



10th CMAPSEEC: BOOK OF ABSTRACTS

**10th Conference on Medicinal and Aromatic Plants of
Southeast European Countries**

May 20-24, 2018, Split, Croatia

10th CMAPSEEC

10th Conference on Medicinal and Aromatic Plants of Southeast European Countries May 20-24, 2018, Split, Croatia

Jointly organized by:



Association for Medicinal and Aromatic Plants of Southeast European Countries (AMAPSEEC), Belgrade, Serbia

and

University of Zagreb, Faculty of Agriculture, Zagreb, Croatia

Institute for Adriatic Crops and Karst Reclamation, Split, Croatia

Centre of Excellence for Biodiversity and Molecular Plant Breeding (CroP-BioDiv), Zagreb, Croatia

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10th CMAPSEEC

10th Conference on Medicinal and Aromatic Plants of Southeast European Countries May 20-24, 2018, Split, Croatia

Dear Friends and Colleagues,

On behalf of the Organizing and Scientific Committees I am pleased to welcome you to the 10th Conference on Medicinal and Aromatic Plants of Southeast European Countries (10th CMAPSEEC) jointly organized by the University of Zagreb, Faculty of Agriculture, Zagreb, Croatia, Institute for Adriatic Crops and Karst Reclamation, Split, Croatia and the Centre of Excellence for Biodiversity and Molecular Plant Breeding (CroP-BioDiv), Zagreb, Croatia in collaboration with Association for Medicinal and Aromatic Plants of Southeast European Countries (AMAPSEEC).

AMAPSEEC was founded in 1998 on the initiative of the Institute for Medicinal and Aromatic Plant Research 'Dr Josif Pančić', Belgrade, Serbia. Ever since the Association served as an important network connecting scientists interested in medicinal and aromatic plants. After the foundation of the Association, the Conferences were organized in different countries every second year greatly contributing to the development of various fields of research concerning medicinal and aromatic plants.

As this is the first time for the Conference to be organized in Croatia it is our great privilege to host the 10th Jubilee Conference in Split and pay tribute to all the past efforts in organizing our meetings:

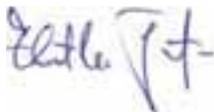
- 2000: 1st CMAPSEEC, Arandjelovac, Serbia; hosted by Mihailo Ristić
- 2002: 2nd CMAPSEEC, Chalkidiki, Greece; hosted by Maria Couladis and Vassilios Roussis
- 2004: 3rd CMAPSEEC, Nitra, Slovakia; hosted by Miroslav Haban and Ivan Šalamon
- 2006: 4th CMAPSEEC, Iasi, Romania; hosted by Gogu Ghiorghita
- 2008: 5th CMAPSEEC, Brno, Czech Republic; hosted by Gabriela Růžičková
- 2010: 6th CMAPSEEC, Antalya, Turkey; hosted by Ilkay Erdogan-Orhan
- 2012: 7th CMAPSEEC, Subotica, Serbia; hosted by Zora Dajić Stevanović
- 2014: 8th CMAPSEEC, Durrës, Albania; hosted by Alban Ibraliu
- 2016: 9th CMAPSEEC, Plovdiv, Bulgaria; hosted by Vassya Bakova

Moreover, in dedication to our late colleague and dear friend Mihailo Ristić (1953 - 2017), the founder and the first president of AMAPSEEC, the Best Poster Award has been established in his honour.

I sincerely hope that the 10th CMAPSEEC will follow the established tradition in gathering scientists, young researchers and entrepreneurs involved in medicinal and aromatic plants production, processing and use. Thus, the scientific programme tends to cover a broad range of topics organized into three sections: (1) Medicinal and aromatic plants ecology, diversity and ethnobotany, (2) Phytochemical analysis, pharmacology, biological activity and toxicity, and (3) Cultivation, breeding and biotechnology of medicinal and aromatic plants.

The Organizing and Scientific Committees would like to thank all the participants for their contributions as well as the sponsors for their financial support.

The 10th CMAPSEEC is a result of effort of a large group of people and everyone should be proud of the outcome.

A handwritten signature in blue ink, appearing to read 'Zlatko Šatović', with a stylized flourish at the end.

Zlatko Šatović

President of the Scientific Committee

10th CMAPSEEC

**10th Conference on Medicinal and Aromatic Plants
of Southeast European Countries
May 20-24, 2018, Split, Croatia**

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I. MEDICINAL AND AROMATIC PLANTS ECOLOGY, DIVERSITY AND ETHNOBOTANY

Monday, May 21st

9:00	Opening Ceremony	
10:15	Coffee Break	
Moderators: Zora Dajić Stevanović, Zlatko Šatović		
11:00	Plenary lecture - Łukasz Łuczaj Ethnobotany of the Adriatic Islands in Croatia	25
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12:00	<u>Ekaterina Kozuharova</u> , Ina Aneva, Hristo Valchev, Dave Goulson Wild Labiates Aromatic Plants and their Bee Pollinators	28
12:15	<u>Marija Jug-Dujaković</u> , Ivana Šolić, Martina Grdiša, Łukasz Łuczaj, Filip Varga The Use of Medicinal Plants in the Knin Area, Croatia	29
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Moderators: Ivan Šoštarić, Zlatko Liber

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II. PHYTOCHEMICAL ANALYSIS, PHARMACOLOGY, BIOLOGICAL ACTIVITY AND TOXICITY (PART I)

Tuesday, May 22nd

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II. PHYTOCHEMICAL ANALYSIS, PHARMACOLOGY, BIOLOGICAL ACTIVITY AND TOXICITY (PART II)

Thursday, May 24th

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III. CULTIVATION, BREEDING AND BIOTECHNOLOGY OF MEDICINAL AND AROMATIC PLANTS

Thursday, May 24th

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**I. MEDICINAL AND AROMATIC PLANTS ECOLOGY,
DIVERSITY AND ETHNOBOTANY**

ETHNOBOTANY OF THE ADRIATIC ISLANDS IN CROATIA

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There are over a thousand islands in Croatia but only 79 have an area of over 1 km² and only 48 are inhabited. The Adriatic islands are very attractive places for tourism. They are influenced both by the influx of well-off new inhabitants from different countries of Europe, and the exodus of the local population to mainland cities. In order to preserve the remaining scraps of traditional knowledge among the local indigenous population, in 2014-2017 we interviewed over 250 key informants on 17 islands on the Adriatic Coast. The main goal of the study was to document wild edible plants, with particular reference to wild vegetables. Using the opportunity to talk to elderly informants, we also interviewed them about other aspects of traditional knowledge, e.g. medicinal plants, and plants used for dyes and tools, as well as ritual plants. One striking discovery was that one island preserved its knowledge significantly better than others, namely Korčula. In every village on Korčula most people know and use numerous wild vegetables and are willing to talk about it. In contrast to this some large islands have almost completely lost their knowledge of plants. This is, for example, the case with Dugi Otok and the Lastovo islands. Our preliminary results suggest that knowledge was better preserved on larger islands with living agriculture and with more lively communities, where the interchange and transfer of knowledge is easier. On smaller islands there are fewer habitats for gathering wild edible plants, and a large proportion of inhabitants are retired people who spent most of their lives abroad, e.g. in the USA, and are not interested in traditional ways of life. Apart from on Korčula, several individuals with considerable reservoirs of knowledge can still be found on Brač, Šolta and Pašman.

Key words: ethnobotany, wild vegetables, wild edible plants, wild food plants

CHLOROPLAST DNA DIVERSITY AND PHYLOGEOGRAPHY OF *SALVIA OFFICINALIS* L. AND *SALVIA LAVANDULIFOLIA* Vahl

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Dalmatian sage (*Salvia officinalis* L.) and Spanish sage (*Salvia lavandulifolia* Vahl) are perennial, evergreen shrubs from the family *Lamiaceae*. Because of the essential oils both species have been treasured as medicinal herbs. The taxonomic position of *S. lavandulifolia* is still controversial since certain authors consider it as subspecies of the *S. officinalis*. The natural distribution area of *S. officinalis* is coastal region of the western Balkan and southern Apennine Peninsulas while *S. lavandulifolia* is native to Iberian Peninsula. Genetic diversity and phylogeographical pattern of Dalmatian and Spanish sage were analysed comparing two chloroplast DNA regions. A total of 29 chloroplast haplotypes were identified in 88 native populations. Twelve chloroplast haplotypes were found on the Balkan Peninsula (*S. officinalis*), six on the Apennine Peninsula (*S. officinalis*) and 13 on Iberian Peninsula (*S. lavandulifolia*). The two species did not share haplotypes while Balkan and Apennine populations of *S. officinalis* had two haplotypes in common. *S. officinalis* exhibited higher haplotype and nucleotide diversity than *S. lavandulifolia*.

Key words: *Salvia officinalis*, *Salvia lavandulifolia*, phylogeography, chloroplast DNA, Balkan peninsula, Apennine peninsula, Iberian Peninsula

WILD RASPBERRY FROM THE CENTRAL BALKAN: FROM ETHNOBOTANY TO BIOACTIVITY

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Raspberry (*Rubus idaeus* L., Rosaceae) is a well-known species, native to Europe and north Asia. Its fruit is used as food and leaves are used in traditional medicine. In this study the leaves, fruits and some traditional fruit products of wild raspberry populations of the central Balkan region were examined to determine the level of secondary metabolites (total phenols, flavonoids, tannins and anthocyanins) and related antioxidant (AOA) and biological activity. The values obtained for total phenols ranged from 59.68 to 96.83 mg of GA/g and 24.29 to 38.71 mg of GA/g in leaf and fruit extracts, respectively. The higher AOA (tested by DPPH) was detected in the leaf methanol extracts than in the fruit extracts. Leaf and fruit extract were the most effective against *Escherichia coli* (ATCC 8739). Anticancer activity was studied on a human colorectal cancer cell line HCT-116. Leaf extracts exhibited anticancer activity with 162.38 µg/mL -IC₅₀/24 h and 95.69 µg/mL - IC₅₀/48 h. The ethnobotanical survey was performed at the nine high-mountain rural sites (corresponding to sites where plant material for phytochemical study was collected) belonging to regions of southwest (SWS) and southeast (SES) part of Serbia. In SWS region, the most frequent use of wild raspberry leaf is linked to gastrointestinal disorders and prostate inflammation. In the SES region, the leaves are mostly used for prevention and healing of gynecological disorders, and during pregnancy and lactation. Local population of SWS region uses fruits mainly for preparation of the syrup juice, the sweet fruit preserve (“slatko”) and in desserts, while in SES the fruits are mostly used for preparation of nonalcoholic and alcoholic beverages. Considering results from this study, wild raspberry traditional products can be recommended as healthy, or even the functional food, but further analyses on its biological activity are needed.

Key words: *Rubus idaeus*, phenols, antioxidant, antimicrobial, anticancer, leaf, fruit, traditional products

WILD LABIATES AROMATIC PLANTS AND THEIR BEE POLLINATORS

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Family Lamiaceae is famous with many aromatic wild plants. Plant reproduction is crucial for sufficient essential oil production, herbal tea production and sustainable development. Particularly important is the pollination process when seed reproduction is the leading way of propagation. Functional flower morphology of Lamiaceae members reveals predominantly bee pollination syndrome. Due to habitat destruction, loss of flower resources, and increased use of pesticides (particularly neonicotinoids), declines in bees' abundance and diversity have been observed over recent decades. The aim of this review study is to evaluate the importance of bees as pollinators of wild Lamiaceae aromatic plants. The object of our study were *Acinos arvensis* (Lam.) Dandy, *A. suaveolens* (S.et S.) G.Don., *Calamintha nepeta* (L.) Savi., *Nepeta cataria* L., *Origanum vulgare* L., *Mentha* sp. div., *Thymus* sp. div., *Micromeria dalmatica* Benth., *Sideritis scardica* Grsb., *Satureja montana* L., *Hyssopus officinalis* L. and *Melissa officinalis* L. which are collected for herbal teas in Bulgaria. We analysed these taxa for their biological type, propagation strategies, pollination ecology and conservation significance. These are mostly perennials, with predominantly propagation by seeds. Both honey and wild bees are necessary for the optimal seed set. Therefore, the sustainable use of wild aromatic labiates requires preservation of both plant component and their pollinators.

Key words: herbal tea, aromatic, bees, Lamiaceae

THE USE OF MEDICINAL PLANTS IN THE KNIN AREA, CROATIA

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We documented the knowledge of local people concerning the use of medicinal plants in the Knin area. Forty-two native inhabitants (35 female and 7 male) were interviewed in 34 semi-structured interviews. Relative frequency of citation (RFC) and Fidelity level (FL) were used to analyze the obtained data. The uses and preparations of 82 plant species belonging to 36 families were recorded. Rosaceae was the most represented family with 14 species followed by Lamiaceae (10) and Asteraceae (9). RFC was the highest for *Hypericum perforatum* L. (0.65), followed by *Plantago major* L. (0.38) and *Rumex pulcher* L. (0.38), while 35 species were mentioned only once. The FL of 1 was scored by *Chelidonium majus* L., *Cydonia oblonga* Mill., *Helichrysum italicum* (Roth) G. Don and *Rumex pulcher* L., and FL of 0.8 was scored by *Equisetum arvense* L., *Sempervivum tectorum* L. and *Crataegus monogyna* Jacq. The diseases recorded in the present study were classified into 14 ailment categories. Most uses for the recorded plant species were for treating digestive system disorders (14), skin/subcutaneous cellular tissue disorders (11), circulatory and blood system disorders (10), and respiratory system disorders (10). The knowledge of medicinal plants use is kept alive mostly by elderly population.

Key words: ethnobotany, medicinal plants, traditional knowledge

ENVIRONMENTAL POLLUTION AND HEAVY METALS ACCUMULATION IN *SALIX ALBA L.* (SALICACEAE), ALONG THE RIVER STREAM OF SITNICA

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Heavy metals are one of the main hazards because of their toxicity to environment and human life. This study describes an investigation of environmental pollution along the river stream of Sitnica, which passes near the Kosovo Thermal Power Plants, and accumulation of selected heavy metals (As, Cd, Cr, Ni and Pb) in water, soil and willow bark samples (*Salix alba L.*, Salicaceae), considering the fact that industrial area of Kosovo Thermal Power Plants is one of most polluted area in Kosovo. Samples were analysed using ICP-MS (inductively coupled plasma mass spectrometry), and the results are from 30 water samples, 30 soil samples, and 30 willow bark samples, collected approximately along the 35 km of river stream of Sitnica, in both directions from the source of pollution. According to the obtained values, it was determined that the content of these pollutants in this study area, differ within elements and samples, and they indicate a possible threat to environment pollution and impact of the industrial sector in the living environment. Chromium, nickel and lead in water samples were above target values, and in some cases above intervention values. Heavy metals content in soil samples varied. While chromium was within permissible limits, max. concentrations of arsenic, cadmium, and lead, were above intervention values. In willow bark samples concentrations of nickel was detected in the upper allowed limits. Cadmium and chromium mostly were above permissible limits. Results indicated a possible threat to environment pollution and impact of the industrial sector in the environment, and if adequate protection measures are not taken, degradation of living environment can continue.

Key words: heavy metals, analysis, accumulation, environmental pollution

STRONG INFLUENCE OF CLONALITY ON THE FINE SCALE SPATIAL AND GENETIC STRUCTURE OF *SALVIA BRACHYODON VANDAS* (LAMIACEAE) POPULATION

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Salvia brachyodon Vandas is a narrowly endemic species restricted to only three known populations in the central Adriatic region and it is treated as a highly vulnerable species in Montenegro and statutorily strictly protected indigenous plant species in Croatia. It is a partially clonal species and reproduces both sexually through seeding and clonally from perennial underground stolons. The species is characterized by rich content of essential oil, of which chemical composition is rather similar to other closely related sage species including well-studied common sage. The studied population of *S. brachyodon*, located on Pelješac Peninsula, Croatia is the largest of all known populations. In order to assess levels of genetic diversity, 687 individuals were georeferenced, sampled and genotyped using eight microsatellite markers. For additional assessment of trade-off between sexual and clonal reproduction, the ramets and inflorescence were counted. Population genetic analysis revealed high levels of genetic diversity with no significant departure from Hardy-Weinberg equilibrium. Although numerous small clones dominated population over only few large ones, clonal reproduction strongly prevail over sexual reproduction. Since different genets virtually did not intermingle with each other, population's clonal architecture relies on phalanx strategy of ramets' spatial distribution. Results revealed that clones of different sizes flower with similar intensity and consequently the larger clones contribute more to sexual reproduction than the smaller ones. The analysis of clone size distribution revealed that studied population was characterized by Repeated Seedling Recruitment (RSR) strategy of new genets recruitment, which assumed constant emergence and establishment of new seedling. Strong spatial segregation was detected among large and small clones implying specific colonization strategy of the population.

Key words: *Salvia brachyodon* Vandas, clonality, genetic diversity, genets, ramets

MICROSATELLITE DIVERSITY OF DALMATIAN SAGE POPULATIONS FROM THE BALKAN AND APENNINE PENINSULA

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Dalmatian sage (*Salvia officinalis* L., Lamiaceae) is a well-known aromatic and medicinal Mediterranean plant that is native in coastal regions of the western Balkan and central and southern Apennine Peninsulas and is commonly cultivated worldwide. We used eight microsatellite markers to investigate genetic diversity and population structure. A total of 1,350 specimens from 50 locations across the Balkan Peninsula as well as from 12 locations from the Apennine Peninsula were sampled and genotyped. The model-based structure analysis revealed the presence of five geographically coherent genetic clusters that differed significantly in allelic richness, observed and expected heterozygosity. The highest level of allelic richness was found in populations located in the central part of the eastern Adriatic coast, while decreases in allelic richness were evident towards the northwestern Adriatic coast and southern and eastern regions of the Balkan Peninsula. Populations from Apennine Peninsula had substantially lower allelic richness than those from Balkan Peninsula. The genetic relationships among the genetic clusters were evaluated based on net nucleotide distance. A Neighbor-Joining tree based on the net nucleotide distances was generated showing that the two Apennine clusters were markedly divergent from each other as well as from the three Balkan clusters suggesting their long-term genetic isolation that led to a reduction in gene diversity.

Key words: *Salvia officinalis*, medicinal and aromatic plants, plant genetic resources, genetic diversity, genetic structure

MEDICINAL PLANTS AS A FOOD SOURCE FOR POLLINATORS – FLUCTUATION OF ATTRACTIVENESS OF SELECTED SPECIES DURING THE DAY

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Less known role of medicinal and aromatic plants (MAPs) is that they provide food for the insect pollinators. When evaluating the attractiveness of individual plant species, it is necessary to realize the fact that different types of plants, due the different reasons, provide nectar and pollen in a different quantity during the day. Thirty-eight species of MAPs as a source of bumble bees grazing were assessed on experimental plots (2x2m²) in 2016. The evaluation took place during the whole period of flowering of each species on working days with favorable weather, six times per day (7:00 – 14:00). It was found that studied species can be clearly divided into several groups according to time of their attractiveness for bumble bees. Although some species (*Papaver rhoeas* L., *Verbascum densiflorum* Bertol., *Althea officinalis* L., etc.) are the most attractive for bumble bees in the early morning and later their attendance drops significantly, for most of the evaluated MAP species the peak of bumble bees traffic in the flowers reflected somewhere between 9:00 and 12:00 a.m. The results clearly show that assessing the significance of MAPs, as well as other plants, as a source of a bumble bee or alternatively honey bee pasture, cannot be based on observations made in only one specific hour of the day or on different days at different times of day. Such a finding does not reflect specific characteristics of nectar and/or pollen production from individual species. The obtained results must be taken into account also when drawing up the various seeding mixtures as a food source for pollinators.

Key words: bees grazing, bumble bee, medicinal plants, pollinator attraction

GENETIC DIVERSITY OF IMMORTELLE (*HELICHRYSUM ITALICUM* /ROTH/ G. DON) POPULATIONS IN CROATIA

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Immortelle (*Helichrysum italicum* /Roth/ G. Don) is a perennial thermophilous subshrub widely distributed in the Mediterranean basin. Immortelle essential oil is highly praised having a wide range of remarkable medicinal properties. In recent years, massive uncontrolled commercial exploitation of wild immortelle populations prompts the Ministry of Environmental and Nature Protection of the R. of Croatia to limit or even ban wild harvesting. Genetic diversity and population structure of 18 immortelle populations from Croatia were examined by using amplified fragment length polymorphism (AFLP) markers. By assuming that the populations were in Hardy-Weinberg equilibrium, Wright's index of genetic differentiation was significant but low suggesting extensive gene flow among populations. The analysis of molecular variance indicated that the most of the genetic diversity was attributable to differences among individuals within populations in concordance with the patterns expected for an outcrossing and moderately long-lived species. Bayesian Analysis of Population Structure (BAPS) resulted in assignment of the investigated samples in two clusters. Samples originating from populations sampled in northern Adriatic were assigned to Cluster A, while the Cluster B included the samples belonging to populations from the middle and southern Adriatic. The results were compared with those obtained from AFLP analyses of Dalmatian sage (*Salvia officinalis* L.) and Dalmatian pyrethrum (*Tanacetum cinerariifolium* Trevir. /Sch./ Bip.) populations from Croatia in which the similar pattern of genetic diversity and population structure has been observed.

Key words: *Helichrysum italicum*, medicinal and aromatic plants, genetic diversity, AFLPs, plant genetic resources

GENETIC DIVERSITY OF NATURAL POPULATIONS OF *SALVIA FRUTICOSA* MILL. AND *SALVIA POMIFERA* L. FROM PELOPONNESE BASED ON SSR MARKERS

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S. fruticosa Mill., known in the international trade as Greek sage and *S. pomifera* L. known as Cretan sage, both called in Greece “faskomilo”, are two *Salvia* species growing naturally in Peloponnese, Southern Greece. They are both used as remedies and spices since ancient times in the Mediterranean. The demand of the aromatic and medicinal plants’ industry for quality products, urges the need for development of plant propagating material for farmers to grow systematically and in compliance with modern cultivation practices. In this frame, the purpose of this study, which is part of a bigger project, was to assess the genetic diversity existing among ten natural populations of *S. fruticosa* and five natural populations of *S. pomifera* growing in Peloponnese, using nine SSR markers that have been previously developed for *S. officinalis*. In *S. fruticosa* all nine and in *S. pomifera* eight out of nine SSR primers examined, were successfully amplified. The total number of revealed alleles was 73 and 70 for *S. fruticosa* and *S. pomifera* respectively. While most of the SSR markers studied revealed 2-5 alleles per locus, there were three that were extremely polymorphic revealing a number of alleles, ranging from 15 to 22. In *S. fruticosa* the observed heterozygosity ranged from 0.05 to 0.95 and the expected heterozygosity from 0.05 to 0.94, while in *S. pomifera* from 0.20 to 0.76 and from 0.19 to 0.87, respectively. Notably, the PCoA was able to separate the different species and moreover to group together *S. fruticosa* populations originating from neighboring geographical regions of Peloponnese. Moreover, as shown by AMOVA, high molecular variance was found within populations and lesser among them in both species.

Key words: *Salvia*, Greek sage, Cretan sage, microsatellites, population genetics

CHEMOSYSTEMATICS OF THE *CICHORIEAE* TRIBE OF THE ASTERACEAE FAMILY

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The *Cichorieae* tribe of the Asteraceae family is well characterized by a combination of milky latex and homogamous capitula with five - dentate ligulate flowers. The latex has a bitter taste, caused by sesquiterpene lactones and, particularly characteristic for *Cichorieae*, their glucosides. The tribe encompasses about 100 genera, 1500 species and additionally a multitude of micro - species in the genera *Hieracium*, *Pilosella*, and *Taraxacum*. *Cichorieae* encompass medicinal plant species, such as *Lactuca virosa* L. and *Hieracium pilosella* L. / *Pilosella officinarum* (L.) F.W. Schultz & Sch.Bip., and many bitter tasting vegetables, such as lettuce (*Lactuca sativa* L.), chicory and radicchio (*Cichorium intybus* L.), and endive (*Cichorium endivia* L.). Additional species are collected as vegetables in the wild, e.g. *Lactuca alpina* (L.) Wallr. Besides sesquiterpenes, triterpenes, coumarins, flavonoids, phenolic acids, and more recently, stilbenoids have been found in the *Cichorieae*. However, chemical diversity is greatest in the class of sesquiterpene lactones. Each genus seems to be characterized by a specific set of compounds, belonging to the sub-classes of eudesmanolides, germacranolides, and/or guaianolides. The *Cichorieae* even feature one group of sesquiterpene lactones, hypocretenolides, i.e. 12,5-guaianolides, which has so far only have been found in members of the *Cichorieae*, and here only in the *Hypochaeridinae* subtribe. Chemosystematic studies can not only be utilized to characterize taxa at different levels such as species or genera, they can also aid in the identification of parental species of hybrids. *Leontodon* x *grassiorum* Zidorn, a new *Leontodon* s.str. hybrid, was recently discovered in Austria. The nothospecies proved to be a hybrid between an Azorean endemic *Leontodon* species, *Leontodon hochstetteri* M. Moura & L. Silva and *L. hispidus* L. The new hybrid was morphologically and phytochemically characterised. Moreover, molecular phylogenetic studies were performed. To unravel the exact origin of the hybrid, a combination of all three data sets was necessary. The observed patterns will be discussed with special emphasis on the importance of chemical markers in the discovery and characterisation of hybrids.

Key words: Asteraceae, chemosystematics, sesquiterpene lactones

AN ANALYSIS ON WILD EDIBLE PLANTS OF THE TURKISH ASTERACEAE TAXA

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Asteraceae is one of the largest families in the Flora of Turkey. Also, it is one of the most important plant families in which there are many popular plants mainly used as food and tea in Turkey, because of the essential oils content. The aim of this study is the analysis of the wild edible plants of the Asteraceae family in Turkey, based on our local ethnobotanical research and available scientific literature. The information about the plants was obtained from the local people through open and semi-structured interviews. According to the list based on our investigations and the literature records, 43 genera (including 121 taxa) of the family Asteraceae is used for food, tea and other purposes. Among them *Achillea*, *Centaurea*, *Gundelia*, *Onopordum*, *Scorzonera* and *Tragopogon* are the most popular genera in the localities of Turkey.

Key words: Asteraceae, wild edible plants, Turkey

GENETIC BACKGROUND OF DALMATIAN PYRETHRUM (*TANACETUM CINERARIIFOLIUM* /Trevir./ Sch. Bip.) INSECTICIDAL POTENTIAL

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Pyrethrin is the secondary metabolite synthesized in Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir. / Sch. Bip.) and recognized for its potent insecticidal activity with no adverse effects on humans and environment. The natural distribution area of Dalmatian pyrethrum expands throughout the Eastern Adriatic coast and the islands. Today, as an endemic species, Dalmatian pyrethrum is threatened by anthropogenic habitat loss and degradation related to factors such as urbanization and habitat conversion and therefore protected by the Ordinance on Strictly Protected Species (Official Gazette 144/13, 73/2016). Despite its historical significance and economic value limited genetic and biochemical data on Dalmatian pyrethrum is available. Therefore, the importance of this project proposal lies in the integration of various scientific fields in resolving important issues concerning genetic and biochemical diversity of Dalmatian pyrethrum. The scientific objectives of the project are: (i) development of microsatellite primers (SSR; Simple Sequence Repeats) for Dalmatian pyrethrum; (ii) utilization of newly developed SSR and Amplified Fragment Length Polymorphism (AFLP) markers in assessing genetic diversity of *D. pyrethrum* natural populations (iii) optimization of Matric Solid Phase Dispersion (MSPD) technique and its utilization in the extraction of pyrethrins, and identification and determination of six extracted pyrethrin components in sampled populations with High - Performance Liquid Chromatography- DAD (HPLC-DAD), (iv) identification of AFLP markers linked to the trait loci related to the content of each pyrethrin component through Association Mapping studies which will facilitate the management of natural populations in future breeding programmes based on Marker-Assisted Selection (MAS). Emerging technologies and methods proposed in this project will lead to the development of new approaches to biodiversity monitoring, plant genetic resources conservation, future breeding programmes and agricultural exploitation of Dalmatian pyrethrum.

Keywords: association mapping, biochemical diversity, Dalmatian pyrethrum, genetic diversity, insecticide, MSPD, pyrethrin, *Tanacetum cinerariifolium*

Acknowledgment: This work has been fully supported by the Croatian Science Foundation under the project 'Genetic background of Dalmatian pyrethrum (Tanacetum cinerariifolium /Trevir. / Sch. Bip.) insecticidal potential' - (PyrDiv) (IP-06-2016-9034).

PREDICTING SUITABLE HABITAT FOR DALMATIAN PYRETHRUM (*TANACETUM CINERARIIFOLIUM* /Trevir. / Sch. Bip.) IN CROATIA

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Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir. / Sch. Bip.) is a thermophilic plant species of the Asteraceae family naturally distributed on the East Adriatic coast and its offshore islands. The size and number of populations in recent years have decreased, prompting the need to determine potential new localities where this species can be found. The aim of this study was to combine all distribution data available for Dalmatian pyrethrum in Croatia and to predict potential localities for future ecogeographical surveys. The data was mainly obtained from field studies conducted in the period from 2006 to 2017, as well as from available scientific literature and herbarium collections. A total of 224 localities of high geographic coordinates precision were recorded, most frequently in garrigue and dry grasslands. MaxEnt software was used to predict ecologically suitable ranges based on 19 bioclimatic variables obtained from the WorldClim data set. The most suitable geographical areas for *T. cinerariifolium* in Croatia are located along the coastline and on the islands with exception of the most northern part of Istria. The model also revealed some suitable areas in Bosnia and Herzegovina, Montenegro and Albania. Isothermality and Mean Temperature of Driest Quarter had the highest influence on the potential distribution of this species. The information obtained will be used in planning future field studies as well as building prediction models for distribution of the species in the future due to climate change.

Key words: suitable habitat modelling, *Tanacetum cinerariifolium*, maxent model, Balkan Peninsula

Acknowledgment: This work has been fully supported by the Croatian Science Foundation under the project 'Genetic background of Dalmatian pyrethrum (Tanacetum cinerariifolium / Trevir./ Sch. Bip.) insecticidal potential' - (PyrDiv) (IP-06-2016-9034).

ASSESSING THE MEDICINAL AND AROMATIC PLANTS IN WATERSHED OF THE MIDDLE SECTION OF DEVOLLI RIVER

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In the period 2015-2017, a special emphasis was put on the inventory and distribution of medicinal and aromatic plants (MAPs) in watershed of the middle section of the Devolli River, located in SE Albania distinguished for the rich flora. More than 30% of the Albanian flora is found in this region. From the study it turns out that about 160 taxa of the flora of this area belongs to MAPs. These plants belong to 132 genera and 57 families, mainly to the Lamiaceae (18), Asteraceae (14), Rosaceae (14) and Fabaceae (10). Hemicryptophytes (54 taxa) followed by Phanerophyte (47 taxa) are the most abundant life forms. A higher percentage of the taxa originated from Euro-Asia (24%). The month with the greatest number of MAPs species in flowering is June (110 taxa). Some of them are included in the group of species of national conservation concern that are protected by National Legislation or in the National Red Data Book. According to the local information and the data obtained through the interviews with the companies which gather plants in this area, larger quantities of these species are exported mostly in USA and Germany, and from them it is generated about 546,000.00 \$ per year. More than 50% of this value comes from exportation of *Lavandula angustifolia* Mill. and from other wild MAPs such are *Primula veris* L., *Juniperus communis* L., *J. oxycedrus* L., *Satureja montana* L. *Origanum vulgare* L., *Cistus incanus* L. and *Sideritis raeseri* Boiss and Heldr. The cultivation of MAPs is becoming increasingly important in this region. Currently, *Lavandula angustifolia* Mill. dominates the cultivated MAPs. Smaller surfaces are also cultivated with *Salvia officinalis* L., *Cyanus segetum* L., *Rosmarinus officinalis* L., etc. This study shows that stocks of MAPs in the watershed of the middle section of the Devolli River have declined in the past decades with some species becoming rare or endangered due to habitat loss, habitat modification and over-exploitation.

Key words: watershed, Devolli River, MAPs

THE MEDICINAL AND WILD FOOD PLANTS OF BATMAN CITY CENTER AND KOZLUK DISTRICT (BATMAN-TURKEY)

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This paper reports an ethnobotanical investigation carried out in 2012 to determine medicinal and wild food plants of Batman city center and Kozluk district. In total, thirty-six plants are used as traditional folk medicine in the region, and eleven of these are also used as a source of wild food. Furthermore, nine taxa are wild sources for other ethnobotanical usages for the area. The plants most commonly used in the region as medicinal remedies are *Malva nicaeensis* All., *Pistacia khinjuk* Stocks, *Plantago major* subsp. *intermedia* Gilib. and *Teucrium polium* L. Plants are mostly used for the treatment of gastrointestinal system diseases, respiratory system diseases and diabetes. The species most commonly used for food are: *Gundelia tournefortii* L., *Pistacia khinjuk* Stocks and *Rhus coriaria* L. This ethnobotanical study conducted in both districts will enable the traditional use of wild plants both as food sources and herbal remedies to be passed on to future generations.

Key words: ethnobotany, medicinal plants, Kozluk, Batman, Turkey

MEDICINAL PLANTS TRADITIONALLY USED IN THE TREATMENT OF DIABETES IN THE REGION OF SUVA PLANINA MOUNTAIN (SOUTHEASTERN SERBIA)

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Medicinal plants traditionally used on Suva planina Mt. are extremely important natural resource for a large majority of the people of this rural region for their primary health care. This is due to the specific geographical position, the high biodiversity, ethnic and cultural characteristics and folk tradition. This ethnobotanical survey was undertaken to investigate and document the anti-diabetic plant species used by the local people living on Suva Planina Mt. and its surroundings, as well as to preserve and utilize traditional knowledge for the potential development of new effective and safe medicines. The study was based on the field survey and details about the herb collection, mode of preparation of herbal remedies, treatment methods, and vernacular plant names were recorded. Information on traditional knowledge were collected from the locals through semi structured interviews, group discussions and observations, and 66 people of within 30–70 age groups of both sexes were interviewed. A total of 128 plant species and two fungi are traditionally used for medicinal purposes in the investigated region, of which 10 (12.8%) are used in the treatment of diabetes. The most frequently mentioned anti-diabetic medicinal plants were: *Alchemilla vulgaris* L. (the whole plant), *Allium ampeloprasum* L. (aerial part), *Centaurium umbellatum* Gilib., (aerial part), *Gentiana cruciata* L. (herb), *Helianthus tuberosus* L. (fresh rhizome), *Juglans regia* L. (internal hardened part of endocarp), *Mentha piperita* L. (aerial part), *Morus alba* L. (leaf), *Petroselinum crispum* (Mill.) A. W. Hill (leaf and root) and *Sorbus domestica* L. (fruit). For medicinal purposes, the fresh or dried aerial or underground plant parts including the rhizome are used, and general method for preparing herbal remedies includes infusion and decoction. Rich plant diversity, combined with local skills, experiences and knowledge of the healing properties of herbs in the region of Suva Planina Mt., represent an invaluable heritage to be preserved for future generations.

Key words: medicinal plants, diabetes, Suva Planina Mountain (Southeastern Serbia)

ETHNOMEDICINAL STUDY OF PREPARATIONS OBTAINED FROM *JUGLANDIS IMMATURE FRUCTUS* FROM THE FOLK AND TRADITIONAL MEDICINE OF REPUBLIC OF MACEDONIA

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Republic of Macedonia has old ethnomedicine traditions, consisting of many recipes with herbal, animal, and mineral original ingredients. The folk and traditional medicine of Republic of Macedonia pays special attention to disease prevention. It is interesting to mention a nice practice in Republic of Macedonia: conserving of *the juglandis immaturi fructus* in honey as "sweet" for service, for sick, weaker children, pale teenagers, anemic households and persons with weak stomach. One little spoon honey and one walnut are used in spring period every morning against weakness, tuberculosis and scrofula at glandular tuberculosis. It is used generally for immunity. Also, *juglandis immaturi fructus* are stored in raki for 4 weeks on sun or in a warm place. After that the liquid is filtrated and used according to the need of the organism, by one little spoon. This raki is used for cleanses of the stomach, liver and blood; for removing the weakness of the organism, removing bacteria from the intestine and for balances the viscous / density of the blood. Walnuts in these recipes are not heat, and there is no harmful effect from heat. These useful customs are continuing to be expanded at every step by the people and valued as good old medical receipts. It should be chemically and clinically examined how vitamins are preserved in that can. In this way "sweet" (vitamin C 165.16 mg 100g⁻¹, vitamin D 7 mg 100g⁻¹, sodium 142.54 mg 100g⁻¹, calcium 3.01 mg 100g⁻¹) and raki (vitamin C 6.15 mg 100g⁻¹, vitamin D 1.7 mg 100g⁻¹, sodium 119.55 mg 100g⁻¹, potassium 58.65 mg 100g⁻¹, calcium 1.12 mg 100g⁻¹) of the walnuts is our best folk can of vitamins and other medicinal ingredients.

Key words: honey, vitamins, raki, minerals

DEMOGRAPHIC CHARACTERISTICS OF THE LOCAL POPULATION IN CERTAIN SETTLEMENTS ON THE NORTH BLACK SEA COAST (BULGARIA) IN THE USE OF MEDICINAL PLANTS

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The purpose of this study was to establish the demographic indicators and to take into account the peculiarities of the medicinal plants use in human and veterinary medicine by the local population from certain settlements on the North Black Sea coast. Two settlements were studied: Aksakovo and Topoli, Varna district. The survey was conducted between June and August 2017. Interviews with the local population were conducted using original questionnaires prepared upfront. The respondents belonged to different gender, ethnicity, age and education groups. The data on how to gather medicinal plants, recommendations for use, drawbacks, frequency of use of medicinal plants are compared.

Key words: medicinal plants, ethnobotany, Northern Black Sea coast

INVESTIGATION OF MEDICINAL PLANTS ON THE TERRITORY OF PETROHAN TRAINING AND EXPERIMENTAL FOREST RANGE (WESTERN STARA PLANINA, BULGARIA)

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Medicinal plants are an important component of plant communities. Their study has a significant scientific and practical importance, related to the conservation of their resources and their sustainable use. The present study presents results of the inventory of medicinal plants on the territory of the Petrohan Training and Experimental Forest Range (Western Stara Planina, Bulgaria). A total of 140 species of vascular plants belonging to 122 genera and 53 families were described. Characteristic of the systematic structure of the studied plant was done and their classification according to biological type, life form, floristic geolements, period of flowering, and ecological group is presented. The ecological and biological characteristic, as well as information on the usable parts of the medicinal plants and the biologically active substances contained are presented. The information gathered during the study is a prerequisite for future resource research. Specifying species composition and resource status will contribute to their assessment as an ecosystem service offered by forest communities. Finally, we present a systematic list of the medicinal plants on the territory of the Petrohan Training and Experimental Forest Range.

