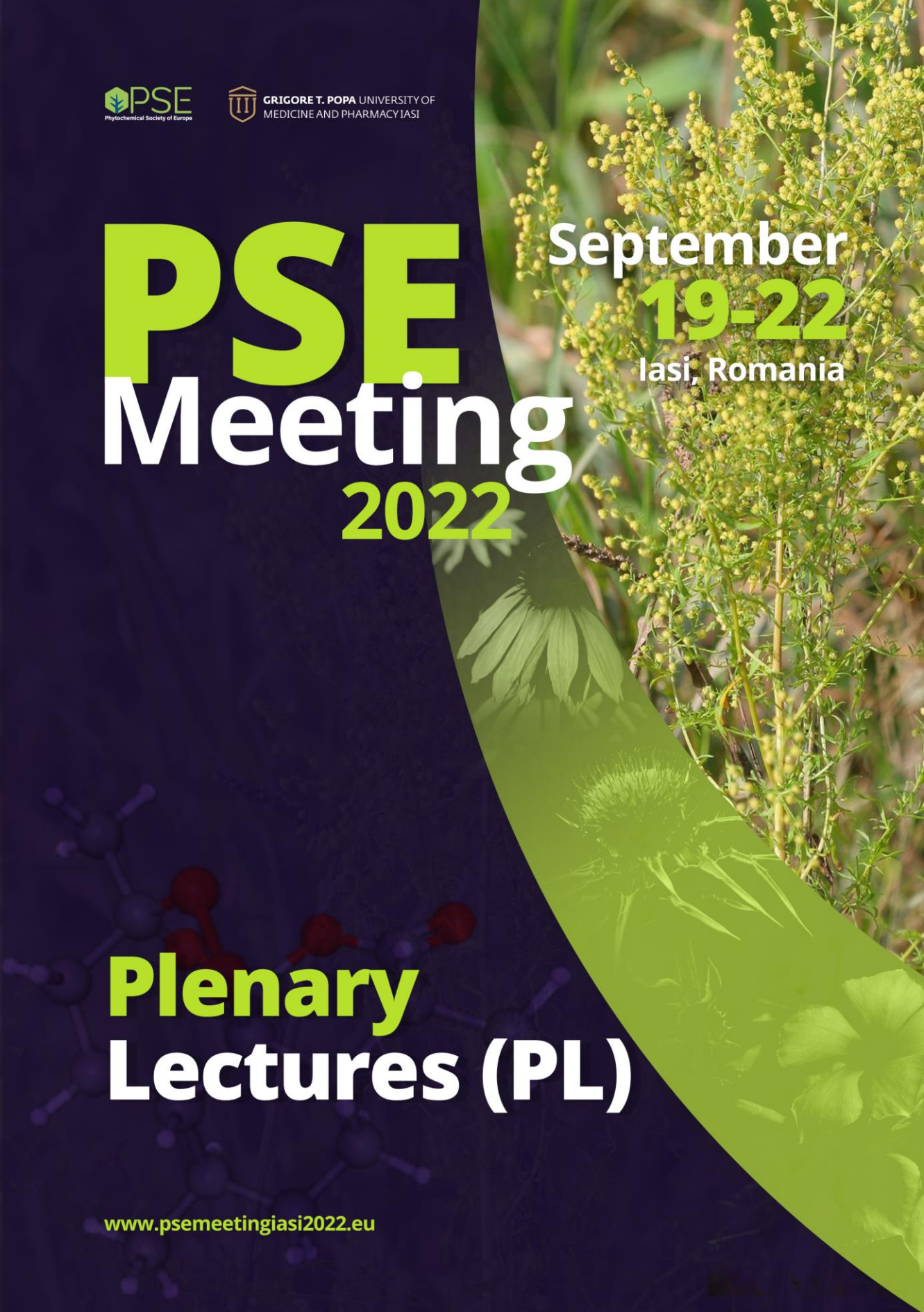

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The leaves of the chirimolla tree as a source of potential cancer prevention therapies

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Aim: Annonaceous acetogenins (ACGs) are fatty-acid derived natural products found only in species of the Annonaceae family. These compounds contain a long aliphatic fatty acid chain with 35–37 carbon atoms, derived from the polyketide pathway, a terminal methyl-substituted alpha, beta-unsaturated gamma-lactone ring and either none, one, two or three tetrahydrofuran rings that can be adjacent or not [1]. A wide range of biological activities, worth noting anticancer properties, have been reported [2]. One of a valuable source of these compounds is *Annona cherimola* Mill., where Spain is one of the major producer of this crop worldwide. Despite the potential of these natural products, their applications are limited by their poor solubility.

Methodology: For this purpose, ACGs were isolated from *A. cherimola* leaves through different chromatographic techniques (where the isolation studies of these compounds in this part of the plant are scarce), the improvement of the physicochemical properties through encapsulation techniques and the evaluation of their cytotoxicity on different tumoral ((human ovarian (IGROV-1) and cervix carcinoma (Hela) cell lines) and non-tumoral (human embryonic kidney 293 (HEK-293)) cells were also performed.

Results: The isolation and structural characterization of five main ACGs from *A. cherimola* deciduous leaves were achieved. The major ACG isolated (annonacin), and also the one that showed the best activity results, was encapsulated to generate a supramolecular polymer micelle structure in an effort to improve the drug release process. An improvement of solubility, stability and activity was achieved.

Conclusions: The presented results suggest that the use of *A. cherimola* deciduous leaves, holding acetogenins, are useful for cancer prevention, and could be a source for the development of promising lead compounds.

Acknowledgements
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Bioprospecting, biosynthetic engineering and synthetic biology towards drug discovery from bacteria

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Although plants and higher fungi have been used by humans for ages as sources for new medicines, drug discovery from bacteria is a relatively new endeavor, its beginning dating back to 1940s. Since then, several decades of bioprospecting in the bacterial kingdom yielded many important anti-microbial and anti-cancer agents (e.g. tetracycline, vancomycin, nystatin, doxorubicin etc), although the rate of discovery declined steeply over the last 20 years. The main reasons for that were high costs of screening coupled to frequent re-discovery of already known bioactive molecules.

It has been demonstrated that certain types of bacteria, namely those belonging to the order *Actinomycetales*, are the most prominent producers of bioactive natural products. Today, although bioprospecting of actinomycetes still remains a viable source of new bioactive compounds, approaches based on post-genomic technologies open completely new possibilities for genome-based drug discovery. Actinomycetes' genomes were shown to harbor dozens of orphan gene clusters that are silent under laboratory conditions, but can be activated via genetic engineering. The latter can also be used to alter natural product biosynthetic genes in order to generate new derivatives with improved pharmacological properties. Moreover, synthetic biology, a new discipline based on the use of engineering principles for the development of novel biological systems, can also be applied to harness bacteria for drug discovery.

In this lecture, examples of successful bioprospecting, genome mining, manipulation of natural products' biosynthetic pathways, as well as synthetic biology-based approaches to engineering actinomycetes for drug discovery will be presented.

Plant-derived indole alkaloids, from an african medicinal plant, as hit/lead compounds against cancer

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Plant-derived compounds, characterized by a great structural diversity and bioactivity, have long been playing a key role in drug discovery and development, particularly in cancer. The discovery of lead compounds from medicinal plants, based on their use in traditional medicine, has been considered an encouraging approach. Aiming at obtaining compounds with anticancer activity for overcoming multidrug resistance (MDR), in our search for bioactive compounds from African medicinal plants, we have been carrying out the phytochemical study of the African medicinal plant *Tabernaemontana elegans* (Apocynaceae), by using both approaches isolation and molecular derivatization.

The ability of compounds as modulators of P-glycoprotein (P-gp), multidrug resistance protein (MRP1) and breast cancer resistance protein (BCRP), the three main ABC transporters associated with MDR, has been assessed in different resistant cancer cells. Several indole alkaloid derivatives were found to be strong P-gp and MRP1 inhibitors and displayed selective antiproliferative activity to MRP1 overexpressing cells [1-4]. Using a different anti-MDR approach, recently we have also identified an indole alkaloid derivative that targeted homologous recombination DNA repair defects, by disrupting BRCA1-BARD1 heterodimer complex, in triple-negative breast and ovarian cancers. Moreover, it showed high in *vitro* and *in vivo* antitumor activity. Significant synergistic effects with anticancer drugs were also observed [5].

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Biological profiling of coumarins

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A great number of secondary metabolites generally found in plants which are daily use in ethnomedicine and as food, may affect the central nervous system (CNS) in mammals because they accumulate in the brain and trigger neuropharmacological effects (desire and/or unwanted). Natural products hold a great promise as potential drug leads to develop neuropharmacological therapies but the lack of critical insights into the translatability related to a knowledge about brain concentration *in vivo* is a serious limitation.

In biological profiling studies, coumarins show a surprisingly broad range of biological activities *in vitro* and *in vivo* and different coumarins, both synthetic and natural, have demonstrated effects on the CNS in preclinical *in vivo* experiments, suggesting their penetration through the blood–brain barrier. Relatively little is known about the pharmacokinetics and specificity of coumarins and how this correlates to activity in different *in vivo* assays. So far we investigate the neuropharmacological effects of coumarins to understand their mode of action in mice and zebrafish and some examples will be presented here. Additionally, latest study on the antiparasitic effects (on *Trypanosoma cruzi* that causes Chagas disease) of coumarins will be presented.

Using state-of the art isolation techniques related to counter current chromatography and a battery of behavioral models in zebrafish and mice we define a workflow that allows to derive conclusions regarding the potential of CNS active natural products and their potential mechanisms of action.

Acknowledgments
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Natural products – paving way from laboratory to cosmetic formulations

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Aim: Cosmetics with natural ingredients as well as being chemical-free have been gradually on demand all over the world. Actually, a natural cosmetic is defined as "*a cosmetic that contains at least 95% natural ingredients*" by CosmeBio. Natural or organic-based cosmetics intend no/minimum harm to human being and also respect to nature including environment and animals. Thus, an immense amount of research is being done on biosources to develop novel, more effective, and safer cosmetic ingredients.

Methodology: Various *in vitro*, *in vivo* and cell culture techniques as well as 3D methods are applied for cosmetic research. In our studies, we have been carrying out large screening assays on plant extracts along with pure natural compounds testing towards a number of relevant enzymes, *i.e.* tyrosinase, elastase, collagenase, lipoxygenase, xanthine oxidase, etc., while also testing the active ones in cell culture assay, *e.g.* human melanoma cell line (SKMEL 30). The active compounds are proceeded to molecular docking experiments as well as *in silico* toxicity. We have been also working on an anti-acne formulation based on plant extracts tested against *Propionibacterium acnes*.

Results: Our extensive screening and afterward studies led to identification of many plant extracts and pure natural compounds inhibiting aforementioned enzymes, which were consistent with data we obtained from cell-based assays. Some examples are the extracts from *Cotinus coggygryia*, *Geranium glaberrimum*, *Garcinia mangostana*, etc., where we designed different formulations such as niosomes or nanoemulsions.

Conclusions: Our outcomes on finding novel cosmetic ingredients from plants and other natural sources led us to possess 3 patents, 4 patent applications, and a final product (commercialized as mouth spray) in addition to several published papers [1-3]. In this talk, details of our recent cosmetic-related studies will be highlighted.

Acknowledgement
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EcoSap: triterpenoid saponins as green solutions for future sustainable food production

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Insect herbivores are damaging to world food production. It is estimated that they consume 20-30% of the potential production; therefore, there is a global need for more effective yet environmentally friendly pesticides. Plant specialized metabolites provide an untapped opportunity to explore and find bioactive compounds as sources for bio-insecticides. Saponins are a class of such underutilized defense compounds. Saponins are triterpenoid derived amphipathic compounds that exhibit detergent-like properties that can disrupt cell membranes of herbivore pests, causing cell death. Through the years the saponin biosynthesis, biochemistry, evolutionary origin and their structure and function relationship was studied in our group. The **EcoSap** project is based on learning from nature and investigating the potential of saponins as bio-insecticides as Green Solutions for future insect pest management. A major bottleneck to utilizing plant specialized compounds as insecticides is their availability and ability to scale up. Accordingly, a number of relevant plant bio-wastes are screened for saponins that are suitable as bio-insecticides. The establishment of a molecular toolbox of genes involved in saponins biosynthesis allow for tailored production of saponins *in vitro* and *in planta.* **EcoSap** will perform a thorough evaluation of the efficacy of selected saponins by studying their effect on target and non-target organisms, going from membrane systems to whole organism, to facilitate their ecotoxicological and environmental safety evaluation. The **EcoSap** project will fully assess the untapped potential of saponins as novel green solutions for sustainable food production going from **mode-of-action**, to **upscaling**, to evaluation of their **efficacy and environmental safety.**

Extraction of phytocannabinoids: an appraisal

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Aim: To present a critical appraisal of published methods and protocols for the extraction of phytocannabinoids.

Methodology: A literature review using the keywords, *Cannabis*, hemp, cannabinoids, *Cannabis sativa*, marijuana and extraction in various combinations, but cannabinoids and extraction being present in all combinations, and utilising the databases, Web of Science, Combined Dictionary of Organic Compounds, PubMed and Google Scholar and other relevant published materials.

Results: Phytochemicals that interact with the cannabinoid receptors and possess similar pharmacological properties as produced by the plant, *Cannabis sativa* L. are known as phytocannabinoids [1-3]. While maceration with organic solvents, *e.g.,* ethanol, is still in use, several other advanced extraction methods like pressurized solvent extraction, solvent heat reflux, Soxhlet extraction, supercritical fluid extraction, ultrasound-assisted extraction and microwave-assisted extraction are routinely used for the extraction of phytocannabinoids from various matrices, e.g., plant materials and consumer products. For forensic analysis, phytocannabinoids are generally extracted from biological samples, *e.g.,* human blood, and also from food and beverages, and wastewater, employing solid-phase extraction (and its variants) and liquid-liquid partitioning. Various computational methods and mathematical modelling tools are now routinely used to optimise parameters for extraction methods for phytocannabinoids.

Conclusions: Whatever may be the chosen extraction method, various physicochemical parameters, e.g., extraction temperature, extraction time, extraction pressure, and polarity of extraction solvent, still play a pivotal role in the overall yield of extraction of phytocannabinoids.

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Cannabimimetic drugs: how natural products inspire drug discovery and development

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The endocannabinoid system, a lipid signalling network involving GPCRs and ion channels, endogenous agonists and modulators, has been discovered thanks to pharmacological investigations on psychoactive cannabinoids from *Cannabis sativa*. Endocannabinoids, which are derived from arachidonic acid, have evolved early in evolution, prior to the classic mammalian cannabinoid receptors CB1 and CB2, and seem to play important functions also in lower organisms. Currently, only two very distant plant taxa are known to exert psychoactive effects via CB1 receptors *in vivo*, but numerous non-psychoactive endocannabinoid system modulating natural products exist. In this lecture, I will discuss the biochemical convergence and how lipids from plants can inspire probe design and help to identify new biochemical pathways in mammals and how this can foster translational research. Results from an ongoing comprehensive "phylo-activity" screening program of plant extract libraries will highpoint the potential link between nutrition and the endocannabinoid system, warranting a more pharmacological inquiry into the relevance of plant secondary metabolites for human health. I will provide an overview about current research on medical cannabis in Switzerland and new endocannabinoid modulators developed in our laboratory. The concept of selective endocannabinoid reuptake inhibitors (SERIs) will be outlined based on the story how natural products can inspire innovative drug development.

Myricetin ameliorated prediabetes via immunomodulation and gut microbiota interaction

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Aim: This study aimed to evaluate the effects of myricetin on prediabetes.

Methodology: RAW 264.7 cells, Jurkat cells and mice were used.

Results: The cell viability, endocytosis and phagocytosis of macrophages were inhibited in RAW 264.7 cells under high glucose (HG) levels, concurrent with an increase in ROS level. Administration of myricetin at a dose of 10 μM restored the immunosuppressive effects mediated by HG. Moreover, the viability of Jurkat cells was also inhibited concurrent with inhibition of IL-2 and IFN-γ expression levels versus increase PD-1 after treatment with myricetin at the dose of 10 μM. In a prediabetic mouse model fed with high fat diet, increase in body weight, fat mass, fasting blood glucose, TG, TC and LDL-C were observed. Flow cytometry analysis revealed a significant decrease in $CD8^+$ T cells in the spleen and blood, whereas the expression of PD-1 on $CD3^+$, $CD4^+$ and CD8+ T cells in the spleen and lymph was significantly elevated. Administration of myricetin showed a potential hypoglycemic and lipid-lowering effect in both prevention and treatment, in addition to restoration of innate and adaptive immune immunity in mice and confirmed the *in vitro* immunomodulation effect. In addition, 16S rRNA showed that myricetin ameliorated prediabetes may be related to decrease in the relative abundance of *Acetatifactor*, *Blautia*, *Intestinimonas*, *Anaerotruncus* and *Peptococcus*.

Conclusions: Myricetin could alleviate dyslipidaemia, as well as the immunosuppressive effects induced by persistent hyperglycaemia *in vitro* and *in vivo* experiments.

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Effect-directed analysis (eda) – how phytochemical analysis and bioassays help assuring quality and safety of herbal products

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Aim: The presentation gives an overview on application of HPTLC based Effect-directed Analysis for herbal products in different categories.

Methodology: Combination of High-Performance Thin Layer Chromatography (HPTLC) with Bioassays delivers chemical and activity information on separated natural multicompound mixtures. Bioassays applied are: DPPH and ABTS radical scavenging over inhibition of *Candida albicans*, SOS-UmuC mutagenicity screening to Yeast estrogen screen (planar YES).

Results: EDA-HPTLC fingerprint comparison delivers active fractions: UmuC shows mutagenicity of estragole and comparison of radical scavenging helps selecting extracts with highest content of antioxidant compounds. Screening for *C. albicans* delivers active fractions and planar YES elucidates endocrine disruptors and contraindications of herbal medicine for patients with endocrine dependent forms of cancer.

Conclusions: Effect-directed Analysis allows for a quick and pre-screening of herbal, plant material and environmental samples and delivers information of quality and safety in one analysis step. The methodology complements sophisticated and expensive LC or GC methods hyphenated with MS for the identification and quantification of active compounds in multicompound mixtures.

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Antibacterial potential of *Rhodiola Rosea*

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Aim: The activity of extracts and fractions prepared from the roots and rhizomes of *Rhodiola rosea* L. (*Sedum roseum* (L.) Scop., Crassulaceae) against *Campylobacter jejuni* strains should be investigated to further understand their antimicrobial activity. Detailed UHPLC-PDA-MS analysis was performed to characterize the qualitative composition of the extracts and fractions of plant material collected from wild habitats and cultivated material ('Mattmark', 'Rosavin').

Methodology: Two different extracts were used in our study: an ethanolic extract (60%, v/v), which was prepared by accelerated solvent extraction (ASE) from plant material collected in Carinthia (Austria) and for the cultivated material an extract, which was pre-extracted with hexane followed by ultrasound assisted extraction with ethanol (96%, v/v). Further fractionation of the latter extract was done on DIAION HP-20 adsorbent resin, an additional fraction was prepared by adsorption to polyvinylpyrrolidone (PVP). LC-PDA-ESI-MS^n analysis, MIC determination, resistance modulation assays, assays on membrane integrity of *Campylobacter jejuni* strains, and assays on AI-2 mediated intercellular signalling were carried out as described previously [1-3], with some modifications.

Results: Depending on the type of extract, seventeen to twenty substances were tentatively identified, including phenylethanoids, cinnamic alcohol glycosides (rosavins), cyanogenic glycosides, proanthocyanidins (PACs) and flavonoids. The 60% ethanolic extract showed resistance modulatory activity when tested in a combination with ciprofloxacin, erythromycin or triclosan. *Rhodiola rosea* preparations from cultivars 'Mattmark' and 'Rosavin' showed a high impact on *C. jejuni* signalling, which was especially indicated for fractions rich in PACs and flavonoids. On the other hand, fractions resulting from PVP adsorption, which were devoid of PACs and flavonoids, were less effective. At a test concentration of 15.625 mg/L, the disruptive impact on membrane integrity was very low.

Conclusions: Aside from phenylethanoids like salidroside and rosavins, more attention should be paid to PACs and flavonoids from *R. rosea*, with a great potential for *C. jejuni* AI-2 -mediated signalling reduction.

Acknowledgements

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Obesity management potential of plant natural compounds: from *in vitro* (human adipocytes) to *in vivo* (*Caenorhabditis Elegans*) studies

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Obesity has outreached the dimensions of a health problem and has established as a global epidemic (named Globesity) over the past decades [1, 2]. Excessive body weight appears among the top five risk factors in terms of attributable deaths and metabolic complications development [2, 3]. Consequently, management of obesity (*i.e.*, prevention and treatment) is subject of undergoing intense research [4].

In this respect, plant extracts and compounds of natural origin attract profound interest as candidates for obesity management. We have examined the potential of plant extracts and their bioactive principles to affect adipogenic differentiation in human adipocytes. The potential mechanisms of action were studied by using transcriptional analysis through real-time quantitative PCR and protein abundance evaluation by Western blotting. The key adipogenic transcription factors – peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT-enhancer-binding protein alpha (C/EBPα) – appeared strongly decreased at a protein level by treatments with plant extracts and pure compounds. Moreover, the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signalling pathway was found to be involved in the anti-adipogenic effect of the plant extracts and pure molecules. Collectively, our findings indicate that selected plant extracts and their active compounds hampered adipocyte differentiation through PI3K/AKT inhibition. Among the selected compounds, betulinic acid and maackiain exhibit the most promising anti-adipogenic activity [5, 6]. Furthermore, the research has been translated from *in vitro* human adipocytes to the *in vivo C. elegans* model.

Acknowledgments

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Natural products - quest for novel intervention strategy in prophylaxis and treatment of *helicobacter pylori* infections

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Helicobacter pylori infection is a major pathogenic factor for gastroduodenal ulcer disease and gastric carcinoma, as well as for other types of gastric and extragastric disease. In fact, *H. pylori* has been included by WHO in the list of pathogens for which identification of novel treatment strategies is urgent. Herbs and spices have been used since ancient times as remedies to treat variety of disorders. It was proven that spices, herbs, and their extracts possess antimicrobial, anti-inflammatory, antimutagenic, anticancer activities as well as gastroprotective and anti-ulcer properties. Our ability to source, extract, produce, characterize, and manipulate natural products has never been greater. Plants, due to broad biological and structural diversity of their components, constitute a unique and renewable source for the discovery of new antibacterial compounds. We hypothesize that in the era when *H. pylori* eradication with antibiotic regiments has a limitation mostly due to antibiotic resistance, natural products provide another choice or opportunity to eradicate *H. pylori* infections. The active compounds could also prevent gastric inflammation, gastric cancer formation and suppress cancer growth, which is prevalent in *H. pylori* infected patients.

Phytochemicals interactions: a tool to improve the bioavailability and health benefits

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Aim: To improve the bioavailability and health benefits of phytochemicals by the interaction with some other major nutrients.

Methodology: The interaction of phytochemicals with carbohydrates, lipids and proteins could increase the bioavailability and provide double or triple health effects.

Results: Royal jelly protein and epigallocatechin gallate (EGCG) interaction led to high bioavailability of EGCG and also showed synergistic health benefits of both royal jelly and EGCG. The esterification of EGCG with fish oil, greatly increased the bioavailability of EGCG, protecting the stability of fish oil. Also showed both health benefits. The interaction between casein and tannin lowers the bitter taste of red wine, while the addition of 10% inulin to tomato sauce causes a significant decline of bioaccessibility.

Conclusions: Numerous epidemiological studies have displayed that phytochemicals show benefits to human health, such as oxidative stress-associated degenerative and chronic diseases like obesity, inflammation, cancer, cardiovascular disease, type-2 diabetes mellitus and aging. But poor bioavailability could lead to different performance. Proper interaction with some other materials could increase and promote the value of phytochemicals.

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Amaryllidaceae alkaloids and their semisynthetic derivatives as inspiration for medicinal chemistry

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Aim: Plants of the family Amaryllidaceae have a long history of usage as herbal remedies all over the world to cure different ailments and diseases. Since the isolation of the first Amaryllidaceae alkaloid (AA) lycorine, more than 600 of AAs have been isolated and studied in terms of their biological activities. More than 100 AAs of different structural types have been isolated within the last 10 years in our research group from the genera Zephyranthes, Narcissus, Nerine, and other species. Isolated compounds have been tested for biological activity connected with the potential treatment of neurodegenerative, oncological, and infectious diseases. Because a number of AAs can be isolated from plants in large quantities, they are also an interesting target for the preparation of semisynthetic derivatives.

Methodology: Structural aspects of AAs (haemanthamine, ambelline, vittatine, galanthamine) allowed us to prepare novel chemical entities by derivatization of its free hydroxyl group and to inspect the structure-activity relationship of the novel derivatives. The hydroxy group was acylated with differently substituted benzoyl chlorides, 1- and 2-naphthoyl chlorides, and 2-furoyl chloride affording the corresponding esters. The structures of the newly synthesized compounds were determined by MS, HRMS, and 1D- and 2D-NMR. Antimycobacterial assay was performed with fast growing *Mycobacterium smegmatis* DSM 43465, *Mycobacterium aurum* DSM 43999, and with an avirulent strain of *Mycobacterium tuberculosis* H37Ra ITM-M006710. The technique used for activity determination was the microdilution broth panel method using 96-well microtitration plates. Modified Elmann´s method has been used for determination of inhibition activity of cholinesterases. Cytotoxicty has been tested using MTT assay on panel of 10 cancerous cell lines.

Results: Derivatization of the hydroxy group of AAs was in some cases associated with significant increase of biological activity. For example, derivatives of galanthamine showed antimycobacterial activity against all studied *Mycobacterium* strains (MIC = 1.56 – 62.5 µg/ml). Derivatives of haemanthamine and ambelline showed promising inhibition activity against cholinesterases. Ambelline derivatives demonstrated selective cytotoxicity against cancerous cell lines.

Conclusions: Within several last studies have been shown that AAs and their semisynthetic derivatives have promising pharmacological potential for further exploration and structure optimalization as new drugs against various diseases.

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Rnaseq as a molecular tool to decipher the pharmacologic activities of hops

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Hops containing, botanical dietary supplements are sold as women´s health supplements primarily to attenuate menopausal symptoms. The recommendation of this application is based on the estrogen-like activity of the constituent 8-prenylnaringenin and the cytoprotective property of another constituent, the chalcone xanthohumol. These properties, in connection with the beneficial influences of 6-prenylnaringenin on steroid metabolism, stimulated the hypothesis to develop this extract towards breast cancer prevention. Regarding composition of the hops (*Humulus lupulus*) extract, 36 % of its constituents are chalcones, with xanthohumol being the most abundant representing 90 % of the chalcones contained. The aim of the study presented here, was to address the role of xanthohumol regarding biological effects of hops extracts in mammary gland tissue. Experimentally, we treated pubertal, ovariectomized female rats with a so-called clinical extract as well as with a DESIGNER knock-out extract representing the clinical extract depleted by xanthohumol. Untreated ovariectomized rats and SHAM operated rats served as controls. Following treatment, we extracted RNA from mammary glands of treated animals and of respective controls. The mRNA obtained was subjected to RNAseq analysis. The major findings were: a) Removal of xanthohumol from the extract selectively abolishes the differential expression of approximately one third of the genes which were up- or down-regulated in response to treatment with the clinical extract. b) In response to the xanthohumol depleted knock-out extract a different gene expression pattern was observed which considerably differed from the one seen for the original extract. Approximately two thirds of the genes regulated in response to the knock-out extract were not regulated in response to the entire extract. In summary and conclusion, RNAseq is a useful tool to decipher the molecular mode of action of botanical extracts in target tissues, here mammary gland tissue. Major constituents of the extract, in this case xanthohumol, not only trigger gene expression, but also mask or suppress bioactivity of other constituents, as visualized by the bioactivity of the xanthohumol-depleted knockout extract.

Use of herbal appetite suppressants to aid weight loss

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Aim: Obesity has become a common health problem worldwide and is a major risk factor for noncommunicable diseases such as cardiovascular diseases, diabetes, musculoskeletal disorders, and some cancers. Thus, weight loss has become a hot topic within the general discussion on health and well-being, and many products are marketed as aids in weight loss. This presentation aims to raise awareness about the role herbal supplements may play in weight management as part of a healthy lifestyle.
Methodology: A systematic review of recent peer-reviewed literature on the application of herbal materials or specialised plant metabolites in weight management.

Results: A range of herbal products and specialised metabolites is used in weight loss: some increase human metabolic rate [1], others act as inhibitors of digestion of either fats or carbohydrates [2], whereas a third group affects the process appetite. The latter can be achieved through creating the sensation of satiety [3] or by other mechanisms that suppress appetite.

Conclusions: Whereas various products are commonly marketed as aids to weight loss, there is currently little scientific consensus on their efficacy or on potential adverse effects of herbal supplements.

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Transcriptome sequencing for individualized natural product-based therapy of multidrug-resistant tumors

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To combat complex systemic diseases such as cancer, single target intervention is proved to be ineffective. Pleiotropic natural products are one of the promising strategies due to their multi-targeting and due to lower side effects. In this presentation, we discuss the application of transcriptomic sequencing and expression analyses of tumor biopsies from cancer patients for natural product-based drug discovery. The efficiency of chemotherapy drugs can be affected by ATP-binding cassette (ABC) transporter expression or by their mutation status. Multidrug resistance is linked with ABC transporter overexpression. We performed RNA-sequencing-based mutation analyses in 18 biopsies of cancer patients for 12 ABC transporters related to drug resistance (ABCA2, -A3, -B1, -B2, -B5, -C1, -C2, -C3, -C4, -C5, -C6, -G2). The mutation rate varied from 27,507 to 300885. In ABCB1, three hotspots with novel mutations were in transmembrane domains 3, 8, and 9. Novel mutations were also found in ABCA2, ABCA3, ABCB2, ABCB5, ABCC1-6, and ABCG2. Diverse nonsense mutations causing premature stop codons were found and compared with the wild-type protein in terms of their protein structure. Nonsense mutations lead to truncated protein structures. Molecular docking and heat map analyses of ABCC1/MRP1 pointed out that Lys498* appeared in a separate cluster branch due to the large deletion, leading to a massive disruption in the protein conformation. The resulting proteins, which are nonfunctional due to nonsense mutations in tumors, offer a promising chemotherapy strategy since tumors with nonsense mutations may be more sensitive to anticancer drugs than wild-type ABCC1-expressing tumors. This could provide a novel tumor-specific toxicity strategy and a way to overcome drug resistance. The ABCB1 nonsense mutation Q856* led to a truncated P-glycoprotein, which may sensitize tumors to anticancer drugs. We also mined the cBioPortal database with 11,814 patients from 23 different tumor entities. We performed Kaplan-Meier survival analyses to investigate the effect of ABC transporter expression on survival rates of cancer patients. 3D-homology modeling allowed virtual drug screening of natural products that are selectively active against wildtype and mutated of ABC transporters. Finally, we present future perspectives on the plausible applications for individualized diagnosis and natural product-based therapy options for cancer.

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The impact of low hyperforin St. John's wort extract ZE 117 on stress induced changes in plasma membrane lipids

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Chronic stress is a key factor in the development of depression as it leads to a hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis which in turn increases the formation of glucocorticoids (GC) [1, 2]. Chronically elevated GC levels disrupt neuroplasticity by unbalancing signalling pathways probably by affecting brain lipid metabolism. Membrane lipids have an important function in the brain as they not only provide a physical barrier segregating the inner and outer cellular environments, but also are involved in cell signalling. It has been shown that the lipid composition influences membrane fluidity which affects lateral mobility and activity of membrane-bound receptors [1, 2].

Since changes in cellular membrane properties are considered to play an important role in the development of depression, the influence of St. John's wort extract Ze 117 on the plasma membrane fluidity of C6 cells, the ratio of PC to PE in whole cell lipid extracts, and the lipid composition of cortisol-stressed peripheral blood mononuclear cells (PBMC) was investigated.

Chronic exposure of C6 cells to cortisol dose-dependently increased membrane fluidity. The observed membrane fluidizing effect of cortisol could be reversed by co-treatment with Ze117 [3]. Further, decreases in phospholipids in terms of number of double bounds and chain lengths under the influence of Ze 117 in cortisol-stressed PBMCs were counteracted by Ze 117.

In summary, Ze 117 normalized the membrane fluidity of cortisol-stressed cells. In particular, the decrease in the number of double bonds under Ze 117 suggests an increase in membrane rigidity, which counteracts the cortisol-stress effect.

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Application of computational methods for alpha-glucosidase inhibitory natural products research

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Aim: Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. Increasing number of computational strategies are being applied to enhance and speed up natural product-based drug discovery. Diabetes mellitus (DM) is a serious chronic disease with increasing prevalence. Type 2 DM is the most common type, which accounts for 90% of all diabetes cases [1]. α-Glucosidase inhibitors, one of the classes of oral antidiabetics used to treat type 2 DM, delay digestion and absorption of glucose, which in turn, has a lowering effect on postprandial blood glucose and insulin levels. Consequently, search for new antidiabetic agents has picked up the pace lately. We tested α-glucosidase inhibitory effects of different types of natural products isolated from selected Turkish medicinal plants to discover natural hits and utilized computational tools to establish structure-activity relationships.

Methodology: We used *in vitro* tests, as well as different computational techniques (molecular docking, molecular dynamics, prediction of druglikeness and pharmacokinetic properties, etc.) to discover natural hits targeting α-glucosidase. Results: We reached compounds approximately 50 times more potent than the positive control, acarbose. We constructed a homology model of α-1,4-glucosidase from *Saccharomyces cerevisiae* according to comparative modelling techniques and used it for structure-based molecular modelling studies [2, 3].

Conclusions: α-Glucosidase inhibition of different compounds isolated from Turkish medicinal plants, their mechanisms of action and structure–activity relationships will be discussed in this presentation. Moreover, enzyme kinetics of the tested compounds *in vitro* and their possible interactions with the enzyme and key residues predicted by structure-based molecular modelling approaches will be provided.

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Rational natural product & drug combinations

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Aim: To strengthen the experimental and therapeutical basis of safe antimicrobial combination applications, especially towards new human pathogenic targets in a broad spectrum.

Methodology: In this review work, *in vitro checkerboard, binary* combinations of mainly pharmaceutical essential oils, fractions, pure compounds as well as standard antimicrobial standard agents of a decade research was elaborated. Different toxicity assays were also performed especially to the antifungal active combinations to ensure the selectivity of the activity towards the target. In addition, some oil combinations and formulations were fortified by micro- or nano-emulsions.

Results: Not all combinations were in bioactivity synergism, also as expected and observed antagonistic or indifferent effects were observed in systematic laboratory experiments. Solubility of lipophilic combinations were also a challenge most of the time. The synergistically active combinations were also verified from the point of *in vitro* cytotoxicity data.

Conclusions: The potential of systematic natural product, active pharmaceutical ingredient, natural/synthetic compound combinations are not only effective in broad sense antimicrobial therapy, but also in cancer and cardiovascular complications among others. The systematic and rational combination of more than two compounds or samples may have some challenges, which may be resolved by potential future applications such as specific *in silico* studies and artificial intelligence.

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Marine bioproducts for discovery in Australia - the Marine Bioproducts Cooperative Research Centre

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The Marine Bioproducts Cooperative Research Centre is a ten year research initiative funded by the Australian federal government and many industry and academic research partners. The Cooperative Research Centre commenced in 2021 and is the largest CRC in the history of the program with over 75 industry partners and 12+ research institutions, including Charles Sturt University. The CRC supports research in three themes including 1) marine organismal discovery, collection and culture 2) bioprocessing advancements for commercialization and 3) product discovery and development. The CRC will eventually support up to 100 postgraduate research students over the next ten years in Australian universities, plus many postdoctoral scholars and technical staff. The total value of the CRC is estimated at over Aus$270M with respect to support for associated research.

The CRC is focused on harnessing Australia's vast and unexplored coastline for marine organisms and biodiversity, enhancement of modular bioprocessing and natural product discovery and development. Australian coastal areas offer tremendous potential for biodiscovery of unique and biodiverse organisms, with a particular focus on the study and use of macroalgae and microalgae for product development. Such products will include human food products, nutriceutics, cosmeceutics, animal feeds, agrichemicals, fertilizers and fine chemicals. A focus on analytical chemistry, metabolomics and morphomolecular techniques for organismal identification, pathways analysis, gene regulation and structural elucidation of lead molecules and mixtures is supported by state-of-the-art instrumentation including numerous Agilent and AB Sciex QToF and triple quad mass spectrometers, NMR spectroscopy plus bioprocessing and off and on-shore culture facilities.

Regional, national and international partnerships will be an important part of the CRC activities, as a means of research collaboration, student capacity building and product development/discovery.

Nepalese propolis alters the metabolic state of *Mycobacterium Tuberculosis*

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Aim: Propolis is a natural product proved to be efficient against *Mycobacterium tuberculosis*. Although it is produced by bees, its active alcoholic-aqueous fraction contains plant derived molecules. To gain some insight into its mechanism of antimycobacterial activity, we studied the metabolic changes in bacterial cells treated with extract of Trigona sp. propolis from Nepal

Methodology: Liquid chromatography-mass spectrometry was used to monitor changed in bacterial metabolome, while qPCR was performed to determine expression of sigma factors genes.

Results: The detailed metabolomic and transcriptomic analysis performed in this study indicated target points in bacterial cells under propolis extract influence. The profile of lipids forming outer and middle layer of the mycobacterial cell envelope was not changed by propolis treatment, however fluctuations in the profiles of amphipathic glycerophospholipids were observed. The enrichment analysis revealed bacterial metabolic pathways affected by Trigona sp. propolis treatment. The early metabolic response involved much more pathways than observed after 48 h of incubation, however the highest enrichment ratio was observed after 48 h, indicating long-lasting influence of propolis. The early bacterial response was related to the increased demand for energy and upregulation of molecules involved in the formation of the cell membrane. Transcriptomic analysis confirmed that bacteria also suffered from oxidative stress, which was more pronounced on the second day of exposure. This was a first attempt to explain the action of Nepalese propolis extract against mycobacteria.

Conclusions: The performed experiment proved the successful application of LC-MS metabolomics combined with targeted transcriptomics in the determination of the mode of action of Nepalese propolis extract.

