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ИНСТИТУТ ПО ОРГАНИЧНА ХИМИЯ С ЦЕНТЪР ПО ФИТОХИМИЯ
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ИНСТИТУТ ПО ОРГАНИЧНА ХИМИЯ С ЦЕНТЪР ПО ФИТОХИМИЯ

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ПОСТЕРНИ ДОКЛАДИ

COMPARATIVE STUDY OF THE SURFACE FLAVONOIDS IN TWO *PRIMULA* SPECIES FROM BULGARIA AND SERBIA

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The genus *Primula* is the largest among the Primulaceae and is widespread mainly in the cold and temperate regions of the Northern Hemisphere. In the folk medicine, the rhizome and root (*Primulae* radix) and the flowers (*Primulae* flos) from *P. veris* and *P. elatior* are used in the form of infusion, decoction or tincture as antitussive and mucolytics/secretolytic remedies. The biological activity of *Primula* species is attributed to as triterpene saponins and phenolic compounds, including flavonoids, phenolic acids, and their glycosides. The surface UV protective nonpolar flavones are also characteristic for *Primula* species.
The aim of study was to characterize the surface flavonoids in two *Primula* species - *Primula veris* and *P. acaulis* and to compare their accumulation in the samples collected from their natural habitats in Serbia and Bulgaria. Fresh leaves were rinsed cautiously with acetone in order to extract the surface flavonoids. Further separation of the obtained extracts by CC and/or PTLC led to isolation of 17 individual compounds. Their structure was determined by NMR and MS. The isolated compounds were flavones differing in the nature (hydroxy, methoxy, and methylenedioxy groups) and the place of the substituents the flavone structure. The comparative study of the Bulgarian and Serbian populations of *Primula veris* and *P. acaulis* revealed some differences in their qualitative profile.

Acknowledgements: The authors are thankful to the bilateral project of the Bulgarian Academy of Sciences and the Serbian Academy of Sciences and Arts "Phytochemical and metabolomics investigation of biologically active compounds from *Primula* species and medicinal mushrooms" and the Centre of Competence (BG05M2OP001-1.002-0012) for running NMR spectra.

INVESTIGATION OF PURSLANE (*PORTULACA OLERACE*A L.) SEED OIL: TRIACYLGLYCEROLS AND STEROLS AS MAIN BIOACTIVE COMPONENTS

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Purslane (*Portulaca oleracea* L.) is a succulent, annual plant of the *Portulacaceae* family which is native to South America and Africa, but nowadays grows in many regions, thus being among the most widely distributed plants in the world. The oil from its seeds has an anti-diabetic effect and reduces the levels of bad cholesterol (LDL) in the blood.

Triacylglycerols (TAG) are a major source of energy in living organisms, and sterols (as free and esterified, St) are important components of cell membranes. These three classes of neutral lipids are main for purslane seed oil. For that reason we aimed to isolate TAG, free (St) and esterified sterols (SE) from oils obtained by four different extraction methods (using hexane, SuperCritical-CO_2 and two chloroform-methanol mixtures) and determine respectively, their fatty acid and sterol composition. For this purpose, after extractions the oils were subjected to Thin layer chromatography on silica gel G for separation, isolation and gravimetric determination of triacylglycerols, sterols and sterol esters. After TAG transmethylation and SE hydrolysis, the fatty acid and sterol compositions were determined by gas chromatography. Sixteen fatty acids in TAG and SE were identified and quantified, the main ones being essential α-linolenic acid (cis-9,12,15-18:3) - 40 and 48% and linoleic acid (cis-9,12-18:2) - 33 and 28%, respectively. Nine sterols in the St class and 13 in StE were identified and quantified, the main ones being sitosterol (77 and 48%, respectively) and campesterol (10 and 20%, respectively). Twenty molecular species of TAG were also determined, the main one being LnLnL (dilinolenoyl-linoleoyl-glycerol) – 30%.

