11th INTERNATIONAL SYMPOSIUM ON CHROMATOGRAPHY OF NATURAL PRODUCTS

LUBLIN (POLAND) June 4th-7th, 2018

BOOK OF ABSTRACTS

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with Medicinal Plant Unit,
Medical University of Lublin

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Welcome!

Dear Colleagues,

On behalf of the Scientific and Organizing Committees, it is our great pleasure to invite you to join us at the 11th International Symposium on Chromatography of Natural Products (ISCNP 2018) that will be held in Lublin, Poland on June 4th-7th, 2018.

The International Symposium on Chromatography of Natural Products (ISCNP) is a meeting covering all aspects related to natural products research. Our meetings began in 1992, when we organized the first national symposium. After a few years we decided to expand our annual national meetings to biennial international conferences. Following the tradition of all of the previous meetings, the aim of upcoming ISCNP symposium is to discuss trends, present the latest results, and exchange ideas relevant to all of the chromatographic and related techniques, as well as hyphenated methods used in phytochemical analysis, sample preparation, and the isolation of biologically active metabolites from medicinal plants, food crops, and other natural sources.

The symposium will include invited plenary lectures, oral presentations, poster sessions, exhibitions, and social activities. Our main goal is to bring together scientists from universities, research centers, and industries from all over the world and offer to all a great and attractive symposium, joining different cultures and knowledge together in the lovely old city of Lublin.

We will be delighted to receive your contribution through scientific papers, exhibitions, and your participation in the discussions and social events at the symposium.

We sincerely hope that you will join us in making the 11th International Symposium on Chromatography of Natural Products a true success. We look forward to welcoming you to Lublin and to ISCNP 2018.

Yours Sincerely,

Prof. Dr. Kazimierz Głowniak

Prof. Dr. Dr. h.c. Günther Bonn

PLENARY LECTURES

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15	**THE MAGIC WORLD OF LIPIDS – NATURAL PRODUCTS AND THE ENDOCANNABINOID SYSTEM** Gertsch J
16	**OLIVE BIOACTIVE COMPOUNDS: CHEMISTRY AND BIOLOGY** Skaltsounis LA
17	**ARE CELL EXPERIMENTS BELIEVABLE FOR PHARMACOLOGICAL STUDIES OF POLYPHENOLS?** Cao H, Xiao J
18	**GPCR PHARMACOLOGY AS A MOSAIC OF LIGANDS, RECEPTORS AND SIGNALS** Jóźwiak K.

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2	**ANTIOXIDANT AND UVA-PHOTOPROTECTING ACTIVITY OF THE EXTRACTS AND CAFFEIC ACID DERIVATIVES FROM *GALINSOGA PARVIFLORA* AND *GALINSOGA CILIATA* HERB** Parzonko A, Bazylko A, Kiss AK
3	**APPLICATION OF GC-MS FOR IDENTIFICATION AND QUANTIFICATION OF TRITERPENOIDS IN DIVERSE MATRICES: EXTRACTS FROM PLANT TISSUES, OILS, OLEORESINS** Szakiel A, Pączkowski C
4	**HIGH-THROUGHPUT SCREENING OF NATURAL PRODUCTS UTILIZING HRMS AND UHPLC-MS/MS TECHNIQUES** Stalica P
5	**DATA DRIVEN DISCOVERING AND DEVELOPMENT OF NEW ACTIVE NATURAL ANTI-AGING INGREDIENT. ENTERING "BIG DATA" AND NETWORK SCIENCE IN PHARMACOGNOSY.** Leonardi M, Visdal-Johnsen L, Österlund C, Mavon A, Fabre S

6	**IDENTIFICATION OF GSK-3 AS A POTENTIAL DRUG TARGET FOR EPILEPSY VIA IN VIVO BIOACTIVITY ANALYSIS OF THE CONGOLESE MEDICINAL PLANT *INDIGOFERA ARRECTA*** Aourz N, Serruys ASA, Nsimire Chabwine J, Byenda Balegamire P, Afrikanova T, Edrada-Ebel R, Grey AI, Kamuhabwa AR, Walrave L, Esguerra CV, Van Leuven F, de Witte PAM, Smolders I, Crawford AD
7	**SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF SOLIDAGO GIGANTEA: OPTIMIZATION AT QUARTER-TECHNICAL SCALE** Wrona O, Rafińska K, Możeński C, Buszewski B
8	**SOLVING PROBLEM OF EMUSLIFICATION SAMPLE PLUG IN CENTRIFUGAL PARTITION CHROMATOGRAPHY BY USING FLOW-RATE GRADIENT** Hodurek P, Jajor P, Skalicka-Woźniak K, Łukaszewicz M
9	**EXPLORING MOLECULAR PROMISCUITY DEGREE AND MULTI-TARGET ACTIVITY SPACE OF DRUGS FROM NATURAL ORIGIN** Ambryszewska KE, Pistelli L
10	**EXPLOITING NATURAL PRODUCTS AS GLYCOGEN PHOSPHORYLASE INHIBITORS: COMPUTATIONALLY MOTIVATED DISCOVERY** Hayes J, Barr D, Chetter B, Snape T, Begum J, Leonidas D, Kun S, Bokor E, Somsak L
11	**USE OF HPLC GLUCOSINOLATE PROFILES TO IDENTIFY MACA PHENOTYPES CULTIVATED IN PERU AND CHINA** Meissner H, Xu L, Wan W, Fan Y
12	**DISTRIBUTION OF SAPONINS IN ROOT OF RED BEET (*BETA VULGARIS* VAR. *VULGARIS* L.) AND THE EFFECT OF PROCESSING ON THEIR ABUNDANCE IN RED BEET PRODUCTS** Mroczek A, Papiernik P, Sukiennik P, Stochmal A, Kowalczyk M

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1	**PURIFICATION OF ANTIFUNGAL COMPOUNDS FROM ARGENTINEAN SPECIES PROSOPIS RUSCIFOLIA (FABACEAE) BY CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC)** Mandova T, Gomez A, Sampietro D, Audo G, Michel S, Grougnet R
2	**PHYTOCHEMICAL ANALYSIS AND MICROBIOTA ASSISTED ISOLATION OF FLAVONOID DERIVATIVES FROM FLOWERS OF MEADOWSWEET *FILIPENDULA ULMARIA* (L.) MAXIM.** Popowski D, Pawłowska Ka, Piwowarski JP, Granica S
3	**TRITERPENOID PRODUCTION IN HAIRY ROOT *IN VITRO* CULTURE OF MARIGOLD *CALENDULA OFFICINALIS*** Alsoufi A, Długosz M, Pączkowski C, Szakiel A

4	***CARPESIUM DIVARICATUM* – A SOURCE OF NEW CARDIVIN WITH CYTOTOXIC ACTIVITY** Kłeczek N, Skalniak Ł, Stojakowska A
5	**HPTLC-BASED METABOLIC PROFILING, A SUPPLEMANTARY ANALYTICAL PLATFORM TO 1H NMR AND GC-MS FOR SPECIES AND SEASONAL CHEMICAL DISCRIMINATION OF PINE RESINS** Salomé-Abarca LF, Van Der Pas Jorik, Kim Hye Kong, Van Uffelen Gerda A, Klinkhamer Peter GL, Choi Young Hae
6	**STUDIES ON NON-ENZYMATIC OXIDATION OF GOMPHRENIN PIGMENT BY HPLC-DAD-ESI-MS/MS** Kumorkiewicz A, Wybraniec S
7	**CHEMICAL CHARACTERIZATION OF VOLATILE COMPOUNDS FROM CAMEROONIAN HONEYS** Makowicz E, Jasicka-Misiak I, Kafarski P
8	**COMPARISON OF TRITERPENOID PROFILE OF GRAPEVINE CV. CABERNET SAUVIGNON NATIVE PLANT AND *IN VITRO* CULTURE** Burdziej A, Pączkowski C, Cluzet S, Szakiel A

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2	**RESPONSE SURFACE METHODOLOGY AS AN EFFECTIVE TOOL IN THE OPTIMIZATION OF EXTRACTION CONDITIONS AND PREDICTING ANTIOXIDANT POTENTIAL OF *MAGNOLIA* X *SOULAGEANA* 'LENNEI' FLOWER BUD EXTRACTS** Adamska-Szewczyk A, Baj T, Zgórka G
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4	**THE ISOLATION OF PHENOLIC COMPOUNDS FROM *CISTUS MONSPELIENSIS* METHANOLIC EXTRACTS BY CPC CHROMATOGRAPHY** Voudour I, Cheilari A, Aligiannis N
5	**MODIFICATION OF STEROL CONTENT IN MARIGOLD *CALENDULA OFFICINALIS* HAIRY ROOTS AS A RESPONSE TO ELICITATION WITH SELECTED BIOTIC AND ABIOTIC FACTORS** Alsoufi A, Długosz M, Pączkowski C, Szakiel A
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8	**ROYAN BIODISCOVERY INITIATIVE USING ZEBRAFISH IN VIVO AND OTHER IN VITRO ASSAYS FOR IDENTIFICATION OF BIOACTIVE NATURAL PRODUCTS** Ayyari M, Tahamtani Y, Pahlavan S, Rezaei M, Karami F, Shahbazi N, Hosseini M, Pourghadamyari H, Fooladi P, Baharvand H
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51	**QUALITATIVE AND QUANTITATIVE ANALYSES OF PHENOLIC ACIDS BY LC-DAD METHOD IN AGAR MICROSHOOT CULTURES OF *SCHISANDRA CHINENSIS* CV. SADOVA** Szopa A, Klimek-Szczykutowicz M, Ekiert H
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LECTURES

PL–1

PHYTONEERING: FROM EMPIRIC TRADITIONAL PLANT-BASED MEDICINE TO EVIDENCE-BASED PHYTO-PHARMACEUTICALS

POPP MA

Bionorica SE, Neumarkt, Germany

The treatment of a wide variety of diseases using plant-based medicines is steadily gaining importance. In terms of their pharmaceutical efficacy, safety and quality, modern research based phyto-medicines have to follow allopathic principles and have to find their place in current evidence-based medicine. Plant-based medicines fulfilling these requirements can be the preferred alternative to chemically and synthetically produced medicines.

Bionorica, located in Neumarkt (Bavaria, Germany) is one of the world's leading manufacturers of scientifically researched herbal medicines. Based on the 'Phytoneering' strategy, Bionorica decodes the extensive active ingredient potential of plants (phytos) using state-of-the-art research and technology (engineering). The result: highly effective medicines with few side effects. Bionorica is using translational science as a rapidly growing discipline in biomedical research, which aims to expedite the discovery of new diagnostic tools and treatments by using a multi-disciplinary, highly collaborative "from bench to bedside and back" approach.

One main focus of the company's work is the research, development and marketing of plant-based medicines for the treatment of infections of the respiratory and the urinary tract.

PL–2

ADVANCEMENTS IN ANALYTICAL CHEMISTRY FOR QUALITY CONTROL AND SAFETY OF HERBAL PRODUCTS – PHYTOVALLEY® WHERE SCIENCE MEETS NATURE IN THE HEART OF THE ALPS

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Herbal preparations offer a unique approach for the development of phytodrugs, phytocosmetics and food supplements. Since herbal preparations like plant extracts are multi-component mixtures, they typically possess a broad spectrum of bioactivities. Their potential applications are generally designed to influence many biological pathways simultaneously. Therefore, the development of herbal products appears promising for applications in which several cell types, organs, biological processes as well as interactions between them might play an important role. However, the achievements in natural product research are largely based on the constant development of highly selective and sensitive analytical technologies. For that novel enrichment and purification methods based on modern solid-phase extraction technologies are applied to reduce the complexity of plant-materials, while HPLC is used for separation, pre-concentration and fractionation. The opportunity to hyphenate these methods to robotic systems permits high-throughput screening. Significant progress has also been made in the development of novel stationary phases which can be tailored to a specific application, allowing endless possibilities in terms of selectivity tuning. Further hyphenation to high-resolution mass spectrometry facilitates the identification and quantitation of active components in natural products. Furthermore, the combination of separation science with spectroscopy represents an attempt to combine different technologies in phytopharmacy and food analysis. Near and mid infrared spectroscopy provides the advantages of fast, non-invasive measurements being also suitable for the imaging of plant tissues. These advances offer new possible quality control strategies in phytoanalysis and enable to get deeper insights into the biochemical background of medicinal relevant questions. In this talk I would like to demonstrate new analytical approaches by several applications in medicine, phytopharmacy, phytocosmetics and nutrition science. Many of the presented methods have already been successfully applied within the recently established Phytovalley® - Tyrol platform, where science meets nature in the heart of the Alps.

PL–3

SYNTHESIS OF UNNATURAL COMPOUNDS BY ENZYME ENGINEERING

MORITA H

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About 60% of the present drugs were developed from natural products owing to their unique chemical diversity and biological activities. Hence, discovery of new bioactive compounds from natural products is still important for the drug development. On the other hand, breakthrough made in synthetic biology has also begun to supply us with many useful compounds through manipulation of biosynthetic gene for secondary metabolites. Theoretically, this approach can also be exploited to generate new unnatural compounds by intermixing genes from different biosynthetic pathway. Considering the potential, we are studying about bioactive compounds in natural sources, as well as the biosynthesis of natural products including engineering of the secondary metabolite enzymes to make new compounds in order to construct the methodological basis of the synthetic biology. In this symposium, bioactive compounds isolated from medicinal plants and marine organism together with unnatural compounds synthesized using plant polyketide-producing enzymes in our laboratory will be introduced.

PL–4

SUNFLOWERS. A NEW HISTORY FROM AN OLD FRIEND

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The agronomic importance of sunflower (*Helianthus annuus* L., Asteraceae), together with its well-documented allelopathic activity, which was first reported in 1931, [1] has led to the extensive study of this species from a chemical point of view. We have been carried out numerous studies on the isolation of sunflower constituents with special emphasis placed on phytotoxic products. Extractions were performed in an effort to mimic natural conditions at different stages of plant growth, using aqueous extracts from leaves and nonpolar solvents by maceration or soxhlet [2]. More recently, we used supercritical fluid extraction. The number and variety of isolated compounds shows the complexity of these extracts and the ability of sunflower to produce a variety of secondary metabolites. These compounds include sesquiterpenes, diterpenes, sesquiterpene lactones, triterpenes, sterols, flavonoids, coumarins, phenolics, and three new terpene skeletons: heliannuols, heliespirones and dimers of sesquiterpene lactones and kaurane acids [3]. Many of these compounds show phytotoxic activity, even at low concentrations, which suggests their implication in the allelopathic activity of sunflower.
We must emphasize that each extraction methods and growth stage afforded different compounds. The first stage of a phytochemical study is the extraction and we cannot avoid the importance of the selected procedure as well as the growth stage [4], since these will determine the nature, amount and activity of the metabolites extracted. Moreover, in SFE there a number of parameters that can be modified as temperature, pressure, co-solvent and fractionation time, and different conditions afford different compositions. So optimization of method should include bioactivity as decision parameter.

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PL–5

WHERE DID MY 'NATURAL' PRODUCT REALLY COME FROM? USING ISOTOPE RATIO MEASUREMENTS TO DISTINGUISH BETWEEN SYNTHETIC, NATURAL AND ARTEFACTUAL ORIGINS

ROBINS RJ

Elucidation of Biosynthesis by Isotopic Spectrometry Group, CEISAM,
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Within the food and pharmaceutical industries, there is an increasing legislative requirement for the accurate labelling of the product's origin. A key feature of this is to indicate whether the product is natural or synthetic. Distinguishing between these options is particularly important when the natural product is costly relative to the synthetic product. We have developed the means to measure by ^{13}C NMR spectrometry (irm-^{13}C NMR) the position-specific distribution of ^{13}C at natural abundance. This technique is well-suited to distinguishing between origins, as the distribution of the ^{13}C isotope reflects the primary source of the carbon atoms and the process by which the molecule was (bio)synthesised.

Two aspects of this problem will be addressed. The first relates to the labelling of commercially available chemicals. To what extent can we rely on the information given in the Certificate of Origin? This will be discussed in relation to a recent investigation of three alkaloids commonly exploited for human use: nicotine, atropine and caffeine [1]. Several samples of each of these molecules was obtained from different sources Our findings indicate that labelling can be misleading, especially in relation to a supplied compound being labelled as synthetic" even though its ^{13}C profile indicates a "natural" origin.

The second will touch on the problem of the possible creation of artefacts originating during the isolation of products from natural sources, and whether irm-^{13}C NMR or irm by mass spectrometry might be useful in distinguishing artefacts from natural products.

Acknowledgements: I am grateful to many colleagues for their advice and support over the years, and to authors and reviewers who have raised concerns about artefacts arising during work-up.

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PL–6

MEDICINAL PLANTS FROM VIETNAM – INVESTIGATION FOR POTENTIAL ANTI-INFLAMMATORY AGENTS

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Vietnam is one of the most biodiverse countries on earth. It is situated on the Indochina Peninsula and stretches over three climate zones 1,650 km from Mong Cai in the north to Ha Tien in the south. It has a varied landscape that encompasses coastal habitats, inland lakes and rivers, tropical rainforests, monsoon savannahs, limestone karst, subalpine scrubland and two important river deltas. The diversity of ecosystems gives rise to a rich variety of plants. The total flora of Vietnam is estimated to contain approximately 12,000 vascular plant species belonging to more than 2000 genera [1]. About 10% of the higher plants are considered as endemic (Tran, 2005). Vietnam has a deep history of relying on plants for remedies and a lot of traditional knowledge is still preserved. It is estimated that 75% of Vietnamese people still use traditional medicine as their primary source of treatment for common health problems [1]. Many of the traditional medicinal plants have not been investigated neither phytochemically nor pharmacologically. In the course of a collaboration project with the University of Medicine and Pharmacy in Ho Chi Minh City we have been studying a variety of Vietnamese plants among these the shrub tree *Eurycoma longifolia* Jack. (Simaroubaceae). The roots of this plant have been used as traditional medicine to alleviate various diseases including malaria, dysentery, sexual insufficiency and rheumatism [2]. Although numerous studies report on various pharmacological properties of *Eurycoma longifolia*, the anti-inflammatory activity has not been investigated so far. One of our aims was to identify the active principles and to determine the mechanism of action. Furthermore, we established an analytical method for quality control purposes and looked for metabolites to identify suitable markers in doping control. Most recent results of our research efforts within this collaborative research project will be presented.

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PL–7

DETERMINATION OF BIOLOGICALLY ACTIVE COMPOUNDS ISOLATED FROM POLISH PLANTS

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A rich source of biologically active compounds in human nutrition is alfalfa or lucerne (*Medicago sativa* L.). During various investigation it has been indicated that besides protein lucerne contains many secondary metabolites – compounds that are not directly involved in the normal plant growth and development. Among secondary metabolites indentified in extracts obtained from alfalfa, the saponins and flavonoids are the most interesting and well characterized. The goldenrod (*Solidago* L.) is a herbaceous plant in the family *Asteraceae*, coming from North America, but well grows in Poland. Honey is obtained from this plant. It serves as a benefit for bees in late summer, when there is a lack of flowers. Goldenrod herb contains essential oils, triterpenoid saponins, diterpenoid acids, phenylacids (phenolic diglycoside-leucocozoid), flavonoids (quercetin, kaempferol and isorhamnetin and their derivatives), derivatives of caffeic acid, polysaccharides, carotenoids and other. Infusions of the goldenrod are primarily used in treatments as a diuretic. Moreover, phenylacids and tannins form goldenrod, make up with harmful metabolic products easily water soluble complexes. Antiseptic and anti-inflammatory properties of this herb is caused by the presence of salicylates and phenylacids.

The main aim of investigations was to develop methodologies for the extraction and determination of biologically active compounds from mentioned plants. Classical solvent extraction and modern methods such as: supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and other allow to obtain extracts rich in biologically active compounds and characterized by a high antioxidant activity. The method of rapid assessment of the qualitative analysis of extracts by means of TLC, HPLC-MS were proposed. Moreover, the application of MALDI-TOFMS for the screening analysis of main sugars in plant extracts has been discussed.

Acknowledgements: This work was financed in the framework of grant entitled: ”Cultivated plants and natural products as a source of biologically active substances assign to the production of cosmetic and pharmaceutical products as well as diet supplements” (nr BIOSTRATEG2/298205/9/NCBR/2016) attributed by the National Center for Research and Development (Warsaw, Poland).

PL–8

INVERTEBRATES AND ASSOCIATED MICROORGANISMS OF THE MARINE MESOPHOTIC ZONE - A UNIQUE SOURCE FOR THE DISCOVERY OF BIOACTIVE SMALL MOLECULES WITH ANTIAGING ACTIVITY

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TASCMAR is the acronym of an H2020 EU-funded project that aspires to develop new tools and efficient strategies on discovering novel marine derived bio-molecules with applications in pharmaceuticals, nutraceuticals and cosmeceuticals with a particular focus on the theme of anti-ageing. The project also focuses on the development of innovative cultivation technologies and equipment for marine invertebrates and associated symbionts aiming their extraction from lab to pilot-scale.
Accordingly, more than 180 existing collection of invertebrates (MACLIB library) and 179 targeted marine invertebrates species (TARMAC library) were collected from the under-investigated mesophotic zone (between 30 and 100 meters depth) of the Indian ocean, the Red sea and the Mediterranean. Furthermore, more than 300 (MICLIB library) and 312 (TARMIC library) associated microorganisms of MACLIB and TARMAC libraries respectively, were collected.
The samples were extracted by optimized protocols in order to collect all the bioactive molecules that they contain. The libraries of extracts were sent for biological evaluation. According to the results for MACLIB library, 5.30% of the extracts showed elastase and tyrosinase inhibitory activity, 7.94% inhibition to Fyn kinase, 6.35% to proteasome and 4.76% to CDK7 kinase. For TARMAC library 16.3% of the extracts showed tyrosinase inhibitory activity, 12.4% elastase inhibitory activity, 5.03% to FYN kinase, 15.64% to CDK7 kinase and 20.67% to proteasome. All active extracts were investigated for their chemical profiling employing UHPLC-HRMS techniques and the metabolites present in each extract were identified using various analytical chemistry techniques and software (dereplication).
These extracts were fractionated by MPLC or flash chromatography using various solvent systems. The fractionation was followed by the preparation of library of fractions for bio-evaluation. Structure elucidation has been performed by NMR and LC-MS methodologies and the compounds of interested have been isolated and send for further investigation.
Furthermore, the biological activity of the associated microorganisms was examined. For MICLIB library, the microorganisms showed 0.5% inhibition activity to tyrosinase and elastase, 0.9% to Fyn kinase, 6.67% to CDK7 kinase and 1.67% to proteasome. For TARMIC extracts, ~80.1% were found to inhibit tyrosinase activity, 22.8% showed elastase inhibitory activity, 14.19% to FYN kinase, 7.67% to proteasome and 7.43% to CDK7 kinase.
Thus a comparison between the profiles of the invertebrate extracts and the profiles of the microbial symbionts was made. According to the results, it was observed that the percentage of the common metabolites between the invertebrates extracts and the symbionts was more than 4% and in one cases it was more than 14%. This overlapping could indicate the contribution of microorganisms to the whole invertebrate metabolome.

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PL–9

METABOLOMICS AND BIOCHEMOMETRICS: TOWARDS ACCELERATED LEAD FINDING

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For centuries plants have long had a central role in the treatment of a wide spectrum of diseases, hence continuously supporting the health of human populations. Nowadays, in excess of quarter of modern medicines are derived (either directly or indirectly) from plants. Artemisinin (antimalarial), paclitaxel (antineoplastic), codeine and morphine (analgesic), andgalanthamine (reversible cholinesterase inhibitor) are good examples in this direction andamongst the best-selling drugs worldwide. Recently in the USA, for instance, two new drug applications have been approved for marketing botanical products as prescription drugs, namelyVeregen (a topical drug for the treatment of genital and perianal warts) and Mytesi (an oral drug for the treatment of HIV/AIDS related diarrhea). These new drug approvals are remarkable examples thatcomplex botanical mixtures can be developedas new drugs in order to meet modern FDA standards [1-3, and the literature cited therein].

At the same time the development of new drugs is rather costly, laborious and time-consuming process, hence platforms for accelerated lead finding/drug discovery, quality control assessment and mode of action of healing herbs, and sustainable production are continuously sought.

An overview of the phytochemical and pharmacological (*in vitro* and *in vivo* studies) aspects of research on selected medicinal plant species towards accelerated lead finding will be given and thoroughly discussed [4-8].

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PL–10

ACCELERATION OF DRUG LEAD DISCOVERY BY BIOACTIVITY-CORRELATED TECHNIQUES IN COMBINATION WITH HPLC-HRMS-SPE-NMR

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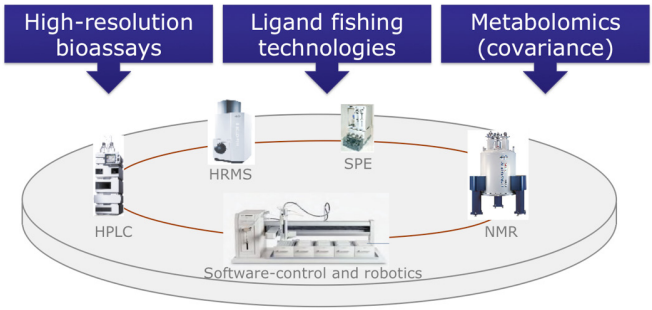
In recent years, hyphenation of analytical-scale high-performance liquid chromatography and high-resolution mass spectrometry with solid-phase extraction and nuclear magnetic resonance spectroscopy, *i.e.*, HPLC-HRMS-SPE-NMR, has proven successful for full structural elucidation of constituents in crude extracts without any prepurification [1]. This even includes acquisition of direct-detected ^{13}C NMR spectra [2] and database-assisted NMR structure elucidation [3]. However, the basic HPLC-HRMS-SPE-NMR setup does not give any information about the bioactivity of individual constituents in the crude extract. Thus, the recent development of microplate-based high-resolution inhibition profiling [4], ligand fishing [5] and bioactivity-correlated metabolomics [6] has allowed targeting subsequent HPLC-HRMS-SPE-NMR experiments towards the bioactive constituents only - as indicated in the figure below.



In this presentation, recent examples of high-resolution polypharmacological profiling, ligand-fishing technologies and bioactivity-correlated metabolomics profiling in combination with HPLC-HRMS-SPE-NMR analysis will be given - with emphasis on key-enzymes in the management of type 2 diabetes.

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PL–11

ON-LINE SUBCRITICAL SOLVENT EXTRACTION AND CHROMATOGRAPHY FOR CAROTENOID FINGEPRINTING IN FOODSTUFFS USING MASS SPECTROMETRY DETECTION

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The awareness of natural bioactive molecules such as carotenoids has indisputably increased over the last decade, for functional foods and food additives; in parallel both the scientific community and the industry have striven for the development of more sustainable extraction processes and analytical methods.
Compared to other solvent-based techniques, supercritical fluid extraction (SFE) and chromatography (SFC) may provide a number of advantages, in terms of organic solvent consumption, costs, and environmental impact. Moreover, SFC provides unmatched analysis speed compared to HPLC, and thus SFE-SFC instrumentation allows for more rapid routine analysis of carotenoids to be carried out.
A new method is here presented, in which extraction and separation of carotenoids in food samples was carried out using a mixture of carbon dioxide-methanol, operated under subcritical conditions.
Multiple extractions, until depletion, were performed on the same sample, in order to evaluate the extraction yield, as a function of the experimental temperatures, investigated in the 40°-80°C range. Carotenoids fingerprinting was attained in tamarillo and pepper samples, in a very fast, green and efficient way, by means of MS detection of intact carotenoids.
The online subcritical solvent extraction and chromatography method developed gave comparable results to traditional solid-liquid extraction and HPLC-MS analysis, while presenting a number of advantages over offline approaches, consisting in improved run-to-run precision, full automation and setting of batch-type applications, reduced risk of sample contamination and deterioration.

PL–12

PHARMACOGNOSY: PAST, PRESENT AND FUTURE?

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This lecture will cover some of the phytochemistry and bioassay-guided isolation work we have conducted over the last 20 years in the areas of antibacterials, anti-TB agents, resistance-modifying agents and latterly prospecting for new antibacterials from spring water.

Our work has mainly focused on Gram-positive bacteria, including resistant variants of *Staphylococcus aureus* and *Mycobacterium tuberculosis*, which still remains a significant global health issue due to multidrug- and total-drug resistant variants. We have delved in to ways to circumvent resistance in these bacteria by characterizing efflux pump inhibitors [1], anti-plasmid conjugation natural products [2] and latterly biofilm inhibitors [3]. The 'Holy Grail' of anti-infective research is currently to find new antibiotics with activity against intrinsically-resistant Gram-negative bacteria, especially *Klebsiella pneumoniae* and its carbapenem-resistant variants such as KPC and KP NDM-1. This is a hard challenge as whilst there are phytochemicals that can kill these bacteria, they generally have poor selectivity and are often cytotoxic against mammalian cell lines [4]. We have turned our attention to characterizing some antibiotic-producing bacteria from springs to address this issue. Springs such as the Roman Baths at Bath are prolific producers of many genera of bacteria, the majority of which have never been studied for antibiotic production.

Finally, this lecture will look at where Pharmacognosy may be directed in the future, given the constraints placed on us by changes in our environment, the petrochemical industry and the need to work in a 'greener', more sustainable and less polluting fashion.

Acknowledgements: I gratefully acknowledge the work of Professor Dr Dr (HC) Yoshinori Asakawa, Professor Dr Geoffrey Cordell, Professor Dr Douglas Kinghorn and Dr Paul Stapleton for their inspirational and vibrant research.

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PL–13

PHARMACOGNOSY IN THE DIGITAL ERA: MOVING TOWARDS CONTEXTUALIZED METABOLOMICS

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The recent rapid innovations made in metabolite profiling and bioassays may lead to a change of paradigm in natural products research. Indeed having at hand full or partial of structure of possibly all metabolites in given natural extract at different quantitative levels open the possibility to perform pharmacognosy studies from a more holistic perspective.

The increasingly amount of accurate metabolome data that can be acquired on massive sample sets, notably through high resolution mass spectrometry data dependent MS/MS analyses (HRMS/MS), allows mapping of natural extracts at an unprecedented precision level [1]. While the acquisition of larger volumes of data is ongoing, contextualizing it is a lagging process. For this the establishment of integrated and open databases ecosystem could be extremely valuable to nurture pharmacognosy in the years to come and have finally general positive societal outcomes [2]. Fast progresses are foreseen bringing together recent computational / analytical approaches linking invaluable published knowledge brought by pharmacognosy mainly based on bioactivity guided isolation studies.

In this context we push forward our applications and further development of UHPLC-HRMS/MS Molecular network (MN) approaches [3,4] to provide enhanced annotation confidence level though multiple scores integrating notably taxonomy information and MN structural consistency as well as other orthogonal analytical data (chromatographic retention, Collisional Cross Section in IMS…). Benchmarking of such approaches is currently assessed by profiling mixtures of herbs with well-studied composition.

Different recent applications of our metabolomics and phytochemical investigations will illustrated these aspects. An ideal workflow will be presented and discussion on what is readily implemented and is still required will be made, notably in term of contextualisation of the data.

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PL–14

PLANT SECONDARY METABOLITES AS ROLE MODELS IN DRUG AND COSMETIC RESEARCH *VIA* ENZYME INHIBITION

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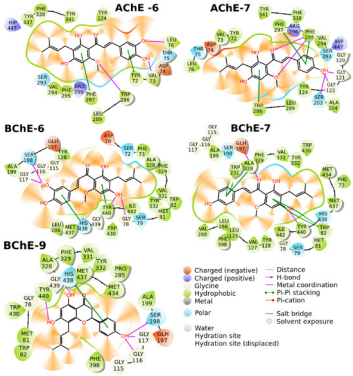
Natural products have been always mainstay of drug discovery and research as many synthetic drugs used currently in clinic are the derivatives of their natural counterparts. In this adventure, enzyme inhibition have been always one of the most popular methods in drug research and many inhibitors are now available for the treatment of various diseases. Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. It is well-known that medicinal plants constitute an attractive natural source with rich secondary metabolite profile that display a wide-range enzyme inhibitory potential.

In this endeavor, many secondary metabolites isolated from medicinal and aromatic plants growing in Turkey and other countries in collaboration as well as their synthetic or semi-synthetic derivatives have been continuously tested by our group against a number of enzymes (*e.g.* tyrosinase, cholinesterases, xanthine oxidase, lipoxygenase, phosphodiesterase, elastase, collagenase, etc). The compounds have been examined by their enzyme inhibitory potential using both *in vitro* and *in silico* (molecular docking) experimental models. Our studies revealed high potential of natural compounds as novel enzyme inhibitors such as resveratrol derivatives, tanshinones, xanthothumol derivatives, coumarins, flavonoids, and some other secondary metabolites pf plant origin. In this talk, the recent results from our group's relevant studies will be highlighted and importance of plant secondary metabolites as enzyme inhibitors will be emphasized.



Detrimental schematic views of the selected xanthohumol derivatives in the interaction with AChE and BChE

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PL–15

THE MAGIC WORLD OF LIPIDS – NATURAL PRODUCTS AND THE ENDOCANNABINOID SYSTEM

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Decades of attempts to isolate the bioactive (i.e. psychoactive) constituent(s) of *Cannabis sativa* L. has led to the first unambiguous proof that Δ9-tetrahydrocannabinol (THC) is the major psychoactive metabolite in marihuana and hashish. In the 1990s the first corresponding receptors and endogenous agonists were identified, giving origin to the concept of the endocannabinoid system (ECS). Today we know receptors, enzymes, transport systems and endogenous allosteric modulators that make up a lipid network. Over the last decade, numerous studies have shown the involvement of the ECS in health and disease. As will be outlined in this talk, research with lipids poses particular challenges and when it comes to lipids, minute changes like the configuration of a double bond, can make big differences. Truly, lipids are magic as they stick to surfaces and integrate in biological membranes but also exert very potent effects, being primary signaling molecules. In our group we are interested in cellular endocannabinoid transport and how selective endocannabinoid reuptake can induce beneficial effects in conditions of ECS deficiencies. In this talk, the evolutionary link between the ECS in mammals and plant natural products will be highlighted, providing a basic framework of the current knowledge.

PL–16

OLIVE BIOACTIVE COMPOUNDS: CHEMISTRY AND BIOLOGY

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The olive tree, closely connected to the Mediterranean region has provided a wealth of goods. Research on the olive has started early but it has proven inexhaustible revealing mainly a vast array of nutritional and health properties. Apart from olive oil and table olives, the by-products coming from olive processing industry have been proven attractive materials for research. The aim of this communication is to present a holistic research strategy towards the multifaceted exploitation of the olive tree including activities such as extraction, fractionation, isolation, analysis of olive tree products as well as investigation of processes related to olive industry and valorization of by-products.

The main products of the olive tree, olive oil and table olives as well as by-products such as leaves, paste, mill wastes and table olive wastewater have been used as sources for the recovery of valuable secondary metabolites. This has been performed with conventional techniques and also by adsorptive resin technology [1, 2]. In addition standardized enriched fractions have been prepared with various techniques, such as MPLC, HPLC, and CCC. Isolation of promising lead compounds with emphasis to olive polyphenols [3] oleuropein (leaves), hydroxytyrosol & tyrosol (olive oil, by-products), oleacein & oleocanthal (olive oil) and lactones (by-products), has been achieved. Additionally advanced analytical techniques and methodologies (UPLC/HPLC-DAD, HPLC-DAD-HR/MS^n, and HPTLC) have been developed and applied for the qualitative and quantitative determination of secondary metabolites in all the above mentioned materials [4]. The lab scale processes have been also adapted to pilot scale systems. The biological profile and the therapeutic potential of olive extracts and compounds is explored and supported by several *in vitro* and *in vivo* studies while their possible application as nutraceuticals, dietary supplements and cosmetics is also investigated.

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PL–17

ARE CELL EXPERIMENTS BELIEVABLE FOR PHARMACOLOGICAL STUDIES OF POLYPHENOLS?

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Their benefits of polyphenols depend on their bioavailability influenced by their stability, the interaction with other components, uptake from the intestine, and metabolism. The *in vitro* cell experiment is one of important approaches to explore the healthy effects of polyphenols. In many cell experiments, the individual polyphenol or the extracts rich in polyphenols are typically incubated with various cell lines for up to a few days. What do really happen for polyphenols during long time incubation with various cancer cells under cell culture conditions? Here, quercetin and 5,7,3',4'-tetrahydroxyflavone were incubated with A549, 231, MCF-7, and Caco-2 cells at 37 °C in 5% CO2 for 24-72 h. We are investigating the structures of these new products by Thermo LTQ XL™ UPLC-MS-MS and Waters Synapt G2-SUPLC-MS-MS [1]. After incubated for 72 h, the new products of polyphenols were found to quite different from different cells. For A549 and Caco-2 cells, the new products of quercetin and 5,7,3',4'-tetrahydroxyflavone are their glucuronides and methylated glucuronides. For 231 and MCF-7 cells, the new products of quercetin and 5,7,3',4'-tetrahydroxyflavone are their methylated forms.
It is time to investigate what really happened for polyphenols and their new products in cells, as well as the related mechanism. It is very important to further check the bioactivities of these new products, which will avoid erroneous conclusions for what's the really bioactive compounds.

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PL-18

GPCR PHARMACOLOGY AS A MOSAIC OF LIGANDS, RECEPTORS AND SIGNALS

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G-protein coupled receptors (GPCRs) are the largest family of proteins responsible for signal transduction into a cell. Since they are involved in most physiological and pathological processes, GPCRs consist one of the biggest group of druggable targets in current pharmacotherapy. Even though the amino acid sequences exercise relatively low level of evolutionary conservation, all GPCRs share very similar overall structural organization and patterns of molecular mechanisms of activation. Classical understanding of GPCR pharmacology where an agonist upon binding to its receptor induces a specific intracellular signaling pathway has been challenged by recent structural and biochemical discoveries. Very complicated network of signaling cascades controls functioning of every cell in the body; a receptor interferes this mosaic of signals usually in more than one endpoint. Biased agonism is a phenomenon where activation of a receptor by different agonists affects the endpoints with different relative intensity what may lead to alternative scenarios of intracellular signaling effects. Every single cell contains the mosaic of diversified membrane receptors, they act in concerted mode where a crosstalk between them may significantly alter the overall effect. Additional level of complication comes from the fact that ligand molecules are not very specific to a particular GPCR; the same compound usually activates a series of different receptors at the physiological concentration. The research in last 20 years keeps revealing much higher level of complexity in actual mechanisms of drug action on the GPCR targets what leads to elaborating novel strategies in future drug development projects.

ORAL PRESENTATIONS

OP–1

PRENYLATED FLAVONOIDS AS ANTIBIOTIC ENHANCERS AGAINST HUMAN PATHOGENIC BACTERIA INCLUDING MRSA

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The use of natural products in combination with conventional antibiotics is an important strategy to minimize antibiotic toxicity and overcome antibiotic resistance [1].
Several prenylated flavonoids were investigated for their *in vitro* antibacterial activity alone and in combination with seven antibiotics against Gram-positive and Gram-negative bacteria. The interactions between prenylated flavonoids and antibiotics were evaluated by the checkerboard assay, the fractional inhibitory concentration indices (FICIs) and surface response (ΔE) models being used to assess the nature of the interactions. The kinetics of the synergistic interactions was further investigated by the time-kill assay [2,3].
The checkerboard assay showed a predominantly synergistic profile against Gram-positive bacteria. Morusin and kuwanon G were synergistic with oxacillin, amoxicillin, gentamicin, ciprofloxacin and tetracycline against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591 and 43300 (FICI ≤ 0.50). The ΔE model confirmed all these synergistic interactions. The time-kill assay indicated bactericidal synergy for the combinations of morusin/kuwanon G with oxacillin and ciprofloxacin against MRSA ATCC 33591 and morusin with oxacillin and gentamicin against MRSA ATCC 43300 (ΔLC_{24} = 2.02 – 2.61$\log_{10}$ CFU/mL). Strong synergistic interactions (FICI ≤ 0.26) were found between morusin and oxacillin, gentamicin, ciprofloxacin and tetracycline against methicillin-sensitive *S. aureus* ATCC 6538 (MSSA) and *S. epidermidis* ATCC 12228. Significant synergistic effects (FICI ≤ 0.26) against the same Gram-positive strains were also generated when combining kuwanon G with gentamicin, ciprofloxacin and clindamycin.
Morusin and kuwanon G are potent antibiotic enhancers against MRSA, MSSA and *S. epidermidis*. The combination of both prenylated flavones with antibiotics is a promising approach for the development of novel treatments for MRSA and other bacterial infections.

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OP–2

ANTIOXIDANT AND UVA-PHOTOPROTECTING ACTIVITY OF THE EXTRACTS AND CAFFEIC ACID DERIVATIVES FROM *GALINSOGA PARVIFLORA* AND *GALINSOGA CILIATA* HERB

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Galinsoga parviflora Cav. and *G. quadriradiata* Ruiz et Pav (*G. ciliata* Raf. Blake) are used in folk medicine as anti-inflammatory agents and accelerators for wound healing. The extracts also have reported potent, concentration-dependent antioxidant activity. Studies performed in cell-free systems confirmed free radical scavenging ability of aqueous and ethanolic extracts against DPPH, reactive oxygen species (superoxide anion and hydrogen peroxide), reactive nitrogen species (nitric oxide and peroxynitrite) as well as inhibitory effect on linoleic acid peroxidation [1]. Phytochemical analysis of the extracts was performed using HPLC-DAD-MS and HPTLC methods and revealed the presence of flavonoids and caffeic acid derivatives [2]. Ethanolic and aqueous extracts from both *Galinsoga* species prevented ROS formation in UVA or UVB irradiated human fibroblasts. However, only aqueous extracts protected cells against UV-induced apoptosis or necrosis [3].

Our recent studies focused on two caffeic acid derivatives: 2,3,5(2,4,5)-tricaffeoylaltraric acid and 2,4(3,5)-dicaffeoylglucaric acid, isolated from *G. parviflora* herb, a major phenolic acids occurring in extracts [4]. In this study, human dermal fibroblasts (NHDF) were used to investigate the ability of tested compounds to inhibit UVA-induced cell damages and to elucidate the molecular mechanisms involved.