Combating cancer: natural products and pseudo natural products-based strategies underpinned by medicinal chemistry

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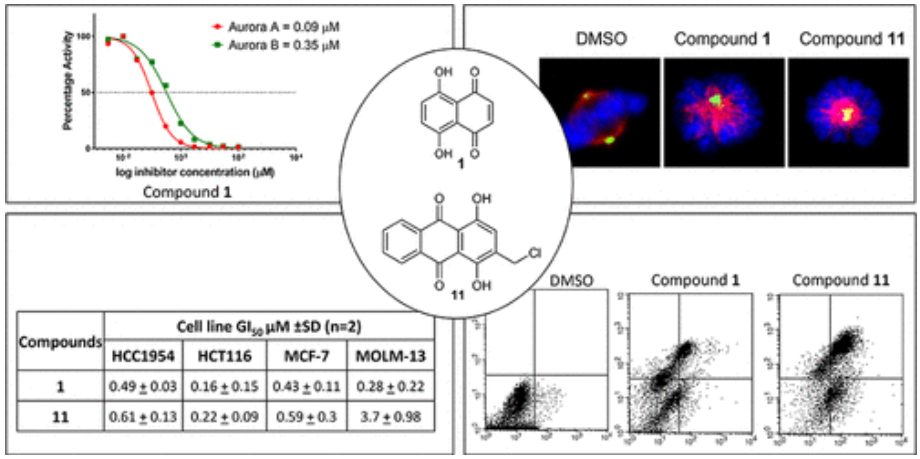
It has been reported that natural products (NPs) have an excellent track record in the discovery of lead compounds to treat various human diseases [1]. A characteristic feature of natural products is their chemical diversity, which is created by a sequence of enzymatic reactions in the producing organisms. The phytochemical investigations of *plants* and *fungi provided* a continuous supply of the natural products of interest but with limited number of their derivatives [1]. Moreover synthetic chemists are applying rational approaches to the generation of lead-like libraries to achieve increased potency, broader biological activity and fewer side effects and thus, a number of clinically important NP-derived derivatives have reached the market [1, 2]. We have isolated a number of natural products (viz., triterpenes, polyketides, and phenazines etc.) from fungi and plants which have interesting chemical diversity. Following the natural product inspired diversity oriented synthesis strategy [3, 4], various derivatives of these bioactive natural products have been prepared with the objective to obtain compounds with greater anticancer effects. A detail discussion about natural and synthetic chemical diversity and their role in anticancer drug discovery will be presented.



Compounds	Cell line GI_{50} μM ±SD (n=2)			
	HCC1954	HCT116	MCF-7	MOLM-13
1	0.49 ± 0.03	0.16 ± 0.15	0.43 ± 0.11	0.28 ± 0.22
11	0.61 ± 0.13	0.22 ± 0.09	0.59 ± 0.3	3.7 ± 0.98

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Natural products against dermatophytes: boosters of terbinafine activity and anti-inflammatory effectors

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Aim: Dermatophyte infections are superficial mycoses that affect the keratinized tissue of the skin, nails, and hair, with different inflammation degrees among immunocompetent individuals. Dermatophyte infections represent a significant public health concern, with an alarming negative impact caused by unsuccessful therapeutic regimens [1]. Combination therapy, associating conventional antifungals and natural products, represents a promising strategy in the treatment of dermatophytosis. Such combinations can exert a multi-target antimicrobial activity, thus increasing efficacy and reducing/reversing fungal resistance, and more importantly, can reduce effective doses of conventional antifungals, being consequently less toxic [2]. Among the plant-derived products, essential oils (EOs) and phenolic compounds are two of the most diverse classes of specialized metabolites that play an important role in plant defensive response to various insults, including microbial attacks [3]. In our study, selected Apiaceae EOs (ajowan, coriander, caraway, and anise) and the main phenolics isolated from *Magnolia* ssp. bark (honokiol and magnolol) were screened for their potential to improve the antifungal activity of terbinafine against dermatophytes.

Methodology: The antifungal screening was performed using dermatophyte standard strains and clinical isolates. The minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC) were determined in accordance with EUCAST-AFST guidelines, with minor modifications. The effects on ergosterol biosynthesis were assessed in *Trichophyton rubrum* cells by HPLC-DAD. Putative interactions with terbinafine against *T. rubrum* were evaluated by the checkerboard method and were interpreted based on the fractional inhibitory concentration index (FICI) values. Their impact on cells' viability and pro-inflammatory cytokines (IL-1β, IL-8 and TNF-α) was shown using an *ex vivo* human neutrophils model [4].

Results: Binary associations of tested EOs with terbinafine were found to be synergistic against *T. rubrum*, with FICI values of 0.26-0.31. EOs did not exert cytotoxic effects, anise EO being the most potent inhibitor of IL-1β

release (46.49% inhibition at 25 µg/mL) and coriander EO displaying the highest inhibition towards IL-8 and TNF-α production (54.15% and 54.91%, respectively, at 25 µg/mL) [5]. Additionally, we found that the main lignans isolated from *Magnolia* bark, magnolol and honokiol, were highly active against tested dermatophytes, with MIC and MFC values of 8 mg/L and 16 mg/L, respectively. The mechanism of action involved the inhibition of ergosterol biosynthesis, with accumulation of squalene in *T. rubrum* cells. Synergy was assessed for binary mixtures of magnolol with terbinafine (FICI=0.50), while honokiol-terbinafine combinations displayed only additive effects (FICI=0.56). Moreover, magnolol displayed inhibitory effects towards IL-1β, IL-8 and TNFα released from lipopolysaccharide (LPS)-stimulated human neutrophils, while honokiol only decreased IL-1β secretion, as compared to untreated control [4].

Conclusions: In conclusion, combinations of terbinafine and natural compounds could be a starting point in the development of novel topical therapies of *T. rubrum*-related dermatophytosis.

Acknowledgements
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Dereplication of natural products using mass spectrometry integrated approach

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Plant metabolites can act as drugs for the treatment of a variety of diseases due to their unique skeletal features. A large number of plant metabolites are used as drugs for the treatment of many diseases. The structural diversity of these plant metabolites formed by complex enzymatically controlled pathways is still not fully explored. Moreover, to preserve the medicinally important plant species and their sustainability, the quantity of plant material has been limited to the analytical level. Therefore, sensitive and high-throughput dereplication methods are required for better, unambiguous and high-throughput analysis of natural products in complex mixtures.

We have developed high-throughput dereplication strategy for the unambiguous identification of different classes of natural products through LC-ESI-MS/MS in the plant extracts and in the herbal formulations using an integrated approach which includes several confirmatory steps such as exact masses measurement, diagnostic fragment ions, databases search, and isotopic pattern. Based on above mentioned integrated approach, we have recently investigated different classes of natural products including withanolides (steroidal lactones), pregnane-type steroidal alkaloids, *Buxus* steroidal alkaloids and many more by using electrospray ionization quadropole time-of-flight mass spectrometry (ESI-QTOF-MS/MS) and LC-QQQ-MS analysis. Moreover, we have also developed a LC-ESI-MS/MS database of unique natural products. Outcome of the study was found to be very effective for their rapid identification of phytochemicals in plant extracts and herbal formulations.

Prenylated phenolics as possible drugs

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Natural substances have parallel anti-inflammatory and antibacterial effects, together with the current antiviral effect. Their mechanism of action is complex. However, the problem of natural substances is often their limited solubility and also problematic bioavailability [1]. Series of prenylated phenols were isolated from Paulowniaceae, Moraceae, and Euphorbiaceae plants [2-5]. As part of the lecture, we will introduce the isolation and identification of prenylated phenols with potential antiviral and anti-inflammatory effects, we will describe their bioactivity, their formulations to increase solubility, and will describe the possibilities of their further development. We described the effects of phenolics *in vitro* in cellular or biochemical systems on the production and release of inflammation-related cytokines, their effects on the inhibition of cyclooxygenases and lipoxygenases, and also some *in vivo* experiments confirming activity. We will also describe the possible semisynthetic compounds based on our prenylated substances. At the end, an improvement of solubility by incorporating tested substances into liposomes was presented.

Acknowledgements
The work was supported by Czech Science foundation, project no. 21-38204L Complexes of selected transition metals with plant-derived compounds with anti-NF-kappa B and pro-PPAR dual activities.

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In vitro investigation of chemopreventive potential of plant-derived extracts on breast cancer models

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In the last decade, several epidemiological studies have demonstrated the existence of a close relationship between a diet rich in natural products and a lower risk of chronic disease development, including some types of cancers. The complex mechanism of phytochemicals bioactivity in humans is far from being unveiled. Currently, red clover is used as functional food and nutritional supplement based to its high content of flavonoids, especially isoflavones. The aim of this study was to evaluate the biological activities of aqueous and ethanolic extracts of *Trifolium pratense* using *in vitro* breast cancer models: MCF-7 (estrogen-dependent) and MDA-MB-468 (estrogen-independent) human cell lines. The total phenolic and flavonoids content were determined, and the phytochemicals profile was established by HPLC. Furthermore, the antioxidant and free radical scavenging activity using spectrophotometric assays were determined and confirmed by Electron Spin Resonance. A biphasic effect of plant extracts on tumor cells was observed: high-dose of ethanolic extract triggered the cell death via oxidative stress; and low-doses of both extracts had a cytoprotective activity against reactive oxygen species (ROS) generation. Moreover, the plant extracts had an impact on antioxidant enzymes activity based on colorimetric and fluorimetry measurements. Aqueous extract showed a stronger estrogenic activity (E-screen test, Western Blot) in comparison with ethanolic one, after prolong treatment on estrogen dependent cell line. Both extracts had a significant anti-proliferative activity on estrogen-independent tumor cells. Moreover, on normal cells (non-tumorigenic mammary gland cell line, MCF12A) no cytotoxicity and a pro-proliferative effect was observed at the same tested concentrations. Our study had demonstrated that *Trifolium pratense* extracts have chemopreventive potential by reducing the viability and proliferation rate of tumor cells and by inducing apoptosis via oxidative stress. Furthermore, they can be a source of dietary phytoestrogens with antioxidant activities.

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New insights into memory-enhancing activities of essential oils (lamiaceae) in the brain: involving the cholinergic system and oxidative stress

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Aim: Essential oils (EOs) and their components have long been used in aromatherapy and conventional medicine to treat a variety of ailments, including dementia. Using a scopolamine-induced zebrafish model of memory impairment, the effects of EOs isolated from *Rosmarinus officinalis*, *Thymus vulgaris* and *Origanum vulgare* (Lamiaceae) were assessed.

Methodology: Zebrafish were given EOs (25, 150, and 300 µL/L) by immersion once daily between 8 and 13 days, along with scopolamine (Sco, 100 µM) 30 min prior to the initiation of the behavioral testing to cause anxiety and memory loss. Employing the novel tank diving test (NTT), the Y-maze, and the novel object recognition test (NOR), anxiety-like responses and memory-related behaviors were assessed. Acetylcholinesterase activity (AChE) and the brain antioxidant status were also evaluated. Detalied phytochemical analyses of the studied EOs were carried out using Gas chromatography-Mass spectrometry (GC-MS) analysis.

Results and conclusions: The studied EOs ameliorated Sco-induced increasing of AChE activity, amnesia, anxiety, and reduced the brain antioxidant capacity. These results suggest that these EOs may have preventive and/or therapeutic potentials in the management of memory deficits and brain oxidative stress in zebrafish with amnesia.

Identification of a hydroxygallic acid derivative, zingibroside R1 and a sterol lipid as potential active ingredients of *Cuscuta Chinensis* extract that has neuroprotective and antioxidant effects in aged *Caenorhabditis Elegans*

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Aim: Traditional Chinese Medicine (TCM) is one of the most important phytomedicine systems used as a treatment for aging-related diseases. Since clinical trials are expensive, protracted, and require substantial organizational efforts, the current study aimed to examine the effect of two TCM extracts from *Cuscuta chinensis* and *Eucommia ulmoides* on the health span of the model organism *Caenorhabditis elegans*.

Methodology: From the fourth larval stage (L4) on, *C. elegans* was treated with *C. chinensis* and *E. ulmoides* organic extract. The impact of these extracts on oxidative stress was investigated by determining the survival rate during paraquat exposure and the level of intracellular ROS. Moreover, a potential neuroprotective effect was tested by evaluating the short-term memory, learning index and mechanical sensory in *C. elegans*. Finally, the chemical composition of the *C. chinensis* and *E. ulmoides* extracts was revealed using UPLC-MS/MS and three main compounds were enriched and tested in further bioassays**.**

Results: *C. chinensis* extract increased the short-term memory of *C. elegans* on the 7th day of adulthood while there was no effect on learning or chemotaxis. On the 12th day of adulthood, *C. chinensis* enhanced the mechanosensory response. Furthermore, both extracts improved the resistance of *C. elegans* towards oxidative stress. Chemical analyses of the *C. chinensis* and *E. ulmoides* extracts revealed the presence of several known bioactive compounds such as chlorogenic acid, cinnamic acid, and quercetin. Furthermore, a hydroxygallic acid derivative and the sterol lipid 4-alpha-formyl-stigmasta-7,24(241)-dien-3-beta-ol are abundantly present in the *C. chinensis* extract, but hardly in *E. ulmoides*. A fraction enriched with zingibroside R1 from *C. chinensis* prolonged the lifespan, the survival after heat stress, and enhanced the locomotion in *C. elegans*.

Conclusions: From the current and our previously published results, we conclude that *C. chinensis* features overall anti-ageing effects in *C. elegans* while *E. ulmoides* improved specifically the physiological fitness. Zingibroside R1, a hydroxygallic acid derivative and the sterol lipid 4-alpha-formyl-stigmasta-7,24(241)-dien-3-beta-ol could be (partly) responsible for the observed health benefits of *C. chinensis*. Our analysis demonstrates that coupling *C. elegans* biotests with metabolomic analyses can help to speed up the identification of compounds of interest for healthy aging applications.

Therapeutic approaches of phytocannabinoids in clinical and preclinical studies

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In the last decades, there has been a growing interest in medical application of *Cannabis* strains components. Phytocannabinoids, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), mainly, have proven multiple physiological implications in experimental studies and a wide variety of preclinical and clinical studies. In addition to these compounds, endogenous cannabinoids and synthetic cannabinoids complete the list of substances whose activity is mediated by the endogenous cannabinoid system (ECS). On the other hand, it is very interesting that multiples researches have shown that changes in the expression, function and density of cannabinoid receptors are associated with diverse pathological conditions. Also, the paradigm according to which cannabinoids act only on specific receptors has been changed. Today, it is generally accepted the fact that substances derived from *Cannabis sativa* strains, regardless of their natural or synthetic origin, have complex mechanisms of action, which it also targets non-cannabinoid receptors. Nevertheless, further studies are needed to elucidate the precise mechanisms of these compounds. Furthermore, cannabinoids have proven beneficial effects such as anti-inflammatory, immunomodulatory, neuromodulatory, antioxidative and cardioprotective effects. Taken together, this information gives us serious reasons to believe that cannabinoids can constitute potential therapeutic, curative or preventive approaches in multifactorial pathologies. Consequently, we focused our attention on our studies to investigate the pharmacological effect of natural cannabinoids in chronic diseases, such as: neurodegenerative, oncological, cardiovascular and musculoskeletal diseases.

Finally, we concluded that phytocannabinoids may represent a promising approach not only for the treatment, but also for the alleviation of some chronic diseases-associated symptoms or for increasing the efficacy of existing drugs [1, 2].

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Alimurgic plants and healthy role of their phytochemical compounds on metabolic disorders

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The alimurgic flora represents, for different countries, a strategic resource and a challenge for future food (diet) with implications in human health due to its ecological, socio-cultural, agri-food, nutritional and healthy positive values [1]. However, this "intangible" patrimony, of inestimable value, is at great risk of extinction as a consequence of the lifestyles imposed by progress and globalization. In modern societies, a new phenomenon associated with the use of wild dietary plants is however emerging because food plants are considered not only in terms of simple nutritional intake, but as a potential source of phytochemicals beneficial to human health [2]. Alimurgic plants are crucial for human diet and can be defined as native plant species that grow uncultivated and reproduce naturally in their habitat. In many countries, has now emerged the opportunity to elaborate and indicate precise dietary behaviours to satisfy the needs of the population and improve health outcomes. One of the opportunities for dietary changes and increase the intake of nutraceuticals relies in the traditional use of wild edible plants both as a source of food and phytochemicals with potential therapeutic activity.

In the last decade, a huge number of alimurgic plant extracts and their constituents have been investigated for their potential to treat metabolic disorders [3]. Among them, many species demonstrated inhibitory activity against the pancreatic lipase enzyme, important in the treatment of obesity and related comorbidities. Wild *Capparis orientalis* Veill. and *C. sicula* Veill. ssp. *sicula, Cichorium intybus* L., *Daucus carota* L., *Leopoldia comosa* (L.) Parl., *Lobularia maritima* (L.) Desv., *Moricandia arvensis* (L.) DC., *Nasturtium officinale* R. Br., *Portulaca oleracea* L., *Silene vulgaris* (Moench) Garcke, are some examples. The breakdown of carbohydrate by carbohydrate-digesting enzymes such as amylase and glucosidase and the subsequent intestinal glucose absorption remain therapeutic targets for the obesity-related disorder diabetes. *Borago officinalis* L., *Clematis vitalba* L., *Echium vulgare* L., *Lepidium sativum* L., *Mentha aquatica* L., *Picris hieracioides* L. have been shown to be active in the inhibition of these specific enzymes. Phenolic acids, flavonoids, terpenes and stilbenes have been identified as responsible for these activities [4-7].

Biochemical knowledge is of crucial importance to evaluate the health benefits and physiological effects of alimurgic plants in order to develop clinical investigations concerning their mechanisms of action, safety, and efficacy.

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Current scientific evidence of the biomolecular potential of selective herbal extracts

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Aim: The chemical aspect and the proper selection of the extract/compound represent the basis for high-quality research in herbal medicines. Several sources of apigenin derivatives were investigated for their pharmacological effects.

Methodology: Chamomile flowers, parsley and celery seeds were used to obtain apigenin rich extracts which were analyzed and standardized by UPLC-PDA and FTIR methods. The obtained samples were assessed in terms of their antioxidant, antimicrobial and *in vitro* anticancer activity.

Results: The calculated EC_{50} value for all the antioxidant tests allowed us to observe differences in regard to their activity. Taken together, all investigated extracts showed a better scavenger activity against free radicals, a good inhibitory activity against lipoxygenase, but a lower sensitivity against iron mechanism. The chamomile extract was active only against tested Gram-positive cocci, while the parsley extract inhibited only the *S. aureus* strain. The celery extract did not show antibacterial activity. All tested preparations showed antifungal activity. At the highest tested concentration, namely 60 µg/mL, parsley extract was the most active in terms of reducing the viability of MCF7 human breast adenocarcinoma cell line and inducing the release of lactate dehydrogenase. In the set experimental conditions chamomile and celery extracts present relevant and moderate antiproliferative and cytotoxic potential. On the other hand, celery extract manifested the most potent anti-migratory effect and was the most active extract in terms of total apoptotic events (both early and late). This preliminary study conducted for the assessment of the various biological effects of screened samples show that further steps need to be undertaken for a clear understanding of the complex mechanisms of this bioactive phytocomplexes.

Conclusions: Our results come in hand for future approach in regard to the development of new therapeutic approaches that use natural compounds as the active drug. Moreover, the identification of rich natural sources is of use for both food and pharmaceutical industries.

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Quantitative structure - activity relationships of prenylated (ISO) flavonoids and isothiocyanates as antimicrobial agents

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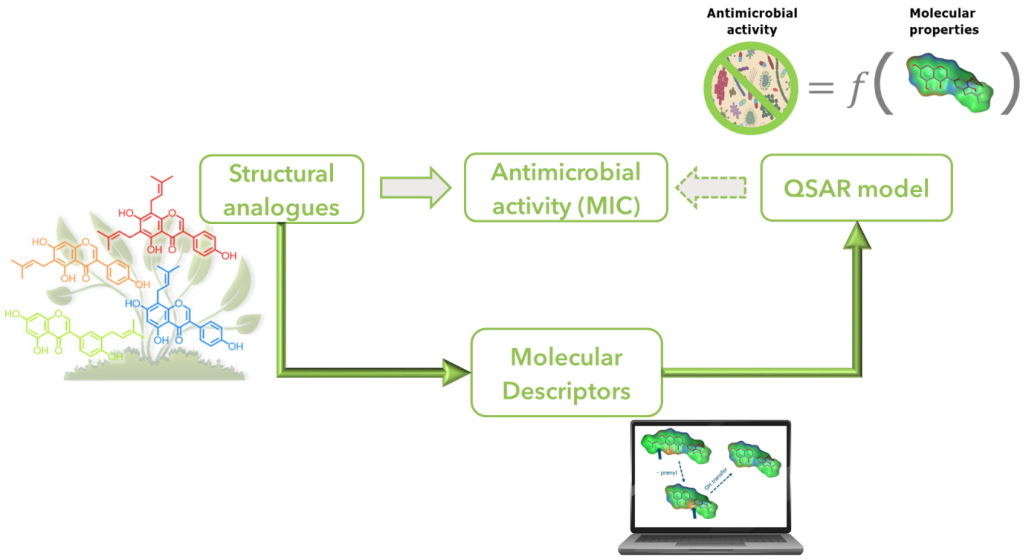
Defence phytochemicals are produced *de novo* in the plant upon (biotic or abiotic) stress. Defence phytochemicals have been acknowledged for their promising antimicrobial activity. Prenylated (iso)flavonoids (bearing a C5-isoprenoid moiety, from the Fabaceae family) and isothiocyanates (ITCs, bearing an -N=C=S group, from the Brassicaceae family) are important classes of defence phytochemicals known for their antimicrobial properties. Evidence shows that rather small structural variations play a key role in the antimicrobial properties and spectrum of activity of these classes of defense phytochemicals. The aim of this research is to understand the structure-activity relationships (SAR) of prenylated (iso)flavonoids and ITCs as antimicrobial agents and to model their activity using an *in-silico* QSAR approach to unravel the most important molecular properties for antimicrobial activity. For this, a collection of analogues of prenylated (iso)flavonoids and ITCs were tested against a broad range of microorganisms and minimum inhibitory and bactericidal concentrations (MIC and MBC) were measured. Molecular properties of the compounds were calculated *in-silico* and QSAR models were developed and validated. Results of SAR showed that diprenylated (iso)flavonoids were among the most active antibacterials against Gram positive bacteria, such as *L. monocytogenes* and methicillin-resistant *S. aureus*, with MICs of less than 10 µg/mL. Furthermore, monoprenylated isoflavonoids, bearing a chain or a ring prenyl group, showed the best anti-yeast activity, with a MIC of ≤ 15 µg/mL against *Z. parabailii*. ITCs with short chained methylsulfinyl (MS) or methylsulfonyl (MSo) groups showed good antibacterial activity (MIC 25 µg/mL) against Gram negative bacteria (*E. coli*), whereas ITCs with a long chained MS/MSo groups showed good antimicrobial activity (MIC ≤ 25 µg/mL) against Gram positive bacteria (*B. cereus, L. monocytogenes*) and fungi (*S. cerevisiae* and *A. niger* spores). Based on the QSAR models, properties such as the extent of hydrophobicity, shape, charge and hydrogen-bonding were found to be important contributors of the antimicrobial activity of prenylated (iso)flavonoids. Partial charge, polarity, reactivity, and shape were key properties for ITCs activity. Overall, prenylated (iso)flavonoids from legumes and ITCs from brassica vegetables can serve as natural potent antimicrobial agents for food preservation and the developed *in silico* QSAR models can be useful for finding new leads for e.g. drug discovery.



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Pyrrolizidine alkaloids as contaminants in herbal drugs and extracts – HRMS based identification and determination including isomeric structures

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Aim: Pyrrolizidine alkaloids (PA) and in particular 1-2 unsaturated PAs are considered liver- and genotoxic. For herbal medicinal products authorized within the EU, an intake limit of 1 µg/d for PA applies, which can get into herbal drugs through contamination with plants containing PA. MS/MS and HRMS methods are available for the identification and quantification of PA in sometimes very complex plant matrices. The conditions for method validation and verification for contaminant PA in herbal drugs and extracts are described in detail in a monograph that has recently come into force in the European Pharmacopoeia (Ph. Eur.) [1]. At about the same time, the European Food Safety Authority (EFSA) published its conditions for determining PA [2]. In contrast to the criteria for PA analysis set out in Ph. Eur., EFSA also requires the differentiated identification and determination of frequently occurring PA isomers.

Methodology: Using the example of an analytical method validated according to the Ph. Eur. criteria, based on UPLC-HRMS-ToF and proven in practice on complex extract matrices, the possibilities and limits of the determination of PA are illustrated.

Results: Possibilities of improving the chromatographic separation performance with regard to stereo- and positional isomeric PA structures are shown and the risks of false negative analysis results, if possible different responses of the isomers are not taken into account, are evaluated.

Conclusions: Ongoing scientific work shows that the different PA show considerable differences in their toxicity. For most of the PA found, a much lower toxicity can be assumed today than was originally found for model substances such as riddelliine and lasiocarpine. In the future, analytical methods should therefore be able to quantify all individual PA individually. This also applies in particular to the isomeric PA structures.

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QSAR models for assessing the biological effects of natural products

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Aim: The number of known natural products increases every year if not every day, today surpassing 400,000. Many of these compounds have little been investigated for their biological (pharmacological, toxicological, others) effects, if at all. A number of logistic and other practical constraints makes wet-lab experiments for many of these compounds virtually impossible. The computing power available today, though, allows researchers to explore and assess their biological and chemical properties through much cheaper and accessible *in silico* tools, among which quantitative structure-activity relationship (QSAR) models play a key role. This lecture aims to provide an overview of the advantages and pitfalls of such models in the assessment of natural products.

Methodology: An overview of what QSAR models are, how they are built, validated and used for making predictions is provided, with appropriate examples.

Results: QSAR models are inexpensive but powerful, if appropriately validated, can be applied to a wide variety of biological effects and virtually to any individual natural product (assessing mixtures is a big challenge).

Conclusions: QSAR models are able to open the door of assessment for many compounds that have never been evaluated for their biological properties.

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Use of disposable analytical tools in natural compounds assessment – versatility and sensitivity versus analytical resolution

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Aim: Current work is discussing the "on-purpose" tailored development of sensors and biosensors as analytical tools to assess natural compounds as occurrence and efficacy, both in raw vegetal materials and conditioned in formulas with enhanced bioavailability.

Methodology: The pathway for tailored design of "on-purpose" electrochemical and impedimetric sensors and biosensors devoted to assessment of polyphenolic poly-carboxylic acids and phytosterols is fully described and their characteristics of analytical performance are presented. The application in detecting, quantifying and efficacy assessment for polyphenols and phytosterols from natural extracts and phytosomes.

Results: Polyphenols and their metabolites were assessed using amperometric-enzymatic biosensors, from various samples, with a limit of detection up to 10^{-8} molL^{-1}. An electrochemical sensor based on immobilized lipoprotein was developed and applied to assess antioxidant effectiveness of formulated plant extracts and other formula based on natural compounds against *in situ* generated lipoperoxides. Sensor was proving its applicability at significant concentration ratios between antioxidant and free radicals thus avoiding misjudging the antioxidant effect by confusing with chemical quenching of free radicals' evolution. An impedimetric sensor Au-MUD-EDC / NHS-ERα-BSA was developed and applied to assess sterols and 17βestradiol with a limit of detection lower than 10^{-9} molL^{-1}. The reliability of sensor response on real samples was confirmed by HPLC-DAD-MS and MALDI -ToF methods. Developed sensors and biosensors proved their versatility in assessing the targeted compounds - phenolics derivatives, sterols and phytosterols – either as major sample components or when occurring in low concentrations in samples with complex matrices, both extracts or formulas with increased bioavailability. Limitation of disposable analytical tools applicability, in terms of sensitivity, interferences and analytical signal resolution are discussed for each category of developed sensor/biosensor.

Conclusions: It is underlined that disposable analytical tools are highly useful and versatile to assess natural compounds in various samples if their limitations are properly considered.

Acknowledgements

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A sticky gold from papaveraceae or just yellow stuff? on the antimicrobial potential of isoquinoline alkaloids from plant *in vitro* cultures

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Aim: Isoquinoline alkaloids are a very diverse class of plant specialized metabolites staining the latex and extracts of Papaveraceae plants yellow, orange or bright red. They have been long considered as potent antimicrobial agents. Some of them, such as berberine and coptisine are made commercially using plant tissue culture. However, much less is known about other compounds that are abundant for example in some traditional medicinal plants of Papaveraceae (encompassing Fumarioideae).

Methodology: Here, we use plant tissue and organ culture for obtaining alkaloids and test them against pathogenic bacteria and candida yeast, including biofilm-forming strains. We also use an innovative cell-support system made of bacterial bionanocellulose to elicit response from partially immobilized plant cells for modified antimicrobial potency [1]. The focus is on *Chelidonium majus* L., a well-known herb used externally against infectious diseases, as well as several related species from *Corydalis*, *Fumaria*, and *Pseudofumaria*.

Results and Conclusions: These results prove a high potential of *in vitro* grown organs and dedifferentiated callus tissue for production of significant amounts of pharmacologically relevant alkaloids. However, other common metabolites, such as flavonoids and hydroxycinnamic acids significantly influenced the overall antimicrobial activity of the studied plants.

Acknowledgements
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Cannabinoids: liquid-liquid chromatography separation and bioactivity evaluation

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Aim: The aim of this study was to develop liquid-liquid chromatography (LLC) separation processes to purify various target cannabinoids from hemp extracts and test their bioactivity

Methodology: For the purification of the target cannabinoids, various LLC operating modes (e.g. isocratic batch, step-gradient, trapping multiple dual mode) were explored in a model-based manner. Thermodynamic models (e.g. COSMO-RS) were used to screen and select the most promising solvent system candidates, whereas the suitable operating conditions were selected by using chromatographic models [1, 2]. The cytotoxicity of the isolated compounds was evaluated in non-tumor (HaCaT keratinocytes, BJ fibroblasts) and malignant melanoma (A375, SH4, G361, SK-MEL-2) cells.

Results: Cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), cannabicyclol (CBL) and cannabidivarin (CBDV) were separated from hemp extracts with purities from 93% to 99%. Of the test cell lines, A375 cells were the most sensitive, with IC_{50} values between 12 and 30 µg/mL, whereas the viability of the non-tumor cells was not affected significantly.

Conclusions: The model-based approach from the current work shows the great potential of LLC in separating high-purity cannabinoids with promising *in vitro* anti-melanoma activity.

Acknowledgements
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Aloe vera extracts - based scaffolds for wound dressing applications

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Aim: The wounds treatment is a challenging biomedical field due to delayed wound healing (microbial infections, underlying physiological conditions, poor nutrition etc.). The traditional wound dressing materials face with weak mechanical properties and poor antimicrobial properties. Therefore, in order to improve antibacterial characteristics, new scaffolds including antibacterial components (silver ions and silver nanoparticles, drugs or combination of drugs) or natural extracts (including *Aloe vera* extracts) have been tested to modulate factors involved in wound healing. The paper is focused on new formulations of *Aloe vera* extracts and biopolymers and evaluates the physical-chemical and biological properties, in correlation with their potential application in wound healing.

Methodology: Scaffolds based on *Aloe vera* extracts and biopolymers (combinations of collagen, gelatine, hyaluronic acid, chitosan) were obtained and correlations between scaffolds engineering and biological properties (biocompatibility, biodegradability, bioadhesion, tissue repair) are discussed. Various formulations with *Aloe vera*, the biopolymer nature and characteristics (average molecular weight, functionality) and the ability to build supramolecular structures are also compared in terms of their biological performances.

Results: The obtained materials absorb biological simulated fluids, are biocompatible, with good bioadhesion properties and retain bioactive compounds. The influences of the *Aloe vera* extracts, biopolymers properties and scaffold structure and morphology, as well as some parameters of the human physiological medium are challenges that must be considered to obtain high-performance wound dressings.

Conclusions: Various studies support the anti-inflammatory and cicatricial potential of *Aloe vera*, which has a wide indication for the treatment of several conditions. The *Aloe vera* combination with biopolymers leads to synergetic processes and has favourable effects in wounds healing. The clinical translation of engineered constructs remains an important challenge, especially when biological components (cells, growth factors, DNA) are involved and constrains by increasing medical procedures costs, regulatory restrictions, and ethical concerns.

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Phenanthrene derivatives from *Juncus Inflexus* as natural antibacterial agents

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This ongoing study aims to limit the spread of bacterial antimicrobial resistance (AMR), a leading global health concern [1], by researching and developing new antimicrobial medicines from plant natural products. On the basis of an ecological approach [2], a screening of the antibacterial activity was firstly performed on a number of *Juncus* species collected from different locations in France. A remarkable antibacterial activity, with a MIC of 39 µg.mL^{-1}, was observed for the methylene chloride sub-extract of *Juncus inflexus* rhizomes, especially against gram-positive bacteria such as *Enterococcus fœcalis* and *E. fœcium*, *Streptococcus pyogenes* and *Staphylococcus aureus*. Bioguided fractionation, using conventional methods (centrifugal partition chromatography and preparative HPLC), was carried out on the active sub-extract and four phenanthrene derivatives were successfully purified and identified. Several antibacterial bioassays were performed on these compounds, like for instance, kill-time curves, anti-biofilm tests, post-antibiotic effect tests and synergy assays between the purified compounds as well as with selected antibiotics. So far, interesting *in vitro* antibacterial results have been acquired on a MRSA clinical isolate: *Staphylococcus aureus* T28, particularly studied for its pathological features. It was isolated from a diabetic foot infection and it harbours genes that are implicated in antibiotic resistance and biofilm formation [3]. On the grounds of these findings, phenanthrene derivatives could be very good candidates for topical application.

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Metabolomic analysis and antimicrobial activity of *Moringa Oleifera* seeds, leaves and oil

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Aim: Moringa (*Moringa oleifera*) is one of the traditional food crops widespread in Asiatic, African and South American regions. It is very important for the ability to grow in harsh conditions, for the high nutritional value, and for its cardioprotective, anti-inflammatory, antioxidant and antimicrobial properties [1]. In this work, we investigated the metabolome of moringa polar and non-polar extracts of leaves and seeds, and of moringa oil, using proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR), Liquid Chromatography Mass Spectroscopy (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS). We also evaluated their antimicrobial activity against four bacterial species.

Methodology: *M. oleifera* leaves and seeds were extracted with dichloromethane and with a methanol/water (1:1) mixture. Polar and non-polar extracts, and oil, were analyzed through 1H-NMR and GC-MS techniques.

Results: The metabolomic profiling provided a wide set of metabolites that were identified and quantified. Moreover, non-polar extracts from seeds showed an antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*, that was associated to the content of specific fatty acids.

Conclusions: Our results remarked the importance of the metabolomic approach for the identification of bioactive molecules by recognizing the molecules responsible for the antimicrobial properties of *Moringa oleifera* seeds extract.

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Cytotoxic triterpene glycosides from the root of *Olax Subscorpioidea* oliv. (olacacae)

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Aim: Cancer is second to cardiovascular diseases in global mortality rate. Medicinal plants remain an affordable and easily available source of anticancer agents [1]. An ethnobotanical survey has identified *Olax subscorpioidea* as part of traditional recipes for cancer treatment in southwestern Nigeria [2]. The present work was aimed at bioactivity-guided isolation of cytotoxic compounds from the root of *O. subscorpioidea*.

Methodology: The methanol root extract *O. subscorpioidea* was fractionated by solid-phase extraction. Cytotoxic potential of the fractions was evaluated by the *in vitro* 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay in HeLa and MCF-7 cell lines. Preparative and semi-preparative HPLC analyses were performed on the most active fractions.

Results: HPLC analyses of the active fractions afforded isolation of oleanane-type triterpene glycosides. The chemical structures of the isolated triterpenes are currently being analyzed by spectroscopic means (1D and 2D NMR, HREIMS). The active fraction displayed dose-dependent inhibition in the HeLa cell line. The IC_{50} values of the active fractions ranged between 25-201 μg/mL and 85-273 μg/mL, respectively, against HeLa and MCF-7 cell lines.

Conclusions: Oleane-based triterpene saponins have been isolated from the active fractions of *Olax subscorpioidea* root bark. The structures of the isolated compounds are being resolved and evaluated for cytotoxicity.

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Anti-inflammatory activity of *Valeriana Phu* and isolation of secondary metabolites

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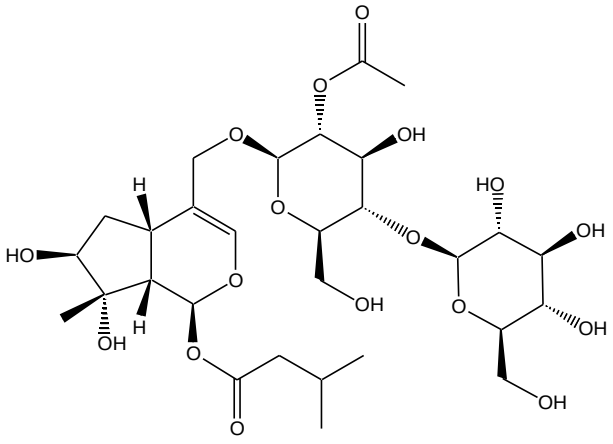
Aim: The genus *Valeriana* consists of more than 200 species distributed worldwide. In previous studies, the extracts or secondary metabolites of several *Valeriana* species were shown to possess anti-inflammatory potential [1,2]. *V. phu* L. has only been investigated in terms of its volatile constituents up to now [3]. The aim of this study is to isolate anti-inflammatory secondary metabolites from the underground parts of *Valeriana phu* L. via activity-guided fractionation.

Methodology: The plant material was extracted with EtOH. The crude extract was dispersed in H_2O and then partitioned with petroleum ether (PE), $CHCl_3$, EtOAc and *n*-BuOH respectively to yield subextracts. The crude EtOH extract and the subextracts were tested for their *in vitro* NO inhibitory activities in LPS-induced RAW 264.7 macrophage cells. Common chromatographic methods such as Diaion HP-20, SiO_2, Sephadex LH-20 CCs and MPLC were used to isolate secondary metabolites. Results: Among the tested extracts, EtOAc and PE subextracts displayed the best inhibitory effect on the production of NO with IC_{50} values of 29.29 and 33.23 μg/mL respectively. Chromatographic separations on the *n*-BuOH subextract yielded a new iridoid glycoside named as phuoside (**1**) along with three known compounds, dioscoridin B, hesperidin and 8-hydroxypinoresinol 4′-*O*-β-D-glucopyranoside. Purification studies on the active subextracts are ongoing in our laboratory.



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Conclusions: This is the first study aiming to isolate anti-inflammatory compounds from *V. phu*. Moreover, all compounds are being reported for the first time from the title species.

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Bioassay guided isolation of anthelmintic compounds

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Aim: Soil transmitted helminthiasis and schistosomiasis are among the most prevalent neglected tropical diseases. There is increased resistance to the few available drugs [1, 2] and therefore the aim was to assess the anthelmintic potential of natural product extracts and isolate the active principles of the most active extracts.

Methodology: Samples were extracted with 80 % methanol and the crude extracts were tested against the free living worm *Caenorhabditis elegans*. Using bioassay guided isolation, anthelmintic compounds were isolated and the structures were elucidated using 1D, 2D NMR and HRMS. Derivatives of some of the compounds were synthesised. Extracts, compounds and synthetic derivatives were then tested against parasitic *Schistosoma mansoni* (adult and newly transformed schistosomula), *Strongyloides ratti*, *Heligosmoides polygyrus*, *Necator americanus* and *Ancylostoma ceylanicum*. In addition, isolated compounds and derivatives thereof were screened for their antiproliferative or cytotoxic activity against two human cancer cell lines.