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EXPLORING THE DEPTHS OF WHEAT BEERS VIA NMR PROFILING

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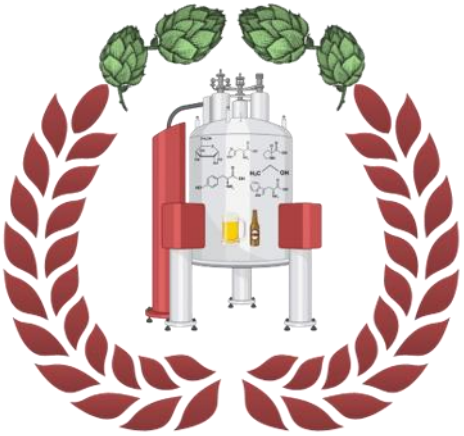
Wheat beer is a top-fermented drink that is often characterised by the use of a higher proportion of wheat compared to malted barley. The most popular varieties of the beverage are German Weißbier and Belgian Witbier, with each having its own features – from the deep-yellow colour and hazy taste to the addition of coriander and orange peel in the Flemish beer.
This study aims to determine the chemical profile of different wheat beer styles, focusing on Weißbier and Witbier. ^{1}H NMR spectroscopy was used to analyse typical German wheat ales, including Hefeweizen, known for its yeast sediment, Dunkelweizen – a dark wheat beer and Kristalweizen, known for its clarity as a result of filtration. Statistical methods (OPLS-DA and Nightingale's diagram) were applied to classify and differentiate each style based on their chemical composition and country of origin.



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MICROFLUIDIC APPROACH FOR THE SYNTHESIS OF MONODISPERSE SILICA MICROSPHERES

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New generation of medications based on peptides and proteins are taking an increasing share in the drug market. Unfortunately, the complexity of these molecules makes their production extremely expensive. The development of synthesis procedures for monodisperse functionalized silica microspheres for highly selective purification of proteins is promising alternative to reduce the price of the manufacturing process and to widen the accessibility of highly efficient medications. The expected performance of these microspheres will be linked to: 1) narrow particle size distribution; 2) suitable porosity allowing diffusion and adsorption of proteins; 3) excellent mechanical stability, and 4) easy functionalization by grafting specific groups with selective affinity to the proteins. Classical synthesis approaches as batch reactions were proved as not suitable to obtain micron-size particles with narrow size distribution. Innovative alternative to solve this problem is given by the microfluidic synthesis approach. In this work, as a synthesis method is applied microfluidic technique, allowing to obtain monodisperse spherical drops in emulsions. The optimal operational conditions were studied. The results for microfluidic synthesis of silica showed formation of spherical drops with size around 200 μm, with short gelation time. The drying of these gel spheres leads to solidification and shrinking of the particles to 90 μm. Considering the obtained results, it can be concluded that the microfluidic approach is suitable method to produce monodisperse silica microspheres, with promising application as adsorbents in purification processes.

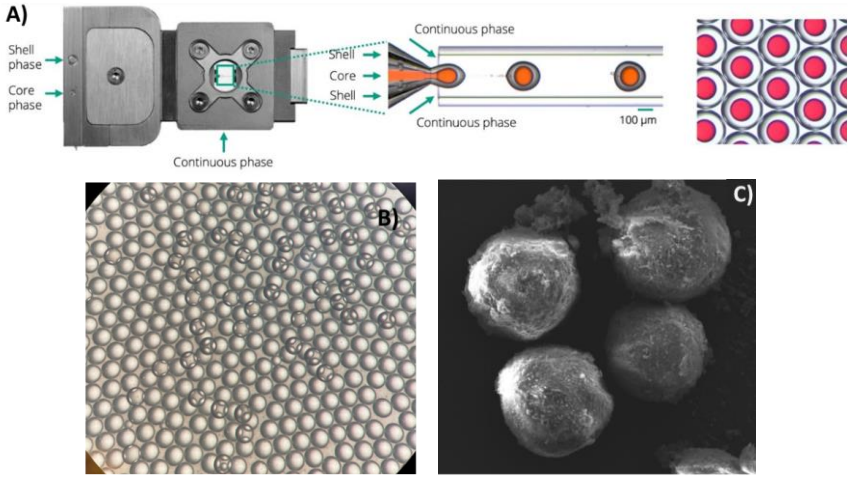

Fig. 1 The Raydrop® microfluidic device (A), just prepared gel (B) and solid silica spheres (C)

Acknowledgments: The authors are thankful to Project MicroSpheres in framework of the BEWARE Fellowships programme, funded by the Marie Skłodowska-Curie Actions of the European Commission and the Public Service of Wallonia Research and Project № КП-06-ДБ/3 “Development of Sustainable Silica Adsorbents for Separation of Naturally Derived Bioactive Molecules” in the frame of National Scientific Program “Petar Beron i NIE” funded by The Bulgarian National Science Fund for the financial support.