Key words: medicinal plants, systematic, ecobiological analysis

PRELIMINARY STUDY OF THE FOLK MEDICINAL PLANTS USED IN TAŞKÖPRÜ (KASTAMONU-TURKEY)

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The aim of this study is to represent the plants used as traditional folk medicine in Taşköprü. For this purpose, the field work was done between May 2016 and November 2017 and our field work will continue for a while in this year. During the research 98 of 127 villages were visited. The specimens of the plants used as folk remedies, have been collected and the information about the local names, the part(s) used, the ailments treated, the therapeutic effect, the preparation, the methods of administration and the duration of treatment were obtained from the native people living in the research area by personal interviews. The plant specimens which are the materials of this study were identified using the “Flora of Turkey and the East Aegean Islands” and they are kept in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE). According to the results of our ethnobotanical study 70 taxa is used as a traditional folk medicine in Taşköprü. According to the majority of the plants which have similar usage, the plants are mostly used for respiratory, integumentary system, circulatory system and digestive system diseases.

Key words: Taşköprü, traditional folk medicine, ethnobotany

GENETIC DIVERSITY OF *HELLEBORUS ODORUS* Waldst. & Kit. subsp. *CYCLOPHYLLUS* (A. Braun) Maire & Petitm. POPULATIONS IN GREECE

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Helleborus odorus Waldst. & Kit. subsp. *cyclophyllus* (A. Braun) Maire & Petitm. is known for its medicinal use since the antiquity. It is endemic to the Balkan Peninsula occurring in woods and thickets. This taxon is renowned for its laxative effects, causing vomit, stimulation of memory and also used as painkiller and antidepressant since the 19th century. Recent studies demonstrate that its main compounds hellebrin and hellebrigenin may have an anticancer effect and can be used as drugs. In the current study, genetic diversity of eight different populations of *H. odorus* subsp. *cyclophyllus*, covering a great range of this subspecies geographical distribution in Greece (Mts Parnassos, Erimanthos, Dirfys and Olympus, regions of Velouhi, Fragma Mesahoras, Naousa and Vourgareli) was estimated using ITS sequences and ISSR markers. While ITS analysis revealed no genetic differentiation among the populations studied, AMOVA based on preliminary analysis of ISSR data showed that within the populations variation was 71%, while only 29% of the total was distributed among them. This suggests a possibly limited gene flow at least among some of the populations studied, which it was also confirmed by the estimated coefficient of gene flow ($Nm=0.7086$). Furthermore, according to PCoA, the populations from Naousa and Parnassos, Dirfys and Olympus Mts are separated from the rest of the other populations studied. The same results were also obtained by the STRUCTURE analysis with the exception of Naousa population. The above conclusions were also confirmed by the UPGMA dendrogram. Moreover, the highest level of genetic diversity was found in Velouhi population ($I=0.3355$, $PPB=66.04\%$, $H=0.3429$), while the lowest in the population from Mt Olympus ($I=0.1957$, $PPB=36.48\%$, $H=0.2578$).

Key words: *Helleborus*, ISSR, genetic diversity

SOME INSIGHTS INTO PROPERTIES OF *BRYOPHYTES* AS NEGLECTED MEDICINAL PLANTS

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Bryophytes are the second biggest group of land plants (after angiosperms). However, they are neglected as medicinal and aromatic plants compared to vascular plants. This is mainly due to their size and small biomass production. However, there are examples in traditional medicine to some extent in different world areas. *Bryophytes* are very diverse group and many representatives harbor very distinctive chemical and medicinal features. However, many have never been studied. The test and analyses of medicinal use and chemical constituents demand biotechnological approaches prior to these with aim to achieve enough biomass. *In vitro* establishment, growth and yield optimization allow us to study these properties in many not only biomass limited but also rare and threatened species. Case studies in various species will be presented; e.g. organic unsaturated acids, sugars, phenols and some other compounds in different species. Some of them will be emphasized as potential source of rare and interested chemical content (e.g. polyunsaturated omega-6 fatty acid in *Mnium hornum* Hedw., trisaccharide - kestose in *Rhodobryum ontariense* (Kindb.) Kindb., or antitumor bis(bibenzyles) - marchantins in thallose liverwort *Marchantia polymorpha* L.). Harvest time or environmental factors can significantly affect the target compound yield, as well as effect of crude extract in tested phenomena. Another significant value of *bryophytes* is the potential use in many biotechnological processes, where bio-pesticides and/or substance with anti-feeding effects can be extracted.

Key words: *bryophytes*, chemistry, medicinal plants

ETHNOBOTANY OF MEDICINAL PLANTS USED IN SOME PARTS OF THE NORTHERN BLACK SEA COAST REGION (BULGARIA)

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This study is part of an ethnobotanical investigation of the medicinal plants in the Northern Black Sea coast region, which includes the area from the village of Durankulak to the town of Obzor. The boundaries were determined using the map of the floristic regions in Bulgaria. Field work was conducted in the period from June to August 2017. Seventy-four people from 2 communities were surveyed. The study was carried out based on the survey methodology. Interviews with the local people were conducted using original questionnaires prepared upfront. The respondents belonged to different gender, ethnicity, age and education groups. This work presents the results of surveys in small communities only, since the field work is not over yet. The data from the different applications of medicinal plants in the life of the local people were processed and summarized.

Key words: ethnobotany, ethnomedicine, ethnoveterinary, North Black Sea coast

REPRODUCTIVE BIOLOGY OF THE ENDANGERED BULGARIAN ENDEMIC *CENTAUREA ACHTAROVII* Urum. (ASTERACEAE)

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Centaurea is the fourth largest genus of the family Asteraceae comprising hundreds of species, many of them used as medicinal plants. *Centaurea ahtarovii* Urum. is a local Bulgarian endemic, distributed in Pirin Mountain, protected by the Biodiversity Act and included in the Red Data Book of Bulgaria as “endangered”. The present study reports the data of current monitoring and the first results on the reproductive biology of *C. ahtarovii* concerning both populations of this poorly studied species: around Orelek summit, and at Kazanite locality. The monitoring was conducted in 2 sampling plots of 1000 square meters in each population. Both populations were in a very good condition. Population of Orelek was larger and with higher density than that of Kazanite: 75 ha, 5.8 individuals per m² (5.6 vegetative and 0.2 generative), and 35 ha, 2.3 individuals per m² (1.6 vegetative and 0.7 generative), respectively. Flower buds and open flowers in different stages of development were treated according to classical paraffin methods in order to reveal the particularities of the reproductive sphere. It was established that the structures in male and female generative spheres were stable, the processes leading to formation of embryos and seeds were balanced, and apomixes did not occur. All these features determined the low plasticity of the species. Pollen viability assessed by acetocarmine test was relatively high – over 50% while seed viability estimated by tetrazolium test was low (17.5%). *In vitro* seed germination was extremely poor. The low plasticity of male and female generative spheres decreased the adaptive mechanisms of *C. ahtarovii* to the environmental conditions. The low viability of seeds, and the small amount of pollen, although with relatively high fertility, determined a low reproductive capacity of the species. Therefore, *in situ* measures like reducing *Juniperus sibirica* Burgsd. coverage is more appropriate for conservation of *C. ahtarovii*.

Key words: embryo and endosperm formation, micro- and macrosporogenesis, monitoring, endemics

ANATOMICAL AND MICROMORPHOLOGICAL INVESTIGATIONS OF *ARTEMISIA ARBORESCENS* L. - WILD-GROWING MEDICINAL PLANT FROM MONTENEGRO

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In this work, anatomical and micromorphological investigations of vegetative organs of *Artemisia arborescens* L. (Compositae), wild-growing medicinal plant from Montenegro, were conducted. The main goal was to examine the anatomy and particular anatomical and micromorphological characteristics which are in relation with production of specialized metabolites. Microscopic slides were prepared following the standard histological procedures. Triarch type of the vascular bundle is present in primary root structure, whereas typical secondary growth occurs in older root. The stem is characterized by collateral vascular bundles, separated by wide parenchyma rays. The largest parenchyma cells occur in the pith. In the older stem, below the epidermis, well developed hypodermis could be noticed, made up of a several layers of enlarged cells arranged in radial rows. Cell walls of the pith region in older stems are lignified. Petiole has trapezoidal shape, unstratified epidermis, alternately arranged by collenchyma and chlorenchyma and one central and two lateral vascular bundles. Concerning leaf anatomy, the isolateral palisade structure and Kranz anatomy (C4 physiology) are observed. Secretory ducts are present in the stem cortical parenchyma and in the leaf and petiole parenchyma. Numerous morphologically variable T-shaped nonglandular, as well as glandular trichomes occur on the surface of all aerial vegetative organs. Earlier histochemical characterization of the oleoresin produced by secretory ducts of some *Artemisia* species showed the presence of terpenoids (essential oils), alkaloids, fatty acids, and polyacetylenes, while in the glandular trichomes, sesquiterpene lactones, steroids and tannins are revealed in addition to essential oils, where all of them are known for their medical properties. Thus, our findings are of an importance for future histochemical and phytochemical investigations of this and related species.

Key words: anatomy, micromorphology, *Artemisia*

PLANTS TRADITIONALLY USED AS ANTITUSSIVES IN TURKEY

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Respiratory disorders are the most common diseases in worldwide. Cough, a symptom of most respiratory illnesses, is a physiological mechanism that clears the airways and is also treated in rural traditional areas with plants. Medicinal plants are a potential source of material with high antitussive efficacy with minimal unwanted effects. Medicinal plants constitute the main therapeutic arsenal for healers in Africa and the most of developing countries. Despite the development of a variety of drugs, cough is a difficult disease to be eradicated. Ethnobotanical studies carried out by traditional methods of treatment are recorded and this information is aimed to contribute to the development of the drug. The information about how to traditionally use these medicinal or wild plants has been transferred from generation to generation. In this study, ethnobotanical studies have been conducted and found a lot of taxa used in traditional treatment against cough in Turkey. The aim was to give information about scientific and local names of these taxa, families, using parts and used as antitussive.

Key words: cough, medicinal plants, traditional treatment, Turkey

MEDICINAL PLANTS IN FOLK TRADITION: A PRELIMINARY ETHNOBOTANICAL STUDY OF PÜLÜMÜR (TUNCELI-TURKEY)

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The goal of this study is to identify the plants used as traditional folk medicine in Pülümür. For this purpose, the field work was done between April 2017-November 2017. During this research all villages of Pülümür district were visited. The specimens of the plants used as folk remedies, have been collected and the information about the local names, the part(s) used, the ailments treated, the therapeutic effect, the preparation, the methods of administration and the duration of treatment were obtained from the native people living in the research area by personal interviews. The plant specimens which are the materials of this study were identified using the “Flora of Turkey and the East Aegean Islands” and they are kept in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE). An ethnobotanical analysis showed that 45 plant taxa are used as a traditional folk medicine in Pülümür. According to the majority of the plants which have similar usage, the plants are mostly used for treatment of wounds, respiratory, digestive and circulatory system diseases.

Key words: Tunceli, Turkey, folk medicinal plants, ethnobotany

FEVERFEW *TANACETUM PARTHENIUM* (L.) Sch. Bip. – NATIVE SPECIES FROM SOUTH EAST EUROPE USED IN LITHUANIAN AND BELORUSSIAN TRADITIONAL MEDICINE

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The rich and diverse traditions of medicinal plants in Lithuania and Belarus countries are the result of the ethnically mixed populations of this region. Investigations into medicinal plants in Lithuania were commenced in the 18th century. Until then, Catholic monasteries played a significant part in spreading phytotherapeutic practises. In Lithuania and Belarus a flower garden used to be a decorative part of the homestead. In former times, both wild and cultivated plants from the garden were used in everyday life too, many plants were used to cure various illness. One of them, feverfew (*Tanacetum parthenium* (L.) Sch.Bip.) native to Balkan's Peninsula, has a long history of use and growing near homestead. It was mentioned in 19th century. The main objectives of this study were to collect information on the medicinal uses of feverfew in past and nowadays. The plant was widely grow in the early of the 20th century throughout Lithuania and Belarus for medicinal and ornamental purposes. The feverfew was important for treatment women's illnesses, suppressing inflammation, spasms and headaches, digestive system disorders, as a sedative. Nowadays the traditional knowledge concerning use of feverfew remains in the memories of older people. This plant can be found in old homesteads, especially in the forested part of South Eastern Lithuanian and Nord Eastern Belarus.

Key words: feverfew, use, traditional medicine

***SIDERITIS RAESERI* Boiss. & Heldr. (ÇAJI I MALIT) DISTRIBUTION IN ALBANIA, CHALLENGES FOR THE FUTURE**

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Sideritis raeseri Boiss.et Heldr., (Çaji i Malit) is included in the Red Book of Albania as an endangered species growing wild in Albania. The aim of this research was to investigate the natural distribution areas of *Sideritis raeseri* and to identify the possible cultivation areas assessing the relationships between environmental conditions and the physiological and morphometric characteristics of *Sideritis raeseri* populations. By identifying the most important natural growing areas of *Sideritis raeseri* populations, this study will contribute in the increasing of the representativeness of Çaji i Malit germplasm in genebank database and the possible quantity of incomes for the local people as the local employments are connected to an increment of *Sideritis* populations in the most natural growing areas of this species. Determining the map impact of cultivation of Çaji i Malit natural populations is an important undertaking, especially in sustainability of the families and individuals situated on the natural growing areas of *Sideritis* populations. Finally, the study has contributed in the development of Çaji i Malit database covering the geographic natural distribution of *Sideritis raeseri* and sparsely cultivation areas near of Natural Parks in Albania.

Key words: *Sideriti raeseri*, Albania, database

***IN SITU* CONSERVATION OF THREE ENDEMIC *VERBASCUM* SPECIES IN BULGARIA BY POPULATION REINFORCEMENT**

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Genus *Verbascum* (Scrophulariaceae) comprises about 250 species, some of them used in folk medicine as tea, ointment, baths; however, many species are endemic and unexplored for their bioactive compounds. *Verbascum tzar-borisii* (Dav. ex Stoj.) Stef.-Gat. and *Verbascum davidoffii* Murb. are Bulgarian endemics, the first one with two populations of about 1000 individuals, the latter with a unique, severely fragmented population. *Verbascum anisophyllum* Murb. is a Balkan endemic with two populations in Bulgaria and one in Serbia. These species are included in the Red Data Book of Bulgaria as “Critically Endangered” and protected by the Biodiversity Act. The investigation deals with *in situ* conservation of the target species, based on monitoring of their populations, seed germination rate, and *in vitro* plant propagation. In nature, all three species are characterized with unstable proportions between vegetative (rosettes) and generative (flowering) individuals, varying from year to year. Observations in the *ex situ* collection of IBER proved *V. anisophyllum* to be monocarp, while plants of the perennial *V. tzar-borisii* reached flowering stage on their second year and bloomed four consecutive years in the greenhouse, twice yearly, forming several flower stalks. Seed germination rate was significantly increased *in vitro* by seed soaking in gibberellic acid prior to cultivation on MS medium: from 1% to 61% for *V. davidoffii*, from 6% to 84% for *V. anisophyllum*, and from 18% to 85% for *V. tzar-borisii*. Plants were *ex vitro* adapted in a phytotron, and after wintering in the unheated greenhouse they were planted in their populations of origin: 42 *V. tzar-borisii* plants in March 2015; 32 *V. anisophyllum*, and 25 *V. davidoffii* plants in June 2017. Monitoring in May 2017 confirmed the successful acclimatization of 32 *V. tzar-borisii* plants (28 rosettes and 4 flowering). Monitoring of the plants from the two other species is foreseen for June 2018.

Key words: mullein, *in vitro* seed germination, monitoring

PLANTS USED IN TRADITIONAL ALCOHOLIC BEVERAGES OF THE ADRIATIC ISLANDS (CROATIA)

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In 2015 - 2017, wild food plants on fourteen of the larger islands in the Croatian Adriatic were investigated. During the project additional ethnobotanical data were also gathered, e.g. about the plants used to flavour traditional alcoholic beverages (TAB). The studied islands were: Brač, Cres, Dugi Otok, Hvar, Korčula, Lastovo, Mljet, Molat, Pag, Pašman, Rab, Šolta, Ugljan, and Vis. Data come from 236 semi-structured interviews. Preliminary results on the basis of 813 citations showed 115 plant species, belonging to 45 families, which are used for TABs. Rosaceae was the most species-rich family (22 species; 18%), followed by Lamiaceae (21 species; 17%), Asteraceae (12 species; 10%), and Rutaceae (6 species; 5%). The most frequently-mentioned species for TABs, also with the most widespread uses from the north to the south of the Adriatic, are: *Myrtus communis* L. (57, 26%, 12 islands), followed by *Salvia officinalis* L. (53, 23%, 13 respectively), *Foeniculum vulgare* Mill. (52 citations, 22% of interviews, 13), *Arbutus unedo* L. (47, 18%, 12 respectively), and *Ruta graveolens* L. (39, 15%, 12 respectively). The above-mentioned plants can be, depending on the species or local tradition, 1) distilled into grappa based on *Vitis vinifera* L. (rakija loza), 2) added after the distillation of grappa (one-species rakija e.g. *Ruta*, *Artemisia absinthium* L. (22, 9%, 8 islands), *Juglans regia* L. (20, 8%, 8), *Ceratonia siliqua* L. (14, 41%, 10), 3) mixed with other plants in rakija travarica (medicinal grappa with more types of plants), 4) used to make a sweet liqueur from fruits e.g. *Myrtus*, *Arbutus*, and *Salvia*, 5) added into mošt (young wine) e.g. *Salvia*, and *Artemisia* or 6) some other species can be used as the base for rakija, e.g. *Arbutus*, *Ficus carica* L. (21, 9, 9) etc. The above-mentioned TABs are used in everyday life, for instance as beverages or medicines against different illnesses, applied internally or externally.

Key words: ethnobotany, Adriatic islands, rakija, liqueur, traditional alcoholic beverage (TAB)

MEDICINAL PLANTS IN THE INTENSIVELY MANAGED AGRICULTURAL LANDSCAPE IN PAZARDZHIK - PLOVDIV REGION OF BULGARIA

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The objective of present study was to assess the diversity of medicinal plants in the agricultural fields and their peripheral parts in the region of Pazardzhik - Plovdiv. Different types of land use were included in the study ranging from cereal crops (the predominant type) through horticultural fields to dirt roads. There were totally 1238 records of 255 plant species in 89 experimental plots belonging to 31 types of landcover. Total 71 medicinal plant species were recorded 315 times. The results indicate that medicinal plants in the agricultural land and its peripheral parts, including field borders, represent relatively small percent of the species composition. The most frequent were 41 species found in 10% or more of the transects. Only about 22% of these species are medicinal plants. The highest diversity of medicinal plants was recorded in the remnants of woody vegetation (Solitary trees and small groups of trees / bushes), and in fruit tree orchards, representing places that are not subjected to regular soil cultivation. However, the diversity of medicinal plant species in the studied land can be evaluated as relatively low. Besides the low level of species diversity in the agricultural land, using of pesticides possess a serious obstacle to the use of these species for medicinal purposes.

Key words: medicinal plants, land use, agriculture

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EFFECT OF ARBUSCULAR MYCORRHIZAE ON PHOSPHORUS DEFICIT STRESS DURING EARLY DEVELOPMENT STAGE OF BASIL (*OCIMUM BASILICUM* L.)

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Basil (*Ocimum basilicum* L.) is one of the most important and widely used medicinal and aromatic plants. Global decline in phosphate rock supplies as a non-renewable source of phosphorus (P) fertilizers represent challenge for the traditional agriculture which requires alternative approaches for P fertilization strategies. Phosphorus is one of the most important but least available plant nutrient in the soil, and P deficit often affects young plants with small root system. One of the widely spread adaptation to low P availability in terrestrial ecosystems is development of mycorrhizal associations. The aim of this study was to determine the effect of arbuscular mycorrhiza (AM) on early root growth, mineral content and photosynthetic efficiency among most used basil cultivars and varieties ('Genovese', 'Sweet Basil', 'Dark Opal' and var. *purpurascens*). AM-inoculated (with *Rhizophagus irregularis*) and AM-free basil accessions were grown for 60 days under low ($1 \mu\text{mol L}^{-1}$) P concentration. Chlorophyll fluorescence measurements were conducted during growth period, and at the end of the experiment root morphological characteristics and shoot mineral content were determined.

Key words: basil, arbuscular mycorrhizae, root traits, chlorophyll fluorescence, mineral content

GENUS *INULA* L. IN BULGARIA – DISTRIBUTION AND NATURAL HABITATS

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Genus *Inula* is represented by 12 species in Bulgaria. Most of them are widely distributed across the country and three species are considered rare. One species – *I. spiraeifolia* L. is protected by the Biodiversity Act and is included in the Red Data Book of Bulgaria. According to their ecological characteristics, the species can be subdivided into four groups. The first group includes species occurring on limestone in open places (for example, *I. oculus-christi* L. and *I. aschersoniana* Janka). Frequently they are part of the habitats 62A0 Eastern sub-mediterranean dry grasslands, 8210 Calcareous rocky slopes with chasmophytic vegetation and 5210 Arborescent matorral with *Juniperus* spp. The second group consists of species which are found mostly on calcareous soils but occur also under wide range of ecological conditions (for example, *I. bifrons* /L./ L., *I. conyza* L.). The third group includes only one species – *I. ensifolia* L. which is found exclusively in forest habitats (mostly 9530 (Sub-) Mediterranean pine forests with endemic black pines and 95A0 High oro-Mediterranean pine forests). However, other species can also be found in the forests, especially in the peripheral parts (for example, *I. helenium* L.). The fourth group includes the most tolerant species, occurring in variety of environmental conditions, like *I. britannica* L., *I. germanica* L. and *I. hirta* L. The results of the study are discussed in the light of the species conservation and sustainable use.

Key words: *Inula*, Asteraceae, medicinal plants, habitats

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COMPARATIVE ANATOMY, MICROMORPHOLOGY AND BIOCHEMISTRY OF *SAMBUCUS NIGRA* L. FRUITS

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Sambucus nigra L. is a popular ornamental shrub and a highly valued medicinal plant, as it is a source of many bioactive compounds. Its flowers and fruits are widely used in the Romanian traditional medicine. Fruit of *Sambucus nigra* is of increasing commercial interest for juice, colorant, and nutraceutical use. The knowledge of the morphology, structure, micromorphology of *Sambucus nigra* fruits and the accumulation of bioactive substances in these organs, is rather poor. The aim of this paper was to analyse the micromorphological, structural and physico-biochemical features of *Sambucus nigra* fruits harvested from five different locations in Iasi county (Romania). Fruit samples were analysed with specific techniques and methods of the light and scanning electron microscopy. Histological features of the epicarp and mesocarp highlighted a diversity regarding the form and dimensions of epidermal cells, the thickness of epidermal cell wall, the thickness of cuticle, the number of stomata, the form and dimensions of hypodermal cells and of mesocarp cells, the thickness of mesocarp parenchyma layers. The micromorphology of the epicarp showed the aspect of cuticle, with numerous parallel oriented crystalline wax platelets, as well as spherical deposits of wax alternating with smooth areas or highly sloped microspheres. In a physical and biochemical point of view, the following parameters have been analysed: the average number of fruits in corymbiform cymes; the average of fruits fresh weight; refractometric index and total content of total soluble solids from the fruits juice, contents in water, dry matter and total mineral elements. The above specified parameters indicate specific variations related to the fruits source location, a high content in water and solid matter, a moderate content in mineral elements, a strong negative correlation between the water content and the soluble solid matter content.

Key words: *Sambucus nigra*, structure, epicarp, mesocarp, micromorphology, biochemistry

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MEDICINAL BOTANICAL KNOWLEDGE AMONG THE LAST REMAINING YÖRÜKS OF THE BALKANS

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Yörüks can be considered a phenomenon in a multicultural and complex environment in the Balkans. This community has nomadic traditions, but nowadays has a settled lifestyle. The aim of this study was to document knowledge concerning local wild and cultivated medicinal plants by study of the current lifestyle of the small and isolated group of Yörüks found within the Republic of Macedonia (Plačkovica Mt.). Forty-two plant taxa were recorded from 35 genera, based on the compilation of more than 120 reports on medicinal plants, as well as some elements relating to animals and minerals. Wild plants were the predominant species listed (36 taxa). Plants are used for a very wide range of complaints and were recorded in more than 50 preparations as home remedies. The largest proportion was used for treating gastrointestinal and upper respiratory tract disorders. The use of home remedies is still active as well as the use of edible plants that until now were known only from past ethnological data for the Balkan region. The most popular group of species that are also used in folk medicine includes *Achillea millefolium* L., *Cornus mas*, *Hypericum perforatum* L., *Juglans regia* L., *Matricaria chamomilla*, *Mentha* spp., *Origanum vulgare* L., *Plantago major* L., *Thymus* spp., *Sambucus nigra* and *Urtica dioica* L. A relatively limited number of plants is used specifically for herbal tea, mostly for refreshment and as a general tonic. *Sempervivum marmoreum* Griseb. as a well-known medicinal plant, for Yörüks has become transformed into a cultural border marker, associated with the space around the house and also use as a medicinal plant, uniquely relieving ear pain. The behavior and customs that still exist today support two processes: adaptation to the flora of the new host country and continued use and acquisition of flora from the homeland of the migrants. Traditional knowledge remains strong and is retained within a well-defined cultural boundary.

Key words: ethnobiology, ethnobotany, Macedonia, Plačkovica Mountain, Yörüks

ASSESSMENT OF POPULATION DIVERSITY IN WILD *RUBUS IDAEUS* L. POPULATIONS FROM SERBIA

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Genus *Rubus* L. (Rosaceae), with more than 700 species, is one of the largest genera in plant kingdom. Raspberry (*Rubus idaeus* L.) is diploid and belongs to subgenus *Idaeobatus*. Raspberry leaves are used in traditional medicine for treatment of various disorders most commonly related to menstruation, parturition, and ailments of the gastrointestinal tract and other purposes, while fruit is used as food. In this work we used AFLP markers to analyse intrapopulation diversity and interpopulation relationships of seven wild growing populations of *R. idaeus* from Serbia, that can be grouped into three spatial groups as well as into two altitude groups. Four AFLP combinations yield a total of 247 polymorphic bands in 133 *R. idaeus* specimens. Data analysis revealed similar levels of diversity across populations. Population from Mt. Ozren had both the highest diversity and the highest frequency down-weighted marker values. AMOVA analyses further showed high intrapopulation diversity, while population differentiation was low. The partition among spatial groups was not significant while the partition between altitude groups was highly significant. BAPS grouped the samples into three clusters of which to cluster C belong exclusively individuals originating from population Ozren. Clusters were almost identical with or without spatial coordinates. In analysed populations altitude had a stronger influence on differentiation than horizontal distance and that can be attributed to different phenology at different altitudes. Our results indicate that AFLP markers are reliable technique for assessing genetic diversity and relations between *R. idaeus* populations.

Key words: Raspberry, AFLP, population variability

THE TREATMENT OF MENORRHAGIA WITH BIOAPIGYN® HERBAL OINTMENT AND PESSARIES

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Menorrhagia is the most common form of abnormal uterine bleeding characterized by prolonged and abundant menstrual bleeding whereby over 80 mL of blood per cycle is lost. The purpose of this work was development and application of new herbal ointment and pessaries for the topical treatment of menorrhagia using macerates and essential oils of medicinal plants. For this purpose, the following ingredients were used: *Capsella bursa-pastoris* L., *Alchemilla vulgaris* L., *Achillea millefolium* L., *Plantago major* L., *Polygonum aviculare* L., *Salvia officinalis* L., *Quercus robur* L., *Olea europaea* L., *Calendula officinalis* L., *Matricaria chamomilla* L., all macerated in olive oil; the essential oils of *Melaleuca alternifolia*, *Eugenia caryophyllata*, *Cymbopogon martini*, *Cinnamomum camphora* ct. cineol, *Thymus vulgaris* ct. timol, *Origanum compactum*. Other ingredients included honey, glycerol, *Cera alba* and Witepsol E75 in the case of pessaries. 50 subjects with proven menorrhagia ranging from 32 to 54 years were divided into two groups (each of 25 participants) of similar age, health status and lifestyle. The first group used 2 g of ointment every eight hours while the second group used pessaries instead. The results were obtained at baseline and following the third menstrual cycle. The improvement was accompanied by reduction in the number of spent sanitary pads or tampons, waking up during the night in order to change the pads or tampons, the duration of bleeding, appearance of the blood clots and the number of painkillers consumed. In both groups a significant decrease was observed for all the symptoms ($p < 0.05$). Significantly better results were obtained in case of the ointment compared to the pessaries as expected due to the higher percentage of the active ingredients. The average duration of bleeding is reduced from 7.4 to 4.7 days in the case of the ointment and from 7.3 to 5.1 days in the group who used pessaries. The average number of painkillers is reduced from 22.1 to 10.6 and from 22.9 to 13.1 in the group using the ointment and pessaries, respectively. Decrease in other symptoms ranged from 71.4% up to 88.2% in the group using the ointment and from 57.3% to 73.7% in the case of pessaries usage. Those results could be correlated with the ingredients with proven haemostatic, vasoconstrictor, astringent and spasmolytic activity.

Key words: olive oil macerates, medicinal plants, essential oils, menorrhagia, herbal ointment, pessaries

EVALUATION OF THE DIVERSITY OF COMMON JUNIPER (*JUNIPERUS COMMUNIS* L.) FROM THE TYPICAL GEOGRAPHIC AREAS IN ALBANIA

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Juniperus communis L., a wild shrub species, growing especially in mountain areas of Albania in association with the other most important coniferous species. It is included in the Red Book of Albania as an endangered species growing wild in Albania. Nowadays, berries of *Juniperus*, are exported from Albania to many countries and especially in EU countries and are considered as the important income source for the local people that live in the poor mountain areas of the country. Assessment of geographic distribution of several populations of *Juniperus*, observed in 10 districts of Albania (Malesi e Madhe, Tropoje, Kukes, Diber, Elbasan, Librazhd, Bilsht, Korce, Erseke, Leskovik), was carried out. Berry fruits were also collected from 18 different natural growing areas of *Juniperus* in Albania. Spatial analysis, using grid square cells of 1 x 1 km, and 10 x 10 km, detects the areas of high diversity of *Juniperus* populations. The geographic areas were separated into small grid square cells, and grid cells of 1 x 1 km, and 10 x 10 km was used to assess the geographic distribution, diversity indices, and richness estimators of oregano populations. The biodiversity monitoring of Juniper populations, growing naturally in different mountain areas of Albania, contributes to the increasing efficiency and enhancement of beverages and food industry.

Key words: *Juniperus communis*, geographic distribution, Albania

MORPHOLOGICAL, AGRONOMIC AND GENETIC CHARACTERIZATION OF *RHODIOLA ROSEA* L. ACCESSIONS FROM THE PROVINCE OF TRENTO, AS PRELIMINARY RESULTS FOR A STUDY ON THE BIODIVERSITY OF THE SPECIES

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Rhodiola rosea L. is a dioecious perennial plant belonging to the family of Crassulaceae which grows in Europe, Asia and North America. In Europe its distribution area encompasses the North of Europe and most of the mountains of Central Europe, including the entire Alpine Arc. Its underground parts, rhizomes and roots, contain active compounds with well established adaptogenic properties which make it a worldwide known medicinal plant. At the moment the market demand of raw material is mostly supplied by the wild collection, and its cultivation is becoming increasingly necessary due to the risk of endangering its long-term survival in the areas in which it is collected, but just a few cultivated varieties of the species are available on the market. The aim of this study was to evaluate the morphological and agronomic characteristics and the genetic variability of eight accessions of the species originating from as many wild populations collected in the province of Trento, North east of Italy, and cultivated *ex situ*. Plantlets of the various accessions were obtained from seeds and transplanted in an experimental trial at 1349 m a.s.l. in the summer of 2013. Morphological traits were recorded from the third to the fifth year of cultivation by the use of descriptors list for the species especially modified for this study and yields of roots and rhizomes were recorded in the fourth and fifth year from transplant. Finally, the plantlets belonging to the selected accessions were genotyped using 20 published SSR (*Simple sequence repeat*) markers. A great variability in morphological traits, genetics and yields was observed among investigated accessions and this could justify, if supported by the currently underway qualitative characterization of the same accessions, the improvement of the species by a breeding program.

Key words: *Rhodiola rosea*, morphological characterization, agronomic characterization, genetic characterization

ESSENTIAL OIL EXTRACT, DETERMINATION OF NANO-EMULSIONS CHARACTERISTICS AND ALLELOPATHIC POTENTIAL OF MEDICINAL PLANT *DUCROSIA ANETHIFOLIA* (DC.) Boiss

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This research aims to identify and determine the chemical composition of the essential oil (EO), characteristics of EO nano-emulsion, allelopathic effects of EO and aqueous extract of the aerial parts of *Ducrosia anethifolia* (DC.) Boiss. The plant material was subjected to hydrodistillation by using Clevenger apparatus and the obtained EO was analyzed by GC and GC-MS instrument. The major compounds of EO were: n-decanal (44.8%), dodecanal (11.56%), n-decanol (8.22%), (2E)-dodecanal (5.7%) and alpha-pinene (5.02%). The EO nano-emulsions were made by the stabilizer solutions of polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP). The characteristics of the nano-emulsion including density, pH, electrical potential, total dissolved solids (TDS), electrical conductivity, electrical resistance, zeta potential, particle size, viscosity and antioxidant were investigated and compared to the control. The results showed that density, pH, electrical resistance, zeta potential and particle size decreased, while electrical potential, TDS, electrical conductivity, viscosity and antioxidant activity of nano-emulsion EO increased. The effects of aqueous extracts on seed germination and growth of *Lepidium sativum* L. and *Amaranthus retroflexus* L. at different concentrations (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4%), the effects of EO on seeds of *A. retroflexus* at the concentrations of 0, 600, 1200, 1800 and 2400 $\mu\text{L L}^{-1}$, and on the seeds of *L. sativum* at the concentration of 0,600,1200,1800, 2400,3000 and 3600 $\mu\text{L L}^{-1}$ were investigated. The measured characteristics included: germination percentage, plumule length, radical length, germination speed, allelopathic index, seeds vigor index and allometric coefficient. The results showed that the aqueous extract had the highest inhibition effect on the measured properties of *A. retroflexus* and *L. sativum* at the concentrations of 2.5% and 3% (germination reached zero), respectively. The *D anethifolia* EO had significant inhibition effect on the measured properties of *A. retroflexus* at the concentration of 2400 $\mu\text{L L}^{-1}$ (germination reached zero). The effect of EO was significant on the following parameters: germination speed, seed vigor index, allometric coefficient, radical length and plumule length of *L. sativum* seeds. however, it did not affect the germination percentage and allelopathic index.

Keyword: *Ducrosia anethifolia*, essential oil, extract, antioxidant, phenolic compound, poly vinyl alcohol, polyvinyl pyrrolidone, allelopathic activity

POPULATION STATUS AND HABITATS OF *ALLIUM URSINUM* L. IN BULGARIA

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Allium ursinum L. is a valuable medicinal plant used also for food. We present results of a study on plant communities and habitats of representative localities in Bulgaria. The species occurs in 10 floristic regions and most localities are in mesophytic forest stands. Floristic composition consists of relatively small number of species typical for the forest habitats. Population status of the species is stable, and in many places, it is subjected to collection with economic purpose and by the local people. The most conserved and numerous populations were established in Stara planina Mts and the scarcest populations were found in Lulin Mts (Vitosha region) due to anthropogenic pressure. Currently *A. ursinum* is not considered endangered and is allowed for sustainable use. The main habitats where the species was recorded are: 9130 *Asperulo Fagetum* beech forests; 91BA Moesian silver fir forests; 91W0 Moesian beech forests; 9170 *Galio-Carpinetum* oak-hornbeam forests; 9410 *Acidophilous Picea* forests of the montane to alpine levels (*Vaccinio-Piceetea*). The highest floristic diversity was recorded in the habitat 9170.

Key words: medicinal plant, habitats, wild garlic

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POPULATION STATUS AND HABITAT CHARACTERISTIC OF TWO *SIDERITIS* SPECIES IN BULGARIA AND GREECE

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Balkan Peninsula is a region of high diversity of *Sideritis*, section *Empedoclia*. The aim of present study is to characterize the population status and habitats of two endemic species – *Sideritis scardica* Griseb. from Bulgaria and Greece, and *Sideritis euboea* Heldr. from Greece. The field observations were performed in Slavyanka Mts, Rhodopes Mts, Pirin Mts (Bulgaria) and Orvilos Mts, Bozdag Mts, Olympos Mts and Dyrfis Mts (Greece). The Greek localities were in better condition, based on a system of indicators, like population size, density, viability and naturalness of plant communities. *S. scardica* is collected for herbal tea both in Bulgaria and Greece, but the studies have shown that Bulgarian localities are subjected to considerably stronger anthropogenic pressure. Also, Bulgarian populations are in the most northern peripheral part of the natural distribution of the species. Natural populations of the narrow endemic *S. euboea* are in good condition which is illustrated by number of different criteria: population size, composition of its natural plant communities, weak anthropogenic pressure. This is illustrated by the presence of numerous unharvest inflorescences, already with mature seeds, in the end of the vegetation season (early September). Seed and pollen viabilities, as measured by tetrazolium and acetocarmine tests, respectively, were similar in the Bulgarian and Greek populations and the differences were not statistically significant. The floristic inventory of the natural communities of the studied species allowed to identify the natural habitats, of which they are part. They included both forest and grassland habitats. Forest habitats were dominated by *Pinus nigra* Arn., *P. peuce* Griseb, *P. heldreichii* Christ and *Abies cephalonica* Loud. The results of the study are discussed in the light of the species conservation and sustainable use.