Results: Among the samples tested, the fruit extract of *Ozoroa insignis,* the root extract of *Echinops kebericho* and the fruiting body extract of the fungus *Albatrellus confluens* showed the best anthelmintic activity against *C. elegans*. The compounds isolated were phenolic lipids (*O. insignis*), thiophenes (*E. kebericho*), and meroterpenoids (*A. confluens*). The stem bark extract of *Commiphora pyracanthoides* displayed the best activity against adult *S. mansoni* (42% activity at 1 µg) while the synthetic meroterpenoid geranylgeraniol-2-orcinol was the most active compound against newly transformed schistosomula (55% activity at 1 µM). Geranylgeraniol-2-orcinol showed also antiproliferative and cytotoxic effects against PC-3 prostate cells (IC_{50} 16.1 µM) and HT-29 colorectal cells (IC_{50} 33.7 µM).

Conclusions: Plants and fungi are still being investigated as sources of anthelmintics and our studies have shown that *O. insignis* and *A. confluens* can be a source of anthelmintic compounds. Further studies need to be done on *C. pyracanthoides* to isolate the active principles.

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Phytochemical profile, antioxidant, antimicrobial, and antiproliferative screening of *Rudbeckia Hirta* flowers

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Aim: *Rudbeckia hirta*, also known as black-eyed Susan, is a popular ornamental plant of the *Asteraceae* family, that is widely cultivated in parks and gardens for its decorative use. Native to North America, the plant can easily grow on most types of soil, given its high adaptability [1]. The species has also been used in traditional medicine for its anthelmintic, anti-inflammatory, and antimicrobial properties[2]. However, few studies have focused on its therapeutic potential. Therefore, we decided to investigate its potential use as a resource of substances with biological actions.

Methodology: The initial chromatographic analysis of phytoconstituents present in a methanolic extract obtained from *Rudbeckia hirta* inflorescences was achieved using high-resolution UHPLC-PDA-CAD-MS/MS. Moreover, the total phenolic and total flavonoid contents have been investigated. The extract was subjected to two different antioxidant assays (metal chelating activity and lipoxygenase inhibition), to *in vitro* antiproliferative assessment on several cancer cell lines, as well as to antimicrobial testing against bacterial and fungal strains.

Results: The obtained results indicate that the methanolic extract contains several polyphenolic compounds such as mono- and diacyl chlorogenic acids, quinic acid and some of its derivatives, and flavonoids such as quercetagitrin, eupatolitin and eupatolin derivatives. Generally, the antioxidant and cytotoxicity assays showed promising results for the investigated extract, which proved to also possess important antifungal properties.

Conclusions: Given the promising results obtained in the *in vitro* biological assays for the methanolic extract obtained from *Rudbeckia hirta* inflorescences, further purification and structural analysis of compounds, as well as further *in vivo* investigations are justified.

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Saponins from *Astragalus Glycyphyllos* and their neuroprotective effects

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Aim: To structurally elucidate triterpenoid saponins, isolated from the overground parts of *Astragalus glycyphyllos* (Fabaceae) and to investigate their *in vitro* pharmacological activity.

Methodology: Repetitive open-column and flash chromatography over various sorbents were used for isolation and purification of the compounds. HRESIMS, 1D and 2D NMR spectroscopy were used for elucidation of the obtained saponins. Their pharmacological effects were investigated *in vitro* on sub-cellular level on rat brain mitochondria, microsomes and synaptosomes.

Results: A new triterpene-type saponin was isolated and structurally elucidated as 3-O-[*α*-rhamnopyranosyl-(1→2)]-*β*-xylopyranosyl]-24-*O*-*α*-arabinosyl 3*β*,6*α*,16*β*,20(*S*),24(*R*), 25-hexahydroxycycloartane. In addition, 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-xylopyranosyl]-cycloastragenol (Astrachrysosid A) was obtained from the plant for the first time. Both saponins displayed statistically significant neuroprotective effects on various models of neurotoxicity at sub-cellular level.

Conclusions: *Astragalus glycyphyllos* contains cycloartane-type triterpenoid saponins with neuroprotective activity and is a valuable and potential species for further phytochemical and pharmacological investigation.

Investigation of the action of Rhoifolin on cognitive processes in zebrafish

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Alzheimer's disease (AD) is a global priority according to the World Health Organization. As the average age of the population increases, the rate of AD also increases [1]. AD affects people from the age of 65, being a neurodegenerative disorder involving language, memory, understanding, judgment, and reasoning [2]. According to reports of AD, the histopathological characteristics associated with Alzheimer's dementia are represented by extracellular accumulations of beta-amyloid leading to the formation of neuritic plaques or senile plaques, but also by internal accumulations of neurofibrillary tangles formed by hyperphosphorylated microtubules associated with tau proteins. Rhoifolin (Rho), a flavone glycoside, exerts many biological activities such as anticancer, antidiabetic, hepatoprotective, antirheumatic, antibacterial, and antiviral properties. The goal of this study was to investigate the improvement impact of Rho (1, 3, and 5 µg/L) on scopolamine (Sco, 100 µM)-induced zebrafish amnesia. Zebrafish have been used as an animal model because it has a high degree of similarity to the human brain model. The behavioral tests used were the Y-maze test that examines the spatial memory of the animal model and their response to novelty, but also the new object recognition test (NOR) - through which we evaluate the memory of zebrafish (*Danio rerio*) recognition of a new object.

Our study data highlighted the beneficial effects of Rho treatment on the spatial memory in the Y maze test and recognition memory in the NOR test, thus suggesting the beneficial effects of Rho on cognitive processes, but also on the amelioration of neuropsychic symptoms of AD in the animal model of dementia represented by zebrafish (*Danio rerio*).

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An acute toxicity study of three synthetic cannabinoids – future directions in chronic neuropathic pain management

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Aim: Neuropathic pain (NP) is a consequence of nerve damage in the somatosensory system, causing symptoms such as mechanical or thermal allodynia, which refers to the perception of normal stimuli, including touch or temperature, as painful ones. These clinical manifestations are impairing life quality, often being poorly managed and unresponsive to traditional analgesics. In this context, the therapeutic potential of cannabinoids in treatment of NP is a growing area of research. Additionally, the successful identification of cannabinoid receptors (CB1 and CB2), as well as endocannabinoids, led to the discovery of a new signalling system, called endocannabinoid system (ES). Therefore, ES could be a potential therapeutic target, knowing that it is involved in regulating pain sensation. On the other hand, both natural and synthetic cannabinoids have shown promising outcomes in pain management research. Interestingly, multiple studies from the last 10 years demonstrated the efficacy of some synthetic cannabinoids in various types of pain, with a special interest in CB2 selective agonists.

Methodology: In this study, three synthetic cannabinoids were selected based on their CB1 and CB2 receptor affinity. The OECD Guidelines for Acute Toxicity Testing were followed, this assessment being the first phase of an *"in vivo"* efficacy study on chronic neuropathic pain in animal models.

Results: The data collected during the monitoring stage showed that only one substance had toxic effects on the central nervous system (such as convulsions, tremor and sedation). Considering the absence of lethality during our study, the median lethal dose (DL50) for the three cannabinoids exceeds 300 mg/kg for oral administration and 50 mg/kg for intraperitoneal administration.

Conclusions: These outcomes reveal a low toxicity for two out of three cannabinoids, allowing us to proceed to the next phase in which we assess their efficacy.

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Metabolomics-guided transcriptomic approach to elucidate the biosynthesis of tanshinones in two species of *Salvia* subg. *Perovskia*

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Aim: Subgenus *Perovskia* of the extended genus of *Salvia* comprises several Central Asian medicinal and aromatic species, of which *S. yangii* and *S. abrotanoides* are the most widespread. Both species are used as a folk medicinal herbs in the areas of natural occurrence [1]. Roots of *S. yangii* and *S. abrotanoides* are abundant in red-colored quinoid norabietanoids called tanshinones and are considered an alternative source of these valuable pharmacologically active compounds. To date, the biosynthetic pathway of tanshinones has been only partially elucidated [2]. Recently, we have performed LC-MS analysis to establish the diterpenoid composition of *S. yangii* and *S. abrotanoides*. We have demonstrated, that their diterpenoid profile differs qualitatively and quantitatively [3]. In this work, we used metabolomics-guided transcriptomic approach, which aimed at discovering candidate genes potentially involved in the biosynthesis of tanshinones.

Methodology: Total RNA was isolated from leaves and roots of *S. yangii* and *S. abrotanoides*. cDNA libraries were synthetized and RNA sequencing was done by Macrogen Inc., (Korea) on the Illumina HiSeq 2500 platform. Transcriptomes were *de novo* assembled and annotated. Differentially expressed genes (DEGs) were selected and functional analysis for DEGs was performed. The expression of tanshinone-related biosynthesis genes was confirmed by quantitative RT-PCR and their transcriptional activity was analyzed during the vegetative season.

Results: NGS has generated over 100 million reads for each cDNA library. *De novo* assembly has generated 372862 potential transcripts for *S. yangii* and 258647 for *S. abrotanoides*. Gene identification revealed a presence of 239327 protein encoding transcripts in *S. yangii* and 158963 in *S. abrotanoides*. Comparative analysis of root and leaf transcriptomes revealed a number of DEGs, out of which several such as: CYP76AH3, CYP76AK1 and CYP71D375, were related to the tanshinone biosynthesis. Novel transcripts of high expression in roots, which might potentially be involved in tanshinone biosynthesis, were reported.

Conclusions: Using the comparative transcriptomic approach, we have generated a dataset of candidate genes as a valuable resource for elucidation of tanshinone biosynthesis. In a long run, our investigation may lead to optimisation of diterpenoid profile in *S. yangii* and *S. abrotanoides*, an alternative source of tanshinones.

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Neglected stereochemistry in amaryllidaceae alkaloids

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Aim: Identification of undescribed structural features isolated from the Amaryllidaceae family with potential for Alzheimer's disease treatment.

Methodology: Basically, structure elucidation employing GS-MS, LC-MS, NMR analysis, and polarimetry. NMR spectroscopy was the key analytical technique (1D and 2D NMR experiments). Two very similar sets of signals in 1H and 13C NMR spectra suggested the presence of diastereomers in the samples. Dynamic NMR analysis was performed with increasing temperature, confirming the restricted bond rotation theory.

Results: Unusual structural motifs were discovered. Both described groups of alkaloids exhibited axial chirality. As it later turned out, stereochemistry was neglected in the literature for galanthindole-type alkaloids.

Conclusions: Although the bulbs of the Amaryllidaceae family may seem to have been largely explored, our work on their alkaloids shows they still have a lot to offer. In Alzheimer's disease research, a new pharmacophore was identified in carltonine-type alkaloids, where galanthindole scaffold is condensed with tyramine. Regarding structural elucidation, the axial chirality was described and compared with the literature. Even though galanthindole alkaloids have long been known and synthesized, atropisomerism was described only once. It shows how important it is to update data in the literature.

Bioassay - guided isolation and evaluation of anticonvulsant and anxiolytic effects of some *Stachys* l. and *Teucrium* l. taxa

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Aim: *Stachys* and *Teucrium* taxa (Lamiaceae) have been traditionally used for the treatment of various disorders, including epilepsy and anxiety [1,2]. The aim of the study was to evaluate the anticonvulsant and anxiolytic properties of extracts and to rationalise its traditional usage on the basis of scientific results by using bio-guided fraction.

Methodology: The dried aerial parts of *S. byzantina, S. officinalis, S. cretica* subsp. *anatolica*, *T. chamaedrys* subsp. *chamaedrys* and *T. montanum* were extracted with the mixture of ethanol:water (70:30). The anticonvulsant and anxiolytic activity of the extracts was investigated in the PTZ model of seizures and elevated plus maze test in BALB-C albino mice, respectively. Liquid liquid extraction was performed using the most effective total extract. The aqueous extract of *S. byzantina*, which was exhibited the highest potential, was fractionated by column chromatography to isolate the effective compounds. One compound (SBMA) was isolated and its structure was elucidated using spectroscopic methods (MS, 1D and 2D NMR).

Results: The most effective total extract in terms of both activities is was found to be *S. byzantina* extract. The results demonstrated a significant increase in latency to PTZ-induced myoclonic seizures provided by *S. byzantina* total extract (60.17±2.66 s), aqueous extract (68.83±4,11 s), active fraction (ACF-3) separated by the column chromatography (68.83±3.52 s), isolated compound SBMA (60.33±2.95) compared to the control (42.17±3.32 s) ($p < 0.05$*). Moreover, *S. byzantina* extracts/fraction/isolated compound significantly decreased the overall seizure score ($p < 0.05$*).

Conclusions: These results showed that the total and aqueous extract of *S. byzantina*, and the compound isolated from aqueous extract exhibited significantly anticonvulsant properties. This is the first study on the anticonvulsant and anxiolytic effects of some *Stachys* and *Teucrium* taxa which are growing in Turkey.

Acknowledgements
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A-Z superfoods: the nature's best

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Aim: To present an overview of various superfoods that we obtain from natural sources, and to appraise their potential health benefits based on published literature.

Methodology: A literature review was performed using the superfood items as keywords and utilising the databases, Web of Science, PubMed and Google Scholar and other relevant published materials.

Results: Foods from natural sources that have high nutritional value because of the presence of various phytonutrients and bioactive phytochemicals [1, 2]. In addition to nutritional importance, these foods also have medicinal values and help with the prevention and cure of certain human ailments. Some of these superfoods increase the metabolic rate and thus boost healthy weight loss. Examples of superfoods may include apples, avocados, beans, beetroot, berries, garlic, honey, oranges and many more. The amounts of published information available on various superfoods are simply huge; some of those have been published in the peer-reviewed literature, while some others are widely available in the popular published and electronic media. It is obvious that not all information that can be found in the media is dependable or can be supported by scientific evidence.

Conclusions: In general, it can be suggested that one should include plenty of superfoods in daily diets for the management of good health and also to prevent various chronic illnesses, like cardiovascular diseases, neurodegenerative diseases and cancers. However, instead of believing in everything that is available in the media, one should rely on the scientific information available in the peer-reviewed literature.

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New sweet-tasting oleanane-type triterpenoid saponins from *Wisteria Sinensis* roots

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The phytochemical study of *Wisteria sinensis* (Sims) Sweet (Fabaceae), commonly known as the Chinese Wisteria, led to the isolation of seven oleanane-type glycosides from an aqueous-ethanolic extract of the roots. After successive purifications by various chromatographic methods, their structures were elucidated by an extensive 600 MHz NMR analysis including 1D and 2D NMR experiments as well as ESI-MS. Among the seven isolated saponins, two have never been reported before: 3-O-α-L-rhamnopyranosyl-(1->2)-β-D-glucopyranosyl-(1->2)-β-D-glucuronopyranosyl-22-O-acetylolean-12-ene-3β,16β,22β,30-tetrol, and 3-O-β-D-xylopyranosyl-(1->2)-β-D-glucuronopyranosyl wistariasapogenol A. Based on the close structures between the saponins from W. sinensis, and the glycyrrhizin from licorice, the stimulation of the sweet testing receptors hTAS1R2/TAS1R3 by these latter was evaluated.

Usage of natural compounds to provide bioactive properties to polymeric materials

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Aim: Natural compounds are largely considered as favourable alternative to synthetic drugs and antibiotics which can have negative side effects on human health or to which microorganisms develop strong resistance. They are also used to provide bioactive properties to materials designed for direct contact with biologic media. One significant advantage of natural compounds, especially those with complex composition (e.g. essential oils), comes from the fact that the biological activity relies not only on the major compounds but mainly on the synergism between all constituents, the function of the main components being regulated by the minor ones. On the other hand, natural compounds have low stability under the action of environmental factors and have to be protected and stabilized in formulations.

Methodology: Clove essential oil and α-tocopherol as model compound for vitamin E were added to chitosan and to poly(ε-caprolactone), respectively, as polymeric matrices. Lyophilisation and electrospinning were applied as different approaches to convert the polymer / natural compound solutions into biomaterials with different architectures.

Results: Super-porous hydrogels with honeycomb pattern and highly hydrophobic meshes with morphologically homogeneous fibers of ~6 μm average diameter were generated – Figure 1. These manifested very good antioxidant activity in DPPH/ABTS tests and microbial inhibition against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, which, in the case of clove essential oil loaded chitosan hydrogel, was preserved after six months of shelf storage.

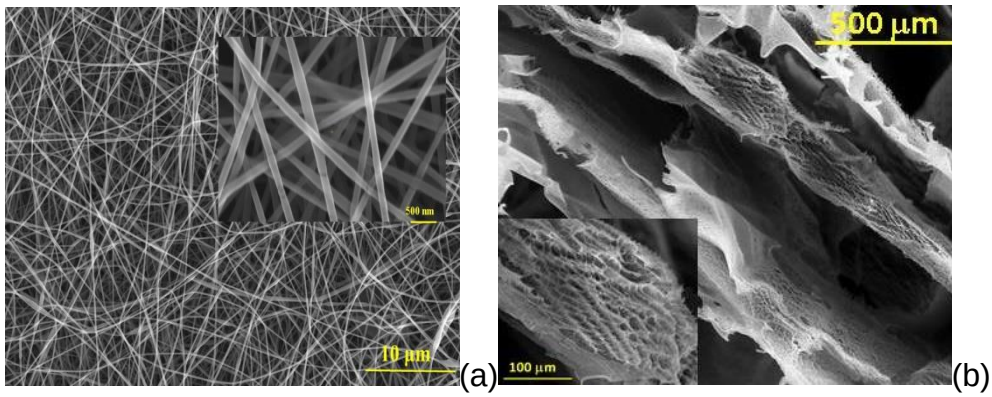

Figure 1. Fibrous and porous aspect of electrospun meshes and hydrogels

Conclusions: Natural compounds can provide favourable bioactivity to polymeric materials while receiving protection and stabilization against environmental factors.

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Anti-inflammatory properties of geranylated flavonoids from *Paulownia Tomentosa* steud. fruit

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Paulownia tomentosa Steud. (Paulowniaceae) is a rich source of secondary metabolites, mainly geranylated flavonoids, which are currently studied for their promising biological activities, such as anti-inflammatory, antioxidant, antimicrobial, or cytotoxic. Aim of our work was to isolate the active constituents from chloroform portion of the ethanolic extract of *P. tomentosa* fruit and to evaluate their anti-inflammatory and antioxidant properties. Compounds were isolated using different chromatographic methods, such as column chromatography, high-performance liquid chromatography, and thin layer chromatography. The structures were elucidated using ultraviolet and infrared spectroscopy, high-resolution mass spectrometry, and one-dimensional and two-dimensional nuclear magnetic resonance spectroscopy. The absolute configurations were determined using circular dichroism spectroscopy. A series of geranylated flavonoids were isolated and several of them were obtained from a natural source for the first time. Selected compounds were evaluated for anti-inflammatory and antioxidant activities on the human monocytic leukemia THP-1-XBlue™-MD2-CD14 cellular model using QUANTI-Blue™ and CAA assays, respectively. The effect of test compounds on the viability of THP-1-XBlue™-MD2-CD14 cells was measured using WST-1 assay. All of the test compounds reduced the activation of NF-κB/AP-1 24 h after the addition of lipopolysaccharide. From the set of 25 geranylated flavonoids, thirteen compounds attenuated the activities of NF-κB/AP-1 in a statistically significant manner and eight compounds were more active than anti-inflammatory drug prednisone. Five the most active compounds were further studied for their effects on the LPS-stimulated secretion of pro-inflammatory cytokines TNF-α and IL-1β using ELISA. In the antioxidant activity assay, only one compound showed statistically significant activity, however, approximately two times lower than quercetin used as a positive control. Some of the tested compounds exhibited more prooxidative activity. Natural compounds, such as geranylated flavonoids from *P. tomentosa*, can still influence the modern healthcare and can be interesting source of inspiration for finding new drugs. The isolated active compounds have to be tested on the animal models to determine their effects on living organisms for their potential usage in the future.

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Biocompatible hydrogels films with the inclusion complex of β-cyclodextrin/curcumin immobilized for biomedical applications

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Aim: This paper aims to obtain hydrogel films based on bovine serum albumin (BSA) and oxidized gellan (OG) with the β-cyclodextrin/curcumin inclusion complex (β-CD/Curc) immobilized used for biomedical applications.

Methodology: The gellan was oxidized in the presence of $NaIO_4$, and the dialdehyde groups formed by cleaving the C2-C3 bond in the glycosidic structure were highlighted by FT-IR and NMR spectroscopy. Hydrogel films based on BSA cross-linked with OG were obtained using different molar ratios. Despite its therapeutic benefits [1], dosing curcumin (Curc) in clinical applications is impossible due to its low bioavailability and poor solubility [2]. A β-CD/Curc, which improves the bioavailability of Curc, was prepared at a molar ratio of 2:1 to increase its solubility, and it was immobilized into the hydrogel films. The conversion index (CI%) of NH_2 groups into Schiff bases was determined using the ninhydrin test. The film's structure was evaluated by FT-IR spectroscopy, and SEM assessed the morphology of the films. The swelling degree (Q%), cytotoxicity, antioxidant activity, and the release kinetics of curcumin from the biopolymers films were evaluated.

Results: The maximum oxidation degree was obtained after 117 hours, being of 57.16%, and the OG molecular weight decreased when the oxidation time increased. The results showed that the CI% increases when the OG amount in the hydrogel film increases. The Q% depended mainly on the samples' hydrophilicity and pH values and were higher at pH=7.4 than at pH=5.5. The antioxidant activity of curcumin-loaded biopolymers films was improved compared to free curcumin. The polymeric matrix has a protective role for curcumin and is not cytotoxic. The release efficiency and skin permeability were higher at pH=7.4, results consistent with the Q%.

Conclusions: Biocompatible biopolymers films based on BSA and OG with the inclusion complex of β-CD/Curc immobilized were obtained with biomedical applications.

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Is it possible to obtain cohesive results of antimicrobial activity of essential oils?

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Aim: The provision of the reliable toolbox for in vitro testing of the liquid and volatile forms of Essential Oils (EOs) against microorganisms, including the infective biofilm. Methodology: The spectrum of conventional methods of EOs testing (including aromatograms, inverted Petri dishes) and recently developed ones (including AntiBioVol) was scrutinized against infective microorganisms in the standard in vitro conditions and these imitating specific niches within human body (including environment of the chronic wound).

Results: The application of conventional methods for testing of EOs antimicrobial activity provides discrepancy in results mostly due to lack of consideration of their mechanism of action, intra-species variability and factual milieu of the infection site. The application of the more recent testing methods allows to overcome these disadvantages and to provide the repeatable, cohesive results.

Conclusions: The increasing resistance of fungi and bacteria against conventional antimicrobials, including antibiotics, requires the introduction of other antibacterial agents. The EOs are considered promising compounds with this regard. Nevertheless, the results on the EOs' antimicrobial activity were for the long time non-cohesive and even opposing. The application of new tests allows to provide the data on factual activity of EOs against microorganisms and to pave the way for their clinical introduction.

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Chemical composition and *in vitro* bioactivities of *Amesiodendron Chinense* (merr.) hu seed oil

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Aim: Seed oil of *Amesiodendron chinense* (Merr.) Hu, (Sapindaceae) has been used by folk healers in southern Thailand for the treatment of wounds and skin disorders. This study aimed to determine the chemical composition and the biological activities of *A. chinense* seed oil.

Methodology: Seed oil was extracted using traditional methods as used locally in Southern Thailand and the chemical constituents analysed by gas chromatography-mass spectrometry (GC-MS). Vacuum liquid chromatography (VLC) was then used to fractionate the oil (10 fractions). To investigate the composition of the seed oil and corresponding fractions, thin layer chromatography (TLC) was carried out. The antioxidant and chemopreventive potential of seed oil and fractions were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and luciferase assays, respectively.

Results: The major component of seed oil was 9-octadecenoic acid (84.82%). DPPH assay analysis revealed that all samples exhibited antioxidant activity as indicated by the presence of white spots on the developed TLC plate. However, quantitative DPPH analysis failed to show antioxidant activity at the highest concentration tested (10 mg/mL). Furthermore, all samples failed to show significant Nrf2/ARE induction at non-cytotoxic concentrations using AREc32 cells.

Conclusions: Seed oil and associated fractions thereof of *A. chinense* were not found to possess chemopreventive potential. Research continues to explore the main active compounds and potential bioactivities of *A. chinense*.

Acknowledgements

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Natural compounds for Sars Cov-2 – what do we know so far?

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Aim: Since the beginning of the pandemic, various natural products or natural compounds have been tested as complementary treatment in SARS-CoV-2 infection. The advantage of natural products is that they are widely available, side effects are less frequent and their severity is minor and are also affordable to a larger group population. The aim of this paper is to highlight the natural compounds that have been tested or used in SARS-CoV-2 infection since the beginning of the pandemic.

Methodology: Studies were identified by searching scientific databases: PubMed, Scopus, Google Scholar and Web of Science. 'Covid', 'SARS-CoV-2' and 'natural compounds' were used as keywords for the primary searches.

Results: Several mechanisms of actions have been proposed, like the disruption of the spike protein, inhibition of Mpro (main protease), ACE2 (Angiotensin-converting enzyme 2) inhibition, and other coronavirus- host protein pathways. It is not surprising that the majority of the compounds that can modulate these targets, are polyphenolic compounds like flavonoids, polyphenol carboxylic acids and tannins. However, other compounds have proved to have beneficial effects: saponins, terpenoids, sterols and coumarins, most of them being well known highly potent compounds with therapeutic applicability in various pathologies (e.g., glycyrrhyzin, curcumin and several essential oils). Still, the low bioavailability of several natural compounds is a major drawback, preventing the therapeutic agent to reach its target.

Conclusions: The current scientific literature clearly supports the therapy with natural compounds as potential effective antivirals against SARS-CoV-2 but these hypotheses need validation in SARS-Cov-2 infection models and COVID-19 patients.

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Fruits of *Hippophaë Rhamnoides* – preventive role in lipopolysaccharide leakage, epithelial inflammation, and glucose transport in CACO-2 and human neutrophils models

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Aim: The aim of the study was an assessment of anti-inflammatory activity of an aqueous extract of *Hippophaë rhamnoides* L. (sea buckthorn) fruit (HRE) expressed as inhibition of cytokine (IL-8, IL-1*β*, IL-10, IL-6, TNF-*α*) secretion by human neutrophils (PMN), peripheral blood mononuclear cells (PBMC), and human colorectal adenocarcinoma cell line (Caco-2) stimulated with LPS (lipopolysaccharide from *Escherichia coli*)/IL-1*β*. HRE influence on LPS-leakage through the Caco-2 monolayer and glucose transporter (GLUT)2 translocation was also evaluated.

Methodology: HRE composition was determined with HPLC-DAD-MS^n. Secretion of cytokines by cell cultures was established with enzyme-linked immunosorbent assay. LPS concentration in the apical and basolateral compartments of the Caco-2 monolayer was evaluated with *Limulus amebocyte lysate* assay. The transepithelial electrical resistance of the Caco-2 monolayer was monitored with a voltmeter. GLUT2 translocation was evaluated using an immunostaining assay.

Results: HRE (100 µg/mL) inhibited the secretion of TNF-*α* and IL-8 in PMN and PBMC. It increased the release of anti-inflammatory cytokine IL-10 in PBMC. The concentration of IL-8 was significantly decreased in the Caco-2 model after the treatment with HRE (50-500 µg/mL). The extract in the concentration of 500 µg/mL significantly inhibited LPS leakage through epithelial monolayer *in vitro* in comparison with non-treated control. GLUT2 expression after the treatment of Caco-2 with HRE in the concentrations of 50 and 100 µg/mL was similar to non-treated control, whereas in the higher concentrations of HRE GLUT2 expression in Caco-2 cells was increased. Conclusions: HRE might prevent low-grade chronic inflammation through the decrease of chemotactic factors released by immune and epithelial cells, which support its use in diabetes-linked complications.

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Fruit waste as a source of active compounds with cosmetic significance

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Aim: Plant by-products derived from the food industry are one of the major environmental concerns worldwide. Due to the high content of bioactive compounds such waste may be considered hazardous due to the interference with the plant growth, deterioration of the drinking water quality or toxic effects on sensitive marine organisms. At the same time plant-derived waste and by-products, with proper handling, may represent a low-cost source of several bioactive compounds potentially important for pharmaceutical and cosmetic industries. Phytochemical analysis of plant by-products and development of sustainable biowaste processing methods might help to meet the growing demand for natural products without increasing the exploitation of plant resources [1,2]. The aim of presented studies was to investigate possible usage of fruit-derived by-products as a source of active compounds with cosmetic significance. The first part of the study presents comparison between phytochemical content and chosen cosmetic properties of flesh and peel extracts obtained from common fruits: *Cydonia oblonga*, *Diospyros kaki*, *Annona cherimola* and *Fortunella margarita*. The second part presents the influence of the solvent on the extraction of phytochemicals and selected cosmetic properties of extracts obtained from peels of five pumpkin (*Cucurbita* spp.) varieties.

Methodology: The extracts were prepared by ultrasound-assisted extraction using "green" solvents: water, water:polyethylene glycol (4:1, *v:v*) and 20% (*v/v*) ethanol. The extracts were compared for the content of total phenolics, flavonoids, antioxidant activity (DPPH and ABTS scavenging), tyrosinase inhibition, *in vitro* sun protection factor and cytotoxicity against human skin cells. The differences between compared extracts were calculated by applying statistical methods.

Results: All analyzed fruit peel extracts contained higher amounts of bioactive compounds and were more active in respect of the antioxidant properties and *in vitro* SPF than flesh extracts. The tyrosinase inhibitory activity was more significant for the flesh extracts. In respect of the *Cucurbita* spp. extracts water was selected as the most effective solvent to obtain extracts with high content of active compound, significant cosmetic activities and low cytotoxicity against human keratinocytes.

Conclusions: The study shows that fruit peels, considered as by-products of the food industry, are a rich source of active compounds with potential application in cosmetic formulations protecting the skin from the negative impact of environmental factors. The extracts might be obtained using simple and eco-friendly extraction methods and "green" solvents.

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Determination of molecular targets of natural compounds: *in silico* and *in vitro* approaches

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Aim: Nature provides a unique resource for the discovery of potential drug candidate molecules. To exemplify, only 13% of the anticancer drugs approved by the United States Food and Drug Administration (FDA) between 1981 and 2014 were reported to be fully synthetic drugs, and the rest were originated from natural sources [1]. The use of natural products in traditional medicine provides a basis for the fact that they are relatively safe [2]. Within the context of our presentation, we plan to mention our two research projects in which *in silico* and *in vitro* methods are jointly applied for the discovery of natural compounds' targets.

Methodology: We used virtual screening and molecular docking methods *in silico* and a variety of *in vitro* methods including resazurin assay, assorted enzymatic assays, western blotting, flow cytometry and fluorescence microscopy techniques for the estimation of the probable activity and relevant modes of action. The bioinformatic approach was further performed in order to prove the potential activity and mechanism of natural compounds of special interest.

Results: Natural compounds/products, offering a wide variety of chemical scaffolds, are of significance in the discovery of new drug candidate molecules and/or in the design of (semi)synthetic derivatives of currently used drugs with high efficacy and low toxicity. In recent years, *in silico* methods have been widely used in pharmaceutical research, reducing cost and saving time. At this point, the joint use of *in silico* and *in vitro* methods makes sense enabling efficient determination of target molecules of natural compounds, and thus the introduction of potential drug leads to the scientific world in shorter time periods.

Conclusions: The importance of integrating *in silico* and *in vitro* approaches in the field of pharmaceutical fields was emphasized, which will pave the way for the discovery of potential therapeutics with natural origin.

Acknowledgements

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Valorisation of licorice (*glycyrrhizae*) roots: antimicrobial activity and cytotoxicity of prenylated (iso) flavonoids and chalcones from licorice spent (*G. Glabra, G. Inflata* and *G. Uralensis*)

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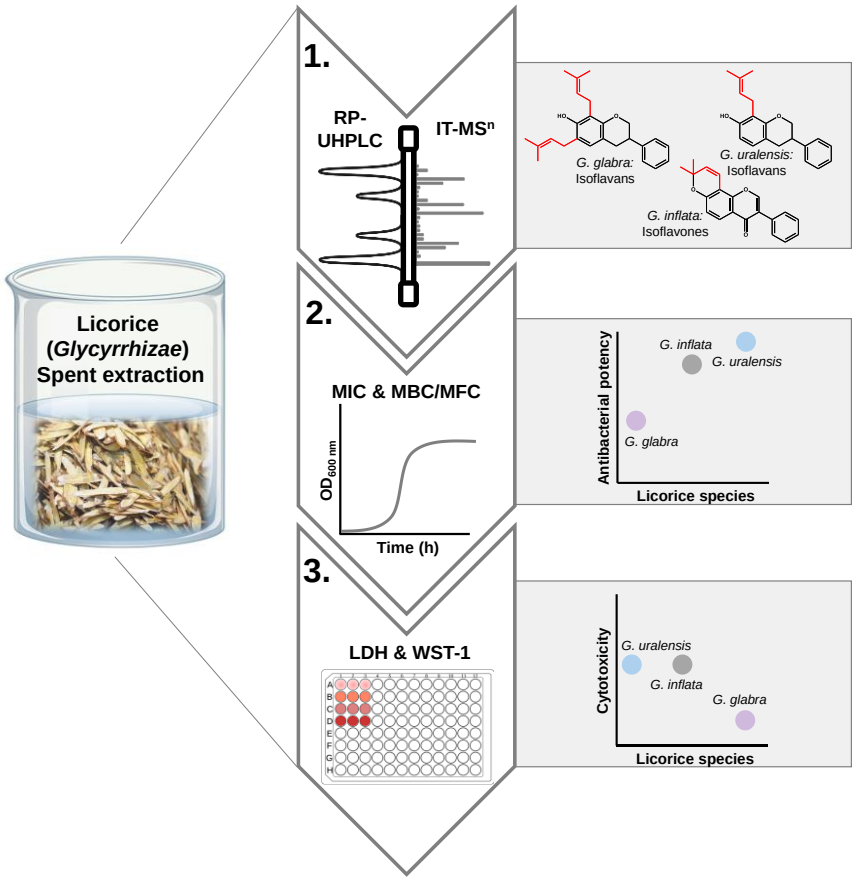
Prenylated (iso)flavonoids and chalcones (phenolics) are phytochemicals with antimicrobial properties found in the Fabaceae family, *i.a.* in licorice roots (*Glycyrrhizae* spp.). Licorice spent is a by-product obtained after commercial aqueous extraction of the roots and is rich in antimicrobial prenylated phenolics. These compounds are currently not valorised. Therefore, in this study we analysed the prenylated phenolics composition of spent from three main licorice species: *G. glabra*, *G. inflata*, and *G. uralensis*, as well as their antimicrobial activity against diverse microorganisms and cytotoxicity towards Caco-2 cells. Phytochemical composition was studied using reversed phase ultra-high pressure liquid chromatography (RP-UHPLC), coupled to photodiode array (PDA), and ion trap mass spectrometry (IT-MS^n) and Orbitrap-MS (FT-MS^n). Ethyl acetate extracts of *G. glabra*, *G. inflata*, and *G. uralensis* spent showed a distinct phytochemical profile with content of prenylated (iso)flavonoids of 96, 171, and 155 mg g^{-1} DW extract, respectively. Licorice species differed in subclass and prenyl configuration: *G. glabra* and *G. uralensis* were rich in isoflavans and *G. inflata* was rich in isoflavones. (Iso)flavonoids in *G. glabra* and *G. inflata* were mainly double chain prenylated, whereas *G. uralensis* was rich in single chain prenylated compounds. Licorice spent extracts showed antibacterial activity against the food spoiler *Lactobacillus buchneri* and against the oral pathogens *Streptococcus mutans* and *Staphylococcus aureus*. The order of antibacterial potency was *G. uralensis* ≈ *G. inflata* > *G. glabra*, with minimum inhibitory concentrations (MICs) of 25-250, 25-250, and 75-1,000 µg mL^{-1}, respectively. The gastro-intestinal pathogen *Escherichia coli* and food spoilers *Zygosaccharomyces parabailli* and *Yarrowia lipolytica* were not susceptible towards the licorice spent extracts up to 2,000 µg mL^{-1}. Licorice spent extracts displayed cytotoxicity in Caco-2 cells at 500 µg mL^{-1} for *G. inflata* and *G. uralensis*, whereas *G. glabra* was not toxic up to 1,000 µg mL^{-1}. Cell viability was reduced between 20-50% at 100-500 µg mL^{-1} for *G. glabra* and *G. uralensis*, and at 50-100 µg mL^{-1} for *G. inflata.* In conclusion, licorice spent is rich in potent antibacterial prenylated phenolics that can be valorised as novel natural antimicrobials.


Licorice
(Glycyrrhizae)
Spent extraction
1.
RP-
UHPLC
IT-MSn
G. glabra:
Isoflavans
G. uralensis:
Isoflavans
G. inflata:
Isoflavones
2.
MIC & MBC/MFC
OD600 nm
Time (h)
Antibacterial potency
G. inflata
G. uralensis
G. glabra
Licorice species
3.
LDH & WST-1
Cytotoxicity
G. uralensis
G. inflata
G. glabra
Licorice species

Anxiolytic, antidepressant and antioxidant profile of cotinine and 6-hydroxy-l-nicotine in a rat model of Alzheimer disease

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Alzheimer's disease (AD) is the most typical form of dementia characterized by memory and cognitive loss and followed by mood changes. The key hallmarks of AD consists of cerebral beta-amyloid (AB) deposition, accumulation of neurofibrillary tangles of protein tau and neuronal loss. Besides progressive cognitive decline, neuropsychiatric symptoms, such as anxiety, depression, and apathy, were also linked to these pathological changes. It was found that nicotine reduce anxiety and improve memory, learning and attention, but the therapeutic use in AD was limited by its cardiovascular and addictive side-effects. Here, we aim to evaluate the anxiolytic, antidepressant, and antioxidant potential of two structural related nicotine derivatives, namely cotinine (COT) and 6-hydroxy-L-nicotine (6HLN) in a rat model of AD induced by intracerebroventricular (i.c.v.) infusion of AB25-35 peptide. For this, COT and 6HLN were chronically and intraperitoneally administered to rats that were previously infused i.c.v. with AB25-35 peptide and their behavior was monitored in specific tasks. Elevated plus maze test (EPM) and open field test (OFT) were used to assess anxiety-like behavior while forced swimming test (FST) was used to evaluate the depressive behavior. The oxidative stress biomarkers were monitored from homogenized amygdala samples. The results revealed positive effects of COT and 6HLN on behavioral changes induced by AB25-35 peptide in rats. Thus, both compounds intensified the locomotor activity and significantly reduced the anxiety-like behavior in EPM and OFT tasks and abolished the depressive behavior in FST task. Also, the treatment reduced the oxidative stress in the amygdala of AB25-35-treated rats by increasing the specific activities of antioxidant enzymes and lowering the level of protein and lipid oxidation products. Taken together, our data suggest that COT and 6HLN could represent viable therapeutic agents for ameliorating AD condition.

Analysis of metformin and five gliptins in counterfeit herbal products: designs of experiment screening and optimization

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Background: Drug counterfeiting is a rising problem due to difficulties with identifying counterfeit drugs and the lack of regulations and legislation in developing countries.