Cells pretreated with tested compounds prior to UVA showed inhibition of intracellular ROS formation and increase of GSH level. Significant increase of cell viability was also observed, as well as decrease of LDH release and a the rate of apoptotic cells in comparison to untreated cells. Further studies revealed, that tested compounds activated the Nrf2 and upregulates the antioxidant genes, such as HO-1 in human skin fibroblasts.

Our results suggested, that caffeic acid derivatives present in Galinsoga herb, in particular 2,3,5(2,4,5)-tricaffeoylaltraric acid, a natural polyphenol, protect the cells from UVA damage mainly by elevating the intracellular antioxidative enzymes throungh the enhanced activation of a transcription factor for antioxidant genes, Nrf2, and significantly induces the expression of the antioxidant genes such as HO-1 following UVA irradiation.

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OP–3

APPLICATION OF GC-MS FOR IDENTIFICATION AND QUANTIFICATION OF TRITERPENOIDS IN DIVERSE MATRICES: EXTRACTS FROM PLANT TISSUES, OILS, OLEORESINS

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GC-MS is a commonly applied method for metabolic profiling of plant extracts with respect to compounds of suitable volatility. Triterpenoids (except for their glycosides) are the group of plant natural products that can be analyzed by this method. These compounds are widely distributed in edible and medicinal plants and they are important as an integral part of the human diet. Formed by the cyclization of the linear squalene molecule, these tetra- or pentacyclic compounds are distinguished by their wide structural diversity and numerous biological and pharmacological activities. There are several possible approaches to the triterpenoid analysis by GC-MS, the majority of them involve derivatization (e.g., silylation) of all analyzed compounds. Nevertheless, the molecules such as steroids and pentacyclic alcoholes, aldehydes or ketones can be subjected to the analysis directly, without derivatization, after chromatographical separation from triterpenoid acids. However, the application of adsorption preparative TLC might not be simple in case of the analysis of oils and oleoresins, e.g., oil from beech tree (*Fagus sylvatica*) seeds, oil from seeds of rugosa rose (*Rosa rugosa*), oleoresin from *Capsicum* spp., which required the two-step chromatography (including preliminary development in chloroform: hexane 1:1 solvent system) to avoid the contamination with triacylglycerols and other lipids. The important factor influencing triterpenoid analysis in plant extracts is the choice of the solvent. Diethyl ether extracts of whole plant parts or chloroform extracts from cuticular waxes contain less contaminants than extracts prepared with the use of more polar solvents, which facilitates precise identification and quantification. For example, the comparison of triterpenoid profiling in diethyl ether and ethanolic extracts of flowers of chamomile (*Matricaria chamomilla*) or fruits (hips) of rugosa rose (*R. rugosa*) revealed significant differences in the total content of triterpenoids, e.g., 2% of diethyl ether extract mass versus 1.4% of ethanolic extract of *M. chamomilla* flowers or 7% of diethyl extract mass of *R. rugosa* hypanthium and 20-times less amount in respective ethanolic extract. The applied solvent influences the extraction yield of triterpenoid classes, i.e. mono- and dihydroxyalcohols, acids, esters; changing their quantified amounts and relative ratios of analyzed compounds. Triterpenoids themselves can appear as contaminants during GC-MS analysis of some alkaloids or polyphenolic compounds in oleoresins (e.g., analysis of capsaicin and curcumin in Capsicum and Turmeric oleoresins, respectively), in which they can constitute up to 0.5% of extract mass.

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OP–4

HIGH-THROUGHPUT SCREENING OF NATURAL PRODUCTS UTILIZING HRMS AND UHPLC-MS/MS TECHNIQUES

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OP–5

DATA DRIVEN DISCOVERING AND DEVELOPMENT OF NEW ACTIVE NATURAL ANTI-AGING INGREDIENT. ENTERING "BIG DATA" AND NETWORK SCIENCE IN PHARMACOGNOSY.

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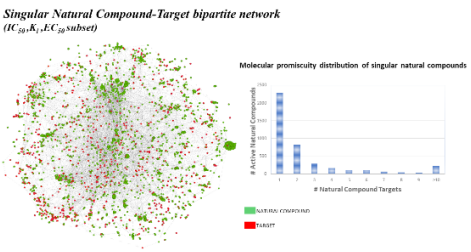
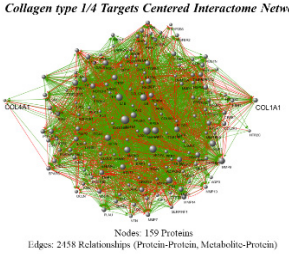
New high-throughput techniques applied to biological, pharmacological and chemical fields have generated in the last decades a large volume of data. In particular, the natural product (NP) origin/sources, molecular promiscuity of singular active natural compounds (SANC), in combination with the emerging of chemo-genomic data, represent an area that is much influenced by big data phenomena [1,2]. In this presentation we report a data-driven based discovery approach for the determination of target biomarkers, phytochemical translation and development of a new natural anti-ageing extract. The determination of the biomarkers was performed in three steps: data mining (by NPL search in the scientific literature and public repository), meta-analysis, omics networks creation and finally the determination of the targets by dynamic analysis of the sub-pathway generated. Starting from SANC-Target and SANC-Source bipartite networks, molecular promiscuity, SAR, availability and patentability criteria were used for the translation and determination of the active compounds and corresponding active extracts on selected targets. Analysis of the extracts by a miniaturized screening platform (micro-separation, UPLC-QTOF, LC-MS and NMR) in combination with targeted *in vitro* biochemical and cellular activity screening have led to the selection of the final extract (from four) ready for formulation work and *in vivo* clinical trials.





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OP–6

IDENTIFICATION OF GSK-3 AS A POTENTIAL DRUG TARGET FOR EPILEPSY VIA *IN VIVO* BIOACTIVITY ANALYSIS OF THE CONGOLESE MEDICINAL PLANT *INDIGOFERA ARRECTA*

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In view of the clinical need for new antiseizure drugs (ASDs) with novel modes of action, we used a zebrafish seizure model [1] to screen the anticonvulsant activity of medicinal plants used by traditional healers in the Congo for the treatment of epilepsy, and identified a crude plant extract, *Indigofera arrecta*, that inhibited pentylenetetrazol (PTZ)-induced seizures in zebrafish larvae. Zebrafish bioassay-guided fractionation of this plant identified indirubin, a compound with known inhibitory activity of glycogen synthase kinase (GSK)-3, as the bioactive component. Indirubin, as well as the more potent and selective GSK-3 inhibitor 6-bromoindirubin-3'-oxime (BIO-acetoxime), were tested in several zebrafish and rodent seizure assays. Both compounds revealed anticonvulsant activity in PTZ-treated zebrafish larvae, with electroencephalographic recordings revealing reduction of epileptiform discharges. Both indirubin and BIO-acetoxime showed anticonvulsant activity in the pilocarpine rat model for limbic seizures and in the 6-Hz refractory seizure mouse model. Most interestingly, BIO-acetoxime was also able to exert anticonvulsant actions in 6-Hz fully kindled mice. Our findings provide the first evidence for anticonvulsant activity of GSK-3 inhibition, thereby implicating GSK-3 signaling as a potential therapeutic entry point for epilepsy. Our results also support zebrafish bioassay-guided fractionation of anti-epileptic medicinal plants as an effective strategy for the discovery of new ASDs with novel mechanisms of action.

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OP–7

SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF *SOLIDAGO GIGANTEA*: OPTIMIZATION AT QUARTER-TECHNICAL SCALE

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The main goal of this research was to obtain the optimal conditions of supercritical fluid extraction of *Solidago gigantea* (goldenrod) with carbon dioxide as a solvent at the quarter-technical plant. Criterion for the selection of optimal conditions was the highest amount of selected bioactive compounds as well as the antioxidant activity and cytotoxicity of the *Solidago gigantea* extract. Goldenrod is widely spread in Poland and has traditional usage as a medicinal plant. Preparations from *Solidago gigantea* have a well-defined diuretic, hypotensive and spasmolytic effect together with bacteriostatic, anti-inflammatory and analgesic properties. It is known to contain flavonoids, clerodane-type diterpenes, saponins, polyacetylenes and triterpenes glycosides.
A Box-Behnken design was used to analyze the effects of three independent variables (pressure - P, temperature – T and flow rate of CO_2 - S) of the goldenrod extraction in a supercritical state on the extraction yield and selected criteria. Box-Behnken design allows to analyze of obtained results by Response Surface Methodology (RSM). RSM is a statistical tool that can be used to evaluate the effect (correlation) between responses and independent variables as well as their interactions which allows to find the levels of input variables (P, T, S) that optimize a particular response (content of phenolics, lipids etc.) of a extraction process.
A second-order quadratic polynomial models were suitable for the experimental data and obtained results (R^2 for phenolics was 0,91, yield – 0,97, Total chlorophyll – 0,97, lipids – 0,98, fatty acid methyl ester - 0,91). Therefore, the response surface methodology can be applied to optimize the supercritical carbon dioxide extraction of *Solidago gigantea.* RSM results indicate that the optimal conditions were achieved by extracting at 62°C, 650 bar and the flow rate 3,5 kg/h.
Extraction of plant material is a complex process that is influenced by numerous factors: the nature of the plant itself, process parameters (temperature, flow rate and/or pressure), physicochemical properties of the solvent (viscosity, density, and/or solubility), and phenomena associated with mass transfer. Hence, there is a need for the proper and/or suitable selection or optimization of the conditions for supercritical extraction of individual plant material. Nevertheless, supercritical fluid extraction using carbon dioxide as a solvent is extremely popular due to the high quality of the extract.

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OP–8

SOLVING PROBLEM OF EMUSLIFICATION SAMPLE PLUG IN CENTRIFUGAL PARTITION CHROMATOGRAPHY BY USING FLOW-RATE GRADIENT

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Centrifugal partition chromatography (CPC), as all countercurrent chromatography (CCC) methods, has a risk of emulsification (and sample plug) that can lead to stationary phase flooding [1]. This risk strongly depends on the sample conditions, especially the concentrations of compounds [2]. The risk is much higher if the compounds in the sample highly decrease the interfacial tension (i.e. surfactants).
In this work, natural surfactants (surfactin from *Bacillus subtilis*) were used as the target for purification as well as fractionation regarding their hydrocarbon chain length (homologues). Due to the problem of emulsification, which was strongly present, several modifications were applied, such as: multiple dual-mode for fractionation, controlled flooding of the stationary phase in the first direction of the multiple dual-mode procedure, and finally adjusting the appropriate flow-rate of mobile phase when the risk of stationary phase flooding gradually decreased.
During CPC procedures, (hydrostatic) pressure was monitored as a factor of stationary phase retention. The separation of surfactin homologues and widening of respective peaks during the chromatography decrease the local concentration of surfactants, which increases the stability of the column. Consequently, the risk of the emulsification sample plug (and the risk of stationary phase flooding) is lowered. Initial low flow-rate (gradually increased) solved the problem of emulsification sample plug. Thus, flow-rate gradient was used for the purification and separation of surfactin with reasonable time of separation; to our knowledge, this is the first report of such approach.

Acknowledgements: Acknowledgements to the company Boruta-Zachem Biochemia Sp. z o.o.

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OP–9

EXPLORING MOLECULAR PROMISCUITY DEGREE AND MULTI-TARGET ACTIVITY SPACE OF DRUGS FROM NATURAL ORIGIN

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Evaluation of molecular promiscuity as the molecular basis of polypharmacology represents useful application for emerging big data in drug discovery [1,2]. Many pharmaceutical relevant compounds/products from natural sources are promiscuous in nature. Polypharmacological profiles of drugs from natural origin are often responsible for their repositioning and/or the withdrawn from the market. For this reason, determination of molecular promiscuity [3] at different stage of the drug development is of considerable interest and, represents the starting point for characterizing the polypharmacological behaviors of several natural products (NP) pharmaceutically relevant. In this work, molecular promiscuity degree (MPD) together with the exploration of the multi-target activity space (MTAS) of pharmaceutically relevant natural compounds is presented. Using the clinical phase stages as criteria (preclinical, phase I, II, III and marketed), the selective mining of activity data of natural compounds from different public repository domains was performed. A total of 309 singular natural compounds active against 1115 unique human single protein targets were determined, composing the “universal” set of this study. Based on IC50, Ki and EC50 described, and classification regarding the development stages, a total of 15 subsets with different confidence levels were created. Analysis of the subsets showed an increase of the average MPD from the preclinical (3.1) to the marketed (4.6) subsets, highlighting the correlation between extensive studies and MDP. CytochromeP450 protein family and estrogen receptors (alpha and beta) were the most frequent targets of the singular natural compounds used in this study. In addition to the MDP determination, a computational and graphical framework for the analysis of multi-targets activity space and MPD patterns are reported. In conclusion, the evaluation of the MPD of singular natural compounds pharmaceutically relevant represent the first step for the analysis of multi-targets activity of NP. All of these represent a rational approach to determine the probability to incur in side-effects and/or drug repositioning possibility in the early stage of active NP development.

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OP–10

EXPLOITING NATURAL PRODUCTS AS GLYCOGEN PHOSPHORYLASE INHIBITORS: COMPUTATIONALLY MOTIVATED DISCOVERY

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The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. Regulation of the glycogen metabolism is a therapeutic strategy for blood glucose control in type 2 diabetes. Glycogen phosphorylase (GP) plays a key role in the glycogenolysis pathway, hence GP has been widely used as a target for compounds that might prevent glycogen breakdown under high glucose conditions. GP is an allosteric enzyme with six different binding sites discovered to date. The majority of inhibitor design efforts to date have focused on the catalytic site and in particular the design of glucose analogue inhibitors but other natural product analogues such as flavonoids and pentacylic triterpenes have revealed considerable potential [1,2]. Computation has played a key role in many of these discoveries [3]. Recent examples of *in silico* guided discovery of GP natural product inhibitors will be presented, where docking and post-docking methods have led to some of the most potent GP inhibitors discovered to date [4-6].

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OP–11

USE OF HPLC GLUCOSINOLATE PROFILES TO IDENTIFY MACA PHENOTYPES CULTIVATED IN PERU AND CHINA

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Since ancient Incas times, Maca (*Lepidium peruvianum*) was cultivated in plateaus of high Peruvian Andes as an energizing and revitalising food additive traditionally recommended for fertility enhancement, improved libido, memory loss, fatigue, mental weakness, insomnia, to regulate menstruation and lessen menopause symptoms. Recent clinical research confirmed that an ingestion of four main Maca phenotypes distinguished by colour of hypocotyls (Yellow, Black, Red and Purple) induces gender-specific physiological responses, thus alleviating effect of several health conditions. This led to development of variety Maca-based dietary supplements for men and women worldwide. Bioactivity of Maca is contributed to the presence of secondary metabolites, namely, glucosinolates and alkaloids, with macaenes and macamides found only in Maca, but their specific physiological targets are not yet precisely defined.
In 2003 Peruvian Maca was introduced to China with cultivation sites established in Yunnan Province. While it has been demonstrated that chemistry and therapeutic functionality of Peruvian Maca are significantly affected by the altitude, environment and geographic location [1], there is no data available to confirm that Peruvian Maca grown in China would project similar analytical and/or therapeutic characteristics as Maca cultivated in Peruvian Andes. Therefore, in this study an attempt has been made to compare Maca phenotypes cultivated in geographically-distant locations: Peru and China using concentrations and HPLC profiles of Glucosinolates considered as the key functional group of active compounds in Maca.
The material for this study was collected in Junin - Peru (4,200m a.s.l) and Shangri La, Lijiang region, Yunnan Province – China (3,180m a.s.l.). Profiles of Glucosinolates were determined by standard HPLC technique with Glucotropaeolin and m-methoxyglucotropaeolin used as external reference standards [1].
Highly significant differences ($P<0.01$) in Glucosinolates both concentrations and shapes of HPLC traces existed between four individual Maca phenotypes grown in Peru (classified as “kimsa kucho” or “Ruyru” shape only) and China (mainly “Achka chupa” resembling Ginseng shape tubers). Depending on the Maca phenotype, statistically significant differences ($P<0.05$ & $P<0.01$) were determined in ratios of individual Glucosinolates (Glucotropaeolin and m-methoxyglucotropaeolin) within four Maca phenotypes grown in each cultivation location and between the locations.
The results confirm earlier assumptions [1] that sums of individual Glucosinolates, their ratios and profiles, may be feasible to explore for analytically identifying individual Maca phenotypes in pulverised Maca products cultivated in Peru or China.

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OP–12

DISTRIBUTION OF SAPONINS IN ROOT OF RED BEET (*BETA VULGARIS* VAR. *VULGARIS* L.) AND THE EFFECT OF PROCESSING ON THEIR ABUNDANCE IN RED BEET PRODUCTS

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Red beet is one of the most important vegetables in Central and Eastern Europe, with roots having substantial levels of vitamins, minerals and nutritients. This plant is well known not only for its nutritional quality and distinctive flavour, but also as a good source of several bioactive plant secondary metabolites. As the consumption of red beet becomes increasingly popular, there is a need to systematically examine the bioactive components such as saponins, phenolics and betalains in the plant, in order to improve and maximise the nutritional quality of this vegetable. While the betalains and phenolic profile in red beet root and food products was thoroughly evaluated, so far nothing was known about the distribution of saponins in red beet root parts as well as the influence of the food processing on the saponin content in red beet products.
Thus in the study represented here, we aimed to evaluate the distribution of saponins in root and the effect of food processing on saponins in different food products. For this purpose, samples were derived from various root parts (lower, elongated part, crown, skin and flesh) as well as food products (juice, borscht, boiled and baked root) and these were subjected to UHPLC-MS/MS quantitative and qualitative analyses in order to monitor the changes in total saponin content, as well as in individual compounds.
It was demonstrated that the saponin content in red beet root exhibits characteristic alterations in different parts, with high abundance of saponins in outer roots part and low abundance in flesh. The differences in the relative proportions of individual saponins present in different parts were observed. Moreover, it was demonstrated that the concentration of saponins depends on the treatment. The content of total saponins decreased during boiling, in baked roots remained unchanged, when for borscht and juice low saponins yield was described. The individual saponins composition was altered significantly in boiled and baked root due to thermal degradation of compounds with higher number of sugars. It was demonstrated that red beet saponins are susceptible to heat, at last one condition present during food processing. Heat degradation of saponins may result in the modulation of nutraceutical properties and a decrease or increase in intensity of biological actions.

YOUNG SCIENTISTS LECTURES

YSL–1

PURIFICATION OF ANTIFUNGAL COMPOUNDS FROM ARGENTINEAN SPECIES *PROSOPIS RUSCIFOLIA* (FABACEAE) BY CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC)

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The aim of this work was to test the antifungal activity of compounds isolated and identified after a CPC (Centrifugal Partition Chromatography) purification from Vinal (*Prosopis ruscifolia* Griseb. (Fabaceae)) on *Aspergillus* species and to identify the responsible metabolites. After successive purification by CPC using Dual mode (DM) and Multiple Dual Mode (MDM) in a small (CPC 250ml) and larger column volume of 1L bioactive compounds were identified. The structures of indolizidine type alkaloids (juliflorine), tryptamine derivative, catechin derivative were ascertained by extensive NMR analyses.

Fungi of the genus *Aspergillus* cause rots in stored cereals and contaminate the grains with aflatoxins. The intake of these mycotoxins in foods is a risk for human and animal health. Chemical control of the *Aspergillus* species is restricted to the use of food preservatives which are fungistatic compounds that can increase mycotoxin accumulation at doses below those required for fungal suppression. New antifungal agents are needed for the control of the *Aspergillus* species. The CPC was used to isolate bioactive compounds from native plant from northwest Argentina. The CPC a hydrostatic CCC column, is a continued liquid-to-liquid solvent partition where the target compounds are competitively distributed between the two-phase solvents due to their different partition coefficient. Using a centrifugal force, one phase is kept stationary in chambers while the other phase is pumped through the stationary one.

Further studies elucidating the mechnism of inhibition the growth of *Aspergillus* by those isolated and identified compounds and the investigation of more compounds purified from the methanolic extract are currently under research.

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YSL–2

PHYTOCHEMICAL ANALYSIS AND MICROBIOTA ASSISTED ISOLATION OF FLAVONOID DERIVATIVES FROM FLOWERS OF MEADOWSWEET *FILIPENDULA ULMARIA* (L.) MAXIM.

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Water infusions from meadowsweet *Filipendula ulmaria* (L.) Maxim. flowers are used in the treatment of fever, rheumatism and traditionally in the urinary tract diseases. The presented research is focused on flavonoid-rich fraction of meadowsweet flower extact, responsible for the diuretic activity [1].
Gut microbiota metabolism of xenobiotics, including natural products, is paid a lot of attention lately. For instance, researchers described gut microbial biotransformation of ellagitannins to urolithins, which are linked to health beneficial activity of ellagitannin-rich products [2].
Preliminary studies, based on UPLC-DAD/MS^n meadowsweet infusion analysis, showed that among other flavonoid glycosides, the infusion contains galloyl derivatives of quercetin and kaempferol glycosides. Incubation of an infusion with human gut microbiota cultures in a small scale and chromatographic analysis were performed to examine changes over the time in a post-growth medium. Differences in dynamics of biodecomposition of mentioned galloyl derivatives in comparison to other flavonoid metabolism were noticed. Scaled up incubation was performed in order to isolate galloyl derivatives. The process was controlled using chromatographic analysis of test samples, collected in several time points and the incubation was terminated when post-growth medium composition was proper for isolation. The medium was extracted using diethyl ether and ethyl acetate. Galloyl derivatives were isolated form ethyl acetate fraction using preparative HPLC and characterized using 1D and 2D NMR techniques. Hydrolysis of glycosides, derivatization and HPLC analysis of hexoside moieties were performed to solve their absolute configuration.
The obtained results showed for the first time that galloyl derivatives of flavonoids are transformed more slowly than simple glycosides. This feature was used for the gut microbiota assisted isolation of three compounds – namely quercetin 3-*O*-β-(2'-*O*-galloyl)-D-galactopyranoside, quercetin 3-*O*-β-(2'-*O*-galloyl)-D-glucopyranoside and kaempferol 3-*O*-β-(2'-*O*-galloyl)-D-glucopyranoside. The presence of all three compounds was confirmed for the first time in flowers of *Filipendula ulmaria*.

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YSL–3

TRITERPENOID PRODUCTION IN HAIRY ROOT *IN VITRO* CULTURE OF MARIGOLD *CALENDULA OFFICINALIS*

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Triterpenoids are a group of plant metabolites widely distributed throughout the plant kingdom. They comprise structurally diverse compounds and in plants they are associated with primary as well as secondary metabolism. The biosynthesis and accumulation of triterpenoids can be affected by various biotic elicitors. Due to many advantages such as rapid growth and genetic/biochemical stability, hairy roots are useful model in plant biotechnology: for research on biochemical, physiological and molecular aspects of plant metabolism. The aim of the present study was to investigate the influence of elicitation with biotic elicitors: jasmonic acid (JA), chitosan and salicylic acid (SA) on the biosynthesis and accumulation of triterpenoid saponins. Established hairy root culture of Calendula officinalis from hypocotyl or cotyledon explants (*Agrobacterium rhizogenes*) in ½ Murashige & Skoog liquid culture were maintained on rotary shaker during 15 days, and afterwards they were treated with jasmonic acid (at concentration 100 µM), chitosan (100 mg/l) and salicylic acid (in concentrations of 50, 100, 250 and 500 µM) and incubated for subsequent 7 days. The liquid media were separated from hairy roots with the use of Büchner funnel and vacuum pump. The after-culture media were extracted by n-butanol and air-dried samples of hairy roots were extracted with methanol in Soxhlet apparatus. Dried extracts were subjected to acid hydrolysis. The hydrolyzates were separated by preparative TLC in the solvent system $CHCl_3$/ MeOH (95:5,v/v), methylated with diazomethane and quantified by GC-FID. After the treatment with JA, the content of oleanolic acid (OA) in the medium increased significantly to 28.46 and 22.71 mg/l for both lines (respectively for CC16 and CH2) in contrast to the control 0.31 and 0.2 mg/l, and OA content in hairy root tissue increased to 41.18 and 52.52 mg/g DW in comparison to 3.75 and 2.56 mg/g DW in control. Application of chitosan at 100 mg/l resulted in slight increase of oleanolic acid content in the medium (CC16 line) to 0.84 mg/l, compared with 0.29 mg/l in the control. Very low amounts of OA were present in the culture medium after elicitation with chitosan line CH2 -0,05 mg/l in contrast to 0.014 mg/l in control. Chitosan at 100mg/l concentration caused an increased secretion of OA only in the line CC16. The effect of chitosan on the tissue was also varying, in the line CC16 OA increased to 5.80 mg/g DW by comparison with control 1.85 mg/g, whereas OA in line CH2 decreased to 0.47 mg/g DW as compared to 2.84 mg/g DW in control. SA affects strongly the secretion of oleanolic acid glycosides into culture medium of both lines at concentrations 50 and 100 µM, but it did not demonstrate any effect at the concentrations of 250 and 500 µM. SA at 50 µM increased the accumulation of OA in the tissue to 1.37 and 1.54 mg/g DW, as compared to 0.78 and 1.05 mg/g DW in control cultures. The obtained results suggest that jasmonic acid is the most promising elicitor among the biotic factors tested in this study.

YSL–4

CARPESIUM DIVARICATUM – A SOURCE OF NEW CARDIVIN WITH CYTOTOXIC ACTIVITY

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Carpesium divaricatum Sieb. et Zucc. (Asteraceae) is a perennial herb which occurs mainly in the mountainous areas of Asia [1]. Plants from the genus *Carpesium* are known for their anti-inflammatory, antipyretic, vermifugic, haemostatic and detoxifying properties [2]. *C. divaricatum* is traditionally claimed to treat e.g.: mastitis, snake bite, acute enteritis, urinary-tract infections, mumps, fever and cold [2]. Some previous phytochemical studies revealed cytotoxic properties of the compounds derived from *C. divaricatum* [2,3].

A concentrated chloroform extract from aerial parts was subjected to CC on silica and eluted by gradient system of Hex/EtOAc (up to 50% EtOAc) and EtOAc/MeOH (up to 50% MeOH). The separated fractions were monitored by TLC and analytical RP-HPLC/DAD. The compounds of interest were separated by HPLC ($MeOH/H_2O$ mixture, either 4:1 (v/v) or 3:2 (v/v); isocratic mode). The procedure led to isolation of *trans*-12-oxo-phytodienoic acid, 10-isobutyryloxy-8,9-epoxythymol isobutyrate and four sesquiterpene lactones: cardivin A [3] and three new constituents named as cardivins E-G. The known compounds were identified by direct comparison of their spectral data with those reported in the literature. Structures of the new constituents were established based on analysis of their MS, ^{1}H NMR and ^{13}C NMR (both 1D and 2D) spectra [3].

To evaluate the cytotoxicity of cardivins A and E two osteosarcoma (U-2 OS and SAOS-2) and one colorectal carcinoma (HCT116) cell lines were used. MTT assay was done to assess the viability of the cells following the treatment with cardivins and doxorubicin as a control. Cardivin A, as it was previously described [3], demonstrated cytotoxic effects with IC_{50} values in a range of 3-9 μM. Similar toxicity was observed for the newly discovered cardivin E. The mechanism of action of the compounds remains unclear and our further investigation will be focused on this issue. Our current research seems to support results of previous studies on cytotoxic properties of the plants from the genus *Carpesium* [2].

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YSL–5

HPTLC-BASED METABOLIC PROFILING, A SUPPLEMANTARY ANALYTICAL PLATFORM TO ^{1}H NMR AND GC-MS FOR SPECIES AND SEASONAL CHEMICAL DISCRIMINATION OF PINE RESINS

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Due to the complexity of the metabolome, a single analytical method is insufficient to cover all metabolites thus the use of multiple protocols has been implemented [1]. Non-polar compounds, especially terpenes, represent a big challenge for some metabolomics platforms such as ^{1}H NMR or MS-based methods [2]. In this study, ^{1}H NMR, GC-MS, and high-performance thin-layer chromatography (HPTLC)-based metabolomics were applied to a set of plant resin samples from five different species that were collected during early and late spring. It was not possible to detect chemical variation associated to the species or time of collection through the use of the ^{1}H NMR. Although, assignments to compounds such as *α*-pinene, *β*-pinene, bornyl acetate and sclareol were achieved. With the results obtained by GC-MS, notable chemical variation by species and collection time was detected. *Abies grandis* was significantly separated from the other species, showing a higher number of monoterpenes. The HPTLC-based profiling method also showed clear separation. Additionally, a broader range of metabolite detection was accomplished with the assistance of post-chemical derivatisation. From multivariate data analysis (MVDA), it can be observed that the variation amongst the species is due to monoterpenes and sesquiterpenes, but the seasonal variation is due to diterpenes (Figure 1). Although the GC-MS and HPTLC data yielded results that appeared similar by comparison, HPTLC provided more discriminant signals than GC-MS and it is considered of high potential for metabolomics with regards to the analytical efficiency and possibility for preparative work.

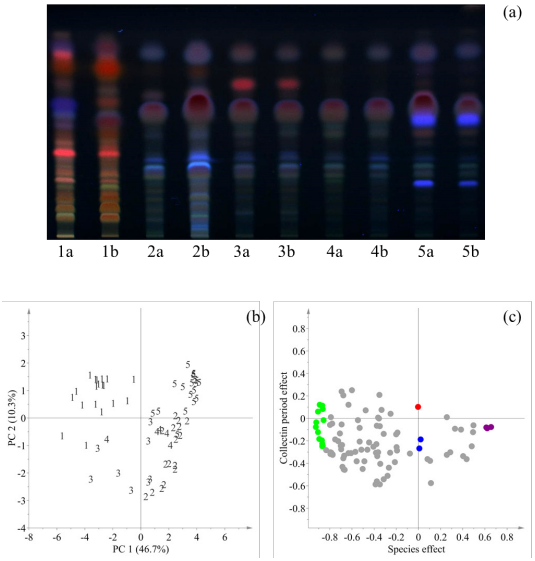

Figure 1. (a) HPTLC's chromatogram from *Abies grandis* (1a, early spring/1b, late spring), *Pseudotsuga menziensii* (2a, early spring/2b, late spring), *Picea abies* (3a, early spring/3b, late spring), *Pinus sylvestris* (4a, early spring/4b, late spring) and *Pinus strobus* (5a, early spring/5b, late spring). (b) Score plot from the PCA numbered by species, 1 (*Abies grandis*), 2 (*Pseudotsuga menziensii)*, 3 (*Picea abies*), 4 (*Pinus sylvestris)* and 5 (*Pinus strobus*). (c) SUS-Plot for species and seasonal effect on resins from five different pine species. Green points: Signals related to *Abies grandis*, purple points: Signals related to *Pseudotsuga menziensii*, *Pinus sylvestris*, *Pinus strobus* and *Picea abies*. Red points: Signals related to late spring, blue points: signals related to early spring.

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YSL–6

STUDIES ON NON-ENZYMATIC OXIDATION OF GOMPHRENIN PIGMENT BY HPLC-DAD-ESI-MS/MS

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Betalains (betaxanthins and betacyanins), water-soluble plant pigments, belonging to most families of the Caryophyllales, are extensively used in the food industry as colorants. Due to the fact that red-purple betacyanins are regarded as highly active natural compounds which exhibit chemopreventive and antioxidative properties, thus potential benefits to human health, accurately established mechanism of betacyanin oxidation is of significant interest. Betanin (betanidin-*5*-*O*-β-glucoside), the principal pigment of red beet root (*Beta vulgaris* L.) was the first and most frequently studied betalain pigment for its antioxidant activity [1]. Additionally, pro-health properties have been also attributed to its isomeric form - the *6*-*O*-glycoside of betanidin (gomphrenin) which is present in high concentration in fruits of *Basella alba* L. and in leaves of its variety *Basella alba 'Rubra'*.

According to our recent studies, because of the presence of the phenolic group at carbon C-6 in gomphrenin, the only possible quinonoid intermediate during oxidation of gomphrenin is a dopachromic derivative. Therefore, gomphrenin enables a unique possibility to observe reaction pathways complementary to betanin reaction routes, which is important for understanding the mechanism of betacyanin oxidation.

We investigated the possible non-enzymatic oxidation pathways of gomphrenin that occurs in the presence of ABTS radicals and established the first tentative structures by means of liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry (LC-DAD-ESI-MS/MS). On the basis of the previous studies on enzymatic and non-enzymatic oxidation of betanidin and betanin [1,2], supported by optical and MS/MS data, the structures of 2-decarboxy-2,3-dehydrogomphrenin and 2,17-bidecarboxy-2,3-dehydrogomphrenin are postulated as the principal products of gomphrenin oxidation. Subsequent oxidation and rearrangement of the conjugated chromophoric system may also lead to the formation of its corresponding 14,15-dehydro derivatives (the "neo" derivatives). The compounds generated this way were detected and tentatively identified as 2,17-bidecarboxy-neogomphrenin and 2-decarboxy-2,3-dehydroneogomphrenin, respectively.

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YSL–7

CHEMICAL CHARACTERIZATION OF VOLATILE COMPOUNDS FROM CAMEROONIAN HONEYS

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Honey is natural food product made by honeybee honeybee (*Apis melifera*) from plant, honeydew or both. It is used not only in food industry but also is popular product in the cosmetics as well as medical industry. Furthermore, consumer interest in this product is constantly increasing. In the market is a wide range of diverse types of honeys with different botanical and geographical origin. Each of them is characterized by unique flavor, nutritional values and therapeutic effects. The chemical composition of honey is variable and strongly depend on huge number of varied factors, such as bee species, climate, soil characteristic, botanical and geographical origin, age of honey, storage methods and honey processing (harvest technology and condition). There is also observed increasing interest of unusual honeys from uncommon geographical origin on international market. Among them are also honeys from Cameroon or other African countries.

The purpose of presented study was evaluation of volatiles chemical content of Cameroonian honeys from different regions of Cameroon. Because of huge ecological diversity of this country chemical composition, honey color, taste and consistency are strongly dependent from the harvesting region e.g. in the center there is humid topical lowland rainforest while on the south and east there is savannah.

During the research three different methods of volatiles extraction were applied (USE, SPE and HS – SPME with two fibers types (PDMS/CAR/DVB and PDMS/DVB)). Each extract was analyzed by GC-MS. Based on the obtained results an unique chemical fingerprint was create. Furthermore, for USE and SPE extracts HPTLC method was applied, which allow us to constructing something like code-bars, which were useful for differentiate honeys from different regions of Cameroon.

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YSL–8

COMPARISON OF TRITERPENOID PROFILE OF GRAPEVINE CV. CABERNET SAUVIGNON NATIVE PLANT AND *IN VITRO* CULTURE

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Grapevine (*Vitis vinifera* L.) represents one of major fruit crop and is commonly studied in regard to polyphenol production ability. In contrast, little information is available about grapevine triterpenoids. The current study aims to investigate triterpenoid profiles of *V. vinifera* 'Cabernet Sauvignon' *in planta* and *in vitro* experimental models. Cell suspension cultures were maintained under continuous fluorescent light (5000 lux) at 25°C on orbital shaker (100 rpm) and weekly subcultured in B5 medium. Cells were harvested by vacuum filtration, frozen at -80°C and lyophilisated. Grapevine plants were propagated from wood cuttings in greenhouse and grown under controlled conditions (25/20°C day/night air temperature, 75% relative humidity and a 16-h photoperiod (350 µmol/m^2/s)). The leaves were harvested from 3-month-old plants, frozen at -80°C and lyophilisated. Plant material was grounded to powder and extracted with diethyl ether in Soxhlet apparathus. The extracts were fractionated by preparative TLC on SiO_2 in a solvent system $CHCl_3$/MeOH (97:3, v/v) and the fractions containing free triterpenoids were directly analyzed by GC-MS/FID on HP-5MS column in temperature programme: initial temperature of 160°C held for 2 min, increased to 280°C at 5°C/1 min and final temperature of 280°C held for further 44 min. The study revealed that some features of the main triterpenoid profile were similar in leaves and in suspension culture cells, i.e. the presence of several steroids (with dominating sitosterol, campesterol, cholesterol, stigmasterol, 24-methylenecycloartanol, tremulone) and a group of oleanane, lupane and ursane pentacyclic triterpenes (α-, β-amyrins, lupeol). However, some qualitative differences were also revealed, including the occurrence of pentacyclic taraxerene alcohol (i.e. taraxerol), lupeol acetate, and two ketones: lupenone and α-amyrenone exclusively in leaves, and the presence of betulin, isofucosterol and stigmastane-3,6-dione only in suspension culture. The ratio of steroids to pentacyclic triterpens differs significantly between the two models tested, equalling almost 9:1 in suspension cultures, and approx. 3:1 in leaves.

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POSTERS

P–1

QUALI-QUANTITATIVE DETERMINATION OF ISOQUINOLINE ALKALOIDS PRESENT IN THE KAZAKH SPECIES OF BARBERRY SHRUBS

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The composition analysis of the root and fruit extracts from two Kazakh barberry species: *Berberis iliensis* M. Pop. and *Berberis sphaerocarpa* Kar. et Kir., was performed, in relation to the European species – *Berberis vulgaris.* As the first species is a rare endemic one with a decreasing natural area under protection [1], it was found interesting to the authors.

To achieve this goal, first an optimization of the extraction protocol was performed using pressurized liquid extraction and green solvents. The efficiency of the recovery process was determined by HPLC, whereas the composition of the extracts was controlled by LC-DAD/ESI-Q-TOF-MS. Several isoquinoline alkaloids were identified in a tailored chromatographic method: berberine, jatrorrhizine, palmatine, columbamine, oxyacanthine, berbamine, obaberine, berberrubine and magnoflorine. For each compounds various fragmentation energies were applied to determine fullest possible fragmentation mechanisms. Interestingly, the two major alkaloids: berberine and palmatine identified in the root extracts were also present in the fruit samples. So far it was believed, that the fruits are sources of polyphenols.

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P–2

RESPONSE SURFACE METHODOLOGY AS AN EFFECTIVE TOOL IN THE OPTIMIZATION OF EXTRACTION CONDITIONS AND PREDICTING ANTIOXIDANT POTENTIAL OF *MAGNOLIA* X *SOULAGEANA* 'LENNEI' FLOWER BUD EXTRACTS

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In the presented experiment, the response surface methodology (RSM) was used to obtain the highest total of phenolic compounds and antioxidant activity in the Magnolia x soulangeana 'Lennei' extract. Dependent parameters, such as the content of the total of phenolic compounds (TPC) was determined using the Folin-Ciocalteu reagent method, whereas the antioxidant activity was determined as a percentage of inhibition (%I) by the DPPH assay. The optimized extraction values (independent parameters) were: extraction time (min), ethanol concentration (%) and solvent/raw material ratio (mL/g). The experiment consisted of 15 experimental sets containing 8 factorial points, 6 axial points and two replicates of central points. The optimum extraction conditions were determined using the desirability function. The highest predicted value for TPC was calculated for the following conditions: extraction time 55.2 min, ethanol concentration 66.8% and 46.8 mL/g solvent/raw material ratio, while the highest antioxidant activity was predicted for extraction conditions conducted over 30 min, ethanol concentration 66.8% and solvent/raw material ratio 30 mL/g. In the case of TPC, the ethanol concentration was the most important ($p < 0.01$), while the extraction conditions were statistically insignificant for antioxidant activity ($p > 0.05$).

Thus, as the optimal extraction conditions for both dependent parameters, the same extraction parameters were adopted as determined for TPC. Using the second degree polynomial equation, it was calculated that at the highest predicted content of phenolic compounds (66.55 mg GAE / g dry extract) the antioxidant activity will be 84.52%. Experimental data for extraction performed under optimal conditions showed slightly higher than predicted TPC values and antioxidant activity, 76.73 mg GAE / g dry extract and 87.10% inhibition of free radicals in the DPPH assay, respectively. The empirical data obtained by the RSM method has been experimentally confirmed, which suggests that this method can be used to optimize the extraction conditions and predict the results of the analysis.

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EXPLOITATION OF BY-PRODUCTS DERIVED FROM ROSE PETALS (*ROSA DAMASCENA* MILL) HYDRODISTILLATION

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The region of Southeastern Europe is well-known for the variety of medicinal and aromatic plants (MAPs). As a common practice, MAPs are used for the production of essential oils, however huge quantities of hydrolates and herbal residues are also produced. Rose oil is one of the most expensive essential oils, often referred as “liquid gold”. During its hydrodistillation from fresh rose flowers, significant amounts of waste water are produced and discarded in the fields. The waste water from rose oil distillation is actually a water extract of rose petals, which is reported to extend the life span of *Drosophila* flies [1], exhibit moderate anti-HIV activity [2], is rich in phenolic compounds, and possesses significant antioxidant activity [3].
Under the frame of the European project „EXANDAS”, our purpose was to study the optimal processes to produce a high-added value product by using liquid-liquid extraction methods in order to enrich the extract in phenols while eliminating containing sugars. Particularly, several samples of rose waste water from different distillation apparatuses and different production days were collected. The aqueous extracts were treated with liquid-liquid extraction technique (Rousselet Robatel, France), using a ratio of EtOAc:EtOH:aq.extract 10:1:10. The dry extracts obtained after the removal of organic solvents were profiled with HPTLC and evaluated for their antioxidant activity (DPPH, ABTS), Total Phenolic Content (Folin-Ciocalteu) and Total Flavonoids Content (TFC). All of the extracts presented similar phenolic content and antioxidant activity. Finally, a selected extract was subjected to Fast Centrifugal Partition Chromatography (FCPC) resulting to the isolation of nine pure compounds, which were identified by NMR and LC-HRMS as quercetin and kaempferol glycosides. As a result, it is rightly expected that the by-product of rose hydrodistillation could be a readily available source of valuable compounds with potential to be applied in food and cosmetics industries.

Acknowledgements: This project has received funding from the European Union’s H2020-MSCA-RISE-2015 under grant agreement No 691247.

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P–4

THE ISOLATION OF PHENOLIC COMPOUNDS FROM *CISTUS MONSPELIENSIS* METHANOLIC EXTRACTS BY CPC CHROMATOGRAPHY

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Cistus species are perennial shrubs known as rockrose plants (Cistaceae family) and are mainly distributed on dry or rocky soils of the Mediterranean basin. The leaves of several species are coated with a highly aromatic resin, known as labdanum and several studies have demonstrated its antimicrobial activities due to the high content of terpenes [1, 2] However, since the aerial parts of the plants have been reported in Greek traditional medicine (Hippocrates, Dioscorides) against a diverse spectrum of skin diseases and as anti-inflammatory agents, the purpose of this research focuses on the exploration of the phenolic content and anti-oxidant potential of five *Cistus* species, *C. monspeliensis*, *C. salvifolius*, *C. parviflorus*, *C. creticus* spp *creticus*, *C. creticus* spp *eriocephalus*, belonging in the Greek flora.