Key words: Mountain tea, *Sideritis*, population status

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TRADITIONAL HERBAL TEAS CONSUMED IN MERSIN (TURKEY)

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Traditional herbal teas have been known and loved for 5000 years. The culture related to herbal teas has been created with myths, idioms, arts and industry. The preparation of tea has been determined by region or country culture and knowledge and by potential of rich flora in Turkey. Either wild or cultivated herbals are evaluated as traditional tea. Herbal teas are made up of single plant species or plant mixtures. They are consumed for their pleasant aromatic flavors and also as ailments e.g., against cold, fatigue and insomnia. Usually, herbal teas are obtained from root, stem, leaf, aerial part and flower, and prepared for drinking in boiling water after being dried. Various scientific studies have shown that phenolic substances in tea composition have antioxidant, antimicrobial and anticarcinogenic properties. Local people of the city of Mersin have been consuming traditional herbal teas. Present study is a review of the ethnobotanical studies that were done by Everest et al. (2005), Eşen (2008), Metin (2009), Saday (2009), Sağıroğlu et al. (2013), Sargın (2015), and Everest et al. (2017) on traditional herbal teas in Mersin and its surroundings. According to the results 16 taxa were identified as traditional herbal teas in the study area belonging to 10 different families. The encountered plant families traditionally consumed as tea were Lamiaceae (5 taxa), Asteraceae (3 taxa), Labiatae (1 taxon), Lauraceae (1 taxon), Oleaceae (1 taxon), Orchidaceae (1 taxon), Rosaceae (1 taxon), Scrophulariaceae (1 taxon), Tiliaceae (1 taxon), and Zingiberaceae (1 taxon). The aerial parts, leaves, roots and fruits were determined as the most favored parts for traditional herbal teas. Traditional herbal teas are consumed in a variety of ways. Ethnobotanically, Mersin is rich in flora due to its geographical position. As a result, traditional herbal teas are still consumed in the city of Mersin (Turkey).

Key words: traditional, herbal tea, Mersin

ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS FROM ASTERACEAE IN BULGARIA

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The present study contains revision of the results on traditional use of wild medicinal plants from Asteraceae in Bulgaria. This is the largest family of vascular plants in the Bulgarian flora with more than 480 species (c. 12% of the total flora). There have been detected 73 Asteraceae species which are being used with ethno therapeutic purpose. Different herbal parts are basis for preparation of infusions, decoct, tinctures. The most significant plants on the field of ethno pharmacology are as follows: *Achillea millefolium* L., *Arctium tomentosum* Mill., *Artemisia absinthium* L., *Artemisia santonicum* L., *Carduus acanthoides* L., *Carlina acanthifolia* All., *Carlina vulgaris* L., *Carthamus lanatus* L., *Centaurea cyanus* L., *Cichorium intybus* L., *Cnicus Benedictus* L., *Helichrisum arenarium* (L.) Moench., *Inula helenium* L., *Matricaria chamomilla* L., *Solidago virga-aurea* Linn., *Sylibum marianum* (L.) Gaertn., *Tanacetum vulgare* L., *Taraxacum officinale* Weber ex Wigg, *Tussilago farfara* L., etc. Majority of herbs is being used for treatment of illnesses of respiratory, gastrointestinal, for treatment of skin conditions, as well as for nervous system and, etc. Some species plays important role as antioxidants in protecting cell membranes against pathological conditions such as carcinogenesis, atherosclerosis, liver disease, prevention and treatment of cancer, aging, mutagenesis. The ecology and populations status of some species have been studied and recommendations have been made for the sustainable use of their resources.

Key words: Asteraceae, Bulgaria, medicinal plants

HOW DO MORPHOTYPES AND CHEMOTYPES RELATE TO GENOTYPES? INTRASPECIES DIVERSITY OF *OCIMUM BASILICUM* L.

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Basil (*Ocimum basilicum* L.) is a widely known member of Lamiaceae family with a long tradition of cultivation. Cultivation for different market requirements led to great intraspecific variation at both morphological and biochemical level. The aim of this research was to assess diversity among basil accessions maintained at the Collection of Medicinal and Aromatic Plants as a part of the National Bank of Plant Genes. Eighty basil accessions belonging to five basil morphotypes (True basil, Small-leaf, Lettuce-leaf, Purple basil A and Purple basil B) and five chemotypes (High linalool, Linalool/trans- α -bergamotene, Linalool/methyl chavicol, Linalool/trans-methyl cinnamate and High methyl chavicol) were analysed using AFLP markers. Sixteen AFLP primer combinations yielded a total of 2,114 polymorphic markers. Analysis of molecular variance (AMOVA) revealed that most of the genetic diversity was attributable to differences among accessions within morphotypes and within chemotypes. Bayesian Analysis of Population Structure (BAPS) resulted in assignment of the investigated accessions to four clusters. The accessions belonging to ‘green’ morphotypes (True basil, Small-leaf, Lettuce-leaf) were assigned to Clusters A or B and Purple basil A accessions to Clusters A or C. Purple basil B morphotype was genetically the most admixed, having accessions belonging to all four genetic clusters.

Key words: accession, AFLP markers, basil, chemotypes, genetic diversity, morphotypes

Acknowledgement: This work is part of the research programme on conservation of medicinal and aromatic plants carried out by the Working Group on Medicinal and Aromatic Plants (<http://cpgrd.hcphs.hr/gb/map/>), which is financed by the National Programme for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture of the R. of Croatia.

TRADITIONAL PREPARATION OF SYRUPS, MARMALADES, LIQUEURS AND HERBAL GRAPPAS IN THE KNIN AREA, CROATIA

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The traditional preparation of syrups, marmalades, liqueurs and herbal grappas were recorded in Knin area in south-eastern Croatia during wider ethnobotanical survey on plant use in the area. Local knowledge was recorded using semi-structured interviews. Thirty-five interviews produced an inventory of 34 species from 13 families and 59 local names from 251 citations. Maximum Relative frequency of citation (RFC) was for *Cornus mas* L. (0.74), following by *Sambucus nigra* L. (0.60), *Prunus domestica* L. (0.60) and *Rosa canina* L. (0.51). Twelve species were mentioned only once. The most frequent use categories were syrups (110), followed by marmalades (86), and liqueurs (43). Fruits (77%) and flowers (11%) were the most used plant parts. The indigenous population of the studied area strongly keeps the tradition of preparing homemade syrups, marmalades, and liqueurs for consumption.

Key words: ethnobotany, Knin, marmalades, syrups, traditional knowledge

II. PHYTOCHEMICAL ANALYSIS, PHARMACOLOGY, BIOLOGICAL ACTIVITY AND TOXICITY (PART I)

CHEMISTRY AND BIOACTIVITY OF *SALVIA OFFICINALIS* L. AND RELATED SPECIES – A MULTI-LAYERED PICTURE

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Garden sage (*Salvia officinalis* L.), Greek sage (*S. fruticosa* Mill.) and apple sage (*S. pomifera* L.) are three closely related *Salvia* species native to the Eastern Mediterranean basin. The three species are widely used as aromatic plants as well as in medicine for several reasons based on different groups of compounds, demonstrating a complex picture of chemical composition of bioactive molecules. Mainly responsible for their aromatic activity are the monoterpenes 1,8-cineole, α - and β -thujone and camphor, which differentiate well the three species. While *S. fruticosa* is rich in 1,8-cineole and *S. fruticosa* is rich in thujones, *S. officinalis* is intermediate with 1,8-cineole and the thujones as major compounds. A long-lasting debate about the toxicity of the thujones is still ongoing. In this context, it is also noteworthy, that the limits for thujones in the European Union for food and pharmaceutical products are different. The high antioxidant activity of sage is a further complex area, based on two different compound groups, the (lipophilic) diterpenes carnosol and carnosic acid as well as on high amounts of (hydrophilic) rosmarinic acid. A further interesting compound is the pentacyclic triterpenoid ursolic acid, which is used as an emulsifier and is active against brain disorders. The many different activities in sage based on many different compounds or compound groups do not permit a simple breeding optimization of one compound (group) only but would justify a differentiation into breeding lines with different maximized activities.

Key words: *Salvia officinalis*, monoterpenes, thujones, antioxidant activity, carnosol, carnosic acid, rosmarinic acid, ursolic acid

ANALYSIS OF AROMATIC AND MEDICINAL PLANTS APPLYING VARIOUS VIBRATIONAL SPECTROSCOPY TECHNIQUES - POSSIBILITIES AND LIMITATIONS

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Quality control of aromatic and medicinal plants as well as products derived from them usually comprises correct botanical identification of the plant material as well as quantification of the individual active principles. Furthermore, residues (e. g. organic solvents) and contaminants (e. g. pesticides and heavy metals) are determined by applying various sophisticated analytical techniques. For this, testing of plant material such as phytopharmaceutical products is usually performed in accordance with validated standard methods described in the Food Chemical Codex, the European Pharmacopeia, the United States Pharmacopoeia and others. Contrary to this approach, there is also a need to apply various rapid high-throughput methods aiming to characterize simultaneously several quality parameters and to reduce efforts for sample preparation to a minimum. In this context new vibrational spectroscopy methods (ATR-IR, NIR and Raman spectroscopy) in combination with various chemometric algorithms are presented which allow efficient monitoring of numerous plant samples within a short time. Raman spectroscopy has especially been found to be a reliable and non-destructive method for rapid discrimination of different plant species or chemotypes if characteristic key bands can be observed in the spectrum. But also, NIR and ATR-IR spectroscopy has made the handling of powdered as well as liquid samples very quick and simple. Today, portable IR and Raman spectrometer systems are available which need only small sample amounts of a few microliters or milligrams for analysis. In most cases, vibrational measurements can be performed directly on plant tissues as well as on fractions isolated from the plant material by hydro-distillation or solvent extraction. Based on individual marker bands, spectroscopic analyses in principle allow the discrimination of different species, and even to classify chemotypes among the same species. Combination of vibrational spectroscopy and hierarchical cluster analysis provides a fast, easy and reliable method for chemotaxonomic characterization. The ability to rapidly monitor various plant components provides the possibility to efficiently select high-quality single plants from wild populations as well as progenies of crossing experiments. Today, vibrational spectroscopy is already introduced in industry in order to perform fast quality checks of incoming raw materials and continuous controlling of production processes.

Key words: quality control, chemotypes, IR, Raman

IN VITRO ANTIFUNGAL POTENCY OF EXTRACT FROM ANISE FRUITS (*PIMPINELLA ANISUM* L.) AND FIVE ESSENTIAL OILS AGAINST SOME PLANT PATHOGENIC FUNGI

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The negative environmental impact of pesticides used for disease control in plants is intensively increasing every day. Antifungal substances which are obtained from plants have no side effect against environment thus, giving a significant advantage. The antifungal activities of extract from anise fruits (*Pimpinella anisum* L.) and five essential oils (α -pinene, β -pinene, estragole, fenchone and trans-anethole) were investigated *in vitro* against five important plant pathogenic fungi (*Alternaria alternata*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Botrytis cinerea* and *Plasmopara viticola*) at different concentrations (0.39 - 50 mL mL⁻¹ 10% DMSO). The plant pathogenic fungi used in the research were obtained from the stock cultures from Department of Microbiology and Microbial Biotechnology, Institute of Biology, Faculty of Natural Sciences and Mathematics in Skopje. Broth-microdilution assay was used for the *in vitro* antimicrobial screening. The methodology used is based on measuring the Minimum Inhibitory Concentration (MIC) as well as the Minimum Fungicide Concentration (MFC). The tested extract has different degree of antifungal activity against five tested fungi. When compared with controls, the highest antifungal activity of extract from anise fruits (*Pimpinella anisum* L.) was recorded for *Fusarium oxysporum*, *Botrytis cinerea* and *Plasmopara viticola* at a concentration of MFC = 0.39 mL mL⁻¹ 10% DMSO, and MIC concentration was < 0.39 mL mL⁻¹ 10% DMSO. These fungi showed same results for α -pinene and estragole. The most sensitive microorganism was *Botrytis cinerea*. The highest concentration of MIC and MFC showed fenchone (25 mL mL⁻¹ 10% DMSO and 50 mL mL⁻¹ 10% DMSO, respectively) with *Plasmopara viticola* and *Alternaria alternata*. The results indicated that all tested substances had antifungal activity against all tested fungi in different concentrations. These substances, or some mixture of them, may be recommended as a potent bio-fungicide against fungal plant diseases. This study paves the way for the development of bioactive natural products with phytosanitary applications, with the added benefits of an environmentally safe and economically viable product.

Key words: antifungal, phytopathogen, essential oil, inhibition

THE CLOVE ESSENTIAL OIL AND ITS MAJOR COMPONENT EUGENOL SUCCESSFULLY RESTORED ERECTILE DYSFUNCTION OF STREPTOZOTOCIN-INDUCED TYPE I DIABETES IN RATS

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Diabetes is one of the major risk factors for erectile dysfunction (ED). Phosphodiesterase-5 inhibitors (PDE5i) are a first-line medical therapy for ED. The risk of ED increases with duration of diabetes and PDE5i are not as effective in the treatment of diabetes-associated ED. Currently, several medicinal plants and secondary metabolites are natural remedies for ED. A tropical plant, *Syzygium aromaticum* (L.) Merrill & Perry or clove from the Myrtaceae family has aphrodisiac activity. In Europe and Asia, it has been used for culinary and medicinal purposes for centuries. In addition, the cloves are reported to be used as sexual invigorators in India. The plant contains polyphenols and eugenol (E) which are the major components of the clove oil (CO) from aromatic flower buds (70 – 85%). In the preclinical study, treatment with 50% ethanolic extract of clove significantly increased the sexual activity of male rats. The present study aimed to investigate the possible beneficial effects of CO and its component, E on streptozotocin-induced diabetic rats. Adult male twenty Sprague-Dawley rats were divided into two groups: control and diabetes induced by a single intraperitoneal injection of streptozotocin (45 mg kg⁻¹). *In vivo* erectile responses were repeated after intracavernosal injection of CO and E in anesthetized rats. The relaxant responses to CO and E (25 - 100 µL) were investigated in rat corpus cavernosum (CC) in organ bath studies. Diabetic rats had significantly decreased *in vivo* erectile responses, which were improved after intracavernosal administration of CO and E. CC strips exhibited remarkable relaxations in a concentration-dependent manner (100%) by the treatments of CO and E in groups. We firstly found that intracavernosal administration of CO and E is beneficial on the recovery of diabetic ED and may be a successful treatment strategy for diabetic patients with ED who do not respond to PDE5i.

Key words: clove, ED, *Syzygium aromaticum*, diabetes

AMELIORATIVE EFFECTS OF *MATRICARIA CHAMOMILLA* L. HYDROALCOHOLIC EXTRACT ON SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT IN RATS: A BEHAVIORAL AND MOLECULAR STUDY

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Matricaria chamomilla L. is a medicinal herb traditionally used as the anti-inflammatory, antimicrobial, antiviral, anxiolytic and antidepressant agent. Nevertheless, supporting evidence demonstrated its memory enhancing activity and antioxidant properties. To investigate the effects of the hydroalcoholic extract of *M. chamomilla* on memory processes in a scopolamine-induced rat model of amnesia and to reveal its underlying mechanism of action. The hydroalcoholic extract (25 and 75 mg kg⁻¹) was intraperitoneally administered to rats once daily for 7 days, and scopolamine (0.7 mg kg⁻¹) was injected 30 minutes before the behavioural testing to induce memory impairment. The phytochemical composition of the extract was quantified by HPLC/DAD analysis. Y-maze and radial arm-maze tests were employed for memory assessing. Acetylcholinesterase activity was measured in the rat hippocampus. Superoxide dismutase, glutathione peroxidase, and catalase specific activities along with the total content of reduced glutathione and protein carbonyl and malondialdehyde levels were also measured in the rat hippocampus. qRT-PCR was used to quantify BDNF mRNA and IL1 β mRNA expression in the rat hippocampus. We first identified the chlorogenic acid, apigenin-7-glucoside, rutin, cynaroside, luteolin, apigenin and derivatives of apigenin-7-glucoside as the extract major components. Furthermore, we showed that the extract reversed the scopolamine-induced decreasing of the spontaneous alternation in the Y-maze test and the scopolamine-induced increasing of the working and reference memory errors in the radial arm maze test. Also, the scopolamine-induced alteration of the acetylcholinesterase activity and the oxidant-antioxidant balance in the rat hippocampus was recovered by the treatment with the extract. Finally, we demonstrated that the extract restored the scopolamine-decreased BDNF expression and increased IL1 β expression in the rat hippocampus. These findings suggest that the extract could be a potent neuropharmacological agent against amnesia via modulating cholinergic activity, neuroinflammation and promoting antioxidant action in the rat hippocampus.

Key words: chamomile, neuroinflammation, memory, behaviour

ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF *PINUS NIGRA* J.F. Arnold AND *PINUS SYLVESTRIS* L. COMPARE WITH ALPHA- AND BETA- PINENE

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In the current work, *in vitro* antibacterial activities of the needle crude extracts of *Pinus nigra* J.F. Arnold and *Pinus sylvestris* L. from Macedonia were analysed. These results were compared with *in vitro* antibacterial activities of α - and β - pinene, as one of the main compounds identified. Broth-microdilution assays were used for the *in vitro* antimicrobial screening, and the tested concentrations of substances were within 50 - 0.390%. As a test microorganism we used a panel of different bacteria, including four Gram negative bacteria (*E. coli* ATCC 8739, *Ps. aeruginosa* ATCC 9027, *S. typhimurium*, *S. enteridis*) and nine Gram positive bacteria (*B. subtilis* ATCC 6633, *B. pumillus* NCTC 8241, *Bacillus* sp., *S. citreus*, *S. albus*, *S. aureus*, *M. luteus*, *L. monocytogenes* and *S. lutea*). Two tested extracts showed antibacterial activity against the various tested bacteria comparing with the standards. Anyway, all Gram positive bacteria showed as more sensitive against all tested substances compare to Gram negative bacteria. Crude extracts from *Pinus nigra* showed as more potent against Gram negative bacteria compare to extract from *Pinus sylvestris* and *Ps. aeruginosa* ATCC 9027 was the most sensitive among Gram negative bacteria (MIC = 0.39% and MBC = 1.562 %). In Gram positive bacteria there are no any significant differences within activity of extracts from *P. nigra* and *P. sylvestris*. From Gram positive bacteria *S. citreus* showed biggest sensitivity against tested substances, with MIC = 0.39% and MBC = 6.25%). From the results from α - and β -pinene, in this screening α -pinene showed better results from β -pinene for both groups of bacteria. Also, β -pinene appeared with the weakest antibacterial activity from all four tested substances. It was concluded that the tested extracts showed promising antimicrobial activity against tested microorganisms. However, this investigation will be completed with evaluation of antioxidative activities of these extracts.

Key words: antibacterial, *Pinus*, extract, broth-microdilution assays

BOTANICALS IN FOOD SUPPLEMENTS AND NOVEL FOOD

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Novel food means all food or its ingredients that were not used in a significant amount until 15 May 1997. The development of new technologies and product formulations with new ingredients takes the lead in the food industry, particularly in the field of food supplements. Based on the development of self-healing awareness, food supplements are experiencing growth, development and its use. Novel foods and food supplements are under the Food Law and all regulations and directives related to this area. The new European Commission Regulation No. 2015/2283 on novel foods and novel foods ingredients has led to new legislative provisions and regulations. The use of botanicals is common in formulations and the production of food supplements. In the Republic of Croatia, use of botanical and other bioactive substances is regulated by the Ordinance on substances that can be added to food and used in the production of food and substances whose use is prohibited or restricted (Official Gazette 160/2013). The type and quantity of botanicals, their standardization forms in combination with other biologically active substances give a certain influence on the organism. Therefore, it is important to pay attention to whether some of the ingredients are in novel foods or are the ingredients of a new food. If they fall into this category, it is necessary to carry out and ensure appropriate risk and safety assessment procedures as well as notification in both the Republic of Croatia and the EU. This paper will show the role of producers, control institutions and competent ministries and the sequence of legislative provisions related to novel food, linking product conformity marking ratings, and check the product category and possible health claims.

Key words: botanicals, food supplements, novel food, labelling, legislation frame, European Union, Republic of Croatia

PHYTOCHEMICAL EVALUATION OF *IN VITRO* CULTURES FROM *ASTRAGALUS GLYCYPHYLLOS* L.

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Astragalus glycyphyllos L. (Fabaceae) is a perennial herbaceous plant, widely distributed in Bulgarian flora. Since medieval times the herb have been used as diuretic, tonic, as a therapy for liver disease, to treat menstrual pain, etc. There are three groups of pharmacologically active substances proved in the species – polysaccharides, saponins and flavonoids. Several pharmacological studies revealed the hepatoprotective and antioxidant potential of extracts from the plant. The aim of the present study was to establish *in vitro* cultures of *A. glycyphyllos* and to investigate the quantity of flavonoids and saponins in extracts from each culture. A highly sensitive UHPLC-HRESIMS was developed for the purpose. Explants from several parts of large intact *A. glycyphyllos* plants were used to form callus. Suspension cultures were established by transferring small pieces of callus to liquid medium which was subsequently placed on a gyratory shaker. Rutin was proved in highest amount in shoot cultures on MS culture medium (8.72 ng mg⁻¹ dw). Camelliaside A was determined in highest quantity in shoots (74.65 ng mg⁻¹ dw). Saponin S1 was quantified as 225.00 ng mg⁻¹ dw for the same culture. All cultures, developed in the dark, proved lowest amounts of analytes, despite of hormones. Calluses grown in light, displayed higher quantity if the three monitored compounds, than suspension cultures. The results of the study indicate that *in vitro* cultures of *A. glycyphyllos* could serve as an alternative way for production of flavonoids and saponins.

Key words: *Astragalus glycyphyllos*, *in vitro* cultures, saponins, flavonoids, UHPLC-HRESIMS

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BIOGENETIC RELATIONSHIP BETWEEN SOME *INULA* SPECIES GROWING IN BULGARIA BASED ON THEIR PHYTOCHEMICAL PROFILE

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Inula (Asteraceae) is a large genus with more than 100 species distributed in Africa, Asia and Europe. *Inula helenium* L., *Inula racemosa* Hook.f. and *Inula britannica* L. are frequently used in ethnomedicine. *Inula* species are continuously the target of scientific investigations to find their active principles and new pharmacological applications, or from chemotaxonomical point of view. Recently we have started phytochemical investigation of *Inula* species growing in Bulgaria. Extracts from *I. helenium*, *I. aschersoniana* Janka, *I. britannica* L., *I. oculus-christi* L., *I. germanica* L., *I. conyza* (Griess.) Meikle, *I. ensifolia* L. and *I. bifrons* (L.) L. were studied. It was found that the methanol extracts of all of them contained chlorogenic, 1,5-, 3,5- 3,4 and 4,5-dicaffeoylquinic acids. Thus, these compounds could not be used as chemotaxonomic markers in this case. Further, the lack of sesquiterpene lactones in *I. conyza*, *I. ensifolia*, and *I. bifrons* distinguished these species from the other four. With exception of *I. germanica* sesquiterpene lactones were studied in *I. helenium* (alantolactone, isovalantolactone), *I. aschersoniana* (parthenolide, diepoxycostunolide, inusoniolide), *I. britannica* (ivalin, britannin, gaillardin, 11,13-dihydroinuchinenolide B, pulchellin C), *I. oculus-christi* (gaillardin, pulchellin E, pulchellin C, 9 β ,10 β -epoxygaillardin, 9 α ,10 α -epoxy-2-epi-gaillardin, 2 α -acetoxo-4 α ,9 β -dihydroxy-1 β -guaia-11(13),10(14)-dien-12,8 α -olide, 4 α ,15 α -epoxypulchellin E). It has been found that species produced different type of lactones: 12,6-olides in *I. aschersoniana* and 12,8-olides in the other 3 species. Another significant feature for *I. aschersoniana* was the production of germacranolides and a 4,5-secoguaianolide. Eudesmanolides (ivalin and its C-3 oxygenated derivatives- pulchellin B and C) and guaianolides (gaillardin and biogenetically related to it) isolated from *I. britannica* and *I. oculus-christi* showed their similarity. The eudesmanolides found in *I. helenium* indicated that it is closer to the latter two species. The obtained results could help in determining the place of these species within the general scheme of the genus.

Key words: *Inula*, Asteraceae, sesquiterpene lactones, biogenetic relationship

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ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES OF METHANOL EXTRACT OF *ARGEMONE MEXICANA* L. AND ITS ANTI-HEPATITIS POTENTIAL

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Hepatitis has been a major plague of mankind. The history of the discovery of causative viruses is one of the most fascinating scientific adventures for half of the century. Individualization of several types of hepatitis only emerged after world war two. Their identification has been associated with milestones which revolutionized medicine and public health. The discovery of HBV brought the first ever vaccine not prepared by tissue culture but initially directly from plasma and soon after that the first vaccine was produced by genetic engineering. Increasing evidences suggest that Viral Hepatitis is a critical disease that kills about 15% to 25% people prematurely due to its infection. Treatment with available interferons only suppresses viral reproduction in about 40% to 90% of patients with chronic hepatitis. Most people do not have a permanent response and relapse is common; the medications do not cure the infection. Determination of inhibition zone on agar media used in this study showed that *Argemone mexicana* L. extract exhibited antimicrobial effects against *E. coli*, *B. subtilis* and *S. aureus*, at 25, 50, and 100 mg mL⁻¹ respectively. The antifungal activity of the extracts of the same concentrations against *A. niger*, *A. fumigatus* and *M.* species was dose dependent. The phytochemical screening of this plant revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids glycosides etc. which are reported to be responsible for numerous medicinal uses of plants. *A. mexicana* is a species whose pharmacological properties such as anti-hepatotoxic activity, anti-malarial, antibacterial, hepatoprotective, anti-HIV and anti-hepatitis have been described by the traditional medical practitioners. In the treatment of jaundice it has been confirmed by several authors hence there is a need for further studies. It is therefore important, for us as scientist responsible for the discovery of noble drugs, to characterize this plant in order to find safer potent drugs for this scourge hepatitis.

Key words: *Argemone mexicana*, bacterial, extract, fungi, hepatitis

HOW DOES ABIOTIC STRESS INFLUENCE A HEALTH-PROMOTING PHYTOCHEMICALS IN *BRASSICA* CROPS?

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Crop plants from the Brassicaceae family, including cabbages, kales, broccoli etc., are among the oldest known cultivated plants and are also commercially important vegetables worldwide. They have an important place in the culture and traditional cuisine of worldwide and are widely used in traditional medicine. Due to affordable price and availability at local markets Brassicaceae stands out as an important source of food, providing nutrients and health-promoting phytochemicals (glucosinolates, phenolic compounds etc.), which particularly attracted much recent scientific attention. In the light of climate changes, *Brassica* crops may be highly influenced by unpleasant environmental conditions or diverse abiotic stresses. Herein we investigated the impact of abiotic stresses on the profile of specialized metabolites in *Brassica* species traditionally grown in Croatia (*Brassica oleracea* L. var. *capitata* f. *alba* cv. 'Varaždinski', and *B. oleracea* var. *acephala*), and compared with *B. rapa* ssp. *pekinensis*, species known as moderate sensitive to abiotic stresses. The results showed that abiotic stress caused qualitative and quantitative changes in specialized metabolites (particularly polyphenolics) and antioxidant activity depend on species and applied stress. These changes in specialized metabolites are discussed in relation to stress tolerance of different *Brassica* species / varieties.

Key words: *Brassica* crops, white cabbage, kale, abiotic stress, phytochemicals

MOLECULAR MODELING AND MOLECULAR DOCKING FOR MEDICINAL PLANT VALORIZATION

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Interest to medicinal and aromatic plants for therapeutic purposes is a highly sought-after area of research and an area that has not yet revealed all its secrets. Around the world, researchers identify the therapeutic virtues of different plants from different regions while identifying the chemical components, in particular the bioactive molecules whose study of their effects for the treatment of certain pathologies remains long and somewhat difficult given the complexity of the procedure. In this context, molecular modeling and Molecular Docking has been adopted as an important step before investigation plant virtues in the treatment of any disease. Molecular Docking is a computational method that saves time and money in studying the effect of bioactive molecules before testing and designing drugs. Most of the pharmaceutical laboratories use Molecular Docking to identify the best bioactive molecules capable to inhibit enzymes responsible of pathologies. This presentation will give an insight to this method as well as its advantages for the valorization of aromatic and medicinal plants via their chemical composition.

Key words: Molecular Docking, bioactives molecules, therapeutic effects, plants valorization

EVALUATION OF THE POLYPHENOLIC AND PHYTOSTEROLIC CONTENT FROM *SCIRPOIDES HOLOSCHOENUS* (L.) Soják

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Scirpoides holoschoenus (L.) Soják (Cyperaceae) is a species known since antiquity and used in the ethno medicine of fishermen and sailors in the Danube Delta and the Black Sea as a decoction for maintaining and preserving the health of the digestive, biliary and hepatic tract but not used in modern therapy (the composition in antioxidant, anti-inflammatory and hepatoprotective principles is little known). The aim of the present study was to identify and quantify several polyphenolic compounds and phytosterols of the *Scipoides holoschoenus* plant (aerial parts and root). The identification and quantification of these compounds was performed using specific phytochemical tests, FT-IR infrared spectrophotometry, HPTLC and HPLC chromatography and validated spectrophotometric UV-VIS methods. The phytochemical study showed the presence of the compounds of interest: flavones, polyphenols, sterols, but with different intensities in the aerial parts and root. FT-IR experimental results indicated the presence of specific functional groups similar in the aerial parts and root: 1026; 1027 cm⁻¹ (C–O–C of glycoside structures)/ 1226; 1240 cm⁻¹ (carbonyl C–O)/1419; 1455 cm⁻¹ (C=C aromatic); 2192; 2939 cm⁻¹ (CH aromatic); 3275; 3276; 3462 cm⁻¹ (OH free alcohol type). In correlation with previous methods, the HPTLC chromatograms revealed a limited range of phenolic and sterolic substances: chlorogenic acid, ferulic acid, rosmarinic acid, apigenin, luteolin, β-sitosterol and stigmasterol. Quantitative determinations revealed that the aerial parts have the highest content of flavonoids (0.0069% g/gv.m. as rutin) and β-sitosterol (0.1 g/100 g v.m.- densitometric) and the root content of polyphenols (0.400% g/gv.m as chlorogenic acid). We found caffeic acid in methanolic extracts of the aerial parts and root (3.36 mg, respectively 0.63 mg/100 mL) and p-coumaric acid (3.74 mg, respectively 0.97 mg/100 mL) by HPLC chromatography method. The obtained results may serve to promote the use of the *Scirpoides holoschoenus* L. species as antioxidant and hepatoprotective agents.

Key words: *Scirpoides holoschoenus* L., antioxidants, FT-IR spectrofotometry, HPTLC chromatography, HPLC chromatography

BIOACTIVE COMPOUNDS IN *BRASSICA OLERACEA* L. VEGETABLES TRADITIONALLY GROWN IN CROATIA

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Cruciferous vegetables are recognized as a functional food because different epidemiological and meta-analysis suggested that consumption of cruciferous has preventive role against a variety of disease due to the presence of the bioactive compounds such as glucosinolates, polyphenols, carotenoids etc. In the present study we aimed to identify bioactive compounds (glucosinolates, polyphenols, carotenoids, ascorbic acid) and to measure antioxidant activity of white cabbage cv. Varaždinski (Croatian name varaždinsko zelje; Latin name *Brassica oleracea* L. var *capitata* f. *alba*) and flat leaves kale (Croatian name raštika; Latin name *Brassica oleracea* L. var *acephala*), two *B. oleracea* vegetables commonly used in Croatian cuisine and traditional medicine. In addition, we also measured activity of endogenous enzymes (peroxidases, myrosinase, and polyphenol-oxidase) associated with phytochemical biosynthesis, stability and bioavailability. For analysis, we used HPLC-MS, HPLC-DAD and UV/VIS methods. In the study, we include vegetables at different developing stages as well as from different seed producers. Results showed the presence of the bioactive compounds associated with health benefits and significant antioxidant activity in both *B. oleracea* varieties. Based on comparative analysis of different cruciferous vegetables, white cabbage and kale show higher amount of polyphenols and glucosinolates than Chinese cabbage or broccoli.

Key words: *Brassica*, glucosinolates, polyphenols, carotenoids, Croatia

PROTECTIVE POTENTIAL OF LYOPHILIZED EXTRACT OF *CLINOPODIUM VULGARE* L. (LAMIACEAE) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHRS)

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Hypertension (HTN) is the most important cardiovascular risk factor, leading to coronary and cerebrovascular diseases. Along with antihypertensive medications, plant-based treatment has been thought to be effective for the prevention and control of HTN. In this study, lyophilized extract of *Clinopodium vulgare* L. (Lamiaceae) (LECV) was analyzed by ultra-high-performance liquid chromatography – Orbitrap high resolution mass spectrometry (UHPLC-HRMS). Based on the accurate mass measurements, fragmentation patterns in MS/MS analyses and comparison with standards, a variety of clinopodic and caffeoylquinic acids, O- and C-glycosides of flavones and flavanones were identified or tentatively elucidated in LECV. We evaluated the neuro-, nephro-, and hepatoprotective potential of LECV in spontaneously hypertensive rats (SHR). LECV has been administered at a dose of 100 mg/kg bw (1/20 LD50) for 14 days. The activity of the brain acetylcholinesterase (AChE) and the following antioxidant enzymes: superoxidedismutase (SOD), catalase (CAT), glutathione-peroxidase (GPx), as well as the biomarkers of oxidative stress malondialdehyde (MDA) and reduced glutathione (GSH) have been measured in the brain, kidney and liver. Histopathological examination of those organs was performed. The levels of those parameters measured in control, non-treated SHRs were compared to their matched normotensive controls (NTRs). In the SHRs, the GSH levels and the activity of antioxidant enzymes were lower while the level of MDA was higher in all investigated organs. The activity of AChE in the brain of SHRs was lower than in the NTRs. When compared to the control SHRs, LECV administration exerted antioxidant activity, discerned by significantly increased activities of the antioxidant enzymes and decreased production of MDA. The AChE activity remained unchanged. The observed effects on tissue level were consistent with the histopathological observations of the organs. Under the conditions of the experiment the LECV showed antioxidant potential in the investigated organs of SHRs.

Key words: *Clinopodium vulgare* L., SHR, oxidative stress, antioxidant enzymes

Acknowledgement: The study was carried out with the financial support by the Council of Medical Science, Project № 8573, Contract D-89/05.2017.

UHPLC-HRMS BASED FLAVONOID PROFILING OF THE AERIAL PARTS FROM *CHENOPODIUM FOLIOSUM* Asch. (AMARANTHACEAE)

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The genus *Chenopodium* (Amaranthaceae) has been estimated to comprise about 150 species. *Chenopodium* spp. have been used as leafy vegetables, important subsidiary grain crops for human and animal foodstuff and as medicinal agents in traditional systems of medicine. *C. foliosum* Asch. also known as “garliche” or “svinski yagodi” (swine's berries) has been recognized by Bulgarian legislation as a medicinal plant. The decoction of its aerial parts has been used for treatment of cancer, as an immunostimulant and antioxidant. An UHPLC-HRMS profiling method was used for a comprehensive study of flavonoid composition of *C. foliosum*. Forty-two flavonoid glycosides of patuletin, gomphrenol, spinacetin, 6-methoxykaempferol, 3,5,3',4'-tetrahydroxy-6,7-methylenedioxyflavone and 3,5,4'-trihydroxy-3'-methoxy-6,7-methylenedioxyflavone were detected. Kaempferol, quercetin and isorhamnetin glycosides were identified as minor components. Flavonoid composition was dominated by di-, triglycosides and acylated flavonoids. Acid hydrolysis and GS-MS analysis confirmed the presence of D-glucose, D-apiose and L-rhamnose. At least 11 flavonoids were reported for the first time.

Key words: *Chenopodium foliosum*, Amaranthaceae, flavonoids, UHPLC-HRMS

PHYTOCHEMICAL EVALUATION OF SOME FOOD SUPPLEMENTS WITH *MELISSA OFFICINALIS* L. FROM ROMANIA

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Melissa officinalis L. (Lamiaceae), lemon balm, is a well-known medicinal and aromatic plant. The dried leaves are long used in traditional and modern medicine for headaches, mental stress, nervous disturbances of sleep, cardiovascular, respiratory and gastrointestinal problems, and as a memory enhancer, cardiac tonic and antidepressant. The hydroxycinnamic acids (rosmarinic, p-coumaric, caffeic and chlorogenic) and the essential oil (monoterpenes and sesquiterpenes) are the main constituents. Citral, citronellal, geraniol, neral and β -caryophyllene are the most significant terpenoid components. It also contains flavonoids (e.g. luteolin and apigenin), tannins and triterpenoids (ursolic and oleanolic acids). The biological activities of *M. officinalis* (antioxidant, antiviral, antimicrobial, anticancer, anti-inflammatory, neuroprotective and sedative) have been attributed mainly to its essential oil (citral A and B) and phenolic compounds (rosmarinic acid). Our work is a result of collaboration with a SME unit (Dacia Plant S.R.L., Brasov/Romania) that supplies, processes vegetal materials and produces food supplements. Four samples of *M. officinalis* from Romania (Perisoru/Calarasi, Turda/Cluj, Rasnov/Brasov and Nadlac/Arad) and different products with lemon balm obtained and commercialized by Dacia Plant were analyzed by qualitative (TLC), semi-quantitative (GC-MS) and quantitative (UV-VIS spectrophotometry) methods, in order to evaluate the bioactive compounds responsible for their phytotherapeutic properties: polyphenols (gallic acid), flavonoids (rutin, luteolin and apigenin), phenolcarboxylic acids (rosmarinic, caffeic, chlorogenic and p-coumaric acids), and volatile fractions. Rosmarinic acid was used as biomarker of quality control for lemon balm samples and the food supplements containing them. Lemon balm sample from Rasnov has the highest content in phenolcarboxylic acids (1.462 g RAE/100 g d. w.), followed by those from Perisoru with 1.329 g RAE/100 g d. w. The evaluation *M. officinalis* essential oils by GC-MS indicated the presence of the main volatile fractions: citronellal (Turda 32.60%, Rasnov 33.70%), β -citral (Perisoru 26.25%, Nadlac 30.5%) and α -citral (Perisoru 32.33%, Nadlac 37.61%).