SYNTHESIS, STRUCTURE AND SPECTROSCOPIC STUDIES OF 2-ARYL SUBSTITUTED BENZIMIDAZOLE DERIVATIVES WITH POTENTIAL PHOTO-PROTECTIVE EFFECT

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The benzimidazole core might efficiently serve as lead structure for the design of sunscreens based on the good UV filtering capacities of 2-phenyl1H-benzimidazole-5-sulfonic acid (PBSA) and disodium salt of phenyl-dibenzimidazole-tetrasulfonate (Neo Heliopan) - benzimidazole-based compounds already used in cosmetics for sun protection [1]. Some 2-aryl benzimidazole compounds are reported to show antibacterial activity [2] which might be favorably combined with UV filtering properties for potential use in sun protection products.

In this context, our efforts were focused on the synthesis of a series of 2-aryl benzimidazoles containing nitro and benzoyl substituents in the benzimidazole core and respectively hydroxyl, methoxy and nitro substituents in the phenyl ring. The synthesis goes through reaction of condensation of the corresponding substituted phenylenediamine with respectively substituted benzaldehyde. The structure of the obtained 2-aryl benzimidazoles was confirmed with IR and NMR spectroscopy. The filtering capacity of the compounds was evaluated by UV-VIS spectroscopy in methanol solution. The molecular structure and electronic characteristics of the compounds were studied by quantum-chemical computations.

Acknowledgments: This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme “Young scientists and postdoctoral students -2” approved by DCM 206 / 07.04.2022

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OCTA-SUBSTITUTED Ga(III)- or Zn(II)-PHTHALOCYANINES' PHOTODYNAMIC ACTION PROMOTED BY A SERINE PROTEASE α-CHYMOTRYPSIN

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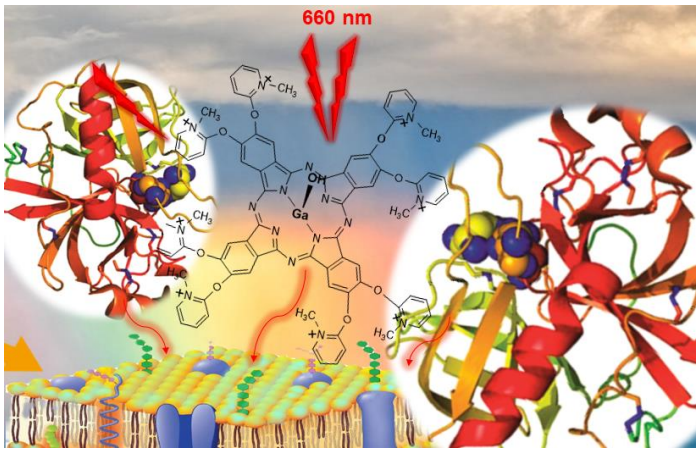
A serine protease α-chymotrypsin (ChT) is known with its curative properties and the potential anticancer effect [1]. The photodynamic efficiency of two octa-substituted Ga(III)- or Zn(II)-phthalocyanines (GaPc2 and ZnPc2) was studied on pigmented melanoma cells versus normal keratinocyte cells (SH-4 vs. HaCaT). Both complexes were prepared following a known synthetic scheme for quaternized compounds. The formation of molecular conjugates with ChT was studied by absorption and fluorescence changes following a titration method. A full inhibition of ChT proteolytic activity was studied as a result of GaPc2 and less for ZnPc2 at 660 nm irradiation. The application of UV-C (185 nm and 254 nm) reduced ChT activity higher then in the presence of GaPc2. The compounds were evaluated as photo-safe on embryonal BALB 3T3 cell line at solar exposure with an exception of ZnPc2 which showed a high factor (PIF~6). Photodynamic efficacy showed no selectivity for GaPc2 and ZnPc2 applied individually. However, the addition of ChT changed the photo- and dark toxicities showing almost double phototherapeutic index (PI) as for example is for GaPc2+ChT (PI=1.71) on SH-4 cells. The positive effect of ChT' was observed also in lower dark toxicity of GaPc2 (PI=1.33) as for ZnPc2 (1.27) on HaCaT cells. These findings suggested that the incorporation of GaPc2 or ZnPc2 into an enzyme such as ChT might have positive impact on the toxicity and PDT efficacy in direction of acting together as a combine enzymic and PDT therapeutic approach against harsh to treat melanomas.