All species were successively extracted with *c*-hexane, ethylacetate, methanol and water by Accelerated Solvent Extraction (ASE, Dionex) and the methanolic extract of *Cistus monspeliensis* was chosen, due to rich content, for further analysis after comparison of extracts profile with HPTLC (CAMAG). Simultaneously, all extracts were tested for their antioxidant capacity by TPC, ABTS and DPPH assays. Fractionation and isolation was achieved by applying a three-phase solvent system in FCPC analysis, which facilitated the separation of the phenolic content (flavonoid glucosides) while retaining the containing tannins. Further purification of compounds was accomplished by off-line coupling of FCPC with sephadex column chromatography and prep-TLC. The purity and identity of isolated compounds was confirmed by NMR spectroscopy.

In conclusion, it was demonstrated that counter-current chromatography is a robust and valuable technique for the successfully enrichment of crude extracts in polyphenols increasing their antioxidant properties while simplifying the separation of natural compounds from complex matrices.

Acknowledgements: We acknowledge Dr. E. Kalpoutzakis for the collection and identification of plant materials.

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P–5

MODIFICATION OF STEROL CONTENT IN MARIGOLD *CALENDULA OFFICINALIS* HAIRY ROOTS AS A RESPONSE TO ELICITATION WITH SELECTED BIOTIC AND ABIOTIC FACTORS

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Triterpenoids can be divided into steroids, i.e. tetracyclic compounds based on perhydro-1,2-cyclopentano-phenantren moiety, and pentacyclic triterpenoids with 5-ring carbon skeleton of various arrangement. Sterols (steroids with a hydroxyl group at C-3) are constituents of plant membranes and they participate in the regulation of their fluidity and permeabilty, they also serve as precursors of brassinosteroid hormones. The trials of stimulation of triterpenoid biosynthesis in plants and plant *in vitro* cultures often concern the possible competition of pathways leading to sterols and other triterpenoids. Both sterols and pentacyclic triterpenoids are synthesized as products of 2,3-oxidosqualene cyclization and it can be expected that biosynthesis of triterpenoids occurs when sterol formation is already satisfied or sacrificed. In the present study performed on established hairy root cultures of *Calendula officinalis* from hypocotyl or cotyledon explants obtained by transformation with wild-type *Agrobacterium rhizogenes*, the symptoms of the competition between biosynthetic pathways of sterols and pentacyclic triterpenoids were the most visible in reaction of *Calendula* hairy roots to stimulation with biotic elicitors: jasmonic acid, salicylic acid and chitosan, whereas less evident in reactions to abiotic stressors, particularly UV radiation that caused the increase of the content of all investigated groups of triterpenoids. The fractions containing neutral triterpenoids in a free form were obtained form diethyl ether extracts from dried hairy roots by preparative TLC in the solvent system $CHCl_3$/MeOH (95:5,v/v) and analysed by GC-MS directly without derivatization. The result obtained in the present study revealed that the total content of sterols was never changed more than 70% in comparison to the control. The sharpest decrease was noted after elicitation with jasmonic acid (62%) and ultrasound (47%), whereas the strongest increase after treatment with high concentrations of salicylic acid (70%) and Cd^{2+} ions (50%). The treatment with chitosan, which mimics the pathogen attack, increased the level of sterols up to 18% and changed the ratio between stigmasterol and sitosterol (increasing stigmasterol content and decreasing sitosterol), suggesting that such phenomenon can be typical for plant response to some fungal infections. Heavy metal ions also appeared as the agents deeply rearranging the sterol metabolism and causing modifications of their basic profile. Surprisingly, no alteration of sterol profile occurred after UV radiation treatment, although such reactions (particularly increased sitosterol to stigmasterol ratio) were observed in some plants exposed to UV irradiance.

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IDENTIFICATION AND QUANTIFICATION OF VARIOUS SUGARS AND INOSITOLS PRESENT IN DIFFERENT PLANTS USING GC-MS

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Inositols are cyclitols with the empirical formula $C_6H_{12}O_6$. They are polyols of cyclohexane or sugar alcohol and there are 9 possible stereoisomers. Among all the known isomers, myo-inositol is the most ubiquitous in nature [1]. Soybeans and peanuts are examples of good sources of inositols. The interest of cyclitols increased rapidly due to their multiple medicinal attributes, through the most important are the anti-diabetic [2], antioxidant [3] and anti-cancer [4] properties. In addition, they are secondary metabolites compounds in plants which playing an important role in self-defiance against unfavorable conditions such as salt and water stress.

The aim of the study was to find a new source of plants which could be rich in these types of compounds. For this purpose, different plants such as medicago sativa L, solidago virgaurea L, phacelia tanacetifolia Benth, lupinus seeds, trigonella foenum-graecum, white onion, Lettuce, carrot, carob, red paprika, elder fruit, blueberry fruit, Mountain Ash, Carawy seeds and chamomile were collected, dried, grounded to fine powdered and then extracted using accelerated solvent extraction (conditions: solvent: water, temperature: 100 C, time: 18 min (3 cycles)). For purification of target compounds, solid phase extraction method was employed (C18 and OASIS cartridges). Prior to the analysis of sugars and inositols using GC-MS instrumentation, a derivatization reaction was performed using trimethylsilylimidazole (TMSI) and pyridine in order to volatilize our target components. The experimental results successfully demonstrated that all analyzed samples contain inositols with variance quantities included (D-pinitol, bornositol, D-*chiro*-inositol, *scyllo*-inositol, *myo*-inositol).

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IDENTIFICATION OF PHTHALIDES IN DIFFERENT PARTS OF *KELUSSIA ODORATISSIMA* MOZAFF. WITH GC/MS

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Kelussia odoratissima Mozaff. from the Apiaceae family is an endemic and endangered plant growing at 2500 m and above in the central Zagros Mountains of Iran. It is one of the most popular wild vegetables used in salads, pickled, soups and yogurt by local people [1]. There is quite a diverse biological activity reported traditionally and pharmacologically for this plant, including treatment for rheumatism, indigestion, hypertension, inflammation, ulcers and cardiovascular diseases [2, 3]. However, the phytochemical background of this plant is limited. Here, the leaves, stem and seeds of *K. odoratissima* were extracted once under sonication by *n*-hexane, EtOAc and MeOH, respectively and then analyzed to GC/MS. The results revealed phthalide structures as main volatile compounds in these extracts. Indeed, 3-butyl phthalide (**1**), 3*Z*-butylidene phthalide (**2**), 3*E*- butylidene phthalide (**3**), *Z*-liqustilide (**4**) as well as *E*-ligustilide (**5**) could be firmly characterized. Table 1 represents a relative quantification of these phthalides. As far as a known active component such as Z/E-ligustilide was concerned, EtOAc appeared as the best extraction solvent.

Table1. The percentages of phthalide structures in leaves, stem and seeds of *Kelussia odoratissima* Mozaff. extracted with hexane, ethylacetate and methanol solvent

Comp.	RI^{cal*}	Hexane extract			Ethylacetate extract			Methanol extract		
		Leaves	Stem	Seed	Leaves	Stem	Seed	Leaves	Stem	Seed
1	1664	0.1	-	-	0.4	-	0.5	-	-	0.5
2	1687	2.7	1.0	0.4	7.4	2.7	2.4	2.0	-	2.3
3	1735	0.6	0.2	-	1.8	0.6	1.4	-	-	-
4	1756	28.2	82.3	23.8	54.4	76. 9	72.3	21.6	10.2	81. 8
5	1817	2.8	3.5	0.7	7.5	5. 8	2.8	3.9	-	3.1

* The retention indices were calculated according to the injection of normal alkanes C9-C24 with the same condition of the injected samples

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ROYAN BIODISCOVERY INITIATIVE USING ZEBRAFISH *IN VIVO* AND OTHER *IN VITRO* ASSAYS FOR IDENTIFICATION OF BIOACTIVE NATURAL PRODUCTS

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Royan Biodiscovery Initiative (RBI) was founded in 2015 with the main objective of conducting research in the role of herbal medicines to find the bioactive natural products using zebrafish as a modern platform. RBI's mission is to use the Iranian traditional medicine and medicinal plants and with use of different disease models especially the zebrafish and with the bioassay guided fractionation to reach the main active compounds of the plants. RBI have had several projects on discovery of bioactive natural products using in vitro and in vivo assays. Our assay include several disease like, diabetes, cardiovascular, cancer, MS and epilepsy. Beta cell regeneration in diabetes uses zebrafish model and cell based systems. Antiangiogenesis on zebrafish and HUVEC cell are the main assay in cancer. Demyelination and remyelination in zebrafish are used for more understanding of MS disease. In zebrafish lab, the new line of transgenic zebrafish for producing disease model has also been established. The extract bank of our group includes several Iranian medicinal plants which is used in screening and evaluating their bioactivity in different fields.

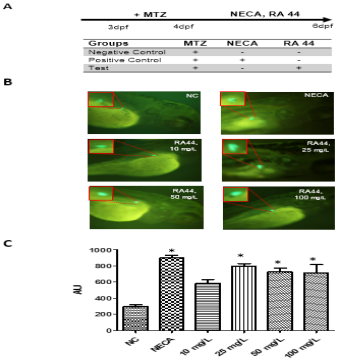

Figure 1. Beta cell regeneration platform in transgenic zebrafish, using NECA as the positive control and RA44 a plant extract

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APPLICATION OF STATISTICAL ANALYSES TO CORRELATE THE CHEMICAL COMPOSITION OF SELECTED ESSENTIAL OILS TOGETHER WITH DETERMINATION OF THEIR ANTI-*HELICOBACTER PYLORI* ACTIVITY *IN VITRO*

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Several studies have been performed in the aspect of searching for natural antibacterial agents of plant origin [1,2].The objective of this study was to analyze the composition of 9 commercially available essential oils (Abies alba, cedarwood, lemon, lemongrass, melissa, oregano, pine, tea tree and thyme) and to estimate their effect *in vitro* on growth of the reference strain *Helicobacter pylori* ATCC 43504. The gas chromatography-mass spectrometry (GC-MS) method was used to determine of the composition of the tested oils. The principal component (PC) and cluster analysis (CA) were carried out to assess the similarities and differences between their components. The estimation of MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration) was assessed by the dilution method. The studied essential oils could be divided into three groups, regarding the differences in their chemical composition. A correlation between chemical composition of the essential oils and their activity against *H. pylori* as well as bacteriostatic or bactericidal effect could be observed. According to CA analysis, the most effective *in vitro* essential oils belonged to the first group (I) characterized by relatively low MIC and bactericidal effect, assessed by MBC/MIC ratio ≤ 4. The obtained data are presented in Table 1.

Table 1. The main ingredients in the tested essential oils and their statistical classification (PC, CA) and microbial activity (MIC, MBC)

Tested oils	The main compounds (>10%)	PC	CA	MIC (μg/mL)	MBC (μg/mL)	MBC/ MIC
Abies alba	bornyl acetate (53.2), α-pinene (15.5)	II	II	7.8	250	128
Cedarwood	α-cedren (22.9), thujopsene (21.8), cedrol (15.1)	I	I	15.6	62.5	64
Lemon	limonene (48.1), β-pinene (17.0)	II	II	1.95	250	32
Lemongrass	geranial (42.8), neral (32.6)	I	I	15.6	15.6	1
Melissa	citronellal (31.2), geraniol (21.2), citronellol (13.9)	I	I	15.6	62.5	1
Oregano	carvacrol (67.7), p-cymene (14.6)	III	III	31.3	31.3	4
Pine	α-pinene (32.1), β-pinene (20.4), 3-carene (15.0), limonene (12.2)	II	II	1.95	125	4
Tea tree	terpinen-4-ol (39.6), γ–terpinene (19.3)	III	I	15.6	62.5	4
Thyme	thymol (45.4), p-cymene (22.5)	III	I	15.6	15.6	1

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ESSENTIAL OIL COMPOSITION OF *PEUMUS BOLDUS*

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Peumus boldus commonly referred to as 'boldo' is a tree or shrub with medicinal properties native to Chile. The leaves contain alkaloids, flavonoids and essential oils.
Infusion of boldo leaves is recommended for the treatment of gastrointestinal spasms, dyspeptic and hepatobiliary disorders. The main bioactive compounds of boldo leaves are flavonoids, alkaloids and essential oils. Several studies have demonstrated the antioxidant activity of flavonoids and alkaloids, particularly the aporphine alkaloid boldine. The antimicrobial, fungicidal or anthelmintic effects of ascaridole, the main compound in the essential oil,is also reported
Boldine, a major alkaloidal constituent found in the leaves and bark of the boldo tree, has been shown to possess antioxidant and anti-inflammatory activity. The German Commission E has approved boldo leaf as treatment for mild dyspepsia (upset stomach) and spastic gastrointestinal complaints.
Boldo is considered one of the best medicinal herbs for many digestive disorders, such as bloating, heartburn, and poor absorption of nutrients in the stomach and intestines. It is also used to enhance detoxification of the liver and protect against liver damage from toxins and drugs which are known to have a detrimental effect on the liver. The herb has a mild diuretic, mucosal protective, antiseptic and slightly calming effect and stimulates the excretion of uric acid.
The purpose of study was to analyze the chemical composition of the oil found in the boldo coming from Polish market.
Distillation of essential oils of boldo carried out by the Polish Pharmacopoeia VIII. Due to the lack of detailed monographs in FP VIII distillation time was set at 3 hours using the indirect method. Qualitative and quantitative analysis of the essential oil was carried out by GC/MS.
Essential oil content in the tested samples of boldo leaves was 1.66 % v/w. In the studied oil of boldo they identified a total of 76 compounds. For the dominant components included the chrysanthenyl acetate (20.82%), o-cymene (18.68%), 1,8-cineole (14.09%), ascaridole (13.41%), β-oplopenone (2.73%), terpinen-4-ol (2.24%), γ-terpineol (2.23%), methyl eugenol (1.82%), α-pinene (1.60%), spathulenol (1.34%), α-chenepodiol (1.16%).

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ISOLATION OF 5-MOP FROM THE PLANT SOURCE AND AN *IN VITRO* ANTIPROLIFERATIVE AND ANTIMIGRATIVE EFFECT ON CULTURED HUMAN TUMOR CELL LINES

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Plant-derived compounds are an important source of anticancer drugs, chemopreventive compounds, or chemotherapy adjuvants [1]. Psoralens (linear furanocoumarins) in combination with UV light are potent modulators of epidermal cell growth and differentiation [2]. It has been shown that methoxycoumarins; bergapten (5-methoxypsoralen=5-MOP) and xanthotoxin (8-MOP), independently of its photoactivation, exert anti-cancer activity [3,4].

The aim of our study was evaluation UV-independent anticancer activity of 5-MOP, isolated from *Peucedanum tauricum* (Apiaceae) fruits, on human cancer cell lines. The effect of 5-MOP on cell proliferation and migration was evaluated on HT29 and SW620 (colorectal adenocarcinoma), Saos-2 (osteosarcoma), RPMI8226 and U266 (multiple myeloma) cell lines. Isolation and purification of 5-MOP (purity assayed by HPLC/DAD was more than 99%) was performed with Soxhlet extraction and combined chromatographic methods (CC, preparative TLC, as well as the CPC method) in various mixtures of CH_2Cl_2, AcOEt, cyclohexane, n-hexane, MeCN and MeOH. Cell proliferation was determined by means of MTT method. The influence of 5-MOP on mobility of cells was evaluated by scratch-wound assay.

It was found that non-photoactivated bergapten showed concentration-depend moderate antiproliferative activity against all of the tested cell lines, except for RPMI8226 cells. The strongest inhibitory effect of this compound on cell proliferation was observed on Saos-2, SW620 and HT29 cell lines. Moreover, 5-MOP inhibited the cell migration above mentioned cell lines.

We can conclude that 5-MOP, independently of photoactivation, can inhibit the growth of osteosarcoma and colon cancer cells. Due to its promising effects in *in vitro* studies, 5-MOP could be used as a chemo-therapeutic agent in the treatment of susceptible cancers.

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P–12

HPLC/ESI-QTOF-MS ANALYSIS OF *LEVISTICUM OFFICINALE* FRUIT EXTRACTS OBTAINED BY DIFFERENT EXTRACTION TECHNIQUES

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Levisticum officinale Koch. (Apiaceae) is an important medicinal plant with a thousands of years of traditional medicinal use. Roots of this species (*Levistici radix*, listed in EMA) and its preparations were used as diuretic (mostly due to volatiles alkylphtalides) and also as an emmenagogue, carminativum, remedy for various skin ailments, and also used in cuisine [1,2,3]. The fruits of this plant have rarely been studied.
In our study, the preliminary quantitative analysis of flavonoids FL (by use of Christ-Muller method) and phenolic acids PhAs (by use of Arnov's method) in lovage fruits were performed for the first time. *L. officinale* fruit extracts, prepared by use of various techniques (water bath extraction, Soxhlet extraction, UAE, ASE/PLE) and with solvents with different polarity, such as chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), ethyl acetate (AcOEt) and methanol (MeOH) were also analyzed by HPLC/ESI-QTOF-MS for the presence of polyphenolic compounds; coumarins, PhAs and FL. LC-MS analysis was performed on Zorbax eclipse XDB-C18 Rapid Resolution column (150x4.6 mm i.d, 3.5 µm), with mobile phase composed of 1% acetonitrile in water, and 95% acetonitrile in water (both with addition of ammonium buffer 10 mM; pH 4.5), in gradient elution (20→100% B, v/v), analysis time 60 min, flow 0.400 mL/min.
As the result we found, that extraction efficiency was the highest for ASE/PLE method. In the investigated extracts flavonoids; apigenin and quercetin, three phenolic acids; chlorogenic, gentisic and p-hydroxybenzoic, furanocoumarins; bergapten, xanthotoxin, isopimpinellin and simple coumarin scopoletin were identified. Apigenin, gentisic and p-hydroxybenzoic acid, isopimpinellin and scopoletin were detected for the first time in analyzed plant material.
Interestingly, in the fruits of *L. officinale*, high amount of FL and PhAs was found, and was as follows; FL: 1.8 mg/g ± 0.027 dry wt. calculated as quercetin, and 2.6 mg/g ± 0.042 dry wt. calculated as kempferol; and PhAs: 2.982 mg/g ± 0.032 dry wt. calculated as caffeic acid.
These results suggest that lovage fruits could be considered as a good source of plant polyphenols.

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P–13

THE TRITERPENE COMPOSITION OF LEAF AND FRUIT CUTICULAR WAXES OF TWO POLISH VARIETIES OF *RIBES NIGRUM* L.

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The cuticle is composed of cutin polymer matrix embedded and coated with wax. In general, plant waxes are a mixture of aliphatic long-chain hydrocarbons and their derivates, however in some species they also contain cyclic compounds, for example triterpenoids. The chemical composition of plant waxes is highly variable among plant species, the organs of one plant (e.g., fruits and leaves), and during organ ontogeny.
The triterpenoid components of leaf and fruit cuticular waxes of black currant varieties (Tines and Tisel) were identified by GC-MS. Surface waxes were removed by immersion of leaves and fruits in chloroform at room temperature. Extracts were separated by preparative thin-layer chromatography (TLC) on 20 × 20 cm glass plates coated with layer of silica gel 60G (Merck) in the solvent system $CHCl_3$/MeOH (97:3, v/v) into two fractions: (i) free (non-esterified) steroids and neutral triterpenes (alcohols, aldehydes, and ketones), and (ii) triterpene acids.
The triterpenoid profile of black currant consists of steroids (stigmasta-3,5,22-trien, cholesterol, campesterol, stigmasterol, sitosterol, 24-methylene-cycloartanol, tremulone) and pentacyclic compounds (α- and β-amyrins, ursolic aldehyde and several acids: oleanolic and ursolic accompanied with their methyl esters, 2,3-dihydroxyursolic and 2,3,23-trihydroxyoleanolic). Ursa-2,12-dien-28-oic and 2,3-dihydroxyoleanolic acids were detected only in Tines variety. The total content of triterpenoids in Tines and Tisel varieties accounted for approximately 85.96 and 41.26 $\mu g \cdot mg^{-1}$ of fruit wax extracts, respectively, and for 88.31 and 57.37 $\mu g \cdot mg^{-1}$ of leaf wax extracts. The fraction of steroids was predominating in fruit and leaf waxes of both varieties, with sitosterol as the prevailing compound (approx. 19-25% of all triterpenoids in fruits, 30-36% in leaves). The results revealed small qualitative and quantitative differences in triterpenoid profile of waxes of the two analyzed varieties, however, some common general features can be described as potentially characteristic for black current wax.

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SCREENING FOR ISOPRENOID DERIVATIVES AND THEIR DISTRIBUTION IN BLACKBERRY *RUBUS FRUTICOSUS* L.

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Triterpenoids occur in plant organs in several basic forms: as free compounds, esters or glycosides. These forms differ considerably in a polarity of a molecule, which determinates their occurrence in plant cell compartments as well as their functions. The forms of low polarity (free compounds and esters) are often secreted to surface layers (mainly to cuticular waxes), where they constitute the first line of plant defense against pathogens and herbivores. More polar glycosidic forms (saponins) are accumulated in the cellular walls and vacuoles in cells of internal tissues, located deeper in the plant organs, thus constituting the second line of plant defense.
Blackberry *Rubus* L. (Rosaceae) var. Gazda extracts of fruit and leaf cuticular waxes (30 s immersion of entire fruits or leaves in chloroform) and extracts of dried organs after wax extraction (obtained after 8 hrs extraction with diethyl ether and methanol in Soxhlet apparatus) were fractionated by preparative TLC on SiO_2 ($CHCl_3$:MeOH 97:3) and analyzed by GC-MS.
Leaf extracts had higher total triterpenoid content than fruit extracts. The content amounted to 90.24 µg/mg of wax chloroform extract and 1 623.06 to 2 390.80 µg/g of dry weight for diethyl ether and methanol extracts, respectively. Six major triterpene acids: oleanolic, ursolic, ursa-2,12-dien-28-oic, 3-oxo-oleanolic, 2,3-dihydroxyursolic and 2,3,23-trihydroxyoleanolic levels were determined in all fruit and leaf extracts and oleanolic acid was found to be dominant among these compounds in analyzed organs. The main steroid profile consisted of stigmasta-3,5,22-triene, cholesterol, campesterol, stigmasterol, sitosterol, cycloartanol and tremulone, whereas isofucosterol and cholesta-3,5-dien-7-one were found only in methanol and diethyl ether extract of fruits, respectively. The neutral pentacyclic triterpenes (alcohols, aldehydes, and ketones) were found in the lowest quantities in all extracts.
The GC-MS analysis showed significant differences in the qualitative and quantitative composition of the analyzed fruit and leaf extracts, pointing to the diverse pattern of triterpenoid distribution among plant tissues. The low amount of triterpenoids in cuticular wax seems to be correlated with high amount of triterpene aglycons in internal tissues, thus supporting the hypothesis of the role of free and glycosylated forms of triterpenoids in the first or the second line of plant defense.

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POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF EXTRACTS FROM DIFFERENT PARTS OF KAZAKH ENDEMIC *CRATAEGUS ALMATENSIS* POJARK

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Hawthorn is one of the oldest popular medicinal plants used for the prevention and treatment of cardiovascular diseases, such as hypertension, angina, arrhythmia, and the early stages of congestive heart failure. Due to its biologically active constituents, hawthorn has shown to have free radical scavenging, anti-inflammatory and antimicrobial activities, with very low toxic effect [1]. *C. almaatensis* is an endemic Kazakh plant with potential to be used as a source of pharmaceutical products on its basis.
The aim of this study is to conduct quantitative analysis of earlier identified phenolic compounds of *C. almaatensis* [2] and determine total phenolic content, along with the antioxidant activity. In order to conduct this study the ethanol extracts (50, 96%) of fruits, leaves and flowers were obtained by ultrasound extraction and subjected to LC-MS analysis under optimized conditions. Total phenolic content and antioxidant potential were determined using the Folin–Ciocalteu assay and the DPPH test respectively.
LC-MS analysis of *C. almaatensis* extracts from different organs revealed a wide range of phenolic compounds present in high concentrations. The 50% extract showed to possess better antioxidant activity. For the 50% ethanol extracts the leaves were the richest in total polyphenol content, followed by the flowers and finally the fruits, which is in agreement with their antioxidant activity profile.
The results show the potential of the endemic herbal plant *C. almaatensis* to be used for the development of new pharmaceutical products in Kazakhstan.

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P–16

LC/MS-BASED DISCOVERY OF METABOLIC MARKERS FOR THE QUALITY CONTROL OF FRUIT AND SEED OILS

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Mass spectrometry based metabolomics is starting to play more and more significant role in food science. Different analytical techniques (GC/MS, LC/MS, CE/MS) can be used, each with their own advantages and disadvantages [1]. In recent years the number of applications of LC/MS, involving both low- and high-resolution mass spectrometry, is rapidly growing. LC/MS methods allow the detection of most organic and some inorganic molecules, ensure very high sensitivity, require low amounts of samples, are very flexible and enable analysis of very complex food samples with minimal sample pretreatment. LC/MS-based discovery of metabolic markers for food quality control was successfully employed among others to the classification of monovarietal extra virgin olive oils [2] and olive oil origin discrimination [3]. However, there is no systematic, comprehensive studies on the metabolomic fingerprints of oils from seeds and fruits, pressed in smaller quantities than the most popular plant oils, e.g. milk thistle, nigella or evening primrose.

The aim of the study is the identification of metabolic markers specific to plant olives involving oils pressed from rapeseed, flaxseed, pumpkin seed, milk thistle, nigella, sunflower seeds, evening primrose, sesame, coconut and hemp seeds. Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry was employed for the analysis of olive extracts and metabolomic fingerprints were obtained. Here we present the primary results on discovery of metabolic markers for the quality control of examined fruit and seed oils. The results of mass profiling and multivariate analyses (Principal Component Analysis - PCA, Partial Least Square - Discriminant Analysis PLS-DA) of the acquired high-resolution mass spectrometry (HRMS) data allowed the determination of metabolomic features differentiating the examined olives indicating that LC/MS-based metabolomic fingerprinting can be used for the quality control and assurance of these products as well as for the detection of adulterations.

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P–17

QUININE TRANSFORMATION – KNOWN AND NEW DERIVATIVES ANALYZED WITH APPLYING LC-MS AND NMR TECHNIQUES

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Although quinine is widely used in food industry as a flavor component but receives also the attention of scientists due to its several medical properties, e.g. fever-reducing, painkilling and anti-inflammatory. According to [1,2], quinine transforms to its structural isomers in the strongly acidic water environment. In the course of detailed investigations we observed that this process occurs also in less acidic water and methanol/water solutions. In addition to structural quinine isomers, hydroxyl derivatives of quinine itself and its structural isomers are formed in these environments. Moreover, methoxy derivatives of quinine and its structural isomers also appear in buffered methanol/water system. All the hydroxyl and methoxy derivatives of quinine constitute newly discovered compounds. The amount of each formed component depends on the heating time, concentration of alcohol and on the solvent pH (see examplary relationships in Fig. 1).

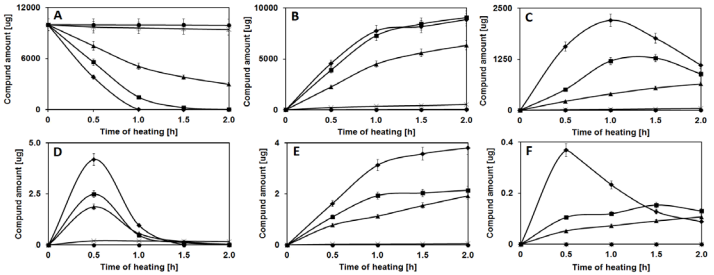

Fig. 1. The influence of heating time on the amount of quinine remaining in the heated quinine solution (Fig. 1A) and on the amount of individual quinine isomers [epiquinine (Fig. 1B), quinotoxine (Fig. 1C), and their hydroxyl derivatives, (Fig. 1D, 1E, 1F, respectively)], all formed during the heating of quinine in phosphoric buffer at pH=2 (diamonds), pH=3 (squares), pH=4 (triangles), pH=5 (cross) and pH=6 (circle).

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TRANSFORMATION OF RUTIN DURING ITS EXTRACTION

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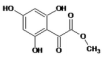
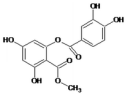
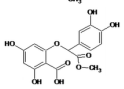
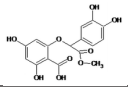
Rutin is one of the most popular polyphenolic compounds found in many plants. Its health-promoting properties still attracts the attention of scientists investigating plant metabolism involving this compound and looking for its new natural sources. Liquid-solid extraction is the most popular method for rutin isolation from plants. We found that at least thirty four compounds (rutin transformation and degradation products and their alcohol derivatives) were formed from rutin during its extraction from plants rich in this compound by alcohol (methanol or ethanol) and alcohol/water mixtures. All derivatives have been identified based on retention times of standards (quercetin, quercitrin, isoquercitrin and rutin), PDA spectra, MS^n data (see Table 1 below), HRMS and literature data [1-4].

Table 1: Examples of new rutin transformation products formed during its high-temperature extraction.

Compound name	Tentative structure of compound	MS^1, base peak (m/z)	MS^2, base peak (m/z)	MS^2, secondary peak (m/z)
methyl oxo(2,4,6-trihydroxyphenyl)acetate		211.1	196.2	153.2 125.1
methyl 2-[(3,4-dihydroxybenzoyl) oxy]-4,6-dihydroxybenzoate		319.2	304.1	168.1 196.1 261.1
2-[1-(3,4-dihydroxyphenyl)-2-methoxy-2-oxoethoxy]-4,6-dihydroxybenzoic acid		349.0	334.2	247.1 291.1
2-[1-(3,4-dihydroxyphenyl)-2-methoxy-2-oxoethoxy]-4,6-dihydroxybenzoic acid		349.0	334.2	247.1 291.1

The performed experiments shows that the amount of each formed rutin transformation product depends on the extraction time, alcohol concentration, extractant pH, and, in addition, on the components of plant matrix from which rutin was extracted.

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THE INFLUENCE OF VARIOUS LAVENDER OILS ON THE FACIAL SKIN MICROBIOTA

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The lavender oil, one of the eldest perfume ingredients, is used for centuries in traditional medicine, also belongs to the most precious aromatherapy oils. Due to its anti-inflammatory and antiseptic properties, lavender oil is used in skin care [1]. The face skin microbiota is mainly composed of gram-positive cocci (*Staphylococcus epidermidis*, *S. haemolyticus*, *S. hominis*, *Enterococcus faecalis*, *Micrococcus* spp., *Streptococcus* sp.), gram-positive bacilli (*Corynebacterium* spp., *Propionibacterium acnes*, *P. granulosum*, *P. avidum*, *Bacillus* spp.), gram-negative bacilli (Acinetobacter spp., Escherichia coli), yeast-like fungi (*Pityrosporum ovale, Candida* spp.) [2].
The most commonly used species of lavender are: *Lavandula angustifolia, Lavandula stoechas* and *Lavandula latifolia.* Studies have confirmed that the antibacterial and antifungal activity of lavender essential oil correspond to its main components such as linalool, linalool acetate, lavandulol, geraniol or eucalyptol [2].The content of this components of oil depends of used species lavandue.
The aim of the study was to assess the antimicrobial activity of two lavender oils of different origin on a mixed microbiota of the face skin.
The commercial lavender oil form ETJA and essential oil derived from the Crimea lavender were analysed by gas chromatography coupled to mass spectrometry. The main components of ETJA lavender oil were linalol (43%), linalyl acetate (33%) and limonene (19%) Crimea lavender essential oil contained mainly: linalool (over 34%), linalyl acetate (23%) and eucalyptol (5%).
The gram-positive bacilli were most sensitive to the influence of ETJA lavender oil, and gram-negative bacilli were most sensitive to Crimea lavender oil. However, none of the tested oils inhibited the development of gram-positive cocci.
The tested lavender oils limited the number of mixed facial skin microbiota, but higher efficiency was noticed for ETJA oil, which contained the higher amount of monoterpenoids (linalol, linalyl acetate) and monoterpenes (limonene) than Crimea lavender oil.

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GC-MS ANALYSIS OF ISOPRENOIDS IN GRAPEVINE CV. MERLOT AND GAMAY LEAVES AFTER EXPOSITION TO UV-B RADIATION

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Climate changes, including increased UV-B radiation, affect grapevine leaf metabolism altering fruit ripening rates which is a growing concern for wine makers. Acclimation to UV-B radiation includes the biosynthesis of phenolic compounds that protect plant tissues from UV-B harming effects. Moreover, it has been shown that UV-B treatment increased levels of membrane-related triterpenes i.e. phytosterols suggesting elicitation of a mechanism of grapevine acclimation [1]. The aim of the current study was to investigate the influence of UV-B radiation on the triterpenoid content in grapevine (*Vitis vinifera* L.) cv. Merlot and Gamay which represent some of the main cultivars destined for French wine production. At least 10 mature leaves were harvested from plants growing in experimental vineyard "VitAdapt" (INRA, Villenave-d'Ornon, France). The lower surfaces of leaves were exposed for 5 min to UV light (λ = 312 nm) from a UV-B tube. Leaves were collected at 48 h after treatment, conserved at –80ºC and lyophilisated. Homogenized plant material was extracted with diethyl ether in Soxhlet apparathus. The extracts were fractionated by preparative TLC on SiO_2 in a solvent system $CHCl_3$/MeOH (97:3, v/v) and the fractions containing free triterpenoids were directly analyzed by GC-MS/FID. The observed changes in metabolism of pentacyclic and tetracyclic triterpenoids differ in two cultivars. The ratio of sitosterol to stigmasterol in Merlot leaves increased markedly after the exposure to UV-B suggesting the enhancement of sterol-structural defense being a part of adaptation to stress. In turn, in Gamay leaves exposed to UV-B the increase of the concentration of sterols (except for cholesterol) was observed, accompanied with the accumulation of saturated derivative of sitosterol, i.e. sitostanol. Also the content of some pentacyclic compounds (α-, β-amyrins and lupeol) increased in Gamay leaves, while the content of taraxerol decreased. The higher levels of α- and γ-tocopherols were observed in both Gamay and Merlot leaves after UV-B treatment. This preliminary approach could provide an information about response capacity of analyzed varieties to climate changes and be the first step in genotype selection in grapevine breeding program.

Acknowledgements: The study was performed owing to the financial support of the French Government and the French Embassy in Poland.

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BULBS OF *GALANTHUS PLATYPHYLLUS* TRAUB & MOLDENKE AS A SOURCE OF BIOACTIVE ALKALOIDS

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The *Amaryllidaceae* family is well known as the source of many different structurally and biogenetically related alkaloids. Many of them have proven biological activity, the most studied of which is the ability to inhibit cholinesterases. The aim of this work is to find and identify a new potential drug that can be used in the treatment of neurodegenerative diseases such as Alzheimer's disease.
Galanthus platyphyllus is a plant belonging to *Amaryllidaceae* family and occurs in Caucasus (Georgia). The plant material (bulbs) was dried, pulverized and extracted.
The extracts were prepared from powderized plant material by a very efficient accelerated solvent extraction using methanol and methanol acidified by tartaric acid. The crude extracts were evaporated under reduced pressure used a rotary vacuum evaporator and purified by SPE procedure. The obtained pure alkaloid extracts were subjected to LCMS analysis using the HPLC/ESI-QTOF-MS method. Extracts containing alkaloids were analyzed qualitatively in positive ion mode using 6530B Accurate-mass-Q-TOF-MS (Agilent Technologies). For analysis Atlantis® HILIC silica column (d_p=3 µm, 2.1x150 mm) and gradient of acetonitrile (95%) plus 10 mM ammonium formate (0.2%) [A], and acetonitrile (55%) plus 10 nm ammonium formate (0.2%) [B] as a mobile phase were used. According qualitative analysis six alkaloid compunds were identified. Acetylcholinesterase inhibition of obtained extracts was evaluated by TLC bioautographic assay on thin layer plate covered with silica gel in derivatization chamber (Camag, Switzerland). The active inhibitors have been identified in the investigated plant species.

Acknowledgements: The work was financed from grant No 4/POLTUR-1/2016.

P–22

ASSAY OF RUTIN IN BULK AND IN PHARMACEUTICAL PREPARATION BY USING UHPLC-DAD METHOD

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Rutin is a flavonoid and is well known for its anti-inflammatory and vasoactive properties as well as the potential to reduce the risk of arteriosclerosis [1].
The aim of this work focused on the development and validation of a UHPLC-DAD (ultrahigh performance liquid chromatography with diode array detector) method for the determination of rutin in bulk (in the presence of impurities and degradation products) as well as in commercial pharmaceutical preparations (Figure 1).
The determination of rutin was possible using the LC system (Dionex Thermoline Fisher Scientific, Germany). Separations were performed on a Kinetex-C18 column (100 mm × 2.1 mm, 1.7 µm). The detection was performed using diode array detector at a wavelength maxima (λ_{max}) of 353nm. The mobile phase consisted of a mixture of 0.1% formic acid and acetonitrile (80:20 V/V) with a mobile phase flow rate of 1.0mL/min.
The method was validated according to the International Conference on Harmonization Guidelines [2]. It comprised selectivity, linearity, accuracy, precision, limits of detection (LOD) and quantitation (LOQ).

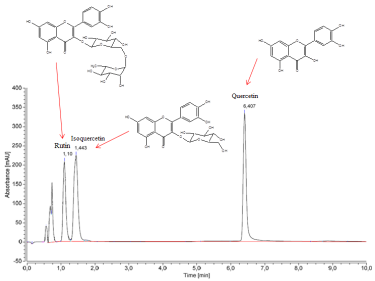

Figure 1: Chromatogram of rutin and its degradation products (isoquercetin and quercetin).

The presented UHPLC-DAD method is useful for determination of rutin and its impurities in pharmaceutical preparations with the benefits of short analysis time and the possibility of determination in the presence of related products (impurities and degradation products).

Acknowledgements: This study was supported by grant from the Poznan University of Medicinal Sciences.

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P–23

THE SCREENING OF ANTI-LIPASE AND ANTI-AMYLASE ACTIVITY OF SELECTED EDIBLE PLANT MATERIALS

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Searching of pancreatic lipase and α-amylase inhibitors from natural products for the treatment of obesity and diabetes mellitus type 2 is justified due to a wide range of adverse effects of presently used drugs, such as orlistat and acarbose.
The aim of the study was the screening of the *in vitro* α-amylase and pancreatic lipase (PL) inhibitory activities of the aqueous (H_2O) and ethanolic (EtOH) extracts prepared from fourteen selected edible plant materials. The phytochemical analysis of extracts was conducted in order to identify their potentially active constituents.
Their effects on digestive enzyme activity were evaluated using an *in vitro* fluorescence method [1]. Orlistat and acarbose were used as positive controls. The chemical compositions of the extracts were analyzed by HPLC-DAD-MS/MS method.
The results of *in vitro* assay for the most active extracts from fruits of *Chaenomeles japonica* (CJ) and *Hippophaë rhamnoides* (HR) as well as flower of *Hibiscus sabdariffa* (HS) are shown in Table 1. Chlorogenic acid and procyanidins were detected in CJ fruits, whereas hexoside of hibiscus acid and sambubiosides of delphinidin and cyanidin were the most abundant compounds of HS flower.
In conclusion, our results indicate the aqueous extract from CJ fruits as the active inhibitor of both digestive enzymes. The extracts from HS flower were particularly active inhibitors of α-amylase. Thus, their supportive role in the metabolic diseases was confirmed.

Table 1: IC_{50} [μg/ml] values of the most active extracts for the inhibition of pancreatic lipase and α-amylase.

Extract	Lipase inhibition	Amylase inhibition
Chaenomeles japonica EtOH	259.5 ± 34.6	48.7 ± 4.9
Chaenomeles japonica H_20	44.9 ± 4.4	53.6 ± 5.1
Hippophaë rhamnoides EtOH	335.5 ± 33.8	89.7 ± 11.3
Hippophaë rhamnoides H_20	59.7 ± 4.6	83.0 ± 7.8
Hibiscus sabdariffa EtOH	151.2 ± 15.2	40.2 ± 2.9
Hibiscus sabdariffa H_20	109.1 ± 5.6	35.8 ± 3.6
Orlistat [ng/ml]	11.6 ± 0.5	-
Acarbose	-	2.4 ± 0.4

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

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P–24

DISTRIBUTION OF TRITERPENOIDS AMONG DIFFERENT TISSUES OF *ROSA RUGOSA* HIP

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The pseudo fruits (accessory fruits) of rugosa rose (*Rosa rugosa* Thunb.) have many traditional uses in folk and herbal medicine as well as in cosmetics and confectionary industry (e.g., for the production of jams). Rose hips are aggregate fruits consisting of several achenes (the actual seed-containing fruits of rose hips) enclosed by an enlarged, fleshy floral cup (hypanthium). The aim of the present study was GC-MS analysis of triterpenoids occurring in diethyl ether extracts obtained from different parts of rugosa rose hip: peel, pericarp (i.e. hypanthium) and seeds, as well as compounds present in chloroform extract from cuticular waxes. The general triterpenoid profile of rugosa rose hip is complex, involving several steroids, i.e. sitosterol (predominant phytosterol in all analyzed extracts), accompanied by campesterol, stigmasterol, avenasterol, isofucosterol, stigmast-7-en-3-ol, sitostanol, some intermediates of sterol biosynthesis accumulated in detectable amounts (e.g., cycloartanol, 24-methylene-cycloartanol, obtusifoliol) and steroidal ketones, i.e. sitostenone, cholesta-3,5-dien-7-one and stigmasta-3,5-dien-7-one. Among pentacyclic triterpenoids, the compounds with ursane, oleanane and lupane skeletons were detected, i.e. monohydroxyalcohols: α-, β- amyrins and lupeol, dihydroxyalcohols: erythrodiol, uvaol, betulin, and aldehydes: oleanolic and ursolic. Particularly in the pericarp, the most abundant compounds were acids: ursolic, oleanolic and betulinic, as well as their derivatives with additional hydroxy groups, including 19-hydroxyursan-type acids (e.g. pomolic acid, and two 2,3,19-trihydroxy epimers: tormentic and euscaphic acids); some of these compounds occurred also as acetates. Ursolic and oleanolic acids, accompanied with small amounts of their polihydroxy derivatives, were also detected in seeds, however, in much smaller amounts. The fraction of acids was the most abundant in cuticular wax, entire peels and hypanthium, whereas in the seeds the fraction of steroids was prevailing. The total content of triterpenoids reached 7% of hypanthium extract mass and 2.5% of seed extract. In hypanthium, pentacyclic triterpenoids constituted 83% of all triterpenoid fraction, in seeds less than 40%. The obtained results revealed significant differences in accumulation of triterpenoids in analyzed rose hip tissues. Moreover, they indicate that the distribution of triterpenoids in different fruit tissues is a selective process, e.g., the triterpenoid profile of cuticular wax is not a simple reflection of the composition of these compounds in hypanthium or the whole fruit.