Key words: lemon balm, food supplements, rosmarinic acid, volatile fractions

VOLATILE COMPOUNDS FROM *FOENICULUM VULGARE* MILL. YELLOW LEAVES OBTAINED BY SUPERCRITICAL FLUID EXTRACTION

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Foeniculum vulgare Mill. grows wild in the Mediterranean region. Yellow *Foeniculum vulgare* leaves were collected in August 2017 in Dobre Vode, Montenegro. Collected leaves were air dried for three weeks and then grinded. After the cell walls were broken by the mechanical stress some essential oil compounds have immediately dispersed in the air. Supercritical fluid extraction with CO₂ was used for the extraction of volatile compounds from yellow *Foeniculum vulgare* leaves powder. The extraction was carried out on a lab-made apparatus at T = 40°C, p = 300 bar, CO₂ flow rate 2 kg h⁻¹ for four hours on 0.64 mm mean particle size of *Foeniculum vulgare* yellow leaves powder. The obtained *Foeniculum vulgare* extract was analyzed by gas chromatography followed by flame ionization detection and gas chromatography-mass spectrometry. A total of 15 compounds was detected and 11 compounds were identified. Two identified compounds contained a five-member aromatic ring with oxygen atom: 2-furanmethanol (3.40%) and cis-2-(1-pentenyl) furan (11.73%). Heterocyclic sulfur compound 2,3,4-trimethylthiophene was detected (1.42%). Oxygenated monoterpenes were present in the amount of 29.11%. Diterpenes present in the extract were neophytadiene (2.11%) and oxygenated diterpene phytol (4.55%). In the yellow *Foeniculum vulgare* leaves extract the most abundant compound was cyclic ketone: 2-acetylcyclopentanone (20.16%). The oxygenated monoterpene diosphenol was present in the amount of 11.14%. The extract can be used for the isolation of the most abundant compounds: 2-acetylcyclopentanone and diosphenol.

Key words: *Foeniculum vulgare*, yellow leaves, SFE

POLYPHENOL DIVERSITY AND *IN VITRO* ANTIOXIDANT PROPERTIES OF TUBERS OF ORNAMENTAL DAHLIA VARIETIES

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Dahlias are popular ornamental plants, but they are also used as alternative food source with appreciated prebiotic properties. Botanically, they are hybrids usually referred to as *Dahlia x hortensis*. As members of the Asteroideae subfamily, dahlias are a potential source of pharmacologically active caffeoylquinic acids (CQAs) but little is known about the diversity and actual content of these compounds in different parts of the plants. In this study, we analyzed the diversity of phenolic compounds in extracts from leaves and tubers of nine differently colored ornamental varieties from the collection of National Botanical Garden in Kiev. For analysis, a high-performance liquid chromatography - diode array detector system was used, coupled with ion trap mass spectrometer with electrospray ion source. The extracts were also tested for the antioxidant activity using *in vitro* spectrophotometric assays (DPPH scavenging, phosphomolybdenum reduction, polyunsaturated fatty acids peroxidation) and inhibition of pro-inflammatory proteolytic enzymes (elastase and collagenase). This approach enabled identification of several caffeoylquinic (chlorogenic) acids and correlated their content with antioxidant properties. The main compounds identified based on the MS3 fragmentation were 3-CQA (chlorogenic acid), 5-CQA, 4-CQA as well as a few dicaffeoylquinic acids (3,4-dCQA, 3,5-dCQA). There was no clear correlation of CQAs content and profile with flowerhead colors. Most of the varieties were relatively weak as antioxidants and proteolytic enzyme inhibitors due to the quite low content of CGAs. However, given the common availability of the plant material, and unproblematic growing, dahlias which are also generally considered as non-toxic could become an alternative dietary source of these natural products.

Key words: Dahlia, Asteraceae, caffeoylquinic acids, antioxidant, LC-MS

Acknowledgment: This study was supported by Wrocław Medical University grant ST-909.

THE EFFECTS OF ORGANIC FERTILIZERS ON CAROTENOID COMPOUNDS AND YIELD IN SAFFRON (*CROCUS SATIVUS* L.)

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In order to assess some physiological and agronomic traits in saffron, a factorial experiment based on randomized complete block design with four replications was carried out in Neyshabour, Iran in 2015 and 2016. The experimental treatments were combinations of three levels of organic fertilizers, including Vermicompost, Spent Mushroom Compost (SMC) and Processed Spent Mushroom Compost (PSMC), and five levels of application amounts (0, 1, 2, 3 and 4 t ha⁻¹). Results showed that all parameters were affected by main effects and interactions, so applying all fertilizer types increased studied traits in comparison with control. In addition, more rates of all fertilizers, showed higher amounts of parameters. For instance, application of 5 t ha⁻¹ PSMC demonstrated the highest percentage of crocin, picrocrocin and safranal (24.3%, 18.1% and 11.7% respectively). Besides, this treatment represented the greatest effect on flower number (191.3 per m²) and stigma dry weight (7.14 kg ha⁻¹). Moreover, results displayed that there are significant positive correlations between percentage of crocin with picrocrocin, stigma dry weight and flower number.

Key words: saffron, organic fertilizers, crocin, picrocrocin, safranal, and dry stigma yield

ANTIBACTERIAL ACTIVITY OF DIFFERENT LEAF EXTRACTS OF *CENTAUREA CALCITRAPA* L. FROM SERBIA

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In this work, we investigated antimicrobial activity of methanolic (MeOH), ethyl-acetic (EtOAc), 70% ethanolic (EtOH) and 50% acetic (Me₂CO) leaf extracts of *Centaurea calcitrapa* L. against bacterial phytopathogenic and human opportunistic and pathogenic strains. Many *Centaurea* species exhibit different biological activities and are often used in folk medicine. The extracts were obtained using standard maceration procedure, and modified diffusion well method, was used as a preliminary screening method for the antimicrobial potential of the different extracts. The obtained data were subjected to analysis of variance (ANOVA), and separation of the means of zone inhibition for treatment with different extracts was accomplished by Tukey's HSD (honest significant difference) test. Out of 13 indicator strains [*Listeria monocytogenes* ATCC 19111, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Shigella flexneri* ATCC 9199, *Escherichia coli* ATCC 25922, *Pseudomonas syringae* pv. *aptata* (P16, P29, and P49), *Xanthomonas arboricola* pv. *juglandis* (IZB 320 and IZB 321), *Xanthomonas campestris* pv. *campestris* 178 and *Agrobacterium tumefaciens*], 10 strains were sensitive to the applied extracts (0.75 mg mL⁻¹) which caused the significant measurable growth inhibition or showed a positive activity. Insensitive strains were *E. coli* and both phytopathogenic strains of *X. arboricola* pv. *juglandis*. The antibacterial potential of the tested extracts was as follows: 50% Me₂CO < 70% EtOH < MeOH < EtOAc extract. The best activity was shown by the EtOAc extract, exhibiting a very strong activity against 10 indicator strains. In general, the statistical significance in relation to all four types was achieved by EtOAc and MeOH extracts. The obtained results showed that *C. calcitrapa* possesses pronounced antimicrobial activity, especially against plant pathogens. Thus, the future investigations should be continued with the aim to characterize chemical constituents of the particular extracts as well as to examine their potential biological activities.

Key words: *Centaurea*, leaf extracts, antibacterial activity

ORIGANUM MAJORANA L. AND O. VULGARE L. FROM SERBIA ARE PROMISING AS FUNCTIONAL FOOD

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Genus *Origanum* L. comprises 44 species, used as spice, medicines, fragrance and ornamental plants. Among them, *O. majorana* L. (sweet marjoram) and *O. vulgare* L. (oregano), are highly valued plants in culinary, especially in the Mediterranean. Since literature survey showed versatile biological activities of *Origanum* spp., the aim of our study was to examine the antioxidant and antineurodegenerative activities of *O. majorana* and *O. vulgare* from Serbia. The plant material was obtained from the Institute for Medicinal Plant Research “Dr Josif Pančić”. Crude extracts were prepared using 70% methanol, 70% ethanol or hot distilled water, and dissolved to the concentrations of 0.5 mg/mL, 0.25 mg mL⁻¹ and 0.1 mg mL⁻¹. The analyses were carried out spectrophotometrically, including the ones of the total phenolic (TPC) and flavonoid contents (TFC). DPPH, ABTS, FRAP and β-carotene bleaching assays were used for the assessment of the antioxidant activity, whereas the antineurodegenerative activity was evaluated using acetylcholinesterase (AChE) and tyrosinase (TYR) inhibition assays. In DPPH, ABTS and FRAP assays, oregano extracts showed slightly better antioxidant activity than those of sweet marjoram, and the activities were concentration-dependent. Also, oregano aqueous extracts showed a higher rate of enzyme inhibition (75.81% in AChE and 63.03% in TYR assay at the concentration of 0.5 mg mL⁻¹) in comparison with sweet marjoram extracts. TPC and TFC were strongly correlated among each other ($r = 0.88$), as well as with all of the antioxidant assays ($r = 0.69-0.93$). Moreover, the correlation among AChE and the antioxidant assays was weak, while TYR assay showed a moderate correlation with all applied tests, except β-carotene bleaching assay. The given results pointed target *Origanum* species as potential ingredients in dietary supplements or functional food products.

Key words: *Origanum majorana*, *Origanum vulgare*, Lamiaceae, biological activities, extracts

ANTIOXIDANT AND ANTINEURODEGENERATIVE ACTIVITIES OF *MELISSA OFFICINALIS* L. AND *MENTHA x PIPERITA* L. CULTIVATED IN SERBIA

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Lamiaceae is one of the largest flowering plant families, consisting of 236 genera and about 7200 species, among which *Melissa officinalis* L. (lemon balm) and *Mentha x piperita* L. (peppermint) are highly important commercial aromatic herbs. Both species are used in traditional as well as in modern medicine and cookery. The aim of this study was to determine the antioxidant and antineurodegenerative potential of samples from Serbia. Crude extracts were prepared with 70% methanol, 70% ethanol or hot distilled water, and dissolved to the concentrations of 0.5 mg/mL, 0.25 mg/mL and 0.1 mg/mL. Total phenolic (TPC) and total flavonoid contents (TFC) were determined spectrophotometrically. The antioxidant activity was evaluated using DPPH, ABTS, FRAP and β -carotene bleaching assays, whereas the antineurodegenerative activity was evaluated using acetylcholinesterase (AChE) and tyrosinase (TYR) inhibition assays. In each antioxidant assay, extracts showed concentration-dependent activity, unlike in the antineurodegenerative tests. In DPPH, ABTS and β -carotene bleaching assays, peppermint extracts, especially methanolic (99.42% inhibition in ABTS assay) and aqueous extracts (99.53% inhibition in β -carotene bleaching assay), showed higher antioxidant activity than those of lemon balm. In general, aqueous extracts of both plants exhibited significant inhibition of AChE and TYR. The highest percentage of enzyme inhibition was noticed for lemon balm aqueous extract in both AChE (77.88%) and TYR assays (57.69%), at the highest applied concentration. TPC was strongly correlated with TFC and antioxidant activity assays ($r = 0.68-0.97$). Furthermore, TFC was strongly correlated with ABTS and β -carotene bleaching assays (0.68 and 0.72, respectively). The correlation between the enzyme inhibition assays and the other tests was moderate to weak. In conclusion, these findings indicate that both species can be considered as strong antioxidant and antineurodegenerative agents.

Key words: lemon balm, peppermint, Lamiaceae, biological activities, extracts

PHYTOTOXIC ACTIVITY OF AQUEOUS EXTRACTS FROM DIFFERENT *ARTEMISIA ABSINTHIUM* L. CHEMOTYPES ON SEED GERMINATION OF LETTUCE AND BASIL

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The aqueous extracts of leaves and flowers of two *Artemisia absinthium* L. chemotypes in various concentrations: 0 (control); 0.1; 0.2; 0.3; 0.4 mg mL⁻¹ were used to carry out a systematic evaluation of phytotoxic activities of wormwood on the germination of *Lactuca sativa* L. (lettuce) and *Ocimum basilicum* L. (basil). The experiment was carried out in both open field and climatic chamber with program of 300C/200C; 16 klx light intensity with 14h day/10h night rhythm in 2016. The two investigated accessions were identified in our previous study: “Hungarian” accession was indicated as non-thujone chemotype and “Belgien” accession was thujone chemotype which was presented with up to 91.55% of thujone in essential oil. The mean germination time of both species varied from 5.9 days (lettuce) to 6.6 days (basil). Different extracts concentrations of both chemotype accessions had significant effects on total germination percent. In case of lettuce, no germination was observed when using the plant extracts at concentrations higher than 0.3 mg mL⁻¹ (non-thujone chemotype) and at the highest concentration of 0.4 mg mL⁻¹ (thujone chemotype) while the highest germination capacity (76.7%) was detected in the control treatment (only distilled water). The germination capacity of basil seeds ranged from a lowest rate of 14% (non-thujone chemotype in concentration of 0.4 mg mL⁻¹) to the highest rate of 79.3% (control). We established that lettuce seeds were more sensitive to the extracts compared to basil seeds. Among the chemotypes of wormwood, the non-thujone chemotype showed stronger inhibition of germination. At a concentration of 0.3 mg mL⁻¹, 22.7% of germination capacity was detected when using plant extracts of thujone chemotype while no seeds could germinate with extracts of non-thujone chemotype. In the case of basil, a two times higher rate of germination (40%) was obtained with 0.3 mg mL⁻¹ extract from the thujone chemotype in comparison to the non-thujone one (20.7%). Aqueous extracts of high concentrations of both wormwood chemotypes demonstrated strong inhibition on the germination of basil and lettuce.

Key words: wormwood, thujone; extracts, chemotype, phytotoxic activity

OPTIMIZATION OF SUPERCRITICAL FLUID EXTRACTION OF CARNOSIC ACID FROM SAGE LEAVES (*SALVIA OFFICINALIS* L.)

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Sage (*Salvia officinalis* L.) is an aromatic and medicinal plant rich in biologically active components, including terpenes and polyphenols. Polyphenols comprise a large group of compounds, including phenolic acids, flavonoids and phenolic diterpenes. Phenolic diterpenes greatly contribute to antioxidant activity of sage extract and some compounds identified within this class are carnosic acid, carnosol and rosmarinic acid. In addition to antioxidant activity, carnosic acid contributes to positive health effects of sage extract as well. The aim of our study was to optimize supercritical fluid extraction (SFE) variables such as extraction pressure, temperature and CO₂ flow rate in order to obtain the maximum amount of carnosic acid in sage leaves extracts. Carnosic acid content was determined by high-performance liquid chromatography (HPLC) and expressed as µg of CA/g of extract. The content of carnosic acid was in the range from 0.29- 120.05 µg mg⁻¹ of extract, depending on applied extraction conditions as stated above. The temperature exhibited the most significant influence on content of carnosic acid ($p = 0.0063$). Optimal extraction conditions calculated by response surface methodology (RSM) for carnosic acid were at pressure 30MPa, temperature 46°C and CO₂ flow rate 3 kg h⁻¹.

Key words: sage, supercritical fluid extraction, carnosic acid, RSM

COMBINATION OF CYTOSTATIC AND ANTIOXIDANT PRINCIPLES IN LEUKEMIA CELLS TREATMENT

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The *Juniperus sabina* L. leaf extract showed a superior anticancer activity on NB4 acute promyelocytic leukemia cells compared to the extracts of some other medicinal plants, such as *Ruta graveolens* L. (leaves), *Inula britannica* L. (flower heads), *Inula helenium* L. (roots), *Betula pendula* Roth. (cortex), *Buxus sempervirens* L. (leaves) etc. In response to demands of the clinical practice in the prevention of harmful action of cytostatic agents on healthy tissues, we aimed our study at estimating the impact of *Rhodiola rosea* L. (Golden root) extract, an efficient antioxidant, on the antiproliferative activity of juniper extracts and podophyllotoxin (PPT) prodrug. The changes in half-maximum growth-inhibitory concentrations (IC₅₀) of the cytostatic agents in the presence or absence of *Rh. rosea* extract were analyzed. IC₅₀ values were calculated from the dose-response curves of MTT-dye reduction assay of NB4 cells, treated with juniper extracts or PPT. The Golden root extract, containing 2.3% rosavin and 0.8% salidroside, was used at a concentration of 30 µg mL⁻¹, assigned to its half-maximum DPPH-radical scavenging concentration (SC₅₀). *J. sabina* L. leaf extract (IC₅₀ 0.5±0.2 µg/mL in NB4 cells) contained 0.5% PPT, identified by ultra-high performance liquid chromatography in tandem with high resolution mass-spectrometry. The remaining juniper representatives, distributed in Bulgaria, contained barely detectable traces of PPT or did not contain it at all. As a result of the simultaneous treatment of leukemia cells with cytostatic and antioxidant agents, statistical analysis (ANOVA) of the IC₅₀ values of the cytostatic agents in the presence or absence of antioxidants determined maintenance of the efficient antiproliferative potential of the studied juniper extracts or podophyllotoxin. This research has potential application in the future clinical trials regarding combination of cytostatic and antioxidant principles in the prevention of harmful impact on healthy tissues of cytostatic agents during intense chemotherapies.

Key words: *Juniperus* L., leukemia, podophyllotoxin, *Rhodiola rosea* L., UPLC/HRMS

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STANDARDIZED *AJUGA GENEVENSIS* L. AERIAL PARTS EXTRACTS WITH ANTITUMOR POTENTIAL

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Ajuga genevensis L. (Lamiaceae) is an herbaceous flowering species used in Romanian traditional medicine for its anti-inflammatory, hepatoprotective and wound healing properties. The anticancer activity of natural extracts is attributed to their synergistically acting complex mixture of phytochemicals. This research aimed to obtain standardized extracts from *A. genevensis* (Ag) aerial parts and to evaluate the cytotoxic potential by testing the effects on the proliferation of B16.F10 murine melanoma cells, as well as of C26 murine colon carcinoma cells. The hydroalcoholic extract obtained by maceration was standardized in main active compounds polyphenols and iridoids. To evaluate the potential cytotoxic action on both cell types, Ag extract dilutions ranging from 225 $\mu\text{g mL}^{-1}$ to 650 $\mu\text{g mL}^{-1}$ were tested. The proliferative activity of the cancer cells after treatment was tested using ELISA BrdU-colorimetric immunoassay. Hydroalcoholic extract was spray dried and a dry Ag extract was obtained. The hydroalcoholic extract contained bioactive compounds with strong anti-proliferative effects on C26 murine colon carcinoma cell line and moderate anti-proliferative effects on B16.F10 murine melanoma cell line. The dry extract obtained was characterized in terms of its physicochemical parameters. The results showed that the adsorption process progressed smoothly and that an adequate quantity of active principles could be incorporated in the final product. The Ag aerial parts dried extract could be a promising intermediate phytopharmaceutical product with antitumor potential.

Key words: *Ajuga genevensis*, polyphenols, iridoids, antitumor potential

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BIOACTIVITY AND CHEMICAL PROFILE STUDY OF ESSENTIAL OILS FROM VARIOUS MEDICINAL PLANTS

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In order to rationalize the use of aromatic and medicinal plants as alternative remedy to antibiotics due to bacterial multiresistance and antibiotherapy failure, we have focused on the study of three aromatic plants (*Salvia officinalis* L., *Lavandula angustifolia* Mill., *Ruta montana* L.) commonly used by the local population. The study consists first in characterizing physicochemical properties and chromatographic profile of the essential oils obtained by hydrodistillation and then in evaluation of their antibacterial and antifungal activities by various microbiological techniques. Microorganisms sensitivity tests were performed on solid Muller Hinton medium using disk diffusion method techniques. CMI, CMF and CMB were evaluated using microdilution techniques. Gas phase chromatographic method (C. P. G) determined most of essential oil components that may be the origin of antimicrobial properties, considered interesting in this study, on the tested fungi and Gram (+) and Gram (-) bacteria. The essential oils used against the tested germs may contributed to the fight against some antibiotics multiresistant bacteria and to offer, eventually, possibility of their use in pharmaceutical and agri-food industries.

Key words: antibacterial activity, antifungal activity, *Salvia officinalis*, *Lavandula angustifolia*, *Ruta montana* L.

EFFECT OF INOCULATION WITH SALT TOLERANT STRAIN OF *RHIZOBIUM* ON GROWTH AND PROTEIN PRODUCTION OF ALFALFA UNDER SALT CONSTRAINTS

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Alfalfa or *Medicago sativa* L., is a species from family Fabaceae. It is characterized by an exceptional chemical composition with various therapeutic properties. Alfalfa is also an ecological plant. Due to its symbiotic association with atmospheric nitrogen-fixing bacteria from genus *Rhizobium*, it can grow on soils deficient in nitrogen, without application of chemical nitrogenous fertilizers. In a current socio-economic context characterized by a renewable production, at lower cost molecules of interest without negative environmental impacts, alfalfa is the species to promote as part of the valorization of natural bioactive substances. However, in some regions, salinity affects alfalfa acting on *Rhizobium* survival. In order to promote alfalfa in these areas, the plants inoculation by adapted and compatible rhizobial strains can constitute an effective way. In this context, the aim of this study was to evaluate the effect of inoculation on alfalfa behavior in salt conditions. The rhizobia nodulating alfalfa was isolated from various soils, and phenotypically characterized in order to select the most appropriate strains for salt conditions. Alfalfa plants grown in pots were then inoculated with the selected strains and irrigated with saline solutions. The results have shown that the inoculation method is an efficient alternative at all levels of the salt stress resulting in higher protein yields and aerial dry biomass when compared to alfalfa plants treated with chemical nitrogenous fertilizers.

Key words: alfalfa, Rhizobium, salt constraints, biomass and protein yields

IONIC LIQUID-SUPPORTED SOLID–LIQUID EXTRACTION FOR THE QUANTITATIVE DETERMINATION OF GALANTAMINE IN *LEUCOJUM AESTIVUM* L. (AMARYLLIDACEAE)

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Aqueous solutions of a series of hydrophilic imidazolium-, pyrrolidinium- and ammonium-based ionic liquids were used as extractants for the effective extraction of galantamine from aerial parts of *Leucojum aestivum* L. The influence of the anion (chloride, bromide, acesulfamate, trifluoroacetate, acetate, thiocyanate, saccharinate, and dicianamide), cation (imidazolium, pyrrolidinium, and ammonium with different substituents) alkyl chain length in the imidazolium cation, extraction time, particles size, temperature, concentration, and plant material/extractant ratio was investigated. Optimum conditions for quantitative extraction of galantamine were found. The data obtained in this study resulted in the development of an analytical procedure for determination of galantamine in plant material of *L. aestivum* by means of its preliminary extraction with an aqueous solution of [C4C1im] Cl, followed by HPLC quantification. This could be of a great importance from an industrial standpoint, due to the faster and safer nature of the proposed method.

Key words: galantamine, ionic liquids, *Leucojum aestivum*, solid–liquid extraction

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PHYTOCHEMICAL EVALUATION AND ASSESSMENT OF BIOLOGICAL EFFECTS OF *AJUGA REPTANS* L. AERIAL PARTS FROM ROMANIA

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In Romanian traditional medicine, several *Ajuga* species are commonly used for their beneficial effects such as hepatoprotective, anti-inflammatory and wound healing effects. The aim of this study was to investigate *Ajuga reptans* L. ethanol aerial parts extracts by evaluating the phytochemical composition and their biological effects. For the assessment of polyphenols (total phenolic content and total flavonoid content) spectrophotometric and HPLC/UV/MS methods were employed, whereas the iridoids identification and quantification was carried out by HPLC/MS/MS. For the investigation of antioxidant potential, DPPH, TEAC and EPF spectroscopy assays were used. The anti-inflammatory effect of three ethanol extract concentrations (25%, 50%, 100%) were evaluated *in vivo* on a model turpentine oil-induced inflammation in rats by determining several parameters (WBC count, differential WBC count, serum total nitrites and nitrates, total oxidative status, total antioxidant response and oxidative stress index). These three extract dilutions were also evaluated *in vitro* for the ability to inhibit phagocytosis. Caffeic and ferulic acids, quercitrin, luteolin and apigenin were identified by LC-MS in all samples. Harpagide, 8-O-acetylharpagide, harpagoside, aucubin and catalpol were identified and quantified in all extracts. The major iridoids constituents were 8-O-acetylharpagide and harpagide. The 25% diluted extract had the best inhibitory activity on phagocytosis and oxidative stress. Compared to diclofenac, the *Ajuga reptans* 25% extract showed better anti-inflammatory and anti-oxidative stress effects, and this could justify the importance of the correlation between the activity and the dose. *Ajuga reptans* extracts contain bioactive compounds which possess anti-inflammatory and antioxidant activity, thus confirming its use in traditional medicine as an anti-inflammatory agent.

Key words: *Ajuga reptans*, polyphenols, iridoids, antioxidant, anti-inflammatory

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SHORT REVIEW OF THE ANTICANCER AND CYTOTOXIC ACTIVITY OF SOME SPECIES FROM GENUS *EUPHORBIA*

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Euphorbiaceae family is a widely spread family that contains more than 2000 species in the genus *Euphorbia*. The plants of this genus have been used for a long time in traditional medicine. Their main active components alkanes, triterpenes, phyosterols, tannins, polyphenols and flavanoids are supposed to be responsible for different types of activity. *Euphorbia formosana* Hayata is a medicinal plant used to treat rheumatism, liver cirrhosis, herpes zoster, along as tumor suppressor. *Euphorbia tirucalli* L. has been used to obtain methanolic extracts. Their cytotoxic activity has been examined against many different types of cancer cells, as well as colon cancer cell line, liver cancer cell line, ovarian cancer cell line and prostate cancer cell line. Leukemic cell lines, THP-1 and HL-60, have been inhibited dose dependent after 24h of treatment with 400 $\mu\text{g mL}^{-1}$ *Euphorbia formosana* Hayata. *In vitro* anticancer activity assays of *Euphorbia formosana* Hayata suggest potent anticancer effects that cause both cell cycle arrest and apoptosis of leukemic cancer cells. The ethanolic extract of *Euphorbia helioscopia* L. inhibited the growth of only three cancer cell lines, Hep-2 (27%), T-47D (7%) and PC-5 (11%). Cell viability assays were conducted on the pancreatic cancer primary tumor cell line to assess the relative toxicity of the *Euphorbia tirucalli* L. extracts. The toxicity of both extracts was found to be dose dependent, with cell viability decreasing with increasing extract concentration. Both aqueous and methanol extracts demonstrated similar activity at 50 $\mu\text{g mL}^{-1}$ with a viability of 50%, while only the methanol extract exerted a significant decrease in cell viability from 25 $\mu\text{g mL}^{-1}$. The pronounced cytotoxic activity of this very few species from the genus *Euphorbia*, suggest that it could be very interesting to investigate more deeply about their potent anticancer ability. So, the aim of this study was to review the anticancer activity of some of the species examined experimentally by now, using different *in vitro* or *in vivo* assays.

Key words: anticancer effects, cytotoxic effects, genus *Euphorbia*, medicinal plants

IONIC LIQUID-ASSISTED MICELLAR EXTRACTION FOR THE QUANTITATIVE DETERMINATION OF SESQUITERPENIC ACIDS IN *VALERIANA OFFICINALIS* L. (CAPRIFOLIACEAE)

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The performance of aqueous solutions of a series of hydrophilic ionic liquids (ILs) in the extraction of sesquiterpenic acids from roots of *Valeriana officinalis* L. was systematically studied by taking into account the influence of several factors such as anion type (chloride, bromide, acesulfamate, trifluoroacetate, acetate, thiocyanate, saccharinate, and dicianamide), cation type (imidazolium, pyrrolidinium, and ammonium) and substitution pattern, concentration, extraction time, temperature, and plant material/extractant ratio. It was found that specific interactions, such as hydrogen bonding, ion-dipole, hydrophobic or π - π interactions, do not affect the extraction yields (EY) and that the extraction of acetoxyvalerenic and valerenic acids is strongly dependent on the ability of IL to form micelles. The proposed micelle-based mechanism of extraction was confirmed by additional experiments with [C10C1im] Cl at concentrations above and below its critical micelle concentration (CMC). Further optimization of the extraction conditions brought about the development and validation of an alternative method for quantification of sesquiterpenic acids in roots of *V. officinalis* by means of its preliminary extraction with an aqueous solution of [C10C1im] Cl, followed by HPLC quantification. The method developed ensures the same EY as the reference procedures but does not require special equipment; it is easy to perform and excludes the harmful organic solvents from the sample preparation step, the latter being of a great importance from an industrial standpoint.

Key words: ionic liquids, solid-liquid extraction, valerian, sesquiterpenes, acids

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MICROSTRUCTURE OF THE COMMON SELFHEAL (*PRUNELLA VULGARIS* L.) NUTLETS AND MUCILAGE ENVELOPE

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Common selfheal (*Prunellae spica*, Ph. Eur. 9) has four brown ovoid nutlets that are retained in the persistent calyx. These nutlets are characterized with presence of myxocarpy and produce mucilage when the nutlets are hydrated. Such hydration occurs when the herbal substance is prepared as an infusion or decoction. This suggests that part of the healing action of herbal preparations is due to the composition of the nutlets and their mucilage envelope, which determines the need for their detailed study. The following microstructural features of the nutlets were studied by light and scanning electron microscopy: (i) morphology of dry nutlets (surface with outlines of the mucilage secreting cells, thick layers of deposited mucilage of the mucilage secreting cells) - pericarp composed of mucilage rectangular secreting cells accompanied by polygonal cells with thickened walls and granular contents; (ii) morphology of hydrated and lately dried nutlets (thin film surrounding the nutlets and membranes observed correspond to dry mucilage on the surface) – homogenous, smooth structure, some wrinkles and fractures of the mucilaginous material, cellulose fibrils are arranged within the mucilage. Upon full hydration filaments became apparent and conformed to a transparent "capsule" attached to the nutlet. The general morphology of nutlet determines the specificity of the process and three main stages were outlined: initial mucilage developing in white hilum at narrow and cute end; form a mucilage around distinctive dark lines run down centre of each nutlet face and around; stiff gelatinous envelope over the nutlet surface with a distinct outer boundary. The study reports the comparative effect of water and ionic liquids for mucilage developing process.

Key words: common selfheal, *Prunella vulgaris*, mucilage, myxocarpy, ionic liquids, nutlets that are retained

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INFLUENCE OF PLANT AGE ON THE CONTENT AND COMPOSITION OF ESSENTIAL OIL OF FENNEL SEEDS

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Fennel (*Foeniculum vulgare* Mill) is a well-known and often cultivated medicinal and aromatic plant. According to various climatic conditions, it can be grown as one- to three-year crop. The effect of plant age on the essential oil (EO) content and composition was the subject of this study. The seeds of 21 genotypes from the Czech collection of plant genetic resources were evaluated in two seasons (2014 and 2015) as a one-, two- and three-years old plants. The evaluated fennel accessions originated from the Czech Republic or former Czechoslovakia as well as from Germany, Hungary, the Netherlands, Poland and Slovenia. The EO content was analyzed by hydro distillation, and the content of the major EO components by GC-MS according to Pharmacopoea Bohemica. All the ascertained values of EO components content are expressed as the average (Av) of all evaluated genotypes, plant ages and vegetative seasons \pm its standard deviation. The two-way analysis of variance without replication was used for the statistical evaluation. We found out that the tested genotypes produced in average of two seasons and three different crop ages 2.85 – 8.28% of EO and the highest EO yield, 7.08%, was discovered in two-years old crop. The statistically significant differences ($\lambda = 0.01$) in EO content were found between the tested genotypes as well as between the plants of different age while surprisingly no statistically significant difference ($\lambda = 0.05$) was found between the vegetation seasons. The content of trans-anethol, the major compound of fennel EO, was found in the amount from 42.81 to 78.25% (Av 68.12 \pm 9.89%) of EO and only a genotype was the factor which statistically ($\lambda = 0.01$) influenced its content. Also, content of estragol (Av 8.45 \pm 8.49%) and fenchol (Av 15.98 \pm 3.07%) were statistically ($\lambda = 0.01$) influenced only by genotypes, while the content of β -pinene (Av 0.13 \pm 0.05%) and β -myrcene (0.65 \pm 0.19%) were influenced by genotype ($\lambda = 0.05$ or 0.01) as well as by the vegetative season ($\lambda = 0.01$). According to our results the EO content was the only characteristic influenced by different age of fennel plants while the EO composition remained stable. Of course, the huge differences can be expected in the yield of plant biomass per unit of area, because the 2-year old plants were mostly observed higher and generally more robust when compared to youngest plants. On the other hand, some genotypes were handicapped by the first and especially by the second bad wintering. The Czech origin variety 'Moravský' seemed the best adapted to the Czech climatic and soil condition, although these results are based only on observations. Unfortunately, the seed yield per unit of area was not weighed.

Key words: *Foeniculum vulgare*, essential oil, plant age, trans-anethol

EFFECT OF *ARTEMISIA ABSINTHIUM* L. EXTRACT AGAINST *FASCIOLA HEPATICA* LARVAE

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Fasciola hepatica is a helminth parasite affecting wild and domestic animals and humans worldwide. Its complex life cycle involves freshwater snails as intermediate host, where development of three larval stages takes place – sporocysts, rediae and cercariae. The suppression of the intrasnailed development of the larvae is an opportunity to break the life cycle of parasite on the intermediate host level. Recently, attempts are being made to identify naturally occurring plants having selectively larvicidal action. This study was conducted to evaluate the effect of methanolic extract from aerial parts of *Artemisia absinthium* L. on the vitality of *F. hepatica* sporocysts, rediae and cercariae, incubated in physiological saline for *Galba truncatula*. Among the applied concentrations, those higher than 0.85 mg L⁻¹ have a negative effect on the vitality of the larval population after 2 hours of experiment with a delay rate exceeding 60%. Metabolic profile of *Artemisia absinthium* extract was analysed by gas chromatography-mass spectrometry (GC-MS) and high-performance thin-layer chromatography (HPTLC). Compounds belonging to fatty, organic and phenolic acids, fatty alcohols, flavonoids, carbohydrates and other were identified. Based on the results, the extract from *A. absinthium* gave potent larvicidal activity against the *F. hepatica* larval stages, associated with its snail host.

Key words: larvicidal activity, GC-MS, metabolite profile

VERONICA CYMBALARIA Bodard – WHEN THE GC-MS PEAKS OF VOLATILE COMPOUNDS APPEAR

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Genus *Veronica* L. belongs to the Plantaginaceae family, and according to morphological traits, it is divided into 13 subgenera. In Croatia, plant species of genus *Veronica* are spread on a variety of habitats from aquatic, marshy, forest, carbonate, mountain pastures and mountain limestone caves to rock and rock cracks, fields and ruderal habitats. The investigated species *Veronica cymbalaria* Bodard. belongs to South-European-Mediterranean terophytes. Plant material was collected from Split (Croatia), in the spring (April) 2015. Water distilled essential oils from aerial parts of investigation plant have been analysed by GC and GC/MS using VF-5ms capillary column and the first peaks appear after 30 minutes. The total yield of oil was 0.1%, based on dry weight of samples. Ten compounds were determined representing 89.5% of the total oil. Composition of this essential oil is characterized by a high concentration of phytol (59.7%), several different hydrocarbons (in total 14.1%) and caryophyllene oxide (5.1%). In our previous research phytol was also identified in the oil of *V. spicata*. In our review, we found one more species, *Veronica thymoides* P.H. Davis subsp. *pseudocinerea*, M.A. Fischer that was investigated with the same methodology. Compound peaks for *V. thymoides* also appeared after 30 minutes of the GC-MS analysis and the most abundant constituent was hexatriacontene (21%). The present study gives additional knowledge about chemosystematic profile of the genus *Veronica*.

Key words: *Veronica*, secondary metabolites, GC-MS, phytochemical

LC-HRMS PROFILING OF SOME STEROID ALKALOIDS FROM LEAVES EXTRACT OF *SOLANUM SCHIMPERIANUM* Hochst.

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The Solanaceae family are well-known for the production of numerous secondary metabolites among which steroidal glycosides are one major class. Herein, the profiling of this class of semipolar metabolites along with approaches for their isolation from complex plant extracts are discussed. Based on high-resolution accurate mass measurements and characteristic fragmentation patterns we were able to tentatively identify and differentiate several solanidine-type and solasodine-type alkaloids that mainly differ in their side chain (cyclic for solanidine-type and open-chain for solasodine-type alkaloids). Such compounds include various nitrogen containing penta-cyclic steroid alkaloids like solanopubamine, solanocapsine, solasodine, rubijervine and other derivatives. Mass analyses were carried out on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific) equipped with a heated electrospray ionization (HESI-II) probe (ThermoScientific) and the targeted acquisition were carried out on parallel reaction monitoring (PRM) mode.

Key words: *Solanum*, glycoalkaloid, LC-MS

PHYTOCHEMICAL ANALYSIS AND ANTIRADICAL POTENTIAL OF METHANOL EXTRACTS OF *HIERACIUM NAEGELIANUM* Pančić AND *H. SCHEPPIGIANUM* Freyn UNDERGROUND PARTS

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The genus *Hieracium* L. is autochthonous in Eurasia, North Africa and North America and the vast majority of the taxa are distributed in Europe. *Hieracium naegelianum* Pančić, belonging to monotypic subendemic section H. sect. Naegeliana Zahn ex Szelağ, is a small, caespitose plant, with long rhizome. It inhabits subalpine and alpine orosubmediterranean screes and mainly is endemic for the mountains in W Balkan, with a single population in S Apennines. *Hieracium scheppigianum* Freyn is a hybridogenous apomictic species probably originated from *H. gymnocephalum* Griseb. ex Pant. (H. sect. Pannosa /Zahn (Zahn) and *H. bupleuroides* C.C. Gmelin, s. l. (H. sect. Drepanoidea Monnier). The species can be found only on screes, rocky places or rocky pastures in subalpine and alpine zone in Dinarides. The underground parts of *H. naegelianum* and *H. scheppigianum* were collected on Mt Durmitor, Republic of Montenegro. Dried and powered plant material was macerated with dichloromethane and methanol, successively. Both solvents were evaporated under reduced pressure and dried methanol extracts were used for further investigation. Using LC-MS method, sesquiterpene lactone glycoside of guaianolide type, crepiside E, and six phenolic acids, i.e. chlorogenic acid and five dicaffeoylquinic acids (cynarin, 3,5-, 1,5-, 3,4- and 4,5-dicaffeoylquinic acids) were identified and quantified in both dried methanol extracts. Crepiside E was the most abundant compound (116.58 and 126.88 mg/g), while among phenolic acids, 3,5-dicaffeoylquinic acid (72.86 and 62.01 mg/g) was dominant in both investigated extracts. Radical scavenging activity was estimated using colorimetric DPPH and OH radical assays, and both extracts showed prominent and concentration dependent activity with SC50DPPH of 25.25 and 29.38 µg/mL and SC50OH of 16.09 and 17.50 µg/mL, respectively. This is the first report on secondary metabolites and antiradical potential of underground parts of *H. naegelianum* and *H. scheppigianum*.