Objective: This study aims to develop a robust and economic reversed phase high performance liquid chromatography (LC) method for simultaneously determining metformin HCl, vildagliptin, saxagliptin, alogliptin benzoate, sitagliptin phosphate monohydrate, and linagliptin to target counterfeiting.

Methods: Plackett-Burman (PB) and Box-Behnken (BB) designs were used to screen and optimize the mobile phase composition. Chromatographic separation was carried out on an Inertsil® ODS-3 C18 column with isocratic elution mode and the mobile phase was a mixture of acetonitrile–methanol–ammonium formate buffer, pH 3.5 (25:10:65, v/v/v). This method was applied to analyze synthetic drugs in three traditional Chinese and Indian herbal medicines. To identify the adulterants, thin-layer chromatography (TLC), nuclear magnetic resonance (NMR), and mass spectrometry (MS) were used on counterfeit herbal medicines.

Results: The developed method is sensitive, simple, rapid, economical, accurate, and highly robust. Student's *t*-test and variance ratio (*F*-test at $P < 0.05$) were used to compare the results statistically with the reference methods.

Conclusion: The study found that the analyzed herbal medicines were adulterated with metformin and the quantification of anti-diabetic counterfeits was therefore applied.

Highlights: This study determined counterfeited anti-diabetic drugs in Indian and Chinese traditional herbal medicines (THMs). Design-of-experiment, PB, and BB designs were used. Method validation was also performed in accordance with the International Conference on Harmonization guidelines.

NMR profiling-guided isolation of potentially bioactive oleanane saponins from Bellis Sylvestris Cyr.

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Aim: Natural products from plants have a recognized potential as drugs or lead compounds [1]. The aim of this work was to identify potential anticancer compounds from *Bellis sylvestris* Cyr., a plant belonging to the Asteraceae family.

Methodology: The leaf extract of *B. sylvestris* was analyzed by NMR-based profiling. A saponin-enriched fraction was obtained and tested on a panel of cancer cell models including several colorectal carcinoma, pancreatic neuroendocrine, and metastatic melanoma cell lines. NMR-based profiling showed the presence of saponins also in flower heads and roots. Therefore, saponins were isolated from leaf, root, and flower head extracts through a series of chromatographic steps. Structural elucidation was carried out by extensive 2D-NMR (COSY, TOCSY, NOESY, HSQC, HMBC, CIGAR-HMBC, H2BC, HSQC-TOCSY) and Q-TOF HRMS² analyses [2].

Results: The saponin-enriched leaf extract *of B. sylvestris* exerted a significant cell growth inhibitory activity. The NMR profiling of the plant extracts showed the distribution of saponins in different plant organs. The phytochemical study of the extracts obtained from the leaf, root, and flower head led to the isolation of several saponins with oleanane skeleton. These included the already known besylvosides [3] and newly isolated saponins which were characterized by a different aglycone, different oligosaccharide chains and acylation at various positions.

Conclusions: Combining a powerful NMR-based profiling approach with classical phytochemical studies, it was possible to identify compounds potentially responsible for the anticancer activity shown by the extract obtained from *B. sylvestris*. Further studies will be aimed at determining the anticancer potential of the pure compounds.

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There is no burdock like a burdock

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Plants of the genus *Arctium* are spread all over the world and their various parts are used frequently in traditional medicine, especially for diuretic and antidiabetic purposes. Although the phytochemical profile of *A. lappa* is well-investigated, further main burdocks widely distributed throughout Europe, namely *A. minus* and *A. tomentosum*, are for some reason overlooked and we don't know much about their content compounds. Therefore, this study is focused on the comparison of the phytochemical profile of different parts of three main burdocks. HPLC-DAD, HPLC-ELSD, and LC-MS have been used for targeted metabolomic analysis. As expected, the dominant compounds in the leaves and roots are caffeoylquinic acid derivatives, while the fruits contain especially lignans. However, the abundance of secondary metabolites varies between species dramatically as arctiiphenolglycoside, arctiiapolignan A, and lappaol D are more abundant in *A. lappa*, whereas matairesinoside is more abundant in *A. tomentosum*. Moreover, *A. tomentosum* seems to be the only burdock containing hyperoside.

Acknowledgements
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Main secondary metabolites of the anise hyssop herb evaluated by different chromatographic techniques

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The genus *Agastache* (*Lophanthus*) includes 22 species of aromatic herbaceous plants common in North America and Southeast Asia [1]. The representatives of this genus are gradually spreading in Ukraine [2]. Species are characterized by the presence of different varieties and chemotypes.

The aim of the study was the analyses of main secondary metabolites in the violet-floral variety of anise hyssop (*Agastache foeniculum* (Pursh) Kuntze) using different chromatographic techniques.

The herb of *A. foeniculum* was harvested during flowering from the experimental plots in Ternopil region (Ukraine) and shade dried.

The hydrodistilled essential oil of *A. foeniculum* was analyzed by the gas chromatography-mass spectrometry method. It contained the abundance of aromatic compound estragole 32.16% as well as monoterpenoids pulegone (20.43%) and isomenthone (17.65%). The contents of six triterpenoids were revealed in the 96% ethanolic extract of raw material by the high performance liquid chromatography (HPLC) analysis; ursolic (0.32%) and euscaphic (0.44%) acids dominated among them. The specific sequences of a number of spots of phenolic compounds in the methanol extract of herb was detected by the high-performance thin layer chromatography method (HPTLC). The HPLC-analysis revealed that rosmarinic acid (1.79%) and apigenin-7-*O*-glucoside (0.71%) dominated among found polyphenols.

The used chromatographic techniques could be regarded as suitable and reliable methods for the identification of main secondary metabolites in the *A. foeniculum* herb and essential oil in the frame of their standardization procedure.

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Comparison of volatile and semi-volatile components in valerian hydroalcoholic extract and essential oil

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Valeriana officinalis L. (Valerianaceae) is a plant native to different temperate regions of America, Europe and Asia. Extracts from Valerianae radix are frequently used today for sleep disorders. Various active substances are thought to be responsible for the calming effect: valepotriates, lignans, valerenic acid, acetoxyvalerenic acid, baldrinals and numerous components of the essential oil.

The aim of the research work was to produce an extract with a broad spectrum of natural substances, using a lipophilic excipient to reduce the loss of volatile substances when the extraction solvent is evaporated. A suggestion about the composition of the lipophilic extract obtained in this way was received by GC-MS in combination with NIST database. The proposed main components were purchased in form of reference standards to prove their presence in the extract. The main proven components served also for the creation of an identity solution for its qualitative control by GC-FID. Finally, the components of the lipophilic extract were compared with those from the essential oil obtained from the same batch of valerian root.

Methodology. The extraction was carried out with ethanol (85% m/m) using a mechanical stirrer. In order to produce the lipophilic extract, the resulting raw extract and 120% lipophilic excipient calculated from the dry residue content were used. Valerian essential oil was prepared according to the European Pharmacopoeia monograph. Volatile components were determined using a gas chromatograph with FID detection.

Results. Comparison of the extract with the essential oil by GC-FID, showed no significant differences in terms of reference substances tested. As a result, the following substances were found in both samples: acetoxyvalerenic acid, alloaromadendren, borneol, bornyl acetate, camphene, isovaleric acid, myrtenyl acetate, spathulenol, valerenic acid. In the same way, carvyl-acetate, 1-α-pinen, palmitic acid, germacrene D, valeric acid and 3-methyl-valeric acid were tested but could not be identified in any of the samples. However, other peaks were observed in the essential oil that cannot be found in the lipophilic extract.

Metabolic control of alkaloids in *Catharanthus Roseus* in response to different root zone temperatures

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Temperature is one of the important factors affecting various physiological processes of plant and specialized metabolites production. We studied the effect of different root-zone temperatures on alkaloid synthesis and production in *Catharanthus roseus*. Plants were exposed to different root-zone temperatures (low, medium and high) for two days. The alkaloid content of the roots and shoots of the plants in response to different root-zone temperatures was then analyzed using LC-MS. The expression level of genes involved in alkaloid biosynthesis pathways was also determined. A high root-zone temperature resulted in lowest vinblastine content in the leaves of plants. The highest expression of genes involved in alkaloid biosynthesis was with a root-zone temperature of 12 °C. The results suggest that root-zone temperature has a direct influence on biosynthesis and accumulation of alkaloids in plant tissues. In addition, a cross talk between the roots and shoots facilitates the transcription of genes involved in alkaloid biosynthesis in different parts of the plant.

Scutellaria Baicalensis (Baikal Skullcap) plant growth and synthesis of secondary metabolites increased by electroporation

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Aim: Achieving plant growth and higher concentration of secondary metabolites (phenolic compounds) in roots of *Scutellaria baicalensis* (plant species recently added to European Pharmacopeia) by delivering microsecond-long electric pulses and permeabilizing cellular membranes.

Methodology: 6 weeks old plantlets of Baikal skullcap were put in cuvettes (4 mm gap between electrodes) and electroporated under applied electric field strength ranging from 12.5 to 50 kV/cm and different duration and quantity of pulses using BTX ECM 830 Square Wave Electroporation System pulses generator, than plants were cultivated in aeroponic systems and observed throughout the next 4 months. Then they were taken out of the systems, the roots were severed and dried in room temperature. Root extracts were prepared using 80% methanol with 0,1% formic acid. HPLC-MS analysis was conducted using Rp18 column and LCMS grade water and acetonitrile. Generated spectra were analysed, peak surface was measured and concentration of particular compounds was calculated.

Results: Plants from all tests survived the electroporation process and exhibited increased growth in comparison to control. First blossoming of electroporated plants was observed 8 days earlier than in control group. Concentration of baicalin, baicalein, wogonin and wogonoside was either higher or lower than in control group, depending on used parameters of the process. Delivering ten, 100 µs long pulses under electric field strength of 17,5 kV/cm resulted in highest baicalin (4,01 mg/mL) and wogonoside (1,04 mg/mL) concentrations, however increasing the field strength to 30 kV/cm while maintaining other parameters resulted in achieving highest baicalein (0,16 mg/mL) and wogonin (0,06 mg/mL) concentrations.

Conclusions: Electroporation is a successful method of shortening time of plant cultivation and increasing yield of phytochemicals.

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Hydroacetonic fruit extracts with potential cardiovascular benefits

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Aim: The prevalence of cardiovascular diseases has almost doubled in the last 30 years. Despite the advances made in the medical field, only 34% of the hypertensive patients have a well-controlled blood pressure (1,2). Thus, there is a growing interest in panning out the cardioprotective effects of plant extracts. The aim of study was to investigate the activity of three hydroacetonic dry extracts obtained from fruits of *Viburnum opulus* L. (VO), *Sorbus aucuparia* L. (SA) and *Cornus mas* L. (CM) on different cardiovascular targets.

Methodology: We performed vascular reactivity studies on rat thoracic aortic rings, *in vitro* arginase inhibition assay and anti-platelet activity in human platelet-rich plasma. Phytochemical profile of extracts was analyzed using HPLC-DAD-ESI-Q-TOF-MS/MS.

Results: VO extract was the most potent inhibitor of arginase activity (67,95% inhibition at 100 µg/mL), while SA and CM extracts had a weak inhibitory activity. All three extracts (VO, SA, CM) produced the relaxation of aortic rings (EC_{50} of 6.31, 78.52 and 100.9 µg/mL respectively). Their effect was blunted by L-NAME, an inhibitor of endothelial nitric oxide synthase. Platelets aggregation induced by convulxin (7 ng/mL), ADP (3 µM) and ristocetin (1 mg/mL) was significantly inhibited by all extracts, VO extract being the most potent (between 55-81% inhibition of aggregation at 0.5 mg/mL). The anti-platelet activity was confirmed by the inhibition of integrin αIIbβ3 (known as a fibrinogen binding receptor) activation and P-selectin (marker of α-granule release) expression. The cardiovascular effects of VO, SA, CM extracts were ascribed to their rich secondary metabolite profiles that consist of phenolic acids, flavonoids, iridoids and proanthocyanidins.

Conclusion: These findings suggest that *V. opulus* fruit extract has a promising cardiovascular potential in diseases associated with vasoconstriction and hyperaggregability.

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Biotechnologically produced *Harpagophytum Procumbens* dc. ex meisn extract: metabolite profiling and cytotoxicity *in vitro*

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Aim: The aim of the study was to perform a metabolite profiling of a *Harpagophytum procumbens* DC. ex Meisn. hairy roots extract (HPE) and to evaluate cytotoxicity of the extract and identified major compounds on *in vitro* psoriatic and non-psoriatic human keratinocytes (HaCaT).

Methodology: The phytochemical analysis was performed through NMR-based profiling [1]. The psoriasis-like model was conducted on HaCaT cells by stimulation with combination of IFN-γ/IL-17A/IL-22 (10 ng/ml each) 1 h prior treatment with the extract or pure compounds. A MTT assay was used to determine the cytotoxicity effect [2].

Results: The HPE accumulate several phenylethanoid glycosides, such as β-OH-verbascoside, verbascoside (VER), leucosceptoside A (LEU), and martynoside, among which VER and LEU were the major ones. The MTT assay revealed that the HPE, and both VER and LEU, were nontoxic for keratinocytes up to 1000 μg/mL and 100 μM, respectively.

Conclusions: The HPE, VER and LEU are nontoxic for HaCaT cells in the investigated concentrations. Further, their anti-inflammatory and anti-psoriatic potential and underlying signalling pathways involved *in vitro* need to be investigated.

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Collagenase and elastase inhibitions by *Rosa Damascena* and *Pelargonium Graveolens* essential oils

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Aim: To evaluate the anti-collagenase and anti-elastase enzyme inhibitory potential of *Rosa damascena* Mill. as well as the *Pelargonium graveolens* L'Hér. essential oils, comparatively.

Methodology: The *in vitro* enzyme inhibition of *R. damascena* and *P. graveolens* essential oils were screened using commercial microplate enzyme kits. Phytochemical analyses of essential oils were evaluated and confirmed both by GC-FID and GC/MS systems.

Results: 27.3% citronellal, and 23.5% geraniol were determined as major components in the *R. damascena* essential oil, whereas the major components of *P. graveolens* essential oil were 38.8% citronellal, and 19.3% geraniol, respectively. While the collagenase enzyme inhibition of *R. damascena* essential oil was calculated as 93.1%, and *P. graveolens* essential oil was 74.2%, respectively, compared to the positive control quercetin - 72% inhibition. The elastase enzyme inhibition results for *R. damascena* and *P. graveolens* essential oils were recorded as 91.1% and 86.3%, respectively.

Conclusions: The tested citronellal and geraniol-rich essential oils showed dermo-cosmetic application potential, where further *in vivo* studies especially against the aging process is needed. However, composition analyses of essential oils are important as especially expensive oils may be adulterated.

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Untargeted screening of plant metabolites based on data-independent and data-dependent acquisition modes using LC-ESI-QTOF-MS: *Tribulus Terrestris* l. as a case study

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Aim: The aim of this study is to utilize a combination of mass spectrometry-based acquisition modes for the putative identification of wide range of phytochemicals for quality control in industrial valued plants and herbal formulations.

Methodology: All acquisitions were performed using LC-ESI-QTOF mass spectrometry. This study describes the utility of two distinctive acquisition modes in combination, data-dependent acquisition (DDA) mode and a data-independent acquisition (DIA) mode for obtaining the mass spectrometry data. *Tribulus terrestris* L. plants from two different regions were used as a case study. Open-access data processing tools were applied to identify wide-range metabolites rapidly.

Results: After combining mass spectrometry results of plant samples from two different regions, both modes are highly complementary and each of them has been utilized to bring qualitative and quantitative information in this study. However, the difference between sensitivity and distribution of metabolites in both modes was very clear. DIA mode achieved more sensitivity and highly qualitative profile of phytochemicals in each plant sample from both regions.

Conclusions: This is a comprehensive untargeted metabolomics study focusing on the generation of wide range phytochemical profile of *Tribulus terrestris* plant based on LC-ESI-QTOF/MS. The outcomes of this study for untargeted plant metabolites identification, validated the remarkable analytical performance of the constructed workflow, proving it to be a sensitive and reproducible pipeline for monitoring quality control of herbal medicines.

Acknowledgements
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illumination spectra effect on metabolic profile of *Salvia Yangii* b.t. drew. *in vitro*

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Salvia yangii B.T. Drew, a medicinal and aromatic plant of Central-West Asia, is a rich source of volatile terpenoids that are produced in different proportions. To reveal the relationship between the relative gene expression level and the metabolome feedback, we introduced this plant species into the *in vitro* culture conditions, where the effect of illumination was examined.

S. yangii shoots were cultured on Murashige and Skoog media under the following LED light spectra: White (W), Photosynthetic Active Radiation (PAR), Blue (B), Red (R), and Red and Blue mix in a proportion of 50:50 (R+B). The data on the morphogenetic and metabolic responses were collected after 6, 24, and 54 weeks of culture. Chromatographic analysis (GC-MS) was used to monitor changes in *S. yangii* volatile compounds profile. The relative level of gene expression for the most abundant compounds such as 1,8-cineole and camphor was examined using the RT-qPCR technique. Also, the activity of key enzymes of the mono- and sesquiterpenes biosynthesis pathway (GPPS and FPPS, respectively) was determined.

The light spectrum significantly affected the morphology of *S. yangii* organs, and the observed changes corresponded to the physiological alterations. The metabolic profile of volatile constituents changed in terms of quality and quantity depending on light spectra treatment. The yield of volatile compounds decreased along with the time of culture in all spectra conditions but PAR. Also, under PAR illumination the 1,8-cineole content was the lowest, while camphor reached the highest amounts. However, camphor was significantly reduced under B and R+B spectra. The GPPS expression was significantly higher than FPPS, corresponding to total mono- and sesquiterpenes content. The relative contribution analysis revealed culture duration being more significant for 1,8-cineole and camphor production compared to the light spectrum.

Based on these results, the comprehensive approach of light spectra influence on *S. yangii* volatile metabolites production may provide a valuable model for metabolic studies.

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How to feed *Komagataeibacter* species for the best nanocellulose yield

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Aim: Depending on the culture media different species of *Komagataeibacter* can produce bacterial nanocellulose (BNS) more or less effectively. BNS is a raw material widely used in the food industry, medicine and pharmacy (i.e. as a carrier of therapeutics). The study's first aim was to identify the species of bacteria isolated from vinegar mothers with molecular tools - 16S rRNA-based DNA Barcoding. A second aim was to estimate optimal culture conditions for 3 studied strains of *Komagataeibacter*: *Komagataeibacter* sp. strain 1.3, *Komagataeibacter* sp. strain 4.2 and *Komagataeibacter* sp. strain 5.3.

Methodology: Molecular identification was done by the 16S-rRNA dideoxy sequencing and phylogenetic analysis based on the Maximum Likelihood method. Then, 3 types of culturing media were chosen for the experiment according to the literature. The culture media optimisation was performed by the cultivation of isolated strains in 3 different conditions (Hestrin-Schramm, Hestrin-Schramm emulsion and apple juice) for 7 days with control of the mass of produced BNS biofilm after 72h, 120h and 168h of growth.

Results: Two strains were identified (*K. saccharivorans*, *K. hansenii*) and one strain couldn't be phylogenetically placed and was described as a potential *Komagataeibacter* sp. nov. However, results of media optimisation revealed completely different media preferences between studied bacteria. The highest BNS yields for *K. saccharivorans* strain 1.3, *K. hansenii* strain 4.2 and *K.* sp. nov strain 5.3 were observed using apple juice, Hestrin-Schramm emulsion and Hestrin-Schramm respectively. The most efficient BNS producer in studied conditions was *K. saccharivorans* strain 1.3.

Conclusions: BNS importance in the pharmaceutical industry increases. Thus this is important to effectively estimate the best conditions of BNS production for the highest yield of product. It also seems important to study new strains and species to find the best BNS producer for the most efficient industrial application.

HPLC-DAD analysis of anthocyanins from *Ribes Nigrum* fruit extracts

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Aim: The aim of the current research was to evaluate and compare the anthocyanin profile in different varieties of black currant.

Methodology: Fruits were harvested in July 2020 from a culture located in Mures county, Romania. Extracts from the fruits of five different varieties of *Ribes nigrum* (Gofert, Tiben, Ceres, Ronix and Ruben) were prepared with a 0.1% HCl solution. The anthocyanins were quantified using a Merck Hitachi L-7100 system with a Merck Hitachi L-7455 diode array detector, and the chromatographic separation was carried out on an Inertsil ODS-3, 3µm, 150 x 4.6 mm column.

Results: The results indicated that the anthocyanin profile is similar among the studied varieties, but there are statistically significant differences in the anthocyanins concentrations between the analyzed samples. The HPLC chromatograms of the analyzed extracts revealed the presence of delphinidin rutinoside, cyanidin rutinoside and cyanidin glucoside. The Ceres variety shows the lowest concentrations of determined anthocyanins (0.654 ± 0.015 mg/g cyanidin rutinoside, 0.328 ± 0.068 mg/g delphinidin rutinoside, 0.197 ± 0.004 mg/g cyanidin glucoside) compared to the other varieties. The highest concentration of delphinidin rutinoside (0.973 ± 0.028 mg/g FW) was determined in Ronix variety. The highest concentration of cyanidin rutinoside and cyanidin glucoside were determined in Tiben fruits (2.301 ± 0.105 mg/g FW and 0.872 ± 0.075 mg/g FW). The highest concentrations of anthocyanins were determined in the Tiben (2.301 ± 0.105 mg/g cyanidin rutinoside, 0.621 ± 0.036 mg/g delphinidin rutinoside), Ronix and Ruben varieties. HPLC-DAD analysis revealed that the dominant anthocyanins in the fruit extracts were delphinidin rutinoside and cyanidin rutinoside.

Conclusions: These results revealed that there are statistically significant differences between the analyzed varieties of *Ribes nigrum*, and also provide an insight on the variety with an optimal phytochemical profile, that can be cultivated for industrial applications.

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Ash leaves (*Fraxinus Excelsior* l.) - phytochemical analysis and *in vitro* evaluation of the anti-inflammatory activity

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Inflammation is the first physiological defence mechanism against external and internal stimuli. The acute phase of inflammation may be sufficient to eliminate the harmful agents but a prolonged or inappropriate response may lead to the persistence of an inflammatory response that can potentially become a basis for chronic diseases e.g. asthma, type II diabetes or cancer. An important role in the alleviation of inflammatory processes, as an adjunct to traditional pharmacological therapy is attributed to phytotherapy, especially to raw materials with a long tradition of use, e.g. ash leaves [1]. Despite their long-term use in phytotherapy, the specific mechanisms of action have not been confirmed in a sufficient number of biological or clinical studies [2].

The aim of the study is a detailed phytochemical analysis of infusion and its fractions and isolation of pure compounds, from the leaves of *Fraxinus excelsior* and evaluation of their effect on the secretion of pro-inflammatory cytokines (TNF-α, IL-6) and IL-10 receptor expression in an *in vitro* model of monocyte/macrophage cells isolated from peripheral blood.

Methodology: Phytochemical analysis was carried out by UHPLC-DAD-ESI-MS/MS method. Monocytes/macrophages were isolated from human peripheral blood using density gradient centrifugation on Pancoll. After 24-h incubation with tested fractions/subfractions and pure compounds, cells or their supernatants were studied respectively on IL-10 receptor expression by flow cytometry and IL-6, TNF-α secretion by ELISA test. Results were presented with respect to Lipopolysaccharide (LPS)- control and positive control with dexamethasone.

Results: The infusion, 20% and 50% methanolic fractions and their subfractions, as well as their dominating compounds e.g. ligstroside, formoside and oleoacteoside isolated from the leaves, show the ability to increase the IL-10 receptor expression on the surface of monocyte/macrophage cells, stimulated by LPS, and to decrease the secretion of pro-inflammatory cytokines, e.g. TNF-α, IL-6.

Conclusion: This study may serve as a basis for confirming the usefulness of ash leaf preparations as a potential component in the treatment of inflammatory-based diseases.

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Contribution to the conservation of traditional uses of wild edible plants in the region Casa Blanca Settat Morocco

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Our ethnobotanical study revealed the central importance of wild edible plants in several types of uses by rural populations of the Casablanca-Settat region of Morocco. The study also reported that in this region, the elderly are the bearers of this cultural heritage, which must now be transferred to the younger generation to avoid the danger of their disappearance. In this framework, this present work comes, which is a logical continuation of our previous works. With a very particular accent on the characterization of traditional use of these plants with integration this time of a group of sellers of these last ones to give an outline on their modes of marketing within an informal sector threatened of disappearance.

The objective of this work was to inventory the uses and marketing of spontaneous edible plants among the survey population. In order to know the edible organ, its mode of consumption (recipe), the frequency, the type of use, its state of commercialization, their availability at the market, the plant organ sold, its average price, number and frequency of sellers for each species. An ethnobotanical survey consisted of interviews with consumers and traders of these plants using a pre-established questionnaire. The study reports a total of 78 plant species belonging to 36 families were identified, only plant species representing a use description or recipe were considered. 48 recipes were cited, of which 24 were for food use, 11 for medicinal use, 14 for food and medicinal use, and 2 for food and other use. Only one recipe for each of the following uses: food/medicinal/cosmetic, food/medicinal and other. Finally, 25 recipes for other uses in the locations studied. The most consumed plant parts were leaves (21.53%), followed by all the aerial part (18.46%), stems (16.92%), tubers or roots (15.38%), seeds (9.23%), fruits (7.69%), flowers (4.61%), rhizomes (3.07%), fillets, stamens and receptacles (1.53%).

The results of this research contribute to document the modes of use of the identified wild edible plants which deserve to be valued in order to fight against their disappearance by their domestication.

Bioactive compounds and antioxidant potential of some *Lonicera Caerulea* l. indigenous berries extracts

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Aim: *Lonicera caerulea* L., known as blue-berried honeysuckle, honeyberries or haskap, a native of Eastern Siberia, Northern China and Northern Japan is mostly cultivated throughout the world for its applications in folk medicine or as a "super-food". Honeyberries have been used for centuries as a medicinal plant especially in Siberia and Tibet, among others, for angina, eye illnesses, periodontal disease and various infections and allergies. Also, haskap extracts exhibit anti-inflammatory activities, anti-diabetic, inhibition of lipid peroxidation, enhance night vision, are cardioprotective, anti-aggregating and limit damage to vascular endothelium. The goal of this paper was to establish the content in some bioactive phytochemicals, such as total polyphenols, total carotenoids, lycopen, beta-caroten, lutein, flavonoids, tannins, anthocyanins and total carbohydrates, correlated with antioxidative capacity of the soluble lipid compounds from some fluid extracts of indigenous *Lonicera caerulea* L., collected from Moldavia area, Romania.

Methodology: Fresh fruits were dried at room temperature, grind to a fine powder and extracted 10% concentration in ethanol 50% and 70% using cold maceration, respectively ultrasonication method, followed by filtration at normal pressure. Haskap hydroalcoholic extracts were analysed by UV-Vis spectrophotometry for determination the bioactive compounds content. For total antioxidant capacity assessment of fruits extracts, the photochemiluminescence method through antioxidant capacity of lipid soluble substances procedure for vegetal products, has been used by comparing with the standard Trolox® solution, using Photochem apparatus Analytik Jena AG, Germany.

Results: Photochemiluminescence analysis emphasized an increased antioxidant capacity of the hydroalcoholic fruits extracts 10% concentration in 70% ethanol, by cold maceration and ultrasonication, which may be attributed to the high level content of polyphenols, flavonoids and tannins registered.

Conclusions: The high values of total antioxidant capacity of the obtained fluid extracts justify research orientation towards the assessment of antioxidant potential and sustain the possibility to use these valuable indigenous fruits for their phytotherapeutical potential in some hepato-digestive disorders and we propose further research, to evaluate in detail the bioactive compounds from extracts of *Lonicera caerulea* L. Romanian berries, to confirm their therapeutical potential.

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Spirulina as a natural emulgator for oil/water emulsions

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Sunflower oil/water emulsions combined with different emulsifiers are often used in the pharmaceutical and food industries and find applications in various technological process. The ability of the dietary fiber in Spirulina (*Arthrospira platensis*) to hold water or vegetable oil makes the alga suitable for use as an emulsifier in colloidal or emulsion systems. The present study aims to investigate the influence of Spirulina (4%, 8%, 12%) on emulsion stability, dispersibility, and thermodynamic stability by determining parameters such as Gibbs energy, enthalpy, and entropy. A spectrophotometric method was used to determine the thermodynamic stability and dispersity of the systems. The emulsion stability was studied by the Cosine method. From the microscopic analysis, it is evident that as the concentration of Spirulina increases, the size of the oil globules decreases. As the quantity of biomass increases, the emulsion stability and thermodynamic stability increase, i.e., the Gibbs free energy decreases. The highest percentage of retained stability of emulsion (100%) was with 12% Spirulina at 40% or 60% oil phase content. From a rheological point of view, emulsions with 20% oil phase have plastic body behavior, and those with 40% and 60% oil phase have pseudoplastic behavior. Due to the high protein content, the oil/water emulsions with 12% Spirulina are stable and viscous at oil phases of 20%, 40%, and 60%. They can be used as natural ingredients in different colloidal systems in pharmaceutical and food industries.

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Olive leaf extracts in green synthesis of ag nanoparticles for wound dressing materials

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Aim: Nanotechnology has recently emerged as a rapidly growing field with numerous biomedical applications. With antibacterial properties, silver nanoparticles have been tested in the treatments for skin and biological ("green") methods to prepare such nanomaterials have been proposed. Our study presents the preparation of silver nanoparticles in presence of olive leaf extracts and the nanoparticles application in formulations as wound dressings.

Methodology: Extracts were obtained from olive leaves (*Olea europaea* species, *Sevillan* subspecies) by boiling in distilled water and purification. Silver nanoparticles have been obtained by mixing extracts and $AgNO_3$/$AgNO_3$+Glucose, at pH 7 and controlled temperature. The nanoparticles were characterized in terms of structure, dimensions and properties and, finally, incorporated into films and porous scaffolds based on xanthan and gelatine. The scaffolds properties have been evaluated in correlation with their potential as wound dressing materials.

Results: The obtained nanoparticles (under 100 nm as dimensions and negatively charged) can be stored as suspension without changes of shape and stability. Uniform films (by drying method) and porous scaffolds (by freeze-drying method) have been obtained from olive extract-based Ag nanoparticles, gelatine and xanthan. The volume of fluids retained in the films/porous materials is strongly influenced by the ratio between polymers and AgNP concentration, respectively. The materials are degraded by enzyme and the degradation rate is slightly dependent on the AgNP concentration. *In vitro* cytotoxicity tests (MTT method and fluorescence microscopy) showed that porous materials are biocompatible.

Conclusions: Olive leaf extracts have been used as protective agent for obtaining silver particles with nanometric sizes. The size distribution and morphology of the nanoparticle are controlled by many factors, such as: the nature of the extract, the pH of the solution and the reaction temperature. Nanoparticles were obtained with high stability, negative zeta potential and antioxidant properties. The nanoparticles have been uniformly distributed in formulations based on xanthan and gelatin. The films/porous scaffolds retain simulated body fluids, are biodegradable and biocompatible.

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Phytosociological study note II. Comparative phytochemical analysis for *Hyperici Herba* and lemon balm folium introduced in the phytosociological study

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Aim: This study aims to evaluate the biosynthetic capacity of medicinal plants grown in common crops *versus* control crops. It also aimed the determination of the polyphenol content for two species *Hypericum perforatum* L. and *Melissa officinalis* L..

Methodology: Phytochemical analyses were performed on plant products from common lots (phytosociological) and control lots. The following phytochemicals were determined by spectrophotometric methods: flavones, phenolcarboxylic acids (PCAs) and total polyphenol content. Methodologies according to European Pharmacopoeia were applied. In addition, the phytochemical profile was investigated by UHPLC-MS and FT-ICR methods. In addition, the antioxidant activity in the acellular system was studied for all samples.

Results: From a quantitative chemical point of view, there are notable differences in the content of active substances: 16.81±1.00 g% PCAs expressed as caffeic acid equivalents for St. John's Wort in the control group, compared with 22.11±1.27 g% PCAs in the common crop; 1.61±0.26 g% flavones expressed as rutoside equivalents in the control group of lemon balm, compared with 2.27±0.51 g% flavones in the common culture; and 6.88±0.49 g% total polyphenols expressed as tannic acid in the control batch of St. John's Wort, compared with 9.02±0,82 g% total polyphenols in the batch of common culture.

The strongest antioxidant activity was presented by the lemon balm from the common group with an IC_{50} value of 0.0349 μg/mL ???. The dominant compound in the phytosociological lemon balm crop is isoquercitrin - 263.83 μg/g compared to the control crop whose value is 170.67 μg/g. For *Hyperici herba* the main compound is quercetin - 11789.84 μg/g in the common culture and 7632.44 μg/g in the control batch.

Another interesting result was obtained by UHPLC-MS analysis – using positive ionization, rosmarinic acid was identified. The presence of rosmarinic acid in *Hyperici herba* has not been mentioned in literature before.

Conclusions: The association of these two medicinal species can be a starting point in conducting new phytosociological studies with potential stimulation of the biosynthesis of polyphenolic compounds.

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An ethnobotanical study of wild medicinal plants in Republic of Moldova

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Aim: Identify, collect and document the native medicinal plants and their traditional uses by local communities in Republic of Moldova for reconsideration of their pharmacological action and nutritive value.

Methodology: Using ethnobotanical methods, the study was carried out in eleven rural settlements from three southern districts (Cimișlia, Comrat and Cahul). Ethnobotanical and ethnopharmacological information were accumulated via informed questionnaires and open interviews with local people (44 informants, between the ages of 43 and 85 years).

Results: The diversity of plant species in the flora of Republic of Moldova is rich and varied, but traditional uses of plants have not yet been systematically recorded [1, 2]. In this study, a total of 96 medicinal species of 31 botanical families, traditionally used for consumption and for treating various illnesses were documented. The most utilized species belonged to Lamiaceae, Asteraceae, Apiaceae and Fabaceae. Good numbers of species were mentioned by respondents as plants commonly used in the past (ex. during famine, after the 2nd World War) but not anymore. Different part plants are used, aerial parts (34.2%) and leaves (22.5%) being the most frequent. Medicinal uses of the species refer to 13 categories; skin problems (37.2%), respiratory infections (28.5%) and gastrointestinal disorders (18.4%) were reported as frequently treated with herbal remedies. There is to be mentioned that the species *Thymus marschallianus, Tanacetum corymbosum*, *Teucrium* sp. would be interesting targets for further investigations and drug finding.

Conclusions: The study reveals that people from investigated area continue to use the knowledge of wild medicinal plants in their day-to-day life and encourages further ethnobotanical and ethnopharmacological studies in rural areas throughout the country.

Acknowledgements

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Verbascum Nigrum l. extracts with selective cytotoxicity and synergy with doxorubicin in A431 human skin cancer cells

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Aim: *Verbascum* L. species have a widespread and long-standing use in traditional medicine, correlated to a rich phytochemical composition and various biological activities, such as antimicrobial, antioxidant, anti-inflammatory and cytotoxic effects, among others. Thus, the purpose of this study aimed to assess the selective cytotoxic effects of various *Verbascum nigrum* L. extracts against A431 human epidermoid carcinoma cells.

Methodology**:** The antiproliferative effects against A431 cells and human immortalized keratinocyte cells (HaCaT) of the crude methanolic extract (VNE), six extractive fractions (VNF1-VNF6) and five extracts (VN1-VN5) obtained from *V. nigrum* L. aerial parts were assessed using MTT and LDH assays and immunofluorescence staining, followed by chemical characterization of active extracts using HPLC-DAD-ESI-Q-TOF-MS/MS analysis. Cytotoxic activity was compared to doxorubicin.

Results: VN1, VNE and VNF4 methanolic extractive fraction showed a significant inhibition of A431 cells proliferation, the cytotoxic activity varying in the order VNF4 > VNE > VN1, with IC_{50} values of 12.27, 81.92 and 118.50 µg/mL respectively (24 h incubation). All extracts and extractive fractions showed good biocompatibility against normal HaCaT cells. The chemical characterization of VNE and VNF4 revealed high abundance of saponin glycosides (songarosaponins A and B, ilwensisaponins A and C, buddlejasaponin I), aucubin type iridoid glycosides and phenylethanoid glycosides (verbascoside, forsythoside B, martinoside). VNF4 induced a high LDH release in A431 cells (IC_{50} = 28.83 µg/mL), as well as high propidium iodide positive stained cells and lower caspase 3/7 activation, compared to VNE. Cytotoxicity of VN1 increased with increasing exposure time (IC_{50} = 58.50 ± 1.19 µg/mL, 72 h incubtion) and exhibited synergistic activity when combined with doxorubicin (D) against A431 cells. Results were best when the VN1:D ratio of 10:1 was used (combination index 0.21), increasing D cytotoxicity from 69.18% to 97.25%.

Conclusions**:** The results revealed that *Verbascum nigrum* L. extracts display selective cytotoxic activity, setting the premises for further studies aiming to isolate active constituents and investigate the detailed cytotoxic molecular mechanisms.

Acknowledgements
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Induction of apoptosis and cell cycle modulation by *Ulmus Glabra* bark extracts in different cell lines

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Aim: To study the effect of some *Ulmus glabra* bark extracts on cell-cycle and apoptosis processes in normal and tumoral cells in order to elucidate the molecular mechanism and selectivity properties.

Methodology: Vero normal and HeLa cancer cells were incubated with various concentrations of bark extracts for different periods of time. The cell cycle analysis was carried out via flow cytometry using NIM-DAPI staining and apoptosis was evaluated by FITC-Annexin-V and propidium iodide (PI) double staining method.

Results: The impact of polyphenolic elm bark extracts was responsible for inducing a G2/M phase arrest and disturbing cell cycle progression. The treatment of HeLa cells caused significant changes, expressing an intensification of the apoptosis by accumulation of apoptotic and pre-apoptotic cells.

Conclusions: Our data suggest that the anticancer effect of elm bark was achieved by increasing the number of apoptotic cells and G2/M arrest in HeLa cells, justifying the possibility of being developed as therapeutic agent against cancer cells.

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Physicochemical properties and moisturizing effect of a cosmeceutical cream with *Centaurea Cyanus* flower water

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Aim: Cosmeceuticals, a combination between cosmetics and pharmaceuticals, offer the skin an improvement of appearance and provide the nutrients needed to prevent its aging. Also, these products contribute to the insurance of biological functions [1]. The demand for moisturizing and anti-aging products has increased significantly in recent years, requiring products with immediate and long-term effects. Although the cosmetics market does not lack moisturizing and anti-aging products, there is great variability in their effectiveness [2, 3]. The aim of this study was to design a cosmeceutical product and to evaluate the effectiveness of the product in correlation with the pH given by the cream components.

Methodology: There were developed two water in oil (W/O) type creams, formulated with 60% hydrophilic phase and 40% lipophilic phase, using a new type of emulsifier. The first formula (FI) contains as hydrophilic phase, *the Centaurea cyanus* flower water, and the second one (FII) contains distilled water as the hydrophilic phase. The physicochemical properties (appearance, pH), and *in vivo* moisturizing effect were evaluated.