Acknowledgements: The study was funded by the Ministry of Science and Higher Education through the Faculty of Biology, University of Warsaw intramural grant DSM 2017 501-D114-86-0115000-11. Analyses were carried out with the use of CePT infrastructure financed by the European Union—the European Regional Development Fund (Agreement POIG.02.02.00-14-024/08-00).

P–25

ACCUMULATION OF TRITERPENOIDS IN CUTICULAR WAXES DURING *ARONIA MELANOCARPA* FRUIT DEVELOPMENT

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Free and ester forms of triterpenoids are often found in fruit epidermis, mainly in cuticular waxes, and therefore they are considered to play a role in the protection against pathogen infections and in the mechanical toughness of the fruit surface. In some fruits triterpenoids occur in the cuticle in very high amounts, dominating the mixture of aliphatic long-chain hydrocarbons, in others they constitute only the minor part. During the fruit development, the triterpenoid content in cuticular waxes displays the characteristic pattern of changes with fluctuations depending probably on the level of the biosynthesis of these compounds, and their availability in the internal tissues. The aim of the present study was the determination of the changes in the triterpenoid content of cuticular waxes of black chokeberry *Aronia melanocarpa* cv. Nero during fruit ontogeny. Fruit samples were collected at three different phenological stages: very young berries in June, full-size green berries at the onset of ripening in late July, and mature ripen berries in the beginning of September. Triterpenoid content was determined by GC-MS/FID method. The obtained results revealed that the principal triterpenoid compounds occurring in black chokeberry waxes were triterpenoid acids: oleanolic and ursolic, accompanied with their polihydroxylated analogues in free and esterified (acetate) forms. The other identified pentacyclic triterpenoids were α- and β-amyrins, germanicol, α-amyrenone, erythrodiol accompanied with its diacetate, uvaol, ursolic aldehyde; however, their content was very small (approx. 25 µg/mg in cuticular wax of fully rippen fruits). Several typical steroids (campesterol, stigmasterol, sitosterol and two ketones: sitostanone and tremulone) were detected in trace amounts. The accumulation of the most abundant triterpenoid acids, oleanolic and ursolic, in cuticular wax changed according to fruit developmental stages. In very young fruits the total content of the two acids accounted for 220 µg/mg of fruit wax, then it increased sharply to 360 µg/mg and finally decreased to 200 µg/mg. Ursolic acid was the dominant isomer in chokeberry fruit wax, with the ratio of ursolic to oleanolic acid being practically stable during fruit growth (from 1:0.37 in very young berries to 1:0,31 in the rippen fruits). The current study confims that the pattern of compositional evolution of cuticular waxes during development and maturation of different fruits is not uniform. In some fruits the constant increase or decrease of triterpenoid content was observed, whereas in other fruits (such as chokeberry or previously analyzed bilberry *Vaccinium myrtillus*) the level of triterpenoids is increasing during the first phase of fruit growth until reaching the full size, and then decreasing during fruit maturation.

Acknowledgements: Analyses were carried out with the use of CePT infrastructure financed by the European Union—the European Regional Development Fund (Agreement POIG.02.02.00-14-024/08-00).

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APPLICATION OF COC-GC-FID SYSTEM FOR GLYCERIDE AND NON-GLYCERIDE OIL COMPONENTS ANALYSIS

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Cold on-column injection mode coupled with gas chromatography-flame ionization detection system (COC-GC-FID) provides an opportunity to simultaneous quantitation and confirmation of triacylglycerides as it supports high quantitative accuracy, and precision with minimal mass discrimination, which is critically important for such a highly-boiling compounds. Previously, the systems of direct sample introduction and use of a high-temperature columns have been developed in attempts to determination of free and total glycerol and mono-, di-, and triglyceride contents and also steroids (TMS-derivatives) [1]. The purpose of this paper is to present the application of cold on-column injection coupled with gas chromatography-flame ionization detection system for a separation and quantitative determination of triglycerides and and also non-glyceride components such as steroids in selected vegetable oils. The studies were performed using Agilent GC-FID system equipped with J&W VF-5ht column (30 meters, diam. 0.25 mm, film 0.10 µm) with low bleed performance which is suitable for the analysis of high boiling compounds. Figure 1 shows a chromatogram of coconut, olive and strawberry seed oils with determined individual components of triglyceride mixtures.

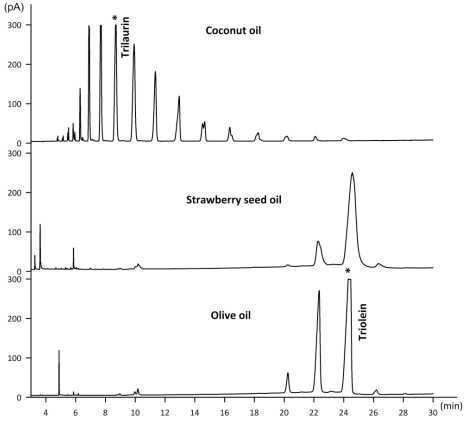

Figure 1. The chromatogram of glyceride oil components by GC-FID with direct injector system.

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P–27

ISOLATION OF ACTIVE AMARYLLIDACEAE ALKALOIDS USING VACUUM LIQUID CHROMATOGRAPHY WITH GRADIENT OF STATIONARY AND MOBILE PHASES

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Species of *Narcissus* belonging to the Amaryllidaceae family are a rich source of alkaloids present in the bulbs and flowering parts. In this parts of plant, a big concentration of alkaloids including lycorine, crinine, angustamine and galanthamine which takes part in the treatment of neurodegenerative illness such as Alzheimer's disease has been found. The action of galanthamine is the inhibition of acetylcholinesterase and butyrylcholinesterase enzymes which leads to increase of acetylcholine concentration in synapses of central-nervous system [1].
Isolation of alkaloids was performed through ASE- Accelerated Solvent Extraction at elevated temperatures up to 140 °C and 100 bars pressure using methanol as polar solvent. Concentrated methanolic extracts after removal of lectins by filtration have been transferred to polypropylene columns filled with different combinations of two polar sorbents: silica gel 60 F_{254} and basic aluminum oxide (150 MeSh) as two-stationary phase systems in various proportions and different order aiming to isolate alkaloidal fractions. Vacuum liquid chromatography (VLC) was carried out with different solvent gradient systems using: chloroform, methanol, acetone, 25% ammonia water solution in various proportions starting from 90:5:5:0.1 (v/v/v/v). The alkaloids of *Narcissus* c.v. 'Hawera' were eluted at a rate depending on polarity of their moieties, starting from non-polar alkaloids and ending with sanguinine and further ones.
The collected fractions have been analysed by TLC method on silica gel plates with two standards for comparison: sanguinine and *nor*-galanthamine. The separated alkaloids were visualized under UV light and by Dragendorff reagent. The chosen methods allowed the separation of individual components and confirmation of the presence of almost pure sanguinine fractions.
The next stage of research included the identification of alkaloidal compounds by LC/ESI-QTOF-MS method, followed by TLC-bioautographic approach towards inhibition of acetylcholinesterase enzyme [2].

Acknowledgements: National Centre for Research and Development (grant No. 4/POLTUR-1/2016).

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P–28

SHOOT CULTURES OF *ARONIA* × *PRUNIFOLIA* CULTIVATED IN PLANTFORM BIOREACTOR – ESTIMATION OF PHENOLIC ACIDS USING LC-DAD METHOD

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Phenolic acids are one of subgroup of polyphenols with very important antioxidant activities. The agar and agitated cultures of *A.* × *prunifolia* are the rich source of these compounds [1,2]. This plant is a hybrid of *A. melanocarpa* and *A arbutifolia,* with North-American origin, cultivated in Poland, with high biosynthetic potential [3,4].
The aim of the study was the establishment of shoot cultures of *A.* × *prunifolia* in Plantform bioreactor.
The shoot cultures were maintained on Murashige and Skoog medium (MS) [5] enriched with 1 mg/l BA and 1 mg/l NAA (3 series). The biomasses and culture media samples were collected after 4 and 8 weeks. In methanolic extracts the LC-DAD analysis [6] of 26 phenolic acids was performed.
The presence of eleven compounds were confirmed. The quantitatively dominating metabolites were: 3,4-dihydroxyphenylacetic acid, 3-phenylacetic acid, chlorogenic acid and isochlorogenic acid. Higher total content of phenolic acids was confirmed after 4-week growth cycles (869.16 mg/100g d.w.). The maximal amounts of metabolites were as follows: 3,4-dihydroxyphenylacetic acid - 150.85 mg/100g d.w. 3-phenylacetic acid - 118.14 mg/100g d.w., chlorogenic acid - 145.67 mg/100g d.w. and isochlorogenic acid - 228.32 mg/100g d.w., respectively. Extracts from the culture media were found to contain no metabolites.
The applied LC-DAD method was very useful for the separation and estimated of analysed compounds.

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P–29

SHOOT CULTURES OF *ARONIA* × *PRUNIFOLIA* MAINTAINED IN RITA BIOREACTOR – ANALYSIS OF PHENOLIC ACIDS WITH LC-DAD METHOD

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Aronia × *prunifolia* is a hybrid of *A. melanocarpa* and *A. arbutifolia* cultivated in Poland of North-American origin. Fruits of this plant contain some subgroups of polyphenol compounds e.g. anthocyanin's and phenolic acids. These compounds are famous antioxidant agents [1,2].
Our previous studies with *in vitro* agar and agitated cultures documented the ability for high production of phenolic acids especially depsides [3,4].
The aim of the present study was the establishment of shoot cultures in temporary immersion system - RITA bioreactor.
The shoot cultures were maintained on Murashige and Skoog medium (MS) [5] supplemented with 1 mg/l BA and 1 mg/l NAA for 4 and 8 weeks (3 series). In methanolic extracts of collected biomasses and culture media samples the LC-DAD analysis was performed [6].
In extracts presence of 11 phenolic acids (from 26 analyzed compounds) was confirmed. The main compounds were: 3,4-dihydroxyphenylacetic acid, 3-phenylacetic acid and isochlorogenic acid (max. 197.39, 141.54 and 161.81 mg/100g d.w., respectively). The total content of phenolic acids was comparable in the extracts collected after 4 and 8 weeks (693.26 and 698.17 mg/100g dw., respectively). Extracts from the culture media were found to contain no metabolites.
The applied LC-DAD method has given the possibility for good separation and quantification of estimated compounds.

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[1] Szopa A., Kubica P., Ekiert H.: *Post. Fitoter.* 2017, 18(2), 145-157.
[2] Szopa A., Kokotkiewicz A., Kubica P. et al.: *Eur. Food Res. Technol.* 2017, 243(9), 1645-1657.
[3] Szopa A., Kubica P., Snoch A. et al.: *Acta Physiol. Plant.* 2018, 40(3), 1-11.
[4] Szopa A., Kubica P., Ekiert H.: *Plant Cell Tiss. Org.* 2018, under review.
[5] Murashige T., Skoog F.: *Physiol. Plant.* 1962, 15, 473–97.
[6] Ellnain-Wojtaszek M., Zgórka G.: *J. Liq. Chromatogr. Relat. Technol.* 2006, 22, 1457–1471.

P–30

THE ISOLATION AND IDENTIFICATION OF THREE NEW COMPOUNDS FROM *PANCRATIUM LITTORALE* JACQ., (AMARYLLIDACEAE)

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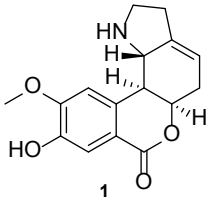
The family Amaryllidaceae – well known for its vast diversity of secondary metabolites and therapeutic potential – is still actively being researched for the identification of novel compounds. Even though over 300 compounds have been already isolated and identified [1], minor alkaloids remain poorly studied. We isolated and characterised a series of alkaloids in a methanol extract from the bulbs of *Pancratium littorale* Jacq., (Amaryllidaceae). For the challenging isolation of minor compounds we used a specific approach, which included Diaion (HP-20) column chromatography for the removal of sugars, alkaloid extraction for enrichment of alkaloids, a pre-separation using diol flash chromatography, and final purification by RP-HPLC using an eluent at pH 9.0 and MS detection. Fifteen isoquinoline alkaloids including three new congeners (**1**, **2**, and **3**) were obtained and structurally characterised using ESI-MS and microprobe NMR analysis. They include 8-demethoxy-lycoranine F (**1**), 8-*O*-demethyl-homolycorine, pseudo-lycorine, 8-*O*-demethyl-lycorenine (**2**), 7-demethoxy-10-*O*-methyl-hostasine, carinatine, lycorine, 10-*O*-methyl-hostasine, 10-deoxy-3-hydroxy-6-hydroxyhippeastidine (**3**), narcissidine, 1-*O*-acetyl-pseudo-lycorine, 8-*O*-demethyl-6-*O*-methyl-lycorenine, 3-*O*-acetyl-narcissidine, 10-deoxy-6(α)-hydroxy-hippeastidine, and 6-hydroxy-hippeastidine. The absolute configuration of compound **1** was established by electronic circular dichroism (ECD) in combination with quantum chemical calculations. In conclusion, plants from the Amaryllidaceae family still represent a source of new secondary metabolites with interesting scaffolds. Linking these compounds to biological activity will contribute to the discovery of new hit compounds.



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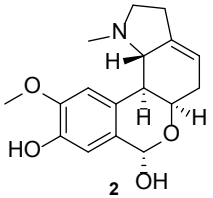

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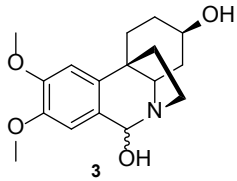

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P–31

CENTRIFUGAL PARTITION CHROMATOGRAPHY: A COST-EFFECTIVE TECHNOLOGY FOR CANNABINOIDS PURIFICATION IN PRODUCTION SCALE

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The use of cannabinoids from *Cannabis sativa* L. in therapeutics is known for long time. Nowadays, it involves various preparations such as raw herbal material, extracts, natural cannabinoid-based medicines and synthetic cannabinoids. These are used in epilepsy, cancer palliation and primary treatment, chronic pain, Parkinson disease, multiple sclerosis, anxiety, irritable bowel syndrome and other diseases [1,2]. Regulations are substantially different between countries: Both the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) do not approve the use of herbal cannabis or extracts. The FDA approved several cannabinoid-based medicines, so did 23 EU countries and Canada [3]. Thus, there is an increasing need for pure cannabinoids, to be used as APIs. Centrifugal Partition Chromatography (CPC) is reputed as being one of the most efficient, high-throughput and selective chromatographic technologies existing in large scale, especially for cannabinoid purification. Academia has evaluated CPC for cannabinoid purification, especially for Cannabidiol (CBD) and Δ-9 Tetrahydrocannabinol (Δ-9 THC), from Cannabis extracts [4]. Nevertheless, there is a strong need for more thorough process development in order to provide the industry with optimized, regulation and GMP-compliant Standard Operating Procedures (SOPs). An industry-driven process development using exclusively ICH (International Council for Harmonization) guidelines Class III (low toxic potential) solvents has been conceived. This is challenging because not a single solvent system family composed exclusively by these solvents has ever been introduced in CPC or generally in liquid/liquid chromatography, due to their physicochemical properties. The developed processes have been proven to scale-up optimally and are semi-continuous. High purities (up to 98%) and recoveries (up to 87%) of the isolated substances have been achieved. Overall, innovative and efficient cannabinoid purification processes have been developed and scaled-up to provide cannabis industry with turnkey solutions.

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P–32

"MICROMETABOLITE" PROJECT: MICROBIAL ENHANCEMENT OF BIOACTIVE SECONDARY METABOLITE PRODUCTION IN PLANTS

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Natural products derived from plant sources - known also as plant secondary metabolites (SMs) - are of great interest for pharmaceutical, cosmeceutical and food supplements industries. Plant-associated microorganisms (such as endophytic bacteria and fungi) are physiologically interlinked with their hosts and, thus essential to plant growth and health. As they may induce or modulate host biosynthesis pathways, they have a critical role in the production of plant SMs. SM biosynthesis in plants is subject to the influence of multiple factors and stimuli such as plant hormones, herbivore and pathogen-derived elicitors, as well as the abiotic environment. The plant-associated microbiota influences plant SM production through various activities, for instance nitrogen fixation, synthesis and modulation of plant hormones, vitamin production, or enhancement of nutrient uptake. Moreover, endophytic bacteria and fungi themselves are known as producers of various SM compounds. In order to further investigate those interactions, in the frame of the EU H2020 project "MICROMETABOLITE", a multidisciplinary consortium consisting of academic and industrial partners, will study plant microbial communities of *Boraginaceae* plants and the influence that might have in the production of bioactive secondary metabolites.
In more details, this study will focus on *Alkanna tinctoria* and *Lithospermum erythrorhizon* roots and on investigating the relationship between their microbiomes and the secondary metabolite production, especially for the enantiomeric naphtoquinones, alkanin and shikonin. Those molecules are well known for their wound healing, anti-microbial, and anti-inflammatory activities. Eco-friendly cultivation systems integrating also essential microorganisms will be developed on plants of *Boraginaceae* family for stimulating the production of secondary metabolite and exploring also new biological activities. All plant materials at several vegetation stages both from the wild collections and cultivations will be extracted and then analyzed with methods based on HPTLC,

HPLC and UHLC-HRMS. The de-replication process of secondary metabolite will lead to a targeted isolation and identification of the most promising compounds associated with selected microorganisms, while the determination of the optimal cultivation conditions for producing the desired naphthoquinones for industrial use will be performed.
Overall MICROMETABOLITE aspires to create an environment that will foster synergistic cooperation between multidisciplinary researchers in relevant, currently hardly inter-linked scientific disciplines and maximize the exploitation of plant biotechnology in accordance with the needs of the European pharmaceutical and cosmetic industries. As the first network in this area, it will train some of the most talented European young scientists (ESRs) to become future leading scientists, technologists and entrepreneurs, who can take on leadership in the required scientific and technological discovery and in the development of next-generation pharmaceutical and cosmeceutical applications.

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USNIC ACID ENANTIOMERS ISOLATION AND ACTIVITY

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Usnic acid is a dibenzofurane derivative, characteristic of lichens. It has been extensively studied due to its antimicrobial, cytotoxic and anti-inflammatory activity. Data on the potential differences in the activity of both usnic acid enantiomers is scarce, thus the aim of this study was to compare their impact on different aspects of skin functioning in an *in vitro* model.

Usnic acid enantiomers were isolated by means of preparative TLC from *Cladonia arbuscula* and *C. uncialis*, and their identity was confirmed by HPLC and polarymetric analysis. The isolated enantiomers were then examined for their anti-inflammatory and anti-hyaluronidase activity. Moreover, the influence of the tested compounds on normal skin cells (fibroblasts, melanocytes etc.) was also defined.

The tested compounds revealed no toxic effect on the normal skin cell lines, at the tested concentration range (up to 100 µg/ml). Moreover, an interesting anti-inflammatory and anti-hyaluronidase activity was observed, although the differences between both enantiomers were mostly not statistically significant.

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POTENTIAL CHEMOPREVENTIVE ACTIVITY OF *FERULA PENNINERVIS* ROOT EXTRACT IN MELANOMA

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Ferula (*Apiacea*) is a genus of flowering plants comprised of about 180 species native to the Middle East, Central Europe and Central Asia. The roots, the leaves and the fruits of these plants are a good source of biologically active compounds including sesquiterpenes, sesquiterpene coumarins, sulfur-containing compounds and sesquiterpene coumarin glycosides [1]. *Ferula* species are used as a spice or in traditional medicine for treatment of various diseases from neurological diseases, rheumatism, headache, arthritis, dizziness, inflammations, dysentery, infant colitis, and digestive disorders [2]. Some species, including *F. latisecta, F. pseudalliacea* and *F. assafoetida* possess also anticancer activity, as shown in studies on human and murine models of colon, cervical, lung and breast cancers [1,3,4].

The aim of this study was to evaluate the activity of the root extract from *Ferula penninervis* as a potential chemopreventive agent against human melanoma. This malignant type of skin cancer might be induced by the exposure to UV radiation, especially UVB (280-320 nm) that increases the production of ROS and cause oxidative stress in the skin [5,6]. *F. penninervis* is a less studied *Ferula* species, containing some unique sesquiterpens with significant antioxidant activity [7,8]. Our studies showed that the methanolic extract from *F. penninervis* absorbs UVB and UVA radiation and possess anti-radical activity, as shown in a DPPH scavenging assay (EC_{50}=0.2007 mg/ml). *In vitro* analysis revealed that the extract is cytotoxic for A375 malignant human melanoma cells at 300, 200 and 100 µg/ml, whereas its toxicity against normal human keratinocytes HaCaT is significantly lower. The content of total phenolics and flavonoids in the extract is relatively low (30.02 ± 0.55 µg/g and 5.09 ± 0.58 µg/g, respectively), suggesting that other compounds are responsible for its anticancer activity. Obtained results indicate that *F. penninervis* might be a valuable source of active compounds with potential chemopreventive activity against human melanoma. To our knowledge, this is the first study on the anticancer properties of *Ferula penninervis*.

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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY AS A SUPPORTING TOOL FOR THE METABOLIC DISCRIMINATION OF *RHEUM* SPECIES

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Metabolomics has been applied to the investigation of the natural product diversity. To accomplish the comprehensive profiling a wide range of analytical platforms are being used for diverse organisms. Of the methods, ^{1}H NMR- and MS-based techniques are the most common metabolomics tools. However, despite the popularity of these two methods, there are still some limitations. Particularly, the identification or selective metabolic group detection following overall metabolic profiling have not been solved yet. To circumvent these problems, here we applied high performance thin-layer chromatography (HPTLC) as a supplementary tool to the conventional NMR- or MS-based method. As a model organism, Radix et Rhizoma Rhubarb, a popular Chinese medicine, is employed in this study. This medicinal plant is associated with many therapeutic uses such as for treatment of hemorrhage, thrombocytopenia, hyperlipemia and infections. The pharmacological activities of the secondary metabolites from rhubarb were found to be related with their secondary metabolites such as anthraquinones, of which contents are assumed to be influenced by diverse factors [1]. To uncover the relationship between biotic and abiotic factors, and metabolome, ^{1}H NMR and HPTLC were applied to various batches of the plant materials. Rhubarb samples from two species (*Rheum palmatum* and *Rheum tanguticum*) collected at different geographical locations and altitudes. Principal component and orthogonal partial least square analyses (OPLS) were used as tools to classify the rhubarb samples. NMR analysis and HPTLC profiles reflected variations in the chemical quality of rhubarb due to both environmental and species effects (Fig. 1a). Interestingly, the most influential factor was collection altitude where the plants grew (Fig. 1b). The compounds that contribute to the altitudinal differentiation are chrysophanol and sennoside A. The clear separation of chemical profiling by ^{1}H NMR and the confirmation and clarification by HPTLC-based method suggests that the latter one is a promising analytical tool to support NMR data in the quality control of herbs used on traditional Chinese medicine.

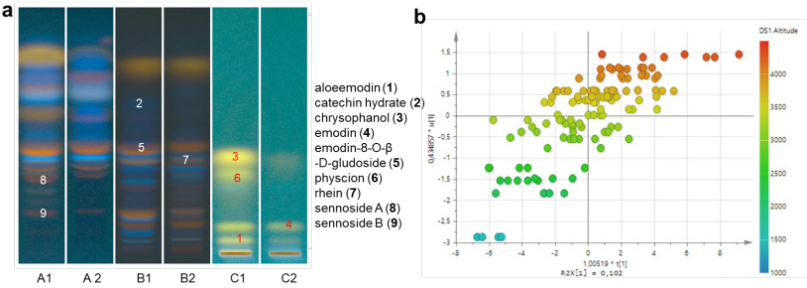

Fig. 1 **(a)** HPTLC derivatised UV366 nm image of two species under three kinds of mobile phases. A1, A2 run in 1-propanol: ethyl acetate: water (4:4:3, v/v/v); B1, B2 run in Ethyl acetate: methanol: water (100:17:13, v/v/v); C1, C2 run in Cyclohexane: ethyl acetate: methanol: formic acid: water (3:1:2:2:0.1, v/v/v/v/v, upper layer). 1: *R. palmatum*, 2: *R. tanguticum.* **(b)** Score of OPLS modeling obtained from HPTLC data in mobile phase 1-propanol: ethyl acetate: water (4:4:3, v/v/v) as X-data and collection altitude of each sample as Y-data, Q^2= 0.35, p= 2.6 x 10^{-7}.

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PHENOLIC COMPOUNDS CHARACTERIZATION AND ANTIOXIDANT POTENTIAL OF *NEPETA HUMILIS* BENTHAM

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Nepeta genus, a member of Lamiaceae, is represented by approximately 250-300 species all over the world. The members of the genus are widely distributed in Eurasia, Africa, America and especially in the Mediterranean region. *Nepeta* species are widely used in traditional medicine for their expectorant, diuretic, antispasmodic and antiasthmatic activities. Terpenic compounds, flavonoids, phenolic acids, iridoids and essential oil have been reported within the genus [1]. *Nepeta humilis* Bentham is an annual plant which grows naturally in the South-East region of Anatolia [2]. The purpose of this study is to evaluate the total phenolic content and radical scavenging activity of *N. humilis* flowers, leaves and roots as well as to perform the qualitative and quantitative analysis of the phenolics using a validated RP-HPLC method [3]. Results revealed that the total phenolic contents of flowers, leaves and roots were 123,18 ± 1.01, 66.20 ± 0.49 and 54,77 ± 1.23 mg GAE/g extract, respectively. The flower extract was able to scavenge DPPH radical with an IC_{50} of 1.29 ± 0.02 mg/mL, and ABTS with an IC_{50} of 0.35 ± 0.01 mg/mL, which the results represent more radical scavenging power compared to the leaf and root extracts. The flower extract having more total phenolic content exhibited more radical scavenging activity. HPLC results showed that rosmarinic acid was detected as one of the main compounds in all the investigated parts of the plant, especially in the flowers. Moreover, chlorogenic acid, luteolin and apigenin were found in significant amount in the flower extract. Any of the investigated flavonoids were detected in the root extract. The high antioxidant potential of the flowers of the plant could be attributed to the substantial amount of rosmarinic acid supported by phenolic acids and flavonoids.

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IN SEARCH OF CHEMOTAXONOMIC MARKERS OF *TILIA* SPECIES USING UHPLC-DAD-MS METHOD

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

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

Almost two hundred samples of flowers of *Tilia* collected between 2013-2016 in different regions of Poland and Europe were investigated. The UHPLC-DAD-MS method using Kinetex XB-C_{18} column was developed. Chromatograms were recorded at 254, 280 and 350 nm. The MS spectra were analyzed in negative ion mode. Over 40 compounds were detected and characterized comprising flavan-3-ols, flavonoids and phenolic acids. The identification was based on the comparison with available chemical standards and on the literature data. Major compounds from groups of flavan-3-ols and flavonoids were quantified using a validated method with epicatechin and isoquercitrin as reference standards.

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

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DETERMINATION OF FLAVONOIDS IN AN ELICITED *CHAMAENERION ANGUSTIFOLIUM* (L.) PLANTS CULTIVATED *IN VITRO*

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The *Epilobium* genus includes over 200 species. The most common species are *Chamaenerion augustifolium, E. hirsutum, E. parviflorum* [1]. *C. angustifolium* (fireweed) plants are used in traditional, folk medicine. Russians, Chinese and American Indians used herb and roots in treatment of benign prostate hyperplasia, rectal bleeding, to relief of menstrual disorders [1, 2].

C. angustifolium plants are rich source of phenolic compounds, especially ellagitannins, flavonoids and phenolic acids [3]. Other minor constituents such as sterols, triterpenoids, fatty acids, and essential oil have been also determined. Oenothein B is the most abundant ellagotannin, and the main active compound. Quercetin-*3*-*O*-glucuronide is the characteristic flavonoid of fireweed [3]. Therefore, analysis of oenothein B and quercetin-3-O-glucuronide content is recommended as markers and bases for raw material standardization.

C. angustifolium cultivated *in vitro* plants were exposed to different UV-B doses to enhance the content of flavonoids. Oenothein B, quercetin-*3*-*O*-glucuronide, naringenin and myricetin were determined in dried samples using HPLC-DAD (Agilent). A chromatography separation was obtained on a column LiChrospher 100 RP-C18e, 5µm 4x250 mm (Merck). Detection of flavonoids were detected at 263 nm and 290 nm. 2.5% acetic acid and mixture 2.5% acetic acid and acetonitril (20:80 V/V) were used as eluents. Peaks were identified by the addition of standards and by UV-VIS spectra.

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DETERMINATION OF FLAVONOIDS AND PHENOLIC ACIDS IN AQUEOUS AND 50% HYDROALCOHOLIC EXTRACTS FROM FLOVERS AND FRUITS FROM *SAMBUCUS NIGRA* L.

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Several reports have indicated the chemical composition of *Sambucus nigra* L. berries, flowers and leaves [1,2,3]. *Sambucus nigra*, commonly refferd to as elderberry, is rich in proteins, free and conjugated forms of amino acids, unsaturated fatty acids, vitamins, antioxidants, and minerals [4]. As the results from literature data suggest, elderberry is a good source of bioactive components, primarily polyphenols, mostly flavonols, phenolic acids, anthocyanins, and proanthocyanidins with the latter giving its fruits the characteristic black-purple colour [5]. The analysis of the content of phenolic compounds in *Sambucus nigra* proves why people have believed in the beneficial effect of the plant (its leaves, flowers, fruits, and bark) and have used it in pharmacy and folk medicine for centuries.

The dry extracts from elder were extracted with 70% ethanol and analyzed for the content of flavonoids and acids. Flavonoids and phenolic acids were determined using HPLC-DAD (Agilent). A chromatography separation was obtained on a column Zorbax Poroshell 120 SB-C18 column, 2.7 mm × 3.0 mm × 100 mm (Agilent). The rutine, quercetin, kaempferol were detected at 250 nm, *p*-coumaric acid at 280 nm, whereas chlorogenic acid, caffeic acid, 7-O-luteoline and apigenin at 330 nm. The gradient mixtures of phase A – water : H_3PO_4 (100:0.02 V/V) and of phase B – acetonitrile : tetrahydrofurane (100:2 V/V) were used as eluents. The identification of peaks was taken place by the addition of standard solutions and by UV–VIS spectra.

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ACCUMULATION OF PHENOLIC COMPOUNDS IN DIFFERENT *IN VITRO* CULTURE SYSTEMS OF *SALVIA VIRIDIS* L.

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Salvia viridis, of the Lamiaceae family, is an annual herb native to the Mediterranean area. This polyphenol-rich species has been used in traditional Turkish medicine as an anti-inflammatory and antiseptic agent [1]. The present study describes the establishment callus and shoot *in vitro* cultures of *S. viridis* as well as production of bioactive compounds in the cultures. The undifferentiated and differentiated cultures were obtained from fragments of 3-week-old aseptically germinated seedlings. Calli were maintained on SH (Schenk-Hildebrandt) and MS (Murashige and Skoog) medium supplemented with 0.1 mg/L NAA, 0.2 mg/L BAP and 0.5 mg/L 2,4-D. Shoots were cultivated on MS medium supplemented with 0.1 mg/L IAA and 0.5 mg/L BAP.
The polyphenol profiling of the hydromethanolic extracts from plant material was performed *via* UPLC-DAD/ESI-MS method in the negative ion modes. Qualitative analysis was performed using an Agilent Technologies 1290 Infinity UPLC apparatus equipped. The analysis was performed on a Zorbax Eclipse Plus C18 column (3x100 mm; 1.8µm Agilent Technologies). The compounds were identified by comparison of their retention time, UV spectra and mass spectra with those of the standard compounds and published data. Additionally, the antioxidant activity of different culture systems of *S. viridis* were evaluated using DPPH and $O_2^{\bullet-}$ radical scavenging, FRAP (ferric reducing antioxidant power) and lipid peroxidation assays.
The UPLC-DAD-ESI/MS analysis revealed the presence of phenolic acid derivatives and phenylethanoids in analyzed samples. Our results demonstrate that the accumulation of the compound depended on the type of culture, medium composition, age of culture and its duration. The predominant metabolite in all samples analyzed was rosmarinic acid. Its content ranged between 1.5 and 11.5 mg/g dry weight. The highest level of rosmarinic acid was found in a half-year-old shoot culture. The content of the compound was 9 times higher than that in shoots of plant grown in the field conditions. Moreover, all samples showed a promising antioxidant potential.

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STEPWISE GRADIENT THIN LAYER CHROMATOGRAPHY OF COMPONENTS OF HIEROCHLOE AUSTRALIS EXTRACTS UNDER CONDITIONS OF CONTROLLED MOBILE PHASE VELOCITY

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Gradient thin-layer chromatography offers many advantages, which enable to solve lots of analytical problems encountered with the separation of multicomponent mixtures with general elution problem, e.g. components of plants extracts. Nowadays, practitioners of TLC can develop gradient chromatograms using only one commercially available device, i.e. AMD 2 system from Camag [1]. AMD 2 has a lot of benefits like good performance and reproducibility. Moreover, this device is fully automated. However, AMD 2 has some disadvantages, such as the long-time of chromatogram development or the lack of adequate and simple optimization and method development model. Furthermore, due to many stages of drying, there is a risk of decomposition of some mixture components. Therefore our research group has been looking for alternative solutions for gradient mode in thin-layer chromatography [2-4]. In this poster we show the results obtained with the new device in which the mobile phase solution is delivered onto the surface of the adsorbent layer with controlled velocity (by moving pipette driven by 3D machine). Delivery velocity of the solvent to the adsorbent layer is equal to or lower than that of conventional development. Therefore chromatograms can be developed with optimal constant linear mobile phase velocity. Furthermore, under such conditions there is no excess of eluent solution on the surface of the adsorbent layer thus higher performance of the chromatographic system can be obtained. The new device was applied for separation of components of *Hierochloe australis* extracts. Results show that satisfactory separation of substance zones (components of plant extracts) is obtained with reasonable time of chromatogram development. It means that presented device brings new approach to planar chromatogram development and is promising for the future.

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CHROMATOGRAPHIC AND SPECTROSCOPIC PROFILING VERSUS ANTIOXIDANT AND CYTOPROTECTIVE POTENTIAL OF LYOPHILISATES OBTAINED FROM AERIAL PARTS OF THREE SCUTELLARIA L. SPECIES

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Lyophilisates obtained from aerial parts of three species belonging to *Scutellaria* L. genus, namely *S. baicalensis* (baical skullcap), *S. orientalis* L. (oriental skullcap), and *S. hastifolia* L. (spear-leaved skullcap) were subjected to simultaneous phytochemical and biological profiling to evaluate and compare their therapeutic potential. At the beginning of the research, ultrasound-assisted extraction (UAE) was carried out to obtain 50% ethanolic-aqueous (v/v) extracts from plant material that were further condensed, dissolved in water, freezed, and vacuum-dried. As a result of this, the skullcap lyophilisates (SBL, SOL and SHL) were obtained. After purification on SPE phenyl BakerBond microcolumns polyphenolic fractions were standardized for the content of bioactive flavonoids using the elaborated LC/PDA method. The separation of compounds was carried out using a gradient mobile phase system with acetonitrile as organic modifier and an Aquasil C18 stainless-steel columns (250 x 4.6 mm i.d.; d_p= 5 μm) as the stationary phase. The identity of particular flavonoid compounds in skullcap lyophilisates was additionally confirmed by means of the simultaneous LC-PDA/Q-TOF/MS-MS profiling.

To evaluate the cytoprotective properties of SBL, SOL and SHL, their effects against 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and tert-butyl hydroperoxide (t-BuOOH)-induced human erythrocyte oxidative damage were estimated in vitro as well as the influence on the human erythrocyte morphology and erythrocyte membrane permeability were examined in the haemolysis assay. The antioxidant and ROS scavenging potential of lyophilisates was investigated using standard antioxidant assays, namely 1,1-diphenyl-2-picryl-hydrazyl free radical ($DPPH^{\bullet}$) scavenging, ferrous ions (Fe^{2+}) chelating activity and total reducing ability determination by $Fe^{3+} \rightarrow Fe^{2+}$ transformation. The protective effect of skullcap lyophilisates was compared with selected flavone standard substances and reference antioxidants. The results of the study revealed the strong free radical scavenging activity and efficient attenuation of AAPH-induced oxidative damage in human erythrocytes which can be explained not only by their antioxidative potential but also by the erythrocyte membrane partitioning of bioactive lyophilisate components. Significant correlation between the content of flavonoid compounds in SBL, SOL and SHL and their biological potential have been also observed.

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OLIVE-NET, BIOACTIVE COMPOUNDS FROM OLEA EUROPAEA: INVESTIGATION AND APPLICATION IN FOOD, COSMETIC AND PHARMACEUTICAL INDUSTRY

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The goal of the Olive-Net project is to introduce a novel approach for the exploration, valorization and marketing of new products based on bioactive compounds from *Olea europaea*. This will be achieved through an extended and well-balanced scheme of researcher's secondments between universities and enterprises from EU & Associated countries as well as universities from Third countries. A mutual scientific project developed on the needs and interests of both sectors exploiting the existing expertise will be the base of this proposal. Products and side-products of the olive tree such as olive oil, edible olive fruits, olive mill waste and olive tree leaves, will be subjected to a series of state-of-the-art extraction and isolation cascades in order to provide extracts, enriched fractions and isolated compounds of high purity. Target chemical categories will involve the well known olive oil polyphenols and secoiridoids, that will be assessed for their safety and pharmacological effects against inflammation, osteoarthritis, cardiovascular disease, etc. in cell-based and in vivo assays. All active ingredients will be identified and characterized with advanced analytical techniques, in order to be integrated in formulations and products in the area of nutraceuticals/dietary supplements. Within this project, core scientific multidisciplinary knowledge from different research areas will be integrated creating valuable synergies. Expertise will be transferred by means of the seconded researchers training in environments with different research orientation where complimentary skills are required. Special attention will be given to dissemination activities aiming to public awareness of benefits of healthy diet(s). Olive-Net aspires to create a successful model promoting considerably researchers' competences and long-lasting collaboration between Industry and Academia.

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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF SEVERAL *ARTEMISIA* L. SPECIES

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The plant materials (blooming herbs) of seven Artemisia species: *A. abrotanum* L., *A. campestris* L., *A. capillaris* Thunb., *A. gmelinii* Weber & Stechm., *A. indica* var. *maximowiczii* (Nakai) H. Hara, *A. umbelliformis* Lam. and *A. verlotiorum* Lamotte (syn. *Artemisia selengensis* Turcz.) was collected in July and September 2016, in the Botanical Garden Chair and Dept. of Pharmacognosy with Medicinal Plant Unit, Faculty of Pharmacy, Medical University of Lublin (Poland), and and then dried in the shade and draught and immediately powdered according to accepted normal procedures.
Using the ultrasonic extraction method (UEA) 21 extracts (extraction solvent: 7:3, 3:7 MeOH: H_2O v/v and water) were obtained, in which the content of phenolic acids was determined and the components of extracts were identified using high-performance liquid chromatography (HPLC) with DAD detection. The antioxidant activity of plant substances was determined using the DPPH• radical method at the λ wavelength = 515 nm Microbiological activity of the tested extracts in the direction of action against the micro-organisms Gram (+) - positive (7), Gram (-) - negative (5) and fungi (of the genus Candida sp.) - 5 was also determined.
VERO (ECACC, No. 84113001, green monkey kidney) and FaDu (hypopharyngeal squamous cell carcinoma) were used for the assessment of cytotoxicity of tested extracts. FaDu is included in the ATCC Head and Neck Cancer Panel and are commonly used for studying cancer on a molecular level and screening for biologically active small molecules in cancer drug development. The MTT tetrazolium method was used to assess the viability of cells and CC_{50} values (concentration decreasing the viability of the cells by 50%) were calculated.
The tested extracts were also evaluated for anthelmintic effects - taking advantage of their effect on the viability of nematodes. The tested extracts were added to the cultures at concentrations of 20 mg/ml, 40 mg/ml and 60 mg/ml. After 24 hours culturing, nematodes were observed for growth inhibition and deformation of the body. To determine the viability of nematodes, methylene blue staining was performed. An untreated culture of nematodes was used as a control [1].

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HPLC-DAD ANALYSIS OF ARBUTIN CONTENTS PRODUCED FROM P-HYDROXYBENZOIC ACID IN A BIOTRANSFORMATION PROCESS IN *SCUTELLARIA LATERIFLORA* L. *IN VITRO* CULRURES

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Arbutin (hydroquinone O-β-D-glucoside) is a compound of plant origin that plays an important role in medicine and cosmetology. Arbutin are widely used for their urogenital disinfectant activity, antitussive effects and as a whitening agent in cosmetology [1].
It is possible to obtain arbutin in *in vitro* cultures via biotransformation of *p*-hydroxybenzoic acid. Previous studies of our team show, that the cells of *Ruta graveolens, Ruta g.* ssp. *divaricata, Schisandra chinensis and Aronia melanocarpa* didn't transform exogenously supplied *p*-hydroxybenzoic acid into arbutin [2].
Agitated *in vitro* cultures of *Scutellaria lateriflora* were cultured on Murashige-Skoog [4] medium with 1 mg/l BAP and 0,5 mg/l NAA for 14 days.
The *p*-hydroxybenzoic acid was added a single dose or divided into 2 to 3 portions, every 24h. The final concentration of precursors were: 96, 144, 192, 288, and 384 mg/l of medium. The biomasses and media were collected after 24 hours after addition of the last dose of precursor. Methanolic extracts from biomass and lyophilized media were analyzed by HPLC method.[5].
After addition of *p*-hydroxybenzoic acid two products were confirmed (arbutin and hydroquinone). The total amounts of hydroquinone were very diverse, from 0,02 to 0,49 g/100g DW, but for arbutin it was 0,11 to 1,45 g/100g DW. The maximum content of arbutin was confirmed after precursor addition at 144 mg/l as single dose.
The results show that *Scutellaria lateriflora* L. seems to be quite promising objects for further investigation on optimisation of biotransformation process. All metabolites were successfully qualified using a RP-HPLC method.