Key words: *Hieracium*, underground parts, phenolic acids, sesquiterpene lactone, antiradical

ANTIOXIDANT, CYTOTOXIC, ACETYLCHOLINESTERASE AND TYROSINASE ENZYME INHIBITORY ACTIVITIES OF SIX *INULA* SPECIES FROM BULGARIA

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Continuing our research on *Inula* species, growing in Bulgaria we have focused our attention on phenolic constituents and antioxidant, cytotoxic and enzyme inhibitory activity of methanol extracts of six *Inula* species - *I. aschersoniana* Janka., *I. conyza* DC., *I. ensifolia* L., *I. oculus-christi* L., *I. bifrons* (L.) L. and *I. germanica* L. HPTLC was used for qualitative analysis of phenolic acids. Total phenolic content was determined by Folin-Ciocalteu method. The free radical scavenging potential was assessed against DPPH[•] and ABTS^{•+} radicals. Acetylcholinesterase inhibition was evaluated using Ellman's method, while tyrosinase inhibition - by the microtiter plate assay reported by Masuda (2005). The cytotoxic activity (10 to 300 µg mL⁻¹) and analysis of cell morphology were determined by crystal violet assay. Only the *I. oculus-christi* and *I. aschersoniana* extracts reached IC₅₀ for the malignant cells (A549) on 48 h, while viability was around 75% for the non-cancer ones (MDCK II). HPTLC comparison of the methanol extracts with commercial standards showed presence of chlorogenic, 1,5-, 3,5-, 3,4- and 4,5-dicaffeoylquinic acids. *I. ensifolia*, *I. conyza* and *I. germanica* (119.92 ± 0.95, 96.87 ± 0.85, 85.81 ± 1.01 mg GAE gE⁻¹, resp.) contained the highest amounts of phenolics and demonstrated the highest percentage of inhibition of DPPH radicals at concentration of 200 µg mL⁻¹ (69.41 ± 0.55, 68.58 ± 0.52 and 60.17 ± 0.3%, respectively). *I. bifrons* and *I. germanica* extracts were found to be the most active *Inula* samples with TEAC values 0.257 ± 0.012 and 0.242 ± 0.011 mg mL⁻¹, respectively. These two samples also showed the best inhibitory potential against tyrosinase enzyme (IC₅₀ 0.123 ± 0.0 and 0.159 ± 0.032 mg mL⁻¹, resp.). All tested extracts demonstrated weak anti-AChE activity (Inh % 4 - 17). A good correlation between total phenolic content and antioxidant, acetylcholinesterase and tyrosinase enzyme inhibitory activities was observed by calculating Pearson's correlation coefficients.

Key words: *Inula*, Asteraceae, phenolic acids, biological activity

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DETERMINATION OF THE TOTAL PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND CYTOTOXICITY OF SELECTED AROMATIC HERBS

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Aromatic plants used as culinary herbs contain wide range of phytochemicals with distinct bioactive properties affecting the population that utilizes them. However, there is still insufficient data on their antioxidant capacity and cytotoxicity. The present study investigated the antioxidant properties and cytotoxic potential of five aromatic herbs: *Allium schoenoprasum* L. (ASPR), *Allium ursinum* L. (AUR), *Anthriscus cerefolium* L. (ACH), *Capsicum annuum* L. (CAF) and *Foeniculum vulgare* Mill (FVH). Folin-Ciocalteu method and AlCl₃ method were performed to determine total phenolic content (TPC) and total flavonoid content (TFC). Antioxidant activity of ethanol extracts was examined by 2,2'-diphenyl-1-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), Non-site-specific- (NSSOH) and Site-specific-2-deoxyribose-degradation (SSOH) assays. The cytotoxic potential of water: ethanol extracts was evaluated by Brine shrimp lethality assay (BSLA). Considerable variations were observed among TPC values from 65.03 to 253.74 mg GAE g⁻¹ crude extract and among the TFC values from 8.02 to 49.58 mg QE g⁻¹ crude extract. Highest quantity of total flavonoids was measured in CAF which is in accordance with the high amount of total polyphenols. According the DPPH assay, highest radical scavenging activity was observed for CAF, while ACH showed lowest antioxidant activity. Moreover, the FRAP capacity is decreasing as follows: CAF > AUR > FVH > ASPR > ACH. Additionally, NSSOH and SSOH assays indicated that CAF has prominent hydroxyl scavenging and chelating properties. Obtained LC₅₀ values by BSLA revealed different cytotoxicity among the extracts. According Clarkson's scale, strong cytotoxicity was observed for CAF, moderate cytotoxic effects for FVH and ASPR, weak cytotoxicity demonstrated AUR, while ACH caused no cytotoxic effects against the shrimps. The obtained data in the present study indicates that all extracts possess notable antioxidant properties and cytotoxic potential. Due to their significant bioactivity, the examined species present promising dietary sources in prevention of pathological conditions associated with accumulation of free radicals.

Key words: dietary antioxidants, antioxidant capacity, cytotoxic potential, brine shrimp lethality assay, LC₅₀

NMR-BASED METABOLOMICS OF *VERBASCUM PURPUREUM* (Janka) Hub.-Mor. AND THEIR TRANSFORMED ROOTS

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The genus *Verbascum* L. (mulleins) comprises of about 360 species of flowering plants within the Scrophulariaceae family. Mulleins have been used in the traditional folk medicine for treatment of a wide range of human ailments, inter alia bronchitis, tuberculosis, asthma, and several others inflammation processes. The aim of the study was to determine the metabolic differences of *V. purpureum* (Janka) Hub.-Mor. mother plant and sonication assisted *Agrobacterium rhizogenes*-mediated genetic transformation (SAArT) induced hairy roots by combining NMR-based metabolomics (1D and 2D) approach and multivariate data analysis. This is the first report on the genetic transformation of *V. purpureum* plants with the application of metabolomics approach to observe the metabolome alterations as a result of the foreign T-DNA transfer. The genetic transformation of two selected fast growing hairy root lines (denoted as VPD2 and VPD11) was confirmed by PCR analysis. The metabolic analysis of *A. rhizogenes* has been performed for first time as well and it was found to contain only primary metabolites, such as sugars and amino acids. The most distinguished feature of the transformed root lines was the identification of verbascoside, a phenylethanoid glycoside with important anti-inflammatory, antitumorigenic and immune stimulating activities. The presence of some primary metabolites, including amino acids, organic acids and sugars was reported as well. The results reported here revealed that the hairy roots from *V. purpureum* have the potential to be used as an alternative source for biosynthesis of biologically active molecules, such as verbascoside.

Key words: *Verbascum purpureum* L., hairy roots, NMR, *Agrobacterium rhizogenes*, Verbascoside

Acknowledgement: This work has been supported by a grant from NSF of Bulgaria and DAAD Germany (contract number DNTS/Germany 01/8).

ANTIOXIDATIVE ACTIVITY OF LEAF EXTRACTS OF *ACHILLEA COARCTATA* Poir. AND *HIERACIUM WALDSTEINII* Tausch FROM MACEDONIA

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In this study the antioxidative capacity of leaf extracts of two potentially medicinal plants: *Achillea coarctata* Poir. and *Hieracium waldsteinii* Tausch from Macedonia, were investigated. It is well known that *Achillea* L. and *Hieracium* L. species are widely used in folk medicine. The extracts were obtained using Soxhlet type apparatus, with two different solvents, ethyl-acetate and methanol. The antioxidative capacity was evaluated using ABTS, FRAP and DPPH assays. Total phenolic and flavonoid content was also determined. The highest antioxidative activity was detected with methanol extracts of *A. coarctata* (DPPH IC₅₀ = 0.331 mg mL⁻¹; FRAP 0.425 mM g⁻¹Fe; ABTS AAEC = 0.172 mg mL⁻¹, IC₅₀ = 1.552 mg mL⁻¹) and *H. waldsteinii* (DPPH IC₅₀=0.515 mg mL⁻¹; FRAP 0.411 mM g⁻¹Fe; ABTS AAEC = 0.118 mg mL⁻¹, IC₅₀ = 2.267 mg mL⁻¹). The total phenolic content varied from 55.11 µg mL⁻¹ to 133.37 µg mL⁻¹, while the total flavonoid concentrations were in the range from 60.71 µg mL⁻¹ to 133.04 µg mL⁻¹. The total phenolic content was the highest in the methanol extract of *A. coarctata* (133.37 µg mL⁻¹) while the total flavonoid content was in the methanol extract of *H. waldsteinii* (133.04 µg mL⁻¹). Obtained results showed a correlation between antioxidative activity and concentrations of the phenolic compounds.

Key words: *Achillea*, *Hieracium*, leaf extracts, antioxidative activity

ANTIOXIDATIVE ACTIVITY OF LEAF EXTRACTS OF THREE *ARTEMISIA* SPECIES FROM SE EUROPE

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In the present study, antioxidative activity of methanolic, 70% ethanolic and ethyl-acetate extracts of leaves from three *Artemisia* (Compositae) species (*A. campestris* L. from Montenegro, *A. lobeli* All. var. *canescens* (DC) Briqu. from Serbia and *A. absinthium* L. from Romania) were investigated. All three species are used in traditional medicine and it is documented to possess medicinal properties. The extracts were obtained using the standard procedure, with MeOH, 70% EtOH and EtOAc, stored in dark at room temperature for 24 h. The antioxidative capacity was assessed using DPPH, ABTS and FRAP assays. Total phenolic and flavonoid content was also determined. The highest antioxidative activity in DPPH assay was detected with MeOH and 70% EtOH extracts of *A. l. var. canescens*: IC₅₀ = 0.196 mg mL⁻¹ and IC₅₀ = 0.197 mg mL⁻¹, respectively. The highest antioxidative activity in ABTS assay was detected with MeOH extract of *A. campestris*: AAEC = 0.371 mg mL⁻¹, IC₅₀ = 0.720 mg mL⁻¹, while in FRAP assay the highest antioxidative activity was detected with 70% EtOH extract of *A. campestris*: 1.199 mM g⁻¹Fe. All extracts of *A. absinthium* showed weak antioxidative activity. The total phenolic content varied from 25.96 µg mL⁻¹ to 176.04 µg mL⁻¹, while the total flavonoid amount was in range from 30.71 µg mL⁻¹ to 41.92 µg mL⁻¹. Total phenolic and flavonoid content was the highest in 70% EtOH extracts of *A. l. var. canescens* (176.04 and 41.92 µg mL⁻¹, respectively). Given results showed a correlation between antioxidative activity and content of phenolic compounds. These results indicate that *A. l. var. canescens* and *A. campestris* have the good antioxidative capacity and could be considered as natural sources of antioxidant agents.

Key words: *Artemisia*, leaf extracts, antioxidative activity

DETERMINATION OF TOTAL HYPERICINS OF *HYPERICUM PERFORATUM* L., FROM DIFFERENT LOCATIONS IN WESTERN R. MACEDONIA

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Hypericum perforatum L. (HP) contains numerous compounds with documented biological activity. The most interested constituents include naphthodianthrones, hypericin and pseudohypericin which have been used for the treatment of neurological disorders and depression. In addition, hypericin, has shown antiviral activity against several types of viruses, including the human immunodeficiency virus (HIV) and has been tested as a photosensitizer in the treatment of cancer. According to this fact, the aim of this study was to determine the level of total hypericins expressed as hypericin from aerial parts of HP collected from different locations in Western R. Macedonia during the year 2016/17. Hypericin content of the samples was measured at 590 nm using UV-Vis spectrophotometry. The analysis showed that levels of hypericin varied depending on the location and the time of collection as well as the part of the plant (herba, flower or leaf) that has been investigated. The lowest level of hypericin (0.07 - 0.16%) was detected in leaves of HP collected in Mavrovo while the highest (0.18 - 0.45%) was detected in HP flowers collected in Tetovo. In conclusion, the aerial parts from Macedonian wild- growing *Hypericum perforatum* can be considered as a good source of hypericins in accordance to the requirements of the European Pharmacopoeia.

Key words: *Hypericum perforatum*, hypericin, spectrophotometry.

EVALUATION OF INHIBITORY POTENTIAL OF *LIGUSTICUM ALATUM* (Bieb.) Sprengel ESSENTIAL OILS AND EXTRACTS AGAINST ENZYMES INVOLVED IN ALZHEIMER DISEASE AND TYPE II DIABETES

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In the present work the essential oils, hexane and methanol extracts obtained from the leaf and fruits of *Ligusticum alatum* (Bieb.) Sprengel were investigated for chemical composition, free radical (DPPH[•] and ABTS^{•+}) scavenging potential and inhibitory effects against acetylcholinesterase (from *Electrophorus electricus*) and porcine pancreatic α -amylase. Acetylcholinesterase inhibition of the samples was evaluated using Ellman's method. Antidiabetic potentials of the extracts and the oils were evaluated via measurement of inhibition of α -amylase enzyme using KI/I2 method. Epoxy-trans-pseudoisoeugenyl angelate (22.4%), (E)-anethole (13.8%), caryophyllene oxide (8.7%), β -caryophyllene (6.3%) and γ -himachalene (6.6%) were detected in the leaf. In the fruits, (Z)- β -farnesene (50.3%), (E)-anethole (13.2%), epoxy-trans-pseudoisoeugenyl angelate (12.8%), zizanene (5.8%), γ -himachalene (4.9%) and α -zingiberene (4.9%) were found as major constituents. The essential oils and the extracts of *L. alatum* demonstrated anti-acetylcholinesterase activity ranged between 43% and 58%. The highest TEAC values were obtained for the extract obtained with methanol (2.47 ± 0.18 mM) and the essential oil (1.78 ± 0.11 mM) from the leaf. The extracts demonstrated weak inhibitory effect (Inh. % up to 20.63 ± 0.37) towards to α -amylase. The present work is the first contribution about biological potential of *Ligusticum alatum* fruit and leaf extracts.

Key words: *Ligusticum alatum*, composition, α -amylase, acetylcholinesterase, antioxidant

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RAMAN SPECTROSCOPIC CHARACTERIZATION OF CAROTENOIDS FROM ROSE HIPS HERBAL TEA MIXTURES

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Dog rose (*Rosa canina* L.), the well known and traditionally used European species has been recently considered as a complex of species (the aggregate) due to genetic and related morphological polymorphism. The plant was broadly studied for its phytochemical, nutritive and medicinal properties, mainly linked to high content of carotenoids, the lycopene, beta-carotene, and rubixanthin, in addition to considerable content of vitamin C, the total polyphenols, tocopherol, pectin, and other metabolites. The aim of this study was to determine different type of carotenoids in rose hip fresh fruit as well as in local Serbian herbal tea products (ingredients: 70% fruit and 30% of rose hip leaf) using Raman spectroscopy to assess the validity of a method for rapid samples screening. Raman spectroscopic analysis was conducted by laser excitation at 532 nm, focusing on direct measurement of inside fruit parenchyma cells. In order to take a possible sample inhomogeneity, at least ten Raman spectra were recorded for each sample, in range between 900 and 1800 cm^{-1} . Spectra were analysed by Origin Pro 8.6 software. For carotenoids comparison, spectra were normalized by the intensity of the band at 1517 cm^{-1} . Analysis of the vibration mode at 1517 cm^{-1} strongly indicates the predominance of a C9 (9-conjugated) carotene. The main characteristic carotene bands appeared at 1517, 1152 and 1007 cm^{-1} , and can be assigned as C=C, C-C, and C-CH₃ stretching modes, respectively, suggesting the presence of beta-carotene as the main constituent of rose hip parenchyma. After spectra normalization, the all carotenoid bands have higher intensity in fresh fruits than in tea products, as expected, in addition to differences in bands intensity among rose hip tea products. The preliminary results show that Raman spectroscopy can be used in carotenoid analysis in rose hip tea and related products for rapid and efficient product quality assessment. Future research should include additional spectral and HPLC analysis of samples.

Key words: rose hip, herbal tea, spectroscopy method, beta-carotene

MICROMERIA CROATICA (Pers.) Schott - CHEMICAL COMPOSITION AND VARIABILITY OF ESSENTIAL OIL DEPENDING ON LOCALITY

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The essential oil (EO) was isolated from *Micromeria croatica* (Pers.) Schott, an endemic plant species of the Dinarides mountain range. The plant material was collected at localities on Mt. Velebit (localities Bojinac, Bačić kuk and Stupačinovo) in Croatia. The EO was subjected to detailed GC and GC-MS analysis to determine chemical composition and the similarity and variability in oil composition depending on locality. The overall composition of EO of *M. croatica* is characterized by a high percentage of sesquiterpenes and monoterpenes. In the oil from Bojinac, sesquiterpene β -caryophyllene (25.2%) is the dominant component, followed by oxygenated sesquiterpene caryophyllene oxide (10.1%). In the oil from Bačić kuk, the cumulative amount of monoterpenes is substantially higher than sesquiterpenes, although the oxygenated sesquiterpene caryophyllene oxide (21.1%) is a major component of this oil. In the oil from Stupačinovo, caryophyllene oxide (20.2%) is the major compound, followed by β -caryophyllene (10.2%). To explore the relationship between the oil samples from various populations and their relation to specific volatile compounds, the GC-MS data were analysed with Principal component analysis. Based on these results, the EO composition in the studied populations of *M. croatica* can be considered to be quite uniform. A somewhat more pronounced degree of specificity was observed in the composition of oil from Stupačinovo. Cluster analysis classifies the number of samples studied into groups according to the chemical composition of essential oil by 'magnifying' their similarities. Multivariate analysis of the samples differentiated two well-defined clusters of *M. croatica*, in which one cluster included the localities Bojinac and Bačić kuk, and the locality Stupačinovo was isolated in its own cluster, indicating the specificity of its EO composition.

Key words: *Micromeria croatica*, essential oil, β -caryophyllene, caryophyllene oxide

INFLUENCE OF *RHODIOLA ROSEA* L. CALLI EXTRACTS ON CD4 T CELLS PHENOTYPE CHANGES

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Rhodiola rosea L. extract and its major bioactive constituents (salidroside, rosarin, rosavin and rosin) have been found to alter CD3 T cell growth and apoptosis via modulation of IFN-gamma production, expression of pro-apoptotic markers and phosphorylation of extracellular signal-regulated kinase. In this study, the effect of various calli extracts from *R. rosea* on CD4 T cell population phenotype was investigated. CD4+ T cells from mouse spleens were stimulated in the presence of the calli extracts (originated from different calli lines and designated as L82, L84, L90, L91), as well as, p-tyrosol. The investigated extracts and p-tyrosol failed to inhibit markedly T cell proliferation at doses below 50 µg mL⁻¹. At a dose of 10 µg mL⁻¹ they affected the production of IFN-gamma and changed the frequencies of IL-17+Foxp3+ cells vs single IL-17+ and Foxp3+ populations. The L82, L84 extracts and p-tyrosol significantly increased the frequency of IL-17+ cells while reducing the frequency of the double positive IL-17+Foxp3+ cells in comparison to control culture. L90 did not to elevate the number of Th17 cells but it decreased the frequency of cells at intermediate state - IL-17+Foxp3+ cells. L91 promoted the generation of IL-17+Foxp3+ cells without an effect on Th17 cells. These data suggest that *R. rosea* calli extracts and p-tyrosol at low dose may have potential to affect the phenotype profile of CD4 T cells and eventually the differentiation and functions of populations (IL-17+Foxp3+ T cells, T regulatory and Th17 cells) playing a role in the pathogenesis of immune-related diseases.

Key words: *Rhodiola rosea*, calli, T cells, Foxp3, immunity

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HPLC/DAD/ESI-MSN ANALYSIS OF PHENOLIC COMPOUNDS IN *HYPERICUM PERFORATUM* L. WILD-GROWING PLANTS FROM PELISTER NATIONAL PARK, REPUBLIC OF MACEDONIA

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The aim of this study was to provide comprehensive understanding of phenolic compounds distribution in roots (RO), non-flower shoots (NFS) and flower shoots (FS) of *Hypericum perforatum* L. wild-growing plants. Chromatographic analysis of plant methanolic extracts revealed the presence of phenolic acids, flavan-3-ols, flavonol glucosides and aglycones, anthocyanins, naphthodianthrone, acyl-phloroglucinols and xanthenes. Among the identified phenolic acids, quinic acid was detected in all tested extracts, while chlorogenic acid and 3-p-coumaroylquinic acid were identified in only in NFS and FS. Interestingly, 3-feruloylquinic acid was detected in RO and FS, but not in NFS extracts. Regarding the flavan-3-ols, (epi) catechin and several oligomeric procyanidins were found in all tested samples, whereas catechin and B-type procyanidin dimer were confirmed in NFS and FS. Four flavonol glucosides (hyperoside, rutin, quercitrin and kaempferol 3-O-rutinoside) were identified in the aerial parts, while guaijaverin and kaempferol 3-O-glucoside were exclusively found in NFS. Additionally, three flavonoid aglycones (quercetin, amentoflavone and I3-II8 biapigenin) were detected only in FS extracts. The presence of two anthocyanins, such as cyanidin 3-O-glycoside and cyanidin 3-O-rhamnoside was confirmed in NFS and FS. Naphthodianthrone were represented with pseudohypericin, hypericin and protopseudohypericin in FS, while only hypericin was detected in NFS. Acyl-phloroglucinols, hyperforin and adhyperforin were identified in NFS and FS, but not in RO. The presence of 11 xanthenes (γ -mangostin, 5-O-methyl-2-deprenylrheediaxanthone B, garcinone C, 3,6-dihydroxy-1,5,7-trimethoxy-xanthone, cadensin G, cadensin C and several xanthone derivatives) was exclusively confirmed only in RO extracts. For the first time, padiaxanthone was detected in NFS, while dimethylmangiferin in FS extracts. The potential interesting finding in this study was the identification of novel xanthenes in NFS, FS and RO from *H. perforatum* that can be used as biologically active compounds in food and pharmaceutical industry.

Key words: HPLC/DAD/ESI-MSn, *Hypericum perforatum* L., phenolic compounds

PRODUCTION OF NITRIC OXIDE, REACTIVE OXYGEN SPECIES AND COX2 BY NEUTROPHILS UNDER THE INFLUENCE OF *CLINPODIUM VULGARE* L.

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Clinopodium vulgare L. possesses important pharmacological activities due to the presence of phenolic acids, flavonoids and triterpenes. In this study the qualitative and quantitative content of chlorogenic and caffeic acids, as well as, catechin of *C. vulgare* from Pirin Mountain, Bulgaria were assessed by NMR (1D and 2D)-based metabolomics and HPLC. The estimated amounts were $116.83 \pm 1.15 \mu\text{g g}^{-1}$, $431.57 \pm 16.74 \mu\text{g g}^{-1}$ and $6.54 \pm 0.32 \mu\text{g g}^{-1}$, respectively. The influence of 50% methanol extract of *C. vulgare* has been investigated on the functions of neutrophils regarding the production of nitric oxide (NO), reactive oxygen species (ROS) and cyclooxygenase (COX2). Neutrophils have been isolated from bone marrow of healthy ICR mice or ICR mice with zymosan-induced shock. Cells were cultivated for 18 hours with the plant extract, chlorogenic acid, caffeic acid and catechin at concentrations from 10 ng mL^{-1} to $10 \mu\text{g mL}^{-1}$.

The production of NO and ROS has been evaluated by colorimetric assays and cell apoptosis. COX2 expression has been assessed by flow cytometry. The plant extract at $10 \mu\text{g mL}^{-1}$ increased apoptosis of healthy neutrophils to $25 \pm 5\%$, and to $45 \pm 9\%$ of the zymosan treated neutrophil population, compared to the control ($8 \pm 2\%$). On the other hand, in low doses ($1 \mu\text{g mL}^{-1}$) the plant extract reduced twice the apoptosis in both neutrophil populations. The chlorogenic and caffeic acids demonstrated similar effect, while catechin increased 1.5 times COX2 expression, NO and ROS levels in PBS and zymosan- induced shock neutrophil populations. The obtained data showed that the extract of *C. vulgare* may have good potential on the neutrophil functions. Nevertheless, this activity may depend on the cellular state, the inflammation milieu and the relative content of the two phenolic acids.

Key words: *Clinopodium vulgare* L., neutrophils, inflammation, phenolics, oxidative stress/free radicals

Acknowledgement: This project for establishment of CPSBB has received funding from The European Union's Horizon 2020 research and innovation programme under grant agreement No PlantaSYST – SGA/CSA: 739582 – under FPA: 664620.

CHEMICAL COMPOSITION AND FREE RADICAL SCAVENGING PROPERTIES OF *PULICARIA* *SALVIIFOLIA* Bunge., *P. GNAPHALODES* (Vent.) Boiss. AND *P. ULIGINOSA* Steven ex DC. ESSENTIAL OILS

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In the present work, three *Pulicaria* species collected in Uzbekistan, *Pulicaria salviifolia* Bunge., *P. gnaphalodes* (Vent.) Boiss. and *P. uliginosa* Steven ex DC. were subjected to hydrodistillation to yield essential oils. Chemical compositions of the oils were analysed with GC-FID and GC/MS techniques. The oils were investigated for antioxidant properties via scavenging of DPPH[•] and ABTS^{•+} free radicals. In *P. salviifolia* oil, terpinen-4-ol (12.2%), δ -cadinene (7.1%), junenol (= eudesm-4(15)-ene-6-ol) (5.8%), β -caryophyllene (5.6%), γ -terpinene (4.6%), T-muurolol (3.5 %) and γ -cadinene (3.3%) were found as the main constituents. The oil of *P. gnaphalodes* was found to be rich with oxygenated monoterpenes, 1,8-cineole (11.2%), cis-chrysanthenol (4.5%), α -terpineol (3.5%), terpinen-4-ol (2.6%) and chrysanthenone (2.4%), as well as oxygenated sesquiterpenes, cadina-1(10),4-dien-8 α -ol (16.1%) and eudesma-4(15),7-dien-1- β -ol (4.2%). The oil of *P. uliginosa* was found to contain α -cadinol (5.9%), menthone (4.5%), hexadecanoic acid (4.5%), 13-nor-7,8-epoxy-eremophil-1(10)-en-11-one (3.2%), T-cadinol (3.7%), δ -cadinene (3.0%), caryophyllene oxide (2.7%), 1(10),5-germacradien-4- β -ol (2.7%) and pulegone (2.5%) as the main constituents. The oils demonstrated moderate inhibitory effects towards to free radicals. The present work is the first contribution about chemical composition and free radical scavenging potential of these *Pulicaria* species.

Key words: *Pulicaria*, essential oil, gas chromatography, antioxidant

COMPARATIVE ANALYSIS OF VOLATILE OILS OBTAINED FROM SEVEN ROMANIAN BRANDS OF CHAMOMILE TEA

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Chamomile tea is one of the most popular teas in Romania for its therapeutic qualities as well as for its taste and smell. Unfortunately, the products from the Romanian trade analysed by us showed extremely different organoleptic characters. Some of them were at the limit of acceptability. The volatile oil is one of the major therapeutic compounds in *Matricariae flos*. It is also responsible for the taste and, especially, for the smell of the vegetable product. That's why we have decided to obtain and analyse the volatile oils of the purchased products. Volatile oils were obtained by the Microwave-assisted hydrodistillation (MAHD) method, adapted and optimized for the analysed product. The sample size of plant product and the amount of hexane were according to the European Pharmacopoeia 5th Ed. The obtained solutions of chamomile oil in hexane were of various shades of blue, from pale to intense. The results of the analyses showed considerable differences between the obtained volatile oils both qualitatively and, especially, quantitatively. Volatile oil concentrations in the purchased products varied between 0.15 and 0.49%. The obtained results show once again the necessity to apply more stringent requirements for medicinal plants and for herbal products.

Key words: chamomile, volatile oil, microwave-assisted hydrodistillation,

PHENOLIC COMPOUNDS IN SELECTED PLANT SPECIES

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Phenolic substances belong to the most important secondary metabolites of plants; they have high antioxidant activity useful for the prevention of cardiovascular diseases, bacterial growth or cancer. Phenolic acids and flavonoids obtained by the means of different extraction methods were studied in elderberry flowers and marigold, namely chlorogenic acid, caffeic acid, rutin, hyperoside, isoquercetin, quercetin and kempferol. The plants were extracted in order to obtain three types of phenolic compounds: free, bound and esterified ones. The compounds of interest were analysed by the means of UHPLC liquid chromatograph with a PDA detector at the Research Institute of Brewing and Malting, PLC. The obtained results indicate that the extraction of free phenolic compounds seems to be the best method for obtaining phenolic substances. Following phenolic substances are used in the food industry: chlorogenic acid, rutin, hyperoside, isoquercetin, quercetin and kaempferol.

Key words: flavonoids, phenolic compounds, phenolic acids, elderberry, marigold

GLYCINE MAX L. Merr. SECONDARY METABOLITES AS NUTRACEUTICALS

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Glycine max L. Merr. is world famous for its high content of phyto proteins (35 - 40%), oils (18 - 20%) and carbohydrates (about 35%). During the last decades soybean attractiveness as source for nutraceuticals is increasing due to its seed richness of many beneficiary secondary substances possessing antioxidant activity such as isoflavones (up to 3 mg g⁻¹ dry weight) and phenolic compounds. Present study describes phytochemical analysis of seeds and seedlings of Bulgarian *Glycine max* varieties for their potential to be used in health care programs.

Key words: *Glycine max*, nutraceuticals, secondary metabolites

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GLYCYRRHIZIN CONTENT IN THE ROOTS OF *GLYCYRRHIZA GLABRA* L. FROM BULGARIAN POPULATIONS

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Licorice (*Glycyrrhiza glabra* L.) is a medicinal plant species from Fabaceae family, with market value and resource deficit. It is protected by the Biodiversity Act and included in the Red Data Book of Republic of Bulgaria as “endangered”. A few localities of licorice are distributed along the Danube River. The healing properties of this perennial plant are due mainly to the triterpenoid saponin glycyrrhizin and the flavonoids, accumulated in the roots (*Radix Glycyrrhizae*) used for treatment of asthma, renal calculus, ulcer, psoriasis, and rheumatism. The aim of the study was the selection of the Bulgarian population with highest content of glycyrrhizin in roots. Root segments of about 20 cm in length and diameter of 10-15 mm, were collected in October from 4 Bulgarian populations of *G. glabra* near the villages of Dolni Vit, Baykal, Koilovci, and Beltsov. In addition, root segments with similar size originating from a Ukrainian population of economic importance were used for comparison of glycyrrhizin content. Root segments were washed, and then cut into small pieces, dried in an oven at 40°C and grinded to a fine powder. Glycyrrhizin content was determined in methanol extracts by HPLC (UV / VIS detector, LC-85B, Perkin Elmer), controlled with Chromulan software (PiKRON), in three samples per population. The four tested Bulgarian licorice populations differed significantly from one another according to their glycyrrhizin contents ($P < 0.001$), the richest one being that near Beltsov (29.6 ± 2.3 mg/g). The population near Baykal had the lowest glycyrrhizin content (11.8 ± 0.5 mg/g) while the other two populations were similar with the Ukrainian one, with intermediate values between 21.2 and 24.0 mg/g. Thus, the licorice population near Beltsov was selected as the most appropriate source of starting material for *in vitro* propagation of the species. Rapid plant multiplication will ensure plants for establishment of pilot field cultivation.

Key words: licorice, *in vitro* propagation, glycyrrhizin

ANTIOXIDANT CAPACITY AND TOTAL POLYPHENOL CONTENT OF LAVENDER CULTIVARS (*L. ANGUSTIFOLIA* Mill., *L. X INTERMEDIA* (L.) Emeric) AT DIFFERENT GROWING AREAS IN HUNGARY

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Lavandula species are worldwide grown essential oil producing medicinal plants with considerable economic value. Beside volatile oil, lavender also contains different phenolic compounds which have been less widely studied till now. Cultivation of lavender has become widespread in Hungary in the recent years, however, growers have limited knowledge on the productivity of cultivars available. It is known from previous literature that these active substances are highly influenced by genetic and environmental factors. In our research we were aimed at studying the antioxidant capacity and total polyphenol content of samples collected in three growing areas (Budapest, Dörgicse, Szomód) from different plant organs (leaves, flowers) of two *L. angustifolia* Mill. and three *L. x intermedia* (L.) Emeric varieties, during the full blooming period of 2017. Antioxidant capacity and total phenol content (TPC) of the samples were determined by FRAP assay of Benzie and Strain (1996) and by a modified method of Singleton and Rossi (1965), respectively. According to our results, correlation was found between FRAP and TPC values of the cultivars. In general, varieties of *L. x intermedia* showed the highest values of antioxidant capacity and of total polyphenol content. Among them, the antioxidant capacity (184.5 mg AAE g⁻¹ DW) and total polyphenol (152.2 GAE g⁻¹ DW) values of 'Judit' flowers from Budapest and those of 'Grosso' from Dörgicse (179.6 mg AAE g⁻¹ DW and 152.4 mg GAE g⁻¹ DW) were the highest, respectively. Significant differences have been found between plant organs in all varieties: flower samples were always superior to the leaves. Concerning the effect of growing area, FRAP values were higher in Szomód at every cultivar, except for 'Grosso' and 'Judit'. However, TPC values were more outstanding in Dörgicse, especially in the case of *L. angustifolia* varieties and of 'Grosso'. Our studies on lavender polyphenols will be completed by qualitative analysis of HPLC and to be continued in the next years.

Key words: lavender, antioxidant capacity, total polyphenol content, growing area

VARIABILITY OF TOTAL ESSENTIAL OILS CONTENT IN LEAVES OF BAY LAUREL (*LAURUS NOBILIS* L.) IN 12 DISTINCT POPULATIONS OF CROATIAN ADRIATIC AREA IN RELATION WITH GEOGRAPHIC POSITION OF LOCATIONS, LEAF LAMINA SURFACE AND GENETIC VARIABILITY

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Within research project “Taxonomy, Ecology and Utilization of Carob tree (*Ceratonia siliqua* L.) and Bay laurel (*Laurus nobilis* L.) in Croatia” (HRZZ-IP-11-2013-3304), a total of 1200 plant samples were collected from 12 distinct populations of bay laurel (*Laurus nobilis* L.) on Croatian Adriatic area. After morphometric analyses of leaves, AFLP analyses of genetic variability, and variability of total essential oils content in leaves of bay laurel populations, significant correlation between leaf surface and essential oils content (Spearman’s Rank Order Corr. Coeff. $r_s = 0.15$ ns) was not found, while the correlation between latitude and essential oils content was strongly negative (Spearman’s Rank Order Corr. Coeff. $r_s = -0.78$; $p < 0.05$). However, the correlation between longitude and essential oils content was strongly positive (Spearman’s Rank Order Corr. Coeff. $r_s = 0.73$; $p < 0.05$). On the other hand, the results of Mantel test showed low, but positive and highly significant correlation between AFLP variability of populations and essential oils content ($r = 0.39$; $p < 0.01$), while the significant correlation between AFLP variability of populations and leaf lamina surface (i.e. expectedly the most influential factor on accumulation of essential oils) was not obtained. Obtained results of these matrix correlations (i.e. Spearman’s Rank Order Correlations and Mantel test) corresponds with the results Friedman’s ANOVA and Kendall’s Coeff. of Concordance for variability of total essential oils content between the populations (ANOVA Chi Square = 21.88; $p = 0.025$ and Kendall’s Coeff. of Concordance = 0.99; Aver. rank $r = 0.98$). According to these results, it is possible to conclude that the populations of bay laurel from locations of South East Croatian Adriatic islands and coastal area accumulate higher quantity of essential oils in the comparison with the populations of North West islands and coastal area.

Key words: bay laurel, *Laurus nobilis* L., Lauraceae, essential oils

ANTIDEPRESSANT-LIKE EFFECT OF SALIDROSIDE AND CURCUMIN ON THE IMMUNOREACTIVITY OF ANIMALS SUBJECTED TO A CHRONIC MILD STRESS MODEL

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Recent studies have suggested altered immune reaction and increased levels of pro-inflammatory cytokines in depression. The present study aimed to evaluate the effect of salidroside and curcumin on immunoreactivity of animals subjected to a chronic mild stress (CMS) model followed by lipopolysaccharide-induced inflammation. Male Wistar rats (n = 48) divided in 6 groups were treated orally with: distilled water 10 mL kg⁻¹ (control group and CMS model group); fluoxetine 2.5 mg kg⁻¹; salidroside 5 mg kg⁻¹; curcumin 20 mg kg⁻¹; salidroside 5 mg kg⁻¹ + curcumin 20 mg kg⁻¹. All groups except the control were stressed daily for four weeks and changes in glucose preference were recorded weekly until the sixth week. On the fifth week treatment was initiated and one week later following LPS challenge ELISA evaluation of IL-6 in rats' sera and of TNF- α in sera and in brain tissue homogenate was performed. The CMS model group showed a decrease in glucose preference compared to the control group. The groups, exposed to stress and treated as described above, increased their glucose preference compared to the CMS model group. In the CMS + LPS model group the TNF- α and IL-6 levels were increased compared to the control group, CMS or LPS alone. In all the experimental groups IL-6 in sera and TNF- α in sera and in brain tissue were decreased compared to the CMS + LPS group. The groups exposed to chronic stress showed anhedonic-like behavioural changes. ELISA tests confirmed that CMS is affecting rats' immunity by increasing the cytokines' levels both in serum and brain tissue. These changes were reversed by combination of salidroside and curcumin suggesting synergic interaction regarding their anti-inflammatory and anti-stress effects.

Key words: salidroside, curcumin, chronic mild stress, immunoreactivity, LPS-induced inflammation

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II. DO PLANT GROWTH REGULATORS INFLUENCE HYSSOP (*HYSSOPUS OFFICINALIS* L.) ANTIOXIDANT SYSTEM DURING MICROPROPAGATION?

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Micropropagation of *Hyssopus officinalis* L. offers the best technique for quality and disease free uniform plant material, but there are no studies have been conducted on the effect of plant growth regulators added to the culture medium on plant antioxidant capacity. *H. officinalis* plants were cultured on Murashige and Skoog (MS) medium supplemented with different cytokines: 6-benzyl-aminopurine, thidiazuron, zeatin in concentrations of 0.5 and 1.0 mg l⁻¹ combined with indole-3-butyric acid during four weeks of culture. The creation of reactive oxygen species, during in vitro cultivation as well as their detoxification, is highly synchronized in plants, and their levels are kept under firm control by a complex antioxidant system. Application of cytokines to the MS nutrient medium lead to increased enzyme activities with increased its concentration. The highest superoxide dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase activities was measured when plants are cultured on the MS medium supplied with 1 mg l⁻¹ 6-benzyl-aminopurine and 0.1 mg l⁻¹ indole-3-butyric acid. The content of water- and lipid-soluble metabolites with antioxidant capacity expressed as ascorbate or α -tocopherol equivalents also increased when cytokines concentration increase but the highest levels were observed in the MS medium without growth regulators, followed by the treatments when 6-benzyl-aminopurine was applied. The content of total phenols and flavonoids was not influenced by the presence of cytokines as well as total antioxidant potential. The antioxidant defence of *in vitro* cultured *H. officinalis* was determined mainly to the enzymatic parameters (the higher activity of antioxidant enzymes) than to the non-enzymatic (declined concentration of low molecular metabolites – water and lipid soluble metabolites with antioxidant capacity, phenols and flavonoids).