Results: Both proposed moisturizer creams present a pleasant texture. The pH differs depending on the proposed hydrophilic phase, FI has a pH of 6.9 and FII has a pH of 5.6. After a single application on dry skin, the proposed formulations can improve the degree of hydration by 70%. FI increases the level of hydration by 83.5% and FII by 72.8% in just one application.

Conclusions: In accordance with the applicable norms in force, proposed formulas have an appropriate pH and increase the degree of hydration. The first formula (FI) that uses *Centaurea cyanus* flower water, has a slightly basic pH and moisturizes the skin more effectively than formula FII, where distilled water was used as the hydrophilic phase.

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The total content of hydroxycinnamic acids in the aerial parts of agrimony and chicory

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Aim: Spectrophotometric determination of total hydroxycinnamic acids in the aerial parts of *Agrimoniae herba* (*Agrimonia eupatoria* L., fam. Rosaceae) and *Cichorii herba* (*Cichorium intybus* L., fam. Asteraceae).

Methodology: The aerial parts of agrimony and chicory were harvested in the flowering period from the collection of Scientific Practical Centre in the Field of Medicinal Plants (SPCFMP) of "Nicolae Testemițanu" State University of Medicine and Pharmacy, during the flowering period (2021). The dry extracts have been obtained through a fractional maceration method and afterwards have been concentrated using the Laborota 4011 rotative evaporator. The quantitative analysis of total hydroxycinnamic acids (THA) was done by three methods using a Metertech UV/VIS SP 8001 spectrophotometer.

Results: The spectrophotometric analysis performed with Arnow reagent (518 nm), according to the European Pharmacopoeia, determined the total hydroxycinnamic acids content (caffeic acid equivalents): 3.67 mg/g for *A. eupatoria* and 1.48 mg/g for *C. intybus*. Our results showed a higher content of total hydroxycinnamic acids in chicory in the extractive solution obtained with 30% ethanol: 13.22% (chlorogenic acid equivalents) than in 20% ethanol extractive solution: 10.93% (caffeic acid equivalents), followed by agrimony (3.78% and 3.10%, respectively). In the last two methods, the optical density of the extracts was read at 325 nm.

Conclusions: The total content of hydroxycinnamic acids was determined by three spectrophotometric methods. Extracts of the aerial parts of *A. eupatoria* and *C. intybus* species from the collection of SPCFMP can serve as sources of phenolic acids.

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Exposure to non-thermal plasma activated water and cold stress to improve the antioxidant potential of sprouted wheat

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Aim: This study was carried out to evaluate the effects of non-thermal plasma activated water (PAW) treatment in combination with cold stress on the antioxidant potential of sprouted wheat.

Methodology: Wheat grains were germinated for 8 days at 15°C. Three different non-thermal PAW treatments were applied, namely PAW25, PAW35 and PAW50 containing 25, 35 and 50 mg/L of nitrate, respectively. Samples collected at days 3 and 8 were investigated for antioxidant and pro-oxidant enzymes activity, free and bound polyphenolic content and antioxidant activity [1, 2].

Results: All non-thermal PAW treatments positively influenced superoxide dismutase, the most potent activity being determined under PAW25 treatment at day 8 (2.2-fold increase *vs*. control). The highest catalase activity was detected in wheat sprouts at day 8 following exposure to PAW50 (1.2-fold increase *vs*. control). Polyphenol oxidase activity slightly increased at day 3 while it was inhibited at day 8. The polyphenols reached high values at day 8: 72.5 mg% free polyphenols *vs*. 60.84 mg% in control (under PAW25 treatment) and 102.43 mg% bound polyphenols *vs*. 93.53 mg% in control (under PAW35 treatment). Free and bound phenolic extracts obtained from sprouted wheats treated with PAW25 at day 8 exhibited the most potent antioxidant activity (52.65% *vs*. 36.02% for control and 82.74% *vs*. 63.94% for control, respectively).

Conclusions: Treatment with non-thermal PAW in combination with cold stress is an efficient approach to enhance the antioxidant potential of sprouted wheat.

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Characterisation of *Artemisia Lancea* ethanolic extract based on antifungal potential and phytochemical profile

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Aim: To evaluate the antifungal potential of ethanolic extract of *A. lancea* Vaniot against eight phytopathogenic and mycotoxigenic fungal strains and characterization of its chemical profile.

Methodology: Plant extract was prepared from aerial parts of *A. lancea* (reported as new alien taxon from Europe) [1] which was collected in North - Eastern Romania (Iasi district). The ethanolic extract was screened for its antifungal activity against *Alternaria* spp., *Fusarium* spp., *Botrytis* spp., *Penicillium* spp., and *Aspergillus* spp., fungal strains (BCCM/MUCL Collection) according to the poisoned food technique [2]. Also, the minimal inhibitory concentrations (MIC_s) and minimal fungicidal concentrations (MFC_s) of the phytoextracts were determined [3]. The alcoholic extract was subjected to LC-MS analysis for identification and quantification of polyphenols, sesquiterpene lactones, phytosterols, and tocopherols.

Results: It was found that the ethanolic extract (5 mg/mL) was more effective against *A. alternata, P. expansum,* and *Botrytis cinerea* fungal strains (100% inhibition of radial mycelial growth), but also against *F. oxysporum f.sp. lycopersici, F. oxysporum f. sp. radicis lycopersici, A. solani, A. flavus* and *A. niger* it was noticed remarkable antifungal activity (86.71 – 52.38% inhibition of fungal growth). The MIC_s values ranged from 2.5 to 10 mg/mL and $MFC_s \geq 10$ mg/mL, depending on the tested fungal strain.

Isoquercitrin, chlorogenic acid and hispidulin were the main polyphenol compounds identified in *A. lancea* ethanolic extract. Sesquiterpenes lactones were represented only by dehydroleucodine, while elevated level of sitosterol, stigmasterol and γ-tocopherol were identified as the main phytosterols and tocopherols, respectively. Conclusions: The results revealed that *A. lancea* could be considered as a promising natural source for the development of new biopesticides, as alternative method to conventional pesticide against selected phytopathogenic fungi.

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Chemical and biological study of some *Teucrium* species from Romania

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Aim: The purpose of this paper is to highlight the chemical composition of extracts obtained from three species of *Teucrium* and the *in vitro* investigation of antioxidant, antimicrobial and / or cytotoxic effects.

Methodology: The aerial parts of each species (*T. chamaedrys, T. montanum* and *T. polium*) were collected in June 2019 from the northeastern region of Romania from the wild flora around the cities of Horlești and Bicaz. The corresponding methanolic extracts were obtained for each plant material. The chemical composition was assessed qualitatively and quantitatively (UPLC-PDA, spectrophotometry). The antioxidant, antimicrobial and cytotoxic activity was evaluated by specific means.

Results: The phytochemical analysis indicated that *T. polium* contains the highest amounts of total polyphenols and total flavonoids, which is supported by the spectrum of components determined and quantified chromatographically. Quantitatively, *T. polium* is followed by *T. chamaedrys* and then by *T. montanum.* The antioxidant potential of the investigated extracts is correlated with the chemical composition. The best scavenger of DPPH radicals was the methanolic extract of *T. polium*, followed by the samples of *T. chamaedrys* and *T. montanum*. Also, all samples were active against lipoxygenase. The antimicrobial potential revealed that the most valuable species remains *T. polium*, followed by *T. chamaedrys* and *T. montanum*, although among the three species investigated, *T. chamaedrys* stands out with the best activity against strains of gram-negative pathogens. The methanolic extract of *T. montanum* has selective cytotoxic effects, the activity being expressed only on the melanoma line. The methanolic extract of *T. chamaedrys* was shown to be active on the melanoma cell line at the concentration of 50 µg/mL, but showed similar effects on the healthy cell line.

Conclusions: Corroborating all the results of biological tests (antioxidants, antimicrobials and cytotoxics) we note the special potential of the three species of *Teucrium* included in the study, which have a variable spectrum of activity from one species to another, while depending on the type of solvent used to obtain extracts.

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Evaluation of the irritative potential of snail (*Helix Aspersa*) mucin extract in the HET-CAM test

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Aim: *Helix aspersa* snail mucin is a natural substance with promising potential. As an ingredient in skin care products, it shows effective properties and provides intensive hydration, regeneration, rejuvenation and protection of the epidermis [1]. One of the snail slime extract stages regulation in finished products is the determination of safe concentrations according to the indicator of irritative activity. The purpose of this study was to evaluate the irritant effect of different snail slime extract concentrations. The raw material was obtained from snails cultivated in Ukraine. Methodology: *H. aspersa* snail mucin extract was studied in a test on the chorioalanthosis membrane of chicken embryo [2]. The chorion-allantois membrane reaction was monitored after 30, 120 and 300 seconds and the occurrence of negative changes was photofixed. The irritation index was calculated as the median value of the all tests' repetitions total scores. Results: The irritative potential of *H. aspersa* snail mucin extract depended on its concentration. In the observation of the CAM vessels after application of native snail mucin extract, there was an increase in vascular blood filling and pinpoint hemorrhages at the 30^{th} and 120^{th} seconds, lysis of small and medium CAM vessels from the 30^{th} second. A solution of 50% snail mucin extract showed lysis of small and medium vessels at the 30^{th} and 120^{th} seconds, pitting hemorrhages at 300^{th} seconds. The analysis of the CAM vessels observation after exposure to a solution of 25% snail mucin extract showed lysis of the vessels at the 120^{th} second. In the solution of 20% snail mucin extract, vascular lysis appeared at the 300^{th} second only in one of the repetitions (Table).

Concentration of snail mucin extract	Irritation index Me (Q1÷Q3)	Category of irritant action
100%	10 (10÷11)	Expressed irritant effect
50%	5 (5÷5,5)	Moderate irritant effect
25%	3 (3÷3)	Weak irritant effect
20%	0 (0÷0,5)	Does not cause irritation

Conclusions: It was found that *H. aspersa* snail mucin extract at a concentration of no more than 20% under the conditions of our studies does not cause adverse effects on HET-CAM and this concentration is recommended to finished cosmetic products.

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New uses for food byproducts

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Aim: Highlighting the most recent trends in ecological recycling of food byproducts.

Methodology: Processing information from more than 10 scientific papers published during the last 3 years, selected based on the following keywords: ecological recycling, bioactive compounds, food composition, green extraction.

Results: Ecological recycling is a current issue which raises the interest of the scientific community. Processing of waste resulting from food of vegetal origin processing in order to extract bioactive compounds represents a growing market sector, that is promising for improving general health through the provision of functional foods, food supplements and natural body care products. Tomatoes and onions are among the most common consumed vegetables in the context of human diets (and also in the context of the food industry, where significant quantities of waste are generated). The date palm (approximate 1500 varieties), due to its fruits, is recognized for its therapeutical uses since ancient times. Byproducts resulting from industrial processing of tomatoes contain carotenoids, phenolic compounds, vitamins, proteins, dietary fiber, essential oils. Onion peels are rich in phenolic compounds, sulfur-containing compounds and dietary fiber. Dates contain phenolic acids, flavonoids and carotenoids, while their seeds contain, in addition, tocopherols, tocotrienols and phytosterols. These bioactive compounds can be extracted either through conventional methods (solvents; enzymatically; ultrasounds; supercritical fluids etc), or through modern methods (hydrocolloidal complexation; microemulsion technique with ohmic heating etc). These bioactive compounds exert various effects (antioxidant, antimicrobial, anticarcinogenic, anti-inflammatory, antidiabetic, prevention of cardiovascular disease, inhibition of enzymatic activity). All these properties extend the field of use of food waste from obtaining biogas and fertilization of crops to food industry (natural food additives for meat products, butter, bakery products, confectionery products, olive oil, sunflower oil, fortified foods; manufacturing of active packaging materials for food products), cosmetics industry (photoprotection; skin whitening treatments) and pharmaceutical industry (pectin is used as a binding agent in tablets etc).

Conclusions: Efficient reuse and with reduced costs of industrial byproducts derived from foods, in order to obtain products with high added value in the context of health and wellbeing promotion represents a real challenge from a technical, scientific, economic or ecological point of view.

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NMR-based metabolomics to study specialized metabolism variability in plants: a case study of seasonal phytochemical changes in selected officinal plants

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Aim: Chemical composition of plant material is highly variable [1]. Unfortunately, this aspect is often overlooked in natural product research. The aim of this study was to explore the seasonality of metabolism in a set of officinal plants of Mediterranean area.

Methodology: The plants chosen for this study were: *Arbutus unedo*, *Myrtus communis*, *Cistus* spp., *Phillyrea angustifolia*, *Pistacia lentiscus*, *Teucrium chamaedris*, *Teucrium polium*, and *Rosmarinus officinalis*. The leaves of these plants were monitored for their metabolite content for a year, with sampling carried out monthly. The plant material was collected, immediately frozen in liquid N_2, lyophilized, and analyzed by a ^{1}H NMR-based metabolomics approach to study the seasonality of specialized metabolites. 2D NMR analyses were performed on selected samples for in-mixture identification of the compounds present in the extracts.

Results: Multivariate data analysis of ^{1}H NMR data allowed us to rapidly highlight the variations in metabolite content along the seasons. A remarkable variability was observed for the analyzed plants. Seasonal fluctuations were observed for most of the known bioactive compounds. In particular, the lowest concentration of phenolic compounds was detected during the summer months. This pattern was observed in most of the analyzed species and was particularly marked in the Labiatae plants. Terpenoids and other compounds showed different accumulation patterns.

Conclusions: A metabolomics approach was here used to study the seasonal phytochemical variations for the analyzed plants, effectively and rapidly. However, the presented approach has potential to be used to easily assess the impact of many different abiotic and biotic factors on the phytochemical composition of plants.

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Anti-adipogenic activity of maackiain and ononin is mediated *via* inhibition of PPARΓ in human adipocytes

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Aim: In the present study, we examined the anti-adipogenic potential of ononin (ONON) and maackiain (MACK) in human adipocytes as an *in vitro* model of obesity.

Methodology: Both secondary metabolites were selected following targeted NMR-based metabolite profiling of the *Ononis spinosa* L. root extract (OSR), based on ethnopharmacological rationale for its application in obesity treatment. Furthermore, *in silico* docking analysis evaluated the molecular interaction between selected compounds and adipogenic regulatory proteins. Estimation of the biological activity was performed in human adipocytes exposed to pure ONON (5, 10 and 25 μM) and MACK (5, 10, 25 and 50 μM). Then, Oil red O lipid staining and assessment of the basal lipolysis were performed. Additionally, the relative mRNA and protein expression of the crucial for adipocyte differentiation transcription factors were evaluated [1].

Results: Quantification of lipid staining revealed inhibition of adipogenesis by ONON and MACK. Moreover, RT-qPCR analysis demonstrated that ONON 10, 25 μM and MACK 5 μM decreased lipid accumulation by upregulation in mRNA expression of *SIRT1* and *AMPK*. Also, MACK in concentration of 50 μM suppressed gene expression of key transcription factors as *SERBP1*, *ACC* and *CEBPA*. Regarding the protein expression, adiponectin was significantly decreased by ONON and MACK in the whole range of the applied concentration. Additionally, western blot analysis confirmed that MACK inhibited adipogenesis through inhibition of PPARγ and C/EBPα [1].

Conclusions: Our results demonstrated anti-adipogenic effect of MACK and ONON reported for the first time in human adipocytes.

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Combination of *Pimpinella Anisum* l. and *Eucalyptus Globulus* l. essential oils and toxicity evaluation

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Aim: The objective of this work was to evaluate the safe and selective combination effects of *Pimpinella anisum* L. and *Eucalyptus globulus* L. essential oils with antibacterial effects.

Methodology: Pharma grade *P. anisum* and *E. globulus* essential oils were obtained from commercial sources. The composition of essential oils was analysed by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS), simultaneously, to confirm the quality. Both essential oils were tested individually and in combined form with tetracycline for the evaluation of potential synergistic activity. Human standard pathogenic strains *Streptococcus mutans* ATCC 25175, *Streptococcus sanguinis* ATCC 10556, *Corynebacterium striatum* ATCC BAA-1293, *Staphylococcus aureus* ATCC 700699 were challenged *in vitro* by microdilution and by checkerboard methods. The acute and lethal toxicity of essential oils were evaluated using the *in vivo* animal alternative method *Caenorhabditis elegans*.

Results: The chromatospectral analyses confirmed (*E*)-anethol (93.5 %) as major component of *P. anisum* essential oil. 1,8-Cineol (80.0 %) was found as major component of *E. globulus* essential oil. Since, *E. globulus* essential oil showed high MIC value (MIC > 6250 µg/mL) against *S. mutans* and *C. striatum* strains, synergistic activity evaluation was not performed. Additive effect was observed against S. *sanguinis* strain (FICI=1.25 µg/mL). *E. globulus* essential oil and tetracycline showed synergistic effects against *S. aureus* strains (FICI=0.46 µg/mL). The combination of *P. anisum* essential oil with tetracycline showed indifferent activity against S. *aureus, S. mutans* and *C. striatum* strains (FICI=1.5-2 µg/mL). Additive effect was observed against *S. sanguinis* strain (FICI=0.75 µg/mL). Lethal concentration of the % inhibition for essential oils were determined >25 mg/mL.

Conclusions: When a number of scientific researches have confirmed the synergistic activity of essential oils and antibiotics certainly, the next step was to investigate the mechanisms of the synergistic action. Further studies will be focused on the mechanisms of synergistic action. In addition, lethal and acute toxicity of major components will be evaluated and reveal the mechanisms of toxicity.

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Exploring the anti-diabetic and anti-inflammatory effects of lignans coming from wood waste materials

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Aim: The study aimed to analyze the potential anti-diabetic and anti-inflammatory effects of lignans isolated from branch wood of silver fir (*Abies alba* Mill.) and Scots pine (*Pinus sylvestris* L.) on *in vitro* cellular models.

Methodology: Plant material was collected from botanical gardens in Poland, from which lignans were isolated using chromatographic methods. Insulin secretagogue properties were assessed on the INS-1 β-cell line. Anti-inflammatory effects were studied on monocytes/macrophages isolated from human peripheral blood by assessing IL-6, TNF-α, and IL-1β concentrations by ELISA test.

Results: Eight lignans were isolated from conifer wood and chemically characterized: nortrachelogenin, pinoresinol, and matairesinol from Scots pine, and secoisolariciresinol, lariciresinol, 7-hydroxylariciresinol, 7-hydroxymatairesinol and cyclolariciresinol from silver fir. None of the compounds was able to modulate basal or glucose-induced insulin secretion. Studied compounds reduced the secretion of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β).

Conclusions: Wood waste metabolites from the group of lignans show anti-inflammatory effects by reducing the secretion of pro-inflammatory cytokines. No impact on insulin secretion has been observed.

Acknowledgments
Plant material was acquired from the Polish Academy of Sciences Botanical Garden in Powsin, Poland.

Yerba mate (*Ilex Paraguariensis*) and matcha (*Camellia Sinensis*) extracts - multifunctional active ingredients of skin protecting cosmetic formulations

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Aim: Plant-derived ingredients are valuable components of modern cosmetic formulation as they contain several bioactive compounds and are widely accepted by consumers, especially those looking for more "natural" and "eco" solutions in their everyday skincare. Green tea (*Camellia sinensis*) is an example of a natural ingredient particularly rich in phytochemicals with multiple health-promoting and cosmetic properties. Yerba mate (*Ilex paraguariensis*) has been also shown as a source of many valuable compounds but the cosmetic use of extracts from this raw material has not been much explored to date. The aim of the study was to compare phytochemical composition and selected cosmetic-related activities of matcha green tea and yerba mate extracts.

Methodology: Aqueous, hydro-glycolic and hydro-ethanolic extracts from matcha and yerba mate were prepared using ultrasound-assisted extraction and compared for the total content of phenolics and flavonoids, the antioxidant activity (DPPH and ABTS scavenging assays), tyrosinase inhibition, *in vitro* cytotoxicity against human fibroblasts BJ and melanoma A375 cell lines and *in vitro* sun protection factor (SPF). The presence of particular active compounds has also been compared between the extracts using HPLC-ESI-QTOF-MS/MS.

Results: All investigated extracts contained a high content of polyphenol compounds, with the water-glycol extract from Yerba Mate having the highest content. Green tea extracts are also strong antioxidants, but the strongest antioxidant properties possessed water-glycol matcha extract and water-glycol extract from Yerba mate. Matcha extracts showed a higher anti-pigmentation potential (the strongest effect showed water-glycol matcha extract). Individual Yerba extracts showed a higher sun protection capacity. Matcha aqueous glycol extract stimulates human fibroblast cells to proliferate at a concentration of 5%. Yerba Mate and Matcha extracts are cytotoxic against the cell line of human melanoma A375.

Conclusions: The results thus demonstrate that the extracts under study could be used as rich sources of active ingredients in anti-aging cosmetics. Matcha extracts are better suited for cosmetics with anti-pigmentation activity. Extracts from Yerba Mate are more suitable in protective cosmetics against sunlight. The best solvent for this type of extracts is a mixture of water and glycol. Water-glycol extracts are characterized by a high content of active compounds and are also safe for cosmetic applications.

Phenolic profile, antimicrobial and diuretic activities of *Thymus Comosus* heuff. ex griseb herbal preparations

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Aim: The present study aimed to evaluate the phenolic profile, antimicrobial and diuretic activities of three herbal preparations obtained from *T. comosus* aerial parts.

Methodology: Maceration, infusion and optimized ultrasound-assisted extraction (OpUAE) were used to obtain the herbal preparations which were further analyzed for their phenolic profile using LC-ESI-DAD/MS^2. *In vivo* diuretic effect of the herbal preparations was evaluated on Wistar rats by monitoring diuretic action and diuretic activity, while microdilution method was employed for the assessment of *in vitro* antimicrobial potential against six bacterial and six fungal strains.

Results: All herbal preparations were characterized by important amounts of phenolic acids derivatives, including rosmarinic and salvianolic acid A, the highest phenolic content being observed for the OpUAE. A moderate antibacterial potential was observed for all the analyzed samples, and the most sensible strains were *E. coli* and *Penicillium* species. Tincture and OpUAE extracts produced a statistically significant and dose-dependent gradual increase of the urine output, the effect being more intense at 24 h.

Conclusions: This is the first study that supports the potential use of *T. comosus* herbal preparations as complementary treatment in urinary tract disorders.

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In vivo & *in vitro*-derived *Corydalis Cheilanthifolia* - a powerful tool against multi-drug resistant human pathogens

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Since strong antimicrobial activity of *Corydalis cheilanthifolia* extracts has been noticed [1, 2], a phytochemically characterized and profiled plant material of the species using biotechnological techniques was highly expected. Hence, *C. cheilanthifolia* plants (Papaveraceae) were introduced into the *in vitro* culture conditions for further manipulations using exogenous plant growth regulators (PGRs).

Surface-sterilized seeds were used to obtain aseptic seedlings, that were cut into explants. Callus tissue and organs were cultured on solid agar media MH3 [3] supplemented with kinetin (KIN)/1-naphthaleneacetic acid (NAA)/2,4-dichlorophenoxyacetic acid (2.4-D) and 6-benzylaminopurine (BA)/indole-3-acetic acid (IAA), respectively. PGRs free medium was considered as control culture conditions. After 28 days of culture data were recorded on the morphogenetic response of explants. Phytochemical characterization of *in vivo* and *in vitro* derived plant material was performed using UHPLC-DAD-TQMS.

Culture media supplementation of BA (1 µM) in combination with IAA (0.5) was the most efficient in terms of shoot biomass production. Higher concentrations of BA resulted in the reduction of the leaf lamina area. Both, callus and shoot cultures were found to produce isoquinoline alkaloids such as protopine, coptisine, berberine, sanguinarine and their derivatives.

Further studies on *C. cheilanthifolia* cell, tissue and organ cultures are needed to explore the regulatory mechanisms of isoquinoline alkaloids as well as other specialized metabolites, and to boost the quality of this highly relevant and medicinally important plant species.

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Characterization of composition and assessment of vascular protective effects of black chokeberry extracts in mice aortas

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Mitigation of oxidative stress has been systematically investigated as adjunct therapeutic approach aimed at preventing the development and progression of cardiovascular diseases. Several bioactive, polyphenol-rich phytochemicals were studied in the past decades for their cardioprotective effects. Particularly, black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) extracts have been reported to elicit vasculo-protective effects, via partially elucidated pathomechanisms. [1, 2] This study was aimed to assess the polyphenolic profile, as well as the inorganic element determination, followed by the *in vitro* evaluation of the cardioprotective effects of black chokeberry extracts (obtained from fruits either in the dried-Dr or frozen-Fr form) collected from the western part of Romania.

The phenolic compound determination of black chokeberry extracts (obtained by HPLC-DAD-ESI+ analysis) revealed, as expected, a high phenolic content. Ten different phenolic compounds were identified, such as: cyanidin-3-*O*-diglucoside, neochlorogenic acid, cyanidin-3-*O*-glucoside, chlorogenic acid, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-xyloside, caffeic acid, rutin, quercetin-3-*O*-glucoside and quercetin. Quantitative analysis revealed that the extract obtained from frozen black chokeberries was very high in anthocyanins (cyanidin-glucoside - 7562.218 mg/g CCE), flavonoids (rutin - 5989.254 mg/g CCE) and hydroxycinnamic acids (caffeic acid - 3752.98 mg/g CCE), while the extract obtained from dried black chokeberries was very high in flavonoids (rutin - 6136.608 mg/g CCE) and hydroxycinnamic acids (caffeic acid - 4224.295 mg/g CCE and chlorogenic acid - 3024.259 mg/g CCE). Inorganic element determination performed on the extracts (by graphite-furnace atomic absorption spectrometry method) highlighted metal concentrations that vary from 0.29 µg/g for Cu to 432.38 µg/g for Fe (µg/g being equivalent to ppm and mg/kg), nickel, plumb, arsenic, cobalt being below detection limit. The next step consisted in the evaluation of black chokeberry extracts effects on vascular reactivity and reactive oxygen species (ROS) production in mice aortic rings after incubation with angiotensin 2 (Ang2), lipopolysaccharide (LPS) and high glucose (Glu). To this aim, mice aorta was prepared and

incubated *in vitro* with Ang2 (100 nM), LPS (1 µg/ml) or Glu (400 mg/dl), in the presence or absence of black chokeberry extracts (in the following doses: 1, 10, 50, 75, 100, 500 µg/ml; 12 h incubation). The vessels were further used for ROS measurement (ferrous oxidation xylenol orange method and dihydroethidium staining) and vascular reactivity (myograph) studies. ROS level was significantly high after Ang2 (0.75±0.13 nM H2O2/mg tissue/h), LPS (0.99±0.03 nM H2O2/mg tissue/h) and Gluc (0.85±0.04 nM H2O2/mg tissue/h) *vs* control (0.34±0.09 nM H2O2/mg tissue/h) exposure together with impairment of endothelium-dependent relaxation. Co-incubation with black chokeberry extracts (Dr and Fr) dose-dependently decreased ROS generation (with 30-80%) and significantly improved the endothelium-dependent vascular relaxation. In conclusion, both characterized black chokeberry extracts elicited vascular protective effects in conditions associated with increased ROS production after Ang2, LPS and Glu stimulation.

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Antibacterial effects of phytochemicals from *Ocimum Gratissimum*

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Aim: The research focused on the investigation of the phytochemical composition and the *in vitro* antibacterial activities of the crude extracts and isolated compounds from *Ocimum gratissimum*.

Methodology: The structures of the isolated compounds were elucidated based on the IR, MS and NMR spectroscopic data.

Results: The investigation resulted in the isolation of stigmast-5-en-3β-ol (1) and cholest-5-en-19-oic, 3β-hydroxyl (2), isolated for the first time in this species. The compound (2) exhibited promising antibacterial activities against *Staphylococcus aureus* with growth inhibition of 70.81%.

Conclusions: The results validate the use in traditional medicine and the isolated compounds could be good candidates for conventional medicine.

Variation of main phenolic compounds in *Agrimoniae Herba*

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Aim: *Agrimonia eupatoria* L. is a widespread in Europe and well-known medicinal plant in the folk medicine of Bulgaria. Its therapeutic effects include: antibacterial, antiviral, anti-inflammatory, antioxidant, hepatoprotective, antidiabetic etc. and it is used in treating conditions such as diarrhea, liver diseases, cholecystitis, cholestasis, pyelonephritis, bleeding disorders, skin defects, inflammations of the oral mucosa, hemorrhoids and others. The effects of a plant, as well as its quality, correspond to its chemical composition, more specifically to its secondary metabolites. Agrimony is known for containing different phenolic compounds, which play an essential role in numerous biological processes and have different effects. By phytochemical quantitative analysis, the quality of the plant material could be determined. Thus, the aim of the current study was to quantify and compare different groups of phenolics in wildly grown agrimony.

Methodology: Agrimony was collected from the nature during its different ontogenetic phases as it flowers from May to August. Two locations in North-East floristic region of Bulgaria were chosen for localities of agrimony for the present study. Collected plant material included aerial flowering parts together with leaves from the lower part of the plant stem. After obtaining the herbal material, it was air-dried in darkness at room temperature and grounded and sieved prior to the spectrophotometric analyses. Analyses were done in accordance with European Pharmacopoeia where the used method for quantifying different classes of phenolic compounds was spectroscopy.

Results: The total polyphenol, tannin and phenolic acids content of agrimony were analysed. The results for both localities showed rise in the polyphenols and tannins quantity in June in comparison with May. The quantity of phenolic acids was close to or even higher than that of tannins which was expected considering its effects. Flavonoids were present in the lowest amounts.

Conclusions: The formation of plant secondary metabolites (SMs) depends on environmental factors and varies during different ontogenetic phases of plants. Due to the simplicity and low cost of spectrophotometry, this method could be integrated as a tool to quality control leading to production of herbal products with clinical curative effects.

New findings regarding phytochemical and bioactive profile of *Hypericum* species from romanian spontaneous flora: case of *H. Alpigenum*, *H. Perforatum* and *H. Rochelii*

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Aim: To fill the research gap which exists in scientific literature, a comparative evaluation between *H. alpigenum* Kit., H. *perforatum* L. and *H. rochelii* Griseb. & Schenk. was made in the present study.

Methodology: Two types of herbal preparations were obtained from the aerial parts of the above-mentioned species using maceration and magnetic stirring assisted extraction (MSA). Their phenolic profile was characterized using a LC-DAD-ESI/MS^2 method, while the *in vitro* antioxidant potential was evaluated using five complementary assays (TEAC, FRAP, DPPH, TBARS and OxHLIA). The extracts were also tested for their enzyme-inhibitory potential (anti-tyrosinase, anti-glucosidase and anti-cholinesterase activities), their antimicrobial activity being assessed against six bacterial and six fungal strains using microdilution method.

Results: Phenolic acids, flavan-3-ols and flavonoid derivatives were found as main constituents of the analyzed extracts. All tested herbal preparations showed a moderate anti-glucosidase and anti-cholinesterase effect, while their inhibitory potential against tyrosinase was weak or inexistent. *H. rochelii* was found as a rich source of phenolic acids and myricetin derivatives, showing an important antioxidant, anti-cholinesterase, and antibacterial activity.

Conclusions: Our work emphasizes for the first time a detailed description of *H. rochelii* phenolic fractions, including their phytochemical and bioactive characterization.

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The biological activity of bark extracts from *Quercus* species

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Aim: The literature shows that many plant species have been tested to obtain extracts with high content of bioactive compounds [1-3]. The aim of this work is to evaluate the biological potential of oak (*Quercus cerris, Q. rubra*) bark extracts.

Methodology: The bark extracts (aqueous / ethanolic) were obtained by two extraction methods, ultrasonic assisted extraction (UAE) and microwave assisted extraction (MAE). The total content of polyphenols and tannins was determined by classical methods.

The antioxidant activity was evaluated by two complementary methods (1,1-diphenyl-2-picrylhydrazyl - DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid – ABTS assays). The antimicrobial activity was evaluated against five bacteria (*Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) and three *Candida* species (*C. albicans, C. parapsilosis, C. krusei*). The effect on biofilm formation and synergism with gentamicin was also evaluated.

The enzymatic inhibitory potential was evaluated for α-glucosidase, tyrosinase and acetylcholinesterase.

Results: The results indicated that the bark extracts obtained by MAE had higher contents of polyphenols and tannins, compared to the UAE bark extracts. All of the oak extracts exhibited antioxidant activities. The antibacterial activity was demonstrated against

Gram-positive bacteria and Gram-negative bacteria with a minimum bactericidal concentration (0.3 mg/mL) against *S. aureus*. The antifungal activity has been shown against *C. parapsilosis* and *C. krusei*. The extracts exert synergic effects with gentamicin against MRSA.

The antibiofilm results show that at the MIC, extracts inhibited the formation of biofilms produced by MRSA. The enzymatic inhibitory effects evaluation showed activity against α-glucosidase, tyrosinase and acetylcholinesterase.

Conclusions: The results show that the oak bark might be an important source of bioactive compounds that can be used for their antioxidant, antimicrobial and enzime inhibitory activity.

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Effect of grape seed powder on noodle quality and its mechanism

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Aim: Grape seeds are one of the main components of wine industry waste and have a high level of nutritional content and biological activity. Here the effect of grape seed powder (GSP) on noodle quality and its mechanism were investigated.

Methodology: Noodles were prepared using wheat flour supplemented with 1%, 3%, and 5% GSP. The farinograph properties, textural properties, edible quality, rheological properties, antioxidant and *in vitro* digestion properties of the noodle, and thermal properties of the gluten were determined. The microstructure was analyzed by scanning electron and atomic force microscopy, and the effects of the addition of GSP on the physicochemical and structural properties of wheat gluten protein and starch structure were analyzed.

Results: Adding 1%-GSP improved the dough's farinograph properties, textural properties, and rheological properties to varying degrees, reducing the cooking losses of the noodles. 1% GSP promoted the aggregation of gluten proteins by promoting hydrophobic interactions and hydrogen bonding, thus enhancing the noodle quality. Whereas, 3% and 5% GSP addition disrupted the disulfide bonds between gluten protein molecules and formed macromolecular aggregates linked to gluten proteins through non-covalent bonds and hydrophobic interactions, which prevented the formation of the gluten protein reticulation structure. Also, XRD, SEM, and iodine binding analyses show that GSP added alters the microstructure of the starch granules, thereby reducing the rate of *in vitro* digestion of starch, and the starch was wrapped in the protein-sugar-oil film.

Conclusions: Our study emphasized on the interaction between wheat proteins, wheat starch, and GSP in the noodle-making dough.

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LC-HRMS/MS analysis and potential antitumor effects of *Vernonia Kotschyana* Sch. Bip. ex walp extracts

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Aim: The present study aimed to investigate the antitumor activity of four extracts obtained from *V. kotschyana* roots (chloroform (VC), ethyl acetate (VEA), ethanol (VE) and water (VA) extracts) on the cell viability, intracellular reactive oxygen species (ROS) levels, cell cycle and apoptosis in human breast adenocarcinoma MCF-7, prostate adenocarcinoma PC3 and alveolar adenocarcinoma A549 cells. To correlate the observed biological outcomes with the phytochemical composition, metabolite profile of the extracts was investigated using LC-HRMS/MS.

Methodology: Dried and powdered roots were extracted successively with solvents of different polarities. Cell viability was assessed using the MTT colorimetric assay. The DCFH-DA assay was performed in order to evaluate the influence of the extracts on intracellular ROS production. Cell cycle analysis was performed using flow cytometry. LC-HRMS/MS phytochemical profiling was performed on an Agilent 1200 HPLC system. A Phenomenex Gemini C18 column was used. MS analysis was done in positive ion mode.

Results: At maximum tested concentration of 100 µg/mL, VC and VEA extracts significantly decreased the viability of cancer cell lines (cell viabilities between 30.92 ± 4.12% and 67.93 ± 2.98%), whereas VE and VA extracts were generally less effective. In addition, VC increased the intracellular ROS levels by 2.1-3.8-fold, leading to a clear accumulation of apoptotic cancer cells at the sub-G1 phase. The LC-HRMS/MS profiling of the four extracts revealed two categories of phytochemicals. Caffeoylquinic acids were majorly observed in VEA, VE and VA, whereas highly-oxygenated stigmastane-type saponin glycosides were predominantly present in VC and VEA.

Conclusions: It can be assumed that the latter class of phytochemicals could play a central role in the observed cancer cell toxicity.

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Influence of pedoclimatic conditions on bioactive compounds content from different plant organs extracts of *Taraxacum* sp.

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Aim: Herbal product-derived compounds present a great structural diversity, which is not commonly seen in synthetic compounds. This plant-based remedies initially provided in forms of tinctures, teas, poultices, powders, now serve as the basis of novel phytopharmaceutical products. Plants from the Asteraceae family have been used everywhere in the world for thousands of years and continue to provide mankind with new remedies. A representative of this family, *Taraxacum* sp. (dandelion), can grow from the sea level to alpine zones, on almost every soil type and it can be collected twice a year, when the nutrient concentration is maximum: spring before flowering and autumn in the beginning. In the present paper we determined the bioactive compounds content in the hydroalcoholic extracts obtained from different plant organs of *Taraxacum* sp., product collected from south Dobrudja area, Romania, in September 2021, respectively May 2022.

Methodology: Fresh plant organs were dried at room temperature, grind to a fine powder and extracted 10% concentration in ethanol 50%, 70% and 96%, using cold maceration, respectively ultrasonication method, followed by filtration at normal pressure. Obtained hydroalcoholic extracts were analysed with Multiparameter Edge, Hanna Instruments, for *p*H and electrical conductivity and by UV-Vis spectrophotometry for total polyphenols, carotenoids, tannins and anthocyanins content determination. Also, for total antioxidant capacity assessment of vegetal extracts, the photochemiluminescence method through ACL procedure of Analytik Jena AG, Germany, has been used.

Results: The obtained results emphasize an almost linear dependence of total polyphenols content and total antioxidant capacity for the hydroalcoholic extracts, more increased values in 70% ethanol extracts, in both harvest periods. Also, the highest values of bioactive compounds content, in case of ultrasonication extraction method, were registered.

Conclusions: Further research is needed to evaluate in detail the pedoclimatic conditions influence on bioactive compounds from hydroalcoholic extracts of *Taraxacum* sp., to confirm the difference between therapeutic potential of different plant organs of this valuable product of Dobrudja spontaneous flora.

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Phenolic content and *in vitro* antioxidant activity of three *Russula* species

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Aim: The ethanolic and hydromethanolic extracts from three *Russula* species growing in Romania, i.e. *Russula cyanoxantha* ((Schaeff.) Fr.), *Russula mustelina* (Fr.) and *Russula xerampelina* ((Schaeff.) Fr.) were analyzed in terms of phenolic content and antioxidant activity (*in vitro* studies).

Methodology: The total phenolics were quantified using Folin-Ciocalteu assay. The antioxidant activity was studied by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals scavenging, reducing power, ferrous ion chelating and 15-lipoxygenase inhibition assays.