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ARBUTIN PRODUCTION VIA BIOTRANSFORMATION OF HYDROQUINONE IN *SCUTELLARIA LATERIFLORA* L. *IN VITRO* CULRURES – HPLC ANALYSIS

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Arbutin (hydroquinone β-D-glucoside) is a plant derived compound medically applied due to its uroantiseptic activity. It is also widely used in cosmetology as whitening agent [1]. Previous study of our Department show, that the cells of different plant species transform exogenously supplied hydroquinone acid into arbutin with a high yield [2].
Agitating shoot cultures of *S. lateriflora* were maintained on Murashige-Skoog medium [3] supplemented with BAP (1 mg/l) and NAA (0,5 mg/l).
Two weeks after inoculation, a substrate - hydroquinone was added into the culture flasks. The final concentration of hydroquinone were: 96, 144, 192, 288, and 384 mg/l of medium. The hydroquinone was added in single dose or doses divided into 2 or 3 portions administered at 24-h intervals.
The content of the reaction product – arbutin in methanolic extracts from biomass and lyophilized medium samples collected 24 hours after the addition of the last precursor dose was determined using an HPLC method [4].
Arbutin was accumulated mostly in the biomass, but small amounts were also detected in the media.
The production of arbutin rose with increasing hydroquinone concentration. The maximum content of the product was confirmed after hydroquinone addition at 288 mg/l as a single dose (5,63 g/100g DW). Biotransformation efficiency varied widely, ranging from 39,92% to 61,99%. Obtained results are interesting from practical point of view and further optimization of culturing and biotransformation process can lead to higher content of this valuable product. The product was successfully qualified using a RP-HPLC method.

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POLYPHENOLIC PROFILE, ANTIOXIDANT EFFECTIVENESS AND PRO-INFLAMMATORY ENZYMES INHIBITION OF FLOWERS, LEAVES, FRUITS AND BARK OF *COTONEASTER INTEGERRIMUS*

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The European Cotoneaster (*C. integerrimus* Medik., Rosaceae), a deciduous shrub with decorative red fruits, has therapeutical background in traditional medicine of Asia and South-Eastern Europe due to its beneficial effects in relieving stomach ailments and symptoms of jaundice. Our previous studies [1] documented that leaves of *C. integerrimus* are distinguished by the abundance of polyphenolic compounds, mainly flavonoids with high antioxidant potential, suggesting that they might be considered as raw materials for development of dietary supplements effective in prevention of oxidative-stress/inflammatory related diseases. However, the available literature has focused mostly on bioactive polyphenols of fruits and twigs [2] with no data considering composition of flowers, leaves and bark. Therefore, the present study includes a comparative evaluation of flowers, leaves, fruits and bark of *C. integerrimus* with respect to their phenolic profile, antioxidant efficiency and potential anti-inflammatory effects. The UHPLC-PDA-ESI-MS^3 analysis of the methanol:water (7:3, *v/v*) extracts revealed a complex (over forty analytes) and diverse composition of the polyphenolic fractions containing flavonoids (flavonol, flavone and flavanone glycosides), flavan-3-ol derivatives (catechins and proanthocyanidins) and phenolic acids. The results of quantitative analysis (UV-photometric methods) indicated a significant content of polyphenols (49.5-113.6 mg/g dw), especially for the flowers and bark (107.0 and 113.6 mg/g, respectively), containing high amounts of proanthocyanidins (96.3-98.7 mg/g) and tannins (28.3-34.3 mg/g). In the HPLC-PDA studies of individual polyphenols the flowers were distinguished by high content of (–)-epicatechin and chlorogenic acid (8.8 and 8.0 mg/g dw, respectively) and the bark samples as rich in eriodictyol hexoside and (–)-epicatechin (9.7 and 7.4 mg/g). The flowers, bark and leaves also displayed the highest antioxidant efficiency, with activity parameters varying in the narrow range of EC_{50} = 20.6-23.8 μg/mL (DPPH, free radical-scavenging test), FRAP = 2.7-2.8 mmol Fe^{2+}/g (ferric reducing antioxidant power test) and IC_{50} = 23.2-28.9 μg/mL (TBARS, linoleic acid peroxidation test). The study on anti-inflammatory properties of the examined samples (inhibitory effects towards lipoxygenase and hyaluronidase) is still in progress.

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CONTRIBUTION OF INDIVIDUAL POLYPHENOLS TO ANTIOXIDANT ACTIVITY OF THE LEAVES OF *COTONEASTER BULLATUS* AND *C. ZABELII*

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Cotoneaster bullatus and *C. zabelii* are rosaceous species native to central and southern China and naturalized in Europe, where they are extensively cultivated as ornamental plants. With other *Cotoneaster* taxa, they are also considered as a valuable source of traditional medicines exhibiting cardiotonic, diuretic, expectorant, antiviral and anti-spasmodic properties [1]. As indicated by the results of LC-MS analysis of the *Cotoneaster* leaves [2], most of these bioactivities might be attributed to low-molecular polyphenols (over thirty analytes identified tentatively), especially B-type proanthocyanidins, flavonoids (kaempferol and quercetin derivatives) and caffeoylquinic acids. The exceptional abundance of these metabolites and high total antioxidant potential were demonstrated for the leaves of *C. bullatus* and *C. zabelii*.
Therefore, the present study was undertaken for full structural characterization of the dominant polyphenols from the leaves of *C. bullatus* and *C. zabelli* and to evaluate their contribution to biological effects of the appropriate leaf extracts. In a course of isolation studies (column (CC), flash (FC) chromatography and prep-HPLC), seven phenolics were obtained, including (–)-epicatechin, procyanidin B2, caffeoylmalic acid, chlorogenic acid, quercetin 3-(2”-xylosyl)galactoside, hyperoside and quercitrin. The structures of the isolates were elucidated with the use of spectroscopic methods (UV-Vis, 1D and 2D NMR). In the second stage of the analysis, the antioxidant capacity of the isolated polyphenols was evaluated using complementary chemical and biological *in vitro* tests. The (–)-epicatechin and procyanidin B2 were found to be the most active phenolics in all chemical models: DPPH free radical scavenging test, ferric reducing antioxidant power (FRAP) assay and linoleic acid peroxidation test (TBARS) and their activity was comparable to that of some synthetic standards. Moreover, the tested polyphenols effectively protected human plasma components against peroxynitrite-induced damage, as monitored by determination of the following biomarkers: 3-nitrotyrosine, free thiol groups, thiobarbituric acid-reactive substances (TBARS) and ferric reducing ability of plasma (FRAP). Based on the statistical analysis of the relation between the activity parameters of the examined polyphenols and their concentrations, the main determinants of the biological effects were indicated.

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OPTIMIZATION OF TOTAL PHENOLIC ACIDS EXTRACTION FROM AERIAL PART OF *NASTURTIUM OFFICINALE R. BR.* AND CYTOTOXICITY ACTIVITIES

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Nasturtium officinale R. Br. grows around clean and cold water. These plants are found in Europe, both Americas and Asia under the name „watercress". Watercress contains many biologically active compounds, e.g. vitamins, folic acid, carotenoids, glucosinolates and minerals [1]. Fractional factorial design was used to optimize of conditions ultrasound-assisted extraction (UAE) to maximize total phenolic acids contents (TPA). The factors investigated were ethanol concentration (%), solvent/ raw material ratio (mL/g), and extraction time (min.). TPA in the analysed extracts were determined by spectrophotometric method with Arnov's reagent according to the procedure described by Tomczyk et al. [2] with slight modifications. The percentage of phenolic acids was expressed as gallic acid equivalent on dry weight. The optimal extraction conditions to maximize TPA content were: 12.4% ethanol, 19.7 mL/g ratio and 31.8 min. extraction time. The TPA content determined in the extract obtained under optimal conditions was slightly lower than the predicted value calculated statistically, 174.8 and 175.4 mgGAE/g raw material respectively.

The cytotoxicity of ethanolic and aqueous-ethanolic (50%) watercress extracts (N100 and N50) were tested on VERO (green monkey kidney) and FaDu (hypopharyngeal squamous cell carcinoma) cell lines. The extracts were dissolved in DMSO (50 mg/ml) and filtered through syringe filters (pore diameter 0,2 µm). Cells were passaged into 96-well plates and incubated overnight. Semi-confluent monolayer was treated with series of dilutions of tested extracts in cell media containing 2% FBS and further incubated for 72h. Subsequently, the MTT technique was used to measure cell viability and CC_{50} (concentration decreasing the viability by 50% when compared to untreated controls) values were calculated.

In case of VERO cells the CC_{50} of both tested extracts was above 500 µg/ml suggesting low cytotoxicity. Interestingly, the cancer line FaDu was more sensitive with CC_{50} for N100 of 270,85 and N50 of 249,28 µg/ml. Further studies will include the evaluation of the activity of the extracts on different cancer and normal human cell lines.

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ACCUMULATION OF PHENOLIC ACIDS IN *NASTURTIUM OFFICINALE* MICROSHOOT CULTURES – ESTIMATION WITH LC-DAD METHOD

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Nasturtium officinale R.Br. (*Brassicaceae*) (watercress) – is an aquatic perennial plant native to western Asia, Europe and Africa. *N. officinale* herb is the valuable medicinal, cosmetic and culinary plant raw material. It possesses scientific proven e.g. antioxidant, hepatoprotective, anticancer, cardioprotective and anti-inflammatory activities. These properties are conditioned by a rich chemical composition, e.g. glucosinolates, carotenoids, phenolic acids and flavonoids [1].

The aim of this work was the initiation and optimization of the growth conditions of microshoot cultures of *N. officinale*. Under the experiment, the variants of Murashige and Skoog (MS) [2] medium supplemented with different concentrations of plant growth regulators: cytokinin - BA (6-benzyladenine) and auxin – NAA (1-naphthaleneacetic acid), were tested. Moreover the different culture growth periods: 10, 20 and 30-days, were tested.

In methanolic extracts from the lyophilized biomasses using the LC-DAD method [3] ten (out of twenty six tested) phenolic acids were estimated: caffeic, o-coumaric, p-coumaric, ellagic, ferulic, gallic, isoferulic, protocatechuic, rosmarinic and syringic acids. The main metabolites were: protocatechuic acid (max. 138.40 mg/100 g DW), gallic acid (max. 61.03 mg/100 g DW) and caffeic acid (max. 16.65 mg/100 g DW). The highest total (237.52 mg/100 g DW) and individual amounts of phenolic acids were found in extracts from the cultures maintained on variant supplemented with 2 mg/l BA and 1 mg/l NAA collected after 20 days of culture growth. This amount was 4.3-times higher than in control samples – extracts from microshoots cultured without plant growth regulators.

The applied LC-DAD method was efficacious for the separation and estimation of analyzed compounds.

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QUALITATIVE AND QUANTITATIVE ANALYSES OF PHENOLIC ACIDS BY LC-DAD METHOD IN AGAR MICROSHOOT CULTURES OF *SCHISANDRA CHINENSIS* CV. SADOVA

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Schisandra chinensis is an East-Asian medicinal plant species which fruits have been known as hepatoprotective, antitumour and adaptogenic raw material [1]. We proved that the *in vitro* cultures of this species were able to accumulate dibenzocyclooctadiene lignans [2] and phenolic acids [3]. Our current studies deal with biosynthetic potential of *in vitro* cultures of Ukrainian cultivar – *S. chinensis* cv. Sadova.
The agar microshoot cultures were maintained under constant artificial light on 6 variants of Murashige-Skoog (MS) medium [4], differing in concentrations of the plant growth regulators (PGRs), as follows: A- control, B- 0.1 mg/l BA and 2 mg/l NAA, C- 0.5 mg/l BA and 2 mg/l NAA, D- 2mg/l BA and 0.5 mg/l NAA, E- 2 mg/l BA and 1 mg/l NAA, F- 2 mg/l BA and 2 mg/l NAA, G- 3 mg/l BA and 1 mg/l NAA, and on one variant without PGRs (control). The cultures were maintained for 30 days (3 series). Quantification of phenolic acids in methanolic extracts of biomasses and leaves and fruits was performed using the validated LC-DAD method [5,6].
Out of 20 analysed phenolic acids the presence of 7 was confirmed. The main metabolites were: hydrocaffeic acid (max. 132.40 mg/100 g DW), neochlorogenic acid (max. 108.63 mg/100 g DW), chlorogenic acid (max. 64.97 mg/100 g DW), protocatechuic acid (max. 53.81 mg/100 g DW) and gallic acid (max. 52.33 mg/100 g DW). The highest total (451.48 mg/100 g DW) and individual amounts of phenolic acids were found in extracts from the cultures maintained on variant D. This amount was 5.4-times higher than in fruit extracts and 1.4-times lower than in leaf extracts of soil-grown plant.

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THE EFFECT OF SELECTED NATURAL OILS WITH MONO- AND POLYUNSATURATED FATTY ACIDS ON THE GROWTH OF DERMATOPHYTES

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The natural oils (lipids) that consist of fatty acids (FAs) amongst the diverse and potent biological activities possess the antifungal activity [1-3]. The aim of our research work was to screen the antifungal activity of selected natural oils against dermatophytes. The activity of tested oils was detected on the basis of the fungi growth inhibition zone (giz, mm) around the well in the Mueller-Hinton agar medium +2% glucose (the agar well diffusion method). FAs composition, after their conversion to methyl esters (FAMEs), was determined by the GC/MS method. Identification of FAMEs was performed by comparing their retention indices and mass spectra with those of reference standards and NIST spectral library. Species dependent antifungal activity of selected natural oils was reported. The highest activity against dermatophytes was detected in prickly pear oil (giz = 15.3-26.7 mm), while the lowest activity was shown in case of strawberry oil (giz = 7.0-14.0 mm). *Microsporum* spp. growth was inhibited only by blackcurrant oil (giz = 8.0-8.5 mm). No activity against dermatophytes was shown for jojoba oil and linseed oil. Most of the analyzed oils were characterized by the presence of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs). The most characteristic compounds of the oils from prickly pear and curcuma were omega-6 PUFA (mainly LA, 18:2, n-6), as well as monounsaturated fatty acids (MUFAs) belonging to omega-9 group. Oleic acid (18:1, n-9) was the major component. Oils obtained from strawberry and blackcurrant were also characterized by the presence of omega-6 PUFAs. In both oils, over 40% of all acids was linoleic acid (LA). More than 80% of all acids in jojoba oil were omega-9 MUFAs, and among them gondoic acid (20:1, n-9) and erucic acid (22:1, n-9) were the major one. Linseed oil contained mainly omega-3 PUFAs, with α-linolenic (ALA, 18:3, n-3) as the major component. The tested natural oils with MUFAs belonging to omega-9 group, especially with oleic acid, can play a role in protection against fungal colonization and may be regarded as promising ingredients in dermal protection products.

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STRAIN DEPENDENT ACTIVITY OF THE COMPOSITIONS WITH *OENOTHERA BIENNIS* L. SEED OIL AGAINST SKIN ISOLATES OF GRAM-POSITIVE BACTERIA

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The evening primrose (*Oenothera biennis* L.) is mainly known as garden plant, but have also w wide range of medicinal properties. Evening primrose oil is widely used as a dietary supplement from which beneficial effects have been reported in rheumatic and arthritic conditions, and it is also used in skin care products [1, 2]. The aim of our study was evaluation of the effectiveness of emulsions based on evening primrose oil against skin opportunistic pathogens. The research was carried out for four variants of samples containing the cold pressed oil from the seeds of the *O. biennis*, evening primrose oil obtained by supercritical fluid extraction and mixtures containing evening primrose oil, collagen hydrolyzate, colloidal silver and emulsifier. All tested oils and mixtures were screened for *in vitro* antimicrobial activity by agar well diffusion method on the basis of the growth inhibition zone (giz, in mm). Fatty acids (FAs) composition, after their conversion to methyl esters (FAMEs), was determined by the GC/MS method. The most characteristic FAs present in the oils were omega-6 polyunsaturated fatty acids (PUFAs), and among them the major components were linoleic (66%) and γ-linolenic (10%) acids. The data showed the strain- and composition-dependent antibacterial activity of the tested oils and mixtures. The highest activity was detected for the pure oils obtained by SFE and cold pressed. The highest inhibitory effect on the growth of staphylococci was observed for emulsions containing evening primrose oil, collagen hydrolyzate and nanosilver without the emulsifier adding (giz = 5.0-12.2 mm). All tested mixtures showed strong activity against *Dermacoccus nishinomiyaensis* (giz = 6.8–15.0). The presence of evening primrose oil and collagen hydrolyzate as well as colloidal nanosilver in emulsions with potential dermo-protective application, positively influenced the antimicrobial activity against skin bacterial pathogens.

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CHEMICAL COMPOSITION AND ACTIVITY OF POLYPHENOLIC COMPOUNDS FROM AERIAL PARTS OF FIVE *CENTAUREA* L.SPECIES

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The plant materials (blooming herbs) of five Centaurea species: *C. alba* L., *C. aspera* L., *C. calocephala* Willd. *(syn. C. atropurpurea* Waldst et Kit.), *C. decipiens* Theill., *C. solstitialis* L. was collected in June and August 2017, in the Botanical Garden Chair and Dept. of Pharmacognosy with Medicinal Plant Unit, Faculty of Pharmacy, Medical University of Lublin (Poland), and and then dried in the shade and draught and immediately powdered according to accepted normal procedures.
Using the ultrasonic extraction method (UEA) 10 extracts (extraction solvent: 7:3, MeOH: H2O v/v and water) were obtained. Before proper extraction, the plant material in the Soxhlet apparatus was removed from the chlorophyll using chloroform, after drying, the plant material has been quantitatively transferred to the round-bottomed flasks and subjected to further extraction.
The scope of conducted examinations included extraction of polyphenolic compounds from plant material with classical methods, antioxidant activity with the DPPH• radical at the λ wavelength = 515 nm, determining the EC_{50} for each extract. The results obtained were related to the activity of trolox and BHT. and the qualitative and quantitative analysis of flavonoids and phenolic acids. Analytical determinations were analyzed using the Agilent 1100 liquid chromatograph with diode – array detector (DAD) (λ=254, 280, 325 nm), XDB-C8 column (150 x 4.6 mm I.D., dp = 5μm) and gradient of acetonitrile (B) - water + acetic acid (1%) (A) as mobile phase.

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EVALUATION OF EXTRACTION PARAMETERS FOR ISOLATION OF TRITERPENOIDS FROM THE FRUITS OF *MOMORDICA CHARANTIA* L.

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Momordica charantia L., a creeper belonging to the family Cucurbitaceae, is commonly known as bitter gourd or bitter melon. All parts of the plant, including the fruit, taste bitter. *M. charantia* has various medicinal properties. It has been used for hundreds of years in Asia, India, Africa and South America to treat and prevent diabetes-related diseases. It has also anthelmintic, antimalarial, anticancer and antioxidant properties. Compounds responsible for the biological activity are triterpene and steroidal saponins. Phytochemical studies showed also the presence of flavonoids, alkaloids, or polypeptides [1-4].
The major purpose of our study was evaluation of extraction parameters (concentration of alcohol and extraction time) of the Japanese bitter melon fruits dried up in various conditions (freeze and oven drying). The HPLC-DAD and HPLC-ESI-Q-TOF-MS/MS techniques were used for profiling of obtained extracts. Measurement of DPPH radical scavenging activity and cytotoxicity on VERO, FaDu, and HEK293 cell lines were also done. The best results were obtained for extracts from lyophilized plant material performed using 70% MeOH in an ultrasonic bath (3 x 20 min). The most characteristic components present in this extract were cucurbitane-type triterpenoids, and among them charantoside I, momordicosides A, K, M and P were identified. Antioxidant activity expressed as a percentage of inhibition of this extract at a concentration 3 mg/ml was also the highest and amounted 60.6%. The cytotoxicity was tested using MTT assay and expressed as CC_{50} value, which is the concentration decreasing the viability of the cells by 50%. The extract showed varied cytotoxicity on tested cell lines. The lowest toxicity was found in case of VERO (green monkey kidney) cells with CC_{50} of 368,87 µg/ml. In case of normal human cells HEK293 (human embryonic kidney) CC_{50} was 106,8 µg/ml, whereas human cancer cells FaDu (hypopharyngeal squamous cell carcinoma) were more sensitive with CC_{50} of 79,8 µg/ml. Further studies are needed to evaluate the activity of the extract and its constituents on different cancer cell lines.

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PHENOLIC COMPOUNDS OF WILD PLANTS OF *HERNIARIA* GENUS. EVALUATION OF THEIR ANTIOXIDANT ACTIVITY *IN VITRO*.

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Plants of *Herniaria* genus from Caryophyllaceae family are widely distributed, their area extending beyond the limits of one floral region. Rupturewort's extracts have been proven effective against urolithiasis, cholelithiasis, diabetes, hypertension and urinary tract infections. According to the literature, *Herniaria* herbs are the source of triterpenoid saponins, but there is limited data on their phenolic constituents [1].
The aim of the study was the characterization of a phenolic profile of purified extracts of *H. glabra* L. (whole plant), *H. polygama* J.Gay (whole plant), *H. incana* L. (herb and root) widely grown in Ukraine and evaluation of their antioxidant activity *in vitro*.
Phenolic constituents were identified by means of HR-QTOF-MS and NMR techniques (1D and 2D), and compared with literature data. The examined species share common components: licoagroside B, rutin, narcissin, isoquercetrin and nicotiflorin. Apiorutin was detected in *H. glabra* and *H. polygama*. *H. polygama* purified extract is a source of *cis* and *trans* 2-hydroxy-4-methoxycinnamic acid derivatives, herniarin, iridoid derivative and simple organic acids. *H. incana* herb additionally contains isomers of cinnamoyl-, hydroxy-methoxy-cinnamoyl- and coumaroylquinic acids, and also 7-*O*-methylkaempferol derivatives. The main constituents of *Herniaria incana* root are UV- absorbing triterpenoid saponins (>99 % due to the Sephadex LH-20 separation fractions).
Antioxidant actions of the purified extracts (1-50 μg/mL) in human blood plasma were evaluated *in vitro*, under the 100-150 μM peroxynitrite-induced oxidative stress. Our experiments indicated that the examined extracts may significantly diminish oxidative damage of blood plasma components. The extracts partly prevented the $ONOO^-$-induced decrease of the antioxidant capacity of blood plasma and reduced the formation of plasma protein 3-nitrotyrosine (even by about 60-70%). Cytotoxicity assays revealed that all *Herniaria* herb extracts (1-50 μg/mL) were safe for PBMCs, while a preparation from *H. incana* roots was non-toxic only at concentrations of $\leq$ 5 μg/mL.

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EVALUATION OF THE ACTIVITY OF METHANOLIC EXTRACT AND MINOR COMPOUNDS OF *PEUCEDANUM LUXURIANS* FRUITS IN A ZEBRAFISH EPILEPSY MODEL

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Genus Peucedanum belonging to Apiaceae family is rich in coumarins. That group of compounds is known for antiseizure properties. In previous studies furanocoumarins like xanthotoxin, imperatorin, and simple coumarins like osthol or umbelliferone were proved to exhibit anticonvulsant effect in the mouse maximal electroshock seizure threshold model [1,2]. Additionally, their acute adverse-effect (neurotoxic) determined in the chimney test showed, that coumarins are good candidates in preclinical study [2]. For these reasons we decided to evaluate the activity of furanocoumarin isolated from Peucedanum luxurians together with the activity of crude extract of this plants.

To isolate pure minor compound of P. luxurians High Performance Countercurrent Chromatography was used. This technique is using immiscible mixtures of different solvents and centrifugal force to held liquid stationary phase [3]. Application HEMWat solvent system in ratio v/v 6:5:6:5 allowed to achieve efficient isolation of officinalin isobutyrate form P. luxurians. The compound and extract were an subject of anticonvulsant evaluation.

Zebrafish epilepsy model is based on the GABAA antagonist pentylenetetrazol (PTZ), which causes zebrafish larvae to exhibit increased locomotor activity, seizure-like behavior, and epileptiform electrographic activity [4]. The locomotor activity of zebrafish larvae was measured for 30 minutes in a dark chamber of an automated tracking device (ZebraBox TM apparatus; Viewpoint, Lyon, France) after overnight incubation. Then 40 mM concentraion of PTZ was added and measurement was repeated. The total locomotor activity was quantified using ZebraLab TM software (Viewpoint, Lyon, France). Officinalin isobutrate exhibited antiseizure activity at dose 150μM, inhibiting 40% of PTZ-induced seizures.

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HPLC-ESI-Q-TOF-MS ANALYSIS OF POLYPHENOLIC CONSTITUENTS PRESENT IN SELECTED *MARRUBIUM* SPECIES

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The *Marrubium* L. genus belongs to the family Lamiacae and comprises about 97 species reported for their medicinal properties. Selected *Marrubium* taxa are widely known in traditional and modern medicine as they are used as choleretic, digestive, anti-inflammatory, antihypertensive, antispasmodic, analgetic, antimicrobial, insecticidal, even antileukemic agents and cytotoxic/cytostatic effects against four human cancer cell lines, specifically HeLa, MCF-7, FM3 and HCT-116. [1-5].
The aim of the present studies was the qualitative and quantitative assessment of a polyphenolic fraction present in the methanolic extracts obtained from the flowering herbs of *Marrubium incanum* Desr., *M. peregrinum* L., *M. thessalum* Boiss. & Heldr., and *M. candidissimum* L. The samples containing free phenolic acids, as well as acids released after acid and alkaline hydrolysis, were investigated by LC-MS. The purified samples were analyzed in negative ion mode using a 6530B accurate-mass-QTOF-MS mass spectrometer with an ESI-Jet stream ion source (Agilent Technologies, Inc., Santa Clara, CA, USA).
For the first time, the qualitative and quantitative analysis of phenolic acids (FAs) present in the flowering herbs of *M. incanum*, *M. candidissimum*, *M. thessalum* and *M. peregrinum* was carried out. By use of the HPLC/ESI-Q-TOF-MS technique eight FAs, namely protocatechuic, *p*-hydroxybenzoic, ferulic, *p*-coumaric, caffeic, gentisic, neochlorogenic, chlorogenic, and five flavonoids namely rutin, kaempherol rhamnohexoside, kaempherol hexoside, isorhamnetin hexoside, apigenin were identified in aerial parts of all *Marrubium* species examined.

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ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACTS FROM INFLORESCENCES OF SELECTED *CIRSIUM* SP.

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In this study, an in vitro antioxidant activity, total phenolic content and the total concentration of flavonoids in 70% (v/v) methanolic extracts from the inflorescences of *C. monspessulanum, C. trachylepsis, C. spinosissimum*, *C. flavispina* were determined using spectrophotometric methods. The antioxidant activity of extracts was expressed as IC_{50} values (mg/ml).

Methanolic extract from of *Cirsium sp*. showed the highest phenolic and flavonoid concentration and the strongest antioxidant activity. The IC_{50} values of the extracts obtained from *C. monspessulanum, C. trachylepsis, C. spinosissimum* and *C. flavispina* were 0.229, 0.223, 0.194, and 0.245 mg/ml, respectively.

The % inhibition of DPPH radicals by the examined extracts were compared with gallic, chlorogenic acid and trolox for which the IC_{50} values where 0.085, 0.453, and 0.302 mg/ml, respectively.

The contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity. The results suggest that the inflorescences of *Cirsium sp*. may serve as an economical and effective source of natural antioxidants.

The identification of active phenolic compounds was performed by means of TLC and HPLC in relation to the reference compounds.

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ENZYME-ASSISTED SUPERCRITICAL FLUID EXTRACTION AS EFFICIENT METHODS OF BIOLOGICAL ACTIVE COMPOUNDS ISOLATION

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The aim of this study was to develop an effective *Medicago sativa* extraction method using enzyme-assisted supercritical fluid extraction.
The response surface methodology (RSM) based on Box-Behnken design was used to define the optimal parameters of extraction (temperature, pressure, % added of co-solvent) for maximum yield of phenolic compounds and antioxidant activity of leaves extract. *M. sativa* leaves were treated with commercial enzyme preparation (kemzyme). Extraction of antioxidant phenolics in optimal conditions from the enzymatically hydrolysed *M. sativa* leaves was carried out by supercritical carbon dioxide (Sc-CO_2) with 96% ethanol as a co-solvent. Qualitative and quantitative HPLC-MS/MS analysis allowed to evaluate the content of individual biological active compounds in obtained extracts.
The experimental results were fitted to a second-order quadratic polynomial model, and they have shown a good fit with the proposed models for the total phenolics content (TPC) (R^2=0.99) and for the antioxidant extraction assayed by DPPH method (R^2=0.99). Obtained optimal extraction parameters for TPC and antioxidant activity were: temperature of 68°C, pressure of 205 bar and 15.5% of co-solvent addition and values of responses of these conditions were 80.3 ± 1.9 mg GAE/g DM and 50.7 ± 0.7 µmol TEAC/g DM, respectively. Then, Sc-CO_2 extractions carried out with untreated (control) and enzymes-digested leaves were compared. The using of commercial enzyme formulation allowed to increase content of phenolics compounds in the leaves extract compared to the control. The total content of phenolics compounds in extracts obtained after enzyme digestion was much higher (142.6 µg/g) than in extracts obtained from non-digested material (97.6 µg/g).
Enzyme-assisted supercritical fluid extraction is an effective, efficient and eco-friendly green method to extract of high-values phenolics and other antioxidants from *M. sativa.* In addition, this study provides constructive information for further investigation different enzyme formulation to improve extraction process.

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GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *MEDICAGO SATIVA* L. EXTRACT

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Zinc oxide (ZnO) has attracted great interest worldwide and is one of the most interesting nanomaterials. A wide band gap, high luminescent efficiency and a large exciton binding energy has triggered intense research on the production of nanoparticles (ZnO NPs) using different synthesis methods [1]. Currently, the development of green chemistry seems to be a scheme generating much interest. Synthesis involving the use of biological systems provides many advantages in comparison to traditional chemical methods, such as not-using toxic and expensive organic solvents, being environmental friendly and allowing to control the NPs size and shape [2]. Within the last years, an increasing number of studies have been focused on the use of plant extracts to obtain metal oxide NPs. Plant extracts consist of a many biocompounds such as e.g. flavonoids and polyphenols which can act both as reducing and capping agents in the process of NPs synthesis [3]. One of the plant, which can be easily adapted to the ZnO NPs formation, is *Medicago sativa* L. Recent studies have demonstrated that lucerne is rich in essential aminoacids, vitamins and secondary metabolites e. g. flavonoids and phenolic compounds [4]. The high content of listed compounds make it a good source of bioactive compounds which can play a crucial role in ZnO NPs biosynthesis. The aim of work was to synthetize ZnO NPs from *Medicago sativa* L. extract by ecofriendly, inexpensive and simple method. Formation of nanoparticles have been confirmed by a wide range of instrumental methods such as UV-VIS spectroscopy, X-ray diffraction, FT-IR spectroscopy and microscopy approach (SEM/EDX and TEM). Additionally, spectrofluorometric analysis and zeta potential and size measurement were performed. XRD data characterized final product as a highly crystalline and hexagonal ZnO. Results of SEM and EDX indicated the formation of nanoparticles and chemical composition of zinc oxide were confirmed. Moreover, phytochemical characterization of *M. sativa* extract was performed and the proposed mechanism of ZnO NPs formation was described.

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LC-DAD ANALYSIS OF VERBASCOSIDE AND ISOVERBASCOSIDE IN *VERBENA OFFICINALIS L. IN VITRO* CULTURES GROWN IN TWO TYPES OF BIOREACTORS

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Verbena officinais L. – common vervain is a plant species with many valuable medicinal activities eg. antioxidant, anti-inflammatory, antibacterial and secretolytic. For the pharmacological properties are responsible different polyphenolic compounds especially iridoids and phenylpropanoid glycosides like verbascoside and isoverbascoside [1].
The experimental *in vitro* cultures were maintained on Murashige and Skoog medium [2] with 1mg/l 6-benzylaminopurine and 1mg/l indole-3-butyric acid as suspension cultures in two types of bioreactors: balloon bioreactor and stirred-tank bioreactor. The cultures were maintained under artificial light 17/24 h and the biomasses were harvested after 2 weeks growth cycles (3 series). The qualitative and quantitative analysis of verbascoside and isoverbascoside were performed in methanolic extracts by LC-DAD method [3].
The quantities of accumulated compounds were varied and depended on the type of bioreactor. The highest amounts of verbascoside (9.18 g/100g dw) and isoverbascoside (2.95 g/100g dw) were obtained in biomas from stirred-tank bioreactor. The amounts of investigated compounds in extract from biomass from balloon bioreactor were lower (7.68 and 2.25 g/100g dw respectively).
Using LC-DAD method with gradient elution it was possible to separate both isomeric compounds (verbascoside 12.5 min, isoverbascoside 15.5 min). The applied LC-DAD method was proved to be good for identification and quantification both phenylpropanoid glycosides.

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VALIDATION OF LC-DAD METHOD FOR EFFECTIVE SEPARATION OF IRIDOIDS: HASTATOSIDE, VERBENALIN AND PHENYLPROPANOID GLYCOSIDES: VERBASCOSIDE, ISOVERBASCOSIDE IN *VERBENA OFFICINALIS* L. HERB EXTRACTS

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The LC-DAD method acc. to Schönbichler et al. [1] was applied for phytochemical qualitative and quantitative analyses of methanolic extracts of *Verbena officinais* L. herb. Under the study four standards: hastatoside, verbenalin (PhytoLab), verbascoside and isoverbascoside were used (ChromaDex).
Separation was performed using a Kinetex C-18 analytical column on Hitachi LaChrom Elite LC system with gradient program. Detection wavelength was set at 240 nm (hastatoside, verbenalin,) and 330 nm (verbascoside, isoverbascoside). Validation of the method was performed by determination of accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ) [2].
The proposed LC method has been characterized by high sensitivity; LOD for hastatoside was 0.0272 mg/mL, 0.0114 mg/mL for verbenalin, 0.0172 mg/mL for verbascoside and 0.0189 mg/mL for isoverbascoside. LOQ values were estimated at 0.0826 mg/mL, 0.0346 mg/mL, 0.0520 mg/mL, and 0.0573 mg/mL, respectively. Percentage recovery of the studied compounds presented as mean values for three concentration levels is high and ranges from 96.43% to 103.37%. Satisfactory precision determined for three concentration levels is confirmed by the values of variability coefficients RSD which are in the range from 0.21% to 2.56%. Linearity of the tested substances was preserved in a wide range: from 0.1500 mg/mL to 0.4500 mg/mL for hastatoside and in the following concentration ranges: trom 0.0625 mg/mL to 0.5000 mg/mL for other substances.
In *V. officinalis* herb extracts estimated amounts of studied metabolites were equal: hastatoside – 0.47 g/100 g dw, verbenalin - 4.02 g/100 g dw, verbascoside - 1.88 g/100 g dw and isoverbascoside - 0.28 g/100 g dw.
The applied LC-DAD method has been effective for separation and quantification of investigated compounds.

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ANTIPROLIFERATIVE EFFECT OF *BERBERIS SIBIRICA* PALL. EXTRACT AND ITS SELECTED ACTIVE CONSTITUENTS AGAINST TRIPLE NEGATIVE BREAST CANCER CELL LINES

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Triple negative breast cancer (TNBC) is the least common form of breast cancer and is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [1]. Due to its aggressive behaviour and the lack of effective specific targeted therapy, successful treatment of TNBC still remains a challenge. Therefore, the identification of novel treatment options is needed. In recent years, the use of herbal medicines has attracted increasing attention due to their varied bioactive components as potential agents in the prevention and cancer treatment. Thus, we investigated the potential anticancer activities of *Berberis sibirica* Pall. extract and its selected active constituents on TNBC. *Berberis sibirica* Pall. (Berberidaceae) is a shrub used in traditional Mongolian medicine as a cholagogue, anti-inflammtory and antipyretic remedy. Its extracts are rich sources of isoquinoline alkaloids (QA's), such as berberine, palmatine, magnoflorine and jatrorrhizine.
The development of a fast method suitable for the purification of QA's was performed by hydrostatic counter-current chromatography (CPC). pH-zone refininig CPC operation mode was used and the application of a solvent system composed of MtBE: H_2O (1:1 v/v) with 10 mM TEA and 10 mM HCl provided fractions containing alkaloids [2]. The viability and proliferation of triple negative breast cancer cells were analyzed by MTT test and BrdU Cell Proliferation Assay, respectively.
Total extract from *Berberis sibirica* and selected its constituents reduced the viability of analyzed human triple negative breast cancer cell lines in a dose-dependent manner. The reduction in cell numbers, represented as viability, was attributed to decreased cell proliferation, as demonstrated by BrdU incorporation assay.
Our findings indicate that protoberberine-type alkaloids might be promising chemotherapeutic candidates for the treatment of TNBC

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THE QUALITY ASSESSMENT OF *ZINGIBER OFFICINALE* EXTRACTS

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Ginger is now one of the most important and popular spices on the international market and the total global production of ginger is estimated at 100,000 tons a year. All over the world, the ginger rhizome is appreciated for its taste qualities; therefore, it is used as a spice, flavoring agent, as an additive in the preparation of meals and dietary supplements, syrups and pills. The ginger rhizome or its extracts have been commonly used in medicine, because of their wide scope of biological effects—confirmed both in various *in vitro* models and in clinical trials.

The aim of the study was to determine the major constituents of ginger extracts of different kinds obtained from Japanese rhizomes from ecological plantations. First, the composition of its volatile components was performed. For this purpose supercritical fluid extracts (CO_2 extracts) were analyzed and compared with diethyl ether extracts in a GC-MS based tailored method. Sesquiterpenes contributed to the composition of ginger volatile fraction mostly. Among them α-zingiberene, β-sesquiphellandrene, (E,E)-α-farnesene, geranial, and *ar*-curcumene were determined as major components.

Also, a thorough study of ginger's phenolic composition and an elemental content analysis were performed by LC-ESI-Q-TOF-MS and AAS spectrometry, revealing the leading content of 6-gingerol (268.3 mg/kg) and potassium (43.963 mg/kg of dry mass) in the studied samples.

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STUDIES ON OXIDATION OF AMARANTHIN DERIVED FROM INFLORESCENCES OF RED *GOMPHRENA GLOBOSA* L. CULTIVARS

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Gomphrena globosa L. is an edible and commercially available ornamental plant commonly known as globe amaranth or bachelor button that belongs to the family Amaranthaceae. Due to betalain pigments content, its inflorescences exhibit different coloration including red, pink, purple and white.[1] Red-colored plants in the family Amaranthaceae are recognized as a rich source of diverse and unique betacyanins which represent one of the most important natural plant pigment classes. The dominant compounds in red cultivars of *Gomphrena globosa* L. are: amaranthin (betanidin-5-*O*-*β*-glucuronosylglucoside) and its diastereomer isoamaranthin (isobetanidin-5-*O*-*β*-glucuronosylglucoside). The plant is also a source of gomphrenin/isogomphrenin (betanidin/isobetanidin-6-*O*-*β*-glucoside) as well as betanin/isobetanin (betanidin/isobetanidin-5-*O*-*β*-glucoside) and their acylated derivatives such a betanidin 5-*O*-*β*-glucuronosylglucoside or betanidin 6-*O*-*β*-glucoside acylated with ferulic, p-coumaric, or 3-hydroxy-3-methylglutaric acids. [2,3]
The betacyanin pigments were extracted from inflorescences of red *Gomphrena globosa* L. cultivars and submitted to purification by preparative high performance liquid chromatography with the aim of amaranthin isolation. Non-enzymatic oxidation of amaranthin in the presence of ABTS cation radicals was investigated by LC-DAD-ESI-MS/MS. Spectrophotometric monitoring of oxidation kinetics was also performed. The effect of the reaction medium and the concentration of oxidant on the reaction course was studied. The oxidation reactions were performed at pH ranging from 3 to 8. The highest oxidation activity during spectrophotometric studies was observed at pH 3. The chromatographic traces of the tested mixtures generated during the pigment oxidation were registered. The presence of prominent oxidation products, tentatively identified as: 2-decarboxy-2,3-dehydroamaranthin, 2,17-bidecarboxy-2,3-dehydroamaranthin and 2,17-bidecarboxy-neoamaranthin was reported mostly at pH 3, 4 and 6. 2,17-bidecarboxy-2,3-dehydroamaranthin was detected in the whole pH range while the presence of 2-decarboxy-2,3-dehydroneoamaranthin, the most hydrophobic reaction product, was observed only at pH 3. The scheme of possible amaranthin oxidation paths is proposed.

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HPLC-DAD-ESI-Q-TOF-MS PROFILING OF *VERBASCUM BLATTARIA* L. AND SEPARATION OF THREE ACYLATED IRIDOID DIGLYCOSIDES BY HIGH-PERFORMANCE COUNTER-CURRENT CHROMATOGRAPHY

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Verbascum blattaria L. (moth-mullein) is a biennial plant of Scrophulariaceae family that grows spontaneously on pastures, meadows and open woods of Europe, Asia, North Africa and North America [1]. Previously, several iridoid monoglycosides and flavonoids have been identified in various extracts of this species [2, 3].
In order to perform a targeted isolation, the initial objective of this study was to perform the qualitative HPLC-DAD-ESI-Q-TOF-MS investigation of extracts obtained from the aerial parts of *V. blattaria* with different polarity solvents (*n*-hexane, dichloromethane, ethyl acetate, methanol and water). Among these, the methanolic extract showed a high abundance of mono-, di- and tri-acyl catalpol-type iridoid diglycosides. Other minor constituents were identified, such as: phenylethanoid glycosides (verbascoside, angoroside C), flavonoids (kaempferol glucoside glucuronide, diosmetin rhamnoside glucoside), phenolic acids (caffeic acid glucoside, *p*-coumaroyl quinic acid) and triterpene saponins (ilwensisaponins A and C, songarosaponins A and B, buddlejasaponin I).
Subsequently, the isolation of three iridoid diglycosides from the methanolic extract of *V. blattaria* by high-performance counter-current chromatography (HPCCC) was targeted. *p*-Coumaroyl 6-*O*-rhamnopyranosylcatalpol (2 mg) and acetyl *p*-coumaroyl 6-*O*-rhamnopyranosylcatalpol (16 mg) were obtained from 300 mg of crude extract with the help of *n*-hexane-ethyl acetate-*n*-butanol-water (1:2:1:2), at a flow-rate of 4.2 mL/min and 1600 rpm. Additionally, acetyl cinnamoyl 6-*O*-rhamnopyranosylcatalpol (13 mg) was separated by HPCCC with *n*-hexane-ethyl acetate-methanol-water (1:19:1:19), at a flow-rate of 6 mL/min and 1600 rpm. None of these compounds have been previously isolated from *V. blattaria*.