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INFLUENCE OF LIGHT AND AUXIN TREATMENTS ON POLYPHENOLCIS ACCUMULATION IN *ARTEMISIA ALBA* TURRA CELL AGGREGATES

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Artemisia alba Turra is an essential oil-bearing shrub, characteristic for the Southeastern parts of Europe. The species is characteristic with the production of essential oils, sesquiterpenoids, flavonoids and phenolic acids which attribute to the anti-inflammatory, antimicrobial, antioxidant and pro-apoptotic activities characteristic for the plant [1].
In vitro cultures of were developed with the purpose to elucidate the effect of different cultivation techniques on the biosynthetic capacity of the plant.
The present work aims at selection of non-differentiated cell aggregates of *Artemisia alba* Turra, cultivated in liquid culture media. Leaf and root explants of the sterile *in vitro* grown stock shoot cultures were compared as starting material for the cell aggregates induction. *In vitro* treatments on the liquid culture lines consisted of comparison of two different auxins (indole-3-butiric acid and 1-naphthylacetic acid), supplemented to one and the same cytokinin (benzyladenine), as well as variation of photoperiod (dark vs. 16 h photoperiod). Polyphenolic productivity, as well as oxidative stress and lipid peroxidation were investigated [2].
Results were indicative that root tissue of the sterile grown shoot cultures of the plant were characterized with lower levels of oxidative stress and lipid peroxidation *in vitro*. This led to the higher potential of polyphenolic production of root induced non-differentiated cell lines as compared with leaf induced ones. Polyphenolics productivity *in vitro* was also stimulated by supplementation with 1-naphthylacetic acid as compared with the indole-3-butiric acid and by photoperiod as compared with dark regime cultivation.

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HPLC PROFILING OF NATURAL DEEP EUTECTIC EXTRACTS FROM COMMON HAWTHORN: A METHOD VALIDATION STUDY

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Recently, there has been significant interest in the utilization of natural deep eutectic solvents (NADESs) for extracting secondary metabolites from raw plant materials. These innovative solvent mixtures are employed not only to achieve a higher yield of pharmacologically active natural substances but also to provide several advantages, including cost-effective production and environmental sustainability [1]. Nevertheless, it is essential to assess the extractant effectiveness by closely monitoring the individual component composition of the obtained plant extract through appropriate and validated methods. For this reason, the present study aims to develop and validate an HPLC-PDA method following ICH guidelines [2] for the quantitative assessment of common hawthorn (*Crataegus monogyna* Jacq.) extracts prepared using both NADESs and conventional extractants. Liquid chromatographic analyses were performed on a Shimadzu Nexera-LC40 XS chromatograph using Restek-Raptor core-shell C18 column. The main derived phenolic compounds, namely chlorogenic acid, hyperoside, vitexin, vitexin-2″-O-rhamnoside, and vitexin-2″-O-(4‴-O-acetyl) rhamnoside were successfully separated with a linear gradient program utilized 0.10 g/L of trichloroacetic acid in water (A) and 40 % of 0.1 g/L trichloroacetic acid in ACN (B). Thus, the compounds' elution occurred over a 90-minute period with a flow rate of 0.25 mL/min. Based on the validation results, the developed method was found to be selective, linear, precise, and sensitive, and successfully profiled individual compounds in both conventional and non-conventional common hawthorn extracts.

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PHYTOCHEMICAL STUDY OF THE ENDEMIC BULGARIAN SPECIES *VERBASCUM URUMOFFII* Stoj. & Acht.

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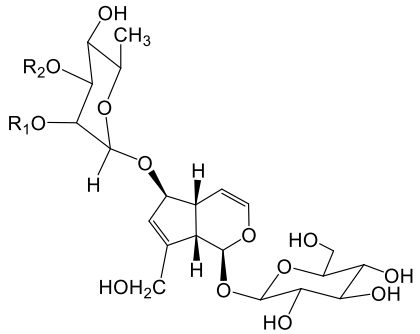
The genus *Verbascum* L. (Mullein) is one of the richest representatives of Scrophulariaceae family. Bulgaria is situated in the zone of speciation of the genus, which has resulted in a considerable number of endemic species, as among the distributed in the country 46 species half are endemic [1]. Mulleins have long tradition of use in folk medicine and are found as a rich source of valuable secondary metabolites. In addition, lack of adequate taxonomic scheme of this genus provides a wide range of research possibilities. *Verbascum urumoffii* is endemic Bulgarian species and its rank as separate species was recently accepted [2]. This rare biennial plant grows mostly in the temperate biome and its area of diversity is Western Bulgaria. The chemical composition of the taxon has never been studied. The aim of this study was phytochemical investigation of methanol extract from aerial parts of *V. urumoffii*. As result main secondary metabolites typical for *Verbascum* ssp. were isolated and identified, namely iridoid glycosides aucubin and its two derivatives, esters of isoferulic acid (Fig.1); triterpene saponins of the monodesmosidic oleanane type – mimengoside B and mulleinsaponin IV; and two flavonoid diglycosides – luteolin-7-O-rutinoside and isorhamnetin-7-O-rutinoside, attached with rutinose moiety. The possibilities and advantages of flash chromatographic techniques were used for separation and purification of positional isomers **1** and **2**.