Key words: *Hyssopus officinalis*, plant growth regulators, antioxidant enzymes and metabolites

Acknowledgement: This study was conducted with financial support from NSF at the BMES, Project DN06/7 2016.

VACCINIUM MACROCARPON Aiton (CRANBERRY) EXTRACT CAUSES THE RELAXATION IN RAT CORPUS CAVERNOSUM TISSUE

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Vaccinium macrocarpon Aiton (Ericaceae), commonly called cranberry, is native to Eastern North America and induces several biological activities, involving antioxidation, anticancer, cardioprotection, attributed to abundant phenolic contents. Numerous cranberry forms (juice, tablets, capsules, and syrup) have been used widely for several decades for the treatment of urinary tract infections (UTIs). While effects of cranberry products may have benefits for the erectile dysfunction (ED) treatment, but the scientific evidence is lacking. This study evaluates the effects of one of the dietary supplements present in the market, containing concentrated extract of cranberry fruit-(E) on rat corpus cavernosum (CC). Male Sprague-Dawley rats (n = 8) were used for organ bath experiments. After phenylephrine (Phe, 10 μ M) contraction, the relaxant responses were determined in the presence of some inhibitors. The relaxant responses of rat CC strips were observed in the presence or the absence of cranberry-E. Cranberry-E induced relaxation of rat CC (maximum response: $76.1 \pm 3.6\%$) after Phe-contraction. Cranberry-E evoked long-lasting relaxations. The nitric oxide synthesis inhibitor N(omega)-nitro-L-arginine methyl ester (L-NAME; 10 μ M), the soluble guanylate cyclase inhibitor ODQ (10 μ M), and endothelial disruption all failed to affect the relaxations. The relaxant responses to acetylcholine (10 μ M), electrical field stimulation (10 Hz), and sodium nitroprusside (0.01 μ M) in rat CC were increased after incubation with cranberry-E. The underlying mechanism of cranberry products is likely independent of the nitric oxide-cyclic guanosine monophosphate pathway. Overall, these *in vitro* studies suggested that consumption of cranberry-E may be efficient and represent an exciting new strategy to prevent and diminish ED in men. The results of the current study could be used by physicians to recommend cranberry extract ingestion to decrease the incidence of ED patients with UTI as an alternative therapy.

Key words: cranberry, ED, *Vaccinium*, Ericaceae

BLOCK COPOLYMER NANOCARRIERS OF CAFFEIC ACID PHENETHYL ESTER: PREPARATION AND ANTIOXIDANT ACTIVITY

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Caffeic acid phenethyl ester (CAPE) is one of the most important constituents of poplar type propolis, well-known for its valuable biological activities: antimicrobial, antioxidant, antitumor, etc. We applied several known procedures for synthesis of CAPE and compared the yields, reaction times, and the ratio E/Z isomers of the obtained product. Next, CAPE was immobilized in block copolymer micelles in order to impart good solubility in biological medium and enhanced bioavailability. Spherical nanosized micelles were prepared from a poly (ethylene oxide)113-b-poly(ϵ -caprolacton)35-b-poly (ethylene oxide)113 (PEO-b-PCL-PEO) triblock polymer, synthesized by “click” reaction. The micellar system comprising a biodegradable PCL core containing CAPE and a hydrated PEO shell exhibited superior colloid stability in aqueous media. Then, the antioxidant activity of CAPE-loaded micelles on a model of hydrogen peroxide – damaged HepG2 cells was studied and compared to that of pure CAPE. Cells were pretreated with the substances in the concentration interval 0.1 – 71.0 $\mu\text{g mL}^{-1}$ and then damaged with 400.0 $\mu\text{M H}_2\text{O}_2$. Significant protective effects of both loaded and non-loaded CAPE were observed at concentration 8 $\mu\text{g mL}^{-1}$, revealing that the micellar carriers retain the antioxidant properties of CAPE.

Key words: caffeic acid phenethyl ester, block copolymer nanocarriers, antioxidant activity

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PROPOLIS FROM VIETNAM – CHEMICAL PROFILES, PLANT ORIGIN AND BIOLOGICAL ACTIVITY

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Propolis is a bee hive product important to social immunity of the bee colony. It is well known to humans for its preventive and healing properties and nowadays it is included in numerous commercial products. As a mixture of plant resinous exudates and beeswax, its chemical composition depends strongly on the local flora and thus on the climatic characteristics at the site of resin collection. The study of propolis from unexplored / scantily explored regions has the potential to uncover new biologically active compounds with important pharmacological effects. We studied propolis from Vietnam: two samples of *Apis mellifera* propolis and four samples from local stingless bees. Chemical profiles of the samples were obtained by GC-MS analysis of their ethanol extracts. The honeybee samples were characterized by the presence of alkylphenols, alkylphloroglucinols, anacardic acids, and triterpenoids, typical constituents of *Mangifera indica* L., a plant well-known as propolis source in tropical regions. On the other hand, the stingless bee propolis samples differed between them and from the *A. mellifera* samples, but all of them had varying amounts of the typical metabolites of the resin of the Vietnamese tree *Cratoxylum cochinchinense* (Lour.) Blume: specific prenylated xanthenes. The samples with highest content of prenylxanthenes demonstrated the highest radical scavenging activity against DPPH. The antimicrobial activity of the samples was also determined. The detailed study of the stingless bees propolis is in progress.

Key words: propolis, plant source, biological activity

CHEMICAL CHARACTERIZATION AND RADICAL-SCAVENGING ACTIVITY OF PROPOLIS FROM PITCAIRN ISLAND

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Propolis (bee glue) is a resinous hive product collected by bees from certain plant sources and known since ancient times for its healing properties. There is a number of scientific evidence that it has antimicrobial, antioxidant, anti-inflammatory, immunomodulating, antiulcer, antidiabetic and anticancer activities. Because of its broad spectrum of biological activities there is an undying interest on the composition of propolis, which depends on the vegetation of the area of collection. Numerous studies on the chemical composition and biological activity of propolis from Europe, South America, Asia and the Pacific region exist in the literature however there is no data about propolis from Pitcairn Islands. In the last years it is of growing commercial interest, because Pitcairn's bee population is disease-free and their products are considered as clean of pollutants. The aim of this work was to determine volatile and non-volatile composition as well as radical-scavenging activity of propolis from Pitcairn Island (South Pacific Ocean), which is not investigated yet. The essential oil were obtained by hydrodistillation extraction of raw propolis in a Likens-Nickerson type apparatus and then analyzed by GC/MS. The main volatile components were monoterpenes p-mentha-1,5-dien-8-ol (4.4 %) and α -pinene (3.4 %), and sesquiterpenes β -caryophyllene (3.3 %), caryophyllene oxide (2.4 %) and verbenone (2.3 %). The major non-volatile constituents according to the GC/MS analysis after silylation were terpenoids (mainly diterpenes and less triterpenes). Phenolic components (cardanols, alk(en)ylresorcinols and anacardic acids) are present in significantly lower amounts, as flavonoids and the usually present phenolic acids are completely absent. On the basis of the overall chemical profile, hypotheses were made regarding the plant sources of the sample. DPPH radical scavenging activity of propolis ethanol extract was tested and the results showed week activity.

Key words: propolis, GC/MS, volatiles, diterpenes, DPPH

Acknowledgement: The authors would like to thank to Pitcairn Island Producers' Co-operative (PIPICO) for providing the sample.

GC/MS BASED METABOLITE PROFILING OF BULGARIAN *GLYCINE MAX* (L.) Merr. VARIETIES

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Soybean (*Glycine max* (L.) Merr.) is one of the most widely cultivated crops in the world for its nutritional and medicinal properties. In Bulgaria series of elite soybean varieties with improved yield and nutrition qualities has been released at the Experimental Station in Soya, Pavlikeni. In the present study Bulgarian soybean seed cultivars were examined and were compared with American variety “Hodson” (world standard) for their metabolite profiles by GC/MS. A variety of fatty acids - saturated and unsaturated, amino acids - indispensable and interchangeable, organic and phenolic acids, a variety of carbohydrates was found. The studied Bulgarian cultivars showed comparable even in terms of extractable lipid components better profiles than world standard “Hodson”. The present results showed that Bulgarian soybean cultivars are promising food with a high nutritional value and beneficial to human health properties.

Key words: Bulgarian cultivars, fatty acids, *Glycine max*

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CHEMOSYSTEMATIC SIGNIFICANCE OF UNDERGROUND PARTS AND FRUIT ESSENTIAL OIL CONSTITUENTS OF NINE *HERACLEUM* TAXA FROM SOUTHEASTERN EUROPE

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Chemical composition data of essential oils from underground parts and fruits of nine *Heracleum* L. taxa (Apiaceae) collected in Serbia, Montenegro, Macedonia and Slovenia were statistically analyzed to evaluate chemosystematic significance of their components. Eight investigated taxa (*H. orphanidis* Boiss. and representatives of *H. sphondylium* group: *H. sphondylium* L., *H. sibiricum* L., *H. montanum* Schleich. ex Gaudin, *H. ternatum* Velen., *H. pyrenaicum* subsp. *pollinianum* (Bertol.) F. Pedrotti & Pignatti, *H. pyrenaicum* subsp. *orsinii* (Guss.) F. Pedrotti & Pignatti and *H. verticillatum* Pančić) belong to the type section of the genus. Additionally, analyzed taxon, *H. austriacum* subsp. *siifolium* (Scop.) Nyman, belongs to *H. sect.* *Wendtia* (Hoffm.) DC. Essential oils were hydrodistilled using Clevenger-type apparatus and analyzed by GC-FID and GC-MS. Chemosystematic significance of constituents was evaluated using multivariate statistics: PCA, nMDS and UPGMA. Statistical analysis included our previously published data on the oils of 11 samples of underground parts and 14 of fruits, as well as additionally analyzed oils of 5 samples of underground parts and 4 of fruits. Underground parts oils of representatives of *H. sphondylium* group were characterized by monoterpenes, with β -pinene, (*Z*)- β -ocimene or limonene being dominant among them, while *H. orphanidis* and *H. austriacum* underground parts oils were dominated by (*Z*)-falcarinol. Fruit oils were rich in aliphatic esters, such as octyl acetate (the members of *H. sect.* *Heracleum*) and octyl hexanoate (*H. austriacum*). Statistical analysis of underground parts essential oils showed that investigated taxa were grouped according to their taxonomic classification. Subspecies of *H. pyrenaicum* were grouped together, and the members of *H. sphondylium* group were well separated from *H. orphanidis* and *H. austriacum*. Regarding fruit essential oils, such relationships were clearly observed in three-dimensional nMDS. PCA revealed that different compounds (not only above mentioned dominant ones) influenced the separation of investigated taxa.

Key words: *Heracleum* underground parts and fruits, essential oils, GC-FID and GC-MS, multivariate statistics, chemosystematics

SCREENING OF CYTOTOXICITY OF *CENTAUREA* SPECIES AQUEOUS EXTRACTS FRACTIONS ON HUMAN FIBROBLASTS

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Centaurea species are used as medical plants or as a food in eastern Mediterranean area. The aim of our study was to screen the cytotoxicity of twelve different aqueous extracts from seven Croatian *Centaurea* species and to compare their HPLC profile to identify toxic fractions. The toxic properties of plant extracts and their constituents may be used in pharmaceutical and cosmetic industry. Cytotoxicity of *Centaurea* extracts and fractions was measured on primary fibroblasts through the MTT assay. The extracts were partitioned using reversed phase HPLC with C8 column and gradient of polar solvents. Aqueous extracts (AE) were prepared from wild growing *Centaurea ragusina* L., *C. scabiosa* L., *C. solstitialis* L., *C. rupestris* L., *C. alba* L., *C. jacea* L. and *C. salonitana* Vis. Among the tested aqueous extracts, the highest cytotoxic activity was found in *C. ragusina*, *C. salonitana*, *C. solstitialis* and *C. alba*. Every other AE showed low toxicity and *C. scabiosa*, wild edible species, showed no toxicity even at 1g/L of extract. Some of the fractions isolated by HPLC were tested for their toxicity based on their HPLC profile. Peaks that were characteristic of each species were collected and tested by MTT on fibroblast at the equivalent for 1g/L of extract. Some of these fractions seem to explain part of the toxicity of the extract. Future investigations of chemical composition should highlight the main compounds in these extracts responsible for those biological activities. As far as previous research showed, it is expected that some of the main groups of compounds could be sesquiterpene lactones, phenolic compounds and organic acids. Sesquiterpene lactones are usually connected with toxic properties of *Centaurea* extracts

Key words: *Centaurea*, extracts, cytotoxicity

GC/MS BASED METABOLITE PROFILING AND ANTIOXIDANT ACTIVITY OF BALKAN AND BULGARIAN ENDEMIC PLANTS

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Balkan and Bulgarian endemic plants *Viola rhodopaea* Becker Violaceae, *Veronica rhodopaea* (Velen.) Degen ex Stoj. & Stefanov Plantaginaceae, *Silene roemerii* Friv. Caryophyllaceae, *Jasione bulgarica* Stoj. & Stef and *Campanula lanata* Friv. Campanulaceae, were examined. Metabolite profiles of methanolic extracts of studied species were analysed by GC/MS and HPTLC. Antioxidant potential was assayed by DPPH method and β -carotene/linoleic acid test. The highest antioxidant potential was estimated for the extracts of *Veronica rhodopaea*, *Jasione bulgarica* and *Viola rhodopaea*. Fatty acids and alcohols, phenolic and organic acids, flavonoids, sterols, carbohydrates were identified. The present data are reported for the first time for the studied species.

Key words: *Viola rhodopaea*, *Jasione bulgarica*, *Campanula lanata*, *Veronica rhodopaea*, *Silene roemerii*

PULMONARIOSIDES A AND B – NEW PHENOLIC COMPOUNDS FROM THE AERIAL PART OF *PULMONARIA OFFICINALIS* L.

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Pulmonaria officinalis L (lungwort) belonging to Boraginaceae family is considered to possess therapeutic properties. It has a long tradition of use in folk medicine as a remedy against various lung disorders including tuberculosis, asthma, and coughs. This herb also has the expectorant, anti-inflammatory, antitussive, and diaphoretic properties. Used externally, it can be very beneficial in the treatment of burns, wounds, cuts, and eczema. However, very few pharmacological studies confirming the effects of the traditional use of *Pulmonariae Herba* are available. Similarly, scientific literature describing secondary metabolites responsible for the biological activity of this herb is very sparse and selective. Although a few studies concerning phenolic compounds are available, other classes of compounds are practically unknown. We employed multistage isolation procedure, including solid phase extraction and few chromatographic steps, to further characterize *Pulmonariae Herba* and to isolate new compounds. Their structures were determined based on its HR-QTOF-MS/MSMS and NMR spectroscopy data, including one-dimensional (¹H, ¹³C and ¹³C-DEPT) as well as two dimensional (¹H-¹H COSY, ¹H-¹H TOCSY, ¹H-¹H ROESY, ¹H-¹³C HSQC, ¹H-¹³C HMBC) spectra. Among the newly isolated compounds are two derivatives of diferulic acid, provisionally called pulmonarioside A (Mw 1014.3005, C₄₈H₅₄O₂₄) and pulmonarioside B (Mw 1000.2849, C₄₇H₅₂O₂₄). The characteristic structural feature of pulmonariosides is diferulic acid residue in aryltetralin form bridged via sucrose to either caffeic or ferulic acid O-rhamnopyranoside.

Key words: *Pulmonaria officinalis*, Boraginaceae, pulmonariosides

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GENUS *THYMUS* IN BULGARIA – A NEW PROJECT AIMED AT REVEALING OF SPECIES' METABOLITE PROFILE AND GENETIC DIVERSITY

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Genus *Thymus* comprises more than 250 species of perennial herbaceous or fruticose plants, classified into eight sections. Total of 66 species with numerous subspecies and varieties are listed in Flora Europaea. Twenty species occur naturally in Bulgaria. One of them is endemic to the country (*Thymus perinicus* /Velen./ Jalas), four are endemics to the Balkan Peninsula (*T. albanus* Heinr. Braun ex Wettst., *T. comptus* Griseb., *T. longedentatus* /Degen & Urum./ Ronniger, *T. stojanovii* Degen) and other three are sub-endemics (*T. atticus* Čelak., *T. moesiacus* Velen., *T. sibthorpii* Benth.). The studies on the genetic variation at species and within-species level will help the elucidation of some unresolved taxonomic issues and at the same time will serve as characteristics of the genetic resources of the species. The species of genus *Thymus* provoke substantial interest worldwide from phytochemical point of view, due to their diverse biological activities with potential for application in pharmaceutical, cosmetic and food industries. They have been used since the ancient times to treat diseases of the respiratory and digestive system, as well as of colds. They possess expectorant, antiseptic, fungicide, spasmolytic, carminative, sedative, diaphoretic and diuretic activity. The main objective of the present project is to perform a complete genetic and phytochemical study on the species of genus *Thymus* in Bulgaria. Distinguishing the taxa according to their morphology, chorology, ecological and genetic characteristics, metabolite content, essential oil composition, expression of antioxidant activity will allow composition of a general picture of the species of genus *Thymus* in Bulgaria. Results of the studies will be a basis for determining the place of each species within the general scheme of the genus. The information gathered will serve as a scientific basis for the initial stages of cultivation of the prospective (with best phytochemical characteristics) species, as well as the endangered ones.

Key words: Thyme, diversity, taxonomy, active compounds

Acknowledgement: This work was supported by the NSF, Ministry of Education and Science, Bulgaria, Project DN 16/3.

II. PHYTOCHEMICAL ANALYSIS, PHARMACOLOGY, BIOLOGICAL ACTIVITY AND TOXICITY (PART II)

NOVEL EXTRACTION TECHNOLOGIES FOR MEDICAL AND AROMATIC PLANTS

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The components of medicinal and aromatic plants are used in many areas of food industry (nutraceuticals, food supplements), pharmaceutical industry (herbal medicinal products and natural products for health care) and bioenergy industries. Extraction is an important process in the isolation of active components from medicinal and aromatic plants using various technologies. Traditional extraction technologies include distillation, solvent extraction and/or cold compression processes. Novel extraction technologies such as ultrasound assisted extraction and microwave assisted extraction enhance the mass transfer, reduce the extraction time, limit the decomposition of extracted compounds and decrease the solvent's consumption. The quality and yield of an extract is influenced by various parameters: the plant matrix, temperature, the solvent used for extraction and extraction technology. Generally, increasing of the ultrasound/microwave power resulted in shorter extraction time and improved extraction yield. However, high microwave/ultrasound power can cause poor extraction yield due to the degradation of thermo-sensitive compounds. Therefore, the process of extraction should be optimized to obtain desirable quality of extract from a specific medicinal and aromatic plant with better yield and selectivity, shorter extraction time.

Key words: ultrasound, extraction, optimization, bioactive compounds

THE INVESTIGATION OF BIOLOGICAL ACTIVITY OF SEVERAL WALNUT (*JUGLANS REGIA* L.) SEPTUM EXTRACTS

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The increasing use of healthy unsaturated fats, more prone to oxidation, the understanding of the pathology of oxidative stress in the organisms, and the negative side-effects of some synthetic antioxidant compounds point to the research of natural antioxidants of plant origin which could be used in food and/or pharmaceutical industry. To investigate the *in vitro* biological activity of walnut septum extracts with high content in antioxidants, and to determine the inhibitory potential of these extracts on some metabolic key enzymes, and the cytotoxic activity on several tumour cell lines. The walnut septum extracts having the highest antioxidant capacity analysed by TEAC method were selected based on optimal experimental design. The inhibitory effect on alpha-amylase, alpha-glucosidase and lipase was further assessed. The cytotoxicity of the walnut extracts was investigated using a resazurin-based assay on three human cancer cell lines: A549 (lung carcinoma), HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma), after 24/48h of exposure at increasing concentrations (1 - 400 µg mL⁻¹). The estrogenic activity was tested on T47D-Kbluc, a cell line naturally expressing ERα and ERβ and stably transfected with a triplet estrogen-responsive element (ERE) – promoter – luciferase reporter gene construct. The analysed extracts presented enzyme inhibitory activity and cytotoxicity on all three cancer cell lines. The same cytotoxic dose response curve was observed for A549 and HepG2: after 24h and 48h exposure, cell viability decreased by 80% and 100%, respectively. On MCF-7, a hormetic response was observed, significant increase in cellular metabolic activity at intermediate concentration levels and cytotoxic activity at the highest tested dose. The estrogenic activity of extracts was confirmed. Our research demonstrates that walnut septum can be a source of bioactive compounds for biopharma or food industry and assert the extension of the investigation in further evaluating the metabolic influence and anticancerous potential.

Key words: walnut, septum, enzymes, cytotoxicity, cancer

THIN LAYER DRYING BEHAVIOUR OF *FOLIUM CUM FLORE CRATAEGI* FROM DIFFERENT PLANT FLOWERING STAGES

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Crataegus L. (hawthorns) is a well-defined genus traditionally defined as belonging to the tribe *Crataegeae*, subfamily *Maloideae* of *Rosaceae*. The number of *Crataegus* species may vary between 150 and 1200. For defining optimal storage conditions, knowledge of the equilibrium relationship between product moisture content (MC) and relative humidity (RH) at a given temperature is fundamental to prevent microbial or enzymatic processes. The moisture sorption behaviour of *Folium cum Flore Crataegi* was investigated by using a dynamic vapour sorption apparatus at temperatures 25 and 60 °C. On practical point of view, knowledge on sorption isotherm would determine the optimal time of drying when using assisted air movement dryers. Drying of *Crataegus* fresh material for determination of optimal drying conditions were investigated for the mixture (*Folium cum Flore Crataegi*) material at air temperature of 30, 45, 60, 75 and 90 °C, specific humidity of 10 g kg⁻¹ and air flow of 0.2 m s⁻¹ and 6 maturity harvesting batches. Loading density of fresh leaves and flowers of 2 kg m⁻², which develops an initial layer depth of about 30 mm, was calculated as optimal density based on previous experience with similar products. It is known that plant materials exposed to water activity below 0.6 or 60% relative humidity can be stored safely at ambient temperature. From the measured data, it can be observed that drying *Crataegus* at a moisture content of 0.12 g g⁻¹ d.b. (12% MC) and storing at temperature of 25 °C is safe and when storing at higher temperatures such as 60 °C it is necessary to dry at MC 0.11 g g⁻¹ d.b. (11% MC).

Key words: *Crataegus*, medicinal plants, drying behaviour, thin layer, sorption isotherm

CURRENT APPROACH AND CHALLENGE FOR THE PROFILING OF GLUCURONIDE OLEANANE-TYPE TRITERPENOID CARBOXYLIC ACID 3, 28-O-BIDESMOSIDES (GOTCAB) SAPONINS. A CASE STUDY OF *GYPSOPHILA* L. (CARYOPHYLLACEAE)

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Glucuronide oleanane-type triterpenoid carboxylic acid 3,28-bidesmosides (GOTCAB) are among the highest glycosylated triterpenoid saponins widespread in the taxonomic groups Caryophyllidae, primitive Rosidae and Asteridae. The study aimed to develop ultra high-performance liquid chromatography-Orbitrap high resolution mass spectrometry (HRMS) acquisition strategy for the peak annotation and dereplication of GOTCABs. A natural variation of GOTCABs, which accumulate to high levels in the roots of *Gypsophila* L. (Caryophyllaceae), is described. Saponins were analyzed in a set of accessions including wild (*G. trichotoma* Wend. and *G. glomerate* Pall. ex Adams) and cultivated (*G. scorzonrifolia* Ser., *G. acutifolia* Stev. ex Spreng, *G. altissima* L., *G. paniculate* L., *G. pacifica* Kom., *G. oldhamiana* Miq., *G. zhegualensis* A.) plants. They were profiled and characterized by classical reverse phase, hydrophilic interaction (HILIC) and two-dimensional (LC × LC) chromatography coupled with hybrid quadrupole-Orbitrap HRMS. The various chromatographic approaches are discussed with respect to their resolution and sensitivity. Based on the accurate mass measurements, fragmentation patterns in MS/MS analyses and comparison with previously isolated authentic references, a total of 54 GOTCAB core structures were identified or tentatively elucidated in the assayed species, forming isobaric and positional isomers. Possible fragmentation pathways for four groups of GOTCABs were suggested. Type I consisted of saponins with C-28 ester-bonded chains with acetyl or/and methoxycinnamoyl group(s); type II and III included sulphated, and acetylated and sulphated saponins, while type IV grouped compounds with unsubstituted tri- to hexa-saccharides. The wild species showed the presence of sulphated GOTCAB, while cultivated accessions displayed a variety of mono- and di-acetylated derivatives of gypsogenin, quillaic and gypsogenic acid. The principal compound analysis (PCA) grouped cultivated *G. paniculata*, *G. oldhamiana* and *G. zhegualensis* in a well-defined cluster. This example of assessing the GOTCAB could be used as a case study to highlight the importance of HRMS and data dependent MS/MS analyses as a rational approach for in-depth metabolome studies.

Key words: triterpenoid saponins, GOTCAB, *Gypsophila*

STATE OF THE ART METHOD ON THE BASE OF GC-ORBITRAP-HRMS FOR SESQUITERPENE LACTONES IN *ARNICA MONTANA* L.

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Arnica montana L. flowers have a long history as herbal medicines for external use on injuries and rheumatic muscle and joint complaints. Esters of the sesquiterpene lactones (STL) helenalin (H) and 11 α , 13-dihydrohelenalin (DH) with short chain fatty acids are considered to be the main active principles of the officinal flower drug. Several high-performance liquid chromatography (HPLC-UV), Gas Chromatography mass spectrometry (GC-MS) and nuclear magnetic resonance methods for the analysis of *A. montana* STLs have been developed. However, all these methods are related either to the total determination of STLs or to the identification of the main compounds. In this study, we aim at developing Gas Chromatography-Orbitrap-High Resolution Mass Spectrometry (GC-Orbitrap-HRMS) strategy for the analyzing of Arnicae STLs with different provenance. The samples originate from Bulgarian, Polish and Finnish cultivated collections and one is purchased in a pharmacy drug store. The sample preparation was done according to the European Pharmacopoeia 7.0 (2011). The subsequent solid phase extraction procedure was performed on Strata C18-E cartridges (Phenomenex, USA) with good recovery. The followed chromatographic determination was carried out by Trace 1310 GC, Exactive Orbitrap GC-MS system (Thermo Fischer Scientific, Bremen, Germany). The data were preceded with Excalibur Software (Thermo Fischer Sci.). NIST Mass Spectral Search Program MS Search 2.0 and NIST Mass Spectral Library were used for STLs identification. Based on the accurate masses and theoretical fragmentations generated with Mass Frontier 7.0 software (ThermoScientific), at least 13 STLs were assessed and quantified, and the fragmentation patterns of both H and DH derivatives were proposed. In conclusion, the reliable GC-Orbitrap-HRMS method would be important for the quantity and quality monitoring of cultivated and native arnica herbal drugs.

Key words: *Arnica montana*, sesquiterpene lactones, GC-Orbitrap-HRMS

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BIOINFORMATIC INSIDE ON *PORTULACA OLERACEA* L. (PURSLANE) WITH BULGARIAN AND GREEK ORIGIN

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Portulaca oleracea L. (purslane) is a green leafy vegetable with worldwide distribution, considered to be a functional food as it provides a health benefit on plant-based diet. Purslane is a rich source of omega-3 and omega-6 fatty acids, vitamins, and minerals as well as high levels of flavonoids, homoisoflavonoids, cyclo-dopa amides and hydroxycinnamic acid amides. This study presents a strategy based on liquid chromatography – high resolution accurate mass spectrometry (LC – HRAMS) and bioinformatic methods to analyze 33 purslane accessions originating from 13 floral regions in Bulgaria together with 5 accessions with Greek provenance. The extraction was performed with microwave with solvent 0.1% formic acid. The aim was to develop a purslane metabolic database that was dedicated to LC-MS metabolic “fingerprints” of assayed samples. LC-MS data were preceded with Software application Compound Discoverer 2.1.0.308, Trial version (Thermo Sci., USA). Principal Component Analysis (PCA) combined with both descriptive and differential analyses were used to find marker metabolites to distinguish North Bulgaria vs South Bulgaria, North Bulgaria vs Greece and South Bulgaria vs Greece. There is no significant difference between the studied Bulgarian samples, while 49 marker metabolites were found in differential analysis between Bulgaria and Greece. Based on accurate masses (mass accuracy 1 ppm), retention times, comparison with commercial standards and literature data these secondary metabolites were identified after detailed analysis of Volcano-plots. Thirty seven of them belonging to phenolic alcohols, phenylacetic acids, acetophenones, cinnamic acid amides, acylquinic acids, lignans, iridoids and tetrahydroxyisoquinolines are reported for the first time. The statistical treatments identified marker compounds that can be used to distinguish origin of accession set. Combining LC-MS data with multivariate statistical analysis was proven effective in studying the purslane metabolites, allowing for integration of chemistry with geographic origin.

Key words: *Portulaca oleracea*, bioinformatic methods, LC – HRAMS, secondary metabolites

OPTIMAL EXPERIMENTAL DESIGN DEVELOPED TO PREPARE WALNUT (*JUGLANS REGIA* L.) SEPTUM EXTRACTS WITH HIGH CONTENT OF BIOACTIVE COMPOUNDS

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Plant by-products can be valuable sources of polyphenolic compounds. Walnut (*Juglans regia* L.) is a very important tree nut filled with biologically active molecules, but its septum was scarcely researched up-to-date. Experimental data indicated the hypoglycemic effect of septum extracts with almost no details about its photochemical composition. To develop an original experimental design with optimum extraction conditions based on which septum extracts rich in bioactive compounds with antioxidant potential can be obtained. Two extraction methods were tested, maceration and turboextraction. The other variables of the experimental design were: temperature, pH, solvent used in the extraction, and percentage of water in the solvent. The first step quantifiable responses were: total phenolic content, total flavonoid content, condensed tannins and antioxidant capacity determined by TEAC assay. The phytochemical profile of lyophilized extracts obtained by turboextraction was further determined by LC-MS/MS analysis of individual polyphenolic compounds and lipophilic compounds: phytosterols and tocopherols. The experimental results proved the net efficiency of turboextraction over maceration in obtaining septum lyophilized extracts rich in bioactive antioxidant compounds. The main parameters favouring extraction were: acidic or neutral pH, the use of acetone as solvent, the presence of water in the solvent in the proportion of 25 - 50%. To the best of our knowledge this study is the first to assay the detailed composition of walnut septum in hydrophilic and lipophilic bioactive compounds. The phytochemical profile of the analysed extracts proves that walnut septum can be a valuable source of biologically active compounds for food and/or pharmaceutical industry, and warrant the continuation of current research in further evaluating its bioactive potential.

Key words: *Juglans regia* L., septum extracts, experimental design, bioactive compounds

ESSENTIAL OIL COMPOSITION OF *TANACETUM PARTHENIUM* L. FROM EASTERN BLACK SEA REGION, TURKEY

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Feverfew (*Tanacetum parthenium* L.), belonging to the family Asteraceae, is a perennial herbaceous plant with small flowers like chamomile. This species is distributed across Europe, America, North Africa and Asia. It is used in the treatment of high fever, migraine headaches, arthritis, stomach aches, toothaches, insect bites in traditional medicine. Nowadays, its dried herbal preparations in capsule and tablets has been sold as food supplement products and disease prevention in world markets. Literature on the essential oil composition of *Tanacetum parthenium* is very limited in Turkey and its essential oil composition can vary largely with different ecological conditions. Therefore, we investigated the essential oil composition of *Tanacetum parthenium* collected from Gumushane province, Turkey. In this study, forty-four components representing 86.04 % of the essential oil of aerial parts of *Tanacetum parthenium* were obtained by hydrodistillation and examined by GC and GC-MS. The main component was camphor (39.47%) and other major components were guaiol (5.21%), caryophyllene oxide (3.91%), bornyl acetate (3.09%), (E)-verbenol (2.68%), camphene (2.29%) and α -cadinol (2.12%). Presence of camphor highlight the potential of this plant as a drug source in the alternative medicine due to its antiseptic, antipruritic, aphrodisiac, contraception and lactation suppressant effects. The results obtained from this study showed differences compared to other studies on essential oil composition.

Key words: feverfew, Asteraceae, essential oil, camphor, GC-MS

AFLATOXIGENIC STRAINS ISOLATED FROM MEDICINAL HERBS

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Fungal contamination of medicinal herbs could influence their chemical composition and the stability of extracts prepared from them. The aim of our study was to assess the level of fungal contamination of 70 samples of medicinal herbs and to evaluate the ability of *Aspergillus* strains to produce aflatoxins. Different samples of medicinal herbs were analysed according to the European Pharmacopoeia, 8th Edition. Samples were mixed with phosphate buffer-peptone solution (pH 7) and the extracts were seeded on Sabouraud broth for fungi, and on agar for total germs. Petri dishes were incubated 5 days at 35°C for total germs, and 7 days at 25°C for fungi development. The number of colony forming units (CFU) expressed in CFU/g has been determined after incubation. For fungi the number of CFU/g was under the limit of 100,000/g and ranged between 100 CFU/g (*Chamomillae flos* - S2 and S4) and 40,800 CFU/g (*Urticae herba* – S5). Fungi represented over 90% of total germs for all samples that were analysed. From *Aspergillus* genus, *Aspergillus niger* was the most frequently identified and *Theae folium* was the most contaminated sample with this strain. *Aspergillus niger* isolated from 5 samples has the ability to produce aflatoxins when sample humidity and environmental temperature are increased. Aflatoxin B1 was identified by TLC and quantified by HPLC after derivatization with beta-cyclodextrin. The amount of aflatoxin B1 ranged between 0.108 µg/sample (*Chamomillae flos* – S6) and 0.152 µg/sample (*Chamomillae flos* - S11).

Key words: fungi, contamination, medicinal herbs

COMMON JUNIPER (*JUNIPERUS COMMUNIS* L.) IN SLOVAKIA

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Common Juniper (*Juniperus communis* L.) is shrub or small tree, 2-20 ft high. Bark is reddish brown, shredding off in papery peels. Leaves (needle) taper to a spiny tip, in whorls of 3's with 2 white bands above (or 1 white band sometimes divided by a green midrib, broader than green margin). Flowering time is April to June. Fruits are on short stalks; round to broadly oval, bluish black, usually with 3 seeds. The fruit is a berry-like cone which is green the first year and ripens to a bluish-black or dark purple color in the second year. The harvest of juniper fruits has a long tradition in Slovakia. Juniper was used to produce the spirit called "Borovička" in many former historical regions of Slovakia. It was also used to prepare jams, herbal teas and sometimes it was consumed fresh. In the kitchen, it was used as a spice. The knowledge of the chemical composition of the raw material of juniper berries is very important for industrial production of the "Borovička" – alcoholic beverage. In regard to the final quality of this Slovakian national liquor, distiller companies prefer the juniper fruits with the high pinene contents, as donors of aroma, odor, and lower essential oil quantity. Samples of juniper fruits were collected from different locations (20) in Slovakia and the content of essential oil and their GC profiles were determined. The content of essential oils ranged between 0.50 ± 0.10 % and 1.80 ± 0.10 %. The qualitative-quantitative characteristics of the juniper essential oil were presented four main components: α -pinene (29.0 ± 0.68 – 61.0 ± 0.60 %), sabinene (8.00 ± 0.30 – 22.0 ± 0.98 %), myrcene (7.5 ± 0.10 – 16.2 ± 0.66 %) and caryophyllene (3.5 ± 0.30 – 17.5 ± 0.33). Ecological diversity of sites, where juniper occurs, its geological age tertiary resulted in great variability, which is the result of adaptation to the specific conditions (ecotypes, chemo types, varieties, etc.).

Key words: Juniper, GC, chemotypes

*Acknowledgement: This research was supported by the Slovak Research and Development Agency (SRDA), the project: APVV-14-0843: "Research of possibilities of growing juniper (*Juniperus communis* L.) for the production of fruits".*

TARGETED METABOLITE PROFILING TO SUPPORT CHEMICAL AND BIOCHEMICAL CHARACTERISATION OF MEDICINAL AND AROMATIC PLANTS (MAPS)

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Cultivation of medicinal and aromatic plants (MAPs) is of growing interest to guarantee high quality products to be used in different industrial applications (food, beverages, phyto-pharmaceuticals, nutraceuticals, veterinary medicine), but also to protect natural habitats from overharvesting, due to increasing demand from consumers and the industry. This increased interest in MAPs is considerably associated with naturally occurring antioxidants and bioactive metabolites, such as polyphenols and terpenoids. Several MAPs have been extensively studied for their antioxidant activity and many indications/applications are known from traditional medicine, while their content and profile of bioactive metabolites as the main source of known, but also new pharmaceutical activities, is rather poorly described. Chemical analysis of natural product pattern (metabolites; metabolomics) and its formation (pathway; biosynthesis) in plants, especially in non-model or crop plants such as MAPs, is a research field with significant potential for breeders, growers and consumers. Secondary metabolites of MAPs are the most important compounds, known for their antioxidant properties, and are often, if not identified as active principle, used as marker compounds in quality assessment of herbal drugs and related preparations (herbal teas, alcoholic extracts etc.). Therefore, offering an efficient, robust and reliable fast tool to determine these quality features of MAPs, will protect the growers, industrial users and the consumers from possible frauds. Metabolic fingerprints of MAPs, together with knowledge on the underlying biochemical pathways can be applied for the development of new DNA molecular markers. SNPs chip, containing the sequences of key genes involved in the metabolic pathways of interest, could be used in future breeding programs, to screen populations segregating the target trait and to produce a linkage map. Using these tools, improved cultivars of different MAPs can be generated, including a solid quality control throughout the production process.