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SELECTIVE CYTOTOXICITY OF VERBASCOSIDE ISOLATED FROM *VERBASCUM OVALIFOLIUM* BY HIGH-PERFORMANCE COUNTER-CURRENT CHROMATOGRAPHY

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Verbascoside (also known as acteoside or kusaginin) is among the most spread phenylethanoid glycosides, being detected in more than 200 species belonging to 23 plant families [1]. Reportedly, verbascoside exerts a wide spectrum of biological activities, including antioxidant, anticholinesterasic, anti-inflammatory, antibacterial and cytotoxic properties [2]. Moreover, it is acknowledged that *Verbascum* genus could serve as an attractive source of verbascoside, as it contains high concentrations of this secondary metabolite [3]. *Verbascum ovalifolium* Donn ex Sims (oval-leaved mullein) is a biennial plant of Scrophulariaceae family, predominantly distributed in Bulgaria, Russia, Greece, Turkey and Romania [4]. The aim of this study was to develop a high-performance counter-current chromatography (HPCCC) method for the efficient isolation of verbascoside from *V. ovalifolium* and to investigate its cytotoxic potential on tumor and non-tumor cells by MTT and fluorescence-activated cell sorting (FACS) assays. The ethyl acetate liquid-liquid partitioning fraction of the crude methanolic extract obtained from the aerial parts of *V. ovalifolium* was initially subjected to flash liquid chromatography over silica gel. The chloroform-methanol (7:3) subfraction was directly injected into the HPCCC system and separated with *n*-hexane-ethyl acetate-*n*-butanol-water (4:10:5:10) at a flow-rate of 4.2 mL/min and 1600 rpm. Verbascoside (7 mg, 98.0% purity) was obtained in less than 35 min from 300 mg of sample.
A 48 h treatment of cells with verbascoside (100 μg/mL) showed a cytotoxicity of 38.54%, 42.17% and 55.39% in A549, MCF-7 and HT-29 tumor cells, respectively, and no significant reduction of non-tumor MCF-10A cell viability. FACS analysis revealed that verbascoside induced apoptosis in A549 cells and led to accumulation of cell population in the sub-G1 phase (11.2%) as compared to control (1.3%).

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THE EFFECT OF *TRITICUM AESTIVUM* GERM OIL ON THE GROWTH OF GRAM-POSITIVE BACTERIA ISOLATED FROM SKIN MICROBIOTA

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Triticum aestivum L. (wheat) germ is widely recognized as a nutritious raw material. Typical applications are in germ-enriched bread, snack foods, and supplements to breakfast cereals, and for production of wheat germ oil. Oil is used for medicinal and therapeutic purposes due to the rich supply of omega-3 and omega-6 fatty acids, vitamins and a lot of other nutrients and antioxidants [1]. It can be also good remedy for eczema, psoriasis and other common skin conditions [2, 3]. The influence of wheat-germ and various mixtures based on this oil on the growth of bacteria isolated from skin microbiota was studied. The mixtures for study, in addition to wheat germ oil, were additionally composed of hydrolyzate of calf collagen, colloidal silver and cetearyl alcohol. The activity was tested by agar well diffusion method on the basis of the bacteria growth inhibition zone (in mm). Fatty acids (FAs) composition, after their conversion to methyl esters (FAMEs), was determined by use of GC/MS method. The major components present in the wheat germ oil were linoleic (LA, 18:2, n-6), oleic (18:1, n-9), and α-linolenic (ALA, 18:3, n-3) acids. The relative percentage of these acids in the examined oil was 72% of all detected compounds. The species and strain-dependent, as well as composition dependent antibacterial activity of tested oil and mixtures was shown. The highest antibacterial activity was observed for the mixture composed of wheat germ oil and hydrolyzate of calf collagen without colloidal silver and cetearyl alcohol. The most sensitive strains were *Micrococcus luteus*, *Dermacoccus nishinomiyaensis* and *Leuconostoc mesenteroides* spp. *cremonium* with the growth inhibition zones between 10 and 16 mm. In conclusion, the occurrence of wheat germ oils and hydrolyzate of collagen in mixtures positively influenced the antimicrobial activity against bacteria isolated from skin microbiota and have potential dermoprotective application.

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OPTIMIZATION OF ULTRASONIC-ASSISTED EXTRACTION OF TOTAL PHENOLIC COMPOUNDS FROM *HYSSOPUS OFFICINALIS* L. BY FRACTIONAL FACTORIAL DESIGN AND ANTIMICROBIAL ACTIVITY

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Hyssop (*Hyssopus officinalis* L.) is a plant with small cultivation requirements. Formerly, hyssop was used in traditional medicine as an antispasmodic, antifungal and antitussive drug. The hyssop revealed the presence of flavonoids and polyphenols, which had an effect on its antioxidant properties [1].

The aim of the presented research was to optimize the conditions of ultrasonic extraction to obtain the maximum content of polyphenolic compounds and determined *in vitro* antimicrobial activity. Extraction of polyphenols was carried out according to the procedure described in Polish Pharmacopoeia XI [2]. Optimization of extraction conditions: time, solvent / raw material ratio and ethanol concentration, carried out using Fractional Factorial Design. The statistical analysis performed for the extracts obtained under different extraction conditions showed that the best parameters were: time 50 min., 30 mL/g solvent/raw material ratio and 50% ethanol as extraction solvent. The graphical dependence of the TPC content on the time of ultrasonic-assisted extraction and solvent concentration is shown in Figure 1.

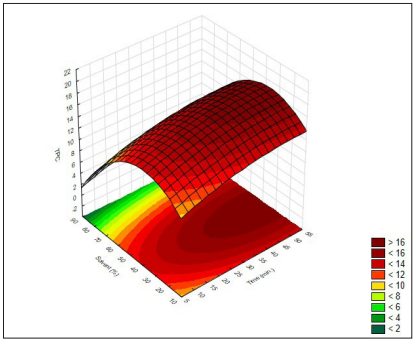

Fig. 1. Response surface graph interaction between extraction time and solvent concentration on content of TPC.

The examined extracts were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines against a panel of reference microorganisms. The values of minimal inhibitory concentration (MIC) and minimal bactericidal/fungicidal concentration (MBC/MFC) were examined using their two-fold dilutions in Mueller-Hinton broth (for bacteria) and RPMI 1640 broth with MOPS (for fungi) prepared in 96-well polystyrene plates. The MBC/MIC or MFC/MIC ratios were calculated in order to determine bactericidal/fungicidal or bacteriostatic/fungistatic effect of the tested extracts.

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EFFICIENT ISOLATION OF PUERARIN AND RELATED ISOFLAVONE COMPOUNDS FROM KUDZU ROOT USING CENTRIFUGAL PARTITION CHROMATOGRAPHY

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Puerarin (daidzein 8-*C*-glycoside) was firstly isolated in the late 1950s from the root of *Pueraria lobata* (Willd.) Ohwi., commonly known as kudzu. It is one of the most popular Chinese herbs used for centuries in a traditional medicine in various cardiovascular and neurodegenerative disorders. Furthermore, kudzu isoflavones, with predominant activity of puerarin, are known as hypoglycemic (attenuating insulin resistance), anti-osteoporotic and anti-inflammatory agents well documented in scientific literature. Some beneficial effects of puerarin on human health have been also reported in alcohol abuse and liver injuries [1].

In order to separate and purify puerarin and related constituents from *P. lobata* root of Chinese origin, the centrifugal partition chromatography (CPC), also known as hydrostatic counter-current chromatography (HSCCC), was performed as the pre-treatment method. Being a unique type of chromatography, this preparative technique is based on the partitioning of constituents between two non-miscible liquid phases. Compared to traditional liquid-liquid extraction, CPC is proved to be a more efficient and less time-consuming separation method. Additionally, the lack of solid stationary phase eliminates irreversible adsorption of separated compounds. To date, HSCCC was successfully employed for the isolation and purifying a wide range of plant phenolics, including isoflavones [2,3]. In our study, various solvent systems composed of the mixture of ethyl acetate, alcoholic and acidic modifiers were optimized in the gradient mode. The obtained, enriched fractions of isoflavone compounds were further subjected to high-performance liquid chromatography coupled with photodiode-array detection and mass spectrometry (LC/PDA–ESI–QTOF-MS/MS) in order to examine in detail the chemical structure of isolated compounds. The presence of six daidzein glycosides (3'-hydroxypuerarin, puerarin, mirificin, 3'-methoxypuerarin, daidzin and 3'-methoxydaidzin) was confirmed.

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EFFECTS OF *SORBUS AUCUPARIA* FLOWER EXTRACTS ON *IN VIVO*-RELEVANT OXIDANTS AND OXIDATIVE/NITRATIVE DAMAGE OF HUMAN PLASMA COMPONENTS

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Sorbus aucuparia L. (rowan, European mountain ash) is a wild rosaceous tree occurring and cultivated across Europe and Asia. The edible fruits, flowers and leaves of the plant are traditionally used for their diuretic, anti-diabetic, anti-inflammatory, anti-atherogenic, vasoprotective, and vasorelaxant properties. The beneficial health effects of rowan are commonly linked with antioxidant activity of its polyphenolic constituents forming unique profiles in particular organs, among which flowers are the least characterised, both in terms of the chemical composition and biological activity. The accumulating research indicated all rowan tissues as strong antioxidants, and the flowers as exhibiting the highest total phenolic content and superior activity parameters [1-3]. However, all activity results have been obtained in simple chemical tests lacking *in-vivo* relevancy.
In this study, the antioxidant effects of the flower extracts of *S. aucuparia* were verified *in vitro* towards the most common *in vivo*-relevant oxidants ($O_2^{\bullet -}$, HO•, NO•, H_2O_2, $ONOO^-$, HClO) and in the model of human plasma exposed to oxidative/nitrative stress generated by $ONOO^-$. All activity assays were performed for extracts standardised by comprehensive phytochemical profiling (UHPLC-PDA-ESI-MS^3, HPLC-PDA, UV-spectrophotometry). In all tests the extracts effects were dose-dependent. As compared to positive controls, the strongest and phenolic-dependent effects were found towards $O_2^{\bullet -}$ and HO• and against nitration of the plasma proteins (formation of 3-nitrotyrosine). At *in vivo*-relevant levels (1-5 μg/ml), the extracts reduced also oxidation of the plasma lipids (formation of hydroperoxides and TBARS) and normalised/enhanced the total antioxidant capacity of plasma (FRAP). The methanol-water (7:3, v/v) extract and its most active fractions were proved rich in polyphenols (221.9-597.6 mg/g dw, 59 analytes), especially caffeoylquinic acids (isomers of chlorogenic acid and cynarin), flavan-3-ols and flavonol glycosides, being thus the most advantageous for future applications and *in vivo* studies.

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IN VITRO EVALUATION OF THE ANTIOXIDANT ACTIVITY OF *PRUNUS SPINOSA* FLOWER EXTRACTS TOWARDS THE MOST COMMON *IN VIVO*-RELEVANT OXIDANTS

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Flowers of *Prunus spinosa* L. due to their vasoprotective, anti-inflammatory, diuretic, detoxifying (blood purifying), and spasmolytic properties have been used as ingredients of compound herbal prescriptions traditionally applied, e.g. to treat intestinal and respiratory tract disorders, but also various cardiac complaints, such as myocarditis, cardiac neurosis and atherosclerosis. The latest studies of our team proved that the antioxidant capacity of *P. spinosa* flower extracts might be counted as one of the mechanisms behind the activity reported by traditional medicine. Not only did the extracts show significant antioxidant effects in chemical models (DPPH, FRAP, TBARS), but they also, at *in vivo*-relevant levels, effectively protected the human plasma proteins and lipids against $ONOO^-$-induced damage and enhanced the total antioxidant status of plasma [1]. Nevertheless, since the antioxidant capacity of *P. spinosa* flower extracts towards reactive oxygen/nitrogen species considered as one of the most important destructive factors etiologically connected with numerous chronic diseases, has not been studied yet, detailed investigations are required.

Therefore, the studies of antioxidant activity of *P. spinosa* flower extracts towards the most common *in vivo*-relevant oxidants ($O_2^{\bullet -}$, HO•, NO•, H_2O_2, $ONOO^-$, HClO) were carried out using spectrophotometric and fluorometric methods acc. to previously optimised procedures. To indicate the main determinants of the tested activity and to approximate the effects expected *in vivo* analogical studies have been conducted for model polyphenols (selected based on the chemical composition of the dry extracts) and compounds considered to be the main polyphenolic metabolites in human body. The results from the analysis showed significant and dose-dependent antioxidant activity of all of the extracts. Regardless of the test, activity of the extracts decreased in similar order, i.e., ethyl acetate fraction ≥ diethyl ether fraction > *n*-butanol fraction > defatted methanol extract > water residue. In a comparison to the positive standards, the antioxidant capacity of the most active extracts, in some tests was higher ($p < 0.05$) than ascorbic acid and Trolox. Moreover, noticeable antioxidant capacity was observed for potential *in vivo* metabolites of the polyphenolic constituents of the extracts.

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PRUNUS SPINOSA FLOWER EXTRACTS AS THROMBIN INHIBITORS – AN *IN VITRO* STUDY

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Blackthorn flower (*Pruni spinosae flos*) is a valued traditional plant remedy from Central and Eastern Europe indicated i.a. for adjunctive therapy of cardiovascular diseases (CVD). Distinct phenolic profile, significant antioxidant activity in both chemical and biological models, and noticeable inhibitory effects on pro-inflammatory enzymes demonstrated in the latest study of our team, seem to confirm the traditional application of *P. spinosa* flowers in CVD [1]. However, the data considering the bioactivity of the plant material is still insufficient, e.g. the influence of blackthorn flowers on haemostasis has not been studied yet.
Haemostasis – physiological process defined as a balance between blood coagulation and fibrinolysis – is maintained by the cooperation of various components i.a. plasma coagulation factors, especially thrombin (central enzyme of the coagulation cascade). The critical role of thrombin in the haemostatic disorders observed in CVD, makes the inhibition of its function a good strategy for prevention of cardiovascular events, often utilised in medical practice and in the search for novel therapies. Interestingly, direct inhibitory effect on thrombin activity has been demonstrated *in vitro* for some natural compounds, e.g. quercetin and their glucosides: quercetin-3-*O*-arabinoside, quercetin 3-*O*-rhamnoside (flavonols occurring also in blackthorn flowers) [2].
Therefore, the aim of the study was *in vitro* evaluation of the inhibitory activity of *P. spinosa* flower extracts on enzymatic properties of thrombin. The results showed high anti-thrombotic properties of all the extracts except water residue. For the most active ethyl acetate and diethyl ether fractions the efficacy of the inhibition of amidolytic activity of thrombin (measured as a hydrolysis of the chromogenic substrate S-2238, Chromogenix, with the use of a kinetic method) at *in vivo*-relevant level of 5 µg/mL attained 58.9 ± 4.3% and 57.0 ± 4.4%, respectively ($p < 0.001$). Furthermore, the extracts were able to reduce the proteolytic activity of thrombin (measurements of fibrinogen polymerization using turbidimetric method, both in suspensions of the isolated fibrinogen and in blood plasma).

Acknowledgements: This work was supported by the Medical University of Lodz, grant No 503/3-022-01/503-31-001, 502-03/3-022-01/502-34-082, and University of Lodz, grant No 506/1136.

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POLYPHENOL CONTENT IN AGASTACHE RUGOSA SHOOT IN VITRO CULTURES GROWN UNDER DIFFERENT ILLUMINATION AND SUPPLEMENTED WITH AMINO ACIDS

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Agastache rugosa (Fischer & C.A.Meyer) O.Kuntze (*Lamiaceae*) is an East Asian medicinal and aromatic plant. It is rich in polyphenolic compounds such as rosmarinic, chlorogenic, ferulic acids and apigenin glycosides. *In vitro* shoot cultures were used to study influence of various factors on polyphenol profile using Ultra Performance Liquid Chromatography coupled to high resolution mass spectrometry (UPLC-ESI-qTOF-MS). Large differences in the morphology and polyphenol profile were observed in experiments with various illumination (white fluorescent lamps withe and photosynthetically active radiation LEDs) and supplementation with plant growth regulators and amino acids. Shoots were cultured on the MS basal agar medium with or without plant growth regulators (6-benzylaminopurine - BA, indole-3-acetic acid – IAA), or supplemented with different concentrations of phenylpropanoid biosynthesis precursor - L-phenylalanine or an amino acid mixture (casein hydrolysate). The composition of polyphenols in methanolic extracts was analyzed using UPLC-DAD-qTOF-MS. The identification was based on retention times (t_R), absorption spectra, (quasi)molecular ions ([M –H]-) and fragments in negative mode. Three phenolic acids: chlorogenic acid (*m/z* 353.069), p-coumaroyl-quinic acid (*m/z* 337.090), rosmarinic acid (*m/z* 359.013), and a rosmarinic acid derivative (*m/z* 373.028), as well as one flavonoid – apigenin (*m/z* 269.071) and its derivative (*m/z* 717.058) were detected. Rosmarinic acid (RA) was the most abundant compound found in the analyzed plant material. Supplementation with amino acids resulted in higher content of RA in shoots cultured for at least 6 months on media containing either low concentration (1 mg/l) of L-phenylalanine or two of the highest - 20, 50 mg/L. The effect of casein hydrolysate supplementation was stronger at the beginning of shoot culture development. However, this did not contribute to the increased rosmarinic acid production (5 mg/g d.w.).On the other hand, shoots that were growing under different illumination regime produced over 20 mg/g d.w. of RA after 70 days of culture.

In conclusion, the production of phenolic compounds in *A. rugosa in vitro* shoots was both related to the age of the shoot cultures and to the influence of light and amino acids supplementation.

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PHYTOCHEMICAL DIVERSITY OF INVASIVE *FALLOPIA* SPECIES AND THEIR BIOACTIVITY CORRELATIONS ELUCIDATED BY LC-MS BASED TARGETED METABOLOMICS

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In China and Japan, the rhizomes of *Fallopia* (syn. *Reynoutria*) *japonica* (*Polygoni cuspidati rhizoma*) are used for treating inflammation, jaundice, skin burns, scald and hyperlipidemia. In 19th century, this East-Asian species was introduced to Europe and North America together with a morphologically similar but not used in TCM - *F. sachalinensis*. In Europe, crossing between these two species produced a hybrid- *F.* x *bohemica*. In 2017, *F. japonica* has been included in European Pharmacopoeia as one of the TCM herbs. All species are closely related and similar morphologicaly. *F. japonica* is considered a richest natural source of resveratrol but the phytochemical composition of two other species is not fully described.
The aim of the study was to examine rhizomes of two *Fallopia* species and the hybrid between them in terms of their phytochemical composition and their antioxidant and anti-elastase activity. Correlation between the phytochemistry and activity of extracts was described using multivariate statistics.
Polar and non-polar extracts and fractions were analyzed by HPLC/UV/ESI-MS and screened for total polyphenols and tannins. Antioxidant activity was tested by DPPH, phosphomolybdenum and linoleic acid assay. Inhibition of elastase was also measured for all extracts and fractions. Relative quantification of metabolites in extracts and fractions was established using mass spectral deconvolution. HPLC/UV/ESI-MS analysis revealed many compounds, many of which had not been detected before. All extracts were rich in proanthocyanidins, from monomers to polymers, including several galloyl derivatives. *F. japonica* had the highest content of stilbenes, which were not detected in *F. sachalinensis*. On the other hand, *F. sachalinesis* was the richest source of hydroxycinnamic glycosides, many of them were detected for the first time. Rhizomes of *F.* x *bohemica* was the source of both stilbenes, anthraquinones and phenylopropanoids, present in intermediate amounts between *F. japonica* and *F.sachalinesis*. There was a strong correlation between polyphenols, mainly proanthocyanidins in the fractions and the antioxidant activity.

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SYSTEMATIC REVIEW OF ETHNOMEDICINAL PLANTS USED IN EPILEPSY – EMPHASIS ON CHROMATOGRAPHY ROLE IN PHYTOCHEMISTRY AND BIOACTIVITY SCREENING

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Epilepsy is one of the world's oldest recognized disorders and a chronic brain disease which affects approximately 50 million people worldwide [4]. Literature evidence for Epilepsy dates back to Assyrian and Babylonian medical texts nearly 2000 years B.C and since then numerous plants have been used to treat Epilepsy. In this review we surveyed the literature from scientific journals and online databases on medicinal plants with anticonvulsant activity used in different traditional medicine systems, systematically organizing them according active compounds and chromatography methods used in their assessment. More than 230 plants were considered to have antiepileptic effect, families Apiaceae, Asteraceae, Fabaceae, Lamiaceae, Rubiaceae and Solanaceae were most frequent. *Salvia miltiorrhiza, Solanum torvum, Berberis vulgaris, Peucedanum ostruthium, Cannabis spp., Tanacetum Parthenium* and *Scutellaria baicalensis* are among most investigated and promising medicinal plants. Regarding potential active phytosubstances, tanshinone IIA [2], steroid glycosides [3], berberine [1] imperatorin [6], osthole [6], cannabinoids [7], apigenin [5] and wogonin [8] demonstrated significant anticonvulsant activity in MES and PTZ seizure assays in zebrafish and rodent models. Further investigation on phytochemical composition by chromatographic methods LC-MS, CC and GC-MS is encouraging to lead to new lead compounds and drug targets for treatment of Epilepsy.

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SYSTEMATIC REVIEW OF ETHNOMEDICINAL PLANTS USED IN ALZHEIMER'S DISEASE – EMPHASIS ON CHROMATOGRAPHY ROLE IN PHYTOCHEMISTRY AND BIOACTIVITY SCREENING

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Alzheimer's disease is a progressive neurodegenerative disorder which causes mental deterioration and accounts for 50-60% of overall cases of dementia among elderly people [2]. Plants are a good source of chemical constituents with neuroprotective, antioxidant and cholinergic effects. In this review we have study the literature from scientific journals, books and online databases on medicinal plants used in different traditional medicine systems to treat Alzheimer's disease, systematically grouping them according active compounds and chromatography methods used in their assessment. More than 130 plants have been evaluated, mostly from Amaryllidaceae, Asteraceae, Apiaceae, Fabaceae and Lamiaceae families. *Huperzia serrata, Salvia miltiorrhiza, Lycoris chejuensis, Teucrium spp., Sideritis spp.,* and *Angelica sinensis* are among most investigated and promising medicinal plants. Regarding potential active phytosubstances, huperzine A [7], salvianolic acid A [1], cryptotanshinone [3], narciclasine [6], hydroxycinnamic acid derivatives [4] and (Z)-ligustilide [5] demonstrated significant neuroprotective activity and cognitive enhancement in different bioassay tests in mouse models of Alzheimer's disease. Further investigation on phytochemical composition by chromatographic methods LC-MS, CC and GC-MS is encouraging to lead to new lead compounds and drug targets for treatment of Alzheimer's disease.

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SEARCHING FOR ACETYLCHOLINESTERASE INHIBITORS IN THE BULBS OF *GALANTHUS KETZKHOVELII* KEM.-NATH.

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Plants from the Amaryllidaceae family contain alkaloids, well known for their acetylcholinesterase (AChE) inhibitory activities. They are widely studied as a potential drugs, that could be used in the treatment of the Alzheimer's disease.
In this work, *Galanthus ketzkhovelii* Kem.-Nath., an endemic species from the Caucasus (Georgia) was examined in search of those compounds.
The bulb extracts were obtained by the use of accelerated solvent extraction (ASE) and purified in the process of solid phase extraction (SPE) in cation-exchange mode. Alkaloid extracts were analyzed qualitatively by the HPLC/ESI-QTOF-MS method in positive ion mode using 6530B Accurate-mass-Q-TOF-MS (Agilent Technologies). For analysis Atlantis® HILIC silica column (d_p=3 µm, 2.1x150 mm) and gradient of acetonitrile (95%) with 10 mM ammonium formate (0.2 %) [B] and acetonitrile (55%) with 10 nm ammonium formate (0.2 %) [B] as a mobile phase were used. In total: six alkaloids were identified from the extract, namely: zefbetaine, tortuosine, ungeremine, galanthine, lycorine and 6-*O*-methylhaemanthidine. Acetylcholinesterase inhibition of alkaloid fractions was evaluated by TLC bioautographic assay using thin layer plate covered with silica gel in derivatization chamber (Camag, Switzerland). The compounds showing acetylcholinesterase inhibitory activity have been identified.

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HPLC-DAD-MS/MS ANALYSIS OF ASH LEAF FROM DIFFERENT COMMERCIAL AND NATURAL SOURCES

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Ash leaf (*Fraxini folium*) obtained, according to the European Pharmacopeia from *Fraxinus excelsior* L. or *Fraxinus angustifolia* Vahl (Oleaceae) is traditionally used 1) for minor articular pain and 2) to increase the amount of urine for flushing in minor urinary complaints [1].
The aim of study was to investigate the phytochemical composition of ash leaf collected from natural (4), as well as commercial sources from Poland (2), Austria (1), France (1) and Portugal (1). In addition, we tested the effect of infusions and 60% ethanolic extracts on interleukin 8 (IL-8) and tumour necrosis factor (TNF-α) secretion.
Methods: Selected ash leaf samples were characterized using a HPLC-DAD- MS^n method. The effects on TNF-α and IL-8 production by neutrophils were measured using enzyme-linked immunosorbent assay (ELISA).
Results: We characterised or partly characterised 48 compounds. The major compounds detected in ash samples were chlorogenic acid, quercetin-3-*O*-rutinoside, verbascoside, oleuropein and ligstroside. However, the sample from Austria contained additionally coumarins derivatives. This suggested an adulteration with other *Fraxinus* species and/or plant's parts. All extracts and infusions were able to inhibit TNF-α production which support the traditional used of this plant material in minor inflammatory complaints.

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SECONDARY METABOLITES FROM COMMON LILAC BARK AND ITS ANTI-INFLAMMATORY ACTIVITIES IN *IN VITRO* MODELS

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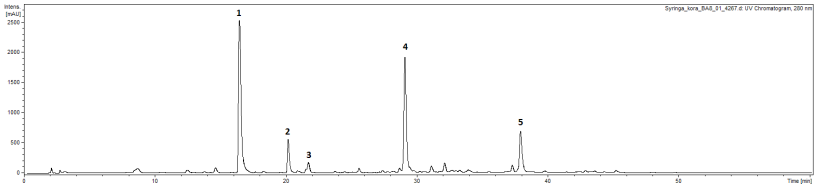
In European traditional medicine common lilac (*Syringa vulgaris* L., Oleaceae) bark in form of infusion, decoction or alcoholic extract was used as antipyretic and to treat cold, cough [1]. Our primary study shoved that bark ethanolic extract was able to decrease the release of interleukin-8 in *in vitro* model [1].
The aim of the study was to isolated the dominating compounds from lilac bark and to investigate their effects on the pro-inflammatory functions of human neutrophils and monocytes-macrophages.
Methods: Compounds were isolated using Diaion HP-20, Sephadex LH-20 and preparative chromatography. The structures of compounds were confirmed based on the UV, MS and ^{1}H and ^{13}C NMR spectra. The effects on cytokines (TNF-α, IL-1β) and chemokines (IL-8 and MCP-1) production by neutrophils and monocytes-macrophages were measured using enzyme-linked immunosorbent assay (ELISA).
Results: Syringin (**1**) (sinapyl alcohol-*O*-glucoside), olivil-*O*-glucoside (**2**), sinapyl aldehyde-*O*-glucoside (**3**) and forsythoside B (**4**) were isolated from the bark extracts. In *in-vitro* models all isolated compounds showed moderate anti-inflammatory activity in comparison with oleuropein (**5**), a widespread compounds in Oleaceae family, with well documented anti-inflammatory effect.
Our result partly support the traditional used of this plant material in common cold and inflammatory complaints.

Fig.1. HPLC-DAD chromatogram of lilac bark extract recorded at 280 nm.



Acknowledgements: This study was financially supported by research grant 2015/17/B/NZ7/03086 from the Polish National Science Center

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SESQUITERPENOIDS AND PHENOLICS FROM *TARAXACUM* SPP.

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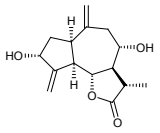
Plants of the genus *Taraxacum* have long been used as medicinal herbs due to their choleretic, diuretic and anti-inflammatory properties [1].
Taraxacum lucidum Dahlst. and *Taraxacum flavostylum* Bäck are classified in the section *Taraxacum* F. H. Wigg., corresponding to *Ruderalia* Kirchner & al., of the genus *Taraxacum* F. H. Wigg. (Asteraceae, tribe Cichorieae, subtribe Crepidinae) [2].
The present study deals with the isolation of eight sesquiterpene lactones and six phenolic compounds from roots of the hitherto unexamined *T. lucidum* and *T. flavostylum*.
Eight sesquiterpene lactones, including four taraxinic acid derivatives, ainslioside, deacetylmatricarin, 3-*epi*-11β,13-dihydrodeacylcynaropicrin (**1**) and ixerin D were isolated from *T. flavostylum* roots, whereas analysis of the extract from *T. lucidum* revealed the presence of three germacranolides and a guaianolide. Moreover, six phenolic metabolites: 3-hydroxy-1-(4-hydroxy-3methoxyphenyl)-1-propanone (**2**), methyl p-hydroxyphenylacetate, 5-methoxy-eugenyl-4-*O*-β-glucopyranoside (**3**), syringin, dihydrosyringin and furofuran lignan syringaresinol-4'-*O*-β-glucopyranoside were isolated from the plant material. The compounds **1**-**3** were found for the first time in *Taraxacum* spp.
All compounds were identified by direct comparison of their spectroscopic data with those of the reference compounds formerly isolated in our laboratory or with literature data [3].



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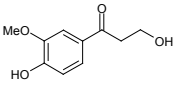

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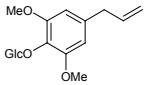

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PHYTOCHEMICAL PROFILE OF *GAULTHERIA PROCUMBENS* STEM EXTRACTS AND THEIR EFFECTS ON THE PRO-INFLAMMATORY AND PRO-OXIDANT FUNCTIONS OF HUMAN NEUTROPHILS

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Gaultheria procumbens L. (American wintergreen, Ericaceae) is an evergreen shrub native to North America and commonly used in traditional medicine as anti-inflammatory, analgesic and antipyretic drug in the treatment of rheumatoid arthritis, influenza and pain of various etiology [1,2]. The active wintergreen components are salicylates, mostly methyl salicylate and its glycosidic precursor – gaultherin, but also other polyphenols, i.e. flavonoids, proanthocyanidins and monocaffeoylquinic acids [2,3]. The plant parts used for medicinal purposes are leaves, stems and fruits, among which stems are the least characterized both in terms of chemical composition and biological activity. Therefore, the present study was conducted for thorough phytochemical profiling of *G. procumbens* stem extracts and measuring their anti-inflammatory and antioxidant effects in model of human neutrophils.

The dry extracts were prepared by direct extraction of the plant material with the use of five solvents of different polarity. The first stage was the UHPLC-PDA-ESI-MS^3 qualitative analysis, that led to the full or tentative structural identification of over forty phenolic constituents. The quantitative standardization was conducted by HPLC-PDA-fingerprinting and by spectrophotometric determination of total polyphenolic (183.7-347.8 mg GAE/g dw) and total proanthocyanidin (51.6-241.6 mg CyE/g dw) contents. In the next stage, the influence of the extract richest in polyphenols on pro-inflammatory and pro-oxidant functions of neutrophils stimulated with LPS and f-MLP was examined in the release tests of elastase, matrix metalloproteinase and pro-inflammatory cytokines, i.e. interleukins IL-8, IL-1β, TNF-α, as well as in the model of oxidative burst.

The results showed that the *G. procumbens* stem extracts are rich source of structurally divers polyphenols, especially proanthocyanidins and gaultherin, and exhibit significant anti-inflammatory and antioxidant activity in *in vitro* cellular models.

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ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF METHYL SALICYLATE GLYCOSIDES FROM *GAULTHERIA PROCUMBENS* FRUIT EXTRACT

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Plant extracts are often indicated as a potential alternative for synthetic anti-inflammatory drugs. Most frequently, the attention turns to plant materials containing natural salicylates, i.e. glycosides of salicylic acid.
Gaultheria procumbens L. (Ericaceae) is an evergreen shrub, that has been used for centuries in traditional medicine of Native Americans as an anti-inflammatory, analgesic and antipyretic drug [1]. Numerous *in vitro* studies conducted on other *Gaultheria* species have demonstrated anti-inflammatory activity of salicylates isolated from aerial parts of the plants [2,3]. Unfortunately, there are no available literature data concerning salicylates in *G. procumbens* plant material. Therefore, the present study was conducted to isolate salicylic acid derivatives and measure their effect on the pro-inflammatory and pro-oxidant functions of human neutrophils.
The isolation of two methyl salicylate glycosides from *n*-butanol extract, prepared by direct extraction of the *G. procumbens* fruits, was carried out by preparative RP-HPLC chromatography. The isolated compounds were identified (by ^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC experiments) as methyl salicylate-2-*O*-(6'-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside (gaultherin) and methyl salicylate-2-*O*-β-D-glucopyranosyl-(1→2)-[*O*-β-D-xylopyranosyl-(1→6)]-*O*-β-D-glucopyranoside.
The antioxidant activity of the salicylates was evaluated in the model of oxidative burst of human neutrophils stimulated with f-MLP peptide. The influence of the isolates on pro-inflammatory functions of neutrophils stimulated with LPS was assessed in the release tests of elastase, matrix metalloproteinase and pro-inflammatory cytokines, i.e. interleukins IL-8, IL-1β, TNF-α.
The results showed that the isolated methyl salicylate glycosides exhibit strong anti-inflammatory and moderate antioxidant properties by inhibiting the production of pro-inflammatory cytokines and reactive oxygen species. The fruits of *G. procumbens* could be, therefore, considered as potentially valuable source of natural salicylates.

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TRITERPENE SAPONINS CONTENT IN *CHENOPODIUM BONUS-HENRICUS*

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The family Amaranthaceae is a widespread and cosmopolitan family that can be found from the tropics to cool temperate regions. Some of the Amaranthaceae plants have economic importance and are used as herbal medicines or vegetables in various parts of the globe. The phytochemical profile of Amaranthaceae plants comprises essential oils, betalains, phenolics and triterpene saponins. Different pattern of triterpene saponin occurrence was characterised in almost 30 species belonging to Amaranthaceae which can be considered as promising and highly available sources of biologically active compunds. The bioactivity of saponin mixtures or individual saponins isolated from the Amaranthaceae plants include cytotoxic, immunomodulatory, hepatoprotective, antidiabetic, hypolipidemic, antiosteoporosis, antiviral, antifungal as well as antihelmintic actions.

So far nothing was known about the occurence of saponins in Good King Henry *(Chenopodium bonus-henricus)*, the edible plant beloning to Amarathaceae family. This herb was used in folk medicine as an antiseptic agent for healing wounds, in treatment of skin mycosis and against skin inflammation. In the contemporary studies extracts from *Chenopodium bonus-henricus* roots showed significant antifungal properties. Due to the wide spectrum of activities saponins could be at last partially responsible for biological activities of this plant. Thus, the aim of the present study was qualitative and quantitative analysis of saponins in *Chenopodium bonus-henricus* aerial parts and roots.

Ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) was used as the analytical method. Results obtained indicated that the arial parts and roots of *Chenopodium bonus-henricus contained* 15 and 5 saponins, respectively, consisting of oleanolic acid, gyspogenin or hederagenin aglycone and varying numbers of sugars. Additionally, it was stated that the plant parts differ in the total concentration of saponins. The saponin concentration in aerial part equaled 6,15 mg/g DW, when in the root was three times lower. Moreover, differences in the relative content of individual saponins were observed.

To the best of our knowledge this is the first report on the occurrence, structure, and content of triterpenoid saponins in *Chenopodium bonus-henricus*.

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PRELIMINARY PHYTOCHEMICAL STUDY ON GREEK ENDEMIC *RINDERA GRAECA* AERIAL PARTS. ANTIOXIDANT ACTIVITY

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In the framework of our phytochemical studies, on endemic Greek plants of Boraginaceae, the species *Rindera graeca* Boiss & Heldr. is studied herein for the first time.

A sample of methanolic extract of its aerial parts, was subjected to quantitative and qualitative LC/MS analysis throughout 12 phenolic metabolites were identified among which 7 phenolic acids: chlorogenic, caffeic, rosmarinic, lithospermic B and salvianolic acid A, together with rabdosiin and rabdosiin disodium salt) and 5 flavonoids: quercetin 3-*O*-rutinoside (rutin), kaempferol 3-robinoside-7-rhamnoside, nicotiflorin, kaempferol 3-glucoside and quercetin 3-*O*-rutinoside-7-rhamnoside. The same extract submitted to various chromatographic separations to afford the flavonoids: rutin and quercetin 3-*O*-rutinoside-7-rhamnoside together with phenolic acids rosmarinic acid and disodium rabdosiin which is a new natural product. The structures of all the isolated metabolites were identified by means of 1D ^{1}H-/^{13}C-NMR and 2D NMR spectroscopy. *Rindera*'s methanolic extract was screened for its content of pyrrolizidine alkaloids (PAs) through Mattocks-Molyneux visualization reagent and SPE clean out method as proposed by BfAr, with positive result, while LC-MS analyses confirmed the presence of PA rinderine and its N-oxide

Moreover, the total phenolic and flavonoid content were estimated by the Folin-Ciocalteu method (TPC 66.5±1.6, TFC mg QUE/g 9.7±0.1) and the free radical scavenging activity was determined by DPPH (% inhibition of 88.6±0.2, 42.8±5.3, 24.2±2.3) and ABTS assays (99.8±0.4, 96.2±2.1, 52.2±1.0) for three different concentrations (200 μg/ml, 100μg/ml, and 50μg/ml respectively)

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POLYPHENOL COMPOSITION OF *VERNONIA AMYGDALINA* DEL. FROM IVORY COAST

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Vernonia amygdalina Del. (*VA*) is one of the most important tropical representatives of family Asteraceae. The plant is commonly known as "bitter leaf" and is widely consumed as drug and vegetable. The total phenolic content of *VA* is a subject of many investigations, while the composition of the polyphenol complex is less studied (1). Usually the plants originate from Nigeria. In the last decade *VA's* medicinal and culinary uses is expanding in other regions of Africa and Asia. Thus, these facts place great value on polyphenol components research. In this study the polyphenols were extracted with 70% methanol and HPLC-PDA fingerprint profile was developed. Separation was optimized by using different columns, mobile phases and gradients. Identification was supported by standardized profile of green coffee polyphenols and quantification was done using rutin as internal standard. Our data reveal that the polyphenol components of *VA* are in lower amounts compared to those of green coffee and wild herbs of the Asteraceae family (2). This is in accordance with the *VA* radical scavenging activity (RSA) evaluated by DPPH assay. Despite of caffeoylquinic acids (CQA) and luteolin glycosides being strong radical scavengers, the RSA of *VA* is not so high IC_{50} 122±0.7 $\mu g.L^{-1}$. The climatic conditions in Ivory Coast are leading to higher content of CQA compared to those in *VA* from Nigeria.

Table 1: Content of caffeoylquinic acids (CQA), luteolin glycosides and luteolin ($mg.g^{-1}$ DM) in *Vernonia amygdalina* Del. from Ivory Coast.

Polyphenols	mg.g-1 DM
Chlorogenic acids	0.24±0.01
1,5+3,5-diCQA	1.53±0.20
4,5-diCQA	0.44±0.03
Luteolin glycosides	0.39±0.04
Luteolin	0.14±0.03

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SUPERCRITICAL AND ACCELERATED SOLVENT EXTRACTION OF JUNIPER CULTIVARS AS NEW POTENTIAL SOURCES OF PODOPHYLLOTOXIN

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Podophyllotoxin (PPT) is used as precursor in the industrial synthesis of efficient anticancer drugs (etoposide, teniposide etc.). Natural sources of PPT are *Podophyllum hexandrum* Royle and *Podophyllum peltatum* L., which are considered already as endangered species because of their intensive exploitation and difficult cultivation. In contrast, junipers are evergreen plants, easy for cultivation and producing a significant amount of plant material all the year. In response to demands of the pharmacy for delivery of high quality plant extracts, *J. virginiana, J. scopulorum, J. horizontalis, J. x media etc.* have been identified as alternative sources of PPT [1,2]. It was determined that cultivars of *J. virginiana* ('Grey Owl' etc.) possess high anticancer activity on NB4 cells (IC_{50} 0.5-1 microgram/ml) [2]. In this study we determined that this antiproliferative activity of 'Grey Owl' juniper corresponded to 0.3-0.4% PPT in the extracts, obtained after classical atmospheric pressure extraction. Our research was aimed also at maximization of the PPT content in the 'Grey Owl' extract as potential new source of PPT using accelerated solvent extraction (ASE) and supercritical extraction (SCE). The PPT concentration was assessed by HPLC-MS/MS method. The extract, obtained by ASE (using MeOH, 3 times for 15 min, 40°C), contained 5.4±1 micrograms PPT per mg dry extract (0.5%). Significantly higher concentration of PPT (21.9±0.5 micrograms per mg dry extract, 2.2%) was achieved under supercritical extraction conditions using CO2 at 30 MPa, 40° for 60 min. CO2 consumption was 100 kg/kg. Thus, the supercritical extraction can be estimated as the best approach to increase the PPT recovery from the studied junipers. Employment of modern technologies in the maximization of the PPT recovery from other juniper representatives as potential new sources of anticancer substances is envisaged in the near future.