1 R1 = H, R2= E-isoferuloyl; Nigroside IV
2 R1 = E-isoferuloyl, R2 = H; Nigroside V

Fig.1 Structures of iridoids isolated from *V. urumoffii*

Acknowledgments: This study is funded by the Ministry of Education and Science through the National Research Program "Young Scientists and Postdoctoral Students - 2", RMS 206/ 07.04.2022. For structural elucidation NMR equipment purchased by Project No BG05M2OP001-1.002-0012 was used.

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EXPLORING MEDICINAL FLORA OF THE BALKANS: PHYTOCHEMICAL CHARACTERIZATION AND BIOACTIVITY ASSESSMENT OF SELECTED BULGARIAN PLANT SPECIES FOR SKINCARE APPLICATIONS

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Based on ethnobotanical studies of medicinal plants traditionally used for treating skin disorders in the Balkan Peninsula, a total of 21 plant species sourced from the rich Bulgarian flora were selected for preliminary chemical characterization and evaluation of biological activity. Harvested from their natural habitats, plant materials were air dried and extracted to obtain dichloromethane, methanol and hydroalcoholic extracts. Employing advanced analytical techniques such as 1D and 2D NMR, as well as normal and reversed phase HPTLC, the main groups secondary metabolites were identified. Total phenolics and total flavonoids content of extracts were measured by the Folin–Ciocalteau and aluminum chloride assays, respectively. Their antioxidant capacity was calculated by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging test. Based on the noteworthy high total phenolic content, ranging from 123.4 to 383.3 mg GAE/g extr, total flavonoid content varied from 76.3 to 204.1 mg QE/g extr and high radical scavenging activity exceeding 90%, methanolic and hydroalcoholic extracts of *Agrimonia eupatoria* L., *Cistus incanus* L., *Potentilla neglecta* Baumg., *Tussilago farfara* L. and *Hypericum perforatum* L. were earmarked for further investigation, involving fractionation, isolation and identification of active compounds. Moreover, the identified promising anti-tyrosinase and/or anti-collagenase activity of these extracts further supports the idea of their comprehensive study. This suggests potential application in the skincare field, particularly as anti-aging and skin-whitening agents.

Aknowledgements: This research has been funded under the European H2020-MSCARISE-2018 (ID 823973) project "EthnoHERBS - Conservation of European Biodiversity through Exploitation of Traditional Herbal Knowledge for the Development of Innovative Products.

PREPARATION OF POLYMER AMPHIPHILES FOR SURFACE MODIFICATION OF NIOSOMES

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Recently one of the fastest growing areas in modern pharmaceutical science and technology is targeted drug delivery [1]. The practical realization of this concept is possible thanks to recent advances in nanotechnology [2]. In the field of nanoscale drug carriers, niosomes attract increasing scientific interest as promising drug delivery systems. They are composed of nonionic surfactants and cholesterol and offer several advantages as vesicles for drug encapsulation, codelivery of lipophilic/water insoluble and hydrophilic compounds, biocompatibility, low-immunogenicity, stability (physical, chemical, and osmotic) and controlled release and targeting properties. By adding appropriately designed polymers, the membranes of niosomes can be modified [3]. This strategy offers many possibilities for fabricating highly effective carriers which, in addition, can release the incorporated drugs in a controlled manner. In this regard, different polymer amphiphiles on the base of a series of non-phospholipid conjugates of polyethyleneoxide, polyglycidol, polyacrylic acid, poly(2-isopropyl-2-oxazoline) and poly(ethyltriethyleneglycolacrylate) were synthesized and characterized in detail using "click" chemistry reactions. Novel noisome formulations using various surfactants and polymer amphiphiles are to be prepared and characterized in terms of size, size distribution, and morphology.