Results: The highest content in total polyphenols was recorded for hydromethanolic extracts (aprox. 20 mg/g for each mushroom species). The hydromethanolic extracts showed higher free radical scavenging and reducing abilities. The hydromethanolic extract from *Russula mustelina* was the most active as scavenger of DPPH radical (EC_{50}=652.2±2.8 µg/mL) while the hydromethanolic extract from *Russula cyanoxantha* was the most active as scavenger of ABTS radical and reducing agent (EC_{50}=97.37±0.55 and 142.24±1.13 µg/mL, respectively). The ethanolic extracts proved to be more efficient in the ferrous ion chelation and 15-lipoxygenase inhibition assays. The ethanolic extract of *Russula mustelina* showed a remarkable ferrous ion chelating capacity, only 7.2 times lower than that of the positive control, EDTA (EC_{50} = 46.1±0.2 µg/mL vs. 6.34±0.06 µg/mL) and 15-lipoxygenase inhibition, only 6.9 times lower than that of the positive control, caffeic acid (EC_{50} = 202.8±1.3 µg/mL vs. 29,1±0,3 µg/mL).

Conclusions: The extracts showed good antioxidant potential in *in vitro* models and therefore they are promising candidates for the development of dietary supplements and pharmaceutical products.

Chemical composition of the ethanolic extracts of the aerial parts and flowers of *Hypericum Perforatum* l. from Republic of Moldova

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Aim: Identification and quantification of flavonoids in dried ethanolic extracts of *H. perforatum* vegetal products using the HPLC-DAD method.

Methodology: The aerial parts and flowers of *H. perforatum* were collected from wild plants and cultivated plants at the Scientific Practical Centre in the Field of Medicinal Plants, "Nicolae Testemițanu" State University of Medicine and Pharmacy. The extracts were obtained with 70% ethanol from *Hyperici herba* and with 80% ethanol from *Hyperici flores* by maceration with stirring. The flavonoids (rutoside, hyperoside, quercitroside, quercetin, I3,II8-biapigenin) were quantified by HPLC-DAD method (Agilent Technologies 1200 series liquid chromatograph, DAD G1315B, Zorbax Eclipse XBD-C 8 analytical column).

Results: In the extracts, the predominant flavonoid was rutoside, followed by hyperoside, quercitroside, quercetin and I3,II8-biapigenin. A higher content of rutoside was determined in *Hyperici herba* extracts (6.41-7.02%) in comparison to *Hyperici flores* extracts (4.25-4.99%). The levels of hyperoside (2.54%), quercitroside (1.47%), quercetin (1.19%) and I3,II8-biapigenin (1.49%) in the extract from flowers (collected from wild flora) were found to be higher in comparison to other extracts. In both flower extracts (collected from wild and cultivated flora), the concentrations of I3,II8-biapigenin (1.49 and 0.62%, respectively) were higher than the ones determined in the extracts obtained from *Hyperici herba* (0.18 and 0.31%, respectively).

Conclusions: *H. perforatum* flowers can serve as a source of I3,II8-biapigenin, which, according to literature data, has antidepressant and anxiolytic activity.

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The antioxidant activity of *Solidago* l. species from the Republic of Moldova flora

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Aim: The comparative chemical study of the antioxidant activity *in vitro* in dry extracts obtained from vegetal products of *Solidago virgaurea* and *S. canadensis* species from the Republic of Moldova flora.

Methodology: Two complementary *in vitro* chemical methods have been used in order to assess the antioxidant activity of *Solidago* species: ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay and the Ferrozine Test (assay of iron-binding antioxidant capacity).

Results: The ABTS assay showed that leaf extracts from both *Solidago* species demonstrated the highest antioxidant activity (*S. canadensis* – 44.17 µM TEAC, *S. virgaurea* – 34.31 µM TEAC), followed by aerial part extracts (*S. canadensis* – 39.32 µM TEAC, *S. virgaurea* – 33.25 µM TEAC) and flower ones (*S. canadensis* – 35.37 µM TEAC, *S. virgaurea* – 30.92 µM TEAC). The comparative analysis of the antioxidant activity by the assay of iron-binding antioxidant capacity demonstrated an insignificant difference in the data results for the vegetal products of *S. canadensis*: the leaves were highlighted with a maximum chelation capacity (81.49%), followed by the aerial parts (80.19%) and flowers (79.65%). The same consecutiveness was maintained for the vegetal products of *S. virgaurea*: the maximum chelation capacity was recorded for leaves (80.19%), followed by aerial parts (79.55%) and flowers (79.01%).

Conclusions: The current study demonstrated a high antioxidant activity for all the analyzed *Solidago* extracts. At the same time, we mention the fact that *Solidago* leaf extracts were distinguished by a significantly increased antioxidant activity in the case of both methods used. These results are starting points for further research that would investigate the antioxidant potential of *Solidago* species from the Republic of Moldova flora.

Effect of non-thermal plasma activated water on the accumulation of bioactive compounds in wheat sprouts

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Aim: This study aimed to evaluate the effect of non-thermal plasma activated water (PAW) on the accumulation of bioactive compounds in wheat sprouts.

Methodology: The wheat caryopses were treated with non-thermal PAW containing 25, 35 and 50 mg/L of nitrate (PAW25, PAW35 and PAW50, respectively). The germination was conducted for 8 days at 20°C. Samples collected at days 1, 2, 3, and 8 were assessed regarding the contents in free and bound polyphenols, total proteins, carotenoids, photosynthetic pigments, and DPPH radical scavenging capacity [1, 2].

Results: A notable increase in free polyphenol content was determined in wheat samples treated with PAW25 at day 8 (121.19 mg% compared to 108.49 mg% in control). All non-thermal PAW treatments significantly enhanced the bound phenolic content in samples at day 3 (125.94 mg%, 119.32 mg% and 129.00 mg% for PAW25, PAW35 and PAW50, respectively compared to 83.81 mg% in control). PAW35 caused an important increase in chlorophyll and carotenoid contents (15.16 mg/g compared to 10.46 mg/g in control and 3.12 mg/g compared to 1.43 mg/g in control, respectively). All PAW treatments enhanced the total protein content. A burst in the antioxidant capacity was noted in the bound phenolic extract from samples treated with PAW25 (day 8, 20°C) (91.12% *vs.* 56.49% for control).

Conclusions: PAW25 and PAW35 treatments showed positive effects on wheat sprouts with respect to bioactive compounds accumulation.

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Anticandidal activity of *Calamintha Menthifolia* host. essential oil

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*A*im: Species of the genus *Candida* are opportunistic pathogens that can become pathogenic and cause infections, especially in immunocompromised patients [1]. These infections can be superficial, affecting the skin or mucous membranes, or invasive, which are associated with high mortality. Due to the development of resistance of many species of *Candida* to antifungal drugs [2], there is a need for new anticandidal drugs that can protect the human or animal body from diseases with minor or no side effects and such an alternative may be plant metabolites [3]. The purpose of this study was to determine the antifungal activity of the *Calamintha menthifolia* essential oil on 5 strains of *Candida.* Methodology: *Calamintha menthifolia* Host. was grown on the experimental plot of the Rice Institute of NAAS of Ukraine (Kherson region) by Dr. Lyudmyla Svydenko and provided to us for research. Oil from freshly harvested and dried flowering shoots of *C. menthifolia* was isolated using the Clevenger-type apparatus. The impact of *C. menthifolia* Host. essential oil on five strains of *Candida*: *C. curvata*, *C. pseudotropicalis, C. parapsilosis, C. tenuis, C. kefir* was investigated. Sensitivity of *Candida* to oil was determined by the standard agar well diffusion method. Lavender essential oil, Fluconazole, Chlorophyllipt, Eucalyptus tincture, Decasan were used as controls.

Results: Comparison of the areas of stunted grows make it possible to show that essential oils both of fresh and dried *C. menthifolia* upground parts inhibit or stop the growth of the studied *Candida* strains. Application of 0.01 ml oil of freshly raw material completely suppressed the growth of all studied strains. The addition of 0.01 ml of dry raw material oil completely inhibited the growth of *C. curvata, C. pseudotropicalis, C. tenuis,* and partially *C. parapsilosis* and *C kefir*.

Conclusions: *Calamintha menthifolia* Host. essential oil possesses pronounced anticandidal effect and is a promising basis for creating drugs.

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Analysis of the elemental composition of *Actinidia Arguta* lindl. leaves

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Aim: Kiwiberry fruits (*Actinidia arguta* Lindl.) are used as a vitamin product and laxative, andtheir leaves are promising raw materials. Minerals are also extracted from plant raw materials during obtaining total extracts. Macro- and microelements affect the accumulation of secondary metabolites in plants and can be used for the prevention and treatment of mineral deficiency. The aim of our work was to study the elemental composition of *A. arguta* leaves.

Methodology: *A. arguta* leaves were harvested in the research area of the Department of Fruit Plants Acclimatization of the M.M. Gryshko National Botanical Garden in August 2020. Determination of the qualitative composition and quantitative content of the elements was performed by inductively coupled plasma atomic emission spectrometry using iCAP 7000 Duo.

Results: The content of 18 macro- and microelements was determined. It is established that the accumulation of macro- and microelements in *A. arguta* leaves occurs according to the following pattern: Ca (15400 mg/kg) > P (11154 mg/kg) > Mg (4007 mg/kg) > Si (195.1 mg/kg) > Ba (140.6 mg/kg) > Fe (93.02 mg/kg) > Al (90.97 mg/kg) > Mn (85.43 mg/kg) > B (83.01 mg/kg) > Zn (34.44 mg/kg) > Cu (18.03 mg/kg) > Ni (1.940 mg/kg) > Mo (0.95 mg/kg) > Se (0.698 mg/kg) > Li (0.58 mg/kg) > Cr (0.497 mg/kg) > V (0.097 mg/kg) > Na (<0.001 mg/kg). The highest content was found for calcium, phosphorus and magnesium. Heavy metals were found in small amounts: Pb (1.18 mg/kg), As (< 0.001 mg/kg), Cd (< 0.001 mg/kg), Hg (0.026 mg/kg). The obtained data do not exceed the permissible content of these elements in plant-based dietary supplements regulated by the State Pharmacopoeia of Ukraine 2.0.

Conclusions: *A. arguta* leaves can be a source of calcium, phosphorus and magnesium, does not accumulate toxic metals, which makes it possible to use it as a medicinal plant raw material to create new dietary supplements.

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Phytopharmacognostic standardization of *chamaenerion Angustifolium* (l.) scop. leaves

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Fireweed leaves (*Chamaenerion angustifolium* (L.) Scop syn. *Epilobium angustifolium* L.) – are a popular herbal drug available on European market. The commercially available plant drug "Epilobii herba" contain the dried aerial parts of several *Epilobium* species [1]. Herbal medicines are fast gaining popularity. However, their acceptability by modern practitioners is low, which is often due to lack of standardization [2]. Aim: Development of standardization approaches for plant raw materials. Methodology: The methods for standardization of fireweed leaves tested using 8 series of raw materials are presented (samples from Ukraine, Lithuania, Poland). Identifications A and B are proposed to be carried out by morphological and anatomical features of raw material. Thin layer chromatography of methanolic extract in a mobile phase containing anhydrous formic acid, water, ethyl acetate (8:8:68 V/V/V) with detection by spraying with a 10 g/L solution of diphenylboric acid aminoethyl ester in methanol and then a 50 g/L solution of macrogol 400 in methanol and examining in ultraviolet light at 365 nm were tested as identification C. Results: Two blue and five yellow fluorescent zones have to be presented in the chromatogram obtained with the test solution. Since ellagitannins are considered to be the major biological active compounds contributing to pharmacological activity of fireweed leaves, determination of tannins in herbal drugs assay (European Pharmacopeia method 2.8.14) was selected as method for qualitative control of raw material. Content of minimum 1.0 per cent tannins expressed as pyrogallol was defined as a quality criterion. Conclusions: The obtained results could be used for development monograph on fireweed leaves for The State Pharmacopoeia of Ukraine.

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Prospects for using species of the genus *Pentaphylloides* duham in phytotherapy

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The use of medicinal plants and their medicinal preparations is due to the fact that, with the correct dosage, they are practically non-toxic, harmless, relatively affordable, effective and, in some cases, due to their complex action, they have no competitors. Significant resources, the availability of raw materials, and the possibility of cultivation make plant raw materials a promising object of research for the purpose of developing new medicinal products of plant origin.

Aim: Study of the chemical composition of species of the genus *Pentaphylloides* Duham and prediction of their pharmacological activity.

Methodology: When evaluating the qualitative composition of raw materials of species of this genus, generally accepted methods were used: identification reactions, chromatography, which allowed to determine promising directions of pharmacological action.

Results: Two types of Shrubby Cinquefoil, which belong to the genus *Pentaphylloides* Duham, were studied: *Pentaphylloides fruticosa* (L.) O. Schwarz and *Pentaphylloides mandshurica* (Maxim.) [1, 2]. The results of our research showed the presence of tannins, ascorbic acid, carotene, flavonoids, organic acids, resins, essential oils, which determine the therapeutic and preventive properties. Plants of the species *Pentaphylloides* Duham have bactericidal, anticoagulant, antiallergic, hepatoprotective, radioprotective, antiviral, immunostimulating, and antidiabetic properties.

Conclusions: Analyzing the obtained research results, we concluded that the complex of substances contained in plants act polyvalently, stimulating various body systems or compensating for their insufficient function. The rationale for the use of phytoremedies from this medicinal plant material is its availability and economic attractiveness, as well as the introduction into medical practice of new drugs with maximum therapeutic effect and minimal side effects is one of the main tasks of modern pharmacy.

Study of neurotropic properties of herbal extracts of *Artemisia* species

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Medicinal products of plant origin, in comparison with synthetic drugs, have a moderate sedative effect on the central nervous system, but at the same time do not cause drowsiness, addictive phenomena, movement disorders manifested by disorders of their coordination. The therapeutic effect of medicinal products of plant origin is related to the content of biologically active substances in them, which belong to different classes of natural chemical compounds [1].

Aim: study of neurotropic properties of herbal extracts of *Artemisia* (*A.*) *absinthium* and *A. vulgaris.*

Methodology: Dry extracts were used for research *A. absinthium* and *A. vulgaris* (the extractant - 70 % ethanol). Study of neurotropic properties of herbal extracts *A. absinthium* and *A. vulgaris* were conducted on 30 non-linear sexually mature male mice weighing 18 - 20 g, which were divided into 5 groups of 6 animals in each. The studied extracts *A. absinthium* and *A. vulgaris* was administered intraperitoneally in doses of 25 mg/kg and 50 mg/kg body weight respectively (I - IV groups of animals) and the comparison drug - St. John's wort tincture at a dose of 50 mg/kg body weight (V group of animals*)* [2].

Results: When evaluating the behavioral reactions of mice in the "Open field" test a significant influence of the investigated extracts on the motor activity of animals was established. The most pronounced approximate research activity was shown by the extract of *A. vulgaris*, which in a dose of 25 mg/kg activated the locomotion of mice against the background of increased psycho-emotional excitement. The indicator of general motor activity increased mainly through a large number of horizontal movements. Grooming in animals that received the extract of *A. vulgaris* significantly increased in comparison with the control. Administration of extract of *A. vulgaris* did not cause at all animals dying down, and the extract of *A. absinthium* in its action approached the action of St. John's wort tincture.

Conclusions: The extract of *A. vulgaris* prevailed over the extract of *A. absinthium* in terms of its neurotropic activity. The most effective was the use of *A. vulgaris* extract at a dose of 25 mg/kg.

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Comparison of the chemical composition of lipophilic extracts of *Lycoperdon pyriforme* Schaeff. and *Scleroderma aurantiacum* L. ex Pers

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Scleroderma aurantiacum L. ex Pers is sometimes mistakenly harvested instead of edible Lycoperdon species (Lycoperdon pyriforme, L. perlatum, L. umbrinum, L. pratense).

Also, Scleroderma species can be mistaken with truffles by inexperienced mushroom hunters. Ingestion of Scleroderma species can cause gastrointestinal distress in humans and animals, and some individuals may experience lacrimation, rhinitis and rhinorrhea, and conjunctivitis from exposure to its spores. According to other data, the use of fungi of the genus Scleroderma cause muscarinic symptoms on the part of the nervous system. The nature of the poisonous substance is not determined. However, it does not disappear when boiling mushrooms and draining the broth. This suggests that this substance is insoluble in water and can withstand temperatures of at least 100°C and may be lipophilic.

Aim: The aim of the study was to compare the chemical composition of lipophilic extracts of Lycoperdon pyriforme and Scleroderma aurantiacum.

Methodology: The fruiting bodies of the dried mushrooms were extracted with hexane in a Soxhlet apparatus. The extract was concentrated, filtered, evaporated and weighed. After dissolution, it was analyzed by gas chromatography-mass spectroscopy (GC-MS).

Results and Conclusions: The weight of dried fruiting bodies of L. pyriforme was 10.58 ± 0.45% of the weight of freshly harvested. The weight of dried fruit bodies of S. aurantiacum was 21.34 ± 0.55%, which is abnormally high for fungi of the Basidiomycota division. The mass of lipophilic substances (% by weight of dried raw materials) for L. pyriforme was 0.75%, and for i was 1.22%. GC-MS revealed 14 substances in L. pyriforme and 25 substances in -. More than 68% of higher fatty acids were found in the lipophilic extract of L. pyriforme, in particular, the content of Linoleic acid was 48.37%. The content of steroids (ergosta-5,7,22-trien-3-ol and ergost-5-en-3-ol) was 15.04%. In S. aurantiacum the content of fatty acids was ≈ 30%, and the content of ergosta-5,7,22-trien-3-ol was 25.99%. Among the toxic components of the lipophilic extract of S. aurantiacum, 4-cyanothiopyran and o-cresol have been identified. Their content was 1.82% and 2.74%, respectively, of the total mass of the extract.

It is concluded that the lipophilic extract from the fruiting bodies of S. aurantiacum does not contain a significant amount of highly toxic components, and their total mass in the lipophilic extract is insufficient for the manifestation of such toxic effects as these fungi.

HPLC analysis of catechins in *Quercus Rubra* and *Parthenocissus Quinquefolia* raw materials

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Aim: to determine the qualitative composition and quantitative content of catechins in *Quercus rubra* L. and *Parthenocissus quinquefolia* (L.) Planch. raw materials for detection of new promising raw material source for the development of phytomedicines and dietary supplements.

Methodology: *Q. rubra* leaves and annual shoots and *P. quinquefolia* leaves and fruits used for investigation were harvested in Kyiv region, Ukraine, in September 2021. Determination of the qualitative composition and quantitative content of catechins was performed by high performance liquid chromatography (HPLC) using an Agilent Technologies 1200 liquid chromatograph (USA) with a diode-matrix detector with signal registration at 280 and 365 nm and fixation of absorption spectra in the range of 210–700 nm [1].

Results: using HPLC, 4 catechins in the *Q. rubra* leaves and annual shoots and *P. quinquefolia* leaves and fruits were identified, namely: catechin, epicatechin, epicatechin gallate, gallocatechin. The results of chromatographic separation are given in table 1.

Table 1
The content of catechins in *Q. rubra* and *P. quinquefolia* raw materials

No	Substance	Quantitative content, mg/100 g in terms of absolutely dry raw materials		
		Q. rubra leaves and shoots	*P. quinquefolia* leaves	*P. quinquefolia* fruits
1	Catechin	2.28	2.85	3.16
2	Epicatechin	1.54	3.34	3.89
3	Epicatechin gallate	25.45	1.32	2.29
4	Gallocatechin	1.67	3.08	1.05

Analysis of the results shows that content of catechins in the studied samples of raw materials of *P. quinquefolia* is similar and rather small. It is worth noting that the highest of quantitative content among catechins is epicatechin gallate (25.45 mg/100g) in the *Q. rubra* leaves and shoots. Epicatechin gallate is characterized by

pronounced antioxidant, anti-inflammatory properties, established its anticancer activity and potentially it also can be used in treatment of Parkinson's disease [2, 3].

Conclusions: the qualitative composition and the quantitative content of catechins have been determined in *Q. rubra* leaves and annual shoots and *P. quinquefolia* leaves and fruits. The results obtained can be used for standardization of *Q. rubra* leaves and shoots and it is advisable to further investigate its antioxidant and anti-inflammatory activity.

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Prenylated flavonoids combating mycobacteria

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Aim: Tuberculosis (TB) is one of the leading causes of death worldwide [1]. Multidrug-resistant *Mycobacterium tuberculosis* and non-tuberculous strains emphasize an urgent need for investigating new antibiotics.

Methodology: Twelve compounds were evaluated for antibacterial activity against three non-tuberculous mycobacterial strains. The minimum inhibitory concentration (MIC) was measured by the microdilution method as the lowest concentration of antimicrobial agent that kills 100% of bacteria. To reveal the mechanism, membrane integrity assays were performed using two fluorescent dyes. The effect on cell viability of THP-1 cells differentiated into macrophages was evaluated using WST-1 assay.

Results: MIC of flavonoids showed significant antimicrobial activity in a range of 2 – 16 μg/mL. MIC values of morusin, kuwanon C and kuwanon U were equal, even lower than MIC of control antibiotic isoniazid. In comparison, four Diels-Alder adducts demonstrated lower antimicrobial potential. Most of the twelve natural compounds did not significantly alter membrane permeability at ¼ of MIC. At MIC, the assay revealed the major membrane disruptive effect of diplacone (40%) and six other compounds showed lower potential. Kuwanon H, albanol B and morusinol possibly interacted with applied fluorescent dyes. All compounds affected the viability of macrophages with their IC_{50} values greater than MIC. Morusin, kuwanon U and kuwanon C showed comparable toxicity against bacteria and macrophages (± 1.5 μM) and are considered to be model agents for the future investigation of less toxic anti-tuberculous drugs.

Conclusions: Natural compounds are promising candidates for novel antimycobacterial agents. The membrane disruption is one, but not the only mechanism of their antibacterial activity. Despite higher toxicity, compounds provide a source of structural templates for potent antimycobacterial treatment.

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Betulinic acid decreased glucose-induced lipid accumulation in *Caenorhabditis Elegans via* modulation of *NHR-49* expression

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Aim: The present study aimed to confirm our prior *in vitro* investigation of the anti-adipogenic potential of betulinic acid (BA) in human adipocytes [1] through *in vivo* validation. Glucose-supplemented media was used for induction of lipid accumulation in *Caenorhabditis elegans*, followed by treatment with BA (10, 25 and 50 μM).

Methodology: Phenotypic analyses for lifespan, locomotion and brood size were performed for assessment of BA effect on the worm physiology. Therefore, accumulated lipids were quantified through Oil red O and Nile red lipid staining techniques and quantification was performed *via* Image J software. Finally, expression of key for *C. elegans* genes and microRNAs associated with lipid metabolism was calculated by $_{\Delta\Delta}$CT method [2].

Results: Betulinic acid did not alter lifespan, the progeny production, or the locomotion of *C. elegans.* Both staining techniques showed similar tendency for dose-dependent and significantly reduced lipid accumulation. Furthermore, on transcriptional level, BA treatment with 10 μM demonstrated increased triglyceride hydrolysis and β-oxidation through upregulation of *nhr-49*, *atgl-1*, *acs-2* and *aak-2*, whereas the treatment with 25 and 50 μM BA revealed lipogenesis inhibition *via* downregulation of *pod-2*, *nhr-49* and subsequent repression of *fat-5*, *fat-6* and *fat-7* gene expression profiles. Interestingly, the expression of *lin-4* and the intestinal *miR-60* were significantly downregulated in all of three concentrations.

Conclusions: Obtained results confirmed our prior proposition that betulinic acid is a promising candidate in obesity management.

Acknowledgements

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Is there a power behind the throne of deep purple? - *Basella Alba in vitro* cultures

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Due to the original phytochemical profile of the Malabar spinach (*Basella alba* L.), classical biotechnology methods were used to obtain cell and organ *in vitro* cultures. Various combinations of plant growth regulators (PGRs) were used to stimulate both the morphogenetic and metabolic response. Additionally, the antioxidant capacity was monitored.

Shoots of *Basella alba* and its red-stem form – *B. rubra*, were cultured on Murashige and Skoog (MS) liquid media supplemented with three cytokinins in the following concentrations: 6-benzylaminopurine (BA): 1.0, 2.0, 4.0 mM; kinetin (KIN): 1.0, 2.0, 4.0 mM or tidiazurone (TDZ): 0.01, 0.05, 0.1 mM, together with one of the auxin - indoleacetic acid (IAA) in the concentration of 0.5 mM. PGRs-free MS medium was considered as a control. After 4, 8 and 12 weeks of culture data on the biomass production as well as metabolites content were recorded. The photosynthetic pigments and the total polyphenolic contents (TPCs) were determined using spectrophotometric technique. The antioxidant capacity was estimated with the use of DPPH, ABTS and FRAP assays. Phytochemical analysis was performed using UHPLC-DAD-QTOF-MS technique.

The addition of BA and KIN significantly increased the multiplication rate of both forms of the species compared to the control media. The highest biomass production was noted for *B.rubra* cultured on media containing BA 1.0 and 2.0 mM, while KIN 1.0 mM supplementation promoted rhizogenesis in both forms. PGRs affected chlorophyll content in a concentration-dependent manner. In all tested conditions, polyphenolic content was 2-5-fold higher in roots than in leaves, with *B. rubra* being the TPCs-richer form. All samples displayed moderate antioxidant potential by the DPPH, whereas in ABTS and FRAP assay plants grown on KIN 2.0 and 4.0 mM displayed significantly higher antioxidant activity.

B.alba shoot cultures can be considered as a rich source of polyphenolic compounds. Further studies on the cell, tissue and organ cultures of the species are needed to optimize the culture conditions for effective specialized metabolites production and their bioactive potential.

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In vitro/in vivo antioxidant and hepatoprotective activity of *Phlomis Tuberosa*

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Aim: To evaluate *in vitro/in vivo* pharmacological effects of a purified extract and a flavonoid fraction from the aerial parts of *Phlomis tuberosa* (Lamiaceae).

Methodology: For the *in vitro* assay, rat hepatocytes were obtained by two-step collagenase perfusion. They were treated with the samples at different concentrations, alone and after oxidative stress induced by *tert*-BuOOH. Cell viability, as well as levels of lactate dehydrogenase (LDH), reduced glutathione (GSH) and malonedialdehyde (MDA) were measured. For the *in vivo* study, rats were treated with purified extract at different concentrations after carbon tetrachloride (CCl_4) intoxication. The levels of MDA and GSH in liver homogenate were determined. A pathohistological study on the livers was performed. Silymarin was used as a positive control in both models.

Results: Administered alone, in isolated rat hepatocytes, both samples did not exhibit a cytotoxic effect on the cell viability, the levels of LDH, reduced GSH and MDA. Under conditions of *tert*-BuOOH-induced oxidative stress in isolated hepatocytes, they exhibited pronounced, concentration-dependent, cytoprotective and antioxidant effects. The *in vivo* study showed that the extract had activity, commensurable to that of Silymarin. It lowered the LDH activity and MDA production and restored the level of GSH, affected by the toxic agent CCl_4. Liver histology was preserved, compared to animals treated only with CCl4 and the findings were similar to those in livers of Silymarin treated group.

Conclusions: The purified extract and flavonoid fraction from the aerial parts of *Phlomis tuberosa* could be used as a potential hepatoprotective agent.

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In silico and *in vitro* studies on the anticoronavirus activity of *Graptopetalum Paraguayense* e. Walter

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Aim: The objective of our work is to evaluate the *in vitro* activity of *Graptopetalum paraguayense* E. Walther (GP) against human alpha coronavirus and to elucidate the mode of action of the plant compounds on entrance protein, human aminopeptidase N, through docking approaches.

Methodology: According to the metabolic profile and GC-MS analysis [1] of the GP total extract, three main fractions: non-polar substances, polar metabolites, and phenolic compounds (PAF), were obtained. To determine the capacity of the extract and its fractions to inhibit the replication activity of human coronavirus strain 229E (HCoV-229E), as well as to evaluate the reduction of viability of infected or uninfected human lung fibroblasts (Lep) cells, the MTT colorimetric assay was used. Acids from the phenolic fraction were analyzed against human aminopeptidase N from 6ATK XRD structure, where it is in complex with HcoV-229E spike protein. Aminopeptidase N was subjected to molecular dynamic simulation in periodic boundary conditions using GROMACS. The equilibrated structure was further used for docking of our ligands using induced fit methodology as it is implemented in MOE software package.

Results: The GP extract and PAF do not have cytotoxic effect on human lung embryonal fibroblasts (Lep). The phenolic acid fraction effectively inhibited HCoV-229E replication in dose-dependent manner. Furthermore, it was a more effective inhibitor of HcoV-229E replication in cultured cells (62%), whereas the GP extract possessed lower effectivity (41%). The IC_{50} values range from 22.4±0.2 to 88.2±7.7μg/mL. We found that most of the phenolic acids form hydrogen bonds with amino acids, that are crucial for the hAPN receptor function. The complexes that demonstrate greater binding affinity are those with *trans*-ferulic acid, *p*-coumaric acid, and *p*-hydroxycinnamic acid.

Conclusions: According to the results obtained, the phytochemicals found in *Graptopetalum paraguayense* E. Walther are potential inhibitors of human coronaviruses and show promising results for discovering new anticoronavirus medicines. The total GP extract and the phenolic fraction did not show toxicity to the cell lines used, which is excellent evidence for safety.

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Immunological effect of modern hydrogels containing polymeric micelles as drug delivery systems for oregano essential oil

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Aim: The aim of this study was to assert the immunological effect of modern hydrogels containing polymeric micelles as drug delivery systems for oregano essential oil (O.v.)

Methodology: The 5% O.v. poloxamer-based binary hydrogel and the corresponding control formulation were prepared using Poloxamer 407 and Pluronic L-31 by the cold method and were characterised in terms of pH, organoleptic and rheological properties. Human peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation, seeded at a density of 7.5 x 106 cells per mL into 6-well plates and cultivated for 2 h in RPMI 1640 + Glutamax supplemented with 50 mM β-mercaptoethanol, 1 mM sodium pyruvate, 100 µg/mL streptomycin, 100 IU/mL penicillin and 2 nM HEPES in the presence of 10% autologous and heat inactivated serum. For the differentiation into dendritic cells (DCs), IL-4 (50 U/ml) and GM-CSF (80 U/ml) were added to the cell culture for 7 days. To test the effects of the O.v. formulation on DCs, different concentrations (0 - 200 µg/mL) of the 5 % O.v. formulation were added in the presence or absence of the inflammatory stimulus lipopolysaccharide (LPS) (250 ng/mL) for 24 h. The potential cytotoxic effects of O.v. on human DCs was assessed by flow cytometry analysis using DAPI staining for cell viability and Annexin V/7'AAD for early and late apoptosis detection. Cell culture supernatants were collected and analyzed by enzyme-linked immunosorbent assay for IL-6, IL-10, TNF-α and IL-23 according to the manufacturer´s recommendations.

Results: The results of pharmacotechnical tests revealed that the evaluated properties of 5% O.v. poloxamers-based hydrogel were in accordance with official requirements for semisolid cutaneous preparations. No harmful effect on the viability of DCs were detected following the incubation with different concentrations (0 - 200 µg/mL) of the 5% O.v. formulation. O.v. treatment in inflammatory DCs (+LPS) indicated a decrease in cytokine production of IL-6, TNF-α and IL-23, but no significant effect on IL-10 in any of the tested O.v. concentrations.

Conclusions: The tested formulation could be used as topical vehicle for O.v. and might have potential immunotherapeutic value to limit the inflammatory response in human DCs.

Polyphenols-loaded gastro-resistant complex particles based on biopolymers with biomedical applications

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Aim: The research aims to prepare new formulations by immobilizing polyphenols (curcumin/quercetin) in biopolymer-based gastro-resistant complex particles to increase their stability, bioavailability, and controlled and sustained release of the active principles in the colon to treat or prevent the inflammatory bowel disease.

Methodology: The polyphenols-loaded complex particles (ComPs) were prepared in two stages. First, the polyphenols-loaded chitosan nanoparticles (chNPs) were prepared by ultrasonication and ionic cross-linking with Na_2SO_4. In the second stage, the polyphenols-loaded chNPs were dispersed in a solution of gellan mixed with bovine serum albumin (BSA) at pH=10. The biopolymers solution was extruded, dropwise, in a solution of pH=2, and the ComPs were obtained by polyelectrolyte complexation. To increase the ComPs' structural stability, they were covalently cross-linked on the surface with aldehyde groups derived from the oxidized gellan (OG). The ComPs were characterized by FTIR spectroscopy, SEM, and their swelling degree (Q%) was determined in three aqueous media of different pH (pH = 2, 6.8, and 7.4). The antioxidant activity was evaluated, and polyphenols were released according to specific kinetic profiles in three different pH media (pH=2, 6.8, and 7.4).

Results: The encapsulation efficiency of the polyphenols was between 63% and 93.5%. It increased when the molar ratio or the weight ratio of gellan/ OG was raised. The average diameter of polyphenols-loaded chNPs varies between 90-200 nm. The Q% and the polyphenols release efficiency increase at pH=7.4 and when the molar report or cross-linking degree decreases. The presence of BSA in the ComPs composition results in a morphology with increased porosity. The antioxidant activity of polyphenols extracted from ComPs was higher than free polyphenols.

Conclusions: It can be admitted that the biopolymers used to obtain ComPs have a protective role on polyphenols, allowing them to overcome the gastric barrier and to be absorbed more efficiently into the colon.

Chemical content and anti-inflammatory potential of sulfated polysaccharides from *Cladophora Vagabunda* seaweed

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Aim: Sulfated polysaccharides isolated from seaweeds are known to possess important pharmacological activities, including antioxidant, antiviral, anticoagulant, anti-inflammatory and anticancer potential [1]. *Cladophora vagabunda* is a macroalgae abundantly found on the coastal waters of the Romanian Black Sea shore, which remains an under-exploited resource of algal biomass. The aim of this study was to characterize the polysaccharides isolated from *C. vagabunda* from the chemical and biological point of view and to evaluate their *in vitro* anti-inflammatory activity.

Methodology: Sulfated polysaccharides were obtained by Soxhlet extraction using water as solvent. Polyphenols and chlorophyll were removed by methanol and acetone extraction, respectively. The crude polysaccharidic extract was precipitated at 50% and 75% ethanol, resulting in two fractions (PzF50 and PzF75). The total hexose content was analysed by anthrone method, the uronic acid level was estimated by the orcinol reaction and sulfate content was measured by barium chloride assay [2, 3]. The antioxidant activity of polysaccharides fractions was evaluated using the ABTS cation radical scavenging assay and reducing power method [4]. The anti-inflammatory potential of *C. vagabunda* polysaccharides was investigated on LPS-stimulated THP-1 cells. The level of IL-1β and TNF-α pro-inflammatory cytokines was determined by ELISA assay.

Results: The chemical composition analysis indicated that PzF50 had a higher content of total hexoses, uronic acid and sulfate content, compared to PzF75. The ABTS radical scavenging activity and reducing power of polysaccharides fractions varied in a dose-dependent manner. However, PzF75 had a stronger scavenging effect on ABTS cation radical and higher reducing power than PzF50, but lower than ascorbic acid used as control. *In vitro* cell culture studies showed that PzF50 significantly reduced the pro-inflammatory cytokines production in LPS-stimulated THP-1 cells.

Conclusions: The obtained results suggested that sulfated polysaccharides from *C. vagabunda* could be further tested and used in the development of new products for improving anti-inflammatory disease conditions.

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Extraction optimization of phycocyanin from spirulina, obtained by green methods

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Phycocyanin is a photosynthetic pigment from the group of phycobiliproteins obtained from the cyanobacterium Spirulina (*Arthrospira platensis*) with possibilities for diverse applications in food and pharmaceutical technologies. Its extraction is essential because of its high antioxidant activity, the presence of antitumor, anticoagulant, antidiabetic, antimicrobial, and antiviral properties, and the possibility of its use as a natural colorant for food and cosmetic products. However, its wide industrial use is limited by its high cost and complex purification procedures during chemical extraction. The present study aimed to use ultrasonic and microwave extraction of phycocyanin and to determine the optimal parameters (temperature, time, and ultrasonic frequency) to obtain maximum purity and yield. For this purpose, ultrasonic extraction of samples of 5 g of convectively dried Spirulina (*Arthrospira platensis*) in 15 ml of distilled water was carried out at temperatures of 20°C, 30°C, and 40°C, respectively, for 1, 2, and 3 hours at an ultrasound frequency of 35 kHz, 40 kHz, and 45 kHz. Microwave extraction was performed for 5 g of biomass obtained under the same conditions in 35 ml of distilled water for 1 min, 2 min, and 3 min, respectively. For each of the obtained samples, three parallel measurements of the antioxidant activity were made by DPPH and FRAP methods, and the yield and purity of phycocyanin were determined spectrophotometrically. The factors' influence on the studied parameters was statistically verified using multifactorial and principal component analysis using SPSS. Ultrasonic extraction produced a higher yield and purity of phycocyanin than microwave extraction. The best ratio yield (14.88 mg/g)/ purity (1.60) is achieved at temperature of 40°C, for one hour, and the frequency of the ultrasonic wave 40 kHz. The obtained purity of phycocyanin is suitable for cosmetic and pharmaceutical application. A relatively low yield (4.21 mg/g), but with a purity (2.67), suitable for biomedical applications and biomarkers, was obtained at a temperature of 30°C, a time of two hours, and an ultrasound wave frequency of 40 kHz. The study proposes ultrasonic extraction as a green method for relatively high yields of phycocyanin with a purity suitable for food, cosmetic, and biomedical purposes, significantly reducing extraction time and product cost.

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Knotweed genetic identity verification for the pharmaceutical industry

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Aim: The present study aimed at investigating genetic diversity and population structure using morphological traits, sequence-related amplified polymorphisms (SRAP) [1] and start codon targeted (SCoT) [2] markers in 17 collections of three invasive knotweed taxa – *Reynoutria japonica* Houtt., *Reynoutria sachalinensis* (F. Schmidt) Nakai and *Reynoutria x bohemica* J. Chrtek & A. Chrtková.

Methodology: Plant material was harvested in sixteen different regions of Wroclaw (Poland) and classified based on their leaf shape and size, trichome type and other morphological characteristics [3]. Young leaves were used for DNA extraction. PCR amplified SCoT and SRAP fragments were detected on agarose gel. Bands were scored as 1 (present) or 0 (absent) and a binary data matrix was generated.

Results: Total thirty-six SCoT primers and sixty-six SRAP primers combinations were screened, out of which eighteen primers were able to amplify reproducible bands. These primers were employed to analyze genetic diversity and population structure. Nine selected SCoT primers produced 149 bands (16.56 bands per primer), of which 130 (86.19%) were polymorphic, whereas nine SRAP primer combinations produced a total of 153 bands (17.0 bands per primer), with 135 (87.11%) of them being polymorphic. Similarity coefficient was used to detect the phylogenetic relationship, subsequently dendogram was constructed for SCoT and SRAP data.

Conclusions: Morphological markers are visual indicators but were often reported to be insufficient in raw material identification. Present investigation suggests the effectiveness of morphological and molecular combination employing SCoT and SRAP marker system to estimate the reliability of the pharmacopoeial raw material.