Key words: metabolite analysis, breeding, MAPs

**AN *IN VITRO* STUDY ON *COMBRETUM*
QUADRANGULARE Kuz., *VERNONIA AMYGDALINA* Del.
AND *CLERODENDRUM INERME* (L.) Gaertn. PLANT
SPECIES FROM THE SOUTH OF VIETNAM FOR α -
-AMYLASE INHIBITORY ACTIVITY**

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The rapid increase of diabetes mellitus worldwide has put enormous burden on the society. Approximately 90% of diabetic patients are type 2. The control of blood glucose levels, not only when fasting but also after eating, must be taken into consideration. The ability of a drug or diet to delay the production or absorption of glucose by inhibiting carbohydrate-hydrolyzing enzymes such as α -amylase and α -glucosidase is one of the therapeutic approaches for decreasing postprandial hyperglycemia. To clarify the indigenous knowledge of use by Vietnamese people in the South, we have carried out an *in vitro* study on *Combretum quadrangulare* Kuz., *Vernonia amygdalina* Del. and *Clerodendrum inerme* (L.) Gaertn. for the α -amylase inhibitory activity. The crude extracts (70% ethanol) and fractions (n-hexane, chloroform, ethyl acetate, n-butanol, water) from the leaves of the above plant species have been prepared and tested. Results showed that all the extracts and fractions exhibited α -amylase inhibitory activity. For the leaves of *Combretum quadrangulare*, the strongest inhibition was attained by the n-hexane fraction with an IC₅₀ value of 81.09 $\mu\text{g mL}^{-1}$. For the leaves of *Vernonia amygdalina* and *Clerodendrum inerme*, the chloroform fractions showed the most powerful with IC₅₀ values of 13.58 $\mu\text{g mL}^{-1}$ and 37.72 $\mu\text{g mL}^{-1}$, respectively. Meanwhile, the IC₅₀ value of acarbose was 214.78 $\mu\text{g mL}^{-1}$. The results suggested that these herbs were potential for the treatment of diabetes as supplements.

Key words: *Combretum quadrangulare* Kuz., *Vernonia amygdalina* Del., *Clerodendrum inerme* (L.) Gaertn., α -amylase inhibition

PRELIMINARY DATA ON THE IMPACT OF *SORBUS AUCUPARIA* L. BARK EXTRACT ON *IN VITRO* CELL VIABILITY

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Polyphenol-rich extracts obtained from different vegetable sources are currently under investigation for their use as alternative therapeutic compounds for different diseases, the most prominent being cancer. Rowan bark extracts were obtained by hydroalcoholic extraction either at room temperature or warm conditions, by reflux, as well as tested on their impact on the viability of normal Vero cells and neoplastic HeLa cells. Assessment of the cell viability was performed by MTT assay in 96 well plates, cells being incubated for 48 hours, with different doses between 100 to 1500 µg/mL equivalent of gallic acid. Impact of the tested polyphenolic phytoextracts on cell viability was dependent by the cell line and the used dose. The reduction of viability 46.4% and 65.2% respectively was registered at doses of 1300 and 1500 µg/mL respectively. The cytotoxic potential was correlated with the polyphenolic content of rowan bark extracts, which depends on the extraction conditions, a higher yield of extraction being registered in the case of extraction for short time (1 hour) in warm conditions, by reflux. The preliminary data confirm the cytotoxic properties of the polyphenolic hydroalcoholic extracts. The further investigations will be employed in order to prove the reproducible character of the cytotoxic effect; establishment of IC₅₀ or IC₉₀ values which highlight the existence of the dose-response relationship; as well as understanding the mechanisms of action by following interaction the polyphenolic phytoextracts with the apoptosis process, cell cycle, healing process, tumor development etc.

Key words: *Sorbus aucuparia* L., bark extracts, viability, cytotoxicity, MTT

Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI – UEFISCDI, project number 188PED/2017, within PNCDI III

PHENOLIC CONTENT AND PROFILE OF THREE *ARTEMISIA* SPECIES FROM REPUBLIC OF MOLDOVA

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The aim of the present study was to assess the phenolic content and profile of *Artemisia* species from Republic of Moldova. We focused on *A. annua* L., *A. absinthium* L. and *A. lerchiana* Weber harvested from field cultures developed at the Botanical Garden (Institute) of the Moldavian Academy of Sciences, Chisinau, R. Moldova. The plant material, consisting of *A. annua* leaves harvested at pre-flowering stage and *A. absinthium* and *A. lerchiana* herba harvested at flowering stage, was extracted with methanol 3 times at 40°C in the ultrasonic bath (40 KHz). Total phenolic content, expressed as mg gallic acid equivalents (GAE) per gram dry extract was determined by Folin-Ciocalteu method and the phenolic profile was achieved by means of HPLC-DAD - identification on the basis of their characteristic UV spectra and comparison with authentic standards. Among the three *Artemisia* species in the study, *A. annua* had the highest content of phenolic compounds (131.10 ± 1.76 - 182.54 ± 2.26 mg GAE/ g dry extract), followed by *A. lerchiana* (114.24 ± 0.00 - 132.06 ± 0.27 mg GAE/ g dry extract) and *A. absinthium* (91.12 ± 1.45 - 99.49 ± 1.19 mg GAE/ g dry extract). The HPLC-DAD analysis identified cynarin as the major phenolic compound in all analysed samples: 7.004 – 21.151 mg/g dry plant material in *A. annua*, 2.400 – 3.895 mg/g in *A. absinthium* and 1.760 – 7.770 mg/g in *A. lerchiana*. Chlorogenic and caffeic acids alongside flavonoids hyperoside, isoquercitrin and luteolin-7-glucoside were identified in the *Artemisia* extracts. Among the three *Artemisia* species, *A. lerchiana* had a more complex flavonoid spectrum. The high phenolic content of these *Artemisia* species suggests their possible use in the food and pharmaceutical industries as an important source of antioxidants.

Key words: *A. annua*, *A. absinthium*, *A. lerchiana*, phytochemical screening

VARIABILITY AND COMPOSITION OF ESSENTIAL OIL CONTENT OF SELECTED SPICES (*OCIMUM BASILICUM* L., *SATUREJA HORTENSIS* L. AND *MAJORANA HORTENSIS* Moench.)

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The thesis Variability and composition of essential oil content of selected spices (*Ocimum basilicum* L., *Satureja hortensis* L. and *Majorana hortensis* Moench.) deals with plant species of whose main ingredient is oil. The process of the isolation of essential oils from plants, individual components of essential oils, their variability and use are described. Selected representatives of the so called green spices belong to the mint family (Lamiaceae), whose typical feature is very high content of essential oils. In the practical part the following species are evaluated: basil (*Ocimum basilicum*), summer savory (*Satureja hortensis* L.) and marjoram (*Origanum majorana* syn. *Majorana hortensis*). Essential oil content was determined in each species by steam distillation and then the composition of essential oils was determined by gas chromatography.

Key words: essential oils, basil, marjoram, savory, steam distillation, gas chromatography

MAIN PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *CARDUUS KERNERI* Simonkai SSP. *AUSTRO-ORIENTALIS* Franco INFLORESCENCES

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Phenolic acid and flavonoid profiles of the Balkan endemic plant species *Carduus kernerii* Simonkai ssp. *austro-orientalis* Franco were investigated for the first time. Ten phenolic acids and eight flavonoids were identified and quantified in the inflorescences, by high performance liquid chromatography. The main flavonoids were found to be: luteolin ($1242.31 \pm 5.22 \mu\text{g g}^{-1} \text{dw}$), apigenin ($714.27 \pm 2.98 \mu\text{g g}^{-1} \text{dw}$), hyperosid ($660.92 \pm 8.0 \mu\text{g g}^{-1} \text{dw}$), and the main phenolic acids were: sinapic acid ($922.78 \pm 3.86 \mu\text{g g}^{-1} \text{dw}$) and chlorogenic acid ($787.32 \pm 3.29 \mu\text{g g}^{-1} \text{dw}$). The antioxidant activity of methanolic extract of inflorescences has been investigated, employing four different established testing systems: scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonate (ABTS) radical cation decolorization assay, ferric reducing antioxidant power (FRAP) and copper reduction antioxidant assays (FRAP). The highest antioxidant activity values ($47.95 \text{ mMTE g}^{-1} \pm 0,12$) were measured by the ABTS assay, among all performed methods.

Key words: *Carduus kernerii*, HPLC, flavonoids, phenolic acids, antioxidant activity

MORPHOLOGIC AND CHEMICAL PROFILE OF TWO *AJUGA* SPECIES CULTIVATED IN NORTHERN ROMANIA

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Ajuga genevensis L. and *Ajuga reptans* L. are two medicinal plants used in Romanian traditional medicine for their anti-inflammatory properties for rheumatism and gout. They are also credited with astringent, tonic and diuretic activities. Although these species are common for the wild flora in our country, our investigation was conducted on specimens harvested in May 2016 from the experimental fields of “Stejarul” Biological Research Centre, Piatra Neamt, Romania. Their introduction in culture as a source of bioactive compounds is intended for pharmaceutical purposes. Therefore, the investigation of their characteristics and benefits is of extreme importance for future use in pharmaceutical preparations and / or food supplements. The aerial parts characteristics were observed with an electronic microscope in several fine coloured sections for each species. Moreover, their chemical profile was assessed on hydro-alcoholic extracts by spectrophotometry and thin layer chromatography techniques. The results confirmed the taxonomic and chemical variability between the investigated samples. The microscopic features (glandular and surface hairs, epidermis structure, the number of and type of stomata) showed similarities and differences at the same time. In terms of compound profile, the polyphenols (flavones and polyphenolic acids) were richer in *Ajuga reptans* (168,3 mg % and 230,4 mg % respectively), whereas *Ajuga genevensis* contained higher quantities of iridoids (1860 mg % as compared to 1250 mg %). Further research is still undergoing to assess the intensity of their biological profile in neuroinflammation model on laboratory rats.

Key words: *Ajuga*, microscopy, chemical profile, polyphenols, iridoids

CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN *HYSSOPUS OFFICINALIS* L. TRADITIONALLY CULTIVATED, *IN VITRO* PROPAGATED AND PLANTS FROM NATURAL HABITATS

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The changes of biologically active substances in methanolic extracts from traditionally cultivated *Hyssopus officinalis* L. plants (from seeds), *in vitro* propagated and adapted under field conditions plants and plants from natural habitats are compared. The content of the investigated non-enzymatic low molecular metabolites – water and lipid soluble metabolites with antioxidant capacity, phenols, flavonoids reached the highest values in the flowers and leaves in the *in vitro* propagated and adapted under field conditions plants. Leaf and flower extracts of *H. officinalis* in all three plant breeding modes showed different antioxidant potential, but the highest values were observed in *in vitro* propagated plants. Differences have been observed between the total content of essential oil and the components of essential oils depending on the way of plants cultivation. Highest content of essential oil in percentage of the drug was measured in plants from natural habitats. Essential oil from plants propagated from seeds have the highest values of the sabinen. Essential oil derived from *in vitro* propagated plants have the highest content of pinocamphon. Oil derived from natural habitats plants have the highest content of β -binen and pinocampen.

Key words: *Hyssopus officinalis*, antioxidant metabolites, essential oil

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INFLUENCE OF MEDICINAL AND AROMATIC PLANTS ON EGG YOLK SENSORY TRAITS IN LAYING HEN

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Many medicinal plants with naturally present pigments have a potential of replacing synthetic pigments used for yolk coloring properties in egg production. Therefore, the aim of this research was to determine the effect of addition of basil (*Ocimum basilicum* L.) (B), pot-marigold (*Calendula officinalis* L.) (C), dandelion (*Taraxacum officinale* Weber ex Wigg.) (D) and marigold (*Tagetes erecta* L.) (M) in diet on egg yolk sensory traits. Control group was fed with a standard commercial diet for layers with synthetic pigments for yolk coloring included. Two levels, 1 and 3% of each plant flower (C, D, M) or herb (B) powder have been added for 4 weeks in trial diets and then sensory analysis of cooked egg yolks was performed. In total, 97 consumers among Faculty of Agriculture staff and students evaluated samples by using 10-point hedonic scales for color, aroma, texture, flavor, off-odors, and overall impression. Differences of median scores between samples were tested using Kruskal-Wallis rank sum test. For traits where significant differences between samples were confirmed, difference of median of each treatment and control group was tested using Steel method. It was found that aroma, texture, off-odors and overall impression were not significantly affected by the addition of medicinal and aromatic plants in feed. Significantly higher rated yolk color score of control group and treatment M at 3% were found when compared to treatments B, C and D at both levels. Flavor scores of all treatments except treatment M at 1% were significantly lower rated when compared to control group. Addition of calendula and dandelion flower powder and basil herb powder in layers feed appeared to be inadequate for egg yolk sensory traits. On the other hand, marigold is found to be a potential replacement for synthetic pigments with emphasis on further research.

Key words: MAPs, sensory traits, egg yolk

CONCERN ON THE PHARMACOPOEIA EXTRACTION PROCEDURE FOR DETERMINATION OF ARBUTIN CONTENT IN BEARBERRY LEAVES

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Arctostaphylos uva-ursi (L.) Spreng., fam. Ericaceae, also known as bearberry or bear's ear, is a small procumbent woody groundcover shrub, widely distributed on a global level and used as urinary tract antiseptic and diuretic, due to the presence of arbutin and hydroquinone. The herb contains hydroquinone glycosides: arbutin (5-16%), methyl arbutin (4%), galloyl derivatives of arbutin (0.05%) and free hydroquinone (<0.3%) as decomposition product of arbutin. The officinal herbal substance consists of whole or cut dried leaves and should not contain less than 7% of anhydrous arbutin according to the European Pharmacopoeia 9.0. The content of arbutin can be determined in plant extracts by spectrophotometry or capillary zone electrophoresis, but HPLC method was found to be the most suitable and included in the Ph. Eur. 9.0 monograph. Several wild growing populations of bearberry from R. Macedonia were assessed on arbutin content and all the results were approximately 50% lower than the required minimum of 7%. According the method described in the Ph. Eur. 9.0., the water extract was prepared from dried bearberry leaves. Extraction procedure comprises two steps water maceration of powdered herbal drug, each of 30 minutes, heated on water bath, under reflux (E1). Apart from this, two independently different extraction procedures were also performed: 60 minutes water maceration on magnetic stirrer on room temperature (E2) or boiling (E3). Obtained results revealed that the content of arbutin is much higher in the extract obtained by E2 but lower in the extract obtained by E3, compared with the pharmacopoeias' method E1. The role of increased extraction temperature is questionable taking into consideration that arbutin has very good solubility in water of 151 g L⁻¹ at room temperature 20 ± 5 °C, as determined using the flask method A6 of Commission Directive 92/69/EEC.

Key words: *Arctostaphylos uva-ursi*, sample-preparation, solubility, arbutin

INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF SOME ANTHOCYANIDIN-RICH EXTRACTS ON CLINICAL ISOLATES OF UPEC

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Uropathogenic *Escherichia coli* (UPEC) is the most common cause of recurring urinary tract infections worldwide. The usual treatment includes antibiotics that stop the multiplication of pathogens. Nevertheless, acquired resistant strains that can adapt easily have spread all over the world due to irrational use of antibiotics, and many pathogens resistant to fluoroquinolones or third-generation cephalosporin were identified in clinical research. Therefore, this study is part of a larger research that aims to identify natural compounds that can be used to prevent or treat urinary tract infections, either on their own or as part of a complex treatment which includes an antibiotic. Anthocyanins are natural compounds well recognized for their health benefits. Several anthocyanin-rich extracts (lyophilized powders from *Aronia melanocarpa* L.) were assessed in terms of their antimicrobial effect on clinical isolates of UPEC. The chemical profile of the extracts was identified by UPLC techniques. The minimal inhibitory concentration (MIC) and minimal biofilm eradication concentration (MBEC) of the extracts against one standard (*E. coli* ATCC) and a few clinical isolated strains (*E. coli* 2041, *E. coli* 1851 and *E. coli* 1992) were determined. The results indicated that the extracts have a good antibacterial activity (5 mg mL⁻¹ MIC or MBEC). Nevertheless, the synergistic effects were modest and a slight increase of the growth inhibition zone diameter for the antibiotic (nitrofurantoin, imipenem and norfloxacin) disks supplemented with the investigated extracts was noted. Further studies are necessary to draw a conclusion regarding synergism between antibiotic and anthocyanidin-rich extracts and its importance.

Key words: UPEC, anthocyanins, *Aronia melanocarpa*, antimicrobial

MICROWAVE AND ULTRASOUND ASSISTED EXTRACTIONS OF POLYPHENOLS FROM DANDELION (*TARAXACUM OFFICINALE* (L.) Weber ex F.H. Wigg) ROOT AND LEAF

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Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) as eco-friendly procedures are widely employed in the analysis and the extraction of bioactive compounds from plants. In comparison to the conventional solvent extraction method, MAE is more advanced as it heats the matrix internally and externally without a thermal gradient thus target compounds can be extracted more effectively and protectively using less energy and solvent. Moreover, the main advantages of using UAE includes more effective mixing of matrix and solvent, faster mass transfer, reduced thermal and concentration gradients, and less energy and solvent consumption. Hence, the aim of this study was to investigate UAE and MAE for isolation of total phenols from dandelion (*Taraxacum officinale* (L.) Weber ex F.H. Wigg) roots and leaves. Several parameters such as ethanol concentrations (50 vs. 70%, v/v), and time (5, 10, 15 min) were studied for both techniques. Moreover, MAE was conducted at various temperature (40, 60, 80°C) while sonication was performed at 20 kHz, at 50% and 100% amplitude of the full power (600 W) in the ambient atmosphere. MAE has given the highest yields for root at 60°C, 15 min, 70% aqueous ethanol (v/v), and for dandelion leaf at 40°C, 10 min and 50% aqueous solution of ethanol (v/v). In addition, high amplitude (100%) and high presence of ethanol in the solvent (70%) under 10 min improved significantly the UAE process of polyphenols recovery from roots, while UAE process with 50% aqueous ethanol (v/v), 50% amplitude in 15 min was found as the optimal for polyphenols recovery from the dandelion leaf. In conclusion, both techniques confirmed suitability for polyphenols recovery from dandelion roots and leaves.

Key words: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), dandelion (*Taraxacum officinale*), polyphenols

EVALUATION OF THE ANTIOXIDANT POTENTIAL OF ALCESEFOLISIDE FROM *ASTRAGALUS MONSPESSULANUS* L. IN *IN VITRO* CELL SYSTEMS

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Astragalus monspessulanus L. (Fabaceae,) is a clump-forming perennial plant, native to the Iberian Peninsula, France, Switzerland, the Apennine Peninsula, the Balkan Peninsula, Eastern Europe, and Northwest Africa. Previous studies showed that a purified saponin fraction, obtained from the plant, possessed cytotoxicity in HepG2 cells and the n-butanol extract of the overground parts exhibited hepatoprotective and antioxidant activities against *in vitro* and *in vivo* CCl₄-induced acute liver damage, comparable to that of silymarin. A new flavonol tetraglycoside, two new flavoalkaloids, and the eight known flavonoids, i.e. alangiflavoside, alcesefoliside, mauritianin, quercetin-3- β -robinobioside, cosmosine, apigenin-4'-O-glucoside, trifolin and rutin were reported from the species. The aim of the current study was to evaluate the effect of the rare flavonoid alcesefoliside (quercetin-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-galactopyranoside), using *in vitro* models of liver injury. In *in vitro* models of CCl₄-induced metabolic bioactivation and t-BuOOH-induced oxidative stress in isolated rat hepatocytes, the flavonoid exerted antioxidant and cytoprotective activity, similar to Silybin, discerned by preserved cell viability and GSH levels as well as by decreased LDH activity and MDA quantity. The effects of alcesefoliside was commensurable with those of Silybin A+B.

Key words: alcesefoliside, *Astragalus monspessulanus*, carbon tetrachloride, hepatotoxicity, oxidative stress, phytochemicals

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CHARACTERIZATION OF INVAZIVE PLANT SPECIES FOR POSSIBLE USE IN PHYTOTHERAPY

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The aim of this study was the research of invasive plant species with possible use in phytotherapy. We chose some plant species that are known to be used as adjuvants / remedies in traditional medicine in Romania. In this study we searched for some solutions to limit the expansion of invasive plant species and also to determine the possible use of their active compounds in phytotherapy. The species were sampled from natural habitats from the eastern area of Transylvania, Romania. Various aspects have been studied, such as: the size of areas with wild populations, the vegetation time, the number of individuals per surface unit, the vegetative propagation capacity and pedoclimatic characteristics. The plant material was phytochemically analyzed for bioproductivity assessment (biomass, active principles). Plant extracts of various types were analyzed by chromatography (TLC, HPLC, GC-MS) and spectrophotometry. The content of active principles with antioxidant activity (polyphenols, flavonoids, triterpenes, essential oil and volatile fractions) was determined. These analyses revealed the presence of variable amounts of active principles, species- and environment-dependent. The presence of the germacren D could explain the invasive capacity of *Impatiens glandulifera* Royle (15.03%) and *Solidago gigantea* Ait. (37.30%). The cytotoxic and genotoxic capacity of germacren D is recognized in various types of essential oils. A feature characteristic of the *Helianthus tuberosus* L. is the presence of polyphenols in all plant organs, with the highest amount found in leaves (1.25 mg gallic acid equivalents per 100 g dried weight). Our research will continue over the next few years to elucidate more aspects of the invasive plant species in Romania.

Key words: invasive plant species, biomass, active principles, phytotherapy

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EVALUATION OF THE EDIBLE FLOWERS OF MEDICINAL PLANTS

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The main aim of this article is to introduce the topic of edible flowers of medicinal plants and to evaluate their chemical substances and favorable effect on people. A broad scale of edible plants and flowers exist and several of them can be regarded as medicinal. Beside the fact, that the edible flowers have been traditionally used in many cuisines around the world, nowadays, their popularity rises because of the knowledge of their nutritional value and beneficial substances. These substances can elicit physiologic and mental responses and prevent and reduce the risk of many diseases. They can e.g. act as antiseptic and antimicrobial agents, positively affect the digestive system, relieve pain, relax spasm, or enhance the immunity system. Forty-one samples of medicinal plants were evaluated. In those samples values of ascorbic acid (AA), total phenolic content (TPC), total flavonoid content (TFC) and the total antioxidant capacity (TAC) were measured. AA was determined by HPLC. The highest amounts were measured in *Primula elatior* Hill. ($2880,70 \pm 50,89$ mg/kg) and the lowest in *Lamium purpureum* L. ($5,10 \pm 3,99$ mg/kg). TPC was determined spectrophotometrically with Folin Ciocalteu reagent. The highest amounts were measured in *Origanum vulgare* L. ($17959,83 \pm 81,68$ mg GAE/kg) and the lowest in *Cucurbita pepo* L. 'Terminator' ($663,21 \pm 9,43$ mg GAE/kg). TFC was determined spectrophotometrically with sodium chloride and aluminium nitride. The highest amounts were measured in *Origanum vulgare* L. ($72,452 \pm 1,417$ mM KAT/kg) and the lowest in *Centaurea cyanus* L. ($0,737 \pm 0,000$ mM KAT/kg). TAC was determined by the DPPH method. The highest amounts were measured in *Rehmannia glutinosa* (Gaertn.) Steud ($18,147 \pm 1,221$ mM TE/kg) and the lowest in ray flowers of *Cucurbita maxima* Duchesne 'Halloween' ($1,658 \pm 0,000$ mM TE/kg). The results prove, that the edible flowers of medicinal plants are worth for further cultivation.

Key words: edible flowers, TAC, TPC, TFC, AA

COMPARISON OF PHENOLIC COMPOSITION AND BIOLOGICAL ACTIVITY OF COMMERCIALY AVAILABLE WHITE WINES ORIGINATING FROM FRUŠKA GORA, SERBIA

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Wine is one of the oldest and most popular alcoholic beverages. Besides pleasure, numerous reports confirm that drinking wine has a beneficial role in preventing conditions that are considered to be triggered by oxidative stress, like cardiovascular and neurodegenerative diseases, cancer, inflammation and ageing. The quality of wine can be estimated by analysing wine phenolics that not only affect organoleptic properties but are also mainly responsible for the exerted health benefits thanks to their strong antioxidant activity. In this study, the phenolic profile and biological activity of five commercial white wines from Serbia have been determined. A quantitative analysis of 41 phenolic compounds was performed using the LC-MS/MS technique. From 21 detected compounds, phenolic acids such as 2,5-dihydroxybenzoic, p-coumaric and caffeic acid were most abundant in the tested samples. The antioxidant activity was evaluated spectrophotometrically by measuring the reducing power and free radical scavenging ability of samples towards diphenylpicrylhydrazyl (DPPH) and nitric oxide radicals, and by inhibiting lipid peroxidation, while the neuroprotective effect was estimated through a potential inhibition of acetylcholinesterase. Even though all white wines failed to inhibit lipid peroxidation, in other performed assays, the samples exhibited moderate to good activity. Fruška Gora is a famous winery region in Serbia. However, not many studies have focused on wines originating from this area. Thus, in this study novel data was collected, focusing on the determination of phenolic profile and biological activity of Serbian commercial white wines, distinguishing them as a promising source of natural antioxidants and neuroprotective agents.

Key words: wine, phenolic profile, caffeic acid, lipid peroxidation, acetylcholinesterase

ANTINOCICEPTIVE ACTIVITY OF METHANOLIC LEAVES EXTRACT OF *CRATEVA ADANSONII* DC. IN ALBINO RAT

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Pain is the most common reason for physician consultation in most developed countries, but treatment is becoming complex because of the side effects of analgesic pharmaceutical drugs. This has led to an increase in the used of medicinal plants for pain and pain related conditions. Previous studies have demonstrated that *Crateva adansonii* DC. stem-bark produced analgesic and anti-inflammatory effect, but no data are available concerning the antinociceptive effect on the leaves. This study was aimed at investigating the antinociceptive effect of the methanolic leaves extract of *C. adansonii* using tail-flick and acetic acid-induced writhing methods in albino rat. The results showed that the pain reaction time (tail withdrawal) following administration of *C. adansonii* leaves was significantly increased ($p < 0.05$) difference in the pain reaction time and number of writhing respectively compared to the standard drug aspirin treated rats. In conclusion, *C. adansonii* leaves exhibited antinociceptive activity against central and peripheral mediated pain sensation. This further justifies the folkloric claim of this plant in pain treatment.

Key words: acetic acid, antinociceptive, Aspirin, *Crateva adansonii*, tail flick

EXTRACTION OF PYRETHRINS FROM DALMATIAN PYRETHRUM (*TANACETUM CINERARIIFOLIUM* /Trevir./ Sch. Bip.) BY MATRIX-SOLID PHASE DISPERSION

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Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir. /Sch. Bip.) is a plant species of the Asteraceae family, restricted to the eastern coast of the Adriatic Sea. It is a perennial plant species known as the source of natural insecticide pyrethrin. The term pyrethrin refers to the six insecticide active components: pyrethrin I and II, cinerin I and II, jasmolin I and II. Insecticidal potential of Dalmatian pyrethrum is measured by the content and composition of pyrethrins which are mainly concentrated in the flower heads. In order to obtain deeper knowledge of its insecticidal potential, content and composition of pyrethrins was assessed by matrix-solid phase extraction (MSPD). The preliminary experiments were carried out to optimize the main parameters affecting MSPD, such as the type of sorbent and the solvent polarity. Results showed that pyrethrins I and pyrethrins II were successfully extracted by florisil as a sorbent using 10 mL of acetone : ethyl-acetate = 1:1 (v/v) as an elution solvent. All experiments were performed using 1:2 ratio of flower heads and sorbent. Extraction efficiency was determined by high performance liquid chromatography with diode array detector (HPLC-DAD). Separation and quantification of the selected components were carried out under gradient elution of mobile phase (0.1% formic acid in water and 0.1% formic acid in acetonitrile) based on Luna C18(2) column, 250 mm x 4.6 mm, particle size 5 µm (Phenomenex).

Key words: pyrethrins, matrix-solid phase dispersion, high performance liquid chromatography

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EVALUATION OF *GLYCYRRHIZA* L. GENETIC RESOURCES IN THE CZECH REPUBLIC

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The aim of this study is to describe and evaluate the Czech collection of genetic resource of licorice (*Glycyrrhiza* L., Fabaceae). Roots and stolons are frequently used in pharmacy and food industry. Since 1995, genetic resources of the genus *Glycyrrhiza* L. have been evaluated at MENDELU in Brno, respectively Faculty of Horticulture in Lednice. The genus *Glycyrrhiza* L. consists of about 30 species and among them the following species were evaluated: *Glycyrrhiza glabra* L., *Glycyrrhiza echinata* L., *Glycyrrhiza pallidiflora* Maxim, *Glycyrrhiza macedonica* Bois. Et Orph., *Glycyrrhiza foetida* Desf. and *Glycyrrhiza uralensis* Fisch. The descriptor list for *Glycyrrhiza* L. includes 54 descriptors and also a content of glycyrrhizic acid (glycyrrhizin). Glycyrrhizic acid was measured by the HPLC according to the Czech Pharmacopeia 2009 (*Liquiriteae radix*). The highest content (2.67%) of glycyrrhizic acid among all analyzed taxa was measured in *Glycyrrhiza glabra* L. and the lowest content (0.05%) was measured in *Glycyrrhiza echinata* L. Finally, the results of accessions evaluation have been transformed to a scale according to the Descriptor list. Based on our evaluation we recommend the accession No 42A4400011 - *Glycyrrhiza glabra* L. for further breeding and cultivation.

Key words: genetic resources, *Glycyrrhiza* L., glycyrrhizin, HPLC, Czech Republic

**PHENOLIC PROFILE, ANTIOXIDANT,
ACETYLCHOLINESTERASE AND TYROSINASE
ENZYME INHIBITORY ACTIVITIES OF ENDEMIC
JURINEA TZAR-FERDINANDII Davidov**

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Jurinea tzar-ferdinandii Davidov (genus *Jurinea*, Asteraceae family) is a Balkan endemic plant with limited distribution in Bulgaria and Romania. Recently, we have started phytochemical investigation of this species. From the chloroform extract obtained from the aerial parts of *J. tzar-ferdinandii* were isolated and identified 26 compounds, belonging to three main groups of secondary metabolites: triterpenoids, sesquiterpene lactones and nonpolar flavonoids. Continuing our research on this species, we have focused our attention on the phenolic constituents, antioxidant and enzyme inhibitory activities of the methanol extract. CC and PTLC of the methanol extract obtained from aerial parts led to isolation and identification of apigenin, luteolin, apigenin-7-O-glucoside, apigenin-4'-O-glucoside, apigenin-7-O-gentiobioside, luteolin-4'-O-glucoside, rutin and narcissin, chlorogenic acid and 1,5-dicaffeoylquinic acid. Structures of all compounds were elucidated using spectral methods (NMR, MS and UV). Antioxidant activity of the extract was tested against ABTS^{•+} radical cations using TEAC (Trolox equivalent antioxidant capacity) assay. Acetylcholinesterase and tyrosinase inhibitions of the sample were evaluated using Ellman's method and the microtiter plate assay reported by Masuda, respectively. The measured TEAC value of the extract was 0.259 ± 0.008 mg mL⁻¹. The methanol extract demonstrated weak anti-AChE activity (8.3 Inh %) and low inhibitory potential against tyrosinase enzyme (IC₅₀ 0.208 ± 0.008 mg mL⁻¹). The present work is the first report on polar phenolic constituents and biological activity of the *J. tzar-ferdinandii* methanol extract.

Key words: *Jurinea tzar-ferdinandii*, flavonoids, phenolic acids, biological activity

CHITOSAN-PECTINE MICROPARTICLES LOADED WITH SAGE EXTRACT FOR EXTENDING SHELL-LIFE OF SAUSAGES

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An expanding tendency of global scale is the commitment of food processors and consumers to reduce the use of synthetic chemicals in food preservation. Recently, there has been a considerable interest in some common culinary herbs representatives (sage, oregano, etc.) with high polyphenolic content and volatile compounds with well-established antimicrobial and antioxidant potency directly to be added to food to improve its taste, smell and other organoleptic properties, to increase the freshness of products and at least to preserve the products in a proper way. However, these days numerous efforts have been made to find alternative solutions (innovative formulations and technological processes) to the aim of avoiding undesirable inactivation, and adulteration of the smell and taste. By applying the spray drying method, sage (*Salvia officinalis* L.) extract was successfully encapsulated into biodegradable chitosan-pectin microparticles (CTP-SE) and physicochemical characterization of the system was performed. TEM images showed that the particles were with spherical morphology and relatively smooth surfaces with a low porosity and uniform size. The mean size of the CTP-SE was from 9.21 to 9.45 μm , with unimodal narrow size distribution. Obtained FTIR spectra suggested possible interaction between the chitosan as coating polymer and rosmarinic acid. High encapsulation efficiency of the active principle was obtained (98.84%). Complete release of rosmarinic acid from the CTP-SE was achieved within 3 h. Dissolution data modeling best fitted to Korsmeyer-Peppas and the Higuchi kinetics. Disc diffusion and disk dilution methods were used in order to investigate the antimicrobial activity. Generally, better antimicrobial activity was observed towards Gram-positive than Gram-negative bacteria. The antioxidant activity was assessed by two methods (β -carotene/linoleic acid and thiobarbituric acid reactive substances assay). CTP-SE showed higher antioxidant capacity compared to ascorbic acid (reference standard substance), but rather moderate activity compared to butylated hydroxyanisole.

Key words: *Salvia officinalis* L., food preservative, biodegradable packing

NUTRACEUTICALS BASED ON BIOACTIVE PRINCIPLES OF PLANT ORIGIN

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“Anca Farm”, a Pharmacy chain in Botosani, located in the North-West part of Moldova Region (Romania), organized in 4 pharmacies, provides quality pharmaceutical services to patients and customers by the continuous research activity of Manager Pharm. Anca Boldea (PhD in Pharmacy) is focused on formulating new products for improving life quality. New formulations for internal (granules, capsules, syrup) and external (stick, essential oil) use were exclusively prepared by Anca Pharmacy. These are based on different mixtures of standardized essential oils (each purchased from manufacturers), e.g. tea tree, *Eucalyptus radiata* A. Cunn. ex DC., *Commiphora myrrha* (Nees) Engl., *Thymus vulgaris* L. ct. thujanol, *Cinnamomum camphora* (L.) J. Presl., *Hyssopus* sp., *Satureja montana* L., *Calophyllum inophyllum* L., *Lavandula spica* L., *Nigella sativa* L., *Abies alba* Mill., *Salvia officinalis* L., *Citrus*, *Laurus nobilis* L., *Melaleuca viridiflora* Sol. ex Gaertn., *Origanum compactum* Benth, *Rosmarinus* ct verbenone. Some formulations also contain different hydroalcoholic extracts from *Echinacea*, *Astragalus*, *Sambucus*. Due to their composition, these formulations claim to be used in phytotherapy to strengthen the immune system (immune-modulators and immune-stimulants) in common cold and flu, respiratory infections caused by different pathogens (rhino pharyngitis, bronchitis, salpingitis, pneumonia, otitis etc.), as natural antibiotics and disinfectant, antiseptic, antibacterial, antifungic, antiviral, anti-inflammatory, antitussive, anti-catarrrhal, mucolytic and antipyretic agents. These formulations were analysed by “Stejarul” BRC, using GC-MS, to evaluate the volatile fractions; it was performed with an Agilent 6890N gas chromatography instrument coupled to an Agilent 5975 mass spectrometer. A capillary column (30 m × 0.25 mm i.d.; 0.25 µm, DB-5) was used for separation. The other GC-MS conditions (inlet mode, injection temperature, separation temperature program, scan range) were optimized. The spectrometer was operated in electron-impact (EI) mode; the quadrupole and ionization source temperature were 200 and 250°C, respectively. The new formulations with standardized essential oils are characterized by the dominance of volatile fractions with significant pharmacological activities.

Key words: nutraceuticals, immunity, essential oils, gas-chromatography, phytotherapy

CARROT JUICE AS FUNCTIONAL FOOD OF PLANT ORIGIN

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Carrot is important vegetable crop that is grown for roots that can be differently coloured. It belongs to group of functional foodstuffs of plant origin. It is rich in minerals, vitamins and other phytonutrients and has beneficial effect on human health. Carrot is juicy vegetable and it is often use as a part of different juice mixtures and baby food. Sugar content and pH of juice play important role in taste of juice, as well as in alkalization of the human body. Although the sugar content in carrot is higher than the most other vegetables, it has low caloric value. The aim of this study was to determine and compare the pH and sugar content in juice of orange coloured carrot roots. Sampling was performed in a triplicate in the city of Zagreb in three selling channels (5 samples in trade chains, 5 samples in markets and 5 samples in stores of ecological products). The average pH value of carrot juice according to selling channel ranged from 6.55 to 6.69. Statistically the highest sugar content was determined in carrot juice from stores of ecological products (8.54% Brix).

Key words: Brix, juice pH, sugar, vegetable

IN VITRO ASSESSMENT OF TWO SPECIES OF THE GENUS *PINUS* GROWING IN ALGERIA FOR THEIR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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A comparative study of volatile compounds from *Pinus pinea* L. and *Pinus pinaster* Aiton obtained by hydrodistillation extraction by GC and gas chromatography mass spectroscopy (GCMS) was conducted. The collective essential oil of *P. pinea* was rich in limonene (56.5 %), followed by α -pinene (6.5 %) and β -phellandrene (6.2 %). The major compounds from the collective essential oil of *P. pinaster* were: α -pinene (36.4 %), β -pinene (12.3 %) and (E)- β -caryophyllene (7.1 %). The results showed that the oils have a great potential as antibiotics against some microorganisms: *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Candida albicans*. The maximum zone of inhibition of *P. pinea* was obtained against *Candida albicans* (23 mm). Antioxidant capacity was assessed by *in vitro* testing using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and significant activity was found for the various oils. These results suggested that essential oils of *P. pinea* and *P. pinaster* processes antimicrobial and antioxidant properties and are therefore a potential source of active ingredients for food and pharmaceutical industry.