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DERMAL CYTOTOXICITY AND TYROSINASE INHIBITION PROPERTIES OF MARCHANTIN A ISOLATED FROM *MARCHANTIA POLYMORPHA* L.

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Liverworts are rich source of cyclic and acyclic bis-bibenzyls, which are very rare natural compounds in the plant kingdom. Marchantin A, a representative of the cyclic compounds, is naturally occurring in some *Marchantia* species [1,2]. This compound has been reported to exert a broad spectrum of biological activities, e.g. antiviral, antioxidant, antimicrobial, cytotoxic, or skeletal muscle relaxant activity [1-3].
The major purpose of this study was to evaluate the safety of usage and effectiveness of marchantin A as a potential active ingredient for dermatologic products. Compound of interest was isolated from the MeOH extract of *Marchantia polymorpha* L. collected in Tokushima (Japan). For this purpose the open column chromatography on silica gel 60 and Sephadex LH-20 were used. The isolation process was controlled by use of TLC and HPLC methods. Isolated marchantin A was significantly cytotoxic against human malignant melanoma cells A-375 (IC_{50}=11.03 µg/ml) and less cytotoxic against immortalized human keratinocytes HaCaT (IC_{50}=27.04 µg/ml) in Neutral Red Uptake Test [4]. To examine anti-hyperpigmentation properties of marchantin A, the inhibitory properties against mushroom tyrosinase activity was assessed [5,6]. The results showed that marchantin A have a direct inhibitory anti-tyrosinase activity, exhibiting only slightly lower effect than the standard tyrosinase inhibitor (kojic acid) for concentrations assessed as safe (12.5 µg/ml and 6.25 µg/ml). Thus it could be concluded, that marchantin A, used in appropriate concentrations might be safe, novel bioactive ingredient for both anti-malignant melanoma and anti-hyperpigmentation dermatological products.

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DEVELOPMENT OF A UHPLC-PDA METHOD FOR STANDARDIZATION OF HIPPOCASTANI CORTEX BY STATISTICAL AND NUMERICAL OPTIMIZATION

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The aim of the study was development of a UHPLC-PDA method for standardization of *Hippocastani cortex*, a plant material used both in traditional and official medicine for the treatment of venous insufficiency, hemorrhoids and skin inflammations [1].
The analyses were performed on Nexera X2 Shimadzu UHPLC system, equipped with Titan™ C18 UHPLC column (1.9 µm, 100 x 2.1 mm, Supelco) and using a gradient of acetonitrile in 0.5% orthophosphoric acid for elution. In the first step of the study, a preliminary UHPLC-PDA-ESI-MS^3 analysis was carried out and led to the identification of 10 main constituents in the methanolic extracts from horse chestnut bark, primarily coumarins (dominating esculin and fraxin) and flavan-3-ol derivatives (dominating (–)-epicatechin and procyanidin B2). Then, the influence of the temperature, flow rate, initial concentration of acetonitrile and gradient slope on the separation was investigated using central composite design combined with response surface methodology. For each plant constituent multivariable quadratic models were developed describing the impact of the aforementioned parameters on the retention time and peak width. Eventually, numerical optimization procedure was applied to find the conditions, that would ensure minimal time of the separation and sufficient resolution between all peaks. The calculated optimal parameters of the analysis were as follows: temperature, 35°C; flow rate, 0.7 ml/min; initial acetonitrile concentration, 9.9%; gradient slope, 2.5 %/min; and allowed for separation of horse chestnut extracts in about 5 min with minimal resolution > 1.5. The experimental data were in good agreement with the predictions of the model and the validation studies showed satisfactory precision (RSD < 1.5%), accuracy (97-103%) and sensitivity (LODs in the range of 0.21-0.76 ng) of the method.
The developed procedure was applied for standardization of commercial samples of *Hippocastani cortex* obtained from four Polish distributors and relevant variability in the quantitative content was observed, both in terms of individual constituents as well as total phenolic content. For example, the level of esculin varied in the range of 23.06-43.40 mg/g dw, fraxin 11.61-17.31 mg/g dw and (–)-epicatechin 9.57-21.86 mg/g dw of the bark. The results emphasize, therefore, the need to introduce quality control studies in production of preparations containing horse chestnut bark and the described method was proved to be suitable for this purpose.

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ISOLATION OF PLANT SPECIFIC METABOLITES FROM FLOWERS OF *YUCCA FILAMENTOSA* VAR. FLACCIDA

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Yucca (*Yucca filamentosa* var. flaccida) is a perennial plant belonging to Agavaceae family. The plant is a native of southeastern N. America to Mexico. It has been introduced and become naturalized in Europe [1]. It occurs in sand dunes and waste ground and is very drought tolerant and frost resistant.
Flowers can be eaten fresh or cooked, also dried and used as flavoring. Some recipes include yucca flower soup, stuffed yucca flowers and apple crumble pie. Flowering stem can be cooked and used like asparagus [2].
The literature data describes exclusively chemical constituents of *Y. filamentosa* leaves and roots, mostly spirostanol-type steroidal saponins, among them tigogenin and yuccosides [3], [4]. The aim of the study was to understand the chemical composition of plant widely grown in Polish gardens.
The procedure of isolation was as follows: lyophilized and ground flowers were exhaustively extracted with 70% acetonitrile, purified with SPE on RP-C18 SPE column and 95% MeOH fraction was subdued to further purification on column filled with Sephadex LH-20 and run with 90% MeOH. The separation produced five main fractions containing various compounds, and fraction 2 and 5 were further separated using either flash column filled with Cosmosil C-18 (12 ×150 mm, 40 µm) or preparative HPLC on Atlantis T3 column (20 × 250 mm, 5 µm) to obtain 3 known flavonoids (narcissin, paeonoside and nicotiflorin) and a new sesquiterpene glycoside (nerolidol β-diglucoside).

Table 1: Fractions obtained after Sephadex LH-20 separation

Fraction	Compds	%
1	steroidal saponins	**19**
2	sesquiterpenoids	**30**
3	kaempferol, quercetin, isorhamnetin triglycosides	**30**
4	kaempferol, quercetin, isorhamnetin diglycosides	**16**
5	kaempferol, quercetin, isorhamnetin monoglycosides	**<5**

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COMPARISON OF TRADICIONAL AND MODERN EXTRACTION METHODS FOR THE ANALISYS OF TILIROSIDE IN TILIA L. FLOWERS BY LC-ESI-MS/MS METHOD

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Tiliroside exhibits a wide spectrum of effects on the human body. Plant materials containing tiliroside exhibit antithrombotic, anticoagulant, antimicrobial, anti-inflammatory [1,2]. Tiliroside has been obtained using different extraction methods, extraction procedures and solvents. However, the extraction process for the highest content was not optimized.

The aim of the present work was to develop extraction conditions for optimal extraction of tiliroside content from the flower *of Tilia* L. For this purpose, various different extraction methods were investigated. The effect of extraction technique, solvent type and temperature on tiliroside extraction efficiency was studied. Classical (maceration, maceration with intensive stirring, extraction under reflux) and modern (ultrasound-assisted extraction, ASE, supercritical carbon dioxide) extraction methods were used. Analysis of tiliroside content in *Tilia* L. extracts were determined by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS).

The results of this study showed that the extracts of lima-tree contain large amounts of tiliroside. The content of tiliroside for modern extraction methods varies between 0,19 mg per g of dry extract (CH 100%,50˚C) to 7,40 mg per g of dry extract (EtOH 70% + 1% acetic acid, 80˚C) for ASE and between 0,83 mg per g of dry g extract (MeOH 50%, room temperature) to 3,37 mg per g dry extract (EtOH 100% + 1% acetic acid, 80˚C) for UAE. Classical extraction technique show much less recovery of tiliroside, 0,14 mg per g of dry extract (maceration with stirring with water) and 9,84 mg per g dry extract (extraction under reflux with diethyl ether).

The ASE method was proved to be the best way to extract tiliroside from Tilia L. flowers, but classical methods, like maceration, also giving high extraction yield of tiliroside.

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OPTIMIZATION OF PHENOLIC COMPOUNDS EXTRACTION FROM *LACTARIUS DELICIOSUS*

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Edible mushroom species possess great potential for both nutrition and therapeutic use. Amongst bioactive compounds occurring in them, phenolic compounds focus much attention due to their antioxidant activity [1].
The aim of presented study was optimization of extraction conditions in order to obtain phenolic compounds from edible mushroom species *Lactarius deliciosus*.
The extractions under different conditions were performed using an accelerated solvent extraction system (ASE). Extractions were conducted at three different extraction temperatures (40, 100, and 180°C) with three ethanol concentrations (50, 70 and 100%). The total phenolic content (TPC) was assayed by the modified Folin-Ciocalteau method. The antioxidant activity was measured using a $DPPH^{\bullet}$ radical scavenging assay and $ABTS^{\bullet}$ decolorization assay. All colorimetric measurements were conducted on 96-well transparent microplates using an Elisa Reader Infinite Pro 200F. Moreover, qualitative and quantitative analysis of different phenolic acids in nine extracts were performed. Phenolic acids contents were determined by reversed-phase high-performance liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS/MS). The QTRAP-MS system was equipped with electrospray ionization source (ESI) operated in the negative-ion mode.
Due to its high extraction efficiency and considerable saving of time and solvent, ASE was selected for effective extraction of phenolic compounds from *L. deliciosus*. Obtained results allowed to choose the optimal conditions to obtain the largest amounts of phenolics. Moreover, the correlation between phenolic compounds and antiradical activity was observed during the study.
Our results indicate that mushrooms might be used directly in diet as an easily accessible source of natural antioxidants.

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ISOLATION OF ELLAGITANNINS' METABOLITES FROM *EX VIVO* GUT MICROBIOTA CULTURES AND HUMAN URINE.

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Gut microbiota metabolism of orally applied natural products has been in recent years indicated as an important factor significantly influencing their therapeutic effects. One of the most extensively studied groups are ellagitannins, which are metabolized to urolithins- compounds having good bioavailability and expressing various bioactivities [1]. Because urolithins and their glucuronide conjugates are required to conduct *in vitro* bioactivity studies, methods for the production and isolation of aglycones using *ex vivo* gut microbiota cultures as well as isolation of II phase conjugates from human urine were developed.

Gut microbiota *ex vivo* cultures were incubated in anaerobic conditions for 24 and 48 h with *Lythrum salicaria* aqueous extract as an ellagitannin source. Simple two-step method using liquid-liquid extraction and preparative HPLC purification was developed allowing obtaining urolithins in a pure form. From 1600 mg of extract incubated with gut microbiota cultures, 7.0 mg of iso-urolithin A and 12.5 mg urolithin B was obtained. For isolation of II phase conjugates, volunteer was asked to supplement his diet with ellagitannin-rich food products. The total urine was collected for five following days and subjected to column chromatography using Diaion HP-20, silica gel and Sephadex LH-20. As a result 20 mg of iso-urolithin A glucuronide, 125 mg of urolithin A glucuronide and 139 mg of urolithin B glucuronide were obtained. For the first time urolithin glucuronides were isolated from human urine, what allowed direct identification of their structure using NMR method.

For the first time iso-urolithin A and its glucuronide were isolated, which together with urolithin A and B are necessary for *in vitro* bioactivity studies of this group of human gut microbiota metabolites. The amounts of urolithins and their II phase metabolites are sufficient to perform future *in vitro* and *ex vivo* bioactivity studies.

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METHOD OF ANTHOCYANIN AND THEIR METABOLITES ANALYSIS IN BLOOD PLASMA AND URINARY OF EWES AFTER CHOKEBERRY ADMINISTRATION

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After plant products intake, thousands of phytochemicals in the various forms are found in the digestive tract of humans and animals. Some of them may be absorbed and in different form occurs in the blood and urine. The substances unabsorbed may undergo transformations in the gastrointestinal tract in results of the conditions prevailing in particular parts of the digestive system and/or the activity of the microflora, and then the resulting metabolites may be absorbed into the circulatory system and may underwent a further metabolism. The aim of the study was to develop a method allowing to determine various forms of anthocyanin metabolites and their concentration in animal physiological fluids after ingestion of chokeberry.
The samples of blood plasma and urine were collected during experiments with ovine model (n = 16) [1] after the anthocyanin preparations administration. The obtained blood plasma and urine were subjected to solid-bed extraction (SPE). Analysis of anthocyanins and their metabolites was performed based on a micro-HPLC system (LC200, Eksigent, Canada) coupled to a QTRAP 5500 mass spectrometer (AB SCIEX, Canada) equipped with an ESI ion source, a triple quadrupole and an ion trap. The separation of anthocyanin compounds and their metabolites was performed on a HALOFused C18, 2.7 µL, 50 x 0.5 mm analytical column (Eksigent, Canada). The analyzes were carried out by two-phase elution method: phase A (99.1:0.9, H_2O:formic acid) and phase B (99.1:0.9 ACN:formic acid). The compounds were identified by means of a comparison of their retention time, MS/MS spectra, and the previous data [2, 3], or through interpretation of the fragmentation spectrum obtained. The quantitative analysis was based on external standards by the MRM method.
In the samples of blood plasma and urine of ewes, native forms as well as methylated, glucuronided and combined derivatives of anthocyanins were discovered. The obtained new, biologically important information will enable the design of further research to explain the potentially beneficial effects of the presence of anthocyanins metabolites in human and animal organisms.

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SYNERGISTC EFFECT OF LYSIMACHIA CILIATA SAPONINS AND DIANTHIN IMMUNOTOXIN AGAINST HER-14 CANCER CELLS

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The specificity of immunotoxins makes them a promising target for the development of anticancer therapies. Dianthin immunotoxins (DE-EGFR) is a conjugate, that consists of epidermal growth factor (EGF) coupled with a toxin (dianthin) that can destroy specific cancer cells expressing human epidermal growth factor (EGFR). Thanks to that, it is possible to kill targeted cells without damaging other cells in the body. Unfortunately, targeted toxin therapies are accompanied by some dose – dependent side effects [1]. Reducing their side effects is therefore the main challenge for their further development. Some triterpene saponins have been shown to increase the efficacy of an EGFR-receptor conjugated toxin - dianthin (DE-EGFR), while limiting the side-effects of their action [2].

According to our previous study, triterpene saponins from *Lysimachia ciliata* L., can act synergistically with mitoxantrone (MTX) and improve cytotoxic activity of MTX against human prostate cancer cells [3]. The aim of the present study was to investigate the effect of these saponins in combination with DE-EGFR toxin on two cell lines: NIH 3T3 cell line (normal cells - not expressing EGF) and HER-14 (cancer cells that overexpress EGF).

The effect of saponin-DE-EGFR combination was determined by MTT cytotoxicity test and crystal violet proliferation assay. Results show that saponins (fraction denoted CIL-1) used together with DE-EGFR significantly increase selectivity of DE-EGFR. We observed strong cytotoxic and cytostatic response of HER-14 cells treated with CIL1/DE-EGFR compilation (IC_{50}=0.5 nM) while the effect on NIH-3T3 was noticeably weaker (IC_{50}=50 nM). Thus, saponins from *L. ciliata* can be considered as compounds that synergistically act with targeted toxins, and increase selectivity of their action, what makes them good candidates for further studies.

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QUANTIFICATION OF CAFFEETANNINS AND FLAVONOIDS IN *THYMI SIRUPUS COMPOSITUS*

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Thymus vulgaris L. and *Thymus zygis* L. are the species of flowering plants from the Lamiaceae family indigenous to southern Europe and Asia. They are woody-based evergreen subshrubs with small, highly aromatic, grey-green leaves and purple or pink flowers in early summer [1]. The thyme herb contains volatile oil with 30% to 50% of thymol, *p*-cymene, *γ*-terpinene and other monoterpenes. In addition, it is rich in polyphenols, especially lamiaceous tannins – caffeetannins (mainly rosmarinic acid) and flavonoids – flavone glycosides [2]. Thyme preparations are used internally for symptoms of bronchitis, as a decongestant (expectorant) for cold-related productive cough and they are classified as traditional herbal medicinal products [3].
The aim of the study was determination of caffeetannins and flavonoids in commercial product of *Thymi sir. comp.* using high performance liquid chromatography.
Materials and methods: Eight samples of *Thymi sir. comp.* from three different manufacturers were analyzed. The individual polyphenolics contents were determined by Knauer Smartline HPLC with DAD detector and EuroChrom for Windows software. As a solvents 5% formic acid in water and 5% formic acid in acetonitrile were used. The wavelength was 254, 280, 320 and 360 nm. Reference standards of rosmarinic acid, chlorogenic acid, caffeic acid, luteolin-7-*O*-β-glucoside, luteolin-3',7-*O*-β-diglucoside and luteolin-4'-*O*-β-glucoside were used. Luteolin-7-*O*-β-glucuronide was isolated from *Thymi herba* by column chromatography on octadecyl and Sephadex LH-20.
Results: Main polyphenols in *Thymi sir. comp*. where rosmarinic acid and luteolin-7-*O*-β-glucuronide. Other components, like caffeic acid, luteolin-7-*O*-β-glucoside and luteolin-4'-*O*-β-glucoside had lower concentration. The amount of chlorogenic acid and luteolin-3',7-O-β-diglucoside in most cases was under detection limit of measuring device.
Conclusions: Content of polyphenols in *Thymi sir. comp*. differed not only between different manufacturers, but also between batches. Phenolic compounds, which concentration was determined in the study are responsible for antioxidant, anti-inflammatory and spasmolytic action of thyme syrup and shows its beneficial role in upper respiratory track inflammation treatment.

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SELECTED LAMIACEAE-SPECIES-POST-DISTILLATION-BROTHS AS AN ALTERNATE SOURCE OF COMMERCIALLY VALUABLE COMPOUNDS

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Species belonging to the Lamiaceae family are among the main sources of distilled essential oils, with cornmint, peppermint and spearmint being in the top five. Approximate recalculation of the literature data shows that there is a need of 2,5-5 Mt of cornmint herb and 375-500 kt of peppermint herb to cover the annual global demand. In the industry, direct boiling of the raw material in water followed by vapours condensation is one of the main ways to obtain volatile oils [1-2]. The waste biomass is mainly used for energy production and for composting. Distillation water may be reused for steam generation or to produce so-called floral waters [1]. The aim of this study was to check, whether a post-distillation broth from water distillation process (WDB), which is generally wasted, can serve as a source of important secondary metabolites.
9 herbal drugs from Lamiaceae family were analysed, namely: lavender, lemon balm, peppermint, rosemary, sage, clary sage, greek sage, wild thyme and thyme. The extracts, both processed (WDB) and unprocessed (RE - reference, room-temperature maceration), were comparatively examined by UHPLC-MS semi-quantitative profiling, which was supported by DPPH radical scavenging test.
The results showed that Lamiaceae-species-post-distillation-broths, with recovery of many compounds exceeding 75% compared to the reference extracts, may serve as important source of pharmaceutically active compounds. Most of Lamiaceae WDB's can provide commercially important substances, like rosmarinic acid or main flavonoids or serve as a source of extracts similar – in terms of composition and antiradical activity - to the original macerates, e.g. for external use in pharmacy or cosmetics. The developed chromatographic method may be used for preliminary scanning of WDB's in terms of commercially important compounds.

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EVALUATION OF CUTICULAR WAX EXTRACTION YIELD FROM GRAPEFRUIT (*CITRUS PARADISI*) PEEL

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Cuticular isoprenoids comprise mainly pentacyclic triterpenes and steroids. In some plant species these compounds occur in wax only in trace amounts, in others they are predominating. The peels of the majority of fruits of the genus Citrus (Rutaceae), e.g., oranges and mandarines, do not contain prevailing amounts of triterpenoids. The exception is the peel of grapefruit (hybrid *Citrus* x *paradisi* Macf.), where these compounds can account for 50% of total wax. Moreover, the triterpenoid profile of grapefruit peel was reported to be characterized by particularly high amount of ketones, what is a rare feature distinguishing this fruit from many others, typically containing triterpene acids or alcohols. Triterpenoids are usually extracted from cuticular wax by short immersion of the fresh fruit in chloroform to avoid the risk of solvent penetration across the cuticle and contamination with compounds present in deeper tissues. The yield of this method is estimated at 30-70%. The aim of the present study was the evaluation of the yield of extraction of triterpenoids from grapefruit peel. Grapefruits of Star Ruby variety were dipped in chloroform and stirred gently for 1 min. Then they were peeled and the peel remaining after wax extraction was dried and extracted with chloroform in Soxhlet apparathus for 8 hrs. Control fruits were also peeled, and the entire dried peels were extracted in Soxhlet apparathus. All extracts were evaporated, fractionated by preparative TLC and the obtained fractions of neutral triterpenoids were analyzed by GC-MS/FID. The same triterpenoid profile was identified in all extracts, however, with remarkable quantitative differences. The keton friedelin (D:A-friedo-oleanan-3-one) was the prevailing compound in wax extract, accompanied with D:A-friedooleanan-2-one, D:A-friedoursan-3-one, D:B-friedo-B':A'-neogammacer-5-en-one, α- and β-amyrenones. The other identified pentacyclic triterpenoids were α- and β-amyrins, lupeol, methyl esters of oleanolic and ursolic acids. In turn, the most abundant compound in whole peel extract was sitosterol, accompanied with cholesterol, campesterol, stigmasterol, sitosterol, 24-methylenecycloartanol and tremulone. The yield of triterpenoid extraction from grapefruit cuticular wax, calculated on the basis of comparison of triterpenoid contents in analyzed extracts, was estimated for 65%.

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TRITERPENOID CONTENT OF PARAGUAYO PEACH (*PRUNUS PERSICA* VAR. *PLATYCARPA*) CUTICULAR WAX

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Paraguayo peach (*Prunus persica* var. *platycarpa*), known as Saturn peach or UFO peach due to its characteristic flattened shape, is a variety of peach created from hybridization. Paraguayo peaches are smaller, flatter and usually less fuzzy than ordinary peaches, with yellow or red peel and pale interior. Fruits of some varieties can be easily peeled and thus they can serve as a suitable model for the analysis of surface wax content. The aim of this study was to assess the yield of triterpenoid extraction from cuticular waxes of Paraguayo peach (variety Samantha, origin: Spain). Control peaches were gently peeled, then the peels were dried and extracted with chloroform in Soxhlet apparathus for 8 hrs. The study group of peaches were immersed in chloroform for 1 min and peeled afterwards. The peels were then extracted identically as the control group. All obtained extracts were evaporated and subjected to preparative TLC chromatography on SiO_2 in a solvent system $CHCl_3$/MeOH (97:3 v/v). Obtained fractions containing free neutral triterpenoids were directly analyzed by GC-MS/FID, while triterpene acids were analyzed after methylation with diazomethan. The fraction of neutral triterpenoids was composed mainly of steroids (campesterol, cholesterol, obtusifoliol, sitostanol, isofucosterol, tremulone, sitostenone and the most abundant sitosterol), however, trace amounts of pentacyclic triterpenoids, i.e. α- and β-amyrins and α-amyrenone were also detected. The fraction of acids was prevailing in Paraguayo peach wax and it contained oleanolic and ursolic acids, their 3-oxo-analogues, and olean-18-en-28-oic and ursa-8-en-28-oic acids. Comparing the content of triterpenoids in entire peels to that in peels remaining after wax extraction, it was calculated that after immersion in chloroform approximately 35-40% and 55-65% of the total content of triterpenoid acids and steroids, respectively, remained in the peel. These results might point to the differences in chloroform extractability of these two groups of triterpenoids differring in polarity, as well as to their accumulation in distinct cuticular layers (e.g., epi- and intracuticular waxes) leading to dissimilar efficacy of solvent penetration and, as a consequence, influencing final extraction yield.

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STUDY OF PHENOLIC COMPOUNDS OF *WISTERIA SINENSIS* LEAVES BY USING COMBINED MASS SPECTROMETRIC AND CHROMATOGRAPHIC METHODS FOR IN-DEPTH ANALYSIS

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The genus *Wisteria*, including ten species of woody climbing vines, are flowering plants belonging to the plant family Fabaceae [1]. The flowers, roots, leaves and seeds of *W. sinensis* are traditionally used in Chinese medicine to treat gastric cancer, stomach, cancer of breast or rheumatoid arthritis patients. The flowers, roots, leaves and seeds of *W. sinensis* are traditionally used in Chinese medicine to treat gastric cancer, stomach, cancer of breast or rheumatoid arthritis patients. The leaves and flowers of *W. sinensis* have several other uses, such as a tea substitute or a local delicacy called “Teng Lo” [2]. As it has been shown that, the extract of *W. sinensis* leaves is a rich source of biologically active compounds including tocopherols and phytosterols [3]. It was found that the extracts of *W. sinensis* leaves have anticancer activity against Hep-G2 cell lines [2], MCF-7 cell lines and HCT-116 cell lines [4].

A survey of the literature showed that in the extract of *W. sinensis* leaves has been identified six known flavonoid, such as orientin, isoorientin, vitexen, isovitexen, apigenin and luteolin [2] and flavone 7-O-[2-O-(6-deoxy-*α*-L-mannopyranosyl)-*β*-D-glucopyranosyl]-5,6,7,4`-tetrahydroxy flavone [4]. Several *Wisteria* species have been also found to contain isoflavones, triterpene saponins, lectins [2].

The main objective of our research has been detection and identification of phenolic compounds in a methanol extract of *W. sinensis leaves*. The goal has been achieved by using of combined mass spectrometry and chromatography methods for in-depth analysis. In the extracts from the *W. sinensis* leaves we found among others 3-p-coumaroylquinic acid (3p-CoQA), p-coumaric acid-glucoside, 4-caffeoylquanic acid (4-CQA) or 3-feruloylqunic acid (3-FQA).

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THE INFLUENCE OF PERUVIAN MACA (*LEPIDIUM PERUVIANUM*) ON BREAST CANCER CELL LINES

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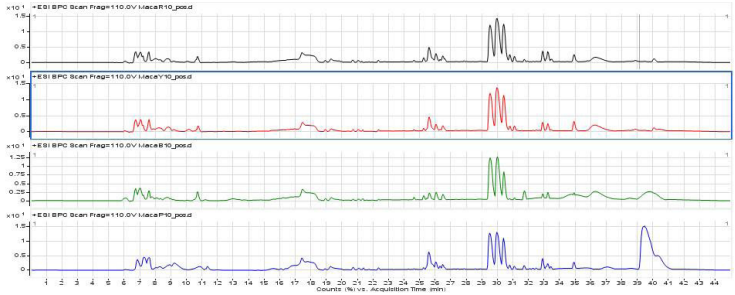
Maca (Brassicaceae) is a common Peruvian crop growing at high altitudes. It has been used in traditional medicine as an aphrodisiac and metabolism stimulant. Maca extracts are rich sources of glucosinolates, alkaloids and macaenes [1].
The aim of the study was to determine the major constituents of the extracts and to evaluate the differences in the composition between different types of maca hypocotyls by LC-ESI-Q-TOF-MS and to determine the anti-proliferative effect of extracts of different varieties of fresh maca.



The MTT assay showed that extracts fresh hypocotyls of black, yellow and red maca (100 μg/mL) inhibited cell proliferation of MDA-MB-468 cells, a triple negative breast cancer cell line, being red maca the variety that showed the highest effect (higher than 60%). Regarding to T47D cell line (ER and PR positive breast cancer cell line), only red maca extract was able to reduce the viability of these cells (up to 50%). To sum up, fresh hypocotyls of red maca can be consider a potential complementary treatment for breast cancer.

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ISOLATION, IDENTIFICATION AND BIOLOGICAL ACTIVITY OF TWO DIGLYCOSIDES FROM *SORBUS DOMESTICA* (L.) LEAVES

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Medicinal plants are an important source of active compounds which can be used in the prevention and therapy of various diseases. Among numerous plants under study, *Sorbus domestica* appears to be a promising target for closer investigation. It is a deciduous tree commonly found in the Southern Europe, where it is a source of ethnomedicinally used plant material with diuretic, anti-inflammatory, anti-diabetic and anti-atherogenic properties [1]. Our previous studies documented that leaves of *S. domestica* cultivated in Poland represent a promising ingredient for the treatment of oxidative stress/inflammation-related pathologies. The UHPLC-PDA-ESI-MS^3 analysis of the leaf extracts led to the detection of forty-four constituents, some of which were only tentatively identified [2].

Thus, the isolation of two quercetin diglycosides from *n*-butanol extract was carried out, firstly by open column chromatography (Sephadex LH-20; eluent: methanol) and then by preparative RP-HPLC chromatography (mobile phase: acetonitrile: 0.5% acetic acid, 13:87, *v*/*v*). The isolated compounds were identified (by ^{1}H NMR, ^{13}C NMR, COSY, HMQC and HMBC experiments) as quercetin 3-*O*-(2''-*O*-*β*-D-glucopyranosyl)-*α*-L-rhamnopyranoside and quercetin 3-*O*-(2''-*O*-*β*-D-xylopyranosyl)-*α*-L-rhamnopyranoside. To our knowledge, this is the first report on these compounds in the *Sorbus* species.

The isolated molecules, together with a series of model *Sorbus* polyphenols, were subjected to *in vitro* evaluation of the protective effects on human plasma exposed to oxidative stress (induced by peroxynitrite). During the studies, the main biomarkers of protein nitration (3-nitrotyrosine), lipid peroxidation (lipid hydroperoxides and thiobarbituric acid-reactive substances), as well as ferric reducing ability of plasma (FRAP) were determined. Finally, the compounds able to significantly diminish nitration/oxidation of plasma proteins and lipids, and to enhance the total antioxidant status of plasma were selected.

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VARIATION IN THE PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF *SORBUS DOMESTICA* (L.) LEAVES DURING FOLIAR DEVELOPMENT

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Sorbus domestica L. (service tree) is a rosaceous species native to the Mediterranean Basin and Central Europe. It is a source of plant materials with reported medicinal properties, i.a. anti-inflammatory, anti-diabetic and anti-atherogenic activities [1]. Our previous studies documented that leaves of *S. domestica* cultivated in Poland represent a promising polyphenol-rich herbal raw material that might be used in the prevention or adjunctive therapy of oxidative stress/inflammation-related pathologies [2]. However, it is known that environmental conditions in a course of plant development have significant impact on production of active metabolites and, therefore, therapeutic efficiency of medicinal plants [3].

Thus, seasonal changes in the polyphenolic composition, antioxidant activity, and their relationship in *S. domestica* leaves harvested monthly across the growing season were evaluated. The phenolic composition of methanol-water (75:25, v/v) extracts were carried out with HPLC-PDA-fingerprint, Folin-Ciocalteau, and *n*-butanol/HCl assays, while antioxidant activity were tested using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging and FRAP (Ferric Reducing Antioxidant Power) assays.

The phenolic levels and antioxidant activity parameters showed parallel seasonal trends with the highest total phenolic content observed in August (73.4 mg GAE/g of leaves dry weight) and the lowest in May (55.6 mg GAE/g DW). The polyphenolic fractions of the samples collected in August presented the antioxidant activity about 2-times higher than Trolox (in both tests) and were rich in proanthocyanidins (30-60% of total phenols, depending on the assay methodology), flavonoids (about 21% of total phenols) and chlorogenic acid isomers (about 17% of total phenols).

Considering both the phenolic contents and antioxidant activity parameters (FRAP: 1.42 ± 0.03 mmol Fe^{2+}/g; DPPH: EC_{50} 31.26 ± 0.81 µg/ml), in Polish climate conditions late summer could be recommended as optimal for harvesting the *S. domestica* leaves for medicinal purposes and cost-effective production of natural products.

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MICROEMULSIONS OF CITRONELLA, EUCALYPTUS AND MINT ESSENTIAL OILS AND THEIR MIXTURE FOR INCREASED ANTIOXIDANT ACTIVITY

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The formulation of microemulsions of essential oils corresponds to a thermodynamic equilibrium between all the compounds of the studied system in order to increase solubility of their lipophilic compounds in aqueous phase [1]. The increased solubility may be correlated with activity of essential oil incorporated in microemulsion. The aim of this work was to prepare stable microemulsions containing essential oils and to compare the antioxidant activity of essential oils with activity of obtained formulations.
Microemulsions were prepared of citronella (*Cymbopogon nardus*), mint (Mentha x piperita 'Multimentha') and eucalyptus (*Eucalyptus globulus*) essential oils (EO) and the mixture of their equal parts. The authentication of essential oils was performed by means of gas chromatography – mass spectrometry (GC-MS) analysis. Nine runs were made in which the aqueous phase, being a mixture of water and polypropylene glycol in a 1: 1 volume ratio, ranged from 10% v/v to 90% v/v. The oily phase was a mixture of the essential oil and soybean oil in a volume ratio of 3:1. Polysorbate 80 with an oil phase in 5 volume ratios varying from 5:1 to 9:1 was mixed in each series. The essential oils constituted from 0.8% to 11% of the microemulsions. The diameter of the microemulsions droplets was measured using DLS technique. The antioxidant activity was determined in 2,2-Diphenyl-1-picrylhydrazyl assay.
GC-MS revealed that main constituents of citronella EO were citronellal, citronellol and geraniol comprising 32.8%, 13.8% and 20.2% in EO, respectively. Eucalyptol (46.9%) dominated in eucalyptus EO, while menthone (42.6%) and menthol (18.9%) were predominant in mint EO. Stable microemulsions were obtained in a range between 10% and 50% of aqueous phase. The measurement of the diameter of the droplets for formulations comprising of 50% of aqueous phase and diluted 10 times with water showed that particles of the oil dispersed in water were in the range between 10 and 20 nm. Antioxidant activity expressed as percent of inhibition and calculated for EO concentration of 5mg/ml ranged from 2.76% to 32.15% for essential oils and from 8.28% to 77.75% for microemulsions indicating the enhanced antioxidant activity of microemulsions. The highest increase (45%) in antioxidant activity was observed for microemulsion containing citronella EO.

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PHYTOCHEMICAL INVESTIGATION OF *POLEMONIUM CAERULEUM* EXTRACTS

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Polemonium caeruleum L. is a perennial, hemicryptophyte from Polemoniaceae family. It is a subcontinental species that occurs in the temperate zone of the northern hemisphere [1,2]. Plants can grow up to 100-150 cm and occur in sunny places, but also tolerate semi-shaded positions and moderate light [2]. Russian traditional medicine recommended this species for treatment of tuberculosis, whooping cough and fever. It was also used as sedative agent [3].
In this work the phytochemical investigation of *P. caeruleum* was undertaken. High performance liquid chromatography coupled with electrospray ionization/quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS) and gas chromatography coupled with mass spectrometry (GC-MS) were applied for chemical characterization of methanol extracts of underground and aerial plant parts.
LC-ESI-QTOF-MS analysis confirmed the presence of triterpene saponins – oleanane derivatives (polemoniumsaponins, glycosides of theasapogenol derivatives and β-amyrin among others), also flavonoid glycosides with most predominant acacetin derivatives were present. Saponins were more abundant and varied in extract from underground parts of the plant, while flavonoids dominated in extract from aerial parts. GC-MS performed after silanization enabled the identification of carbohydrates, fatty acid esters, amino acids and carboxylic acids. Carbohydrates were the major group of compounds in both extracts, mainly represented by α- and β-glucopyranose, and β-fructofuranose. Among esters of fatty acids, palmitic acid, linoleic acid and oleic acid methyl esters were distinguished. Carboxylic acids dominated in the underground part of *P. caeuleum* and were represented by malic, fumaric, 2,3,4-trihydroxybutyric, glycolic and lactic acid.

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THYMOL DERIVATIVES FROM ROOTS OF *XEROLEKIA SPECIOSISSIMA* (L.) ANDERB. AN ENDEMIC SPECIES OF PREALPINE AREA

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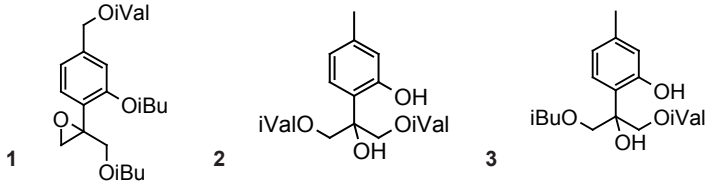
Xerolekia speciosissima (L.) Anderb. (synonyms: *Buphthalmum speciosissimum* Ard., *Telekia speciosissima* (L.) Less.) is the only species which belongs to the newly created *Xerolekia* genus, introduced by Anderberg [1], and is considered as a member of the tribe Inulae, subtribe Inulinae, of the family Compositae (Asteraceae). The plant naturally inhabits crevices on limestone or dolomite rocks, 1,000 – 1,900 m a.s.l. Its distribution is restricted to the area from Lake Como to Lake Garda and to the Ledro Valley [1, 2]. Roots of the plant were extracted with chloroform. The extract was concentrated in vacuo and initially fractionated by CC on silica gel using n-hexane-EtOAc gradient solvent system (up to 100 % EtOAc). Fractions eluted with n-hexane-EtOAc 4:1 (v/v) were further separated by preparative RP-HPLC (column: Synergi 4µ Fusion-RP, 80A, 250 x 10 mm; eluent MeOH-H_2O mixture, 7:3, v/v, isocratic mode). Five thymol derivatives were isolated from the selected fractions. Two of them: 7,10-diisobutyryloxy-8,9-epoxythymol isobutyrate and 8-hydroxy-9,10-diisobutyryloxythymol – the compound of antimicrobial and moderate anti-inflammatory activity were formerly described from other taxa of the Inulae tribe [3-6]. To our knowledge, the three remaining compounds are new thymol derivatives: 10-isobutyryloxy-7-isovaleryloxy-8,9-epoxythymol isobutyrate (**1**), 8-hydroxy-9,10-diisovaleryloxythymol (**2**) and 8-hydroxy-9-isobutyryloxy-10-isovaleryloxythymol (**3**).



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EVALUATION OF THE ANTI-SEIZURE ACTIVITY OF ARTEMISIA SPECIES IN A ZEBRAFISH EPILEPSY MODEL

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Epilepsy is a chronic neurological disease characterized by recurrent seizures that affects around 50 million people worldwide. In 40% of the cases patients have seizures despite medical therapy. Additional therapeutic options are therefore needed for treatment-resistant epilepsy patients. An attractive source of drug-like, neuroactive small molecules for anti-epileptic drug discovery are medicinal plants used in traditional medicine for the treatment of epilepsy

Artemisia (Asteraceae) is a genus that consist about 500 species occurring throughout the world, several of which are used traditionally in Chinese, Iranian and Lebanese medicine for the treatment of a spectrum of diseases such as malaria, hepatitis, cancer and epilepsy . Plants of this genus are known to contain terpenoids, sesquiterpene lactones, coumarins, flavonoids, and sterols, some of which may exhibit anti-seizure activity. Previous studies on rodents showed that some species of Artemisia such as *A. annua*, *A. dracunculus* inhibit clonic seizures induced by the GABAA antagonist pentylenetetrazol (PTZ).

In this study, using a zebrafish seizure model based on acute exposure of larvae to PTZ, we investigated different extracts of the Artemisia genus with respect to their anti-seizure activity. Zebrafish larvae were incubated for 18 hours with plant extracts and analyzed by an automated video tracking system for the quantification of their locomotor activity and seizure-like behavior, before and after the addition of seizure-inducing PTZ. Using this assay, we have investigated 12 extracts of Artemisia species to date that were obtained using different solvents such as ethanol and dichloromethane. So far only the ethanolic extract of *A. abrotanum* in concentrations of 60-70 µg/ml decreased PTZ-induced seizures by 40-50%, compared to untreated controls. This Artemisia extract decrease seizures almost as well as valproic acid, an approved anti-seizure drug, used in this animal model. Further steps of this study will be the isolation and identification of the main compounds of these extracts that are responsible for the anti-seizure activity.

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HPLC-DAD-ESI-Q-TOF-MS ANALYSIS OF TWO *HAPLOPHYLLUM* SPECIES (*H. VULCANICUM* AND *H. SAHINII*) AND THEIR ANTICHOLINESTERASE ACTIVITY

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The genus *Haplophyllum* (Rutaceae) comprises about 70 perennial herbs, mostly distributed around Mediterranean region of Europe and through western Asia, up to Siberia [1]. The aerial parts of *Haplophyllum* species are used in folk medicine for treating eye infections, rheumatic pain, herpes, warts, skin diseases and cancer [2]. Previous works on *Haplophyllum* species have revealed the presence of numerous aromatic compounds, such as lignans, coumarins, flavonoids, and alkaloids [3]. The aim of this work was to investigate the phytochemical profile of *H. vulcanicum* Boiss. et Heldr. and *H. sahinii* Tugay et Ulku□, two endemic species to Turkey, as well as to evaluate their anticholinesterase activity, which is relevant to Alzheimer's disease as the most common treatment strategy at the moment.

HPLC-DAD-ESI-Q-TOF-MS analysis of the extracts from flowers, roots and stems revealed the presence of furoquinoline (β-fagarine, γ-fagarine) and amide (tubasenicine, tubacetine) alkaloids; furano- (rutamarin), pyrano (xanthyletine) and oxygeranylated coumarins; phenylpropanoid (secoisolariciresinol), arylnaphthalene (mono-*O*-acetyl-diphyllin apioside) and dibenzylbutyrolactone (kusunokinin, haplomyrfolin) lignans. Several important differences between analyzed samples were observed: β-fagarine was the major alkaloid in *H. vulcanicum*, whereas γ-fagarine was present only in the roots of both *Haplophyllum* species; moreover, secoisolariciresinol and secoisolariciresinol dimethyl ether were the main lignans in stems and flowers. All six extracts displayed remarkable inhibition towards BChE over 50% (ranging between 51.05 ± 5.76% and 79.05 ± 4.34%), tested at 200 μg/mL. Considering AChE, only the root extract of *H. vulcanicum* exerted inhibition over 50% (50.71 ± 5.50%). Therefore, *H. vulcanicum* and *H. sahinii* seem to contain promising cholinesterase inhibitors.