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Influence of extraction solvent on the chemical profile, antioxidant and antifungal activities of two *Artemisia* species

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Aim: The main purpose of this research was to investigate the efficiency of ethanolic, hydro-ethanolic 70% and methanolic extracts from *Artemisia argyi* H. Lév. et Vaniot and *Artemisia absinthium* L. plants as new antifungal bioproducts, and characterisation of their chemical profile and antioxidant activity.

Methodology: The phytoextracts were obtained from the dried aerial parts and analyzed by LC-MS for determination of polyphenols, sesquiterpene lactones, phytosterols, and tocopherols [1]. The antioxidant activity of plant extracts was investigated by DPPH radical scavenging assay and reducing power assay. The extracts were screened for their antifungal activity against *Aspergillus niger*, *A. flavus* and *Penicillium expansum* fungal strains according to the poisoned food technique [2]. The minimal inhibitory concentration (MICs) and minimal fungicidal concentration (MFCs) of the phytoextracts was determined by broth microdilution method [3].

Results: The polyphenols were identified in higher amounts in *A. argyi*, especially chlorogenic acid, rutoside, isoquercitrin, eupatilin, eupatorin and hispidulin. Phytosterols and tocopherols were better extracted in ethanol, as were sesquiterpene lactones and methoxylated flavonoids. In the antioxidant tests, the methanolic extract of *A. argyi* manifested the best activity: EC_{50}=82.60±1.2 µg/mL in DPPH radical scavenging assay and EC_{50}= 64.87±0.43 µg/mL in the reducing power assay.

The alcoholic extracts (5 mg/mL) exhibited different antifungal activity, depending on the plant species, the solvent used and the target pathogen. The most pronounced antifungal activity was exhibited by ethanolic extracts of *A. argyi* against all tested fungal strains, as follows: *P. expansum* (100% inhibition), *A. flavus* (75.25% inhibition) and *A. niger* (68.25% inhibition). The MIC values of ethanolic extracts from *A. argyi* and *A. absinthium* ranged from 2.5 to 10 mg/mL and >10 mg/mL, respectively. Only *A. argyi* ethanolic extract exhibited MFC against all tested fungal strains (2.5 – 10 mg/mL).

Conclusions: The *A. argyi* ethanolic extracts can be used to develop effective antifungal bioproducts against two important human (*A. niger*) and agricultural pathogens (*A. flavus* and *P. expansum*), as environmentally safe and sustainable alternative in medicine and agriculture (post - harvest disease control).

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Natural products in dermatophytosis: the case of Honokiol and Magnolol

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Aim: Dermatophyte infections represent a significant public health concern, with an alarming negative impact caused by unsuccessful therapeutic regimens [1]. Natural products have been highlighted as a promising alternative, due to their long-standing traditional use and increasing scientific recognition [2]. In this study, honokiol and magnolol, the main bioactives from *Magnolia* spp. bark, were investigated for their antidermatophytic activity.

Methodology: The antifungal screening was performed using dermatophyte standard strains and clinical isolates. The minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC) were determined in accordance with EUCAST-AFST guidelines, with minor modifications. The effects on ergosterol biosynthesis were assessed in *Trichophyton rubrum* cells by HPLC-DAD.

Putative interactions with terbinafine against *T. rubrum* were evaluated by the checkerboard method. Their impact on cells' viability and pro-inflammatory cytokines (IL-1β, IL-8 and TNF-α) was shown using an *ex vivo* human neutrophils model [3].

Results: Honokiol and magnolol were highly active against tested dermatophytes, with MIC and MFC values of 8 and 16 mg/L, respectively. The mechanism of action involved the inhibition of ergosterol biosynthesis, with accumulation of squalene in *T. rubrum* cells.

Synergy was assessed for binary mixtures of magnolol with terbinafine (FICI = 0.50), while honokiol-terbinafine combinations displayed only additive effects (FICI = 0.56). In addition, magnolol displayed inhibitory effects

towards IL-1β, IL-8 and TNF-α released from lipopolysaccharide (LPS)-stimulated human neutrophils, while honokiol only decreased IL-1β secretion, compared to the untreated control [3].

Conclusions: In conclusion, our results can be regarded as a starting point in the development of novel therapy strategies against *T. rubrum*-related dermatophytosis.

Acknowledgements

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Pharmacognostic profile of *Cassia Occidentalis* (l.) link species grown in the climate conditions of the Republic of Moldova

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Aim: Macromorphological, microscopic and phytochemical testing of the lead compounds in the plant of *C. occidentalis* species.

Methodology: Macromorphological, microscopic and phytochemical tests for some chemical compounds (flavonoids, phenols, tannins, saponins, alkaloids, terpenoids and anthraquinones) were applied on plant of *C. occidentalis* according to standard methods.

Results: Organoleptic and macromorphological features: fresh and leaf powder has foetid smell and a bland taste; leaf is pinnate compound with entire margin, glabrous surface, and strong rachis; pods are brown and cylindrical; seeds are lenticular to cordate, mat, and brownish. Microscopy features: dorsoventral and amphistomatic leaf with paracytic, anomocytic and anizocytic stomata, rarely non-glandular trichomes; a lot of glands with brown content on the aerial part of plant; oxalate crystals as druses and prismatic in the vascular sheath. Phytochemical profiles: screening of the results of specific analytical reactions applied to the different classes of chemical compounds shows the presence of flavonoids, tannins, phenols, saponins, terpenoids and anthraquinones in all the analyzed extracts, but with a different degree of expression depending on the plant organ.

Conclusions: The current study has established organoleptic, macromorphological, microscopic and phytochemical features which can serve as diagnostic indices in the identification of *C. occidentalis* species. The phytochemical testing denotes that *C. occidentalis* represents the prospect medicinal species for the Republic of Moldova as source of useful chemical compounds.

Acknowledgements

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Helichrysum species from the Republic of Moldova

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Aim: Assessment of the spread of genus *Helichrysum* in the world flora and flora of the Republic of Moldova, in particular the active compounds and pharmacological activity of the species, according to the scientific literature.

Methodology: Were evaluated the literature and database of *Helichrysum* species, their chemical composition and pharmacological activity.

Results: The genus *Helichrysum* (Asteraceae) includes 600 species distributed mainly in dry forest areas, in Europe, Asia and North America. In the Republic of Moldova only one species *H. arenarium* (L.) Moench- (popularly known as everlasting), occurs sporadically on the cliffs of the right bank of the Nistru River, in the landscape reserve "Climăuții de jos", in the Steppe Hills steppes, on the limestones of Naslavcea, with a declining population due to collection, being a vulnerable species. Many research works focus on the investigation of constituents such as phenolic compounds, flavonoids, phytosterols present in the flowers of *H. arenarii*, indicating that the most important group of compounds responsible for pharmacological activity are flavonoids (naringenin-5-*O*-glucoside or helichrysin A, kaempferol, quercetin, luteolin, apigenin). In the collection of the Scientific Practical Centre in the Field of Medicinal Plants "Nicolae Testemițanu" SUMPh. It has been introduced *H. italicum* (Roth) G. Don., rich in phenolic compounds and essential oils (α-cadren, α-pinene, geranyl acetate, limonene, nerol) with cholagogue, antimicrobial, anti-inflammatory properties, also used in the cosmetic industry due to the characteristic odour.

Conclusions: The species of *Helichrysum* are well-known for their secondary metabolite content and the study contributes to increasing knowledge about the spread of genus *Helichrysum* in the world and the Republic of Moldova flora, which can serve for future investigations on the species (*H. arenarium* and *H. italicum*).

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Secondary phenolic metabolites quantified in extracts obtained from *Lysimachia Nummularia* l.

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Aim: The aim of this paper is to determine and identify, by a HPLC method, some phenolic compounds in extracts obtained from the species *Lysimachia nummularia* L.

Methodology: To obtain the extracts, we used as vegetal products *Lysimachiae herba, Lysimachiae radix* and *Lysimachiae flores*. Thus, the plant products *Lysimachiae herba, Lysimachiae radix and Lysimachiae flores* were extracted by maceration with 40% ethanol for 14 days. The extractive solutions were brought to the residue of a TURBOVAP rotary evaporator. The identification of the phenolic compounds was done by a HPLC method, adapted from the USP 30-NF25.

Results: The lowest amount of extract was obtained from the plant product *Lysimachiae flores.* By HPLC analysis, the following secondary metabolites, polyphenolic compounds, were constantly determined: caffeic acid, chlorogenic acid, resveratrol. The highest amount of resveratrol was detected in the extract obtained from *Lysimachiae radix*: 0.445 mg /g.

Conclusions: The active principles, determined by HPLC analysis, demonstrate the possibility of using the three extracts obtained from plant products (*Lysimachiae radix, Lysimachiae herba, Lysimachiae flores*) in phytotherapy, for their properties especially, antioxidant and antimicrobial effects. The presence of resveratrol is an advantage in terms of antioxidant properties of plant products, because it is considered among the most active antioxidant ingredients known in the literature. The HPLC method for the determination of polyphenols can be used to standardize the extracts.

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Comparative polyphenolic, flavonoid and anthocyanin content and antioxidant activities of pomegranate juice and seeds

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Aim: The pomegranate, *Punica granatum* L., Punicaceae is cultivated throughout tropical and subtropical regions of the world. The pomegranate fruit is one of the oldest known to man and has featured in mythology, and as a food and medicine from ancient civilizations of the Middle East to its present. The juice from fruits and the seeds also has medicinal use. The pomegranate juice as nutraceutical is rich in punicalagin (ellagitannin). It also contains ellagic acid, gallotannins, anthocyanins and flavonoids. The seeds have been studied for their bioactive constituents (monoacylglycerols, glycerides, proteins, pectins and sugars).

Methodology: To obtain the maximum yields of the compounds which influence the free radicals scavenging activity, we used distilled water, 50% ethanol and methanol for seeds extraction. The pomegranate juice and the methanolic, 50% ethanolic (v/v) and aqueous extracts of pomegranate seeds have been investigated for the polyphenolic, flavonoid and anthocyanin contents.

Results: Comparison of the major constituents in the seed extracts and the commercially available pomegranate juice. A link between the antioxidant activity of juice polyphenols, flavonoids and anthocyanins has been confirmed. The antioxidant activity of extracts decreased as follows: juice > water extract > methanol extract > 50% ethanol extract and it is not correlated with the total polyphenol content (juice: 0.047 mg/ml; water extract: 8.81 mg/100 g; ethanolic extract 50%: 8.29 mg/100 g; methanolic extract: 7.01 mg/100 g) and total anthocyanin content (juice: 0.039 mg/100 g; seeds: 0.064 mg/100 g). The flavonoid content is similar in all extracts: 41.6 mg/100 g in methanol extract, 45.6 mg/100g in ethanol 50% extract and 57.33 mg/100 g in the water extract. In the juice the flavonoid content is low: 0.705 mg/100 g.

The HPLC-UV-VIS analysis (RP C-18 stationary phase, elution 8-35% acetonitrile-phosphate buffer pH=2.5) confirmed the presence of the following polyphenols in the pomegranate juice (given in µg/ml) and seed (given in µg/mg): chlorogenic acid (43.13 µg/ml and 53.59 µg/g), caffeic acid (1.23 µg/ml and 12.95 µg/g), catechin (0.08 µg/ml and 42.57 µg/g), epicatechin (3.06 µg/ml and 30.55 µg/g), quercetin (2.33 µg/ml and 31.05 µg/g), isoquercitrin (0.49 µg/ml and 10.20 µg/g) and kaempferol (2.26 µg/ml and 42.26 µg/g).

Conclusions: Based on these results, the pomegranate juice is a source of phenolic, flavonoid and anthocyanin compounds and the seed extracts need further research.

Polyphenolic profile and biological activities of some bark extracts obtained from *Sorbus Aucuparia* l.

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Aim: To obtain polyphenolic extracts, through validated technologies, from native *Sorbus aucuparia* L. bark, with practical use in medicine, pharmacy and cosmetics.

Methodology: The rowan phytoextracts were obtained by reflux extraction under cold and hot conditions and the following *in vitro* parameters were investigated: cytotoxic, antioxidant, and wound healing activity, as well as the mechanisms of action, on normal Vero and neoplastic HeLa cells.

Results: The polyphenolic treatment led to: decrease in cell viability, moderate wound healing action, accumulation of reactive oxygen species (ROS), DNA fragmentation, increase of apoptotic and pre-apoptotic cell number, all responses varying with dose, type of extracts, and cell line.

Conclusions: Our results demonstrated that the rowan extracts, especially those obtained in heat condition, showed selective cytotoxic/cytostatic activity against HeLa cell line, being involved several mechanisms of activity, and also *in vitro* healing potential, was established.

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Electrospun pullulane/polyvinylpyrrolidone nanofibers loaded with green synthesized silver nanoparticles

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Aim: In this paper, colloidal solutions of silver nanoparticles obtained by green synthesis using an aqueous extract of *Abies alba* bark (AgNPs - *A. alba*) were used in combination with pullulan (Pu) and polyvinylpyrrolidone (PVP) solutions with the aim of developing novel Pu/PVP - based nanofiber mats loaded with phytotherapeutic agents via the electrospinning technique.

Methodology: Spherical AgNPs, obtained by bioreduction using *A. alba* bark aqueous extract, were characterized by dynamic light scattering technique (DLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and electron dispersive spectroscopy (EDX). The chemical structure and morphology of the polymeric nanofibers loaded with AgNPs were investigated using UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), SEM, and EDX [1, 2]. Dynamic water vapor measurements were used in order to investigate the moisture sorption and desorption behavior of the developed electrospun materials. The biological activities of these products were also evaluated [3].

Results: Pu/PVP/AgNPs - *A. alba* smooth nanofibers were successfully produced with an average diameter of 0.18 ± 0.03 μm. The presence of metallic silver in the nanofiber mats was confirmed by UV-Vis and EDX spectroscopies. The nanofibers developed promising antimicrobial effects.

Conclusions: This study reports a simple and rapid method for the synthesis of Pu/PVP/AgNPs - *A. alba* nanofibers with potential medical applications.

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Benefits of sea buckthorn oil supplementation on clinical outcomes, physical function and inflammatory biomarkers in patients with active rheumatoid arthritis: a clinical study

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Aim: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease, with fundamental joint damage. Based on the anti-inflammatory properties of sea buckthorn oil (SBTO), the current study was conducted to evaluate the sea buckthorn oil supplement effects on serum levels of inflammatory markers, clinical symptoms, and physical function in patients with active rheumatoid arthritis.

Methodology: In this randomized controlled clinical trial, 80 RA patients (64 female, 16 male) were enrolled randomly and divided into two groups, each of 40 patients: a SBTO-treated group that received a daily dose of 900 mg SBTO, twice a day, additional to conventional treatment (Methotrexate-MTX) and a control group that was treated only with the conventional treatment-Methotrexate. The duration of the study was 3 months.

At baseline and at the end of the study, clinical symptoms, physical function, serum level of inflammatory biomarkers and oxidative stress markers were determined.

Results: It was found that the clinical markers (i.e., the 28-joint count for swelling and tenderness, the 68-joint count for tenderness and 66-joint count for swelling) and the disease activity score assessment for 28 joints, Clinical Disease Activity Index, Simplified Disease Activity Index were significantly lowered in the SBTO-treated group. Moreover, serum levels of certain biochemical markers (i.e., C-reactive protein) were also significantly decreased in SBTO-treated patients but not some inflammatory mediators level.

Conclusion: SBTO supplementation can be considered as a potential adjunct treatment in patients with RA, improving inflammatory mediators and clinical symptoms. However, further studies with longer duration are needed.

Enhancing the nutritional and bioactive potential of sprouted grains: a focus on non-thermal plasma activated water technology

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Aim: To review procedures used to elicit the nutritional and bioactive compounds in sprouted grains, emphasizing the benefits of the treatment with non-thermal plasma activated water (PAW).

Methodology: A literature review based on the following keywords: wheat sprouts, nutrient status, health promoting compounds, controlled germination.

Results: Whole grains are rich in nutrients (proteins, lipids, fibers, vitamins) but also bioactive compounds (polyphenols, gamma-aminobutyric acid, beta-carotene). Both nutritional and bioactive compounds have been reported to increase during germination. Therefore, various procedures have been developed to enhance the nutrient status and functional properties of sprouted grains such as exposure to various stress factors, physical energy, magnetic field, ultrasounds or non-thermal plasma. The latter can be also used as non-thermal PAW which is obtained by spraying water into plasma zone. Non-thermal PAW increases the germination rate, growth parameters, carotenoid, chlorophyll and protein contents, but also the activity of antioxidant enzymes in germinated wheat. These positive effects are attributed to its content of nitrates and reactive oxygen and nitrogen species. Nitrates are essential for protein synthesis whereas reactive species trigger the production of antioxidant metabolites such as polyphenols and carotenoids [1-3].

Conclusions: Treatment with non-thermal PAW is a promising strategy to improve the nutritional and bioactive potential of sprouted whole grains.

Acknowledgements
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Myconoside, isolated from *Haberlea Rhodopensis*, ameliorate UVA/UVB-induced changes in human keratinocytes

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Aim: Chronic and repeated exposures to UV radiation triggers deleterious consequences in skin cells leading to sunburn, photoaging and photocarcinogenesis. An approach to protect human skin is by using phytochemicals as photoprotectants [1]. In the present study, we examined the photoprotective effects of *Haberlea rhodopensis* Friv. (*Gesneriaceae*) extract (HRE) and its biologically active molecules calceolarioside E (CAL) and myconoside (MYC), against the UVA/UVB radiation-induced photoaging by monitoring the expression leveles of matrix metalloproteinase-1 (MMP1), tissue inhibitory metalloproteinase (TIMP1) and collagen type I (COL1A1) in HaCaT cells. Further, the underlying signaling pathways involved have been investigated.

Methodology: The keratinocytes were cultured following the previously described conditions [2]. The UV irradiation chamber (Opsytec Dr. Grobel. GmbH, Germany) was used as source of UVA/UVB radiation. The cells were pre-treated with HRE (1-5 µg/mL) or MYC or CAL (both 1-5 µM) for 1 h and then exposed to UVA/UVB (2.5 J/cm^2, ratio 95/5%) irradiation (Figure 1).

Results: In our model, the MYC upregulated significantly UVA/UVB-decreased expression of *COL1A1* and *TIMP1* in keratinocytes, while mRNA level of *MMP1* three times lower than this of *TIMP1* was not influenced.

Conclusions: The findings suggest that MYC has photoprotective effects due to promoting collagen synthesis in the UVA/UVB-irradiated keratinocytes, therefore it may be promising ingredient for skin protection and/or preparation of skin care products.

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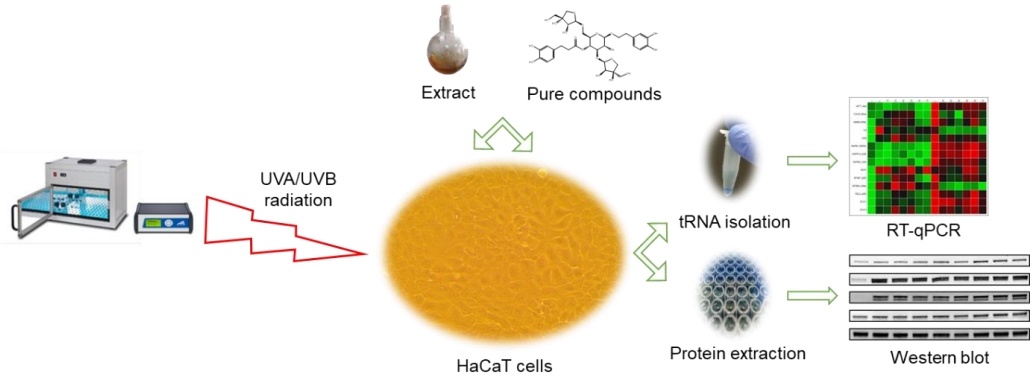

Figure 1. Scheme of experiment

Endophytic fungi of *Salvia Abrotenoides* and *Salvia Yangii* – a potential alternative source for plant bioactive metabolites

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Aim: Endophytic fungi develop within tissues and organs of healthy plants without causing apparent disease symptoms. They are reported to improve plant fitness by supporting plant nutrition and participating in defensive mutualism. Attention by the scientific community is boosted by the opportunity to exploit the unique properties of these associates of plants. These fungi may also participate in metabolic pathways and boost their own natural biosynthetic activity, or may acquire some genetic information to synthesize biologically active compounds closely related to those produced by their host. With reference to the pharmaceutical importance of natural products from *Salvia* subgenus *Perovskia* [1], we investigated the capacity by endophytic fungi recovered from *S. abrotanoides* and *S. yangii* to directly produce these compounds via *in vitro* cultures.

Methodology: Healthy *S. abrotanoides* and *S. yangii* were collected from Botanical Garden of Medicinal Plants at the Wroclaw Medical University (Poland). Endophytic fungi were isolated from their leaves, stems, and roots. Representative strains have been identified and characterized, using morphological characters and phylogenetic analysis of molecular data. The isolates were *in vitro* cultured in the PDB and Czapek-Dox broth. After incubation, the mycelium was separated from the culturing medium via filtering. The biomass and the medium, separately, will be analyzed using LC-MS for metabolic profile, mainly targeted at diterpenoids and phenylpropanoids.

Results: Over hundred isolates belonging to eight different genera were recovered from leaves, roots and stems of both *Salvia* species (Fig 1). The species *Alternaria arborescens, Colletotrichum godetiae, Boeremia exigua, Diaporthe eres, Diaporthe novem, Epicoccum nigrum, Fusarium acuminatum* and *Fusarium avenaceum* were recovered and identified according to morphological characters and molecular data, and represent new records as endophytes of *Salvia spp* [2].

Conclusions: Endophytic fungi are prospective producers of both novel and known bioactive compounds, including those common to their host plants. Isolation of culturable strains of endophytic fungi from *S. abrotanoides* and *S. yangii* may enable production of valuable plant metabolites in *in vitro* cultures. In the long

term, the fungal endophytic strains may become an alternative source of plant-derived bioactive compounds to be used for biotechnological and industrial applications.

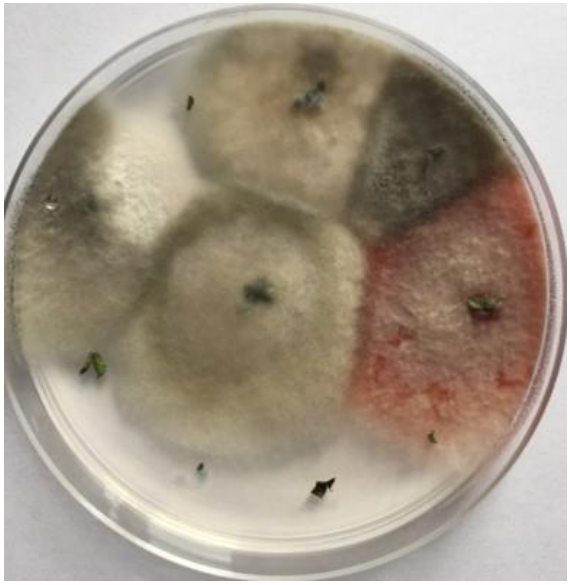

Fig. 1. Isolation of endophytic fungi from leaves of *Salvia yangii*.

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Lalley leaves: its antibacterial effects on vaginal infections

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In traditional medicine, henna plant is used to treat many diseases like oedema, bronchitis, menstrual disorder, rheumatism, hemorrhoids, and even jaundice, leprosy, pain, spleen enlargement, dysentery, and skin problems. This present study was aimed at evaluating the antimicrobial activity of *Lawsomia inermis* leaves extracts on vaginal isolates. The *Lawsomia inermis* leaves were collected on a farm in Kaduna State, and air dried for about two weeks. Thereafter, the leaves were pulverized, extracted, and fractionated into aqueous, ethanol, and chloroform fractions. The extracts obtained were filtered under a vacuum and the various filtrates were concentrated to dryness by evaporation in a rotary evaporator. The residues obtained were stored at 4°C. Microorganisms were isolated from coursemates, university workers, and hostel residents. Biochemical tests were carried out according to established protocols. The broth culture was prepared and the antimicrobial activity was evaluated using the agar diffusion method. The yield obtained was 9.46 %. Phytochemical screening indicated the presence of saponins, tannins, alkaloids, terpenoids, and steroids. *Klebsiella* spp., *Proteus* spp., *E.coli*, *Pseudomonas* spp., *Proteus* spp., *Staphylococcus* spp. were identified. The antimicrobial activity was concentration dependent and it varied with the organisms encountered. The Minimum Inhibitory Concentration (MIC) of the crude extract and aqueous extract was 50 mg/ml for *Proteus* spp. and *Staphylococcus* spp. as well as 25 mg/ml for *Klebsiella* and *Pseudomonas*. The aqueous extract had a higher MIC of 100 mg/ml for all the organisms isolated. The aqueous extract had no MIC for *Klebsiella*. The best minimum inhibitory concentration profile was obtained with the chloroform extract (12.5 mg/ml) for *Proteus* spp., (8.0 + 1 mm), *Staphylococcus* spp. (12.0 + 0 mm), *Klebsiella* spp. (8.5 + 1.5 mm) and *E. coli* (7.5 + 1.5 mm) as well as 6.25 mg/ml for *Pseudomonas* spp. (6.0 + 0 mm). This research had shown that *Lawsomia inermis* leaves had phytoconstituents that can elicit antimicrobial activity.

In vivo evaluation of the wound healing potential of some ointments based on vegetal extracts

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Aim: The aim of this paper was the preparation, characterization and *in vivo* investigation of the wound healing potential of four ointments based on vegetal extracts (*Hippophäe fructus, Calendulae flos, Bardanae folium, Millefolli herba*).

Methodology: The wound-healing capacity was investigated on three experimental models of incision, excision and heat burns induced on Wistar rats. The treatment was applied topically on wounds once a day, for 21 days.

Clinical and macroscopic evaluation was performed throughout the experiment, with the recording of the re-epithelialization period and determination of wound contraction rate on days 6 and 9. The histopathological examination was also achieved on days 3, 6, 12, and 21. Additionally, for the hydro-alcoholic extracts, HPLC analysis was performed.

Results: The phenolic profile of extracts determined by HPLC revealed the presence of caffeic acid, chlorogenic acid and rutoside in all extracts.

The analysis of methoxylated flavonoids allowed their identification only in *Achillea millefolium* extract, while the dominant sterol in the hydro-alcoholic extracts was beta-sitosterol.

The re-epithelialization period for excision was observed on the 12th day of treatment for all ointments, the best result being recorded for the *Arctium lappa* ointment (1 × 2 mm). The *Arctium lappa* group showed a wound contracture of 94.29 ± 0.26%, which is comparable to that of the *Calendula officinalis* group (92.70 ± 0.47%).

The skin lesions with loss of substance evolved in a mature, supple, foldable scar, in comparison with the untreated group that presented a rigid, hypertrophic scar. In addition, the vascularization, through the process of angiogenesis, highlighted by the anatomical–pathological examination, was resumed.

Conclusions: All herbal ointments showed histologically efficient dermal collagenization with the appearance of normal granulation tissue after 21 days of topical application. The most effective ointment proved to be the one based on *Arctium lappa*, followed by that of *Calendula officinalis*, both in terms of area of injury and in terms of wound contraction rate.

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Influence of pulse electric field (pef) on growth and compounds concentration in *Iris Domestica* species (syn. Belamcanda Chinensis), iridaceae

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Aim: The aim of this work was to develop and optimize pulse electric field (PEF) treatment for the first time from the seedlings of Iris domestica, cultivated in aeroponic medium.

Methodology: Iris domestica (syn. Belamcanda chinensis L. DC., Iridaceae) occurs naturally in South Asia in China, Japan and India. Roots and rhizomes are pharmaceutical raw materials with antibacterial, antifungal, estrogenic properties. In addition to the Chinese Pharmacopoeia, the European Pharmacopoeia has also had a monograph on the raw material of this plant for several years, which proves its great healing potential. The main compounds responsible for such a broad spectrum of activity are polyphenols, including isoflavones, xanthonic glycosides, stilbenes, phenolic acids and triterpenes with iridal structure. The most active of the isoflavones are irigene, tectoridine, tectorigenin [1]. We examined 8 weeks old seedlings of I. domestica in 4 mm electroporation cuvete used BTX EMC 830 electroporator from 12,5 to 50 kV/cm voltage, with different time and duration of pulses. Plant growths were continued in aeroponic systems for next 2 months and finally raw material (rhizoma and roots) was collected and HPLC-MS examined.

Results: All plants survived electroporation treatment, but differences in particular compounds were observed. Irigenin and irisflorentin as main compounds with highest concentration in 50 kV/cm, 25µs, 40 pulses (0,712 and 0,426 mg/ml respectively) and lowest in 30 kV/cm, 100µs, 10 pulses (0,316 mg/ml for irigenin), 12,5kV/cm,100, 10 for isoflorentin.

Conclusion: The factors influencing growth performance under aeroponic conditions and accumulation of bioactive metabolites will be explored to understand the regulatory mechanisms.

Acknowledgements
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Absolute configuration and protein Tyrosine Phosphatase 1B inhibitory activity of Xanthoepocin, a dimeric Naphtopyrone from *Penicillium* sp. IQ-429

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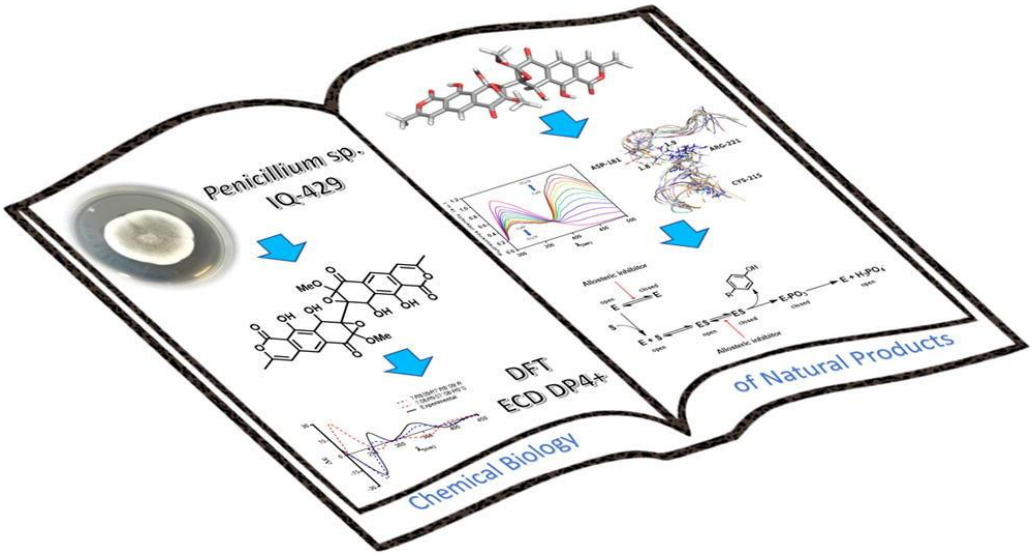
Aim: Discover non-competitive inhibitors of *h*PTP1B$_{1\text{-}400}$ (an active target for developing drugs to treat type II diabetes, obesity, and cancer) from fungi harvested in semi-desert areas in Mexico.

Methodology: The fungal extracts were screened *in vitro* as *h*PTP1B$_{1\text{-}400}$ inhibitors. Based on its potent inhibitory properties, the extract of *Penicillium* sp. (IQ-429) was selected for chemical investigation. Fractionation of this sample using chromatographic methods allowed the isolation of xanthoepocin (**1**) [1]. The absolute configuration of **1** was determined by comparing the theoretical and experimental ECD spectra, and by GIAO-NMR DP4+ statistical analysis. The *in vitro* inhibitory properties of **1** against *h*PTP1B$_{1\text{-}400}$ were determined using a spectrophotometric method. Molecular docking and molecular dynamics simulations (MD) were carried out to get insight onto the presumptive molecular mechanism of inhibition. Finally, quenching fluorescence experiments were carried out.

Results: The absolute configuration of **1** was determined to be 7R8S9R7' R8'S9'R. Xanthoepocin (**1**) inhibited the phosphatase activity of *h*PTP1B$_{1\text{-}400}$ (IC_{50} value of 8.8 ± 1.0 μM) in a mixed type fashion, with k_i and αk_i values of 5.5 and 6.6 μM, respectively. Docking results indicated that **1** binds in a pocket different from the catalytic triad. MD simulations showed that **1** locks the WPD loop of *h*PTP1B$_{1\text{-}400}$. Intrinsic quenching fluorescence experiments indicated that **1** behaves like a static quencher of *h*PTP1B$_{1\text{-}400}$ ($K_{SV} = 1.1 \times 10^5\ M^{-1}$) and corroborated that it binds to the enzyme with an affinity constant (k_a) of $3.7 \times 10^5\ M^{-1}$.

Conclusions: The putative binding site of **1** with *h*PTP1B$_{1\text{-}400}$ predicted that this molecule mainly interacts with residues of the C-terminal domain and Pro_{180} and Phe_{182}. MD indicated that **1** modifies the PTP and WPD loops' dynamics, suggesting an allosteric inhibition triggered by large-scale conformational changes. Fluorescence quenching experiments confirmed that **1** binds to *h*PTP1B$_{1\text{-}400}$ and quenches its intrinsic fluorescens. Furthermore, **1** was determined to be the fungal metabolite with the highest binding affinity to *h*PTP1B$_{1\text{-}400}$ reported hitherto. Moreover, we suggest that xanthoepocin (1) inhibits *h*PTP1B$_{1\text{-}400}$ via different allosteric inhibition mechanism that targets the C-terminal domain of the full-length PTP1B and disables the WPD loop dynamics in the apo- *h*PTP1B$_{1\text{-}400}$.



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In vitro anti-inflammatory activities of *Mentha Spicata, Rosmarinus Officinalis* and rosmarinic acid

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Aim: To evaluate the COX-1 and COX-2 enzyme inhibitory potential of *Mentha spicata* L. as well as the *Rosmarinus officinalis* L. extracts and rosmarinic acid, comparatively.

Methodology: In this study, *in vitro* enzyme inhibition of extracts was investigated using commercial kit systems. Phytochemical analyses of extracts were performed and confirmed by HPLC.

Results: The major component of both extracts was rosmarinic acid. The IC_{50} values for COX-1 enzyme inhibition were calculated as 66.26 µg/mL for *M. spicata* extract, 20.11 µg/mL for *R. officinalis* extract and 10.09 µg/mL for rosmarinic acid. The IC_{50} values for COX-2 enzyme inhibition of *R. officinalis* and *M. spicata* extracts were recorded as 7.21 and 13.02 µg/mL, respectively. Rosmarinic acid showed 2.07 µg/mL IC_{50} value for COX-2 enzyme.

Conclusions: The tested rosmarinic acid-rich extracts showed anti-inflammatory potential and further *in vivo* studies are needed.

Phytochemical analysis of phenolic compounds from *Quercus Cortex*

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Aim: Oak bark is known for accumulating large amounts of phenolic compounds (mainly tannins) which exhibit a wide range of biological effects, including antioxidant, antibacterial and anti-inflammatory activity. Due to its valuable chemical composition, it has been used in traditional medicine for the treatment of skin diseases, wounds, and many types of external inflammations. The content of main phenolic components in naturally harvested and purchased commercial *Quercus cortex* was investigated. The quantity of tannins, flavonoids and phenolic acids in the two types of substances was compared and the dynamics in the accumulation of the mentioned components during the different months of the year was analysed.

Methodology: The study used methods from European Pharmacopoeias 10 for quantitative spectrophotometric determination of the three groups of phenolic compounds.

Results: As a result of the conducted research, the phase in which the oak bark should be collected in order of supreme accumulation of phenolic components. A maximum of polyphenols and related with them, tannins is observed in the spring, followed by a decrease in their amount in the other periods. Similar is the tendency for the accumulation of the other two groups of phenols - flavonoids and phenolic acids. The comparatively lower content of tannins in the substances purchased from the stores was established too. A minimum of tannins content (min. 3.0%) in *Quercus cortex*, according to Ph. Eur. 10 was viewed only in one of all tested commercial substances.

Conclusions: It is necessary to select precisely the phase in which the substances are collected, as well as to increase the quality requirements for the substances available in the stores.

Plant metabolite-enriched bacterial-nano-cellulose as efficient carrier system against microbial pathogens

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Bacterial-nano cellulose (BNC) was used as a carrier-system for *Chelidonium majus* metabolites. The investigations focused on suitability of BNC matrices for plant cell growth and production of biologically active compounds. The antimicrobial and anti-inflammatory effect of plant metabolite-enriched BNC was verified.

Three types of BNC carriers were used in the experiments: NC (intact form), H_2O (sterile water purification) and NaOH (0.1M NaOH purification). For each type 3-, 5- and 7 day-old bacterial culture (*Komagataeibacter xylinus*) was used. *C. majus* cells were submerged in liquid media and cultured *in vitro* on all types of BNC matrices.

The phytochemical profile of BNC was determined using HPLC. The effect of plant metabolite-enriched BNC on the cytokines secretion (IL-1β, IL-8, TNFα) and its potential cytotoxic effect was verified using human LPS-stimulated neutrophils. Furthermore, the antimicrobial properties of the BNC were tested against planktonic and biofilm-forming human pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

Polyphenols and isoquinoline alkaloids were the most abundant group of specialized metabolites secreted by *C. majus* cells into the BNC. 3- followed by 5-day-old NC cellulose were found to possess the richest phytochemical profile. Among non-alkaloid compounds, organic acids, benzoic acid derivatives, hydroxybenzoic acid, hydroxycinnamic acids as well as one flavonoid and one phenolic aldehyde were identified. As for the isoquinoline alkaloids, there were protopine derivatives, protoberberine derivative and phenanthridine derivatives detected.

Based on the plant cell growth parameters and the phytochemical analysis, 3-day-old NC carrier was chosen for anti-inflammatory and antimicrobial assays. Cytokines secretion decrease was observed in terms of TNF-α and IL-8, while in case of IL-1β the overproduction was noted. Additionally, there was no cytotoxic effect of BNC

observed. Minimal Antimicrobial Concentration of 3 day-old NC was 12.5, 25, 25% and Minimal Biofilm Eradication Concentration was 50, 100, 50% against *S. aureus*, *P. aeruginosa*, and *C. albicans*, respectively.

In conclusion, the NC-type of bio-nano-cellulose can serve as efficient carrier for plant-biotechnologically derived pharmacologically active substances. This natural system was found suitable both for *C. majus* cells growth as well as specialized metabolites production of which the quantities were sufficient enough to display antimicrobial and anti-inflammatory effect.

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Comparative evaluation of phenolic profile and bioactive potential of five wild thyme species from Romanian spontaneous flora

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Aim: This study aimed to analyze the phenolic profile, antioxidant and enzyme-inhibitory potential of the hydroethanolic extracts obtained from *Thymus alpestris*, *T. glabrescens ssp. glabrescens*, *T. panonicus ssp. auctus*, *T. pulcherimus* and *T. pulegioides ssp. pulegioides*.