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INFLUENCE OF EVOLUTIONARY DEVELOPMENT AND CULTURE MEDIA COMPOSITION ON THE PHENOLIC PRODUCTION OF SPECIES OF THE GENUS *HYPERICUM IN VITRO*

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St. John's worth (*Hypericum perforatum*) is one of the most widely studied and utilized plants in both traditional and conventional medicinal practices. This is due to the wide array of secondary metabolites characteristic for the representatives of the genus. Pharmacological activities established for the different *Herba hyperici* preparations include antidepressive, antitumor, antiviral, and antibiotic amongst others, determined by the presence of a remarkably broad spectrum of phytochemical constituents such as polyphenolic compounds, flavonoids, naphthodianthrones (hypericin and pseudohypericin), phloroglucinols and terpenes [1]. Except to the widely studied *H. perforatum*, the genus contains nearly 500 representatives, classified into 36 sections according to their evolutionary development. Intragenic studies have shown that representatives of the evolutionarily most developed species are superior in terms of production of hypericins [2].

The present work compares shoot cultures of four *Hypericum* species belonging to different sections of the genus. The hypericin non-producing *H. calycinum* L. is a representative of the evolutionarily most primitive section *Ascyreia*. *H. perforatum* L. and *H. tetrapterum* Fries belong to the evolutionarily younger section *Hypericum* and *H. richeri* Vill. (section *Drosocarpium*) is the evolutionarily youngest in the present study.

Shoot culture experiment included treatments with plant growth regulators (PGRs) and activated charcoal (AC) alone and in combinations. Comparisons were made with the PGR- and AC-free controls for each respective species as well as for each treatment between species.

The intrageneric comparison of obtained results evidenced the evolutionarily oldest hypericin non-producing *H. calycinum* as a remarkably higher phenolic and flavonoids producer *in vitro* as compared with the other three species, followed by *H. perforatum.* Lowest polyphenolics productivity was established for *H. richeri.* PGRs treatment significantly enhances biomass formation, but led to lowering of polyphenolics production. This effect was alleviated by AC supplementation which enhanced phenolic and flavonoid levels, as compared with both PGR-free and PGR-treated media modifications.

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USEFUL TRICKS IN KBr TABLET TECHNIQUE FOR RECORDING GOOD QUALITY IR SPECTRA

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The present poster presents two innovations for quality improvement of FTIR spectra of liquid and solid samples using only KBr tablet technique.
The first one is an improved method aimed at the removal of asymmetric bands and baseline distortion effect observed in routine KBr tablet spectra. This technique is based on a suspension preparation, using a volatile solvent, from KBr powder mixed with target organic substance. This solvent traces from the sample are entirely eliminated through vacuum application, before and during tablet compression. Suspension technique used in present work is mainly applicable for tableting of organic substances which are well dissolved in a selected volatile solvent.
The second ingenuity represents a simple tool for the recording of good quality IR spectra of polar liquids by means of capillary technique using two *expendable* KBr windows (tablets) fixed inside a duralumin mini-cuvette. This appliance can contribute to overcoming difficulties deriving from the use of expensive or chipped KBr windows, while recoding polar liquids IR spectra in mid-IR region.
These two KBr technique improvements can considerably facilitate research work increasing recorded IR spectra quality without any other spectral techniques application.

PHYTOCHEMICAL CHARACTERIZATION OF *VALERIANA OFFICINALIS* L. ROOTS AND THEIR EXTRACTS

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Valeriana officinalis is a perennial plant that belongs to the Valerianaceae family. It is commonly used to treat various nervous and cardiovascular disorders. The plant is a rich source of biological active substances. It mainly contains sesquiterpenic acids, especially valeric acid and its derivatives, including acetoxyvalerenic acid and hydroxyvalerenic acid, which are responsible for anxiolytic effect on human body. In addition, it contains phenolic compounds such as hydroxybenzoic, caffeic, gallic and chlorogenic acids [1]. The roots have hypotensive, spasmolytic, diuretic and anticonvulsant effects [2]. The current study aimed to perform phytochemical characterization of valerian (*Valeriana officinalis*) roots, their extracts (water, 40%, 50%, 60% ethanol and 60% glycerol) and prepared from them polysaccharide-containing complexes (PSC). This characterization was carried out by gravimetric, spectrophotometric and chromatographic methods. Valerian roots were characterized by a high carbohydrate content (48%). Proteins also occupied a large part of the dry matter (15%). Among the extracts, the highest yield was obtained using water as a solvent (38%) and it had the highest carbohydrate content. Sesquiterpenic acids were found in larger quantity in the ethanolic extracts (0.2-0.3%). The 60% glycerol PSC was yielded in the highest amount (3.3%) while the others were found in smaller amounts. The results from this study show that Valerian roots contain a large amount of biological active substances, and that these data can be used for the preparation of extracts having different composition and thus easily be incorporated into commercial products.