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GC-MS ANALYSIS OF *TAMUS EDULIS* LEAF AND TUBER EXTRACTS. I. NON-GLYCOSYLATED STEROIDS

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Tamus edulis Lowe, known as norça, is a member of Dioscoreaceae family native only to Canary Islands and Madeira. It is a liana growing to 4 m tall, with a tuber edible after processing. Tubers and young shoots of *T. edulis* were used traditionally for nourishment and as herbal medicine. The aim of the present study was to determine the triterpenoids occurring in a free, non-glycosylated form in leaves and tubers of this plant. Samples were collected in September 2017 in Madeira. Plant material was dried, homogenized and extracted with diethyl ether in Soxhlet apparathus. Obtained extracts were separated by preparative TLC on SiO_2 in a solvent system $CHCl_3$/MeOH (97:3 v/v) and the fractions containing free triterpenoids were directly analyzed by GC-MS/FID. The GC separation was made on HP-5MS UI, 30 m x 0.25 mm, 0.25 µm film column in the temperature programme: initial temperature of 160°C held for 2 min, increased to 280°C at 5°C/1 min and the final temperature of 280°C held for further 44 min with helium flow rate of 1 ml min^{-1}. Several sterols, i.e. dominating sitosterol (accompanied by saturated derivative, sitostanol) followed by stigmasterol, isofucosterol, campesterol, cholesterol and several steroids, i.e. cycloartanol and its acetate, 24-methylene-cycloartanol, 9,19-cyclolanost-24-en-3-ol, two derivatives of cholest-7-en-3-ol (2,2-dimethyl- and 4-methyl-cholest-7-en-3-ol) and two ketones, tremulone and sitostanone, were identified in the analyzed extracts. Small amounts of pentacyclic triterpenoids, i.e. α- and β-amyrins were detected exclusively in tuber extract. As in the previous report [1], no free form of diosgenin, the aglycone of the saponin dioscin typical for numerous *Dioscoreaceae* species, was detected in *T. edulis* samples analyzed in this study.

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GC-MS ANALYSIS OF *TAMUS EDULIS* LEAF AND TUBER EXTRACTS. II. AGLYCONES OF TRITERPENOID GLYCOSIDES

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Leaves and tubers of *Tamus edulis* Lowe (norça), an endemic plant occurring naturally in Canary Islands and Madeira, were extracted first with diethyl ether, and afterwards with methanol in Soxhlet apparathus. Obtained methanolic extracts were subjected to acid hydrolysis by boiling under reflux with 10% hydrochloric acid in methanol for 120 min. The aglycones liberated from glycosides were extracted from hydrolysates with diethyl ether. The conditions of subsequent chromatographic separation and GC-MS analysis were described previously [abstract: GC-MS analysis of *Tamus edulis* leaf and tuber extracts I. Non-glycosylated steroids]. From several steroids detected in free forms in diethyl ether extracts, in hydrolyzed methanolic extracts only cholesterol and sitosterol were found. The prevailing compounds identified in the fractions of aglycones were diosgenin, (25*R*)-spirost-5-en-3-ol, and its isomer, yamogenin, (25*S*)-spirost-5-en-3-ol. They were found in both analyzed extracts, pointing to the occurrence of significant amounts of steroid saponins not only in the tubers, what is typical for Dioscoraceae plants, but also in the leaves of *T. edulis*. The occurrence of several structurally related steroidal saponins in leaves and twigs of *T. edulis* was reported before [1,2], but only very small amounts of sapogenins other than diosgenin and yamogenin were detected in the present study.

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GC-MS PROFILING OF TRITERPENOIDS FROM CUTICULAR WAXES OF KIWIFRUIT (*ACTINIDIA DELICIOSA* AND *ACTINIDIA ARGUTA*) VARIETIES

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Kiwifruit (often abbreviated as kiwi) or Chinese gooseberry is the edible berry of several species of woody vines in the genus Actinidia. The most common kiwifruit is the “fuzzy” kiwifruit from the species *A. deliciosa*. Another species gaining a growing popularity is hardy kiwifruit *A. arguta*, bearing smaller fruits with thin smooth skin. They are referred to as mini kiwi, kiwi berry or baby kiwi. The aim of this study was the comparison of triterpenoid profile of surface waxes of *A deliciosa* kiwifruit var. Hayward (origin: Chile) and *A. arguta* mini kiwi var. Geneva and Weiki (origin: Poland). Waxes were extracted by immersion in chloroform for 1 minute, the extracts were fractionated by preparative TLC and the obtained fractions were analyzed by GC-MS (the fraction of neutral triterpenoids directly, triterpene acids after methylation). In *A. deliciosa* kiwifruit wax the typical steroids (campesterol, sitosterol, stigmasterol, cholesterol and the two ketones: sitostanone and tremulone) were identified. The composition of neutral pentacyclic triterpenoids included alcohols and ketones of lupane, oleanane, ursane and D:A-fridooleanane skeletons (lupeol, lupenone, β- and α-amyrins, friedelinol and friedelin). The fraction of acids was represented by oleanolic and ursolic acids and their acetates. The total amount of triterpenoids in *A. deliciosa* fruit wax was relatively low (68 µg/mg wax extract), whereas in both *A. arguta* mini kiwi varieties the triterpenoid content was remarkably higher (105 and 115 µg/mg in Geneva and Weiki, respectively). Triterpenoids of D:A-fridooleanane skeletons were also found in mini kiwi, in Geneva variety only friedelin, whereas in Weiki friedelin, friedelinol and epifriedelin. However, the detailed profile of the pentacyclic triterpenoids was richer in Geneva than in Weiki, because lupenone, lupeol and its acetate were detected in Geneva variety (and not in Weiki), moreover, exclusively in Geneva also two other compounds were identified (not occurring in previously tested *A. deliciosa* variety Hayward), i.e. moretenol and taraxasterol, the latter accompanied by its acetate. In turn, fruit waxes of both mini kiwi varieties did not contain any triterpene acids. Thus, our results revealed the occurrence of several qualitative and quantitative differences not only between *A. deliciosa* and *A. arguta*, but also among tested mini kiwi varieties.

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THE PROFILE OF ANTHOCYANINS METABOLITES IN HUMAN AND SHEEP AFTER CHOKEBERRY INTAKE IN THE CONTEXT OF PROTECTIVE EFFECTS ON NERVE CELLS

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A central nervous disorders are closely associated with oxidative stress in nerve cells. Anthocyanins, the common natural pigments with recognized strong antioxidant properties which are consumed with foods of plant origin may favourably influence on nerve cells. It is not known whether anthocyanins and their metabolites present in the physiological fluids after consumption foods rich in these natural pigments are able to permeate the brain barriers and at the site of potential impact achieving a sufficiently high level to play a protective role toward the nerve cells. In addition, it is difficult to investigate this process on the human model. Therefore, the aim of the study was to compare the profile of anthocyanins of chokeberry and their metabolites in human [1] and sheep in context of permeation through the blood-cerebrospinal fluid barrier.
The formulations of chokeberry rich in anthocyanins were the plant materials. Study was performed on ovine model that allowed for repeated sampling of blood samples from the jugular vein and urine after administration of the preparations of chokeberry via intra-rumen intubation [2]. Anthocyanins and their metabolites in biological samples were measured by HPLC-MS/MS method [3].
Cyanidin-3-galactoside which covered 70% of the total content of anthocyanins in chokeberry was a predominant cyanidin derivative found in this berry. In the physiological fluids collected, native forms as well as methylated, glucuronided and combined derivatives of cyanidin were found. This indicate that the profile of anthocyanins in biological fluids of sheep is similar to that found in human which shows that the ovine model can be used for the study of anthocyanins permeation through the blood-cerebrospinal fluid barrier in the context of protective effects of these pigments on nerve cells.

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COMPARISON OF THE ESSENTIAL OIL COMPOSITION OF SELECTED *HEMEROCALLIS* CULTIVARS

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Hemerocallis cultivars belong to the Asphodelaceae family and they are important perennial ornamental plants with large flowers and long flowering duration [1]. Phytochemical studies conducted on various organs of *Hemerocallis* have revealed the presence of flavones, anthraquinones, steroidal saponins, lactams, and carotenoids [2-4]. Numerous horticultural cultivars of *Hemerocallis* have been used to treat diseases such as insomnia, inflammation and depression [5,6], and as a vegetable in eastern Asia [3].

Taking into consideration the fact that the volatile compounds in *Hemerocallis* cultivars have not been investigated to date, we decided to study the composition of the essential oil from the aerial parts of ten varieties collecting from Botanical Garden in Lublin (Poland). The essential oils, obtained by hydrodistillation, were analyzed by GC and GC/MS. The GC and GC-MS methods resulted in identification of about 50 volatile compounds comprising from 97.5%–100.0% of the total amount. The essential oils differed in their composition. Two major groups of the constituent were monoterpene hydrocarbons and their oxygenated compounds. The main component was monoterpene cyclic ether 1,8-cineole (eucalyptol).

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SECONDARY METABOLITES FROM THE AERIAL PARTS OF *IMPATIENS GLANDULIFERA* AND THEIR ANTIOXIDANT ACTIVITY

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The genus *Impatiens*, belonging to the Balsaminaceae family, encompasses more than 480 accepted species [1]. *I. glandulifera* Royle (Himalayan balsam) is perennial plant growing in riparian zones along rivers on humid soils and in wet woodlands [2]. The species is among the invasive plants originally native to Asia that is rapidly spreading across Europe. In Poland, this is one of the top 20 invasive alien plants [3]. Phytochemical studies conducted on various organs of *Impatiens* have revealed the presence of quinones, flavonoids, phenolic acids, anthocyanins, coumarins, saponins, phytosteroids, and essential oils [4-7].

In our previous study, we confirmed that the extracts from species of *Impatiens*, especially *I. balfourii* Hook.f., *I. glandulifera* and *I. parviflora*, contained significant amounts of phenols and flavonoids and have interesting multidirectional biological activity, such as antimicrobial and antioxidant abilities [4].

The present study deals with the isolation and structure elucidation of ten compounds from the aerial parts of *I. glandulifera*, which were collected from natural state in Józefów near Biłgoraj (Poland).

Seven flavonoids, eriodyctiol, eriodyctiol 7-*O*-β-glucoside, kaempferol 3-*O*-β-glucoside, kaempferol 3-*O*-β-galactoside, kaempferol 3-rhamnosyl-di-glucoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-β-galactoside, two phenolic acids – p-hydroxybenzoic acid and protocatechuic acid, and 2-methoxynaphthalene-1,4-dione, were isolated. The structures of these compounds were established by analysis of their spectroscopic (1H and 13C NMR) and spectrometric (MS) data, as well as by comparison of these with those reported in the literature. In addition the antioxidant activities in different tests of selected compounds were evaluated.

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SCHISANDRA RUBRIFLORA, SOIL-GROWN PLANTS AND ESTABLISHED *IN VITRO* MICROSHOOT CULTURES, AS A POTENTIAL SOURCE OF THERAPEUTICALLY VALUABLE DIBENZOCYCLOOCTADIENE LIGNANS – UPLC-MS/MS AND LC-DAD DETECTION AND QUANTIFICATION

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Schisandra chinensis it is pharmacopoeial plant species in Asia, Europe and USA derived from TCM with well documented e.g. adaptogenic, hepatoprotective, immunostimulant and anticancer properties. They are attributed to dibenzocyclooctadiene lignans, called the 'schisandra lignans' (SL) [1].
Schisandra rubriflora is dioecious, endemic species, grown in Southwest China, recently, successfully, commercially cultivated in Middle Europe (in Poland). There are only poor information about its chemical composition [2].
The aim of the study was the analysis of SL in: fruit, leaf and shoot extracts of male (♂) and female (♀) plants collected in Poland using UPLC – MS/MS and LC-DAD [3]. Moreover the initiation of *in vitro* cultures and evaluation of SL contents in microshoot lines (♂,♀) growing on agar MS [4] medium supplemented with 1 mg/l BA and 1 mg/l IBA was performed.
In the all analyzed methanolic extracts the presence of ten SL was confirmed. The total contents (mg/100 g DW) of SL were as follows: 323.01 in fruits, 102.87♂ and 188.34♀ in leaves, and 105.40♂ and 142.95♀ in shoots. The main compounds were: schisantherin A, B and schisanthenol, max. 83.35, 22.38 and 18.94 (mg/100 g DW), respectively. The total content of SL in microshoot extracts were equal 156.26 and 142.70 (mg/100 g DW), ♂ and ♀ lines, respectively. The main SL were: schisandrin, schisanthenol, deoxyschisandrin and gomisin A max. 38.25, 29.15, 23.09 and 18.64 (mg/100 g DW), respectively.
Our results documented for the first time, that both, *S. rubriflora* soil-grown plants and microshoots cultured *in vitro,* could be a rich potential source of SL, compounds with high medicinal value.

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PRODUCTION OF *SCHISANDRA* CHINENSIS CV. SADOVA LIGNANS IN PLANTFORM BIOREACTOR AND THEIR SIMULTANEOUSLY QUANTIFICATION BY LC-DAD METHOD

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Schisandra chinensis is the valuable Far-East medicinal plant species; has been used in official European therapy since 2008. Schisandra fruit extract shows, among others hepatoregenerative, adaptogenic, antioxidant and antitumor properties. The main constituents of extracts are dibenzocyclooctadiene lignans (SL) [1]. The biotechnological possibilities of production of SL in vitro cultures cultivated in different types of bioreactors, had been documented by us earlier [2]. The aim of the present study was to investigate the accumulation SL in *in vitro* cultures of Ukrainian cultivar - *S. chinensis* cv. Sadova No. 1 maintained in Plantform bioreactor.
Shoot cultures were maintained on Murashige-Skoog [3] medium supplemented with 3 mg/l BA and 1 mg/l NAA for 30 days growth periods. In the methanolic extracts, from lyophilized biomasses and media, identification and quantification of fourteen SL were performed by LC-DAD method [4, 5].
The principal metabolites were: schisandrin (max. 115.34 mg/100 g DW), schisantherin B (max. 35.64 mg/100 g DW), angeloylgomisin H (max. 30.03 mg/100 g DW) and gomisin A (max. 27.85 mg/100g DW). The total content of SL was equal 313.51 mg/100 g DW. Extracts from the culture media were found to contain only trace amounts of SL (< 0.1 mg/l). The applied, validated [5] LC-DAD method was effective for separation and quantification of studied SL. The established systems should be considered in the future as a plausible, alternative way of receiving SL.

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CHALLENGES IN QUANTIFYING POLYCYCLIC AROMATIC HYDROCARBONS FROM PLANT MATRICES

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants that raise environmental concerns due to their toxicity. Their metabolites induce mutations in oncogenes and act as tumor promoters. PAHs are primarily found in natural sources like creosote. They are also formed geologically during the chemical transformation of organic matter into fossil fuels. Another source of PAHs is the incomplete combustion of organic matter. Therefore, PAHs are omnipresent in the environment. Due to their importance, the European commission set maximum residue values for benzo[a] pyrene and a sumparameter of benzo[a]pyrene, benz[a]antracene, benzo[b]fluoranthen and chrysene in food, herbs and dietary supplements. In the near future also phytopharmaceuticals will receive maximum residue values. Therefore, an accurate quantification method is mandatory whose performance relies on an efficient extraction and a selective enrichment procedure.
Challenges are efficient and fast extraction, low concentrations in the ppb range and high complexity of the matrix. The poster will deal with GC-MS and HPLC-FLD measurement methods of PAHs and a comparision of performance of these two. Another main topic is the efficient enrichment with highly efficient SPE sorbents especially synthesized for the enrichment of PAHs.

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CYTOTOXICITY AND ANTIVIRAL ACTIVITY OF 14-ACETOXYBADRAKEMIN AND UMBELLIPRENIN ISOLATED FROM *HEPTAPTERA ANISOPTERA* (DC.) TUTIN

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In the presented research the in vitro cytotoxicity and antiviral activity of 14-acetoxybadrakemin and umbelliprenin isolated from *Heptaptera anisoptera* was evaluated. Cytotoxicity was tested using normal (VERO) and cancer (FaDu and SCC-25) cell lines. The antiviral activity was assessed towards HHV-1 (Human Herpesvirus type 1) propagated in VERO cell line.

Heptaptera anisoptera was collected from the Kahramanmaraş province of Turkey. Air-dried and coarsely powdered roots of *H. anisoptera* was extracted with dichloromethane at room temperature. The dichloromethane extract was concentrated in a rotary evaporator under reduced pressure and subjected to chromatographic separations using a Sephadex LH-20 column and then preparative thin-layer chromatography. Structures of the purified compounds were elucidated by spectroscopic methods.

The cytotoxicty was tested using MTT assay and expressed as CC_{50} value, which is the concentration decreasing the viability of the cells by 50%. For antiviral assays, tested substances in non-toxic concentrations were incubated with HHV-1 infected Vero cell line until the cytopathic effect (CPE) was observed in the positive control.

The 14-acetoxybadrakemin showed higher toxicity towards all tested cell lines than umbelliprenin. In case of 14-acetoxybadrakemin the CC_{50} values were similar for VERO (17.95 µg/ml) and FaDu (18.34 µg/ml) cells and higher for SCC-25 (24.88 µg/ml). Whereas, the umbelliprenin showed varied toxicity towards VERO, FaDu and SCC-25 with CC_{50} of 53.88, 25.18 and 78,43 µg/ml, respectively.

Antiviral activity against HHV-1 was tested for 14-acetoxybadrakemin (15.62 and 7.81 µg/ml) and umbelliprenin (31.25 and 15.62 µg/ml). Both tested coumarins inhibited the formation of CPE in HHV-1 infected VERO cells.

Umbelliprenin showed selective toxicity towards FaDu cells suggesting possible anticancer properties, therefore future studies will involve the influence on other types of cancer cells. Furthermore, the end-point dilution assay will be used to assess the reduction of the viral titre in analysed samples.

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CYTOTOXICITY OF PTERYXIN AND HYUGANIN C ISOLATED FROM *MUTELLINA PURPUREA* (APIACEAE)

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The *in vitro* cytotoxicity of pteryxin and hyuganin C isolated from *Mutellina purpurea* (Poir.) Reduron, Charpin & Pimenov (Apiaceae) was evaluated. Cytotoxicity was tested using VERO (ECACC, No. 84113001, green monkey kidney), FaDu (ATCC, HTB-43, hypopharyngeal squamous cell carcinoma) and SCC-25 (ATCC, CRL-1628, tongue squamous cell carcinoma). FaDu and SCC-25 are commonly used for testing biologically active small molecules in the development of drugs with potential application in the treatment of head and neck squamous cell carcinomas (HNSCC).
Petroleum ether extract from dried fruits of *Mutellina purpurea* was subjected to separation using high-performance countercurrent chromatograph (Dynamic Extraction Co., Ltd.) equipped with a Sapphire UV detector and Alpha 10 pump (ECOM). Compound isolation was carried out in the reverse mode at the preparative scale at 30°C. Identification of the isolated compounds was performed by HPLC coupled with DAD and ESI TOF-MS (Agilent G 6210 MSD TOF), as well as ESI QTOF-MS (Agilent 6530B QTOF).
Isolated compounds were dissolved in DMSO to obtain stock solutions (50 µg/ml). Stock solutions were further diluted with culture media containing 2% FBS to obtain series of dilutions used for in vitro studies. A semi-confluent monolayers seeded in 96-well plates were incubated (37°C, 5% CO_2) with serial dilutions of tested samples for 72 hours. Subsequently, the MTT tetrazolium method was used to assess the viability of cells and CC_{50} values (concentration decreasing the viability of the cells by 50%) were calculated. *Mutellina purpurea* crude extract showed higher toxicity on SCC-25 cells (CC_{50} 107.08 µg/ml) than on VERO (CC_{50} 133.7 µg/ml) or FaDu (CC_{50} 165.3 µg/ml) cells. The hyuganin C showed higher toxicity towards all tested cell lines than pteryxin. In case of hyuganin C the CC_{50} values were comparable for all tested cell lines (VERO 13.52 µg/ml, FaDu 12.81 µg/ml and SCC-25 10.41 µg/ml). Whereas, the pteryxin showed similar toxicity on VERO (CC_{50} 47.56 µg/ml) and FaDu (CC_{50} 50.13 µg/ml) and higher on SCC-25 with CC_{50} of 33.43 µg/ml.

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DETERMİNATION Of ESSENTIAL OIL COMPOSITION, TOTAL ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF ANATOLIAN SAGE (*Salvia fruticosa* Mill.) POPULATIONS IN MARMARA REGION IN TURKEY

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The genus *Salvia* L. is the largest genus in the Lamiaceae, comprising nearly 1000 species. *Salvia* L. has radiated extensively in three regions of the world, Central and South America (500 spp.), West (200 spp.) and East Asia (100spp.) [1]. This genus is represented, in Turkish flora, by 99 species and 14 subspecies totally 113 taxa, 58 of which are endemic [2].
Salvia L. species contain various secondary metabolites such as sterols, flavonoids, sesquiterpenoids, sesterpenoids, diterpenoids, triterpenoids, essential oils, and flavonoids [3,4].
This study was carried out determining the essential oil yield and composition, total antioxidant activity, total phenolic and flavonoid contents of the Anatolian sage (*Salvia fruticosa* Mill.) Populations distributed in the Marmara Region, Flowering plant samples of 20 populations collected from Tekirdağ and Balikesir provinces were used in the study.
As a result of the study, it was found that the essential oil content was between 2%-3%, the main constituents of essential oil were changed between 20,7-46,9% of 1,8-cineole, 2,8-17,5% of camphor and 5,3-11,3% of β-pinene. The total antioxidant activity according to the samples ranged from 820,00-876,79 (µmol Trolox Equivalent/100 g DM), the total phenolic between 8,47-13,45 (mg GAE/g DM) and the flavonoid content was found between 5,52-7,93 (mg KE/g DM).

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SEPARATION OF FLAVONOIDS AND PHENOLIC ACIDS BY TLC AND PPEC IN REVERSED PHASE SYSTEMS WITH SURFACTANT

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Plants are the source of many secondary metabolites containing a phenolic moiety in their structure. Phenolic acids and flavonoids are examples of such compounds. The former are derivatives of benzoic or cinnamic acids. They possess a considerably simpler structure in comparison with the latter. Whereas the flavonoids usually consist of two benzene rings linked via pyrene ring bearing an oxygen atom (chromane ring). Such molecules may be hydroxylated at various positions. Additionally, sugar residuals can be bounded to these molecules and then flavonoid glycosides are formed.
Flavonoids and phenolic acids often appear in plants simultaneously. They also show beneficial effects on the human body. This fact makes necessary to investigate the content of phenolic acids and flavonoids both in plants extracts as well as in pharmaceutical prescriptions. In our research, we applied planar chromatography (TLC) and pressurized planar electrochromatography (PPEC) to this purpose. The latter technique involves both electrophoretic effect (migration of compounds under the influence of applied potential difference) and partition of substances between stationary and mobile phases. While the mobile phase movement in PPEC is the result of electric field action (electroosmosis). Whereas in TLC separation of the mixture component is the result of differences in their partition between mobile and stationary phases. Whilst the mobile phase movement in planar chromatography is the effect of capillary action.
PPEC compared to traditional planar chromatography (TLC) has a few advantages. These include shortening the duration of the experiment time as well as increasing the efficiency of the system.
In our paper we present features which affect the retention and migration distances of the phenolic acids and flavonoids in PPEC and TLC systems. They are the mobile phase buffer pH, concentration of butanol and surfactant. The optimal composition of the mobile phase is applied for the separation of the mixture of phenolic acids and flavonoids and then separation of plant extracts with both abovementioned techniques.

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SUPERCRITICAL FLUID CHROMATOGRAPHY IN SEPARATION OF BIOACTIVE COMPOUNDS FROM PLANT MATERIALS EXTRACTS

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Supercritical fluid chromatography (SFC) has found numerous applications in food, cosmetics and pharmaceutical industry, due to some advantages of CO_2 in a supercritical state which enables to perform relatively low cost and shortened analyses. It is a separation technique which provides an opportunity to work in a reversed phase and normal phase mode. SFC system is equipped with a back pressure regulator, which keeps CO_2 under supercritical state [1,2]. The characteristic features of CO_2 are critical pressure of 7,4 MPa and low critical temperature of 305,45 K [3].
The aim of the paper is to present the applications of SFC method in a separation of bioactive compounds, such as fat-soluble vitamins, fatty acids, volatile compounds as well as phenolic compounds in selected plant material extracts obtained with the use of supercritical fluid extraction (SFE). The studies were performed using Waters Acquity UPC^2 system equipped with PDA detection on different stationary phases under different conditions to obtained satisfactory results. Recently, UPC^2 system is a successful method for the separation of volatile compounds, which compared to traditional method, such as gas chromatography, allows to perform even several times shorter analysis. Figure 1 presents the chromatogram of *Humulus lupulus* extract in terms of myrcene and α-humulene on HSS C18 SB (100 x 3,0 mm; 1,8µm) stationary phase.

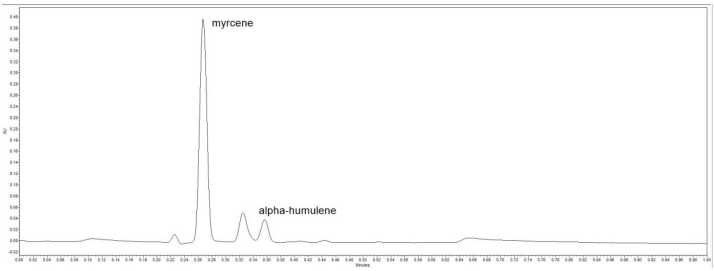

Figure 1. The chromatogram of myrcene and α-humulene by UPC^2 system.

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ROSMARINIC ACID AND SALVIANOLIC ACID B IN SHOOT OF *DRACOCEPHALUM FORRESTII* W. W. SMITH CULTURED IN THE NUTRIENT SPRINKLE BIOREACTOR

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Dracocephalum forrestii (*Lamiaceae*) is plant endemic to northwest region of Yunnan province, China. The plant, important for traditional Tibetan medicine, is widely known as “Quinglan” and usually used as a astringent, antipyretic or diuretic agent. The plant shows significant anti-inflammatory and antioxidant properties.The study focused on production phenolic acids, pronominally rosmarinic acid and salvianolic acid B in a shoot culture of *D. forrestii*.

The shoots were cultivated in the nutrient sprinkle bioreactor in liquid Murashige and Skoog medium with 0.2 mg/l BAP and 0.2 mg/l IAA. The culture period was 4 weeks. The identity of the phenolic compounds present in methanolic extracts from *D. forrestii* shoots was confirmed using UPLC-PDA-ESI-MS method. Analysis was performed using a UHPLC-3000 RS system (Dionex, Germany) with PAD detection and an AmaZon SL ion trap mass spectrometer with ESI interface. Separation was performed on a Zorbax SB C18 column (150 x 2.1 mm, 1.9 µm). Compounds were analyzed in negative ion mode The compounds were identified by comparing their data (retention times, UV-Vis spectra, and MS/MS fragmentation patterns) with those of standards. The content of the phenolic acids in the extracts were determined with UPLC method. Additionally, antioxidant potential of plant material was characterized using *in vitro* spectrophotometric methods (FRAP, DPPH and $O_2^{\bullet-}$ radical scavenging).

After 4 weeks of culture, about 700 shoots of *D. forrestii* were harvested, which gives a multiplication rate about 27 shoots/explants. The biomass of shoots in bioreactor was about 120 g/l FW and 9 g/l DW. The UPLC analysis of methanolic extract revealed the presence of nine compounds identified as caffeic acid derivatives. The highest level of predominant metabolites: rosmarinic acid and salvianolic acid B was about 18 mg/g DW and 6 mg/g DW, respectively. The *D. forrestii* methanolic extract was able to scavenge both DPPH and O_2^{-} radicals with IC_{50} values 0.67 µg/mL and 69.06 µg/mL, respectively. The sample showed FRAP value 1319 µM Fe(II)/g DW of extract.

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ANXIETY-RELATED BEHAVIOURAL RESPONSE TO LIGHT-DARK TRANSITIONS OF *ANGELICA ARCHANGELICA* AND ITS PURE COMPOUNDS FOR POTENTIAL ANXIOLYTIC ACTIVITY USING THE IN VIVO ZEBRAFISH MODEL

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Angelica archangelica is known for centuries for its multidirectional pharmacological properties, resulting mainly from the presence of biologically active compounds, such as coumarins. However, their activities in the central nervous system (CNS) have not, so far, been adequately understood and still remain in study.
The purpose of our experiment was to examine the influence of methanolic extract obtained from fruits of *Angelica archangelica,* as well as pure isolated compounds (imperatorin, xanthotoxin, bergapten) on anxiety-like behaviors in *Danio rerio* larvae. Zebrafish larval behaviour analysis was evaluated using the ZebraBox system manufactured by ViewPoint. Thigmothaxis was used as an index of anxiety. Zebrafish larvae displaying thigmotactic behaviour avoid the centre of an arena (inner zone) and prefer to stay close to the boundaries of a well (outer zone). In the present study continuous light condition and light on/off assay (stressful conditions) was applied to evaluate the influence of extract and pure compounds on thigmotactic behaviours. Moreover, larval locomotion was measured using simple locomotor assay.
Our results indicate that incubation in different concentrations (3-12.5 µg/ml) of extract did not influenced on locomotor behaviors of larvae and thigmothaxis of the larval zebrafish during the light phase whereas at the concentration of 3 µg/ml reduced thigmotactic behaviour during light on/off phases defined as anxiolytic-like behaviours. We also revealed that imperatorin (3-15 µM), xanthotoxin (6-15 µM) and bergapten (60-75 µM) exerts marked anxiolytic profile observed in the *Danio rerio* model of anxiety.
In summary, results obtained in present studies should contribute to explore the coumarins' activity. Anxiolytic effects of coumarins requires further research to determine mechanisms underlying these effects.

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LYCOPODIUM SPECIES AS A SOURCE OF ACETYLCHOLINESTERASE ALKALOIDS – TLC BIOAUTOGRAPHY SCREENING

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One of the most important approaches in treatment of Alzheimer's disease is increasing levels of acetylcholine by inhibition of enzymes hydrolyzing this key neurotransmitter, like acetylcholinesterase (AChE) and butyrylocholinesterase [1].
Plants belonging to *Lycopodium* contain numerous alkaloids, which are potent, reversible and highly selective AChE inhibitors.
Anticholinestrease activity of several species belonging to *Lycopodium* (among them *L. clavatum*, *L. annotinum*, *Huperzia selago*) as well as qualitative analysis of alkaloids, were performed.
The crude plant samples, after drying and pulverizing were extracted using ASE (Accelerated Solvent Extraction) system with different solvents.
Obtained extracts were purified by SPE on Oasis HLB columns and analysed qualitatively in positive ion mode by LC/ESI-QTOF-MS. Analysis was performed using 6530B Accurate-Mass-QTOF-MS (Agilent technologies) on HILIC silica column (d_p=3 µm, 2.1x150 mm) and gradient of acetonitrile (1%) with 10 mM formic acid (0.2 %) (phase A) and acetonitrile (95 %) with 10 mM formic acid (0.2 5) (phase B) as mobile phase.
Acetylcholinesterase inhibition of obtained and analyzed different extracts (methanolic, ethyl acetate, cyclohexane, dichloromethane and methanolic with 1% of tartaric acid) was evaluated by TLC bioautographic assay on thin layer plates covered with silica gel. As a mobile phase $CHCl_3$:MeOH:NH_4OH (70:7:1, v:v:v) system was used. Huperzine A and galanthamine were used as reference compounds.
All tested extracts showed presence of alkaloids belonging to *Lycopodium type* and acetylcholinesterase inhibitory activity.

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ANXIOLYTIC ACTIVITY OF COUMARINS FROM *PEUCEDANUM LUXURIANS* AND *SESELI DEVENYENSE*

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The coumarins are natural compounds widely distributed in plants, which are point of intense interest in the last years, according to their pharmacological activity concerning effects on the central nervous system.
Seseli and *Peucedanum* genera, belonging to *Apiaceae* family are rich in different class of coumarin compounds (pyranocoumarins, furanocoumarins and simple coumarins).
Methanolic and dichloromethane extracts of matured fruits of *S.devenyense* and *P.luxurians* (accelerated solvent extraction – ASE) were submitted to HPCCC chromatography. Application of mixture of *n*-hexane, ethyl acetate, methanol and water 6:5:6:5 (v/v) was used.
The isolation was carried out by high performance counter-current chromatography using a modern HPLCC Dynamic Extraction Spectrophotometer (Slough, UK) equipped with multilayer PTFE analytical and preparative columns (respectively 0.8 mm Ø, 22 mL volume and 1.6 mm Ø, 136 mL volume).
Screening of anxiolytic activity of plant extracts as well as isolated compounds was determined on animal model (five-day larvae of zebrafish), because of it's low cost and ability to perform simple motor tasks [1].
Larvae were placed singly in the well (24-well plate) filled to half the medium or the solution to be tested. The hole was divided into two outer and middle zones. The anxiogenic effect was proved by an extension of time spent in the inner zone in comparison to the control group. Variable lighting was used for anxiety stimulation. Larvae have been placed in tested solution, on a plate 30 minutes before test, which will last 1.5 hours. Diazepam was used in a study as a reference compound (anxiolytic activity).
Results of experiments showed modarate anxiolytic effect of both extracts in in the *Danio rerio* model of anxiety.

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PREPARATION OF FLAVONOID COMPOUNDS AND PHENOLIC ACIDS FROM ROOTS OF SEVERAL *CENTAUREA* L. SPECIES

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Centaurea L. is the fourth largest genus of the family Asteraceae comprising between 400 and 700 species. Members of the genus are found particularly in Mediterranean region and in West Asia [1,2].
The plant materials (roots) of ten Centaurea species: *C. jacea* L.,*C. karabaghensis* (Sosn.) Sosn. (*Psephellus karabaghensis* Sosn.), *C. kotschyana* Heuff., *C. nigra* L., *C. nigrescens* Willd., *C. stoebe* L., *C. nogmovii* (Koss ex Tschuchr) Czerep. (*Psephellus dealbatus* (Willd) K.Koch.), *C. alba* L., *C. biebersteinii* DC, *C. macrocephala* Muss. Puschk. ex Willd. was collected in September 2017, in the Botanical Garden Chair and Dept. of Pharmacognosy with Medicinal Plant Unit, Faculty of Pharmacy, Medical University of Lublin (Poland), and and then dried in the shade and draught and immediately powdered according to accepted normal procedures.
The plant material was extracted by means of ultrasonic extraction with chloroform, methanol and mix methanol-water (7:3 v/v).
The components of extracts (polyphenolic compounds) were identified using high-performance liquid chromatography (HPLC) with DAD detection and LC-QToF method for the identification of the isolated compounds.
Out of the tested extracts, 3 plant substances with the highest content of polyphenols compounds were selected for further research. The fractions with flavonoids and phenolic acids were evaporated at 50^{0}C and purified by use flash chromatography and preparative TLC. Separation by flash chromatography (Isolera One) was performed on reverse phase column (BIOTAGE SNAP 12g, 25g, 60g C_{18}), using stepwise gradient of methanol and water, while prep -TLC method on silica gel plates and *n*-heptane: methylene chloride: ethyl acetate (4:4:2 v/v) – as mobile phase.
Samples were analyzed using the Agilent 1100 liquid chromatograph with diode – array detector (DAD), XDB-C8 column (150 x 4.6 mm I.D., dp = 5µm) and gradient of acetonitrile (B) - water + acetic acid (1%) (A) as mobile phase.

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SEARCHING FOR OPTIMAL EXTRACTION CONDITIONS AND EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF POLYPHENOLIC FRACTIONS OBTAINED FROM *SIDERITIS SCARDICA* GRIESEB.

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Sideritis scardica Grieseb. (Lamiaceae) is one of the medicinal plant species widely distributed and exploited as a curative herb in the Mediterranean region. It is known under the common name “ironwort”. To date, a lot of phytochemical studies have been done that mainly concerned hydrophobic constituents (mono-, sesqui- and diterpenes) occurring in aerial parts of numerous representatives of the *Sideritis* genus [1]. The decoctions of ironwort stems, leaves and flowers have been traditionally used as anti-microbial, anti-inflammatory, gastroprotective, strengthening immunity and even cytotoxic agents [2]. In our study, we decided to optimize the process of obtaining enriched fractions of polyphenolic constituents (PC) and to evaluate their qualitative and quantitative profiles based on the plant material of Turkish origin. For this purpose, dried flowering tops of *S. scardica* shoots were pulverized (0.75 mm) and subjected to the ultrasound-assisted extraction procedure using various extractants (aqueous and ethanolic-aqueous, with the increasing concentration of EtOH), followed by lyophilisation. Then, freeze-dried extracts were standardized using an optimized LC/PDA technique to establish the qualitative profile and the content of bioactive compounds. Three main groups of PC, including flavones (glycosidic derivatives of luteolin), phenylethanoids and phenolic acids have been identified. A very high total content of PC, ranging from 9.8% to 18,3% of dry weight, has been documented for all lyophilisates. The lowest PC concentration was reported for the lyophilisate obtained using water as the extraction solvent and the highest one, for that prepared using 75% (v/v) EtOH. In the second stage of the research, both antioxidant (Folin-Ciocalteu = FCR) and antiradical (DPPH• and ABTS•$^{+}$) assays, using spectrophotometric Vis methods, have been performed to assess a bioprotective potential of standardized extracts in relation to the PC amounts. The results of FCR tests were calculated as gallic acid (GAE) equivalents. For antiradical assays, caffeic acid (CA) and trolox (Tx) were used as standard reference materials. The IC_{50} values obtained in the DPPH□ and ABTS•$^{+}$ free radical assays were highly correlated with each other, as well as with the quantitative results obtained in the phytochemical standardization of the lyophilisates examined. Our research proved that not only water extracts obtained from *S. scardica* but also those prepared using aqueous-alcoholic extractants should be recommended, as rich natural sources of plant antioxidants, to patients suffering from various types of biodegenerative diseases.

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IMPACT OF XANTHAN GUM ADDITION ON PHENOLIC ACIDS COMPOSITION AND SELECTED PROPERTIES OF GLUTEN FREE CORN FIELD BEAN PASTA

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One of the most important dietary product is pasta that is basis of nutrition in many world regions. Taking into account the growing demand on gluten-free products, science and industry make every efforts to extend food asortyment dedicated to people with gluten intolerance. An example can be products based on free-gluten corn. Nevertheless, the absence of gluten results in technological and quality problems. Replacing the gluten network to produce high quality pasta is a great technological challenge. One of used solution is addition of xanthan gum. Nontheless, the influence of the additive on healthy properites and product quality is very important and should be considered. The presented paper is focused on the influence of xanthan gum on phenolics content, antioxidant activity, cooking quality and textural characteristic of gluten-free corn-field bean pasta. The obtained results revealed that 0.25, 0.50 and 0.75 % addition of xanthan gum to the pasta did not have significant influence on phenolic content and antioxidant activity, whereas 1.00 % addition caused reduction of the tested parameters. Slightly different influence of gum on cooking quality and texture characteristic was observed. Addition of xanthan gum in the formulation reduced leaching of components into cooking water and pasta prepared with 1.00 % xanthan gum showed the highest firmness and the lowest adhesiveness. These results revealed significant influence of xanthan gum content on pasta properties.

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PHENOLIC ACID CONTENT AND ANTIOXIDANT ACTIVITY PROPERTIES OF EXTRUDEDCORN SNACKS ENRICHED WITH KALE

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Pro-health food contains specific components which have positive influence on the health and well-being of the consumer. An important position among bioactive compounds occurs for polyphenols. Many results have indicated that an increased intake of phenolic compounds may reduce the risk of cardiovascular diseases and type 2-diabetes. The objective of the study was production of extruded corn snacks with addition (0, 2, 4, 6, 8%) of kale (*Brassica oleracea* L. var. *sabellica)* – polyphenols-rich plant. Afterwards high-performance liquid chromatography-mass spectrometry (LC-ESI-MS/MS) and antioxidant activity analyses of snack's extracts were performed. In the corn snacks enriched with kale fifteen phenolic acids were indicated. These were: protocatechuic, 4-OH-benzoic, vanilic*,* *trans*-caffeic, *cis*-caffeic, *trans*-p-coumaric, *cis*-p-coumaric, *trans*-ferulic, *cis*-ferulic, salicylic, gentisic, syringic, 3-OH-cinnamic, *trans*-sinapic and *cis*-sinapic acids. Both the qualitative and quantitative content of polyphenols increased with the addition of *B. oleracea*.

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HPLC-DAD-ESI-Q-TOF-MS/MS ANALYSIS OF FLAVONOIDS AND PHENOLIC ACIDS FROM *CRATAEGUS PENTAGYNA* WALDST. ET KIT.

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With a long history in preventing or treating cardiovascular pathologies, *Crataegus* species are among the most known medicinal plants in Romania, mainly due to their antioxidant, antiarrhythmic, hypotensive and anti-atherosclerotic effects [1]. However, information on the chemical profile and bioactive compounds of *Crataegus pentagyna* Waldst. Et Kit (Rosaceae), one of the major hawthorn species in southeastern Europe, is still very limited [2].

The aim of this study was to investigate the HPLC-DAD-ESI-Q-TOF-MS profile of methanolic fractions obtained from the ethyl acetate extracts of *C. pentagyna* leaves, flowers and fruits, after Sephadex LH-20 column fractionation. The analyses were carried on a Hypersil ODS C18 (250 ×4.6 mm, 5 µm) column; mobile phase 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B); elution 0% B (0 min), 16% B (25-35 min), 28% B (55 min), 70% B (70 min), 80% B (75-80 min); flow rate 0.5 mL/min; DAD 280 nm; negative ionization mode; *m/z* 50-2000; capillary voltage 4200 V, skimmer: 60 V, N_2 flow rate 7 L/min, gas temperature 220 °C, nebulizer pressure 15 psig, fragmentor 200 V, CID 20 eV.

More than 50 compounds were identified in leaves, flowers and fruits *of C. pentagyna,* such as: organic (malic, citric, vanillic, quinic, and protocatechuic acids) and hydroxycinnamic (chlorogenic, caffeic, ferrulic and coumaric acids) acids, flavones (mainly apigenin and luteolin glycosides), flavonols (quercetin, isorhamentin and kaempferol derivatives), flavanols and polymers (epicatechin, procyanidin dimers). Important flavonols were distributed mainly in leaves and flowers extracts, while organic acids were found predominantly in the fruits. Methanolic fractions from fruits showed the richest secondary metabolite profile.

The qualitative analyses of the methanolic fractions of *C. pentagyna* suggest that they might be a promising source of bioactive phenolic constituents; the bioactivity-guided isolation might enlarge the knowledge of hawthorn pharmacological effects.

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