Methodology: Aerial parts of each species were extracted through maceration using 70% *v:v* ethanol, concentrated under reduced pressure and freeze-dried, obtaining dry extracts. Qualitative and quantitative analysis of individual phenolic compounds was done through LC-ESI-DAD/MS^2. For the *in* vitro evaluation of antioxidant potential TEAC, FRAP, DPPH, TBARS and OxHLIA assays were employed. Additionally, the enzyme-inhibitory activity was tested *in vitro* against tyrosinase, *α*-glucosidase and acetylcholinesterase.

Results: Rosmarinic acid was found in high amounts in all analyzed species; phenolic acids derivatives (including salvianolic acids) were the main constituents of *T. alpestris* and *T. glabrescens ssp. glabrescens*, while the flavonoids (eriodictyol derivatives) were dominant for *T. panonicus ssp. auctus, T. pulcherimus* and *T. pulegioides ssp. pulegioides*. All species exerted a strong antioxidant potential and a mild enzyme-inhibitory effect against *α*-glucosidase and acetylcholinesterase, showing no anti-tyrosinase activity.

Conclusions: The analyzed species could be considered as valuable sources of bioactive compounds with health promoting benefits.

Acknowledgements

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Phytochemical and biological characterization of two *Cynara Scolymus* l. varieties: a glance into their potential large scale cultivation and valorization as bio-functional ingredients

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Aim: Artichoke leaf extracts are used as traditional herbal medicinal products to treat a wide range of human ailments, being widely commercialized as nutraceutical or pharmaceutical preparations. In the current study, the hydromethanolic dried leaf extracts of *Cynara scolymus* L. var. *major* Brotero and *C. scolymus* L. var. *redonensis* N.H.F. Desp. were phytochemically and biologically investigated.

Methodology: The phytochemical characterization of artichoke leaf extracts was assessed by liquid chromatography hyphenated with tandem high-resolution mass spectrometry (LC-HRMS/MS) analysis. The biological profile screening was achieved by *in vitro* testing of their antioxidant effects (free radical scavenging, metal chelating and reducing power, total antioxidant capacity) and by *ex vivo* testing of their ability to inhibit the release of pro-inflammatory cytokines [interleukin (IL)-1β, IL-8 and tumor necrosis factor (TNF)- α] from lipopolysaccharide (LPS)-stimulated human neutrophils.

Results: LC-HRMS/MS metabolite profiling showed a complex composition, with phenolic acids (mostly mono- and di-caffeoylquinic acids), flavonoids and sesquiterpene lactones as the most representative classes. The strong antioxidant activity of the two *C. scolymus* varieties was evidenced in DPPH [64.84-65.21 mg trolox equivalents (TE)/g] and ABTS (86.39-95.55 mg TE/g) radical scavenging, cupric (160.49-171.07 mg TE/g) and ferric (71.47-78.95 mg TE/g) reducing capacity, metal chelating and phosphomolybdenum assays. In addition, the artichoke samples (at the concentration of 20 µg/mL) proved a very potent inhibition of the production of several pro-inflammatory cytokines, namely IL-1β (7.55-15.75% of LPS+ cells), IL-8 (11.72-13.46% of LPS+ cells) and TNF-α (4.07-10.35% LPS+ cells), in LPS-stimulated human neutrophils.

Conclusions: Overall, the results of our study indicate that the two *C. scolymus* varieties could be regarded as a rich source of biologically active compounds, opening thus the perspectives for their future large-scale cultivation and valorization as bio-functional ingredients with putative antioxidant and anti-inflammatory effects.

Evaluation of the antioxidant activity and uv protection capacity of an extract of *Helichrysum Arenarium* (l.) moench flowers

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Helichrysum arenarium (L.) Moench (dwarf everlast) is a perennial herbaceous plant of the Asteraceae family, common in Eastern Europe, the Near East and Central Asia [1]. Due to its successful use in traditional medicine, the monograph of the plant product *Helichrysi arenarii flos* has been included in the World Health Organization, German Comission E, Phisician's desk reference for herbal medicine and German pharmaceutical Codex [2]. The aim of the research work is to analyze and characterize the antioxidant action of the hydroalcoholic extract from dwarf everlast flowers, correlated with its composition, and to evaluate the UV-screening effect of the polyphenolic extract from *Helichrysum arenarium* flowers.

A hydroalcoholic extract of *Helichrysum arenarium* flowers was prepared. The analysis of the extract was done by determination of the total polyphenol content (Folin-Ciocalteu method) and the total flavonoid content. Antioxidant activity was measured by two methods, namely ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity. UV protection activity was evaluated by determination of the SPF by an *in vitro* spectrometric method.

Chemical analysis of the hydroalcoholic extract of dwarf everlast flowers showed a high percentage of polyphenolic compounds in the extract (67.7%). Determination of the total flavonoid content resulted in a value of 0.426 g rutoside/100 ml extract.

Both methods of antioxidant activity evaluation showed remarkable activity and the UV protection effect showed high value in the UVB range.

The polyphenolic extract from the flowers of the *Helichrysum arenarium* plant contains a high amount of polyphenols and shows antioxidant and UV-screening activity against harmful UV radiation for the human skin.

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The antiradical and antibacterial activities of *Usnea Barbata* (l) Weber ex f.h. Wigg ethanol extract

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Aim: The study aims to identify and quantify phenolic compounds in ethanol extract of *Usnea barbata* (L.) Weber ex F.H. Wigg (UBE) from Călimani Mountains, Romania, and to investigate its antiradical and antibacterial activities.

Methodology: A spectrophotometric method evaluated the total phenolic content (TPC) using Folin–Ciocâlteu reagent. Pyrogallol was selected as standard, the TPC values being calculated as mg of pyrogallol equivalents per gram extract (mg PyE/g). The phenolic secondary metabolites identification and quantification were achieved using a standard HPLC method, described by the USP 30-NF25 monograph and previously validated [1]. The antiradical activity was determined spectrophotometrically through the DPPH free-radical scavenging assay. The antibacterial effects were evaluated by an adapted disc diffusion method (DDM) from the Clinical and Laboratory Standard Institute (CLSI) on two Gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae)* and two Gram-negative *(Pseudomonas aeruginosa* and *Klebsiella pneumoniae)* bacteria [2].

Results: The TPC value (mg PyE/g extract) in UBE was 276.603 ± 15.025. Seven phenolic metabolites were identified and quantified (mg/g): caffeic acid (0.414 ± 0.005), *p*-coumaric acid (0.312 ± 0.001), ellagic acid (230.819 ± 0.264), chlorogenic acid (0.512 ± 0.006), gallic acid (27.487 ± 0.459), cinnamic acid (17.948 ± 0.114), and usnic acid (0.257 ± 0.002). The UBE DPPH-Free Radical Scavenging value (%) was 12.162 ± 0.396. UBE displayed an inhibitory activity on *S. aureus*, *S. pneumoniae,* and *P. aeruginosa;* only *K. pneumoniae* was resistant.

Conclusions: The presence of the phenolic secondary metabolites could explain the significant antiradical and antibacterial effects. In addition, according to the Green Chemistry concept, the extraction solvent used to obtain is "preferrable", having a low toxicity.

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Phenolic profile and antioxidant properties of *Momordica Charantia* l. grown in Romania

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Aim: In this study, we proposed to perform an analysis of phenolic compounds of *Momordica charantia* "Enaja" (bitter melon) variety, cultivated in Targu Mures (Romania), and to compare it with those originating from India.

Methodology: Bitter melon's phenolic compounds were analyzed by UPLC-DAD in the ethanol extract of aerial parts (stem and leaves, M1), young green fruits (M2), and ripe fruits (fully yellow, M3) grown in Romania, and were compared to a vegetable product originating from India (M4). Eleven reference compounds were used: gallic acid, eleutheroside B, chlorogenic acid, catechin, epicatechin, luteolin di-glucoside, vanillic acid, luteolin glucoside, sinapic acid, taxifolin, quercetin. The identification of the compounds peaks was carried out by comparing the retention time of the peaks and their UV–Vis spectra with that of the reference compounds. Additionally, total polyphenol content (TPC), total tannin content (TTC), and total flavonoid content (TFC) were determined along with antioxidant activity tests (DPPH and ABTS methods).

Results: The UPLC-DAD analysis led to the identification of catechin, epicatechin, luteolin-di-glucoside, luteolin-glucoside, and vanillin in the stems and leaves of the plant. Epicatechin (859 μg/g) and catechin (1677 μg/g) were the most abundant compounds in the aerial parts, while in the ripe fruits luteolin-glucoside (310 μg/g) was the main phenolic. The highest TPC, TTC, and TFC were observed in the aerial parts (stem and leaves) of the bitter melon. The DPPH scavenging activity shows a good correlation with the phenolic content, and the IC_{50} values are in the range published earlier.

Conclusions: This is the first study describing phenolics of *Momordica charantia* "Enaja" variety grown in Romania. The TPC and ABTS scavenging activity of fruits grown in Romania is similar to those originating from India.

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Tanacetum Vulgare l. - a promising alternative to allopate therapy

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Aim: Giardiasis is currently a common condition among Romanian patients, being addressed by limited therapeutic alternatives, with an increased incidence of adverse effects [1]. Therefore the identification of a safe therapeutical alternative is needed. *Tanacetum vulgare* L., is a widespread species, wildly growing in Romania, known for its good antihelmintic activity [2]. Therefore, this study aimed to investigate the anti-giardia activity of the hydroalcoholic *Tanacetum vulgare* L. flowers extract and to identify and compare the phenolic compounds of *Tanacetum vulgare* L. flowers harvested from Sibiu, a mountain area and Alba, a lowland area. These compounds might be responsible for the therapeutic activity.

Methodology: The major phenolic compounds were identified and quantified using an LC-MS method. The anti-giardia activity of tansy flowers hydroalcoholic extract was evaluated on mice infected with *G. muris*, using metronidazole and Giardiplant® (a commercial phytotherapeutic product containing *Calendula officinalis* hydro-alcoholic extract, *T. vulgare* hydro-alcoholic extract and *Thymi aetheroleum*) for comparison. The efficacy of the therapy was assessed by direct microscopic examination (200xmagnification) of duodenal scraping to reveal *Giardia* trophozoites.

Results: The phenolic profile of tansy flowers harvested from Sibiu showed a higher amount than those from Alba, such as, chlorogenic acid (4334.32 ± 11.79 µg/g and 3673.75 ± 15.70 µg/g, respectively), flavonoid glycosides, such as rutin (350.15 ± 7.30 µg/g and < 0.2 µg/g, respectively) and quercitrin (112.09 ± 2.91µg/g and 13.00 ± 0.04 µg/g, respectively).

Regarding the anti-giardia activity, after 5 days of treatment, *T. vulgare* tincture reduced the count of trophozoites of *G. muris* in the *duodenum* (from 51.6 ± 1.40 to 2.11 ± 0.38 trophozoites/field), comparable with metronidazole (from 28.6 ± 1.20 to 2.45 ± 0.68), and more effective than Giardiplant® (from 39.8 ± 2.20 to 6.08 ± 0.15, $p < 0.01$).

Conclusions: The *in vivo anti-Giardia* effect was demonstrated, indicating that the hydroalcoholic extract of tansy flowers harvested from the mountain area, with a higher amount of therapeutic active compounds, might become a promising natural alternative for giardiasis treatment.

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Researches concerning antiulcer effects of *Vernonia Kotschyana* sch. bip. ex walp extracts

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Aim: The current study investigated the protective effect of two extracts obtained from powdered roots of the plant: VK1 - contains active principles soluble in ethyl acetate; VK2 - obtained using infusion followed by prolonged maceration.

Methodology: Both extracts were investigated by FT-IR analysis and acute toxicity was determined. The gastroprotective effect was evaluated using indomethacin ulcer model in mice. Histopathological evaluation of the samples followed the specific steps of technique. The experiments were conducted in accordance with the international bioethic rules.

Results: FT-IR analysis showed the presence of sterols in both extracts, polyphenol compounds in VK1 and polysaccharides in VK2. Experimental data have shown no acute toxicities for both extracts. Pre-treatment of the mice with VK1, VK2 (800 mg/kg bw) and ranitidine (positive control) decreased the damage scores by 65.2%, 64.5% and 75.5% respectively. VK1 presented a marked inhibition of ulcers, starting with a dose of 400 mg/kg bw. Gastroprotective activity was also partially confirmed by the histopathologic study of ulcerous stomach fragments, sampled from mice treated with both extracts.

Conclusions: This study underlined the antiulcer potential of *Vernonia kotschyana* extracts. In addition, the ethyl acetate extract was proven to have superior gastro-protective activity than aqueous extract, obtained by a method similar to that used in traditional medicine.

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Essential oils in the management of skin conditions

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Aim: To review the scientific literature in order to assess the potential of essential oils (EOs) in the management of skin conditions.

Methodology: A PubMed and ScienceDirect search was conducted in order to identify articles related to the beneficial effects of essential oils in promoting skin health, by using several specific key words.

Results: Dermatological problems affect a large number of individuals worldwide. Fungal and bacterial infections worsen many skin conditions delaying wound healing and exacerbating the scarring. Considering the prevalence of multi-drug resistant microorganisms, natural remedies and especially essential oils could be an important tool in the management of skin conditions. Literature abounds in studies highlighting the beneficial effects of essential oils isolated from over 100 plant species (e.g. sage, curcuma, oregano, lavender, chamomile, immortelle, citrus species, frankincense, tea tree, cinnamom, camphor, peppermint, lemongrass, basil, thyme, black pepper, juniper, patchouli, geranium, clove, yarrow, ylang-ylang, myrrh, lemon balm, myrtle, marjoram, geranium, rosemary, coriander etc.) in the management of a variety of skin conditions such as wounds, psoriasis, melasma, acne, fungal and bacterial infections, eczema, dermatitis. Their beneficial effects are exerted via multiple biological activities: anti-inflammatory, antibacterial, antioxidant, photoprotective, promoting re-epithelization rate and collagen deposition, restoring physiological cell homeostasis during wound and inflammation phases [1,2]. Multiple nanostructures (liposomes, niosomes, nanoemulsions, solid lipid nanoparticles etc.) and composite materials were developed as delivery systems in order to overcome several limitations of essential oils use, regarding availability, dosage and stability [3].

Conclusions: Essential oils have a huge potential in the treatment of skin conditions, therefore should be further explored in this respect.

Acknowledgements
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New qualitative HPTLC method and new marker compounds for an herbal medical product including 10 different plant extracts

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High-performance thin layer chromatography (HPTLC) analysis is one of the key tests used in pharmacopoeial monographs of herbal drugs and extracts to establish and confirm sample identity. Its simplicity, selectivity, and cost-effectiveness make HPTLC an efficient tool for qualitative analysis of natural products, especially in the industry where time-saving methods are essential [1, 2]. Since Good Manufacturing Practices require documenting the presence of each botanical in a blend, this study aimed to develop an HPTLC fingerprint profile for a medical product including 10 different plant extracts. Several mobile phases were tested to find markers in the polyherbal product corresponding to the fingerprints of the extracts obtained from the raw materials. Also enhancing the concentration of substances applied on the HPTLC plate and removing components such as sugars and glycerol that interfered in the visualization of the patterns, was achieved through solid phase extraction (SPE). The raw materials diversity and differences in the polarity of component extracts led to the use of two mobile phases: a hydrophilic and a lipophilic one, which allowed us to observe the constituent profile and identify specific patterns of each individual herbal drug.

Preliminary results suggested that reference substances described literature, for each botanical ingredient lacked to appear in detectable concentrations. Therefore triterpenoids, flavonoids, and phenolic acids had been applied on the plate as markers, or substitute height markers for each crude extract, resulting in an effective method for identification of individual herbal drugs within the final product. To ensure reproducibility, the method was tested on different batches of the medical product followed by a process validation protocol.

To conclude, we present herein a facile route to authenticate, evaluate and ensure the consistency of a complex herbal medical product while avoiding costlier instrumental analysis.

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Investigation on the allergenicity of *Vaccinium Corymbosum* l. shoots aqueous extract

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Aim: Previous studies have found that by-products of cultivation of *Vaccinium corymbosum*, including shoots, are rich in polyphenols, flavonoids, proanthocyanidins, arbutin [1] and can serve as raw materials for new nutraceuticals and cosmetic ingredients. The purpose of this work is to conduct of allergoscreening of *V. corymbosum* shoots extracts under *in vivo* experimental conditions.

Methodology: The experimental work was performed on 24 white outbreds male Guinea pigs, which were sensitized epicutaneously by aqueous *V. corymbosum* shoot extract (Elliott variety). Aqueous extracts (1: 10 / weight: volume / g:ml, prepared on a boiling water bath for 30 minutes) were injected intradermally into the outer surface of the ear of guinea pigs (a total of 20 applications of 1 ml for 4 weeks). At the end of the exposure, peripheral blood parameters were determined; content of populations and subpopulations of lymphocytes, indicators of humoral immunity - immunoglobulins were conducted *in vitro* by immunological tests.

Results: An increase in the total number of neutrophils was observed in the blood of animals of the experimental group, of which the number of segmental neutrophils increased almost twice. In addition, there was a 15-20% decrease in the number of lymphocytes in the experimental group, which indicates the inhibitory effect of the studied extract on the specific immunity of guinea pigs. In animals of the experimental group there was an increase of Ig M, Ig E, as compared to the control. Due to the function of neutrophilic granulocytes, their participation in adaptive and innate immune responses and the ability to interact with antigens and antigen-induced humoral factors, aqueous extract of *V. corymbosum* shoots can provoke latent sensitization of the body.

Conclusions: The results obtained indicate the development of type 1 hypersensitivity reactions in Guinea pigs under the action of aqueous extracts of *V. corymbosum* shoots.

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Food antioxidants – a source of health and beauty

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Aim: Identification of the most recent research regarding the use of food antioxidants in products designed for health promotion.

Methodology: Selection and systematization of information from 20 scientific papers published in the last 3 years, selected according to the following keywords: food antioxidants, nutraceuticals, nutricosmetics, cosmeceuticals, biotechnology, anti-aging.

Results: An adequate diet is one of the components of a healthy lifestyle, that induces wellbeing in the organism, which is associated with beauty. Food science, food supplements industry and cosmetics industry are areas of convergence that led to the development of nutraceuticals, nutricosmetics and cosmeceuticals as products for maintaining a young look and for prevention of premature aging and prevention of pathologies correlated with inflammatory processes. The human organism possesses endogenic antioxidants among its defense systems, which are joined by exogenic antioxidants that come from food. Antioxidant activity of food products of vegetal origin rarely represents only the sum of the antioxidant activity of the contained bioactive compounds (phenolic compounds, carotenoids, vitamins, some minerals, proanthocyanidins, flavonoids, coumarins, essential fatty acids); in most cases, these act synergically, antagonistically or as potentiators. Among natural products with nutricosmetic potential the following can be mentioned: marine algae, edible mushrooms, ferns, plant extracts (tea, bamboo, grapes, apples, brown rice etc). Anti-aging products must inhibit collagenase, elastase and hyaluronidase and must be certified from the point of view of safety and efficacy. The current trend is to employ biotechnologies for producing natural compounds from agro-industrial byproducts, but their extraction and ensuring an adequate stability and bioavailability represent real challenges.

Conclusions: Nutraceuticals, nutricosmetics and cosmeceuticals have a great potential in the prevention and management of pathologies related to inflammatory processes, including the aging process. In order to ensure optimal bioavailability, solubility and controlled release, harmonization between extraction methods and the ways in which the active principles are provided is required, as well as international correlation of the systems for the certification of authenticity, safety and quality.

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Plant-derived polyphenols: chemical structure and biological activity

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Aim: Over time numerous studies have confirmed the involvement of oxidative stress in the etiopathogenesis of many conditions such as neurodegenerative diseases, cardiovascular diseases and even cancer, which is the reason why the scientific world has gained a special interest in studying antioxidant molecules [1]. Additionally, the threat of running out of effective antibiotics becomes more and more real as multi-drug resistance increases both in hospital and community acquired infections which led numerous scientists to search for alternatives [2]. In this context, this work aimed to review the botanical sources, isolation methods and formulation of the most relevant polyphenols and the correlations between their biological activity and chemical structure, which represents the premise of developing novel therapeutic agents.

Methodology: literature search using ScienceDirect, PubMed, Google Scholar as databases searching by different keywords like 'polyphenols', 'flavonoids', 'phenolic acids', 'antioxidant', 'antimicrobial' etc.

Results: Polyphenols of plant origin present a wide range of biological actions, in correlation with their chemical structure, but have several drawbacks in terms of stability or bioavailability.

Conclusions: Polyphenols have promising potential as medicines or as structural models for innovative drugs, but further optimization is required to overcome some of their disadvantages.

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Total polyphenol content in *Galium Verum* l. species by different extraction methods

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Aim: To investigate methods of extraction of polyphenol compounds in the aerial parts of *Galium verum* using methods such as water bath, ultrasound assisted and magnetic stirring extraction.

Methodology: *Galii veri herba* was harvested from the spontaneous flora of the Republic of Moldova in the Bugeac steppe in 2021, during the blooming period. Polyphenol compounds were extracted with 60% ethanol solution for 30 min at 80°C, respecting the ratio of 1:10 for all three methods: water bath with condenser, ultrasound assisted and magnetic stirring extraction. Total polyphenol content was determined spectrophotometrically, by six consecutive measurements according to the Folin-Ciocalteu method, at 765 nm, expressed as gallic acid equivalents (GAE, mg/100 g vegetal product), with statistical processing of the data.

Results**:** It is known that the diversity of phenolic compounds found in vegetal products makes it difficult to develop a universal extraction method, and of the three methods mentioned above, ultrasound-assisted extraction has proven to be the optimal method for the extraction of polyphenols from *G. veri herba* (29.21 ± 0.04 GAE), followed by water bath with condenser (27.56 ± 0.03 GAE) and magnetic stirring extraction (28.18 ± 0.03 GAE) mg GAE /100g vegetal products.

Conclusions: The results confirm that *Galium verum* can be used as a source of polyphenols, and the total polyphenol content depends on the extraction method, the nature and concentration of the solvent, and the nature of the vegetal product.

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Natural phenolic compounds as regulators of bacterial metabolism

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Phenolic compounds are one of the largest naturally occurring group of chemicals, with over 8000 structures described so far. Besides their intrinsic role in the producing organisms, their broad array of biological activities makes them important drug candidates or lead compounds for various derivatives. Due to reactive hydroxyl groups and various other functional groups, phenolics interact with numerous metabolic pathways, including those in microorganisms and thus, they are suitable as control agents for microbial growth in general and as antibacterials, in particular. While the natural forms have significant activities, chemical functionalisation enhances significantly their interactions. Since phenomena such as antimicrobial resistance occurs frequently and new lead compounds are required for the development of efficient strategies, phenolic compounds may serve as a valuable pool for such drug development. As such, summarization of information pertaining to molecular mechanisms becomes useful as a reference for guiding further research in the field.

Biological evaluation of the antioxidant and antimicrobial activity of the ethanolic extracts and agnps from *Equisetum Pratense, Equisetum Sylvaticum* and *Equisetum Telmateia*

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Equisetaceae family has approximately fifteen species and hybrids spread all over the world. In the Romanian flora there are nine of these, commonly known as horsetail [1]. The *Equisetum* species are perennial plants found in forests, wet meadows and swamps which develop spontaneously [2]. Sterile stems belonging to the *Equisetum* genus are often used in the traditional medicine for treating urinary tract infections, cardiovascular diseases and respiratory tract infections. All these actions are due to its content of polyphenolic derivatives that have been isolated [3, 4].

Aim: The purpose of this work is to investigate the biological activity and compare the ethanolic extracts from three different *Equisetum* species (*E. pratense* Ehrh., *E. sylvaticum* L. and *E. telmateia* Ehrh.) with the synthetized silver nanoparticles (AgNPs) both in terms of antioxidant and antimicrobial activity.

Methodology: *In vitro* evaluation of the antioxidant activity of the plant ethanolic extracts and AgNPs was determinated using four tests: the chelating capacity of ferrous ion, the lipoxygenase inhibition capacity, the scavenging capacity of hydroxyl and superoxide anion radicals. The antimicrobial action was highlighted by the diffusimetric method and the determination of the minimum inhibitory concentration.

Results: Regarding the antioxidant activity, *E. sylvaticum* ethanolic extracts and AgNPs presented the best activity in all four tests performed. Also, the antioxidant activity was higher for AgNPs than the ethanolic extracts. The study showed that all the analyzed extracts showed antimicrobial activity, the antifungal activity being more important than the antibacterial one.

Conclusions: Given the promising results obtained in the antioxidant and antimicrobial *in vitro* tests, further isolation of certain flavonoid subfractions and the evaluation of their complex biological potential are justified.

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Screening studies on the antimicrobial properties of *Gentiana Asclepiadea* root extract

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The search for new plant sources is relevant for the production of phytopreparations. It's important to study the antimicrobial activity of plant extracts. *Gentiana asslepiadea* L. is a perspective plant, which contains a complex of biologically active substances with various pharmacological activities.

Aim: conducting screening studies of the antimicrobial activity of a 40% ethanol *Gentiana asslepiadea* root extract.

Methodology: A solid extract of *Gentiana asslepiadea* roots (extractant - 40% ethanol) was used for the research. The study of the antimicrobial activity was performed on clinical isolates of antibiotic-sensitive and antibiotic-resistant microorganisms. Screening was carried out of the antimicrobial action of extract of *Gentiana asslepiadea* roots using the micro-method of diffusion in agar, which is characterized by high sensitivity and discriminating ability and allows reliable differentiation of active extracts from inactive ones.

Results: The results of the research show that the *Gentiana asclepiadea* extract showed moderate antimicrobial activity against Gram-positive microorganisms (staphylococci, streptococci, bacilli). The studied extract shows high fungicidal activity against *Candida* non-*albicans*. The studied extract does not effect on mycelium growth and sporulation of filamentous fungi *Aspergillus niger* and *A. flavus*.

Conclusions: the studied *Gentiana asslepiadea* root extract showed a moderate bacteriostatic effect against rod-shaped and cocci-like microflora and a high fungicidal activity against *Candida* non-*albicans*. Thus, the investigated *Gentiana asslepiadea* root extract can be used to formulate new herbal medicines.

Crocus and *Iris* extracts with anti-inflammatory properties

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Medicinal plants are being increasingly used as antioxidant and anti-inflammatory agents. The therapeutic use of *Crocus sativus* and *Iris hungarica* (Iridaceae) extracts as anti-inflammatory agents is discussed. The main metabolites of plants are phenolic compounds, polysaccharides, amino acids [1]. Aim: Evaluate the anti-inflammatory efficacy of water and ethanolic extracts from *C. sativus* corms, stigma, perianth and *I. hungarica* rhizomes. Methods: Plant dry extracts were used to evaluate their anti-inflammatory properties on models of acute exudative foot inflammation in rats caused by zymosan, histamine, and carrageenan [2]. Results: Extracts of *Crocus* corms (200 mg/kg) showed the greatest anti-inflammatory properties, the mechanism of which is an inhibitory effect on the synthesis of leukotrienes (23 and 20%), the activity of biogenic amines – histamine and serotonin (14 and 20%) and a minor effect on the activity of prostaglandins (12 and 14%). The water extract of *Iris* rhizomes showed only a slight effect upon the synthesis of leukotrienes – 15%, as assessed in the model of zymosan edema. ed Administration of *Crocus* corms and stigma extracts (at a dose of 25 mg/kg) to animals also showed significant anti-inflammatory activity (73.24 and 78.61%, respectively) in the model of carrageenan edema in rats. According to our results, all plant extracts are assigned to the VI class of toxicity (practically non-toxic substances) according to the classification of K.K. Sidorova (LD_{50} >5000 mg/kg) [3]. The ethanolic extract (70% v/v) of *Iris* rhizomes (150 mg/kg) also showed a pronounced anti-exudative effect, suppressing the inflammatory process by 41.6%. The anabolic activity of *Iris* extract (150 mg/kg) had a moderate effect, but a fairly targeted anabolic effect (due to the high content of isoflavonoids and amino acids). Conclusion: Our study demonstrated that the complex of active compounds of *Crocus* and *Iris* plants improved the anti-inflammatory efficacy. However, further research is needed to further characterize the potential benefits of these plant extracts.

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Emulsion development for dry hair care

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It is important to choose the right products according to the type of hair, which will not only clean, but also eliminate the flaws and problems of dry hair.

Aim: development of the composition and technology of an emulsion based on *Oleum Brassicae napus* for the care of dry hair.

Methodology: We chose an emulsion as a cosmetic form, which makes it possible to combine hydrophilic and lipophilic substances. To ensure the therapeutic effect, *Oleum Brassica napus* and infusion of herb of *Thymus serpyllum* were used, which contain various biologically active substances and have different effects on the hair and scalp. *Oleum Brassica napus* moisturizes the scalp, activates blood circulation, accelerates the regeneration of damaged hair, nourishes hair bulbs [1, 2]. During the development of emulsion technology, organoleptic, microscopic and physical research methods were used, which made it possible to assess the quality and stability of the finished product.

Results: Based on the results of the research, the composition of the oil/water type emulsion was developed. The dispersed phase is *Oleum Brassica napus* in the amount of 10%, and the dispersion medium is the infusion of the herb of *Thymus serpyllum*. Tween-80 and PEO-40 emulsifiers, potassium sorbate preservative, and Ylang-Ylangae oil were used to ensure the necessary cosmetic and consumer properties. The finished emulsion is a white, homogeneous, oily liquid with a pleasant smell, stable during storage, easy to apply and wash off from the hair.

Conclusions: On the basis of technological, organoleptic, microscopic and physical studies, we selected the composition of the emulsion for dry hair care and worked out the production technology.

Purification of fatty oil, lectin and a fraction rich in triterpenoids from the seeds of *Amaranthus Caudatus* l. in one technological cycle

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Aim. The aim of the study was to develop a scheme for the production of fatty oil, lectin and a substance rich in triterpenoids in one technological cycle from the seeds of *Amaranthus caudatus* L. (amaranth).

Methodology. Amaranth seeds contain 4-7% of fatty oil, rich on triterpene compounds and squalene, as well as valuable lectin, which has found application in biomedical research. However, lectin is a labile compound that does not withstand heat above 70° C, precipitation with alcohol and acetone, and pH values below 4.0 and above 11.0. Therefore, in order to obtain a complex of triterpene substances and lectin in one technological cycle, two modes of their purification were investigated. They included different sequences of purification of amaranth seed triterpene compounds by methanol extraction. Fatty oil from the seeds was extracted with petroleum ether.

The best result with complete preservation of lectin activity was obtained by extraction of low-fat seeds according to the scheme: 1) extraction of low-fat seeds with 1% NaCl; 2) purification of lectin from the extract by affinity chromatography; 3) extraction of triterpene compounds from oilcake with methanol. The chemical composition of methanol extract of amaranth seeds under different purification regimes was investigated by gas chromatography mass spectrometry (GC-MS). Carbohydrate specificity, interaction with human and animal erythrocytes, and physicochemical characteristics were investigated in the lectin purified by affinity chromatography.

Results. Chromatography of amaranth oil on silica gel column for squalene was developed. Purified by affinity chromatography, the lectin was homogeneous under disk electrophoresis in alkaline (pH 8.9) buffer system, and under denaturing conditions migrated by one zone with mol. mass ≈33 kDa. Lectin interacted with N-acetyl-D-galactopyranoside, N-acetylneuraminic acid and D-glucuronic acid, but the inhibitory power of these carbohydrates is low. Lectin interacted with native bovine submandibular mucin better than its desialized counterpart, and agglutinated native human erythrocytes better than desialized. Lectin agglutinates in low concentrations of erythrocytes of horse, dog, slightly higher - human and very weakly agglutinates erythrocytes of sheep, rabbit, frog and trout.

52.62% of the sum of fatty acids and 41.64% of the sum of triterpenoids were detected in the methanol extract by GC-MS. The main component of the methanol extract was 24-hydroxy-3,4-Secolanosta-4 (28), 8-diene-3-nitrile ($C_{30}H_{49}NO$).

Conclusions. We have developed a scheme for obtaining fatty oil, lectin and the amount of triterpene substances from the seeds of amaranth tailed in one technological cycle. It was found that amaranth seed triterpenoids are not extracted with water, which allows to obtain their amount without loss after purification of lectin.

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Study on the phenolic compounds from the herb of *Alchemilla Phegophila* juz.

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Alchemilla L. is a numerous genus of the *Rosaceae* family whose plants are capable of polymorphism. It has about 1000 species in the world flora where 35 of which grow on the territory of Ukraine. One of the most common members of this genus is *Alchemilla phegophila* Juz. It is a perennial herbaceous plant of grey-green colour with an upright densely pubescent stem, 35-50 cm high, which has been used in the folk medicine [1, 2].

Aim: The purpose of the work was to characterize the phenolic compounds of the aerial parts collected from *Alchemilla phegophila* Juz.

Methodology: The presence of flavonoids, hydrochloric acids and coumarins was established with the help of qualitative reactions and chromatographic analysis in the herb of *Alchemilla phagophilia* Juz.

For the separation of phenolic compounds, adsorption chromatography on polyamide columns was used, followed by repeated chromatography, fractional crystallization, and paper preparative chromatography. Chromatographic separation was subjected to water and ethyl acetate fractions [3].

Results: As a result of the study on *Alchemilla phegophila* Juz. the following phenolic compounds were isolated: 4 hydroxycinnamic acids (n-coumaric, caffeic, ferulic, chlorogenic acids), 3 coumarins (umbelliferone, esculetin, scopoletin) and 6 flavonoids (luteolin, kaempferol, astragalin, quercetin, hyperoside, rutin).

Conclusions: The obtained data indicate the prospect of using the aerial parts from *Alchemilla phegophila* Juz. as sources of biologically active substances of phenolic nature.

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Distribution of species from *Melampyrum* genus on the territory of Ukraine

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People have been saving their lives by using medicinal substances for a long time. Since the pharmacy tries to meet the needs of consumers, the research of medicinal plant raw materials for the development of new medicines is an urgent task.

Aim: to establish the distribution of species of the *Melampyrum* genus (reed) and to identify promising medicinal species.

Methodology: The work used cartographic materials, geobotanical descriptions, forestry materials, GARMIN GPS 12 device. Methodical materials of I. Krylova, A. Shreter on the study of reserves of wild medicinal plants were also used.

Results: Taking into account the distribution of species of the *Melampyrum* genus on the territory of Ukraine, the possibility of cultivation, it is promising to study their raw material found in the western regions of Ukraine. The *Melampyrum* genus includes about 50 species distributed in the Eastern and Western Mediterranean, temperate latitudes of Europe, North Africa, and Asia. In Ukraine, 10 species of reed are common: *Melampyrum cristatum, Melampyrum arvense, Melampyrum argyrocomum, Melampyrum nemorosum, Melampyrum vulgatum, Melampyrum pratense, Melampyrum Herbichii, Melampyrum silvaticum, Melampyrum laciniatum*.

Conclusions: *Melampyrum saxosum, Melampyrum silvaticum* and *Melampyrum herbichii* can be found in the subalpine zone of the Carpathians and rarely in the highlands, menawhile *Melampyrum laciniatum* is located in the Carpathians and Polissia.

The most common are *Melampyrum cristatum, Melampyrum nemorosum* and *Melampyrum pratense*. These species are distributed in Precarpathia, Carpathians, Roztochka, Transcarpathia, Polissia, Forest Steppe, Steppe. They are rarely found on the Left Bank of Ukraine, the South and the Crimea.

So, analyzes of literary sources show that plants of the *Melampyrum* genus are promising raw materials and are widely distributed on the territory of Ukraine.

Influence of a maitake mushrooms thick extract on oxidative stress indicators in rats with acetaminophen hepatitis

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Aim: to investigate the anti-inflammatory properties of a thick extract from maitake mushrooms in an experiment on rats with acetaminophen-induced hepatitis.

Methodology: Experiment was conducted on 60 white male rats, which were divided into 10 groups, each included 6 animals. Acute hepatitis was simulated by the intragastric administration of acetaminophen in the dose of 1250 mg/kg once per day (for 2 days). Correction of the pathology induced was performed with a thick extract of maitake mushrooms in the dose of 150 mg/kg of the animal body weight (b.w.). The reference drug "Silybor" was administered in the dose of 20 mg/kg/b.w. On 3rd, 7th and 10th day from the beginning of toxic agent administration, the liver homogenate and blood serum were taken for the studies. The activity of free radical oxidation processes under the conditions of acetaminophen hepatitis and after the introduction of studied extract and reference drug was assessed by superoxide dismutase, catalase activity, the content of TBA-AP and OMP products.

Results: The development of toxic hepatitis in rats is evidenced by a significant increase in the content of TBA-active products, products of oxidative modification of neutral and basic proteins in the liver and serum of animals. We also noted a significant decrease in the activity of catalase and superoxide dismutase. It has been experimentally proven that the use of thick extract of Maitake mushrooms under the conditions of acetaminophen hepatitis caused a significant increase in catalase and superoxide dismutase activity, a decrease in TBA-AP, neutral and basic 2,4-DNPH in the serum and liver of rats. The results of the experiment were confirmed histologically.

Conclusions: Our study revealed a considerable positive effect of a thick extract of maitake mushrooms on the activity of lipoperoxidation and oxidative modification of proteins under the conditions of acetaminophen hepatitis in white rats, which indicates its antioxidative, hepato-, membrane protective properties and the relevance of further study for development of new effective medications.

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Development of honokiol – loaded mesoporous silica systems

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Aim: The aim of the study was to develop and formulate honokiol-loaded mesoporous silica systems with applications in the management of diabetic disease. Honokiol is a naturally occurring pleiotropic lignan isolated from *Magnolia grandiflora* with antidiabetic, anti-inflammatory, antidepressant, anxiolytic, antithrombotic, analgesic, neuroprotective and many other therapeutic actions. The *in vitro* studies conducted for antidiabetic action reported that honokiol reduces oxidative stress, modulates insulin sensitivity and suppresses high glucose-induced human endothelial cell apoptosis (1,2).

Methodology: Two types of silica matrices, KIT-6 and MCM-48, were synthesized by ultrasonication using Sonics Vibra Cell TM equipment for 2 hours; afterwards the supernatant was removed by centrifugation and then the white solid product was calcined at 550 °C. The calcined matrices were suspended in honokiol hydroalcoholic solution (concentration 2 mg/mL) and magnetically stirred for 24 hours. Then the suspension was centrifuged to facilitate the separation of the honokiol-loaded silica matrix which was dried in hot air at 40 °C. The loading degree was determined by difference from the supernatant.

Results: The retained honokiol quantity was determined using a spectrophotometric UV-VIS method (λ = 293 nm), resulting in a loading degree of 21.05 mg/g KIT-6 and 15.94 mg/g MCM-48. The IR spectra were also recorded for the active substance (honokiol, purity 98%), the simple matrices (KIT-6 and MCM-48), and the loaded mesoporous silica systems using a Bruker FTIR spectrometer. The formation of honokiol-loaded mesoporous silica systems was confirmed by FTIR spectroscopy.

Conclusions: The use of silica matrix systems are very attractive and advantageous in terms of specific surface area and pore volume in the loading process of honokiol, being able to influence the release of the drug substance and increase oral bioavailability.

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