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PHYTOCHEMICAL PROFILE AND BIOLOGICAL ACTIVITY OF BULGARIAN ENDEMIC PLANT *ACHILLEA THRACICA* VELEN.

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Achillea thracica Velen. (Asteraceae) is a Bulgarian endemic plant under protection of the Bulgarian Biodiversity Law with national conservation status: critically endangered.
In the present work we aimed to explore the secondary metabolites and the pharmacological potential of *in vitro* (AI) cultivated and wild-grown (leaf (AL) and flower heads (AF) plants.
The aerial parts were extracted with chloroform and methanol. Further CC (Sephadex LH-20 and Silica gel) and PTLC purification of the extracts afforded to 7 flavonoids and their glycosides (isorhamnetin, diosmetin, kumatakinin, quercetin-3,7-dimethyl ether, quercetin-3-methyl ether, patulitrin and isoquercetrin), phenolic acids (mono- and dicaffeoyl esters of quinic acid) and one irregular monoterpene alcohol santolynilol. Their structure was determined by NMR. Spectrophotometric methods based on different mechanisms (DPPH and Total Antioxidant Activity) were applied to investigate the antioxidant capacity, as well as total phenolic (TPC) and flavonoid content (TFC) of the methanolic extracts obtained from AI, AL and AF.
The obtainted results showed that methanolic extract from leaves (AL) was the richest in TPC, TFC (283.63±11.76 μg GA/mg extract and 37.01±0.66 μg QE/mg extract, respectively) and displayed the highest antioxidant activity.
The anti-inflammatory activity of the methanolic extracts from *in situ* and *in vitro* cultivated plants was tested by hemolytic assay and the highest inhibition of the complement system was observed in the extracts of AI and AL with a maximum inhibition of 73% at a concentration of 2 mg/ml.

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ANTITUMOUR ACTIVITY AND SELECTIVITY OF A SERIES OF BETULINIC ACID-BASED IONIC LIQUIDS

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Some natural products are showing excellent results in the treatment and prevention of cancer, as chemosensitizers, immunotherapeutics and in combination with other anticancer drugs. Due to their unique molecular properties, they may offer superior efficacy and safety compared to synthetic drugs. Despite their remarkable health benefits, the full clinical potential of natural products has not yet been realized due to low aqueous solubility, poor absorption and solubility, poor absorption, lower bioavailability and stability. To overcome these problems, novel formulation strategies are being employed and the conversion of active pharmaceutical ingredients (APIs) into ionic liquids or organic salts is one such promising approach.
The focus of the present study is betulinic acid (BA), a pentacyclic triterpene known for its anticancer, antiviral, antimicrobial, immunostimulatory, anti-diabetic and some other activities, the application of which is however limited due to low water solubility and bioavailability. Here we report the synthesis of 15 novel ILs containing an amino acid ethyl ester cation and anion – BA. The compounds were structurally and physico-chemically characterised in detail. A preliminary screening of the effect of the modification on anti-tumour activity was carried out. When tested against melanoma, breast cancer, colorectal adenocarcinoma and hepatocarcinoma cells, the compounds showed IC50 values in the range of 3 to 150 μM. The compounds were not toxic to normal mouse fibroblasts at concentrations up to 200 μM.

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VOLATILE ORGANIC COMPOUNDS IN BULGARIAN RED WINES – OPTIMIZATION OF ANALYTICAL METHOD USING SOLID-PHASE MICROEXTRACTION AND HEADSPACE GAS CHROMATOGRAPHY

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The content of volatile aroma compounds in wine depends on the varietal characteristics, viticulturally practices and wine-making technology. The aim of this study was to develop sensitive and reproducible procedure using headspace solid-phase micro extraction (HS-SPME) combined with gas chromatography/mass spectrometry (GC/MS) for fast profiling of the free volatile organic compounds (VOCs) in Bulgarian red wines.

A semi-quantitative HS-SPME-GC/MS method has been developed for the red wine aroma profile analysis. A semi-polar divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers was used for extracting the free wine VOCs. An optimization of the experimental conditions (conditioning temperature, conditioning and extraction time) was performed, in order to study their influence on the qualitative and quantitative composition of the wine samples. The effect of addition of NaCl was also studied.

More than 50 individual free volatile and semi-volatile compounds were identified, with the representatives of terpenes, esters, alcohols, fatty acids, sulfur compounds, etc. The study revealed that 3-methyl-1-butanol was the most abundant component, ranging from 55.23% - 28.76%, followed by octanoic acid, ethyl ester (26.34% -7.52%), phenylethyl alcohol (18,61% - 8,04%) and butanedioic acid, diethyl ester (7.34% - 4.71%). The best HS-SPME conditions were as follows: conditioning time 45 min, conditioning temperature 45^{o}C and extraction time 60 min, with addition of 4 grams NaCl. All the samples were analysed in triplicates, with St Dev <5%.

The developed easy and solvent-less HS-SPME-GC/MS method is suitable for fast, sensitive and reproducible analysis of VOCs and can be used for comparative aroma profiling of Bulgarian red wines.

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IR AND MICRO-RAMAN STUDY OF THE INORGANIC AND ORGANIC PAINTING MATERIALS USED IN THE MURALS OF ORLITSA CONVENT, RILA MONASTERY

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Orlitsa convent is one of the oldest convents of Rila monastery. It was first mentioned in a document issued by Tsar Ivan Shishman in 1378. During the Bulgarian National Revival (XVIII-XIX century) it was one of the numerous large convents which provided a shelter for the pilgrims traveling to the Rila Monastery.
Within our ongoing study we have investigated by vibrational spectroscopy the organic and inorganic painting materials used for executed the wall painting decoration from both periods – XV and XIX centuries The pigments identified in the XV century wall paintings encompass red lead, cinnabar, red and yellow ochre, green earths and ultramarine. The wall paintings are executed by egg tempera technique on wet plaster. The analysis of XIX century wall paintings showed the presence of the same earth pigments of red, yellow and brown color, as well as ultramarine and calcium carbonate but in this case the oil binder were used instead of egg. The ATR infrared spectra allowed the identification of beeswax mixed with calcium carbonate and gypsum applied as filler material in the XV century wall painting decoration. The ATR-IR spectra revealed also the presence of metal oxalates in several microsamples from the painting layers, indicative of binder degradation processes.
The information provided by the study allows reconstruction of the color palette of the painters from the different periods and contributes to the assessment of the wall paintings preservation state and the necessary treatment.

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POTENTIAL OF GOLDENBERRY (*PHYSALIS PERUVIANA* L.) SEEDS AND PEELS AS A LOW-COST VALUABLE RESOURCE OF BIOACTIVE COMPOUNDS

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Goldenberry (*Physalis peruviana* L.), native to Chile and Peru, has commercial promise for many regions. It is fairly adaptable to a wide variety of well-drained soils, and very good crops are obtained on rather poor sandy ground. A single plant may yield 300 fruits and carefully tended plants can provide 20-33 tons per hectare [1]. Unfortunately, up to 30 % of this amount corresponds to the pomace obtained after pressing, consisting mainly of skins and seeds. This study aims to evaluate the potential of goldenberry pomace for possible further utilization. Imported Colombian fruits (*Physalis peruviana* L.), available on the Bulgarian market, as well as fruits grown under the ecological conditions of Bulgaria (in the Bankya region) were provided for the present research. The oil extracted from the seeds of physalis fruits grown in Bulgaria has a significantly higher total content of tocopherols with *alpha*-tocopherol as a major component. *Beta*-tocopherol is the main constituent in the Colombian seed oil. The total amount of carotenoids in the skin of Colombian berries (295 mg/g) is threefold lower than that in the skin of Bulgarian (871 mg/g). Concerning analysis of fatty acids, there are no differences in the lipid profile of the oils extracted from the fruit seeds, regardless of their origin. The oxidation stability of oils has also been studied. As a first step toward developing goldenberry as a commercial crop, the data obtained would add to the otherwise scanty literature available on this interesting member of the Nightshade family (Solanaceae).

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