Key words: Pinaceae, essential oils, GC, GCMS, biological activities

INFLUENCE OF HARVEST TIME AND GROWING LOCATION ON THE PHENOLICS AND ANTIOXIDANT ACTIVITY OF MASTIC TREE LEAVES

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The objective of this study was to evaluate the effect of growing location and harvest time on total phenolics (TP), flavonoids (TF), hydroxycinnamic acids (THCA) and flavonols (TFlav) of mastic tree (*Pistacia lentiscus* Linn.) leaves as well as on its antioxidant activity (AA). Leaves were sampled at four different growing locations (Barbariga / Lun / Hvar / Korčula) at three harvest periods (May / August / October 2017). Quantitative determinations of phenolics were conducted spectrophotometrically and AA by ferric reducing antioxidant power method (FRAP). Our findings suggest that phenolic content and AA varied according to growing location and harvest time. The mean TP content ranged from 63.14 to 104.02 mg g⁻¹, TF from 3.81 to 16.49 mg g⁻¹, THCA from 4.87 to 10.19 mg g⁻¹ and TFlav from 4.41 to 9.09 mg g⁻¹. The highest TP, THCA and TFlav content was determined in Mastic tree leaves harvested in May at Korčula but also almost all analysed parameters were the lowest in leaves harvested in August at same growing location. The same trend was observed in samples from Lun and Barbariga but harvested in August and October, indicating decrease in phenolic content and antioxidant activity of mastic tree leaves at later harvesting periods. ANOVA showed a significant effect of growing location on all phenolics while harvest time affected more TF content and AA. The highest (444.30 mg g⁻¹) and the lowest (209.50 mg g⁻¹) AA was determined in leaves from Barbariga (Istria) harvested in August and October, respectively.

Key words: antioxidant activity, harvest time, growing location, mastic tree leaf, phenolics

ANTIOXIDATIVE POTENTIAL OF MACEDONIAN PINE

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The importance of oxidation in the body has been widely recognized as oxidative metabolism is essential for the survival of cells. A side effect of this dependence is the production of free radicals and other reactive oxygen species that cause oxidative changes as well as destructive and lethal cellular effects by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down the cellular respiration. The only defense mechanism against the effects of excessive oxidations is provided by the action of various antioxidants. Although it is well known that some of pine species are characterized by significant antioxidant activity, very little is known about the antioxidative potential of endemic Macedonian pine (MP), *Pinus peuce* Griseb., Pinaceae. In this order, antioxidative activity of MP's essential oil and methanol extracts of needles and bark has been investigated by DPPH, TBARS and FRAP assay. Methanol extracts of MP's needles have showed considerable radical scavenging activity in DPPH assay as the percentage of inhibition and IC50 values ranged from 87.44-98.57% and from 4.80-20.30 mg mL⁻¹, respectively. The estimated values for percentage of inhibition and IC50 for the same extract in TBARS and FRAP assay ranged from 81.77-94.77% and 4.64-17.49 mg mL⁻¹ for TBARS and from 96.89-97.01% and 4.97-6.37 mg mL⁻¹ for FRAP assay, respectively. MP's bark methanol extracts have showed significant radical scavenging activity in DPPH assay as the percentage of inhibition ranged from 88.89-91.43% while the IC50 values ranged from 7.96-11.75 mg mL⁻¹. The percentage of inhibition and IC50 values for these extract in TBARS and FRAP assay were from 85.62-89.55% and 7.49-11.46 mg mL⁻¹ for TBARS and from 88.21- 90.25% and 8.45-11.03 mg mL⁻¹ for FRAP assay, respectively. The same parameters for MP's essential oil were from 71.61-76.01% and 2.08-2.37 mg mL⁻¹ for DPPH, from 70.97-76.02% and 2.03-2.24 mg mL⁻¹ for TBARS and 77.99% and 3.12 mg mL⁻¹ for FRAP assay, respectively.

Key words: *Pinus peuce*, essential oil, extract, bark, needles

***PELARGONIUM RADENS* H. E. Moore:
PHYTOCHEMICAL PROFILE AND *IN VITRO*
ANTIOXIDANT ACTIVITY**

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Pelargonium radens H. E. Moore is a species that belongs to Pelargonium family and represents a taxonomic importance for its class. Nowadays, its extracts have great importance for perfume industry. The macroscopical and organoleptic analysis indicated that crushing the leaves allows a stronger citrus fragrance to release, probably due to glandular hairs structure alteration. The chemical characterization of the hydroalcoholic extract from leaves was obtained by UHPLC technique. The chemical profile included: rutoside (3.325 $\mu\text{g}/\text{mg}$), cinaroside (0.017 $\mu\text{g}/\text{mg}$), cvercetol-3-arabinoside (10.301 $\mu\text{g}/\text{mg}$) and kaempferol (0.147 $\mu\text{g}/\text{mg}$); also, catechin (8.041 $\mu\text{g}/\text{mg}$) and epicatechin (1.487 $\mu\text{g}/\text{mg}$) and polyphenolic derivatives mainly caffeic acid (2.263 $\mu\text{g}/\text{mg}$). The biological profile of the investigated extract showed a good antioxidant activity against the DPPH and ABTS radicals. Moreover, a strong scavenger potential was noted against superoxide anion. For the proper assessment of the antioxidant potential we calculated the efficient concentration 50% for each assay. The results indicated that the extract was most active against ABTS ($21.63 \pm 0.78 \mu\text{g mL}^{-1}$) followed by DPPH ($98.97 \pm 2.38 \mu\text{g mL}^{-1}$) and superoxide anion ($149.85 \pm 0.34 \mu\text{g mL}^{-1}$). The concentration of the active compounds and their reducing character has a great impact in the actual antioxidant activity of plant extracts. Most commonly plants create such secondary metabolites to adapt and survive the environmental changing conditions. We can only benefit as humans by using high quality plant material.

Key words: *Pelargonium*, antioxidant, polyphenols

BUNIAS ERUCAGO L. : CHEMISTRY AND BIOLOGICAL ACTIVITY

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Volatile sulfur compounds are found in many species of 43 plant families and 173 plant genera, including the most important family, Brassicaceae. Glucosinolates, β -thioglucoside-N-hydroxysulfates with variable side chains are secondary metabolites identified in over 16 different plant families. These compounds are found particularly in the Brassicaceae family. *Bunias erucago* L. (Corn Rocket, Crested warty cabbage) is wild-growing plant in Southern Europe. Leaves, young stems as well as roots are edible parts eaten raw or cooked. "Pazija", (a Turkish name for beet; Beta) or "pakoleć" is traditional food prepared as a mixture of wild vegetables boiled in a mixture where the dominant species is *B. erucago*. Samples of *B. erucago* were collected from two different locations: Marjan hill (Split) and island of Brač (Škrip). The glucosinolate identification was carried out indirectly by their degradation, volatile products were obtained by hydrodistillation and organic solvent extraction, mostly isothiocyanates and nitriles using GC-MS technique. Analysis of *B. erucago* from island of Brač has shown the presence of glucotropaeoline and glucodehydroerucin while in the plant collected on Marjan hill five types of glucosinolates have been identified: gluconapin, glucotropaeolin, heptyl glucosinolate, glucodehydroerucin and glucoerucin. Volatile sulfur isolates were also tested for their antioxidative, anticancer, and cholinesterase inhibitory activities.

Key words: *Bunias erucago* L., glucosinolates, GC-MS, biological activity

III. CULTIVATION, BREEDING AND BIOTECHNOLOGY OF MEDICINAL AND AROMATIC PLANTS

METABOLOMICS, PLANT BIOTECHNOLOGY AND LEAD FINDING: PERFECT HOLISTIC MATCH?

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Per definition metabolomics represents a comprehensive holistic approach, comprising of systematic identification and quantification of all metabolites in an organism, at given conditions. The comprehensive analysis of the chemical fingerprints left by metabolic processes started to play a crucial role in the personalized medicine. Since the rise of the omics age several platforms for high throughput analyses of targeted metabolites have been developed. Nuclear magnetic resonance (NMR) appears very suitable and adequate platform to carry out metabolomics analyses, as it allows simultaneous detection of diverse range of abundant (primary and secondary) metabolites, which opens novel avenues to fully explore the total biochemical machinery of plants. A great advantage of ¹H NMR-spectrometry over the other analytical platforms is the possibility for quantification and hence the direct comparison of concentrations of all compounds present in the sample. Some case studies, from author's laboratory, on the application of NMR-based metabolomics concept in natural products research, plant biotechnology and lead finding will be thoroughly presented and discussed.

Key words: NMR, natural products, sustainability, lead finding

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CHOOSING THE RIGHT APPROACH FOR CONDUCTING MEDICINAL PLANTS CULTIVATION TRIAL

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Pioneer attempts in the cultivation of wild plants as well as improving the genetic base or agro-technology, can often be unsuccessful or misleading unless researchers consider some of the basic prerequisites for cultivation experiment. Essentially the idea for cultivation should be generated from market demand in the way that researcher should be confident that results of the experiment aiming to solve some of the long-term problems. Endangered medicinal plant species that are traded in tonal quantities are usually the best starting point for narrowing the choice of the plant of interest. Sometimes the length of a project that provides funding is a limiting factor in selecting a plant species for research. Moreover, the necessity of rapid scientific publications could also favour annual against perennial plants. Regulations concerning the introduction of allochthonous plant species are sometimes very complicated and difficult to circumvent even in very early experimental phases. Placing wild plants in an artificially and ecologically disturbed assembly such as field trial requires a lot of careful planning. Some basic knowledge about plant ecological parameters such as soil type, soil acidity, insolation and water requirements prior to trial conduction would be advisable. Experimental design and introduction of factors in the field trial is often very complex task, whereby the feasibility of post-harvest chemical profiling should be taken into account. Labor consumption in weeding and harvesting could limit the number of plants and dimensions of the trial, and therefore it should be carefully considered before trial conduction. Statistical analysis of obtained results is usually very challenging task and some basic parameters such as sample size, repetitions and statistical power should be also considered prior to trial conduction. Different approaches could be applied in dealing with outliers, population distributions and statistical tests. Moreover, multivariate statistics could reveal some hidden relations among observed parameters. The dissemination of key results can be presented on modern and more informative graphs using available statistical software packages.

Key words: cultivation, dissemination, experimental design, medicinal plants.

EFFECT OF IRRIGATION ON THE PRODUCTION AND SECONDARY METABOLITES OF SUMMER SAVORY (*SATUREJA HORTENSIS* L. 'BUDAKALÁSZI')

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As consequence of the predicted climatic changes analysis of the effects of drought stress on different plant species seems to be essential. In our study summer savory (*Satureja hortensis* L. 'Budakalászi') was investigated in an open field experiment in the Experimental and Research Farm of the Szent István University in Soroksár. To identify the effect of water supply, irrigated (W) (additional 2 × 20 mm water per week) and non-irrigated control (C) treatments were applied on the plants with 50 x 30 cm growth distance. During the vegetation period water potential (pressure chamber) and the chlorophyll content (SPAD-502) were measured. Fresh-, dry mass and leaf ratio was determined when harvesting plants at full flowering stage. The essential oil content was measured with a Clevenger-type apparatus according to the Hungarian Pharmacopoeia (7th ed.). The essential oil composition was identified by GC-MS. The effect of the irrigation was obvious to most of the examined traits. The chlorophyll content decreased (W: 32.58 SPAD unit; C: 35.70 SPAD unit) while the water potential increased (W: -12.85 bar; C: -21.35 bar) significantly with water supply. The fresh mass (W: 104.00 g plant⁻¹) and dry mass (W: 14.09 g plant⁻¹) of the watered plants were higher compared to the untreated control (fresh: 60.55 g plant⁻¹; dry: 6.58 g plant⁻¹). The leaf ratio did not change significantly (W: 51.04%; C: 48.40%). The essential oil composition of savory seems to be independent from the water supply. The main components of the essential oil were p-cymene (3 - 4%), γ -terpinene (35 - 36%) and carvacrol (50 - 51%) in both oils. Irrigation decreased the accumulation level of the essential oil (W: 4.289 mL 100g⁻¹ dm.; C: 4.859 mL 100g⁻¹ dm.). However, due to higher biomass, the essential oil yield of well-watered plants is higher. Based on this information we may declare that during the cultivation of savory, additional irrigation seems to be necessary.

Key words: savory, stress, drought, water supply, irrigation

IMPACT OF NITROGEN FERTILIZATION AND SOWING NORMS ON CHLOROPHYLL FLUORESCENCE OF FENUGREEK (*TRIGONELLA FOENUM-GRACEUM* L.) GENOTYPES

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Fenugreek (*Trigonella foenum-graceum* L.) is an important spice plant with valuable therapeutic and medicinal properties. In traditional medicine, it is especially used for diabetes, anemia, and treatment of respiratory disorders. In Turkey it is widely used among people due to its effects as expectorant, chest emollient and laxative. The fundamental mechanisms in photosynthesis play a critical role in understanding the reactions of plants to environmental changes and ecological diversity. Chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to learn about Photosystem II (PSII). In this context, the maximum quantum yield of PSII photochemistry (Fv/Fm) has been investigated as basic parameter in chlorophyll fluorescence studies. This study was conducted to investigate the effects of different nitrogen fertilizer rates (0, 20, 40, 60 and 80 kg ha⁻¹) and sowing norms (20, 40, 60 kg ha⁻¹) on the chlorophyll fluorescence of fenugreek (Turkey and Iran genotypes) grown at Erzurum, Turkey in 2016 and 2017. The results of this study showed that the Iranian genotype gave higher maximum quantum yield of the PSII photochemistry than the Turkish genotype. The lowest maximum quantum yield of PSII photochemistry (Fv/Fm) value was obtained from the combination of the sowing rate of 60 kg ha⁻¹ and the unfertilized control treatment. The maximum quantum yield of PSII photochemistry (Fv/Fm) values was observed to be reduced at low nitrogen and high sowing norms. The decrease in this value is associated with the exposure of plants to stress, indicating that most of the captured light is not used in photosynthesis. In conclusion, this study clearly suggests that the maximum quantum yield of PSII photosynthesis in fenugreek is significantly influenced by genotype differences, nitrogen stress and plant density.

Key words: fenugreek, nitrogen fertilization, sowing norms, chlorophyll fluorescence

THE SUCCESSFUL BREEDING WORK OF MEDICINAL PLANTS IN SLOVAKIA

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The large-scale cultivation of medicinal, aromatic and spice plants in Slovakia belongs to the special agricultural production. It is the only way of supplying the contracted volume and quality of these crops. The current state of medicinal, aromatic and spice plants cultivation in Slovakia, shows a partial increase of growing areas on arable land [ha], total production [kg] and yield per hectare [kg. ha⁻¹]. Presented data were obtained directly from the producers from the period of 2010 to 2017. The data shows that milk thistle (*Silybum marianum* /L./ Gaertn), plantain (*Plantago lanceolata* L.), german chamomile (*Matricaria recutita* L.), mint (*Mentha ×piperita* L.), and lavender (*Lavandula angustifolia* L.) are the most cultivated medicinal plants from the acreage of arable land viewpoint. Slovakia is one of the European countries in which particular attention has been devoted to the research of medicinal, aromatic and spice plants in all its aspects, including breeding and selection. Based on the study of pharmacodynamics properties of several medicinal crops, the chamomile variety “Lianka” and the peppermint variety “Kristinka” were bred at the University of Presov, Slovakia, between the years 2008 – 2013. Currently, both varieties have the certificates by the Community Plant Variety Office in Angers, France. The chamomile variety is characterized by its high percentage of sesquiterpenes (1- α -Bisabolol [52 – 55 %], Chamazulene [18 – 19 %], the low contents of 1- α Bisabololoxides A and B [

Key words: new variety, chamomile, mint

DRYING PERFORMANCE OF AMARANTH LEAVES (*AMARANTHUS* spp.) USING AN INFLATABLE SOLAR DRYER

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The potential of amaranth leaves (*Amaranthus* spp.) for its nutritional components used in medicinal applications is gaining attention. However, current drying practices of the blanched leaves has become a challenge for processors due to its exposure to contamination and high moisture content. The performance of a mobile inflatable solar dryer (ISD) was evaluated and compared to a cabinet dryer and traditional open sun drying in Kenya, Africa. The quality of the leaves from the drying treatments was determined. The textural alteration of the leaves and the ambient condition during drying was observed. Normal operational range of solar radiation during good drying practice was recorded between 510 and 950 W/m². The highest operating temperature in the ISD was 69.4 °C, while at the same time the temperature of ambient, in the cabinet dryer and under open sun was 28.8, 53.2 and 53.9 °C, respectively. During night, the ambient temperature and relative humidity could reach 14 °C and 100%. No substantial weight change of material was identified during the night, when all dryers were switched off and covered. The leaves dried in ISD showed higher folic acid than the other treatments. The application of ISD showed potential to prevent quality loss and provided protection against contamination like dust and animals.

Key words: solar drying, leaf drying, solar bubble dryer, plant

ASSESSMENT OF THE PROFILE AND ACCUMULATION DYNAMICS OF SECONDARY METABOLITES IN FERTILIZED ORNAMENTAL ASTERACEAE

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The ornamental species represent an interesting potential source of secondary metabolites for pharmaceutical and cosmetic industries. Our research included Asteraceae species: *Chrysanthemum indicum* L., *Rudbeckia hirta* L., *Echinacea purpurea* (L.) Moench, *Tagetes erecta* L., *Carthamus tinctorius* L. and *Calendula officinalis* L. The profile of polyphenolcarboxylic and flavonoid acids included both common and species-specific compounds. The content of the secondary metabolites varied depending on species, organ, phenophase and fertilization. The results indicated that the studied floral species are a potent source of antioxidants. Under the influence of fertilization, the studied species can gain or lose flavonoids and polyphenolcarboxylic acids or may present quantitative differences in biosynthetic capacity for the phenolic derivatives. Moreover, concentrations of total polyphenols were negatively correlated with branching and biomass in annual plants. In conclusion, for such species intended for pharmaceutical use fertilization should be taken into account only when its impact is positive on the secondary metabolites concentration.

Key words: fertilization, secondary metabolites, Asteraceae

TWO NEW CHEMOTYPES OF BALM (*MELISSA OFFICINALIS* L.)

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Lemon balm (*Melissa officinalis* L.) is a well-known aromatic and medicinal plant. Due to proven sedative, spasmolytic and antiviral effects, it is often used in watery or alcoholic extracts for self-medication or pharmaceutical and medical purposes. The therapeutic effect is caused by essential oil and rosmarinic acid, a phenolic acid derivate. For medicinal use appropriate levels of essential oil with lemon fragrance and rosmarinic acid are required. In this study, accessions fulfilling these requirements were called lemon balm, others simply balm. Three sets of in total 120 balm and lemon balm accessions were evaluated for the variability of essential oil content and composition. In addition, ploidy was determined for all accessions, received from LfL, IPK and VIR. For analysis of essential oil distillation, and gas chromatography (GC) was conducted, as well as extraction as method adapted for small amounts of material. All grown lemon balm accessions belong to diploid *M. officinalis* ssp. *officinalis* and have $2n = 2x = 32$ chromosomes. Developed autotetraploid material from lemon balm also displayed the citral chemotype. In contrast the tetraploid ssp. *altissima* (Sibth. & Sm.) Arcangeli has $2n = 4x = 64$ chromosomes and no lemon fragrance. Kittler et al. (2015) also mentioned triploid accessions ($2n = 3x = 48$), which are genetically stable but sterile and also have no lemon-like scent. The evaluation revealed two new chemotypes (ct.), which are germacrene D ct. and a β -caryophyllene oxide ct. In addition, diverging amounts of essential oil content were observed providing genetic material for future breeding progress.

Key words: Lemon balm, essential oil, chromosome number, evaluation, chemotype

INITIATION OF *IN VITRO* CLONES OF *TANACETUM CINERARIIFOLIUM* Trevir. Sch. Bip. (ASTERACEAE)

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Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip. (*Chrysanthemum cinerariifolium*) is a perennial herbaceous plant belonging to the Asteraceae family, endemic to the East coast of the Adriatic Sea. It is commercially grown worldwide because of its valuable compounds pyrethrins used as natural insecticides. Pyrethrins are non-toxic to mammals and other worm-blooded animals; they are environmentally safe as highly biodegradable, unstable in light, oxygen, water and high temperatures. Due to these properties products on their base are leading insecticides in organic farming. The aim of the present study was to initiate *in vitro* clones of *T. cinerariifolium* as a first step for selection of high productive pyrethrin clones, appropriate for further field cultivation under the agro-climatic conditions of Bulgaria. Seeds from *ex situ* collection of pyrethrum in Bulgaria were used as a starting material. After standard surface sterilization seeds were germinated on three nutrient media on MS base: medium MS free of plant growth regulators, and two media supplemented with kinetin (Kin) and indole-3-butyric acid (IBA) in concentrations 1.0 mg L⁻¹ and 0.5 mg L⁻¹ (medium KI), and 0.2 mg L⁻¹ and 0.1 mg L⁻¹ (medium K2I1), respectively. Seedlings were subcultured on the same media after 8 weeks. Nineteen clones were obtained by consecutive subcultivations: shoots formed at the base of the explants were separated, and the longer ones were additionally cut to segments. Each explant developed in new plantlet. The presence of plant growth regulators enhanced shoot formation and elongation; however, endophytic bacteria were observed that caused necrosis of plantlets and loss of entire clones. Propagation coefficient (PC) calculated as the average number of plantlets obtained from one seed depended on the medium. After 5 months, the highest PC was observed in medium K2I1 where 26.5 plantlets per seed were obtained while the number of plantlets on MS medium was 8.5.

Key words: Dalmatian pyrethrum, pyrethrins, bioinsecticides

GROWTH AND ESSENTIAL OILS OF *SALVIA OFFICINALIS* L. PLANTS DERIVED FROM CONVENTIONAL OR AEROPONIC PRODUCED SEEDLINGS

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Salvia officinalis L. (Lamiaceae) is cultivated in many countries as a valuable medicinal and aromatic plant with antiseptic and spasmolytic effects due to its essential oils, used as ingredient in many phytopreparations. The present study was aimed at enhancing the growth of *Salvia officinalis* plants and to improve the essential oil quality, applying hydroponic technology. Seeds of cultivated *S. officinalis* were used as initial material. Conventional seedlings production, used as a control, began in November (in terrines with ordinary soil) while that on aeroponic vertical system began the next year in April (seeds sown into peat cubes in pots with keramzite); seeds germinated for 3 and 2 weeks, respectively. In July, all seedlings were transferred into pots with sterilized compost and universal soil mixture in proportion 2:1. Aeroponically obtained seedlings developed vigorous roots (average length of 12.6 cm). After two months in the greenhouse, the morphometric parameters of 80 seedlings of each group were compared, and then they were planted on the field plot. Herbage of 9 randomly chosen plants per group was harvested during the full flowering stage. The essential oils were extracted on Clevenger apparatus and their composition was analyzed by GC/MS. Although conventionally obtained seedlings were significantly higher ($P < 0.001$), and the ramification of the two groups was similar, the size of all plants at the harvest stage was uniform, with 1.1% w/v essential oils. Aeroponically derived plants developed numerous generative stems (aeroponic: 172.3 vegetative and 120.6 generative stems per plant; conventional: 200.0 vegetative and 57.5 generative stems per plant) which increased their biomass by 20% and was a precondition for variation in the essential oils composition. It can be summarized that the application of aeroponic system shortened the period from germination to harvest for 5 months, enhanced plants flowering and reflected on the composition of the essential oils.

Key words: sage, medicinal and aromatic plant, hydroponic technologies, cultivation

ROOTING OF LEAVES OF *HABERLEA RHODOPENSIS* Friv. (GESNERIACEAE) USING A VERTICAL AEROPONIC SYSTEM

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Haberlea rhodopensis Friv. is a tertiary relict and endemic species to the Balkan Peninsula, distributed only in Bulgaria and Greece. This perennial herbaceous plant is growing in shady rock crevices on limestone at altitudes of 100 – 1700 m. It belongs to the so called “resurrection plants” as its vegetative parts are able to withstand several years of almost complete water loss and to recover fast upon rewatering. Plants were used in the past for its healing and rejuvenating properties. The species is protected by the Biodiversity Act and the Act of Medicinal Plants. The aim of the study was to stimulate the rooting of *H. rhodopensis* leaves by applying hydroponic technologies, testing different substrates and IBA treatment, as a first step toward plant propagation. Leaves from one Bulgarian locality were used as a starting material. Leaf rooting was performed by applying vertical aeroponic system Green Diamond (GHE). Four variants with three substrates in meshy pots were compared; peat cubes, mineral wool, and perlite in a semi-permeable tissue surrounded either by keramzite or agrolava pebbles, 30 leaves per variant. Half of the leaves in each variant were treated with IBA powder prior to leaf embedding in the substrate. Best results were obtained with IBA-treated leaves on perlite surrounded with agrolava, where 100 % of the leaves developed vigour root system for 12 weeks. Both substrates and IBA influenced the rooting process. The effect of IBA was positive except for the variant with keramzite, probably due to its property to decrease pH in the pots. The lowest percent of rooted leaves (20%) was obtained with peat cubes without IBA treatment. In the variant with mineral wool intermediate success was observed. Single rosettes of *H. rhodopensis* began to appear after 3 months of the leaf cultivation.

Key words: endemics, relicts, resurrection plants, hydroponic cultures, medicinal plants

**IN VITRO ESTABLISHMENT AND CULTURE
OF ENDANGERED MEDICINAL PLANTS WITH
ORNAMENTAL VALUE *CAMPTOTHECA ACUMINATA*
Decne (NYSSACEAE)**

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Camptotheca acuminata Decne (happy tree, cancer tree, or tree of life) is a deciduous and endangered tree species endemic to east Tibet and southern China. Camptothecin is a monoterpene indole alkaloid isolated from the bark and stems of *C. acuminata*. Camptothecin and its derivatives are used for the treatment different cancers and have potential for treatment of a wide range of viruses and parasites. The aim of the present study was to develop methods for *in vitro* micropropagation of this valuable plant. Different cultural media based on both MS (Murashige and Skoog, 1962) or DKW (Driver and Kuniyuki, 1984) formulations with different cytokinins (BAP or 2-isopentenyladenine or meta-topolin) have been involved in serial experiments.

Key words: *Camptotheca acuminata*, camptothecin, micropropagation

ANTIOXIDANT ENZYMES AND OXIDATIVE STRESS MARKERS IN *HYPERICUM PERFORATUM* L. TRANSGENIC SHOOT CULTURES

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The up-regulation of antioxidant enzymes is a well-known defence mechanism for neutralization of reactive oxygen species (ROS) in plant tissues upon biotic stress. The objective of this work was to investigate the correlation between antioxidant enzyme activity and oxidative stress marker contents in *Hypericum perforatum* L. transgenic shoots (TS). The TS clones were regenerated from the corresponding hairy roots induced by *Agrobacterium rhizogenes* A4-mediated transformation. In addition, control roots were used for regeneration of non-transgenic shoots (NTS). The activity of antioxidant enzymes, peroxidase (PX), catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD), as well the contents of oxidative stress markers such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$) and malondialdehyde (MDA) were determined in fifteen TS clones and NTS. Present results demonstrated that the up-regulation of PX activity ($0.04 - 0.29 \text{ nkat} \cdot \text{mg}^{-1} \text{ proteins}$) in TS clones greatly contributed to the reduction of H_2O_2 amounts ($0.38 - 1.18 \mu\text{M g}^{-1} \text{ fresh weight}$). On the other side, the activities of CAT ($0.11 - 1.17 \text{ nkat mg}^{-1} \text{ proteins}$) and APX ($13.06 - 124.69 \text{ pkat mg}^{-1} \text{ proteins}$) in TS clones did not correlate with H_2O_2 levels. The inconsistent activity of CAT and APX in TS clones may be compensated by the activity of PX that act concurrently to remove H_2O_2 . The TS clones exhibited an enhancement in SOD activity ($0.47 - 7.51 \text{ U mg}^{-1} \text{ proteins}$) that coincided with decreased $O_2^{\cdot-}$ production rate ($0.24 - 1.22 \text{ nM min}^{-1} \text{ g}^{-1} \text{ fresh weight}$) highlighting its additional protective role in ROS neutralization. In addition, MDA amounts ($0.41 - 1.75 \text{ nM g}^{-1} \text{ fresh weight}$) in TS clones correlated with suppression of H_2O_2 and $O_2^{\cdot-}$ levels. The down-regulation of MDA in TS cultures was attributed to antioxidant enzymes efficiency in ROS scavenging that consequently delayed lipid peroxidation. Thus, the activation of antioxidant defence system significantly contributed to the improvement of oxidative stress tolerance in *H. perforatum* transgenic plants.

Key words: antioxidant enzymes, *Hypericum perforatum* L., oxidative stress markers, transgenic shoots

SEASONAL VARIATION OF THE ESSENTIAL OIL AND POLYPHENOL/FLAVONOID CONTENT OF LEAVES OF *SALVIA FRUTICOSA* Mill. AND *S. POMIFERA* L. PLANTS CULTIVATED IN THE BOTANICAL GARDEN OF UNIVERSITY OF PATRAS

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Salvia is the largest genus of the Lamiaceae family and includes about 700 taxa (sp and ssp.) throughout the world. *S. fruticosa* Mill. (Greek sage) and *S. pomifera* L. (Cretan sage) are common in Greece and are used in traditional medicine and cooking. The purpose of this study was the analysis of seasonal variation of the volatile and non-volatile secondary metabolites of *S. fruticosa* and *S. pomifera* clones cultivated in the botanical garden of University of Patras. These clones originate from plants collected at Messenia and Achaia regions (*S. fruticosa*), and from Arcadia region (*S. pomifera*, n=5, 5 and 4, respectively). Dried leaves collected in April, July, December and October were subjected to steam distillation for 2 h and analysis was performed by GC-MS. Non-volatiles were extracted with 70% methanol after defatting in an ultrasound bath. The total polyphenol and flavonoid content varied significantly among seasons; the higher values were observed during springtime. Statistically significant differences of the essential oil yield were also recorded; the highest yield was recorded in summer, while the lowest in spring. The main *S. fruticosa* essential oil components were eucalyptol (30.4-41.9%), camphor (5.0-9.1%), and viridiflorol (2.3-6.5%), whereas in *S. pomifera* oil were thujone-b (11.6-33.5%), eucalyptol (3.7-13.1%), and cubebol (4.0-9.8%). The oil composition differed not only among species but also among the different populations and among seasons. In both *S. fruticosa* populations, the levels of alpha-pinene were higher in summer, while in spring caryophyllene and its oxygenated derivatives were significantly elevated. Regarding *S. pomifera* the lowest content in thujone was observed in spring. In conclusion, we observed population- and seasonal-variation in the chemical profile of the essential oils of the clones studied. This work contributes to delineating the metabolic mechanisms and to identifying the parameters critical for isolating high-quality *Salvia* essential oils.

Key words: *Salvia*, cultivation, essential oil, polyphenols

SHOOT MULTIPLICATION SYSTEM OF HYSSOP (*HYSSOPUS OFFICINALIS* L.)

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An efficient method for the micropropagation of hyssop (*Hyssopus officinalis* L.) has been developed. The plants were cultured on Murashige and Skoog medium (MS) supplemented with 6-benzyl-aminopurine, thidiazuron, zeatin and indole-3-butyric acid during four weeks of culture. The above mentioned cytokinins were applied in concentration 0.5 and 1.0 mg l⁻¹. Maximum multiplication frequency, numbers of shoots, shoot length, fresh and dry weight were established at 1.0 mg l⁻¹ 6-benzyl-aminopurine combined with 0.1 mg l⁻¹ indole-3-butyric acid. The other tested cytokinins thidiazuron and zeatin were less effective for the micropropagation of hyssop. For root induction, the micropropagated plants from the most effective variant (MS supplied with 1.0 mg l⁻¹ 6-benzyl-aminopurine and 0.1 mg l⁻¹ indole-3-butyric acid) were cultured on root development medium. The uniform micro shoots were excised and transferred to the rooting medium contained half strength MS medium supplemented with three types of auxins: indole-3-butyric acid, and indole-3-acetic acid, applied at a concentration of 0.1 mg l⁻¹ with addition of 2.0% sucrose. The obtained results suggest that all three investigated auxins could be used separately in ½ MS medium for rooting of *H. officinalis*. Indole-3-butyric acid was more effective to produce plants with well-developed roots; therefore, this auxin was found to be the best rooting hormone as opposed to α -naphthalene acetic acid and indole-3-acetic acid. The multiple plants were successfully *ex vitro* adapted with 90% survival. The described protocol allows the establishment of numerous micropropagated plants of *H. officinalis*.

Key words: *Hyssopus officinalis*, micropropagation, plant growth regulators

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THE EFFECT OF IRRIGATION AND ORGANIC FERTILIZATION ON THE ESSENTIAL OIL COMPOSITION OF *MATRICARIA CHAMOMILLA* L.

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Chamomile (*Matricaria recutita* L., fam. Asteraceae), is an important medicinal plant, wild grown and cultivated worldwide. The drug is constituted from the dried flower heads and it is used for the preparation of herbal teas, in phytotherapy, cosmetics, in veterinary medicine, etc. Chamomile essential oil and extracts contain more than 100 secondary metabolites, which contribute to its pharmacological properties. Sesquiterpenes, flavonoids, coumarins and polyacetylenes are the most important group of constituents. Chamomile's blue essential oil, contains mainly sesquiterpene derivatives and among them α -bisabolol, chamazulene, α -bisabolol oxides A and B are the main compounds. Chamazulene and α -bisabolol are considered the most bioactive constituents and known for their anti-inflammatory, antiseptic, antiphlogistic and spasmolytic properties. The essential oil yield and composition is affected by the genotype, the environmental conditions, the cultivation practices, etc. The increasing demand for chamomile-based products, resulted in the development of high yielding varieties, concerning the essential oil yield and the content of bioactive compounds, and the increase of chamomile cultivation all over the world. Chamomile crops, adopting low input farming and organic agricultural practices, is a challenge for the production of high quality raw material and in accordance to the industry demands. In the present study, the effect of irrigation and organic fertilization on the essential oil composition of a local Chamomile cultivar, cultivated in experimental field in Thessaly (C. Greece) was investigated. The results showed that α -bisabolol content was higher under irrigation conditions, though the effect of fertilization was not significant, compared to the control (no treatment). On the other hand, the chamazulene yield in the essential oil was increased under rainfed conditions and application of the lower levels of organic fertilization.

Key words: Chamomile, essential oil, fertilization, irrigation

PHENOTYPIC EVALUATION OF *SIDERITIS SCARDICA* Gris. SELECTED CLONES, AND PRE-BREEDING EVALUATION FOR YIELD POTENTIAL AND QUALITATIVE TRAITS

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Sideritis scardica Gris. (Lamiaceae), commonly known as Mountain tea is an endemic to Balkan countries medicinal and aromatic species. It mainly thrives in low fertility soils, in mountainous areas and at altitudes above 800-1000 m, while is considered to be threatened due to overexploitation resulting on shrinking the population's size in natural habitats. Lately, the increased number of reports on *Sideritis* sp. bioactivities i.e. antioxidant, antibacterial, anti-inflammatory, protective to neurodegenerative diseases etc and its traditional uses against gastrointestinal disorders, triggered the industrial demand for high quality raw material with superior qualitative characteristics. The present study reports the pre-breeding evaluation of a promising *S. scardica* population, selected among several others native to Greece, for expressing superior phenotypic traits. Three clones were developed from three individual plants, respectively, which were initially selected for their superior morpho-metric traits, in order to assess their morphological and agronomic characteristics, yield potential and qualitative traits. All the measurements were conducted at the stage of full blooming on 36 individuals / clone. A statistical significant variation was detected between the clones concerning the time of blooming, the length of stems and inflorescence, the fresh and dry weight of biomass production, indicating high phenotypic diversity of the starting population. The genetic and chemical characterization of the individual plants in the clones, in combination with phenotypic, will determine superior genotypes, for future breeding purposes.

Key words: *Sideritis scardica*, pre-breeding, clones, phenotyping

PHYTOCHEMICAL INVESTIGATIONS OF THREE DIFFERENT ACCESSIONS OF *PERILLA FRUTESCENS* (L.) Britt.

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Perilla frutescens (L.) Britt. is an annual aromatic plant that belongs to Lamiaceae family and has been used traditionally to cure a number of human ailments as well as culinary herb and spices. The aim of present study was to compare the production and secondary metabolites of three morphologically different *Perilla frutescens* accessions (Pf1 dark green leaves), (Pf2 bright green leaves) and (Pf3 purple leaves). Plants have been grown in climatic-chamber, under controlled condition (17/7 h day/night; 25/19 °C, 65% RH). The essential oil content and composition, total polyphenol content (TPC) as well as antioxidant capacity (AOC) of the leaves were investigated. The Pf1 showed the highest essential oil content 1.536 mL 100 g⁻¹, while others showed lower accumulation level: 0.436 mL 100 g⁻¹ (Pf2), 0.326 mL 100 g⁻¹ (Pf3) respectively. The essential oil composition was identified by gas chromatography–mass spectrometry (GC-MS). We found perillaldehyde (77.47%) and limonene (5.71%) as a major essential oil component of Pf1, while in Pf2 perilla ketone (75.09%) and limonene (10.47%) and in Pf3 β-dehydro-elsholtzia ketone (67.79%) and shisofuran (7.71%) were detected in the highest ratio. The highest TPC (211.4 mg g⁻¹ GAE dm.) was measured in Pf3, followed by Pf2 (186.4 mg g⁻¹ GAE dm.) and the lowest value was detected in Pf1 (171.8 mg g⁻¹ GAE). In terms of AOC Pf3 (178.1 mg AAE g⁻¹ dm) showed higher values compared to Pf2 (133.7 mg AAE g⁻¹ dm), and Pf1 (129.2 mg AAE g⁻¹ dm). Based on the results, for essential oils Pf1 and for polyphenols the Pf3 could be considered for further pharmacological studies.

Key words: antioxidant, essential oil, Lamiaceae, polyphenol

OPTIONS OF HERBICIDES PROTECTION OF MARIAN THISTLE (*SILYBUM MARIANUM* L.)

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Silybum marianum L. is a sufficiently robust and competitive plant against most of weeds. But, especially in the early growth and development stages of growth it can be significantly suppressed by quickly emerging annual monocots and broad-leaved weeds. At the same time perennial weeds can be a big problem. In our conditions, couch grass and thistle sown are problem. Although in the past there was also performed mechanical cultivation (hoeing), currently in the case of occurrence of weeds chemical protection is chosen, i.e. using a suitable and registered herbicide. Currently, for application on *Silybum marianum* permitted herbicides are STOMP 330 E with a.i. pendimethalin applied pre-emergently, then REFINE SX 50 with a.i. thifensulfuron-methyl, and Betanal maxxPro respectively Betanal Maxx Pro 209 OD with a.i. desmedipham, ethofumesate, phenmedipham and lenacil. These formulations are designed for post-emergence, respectively early post-emergence treatment. It is interesting that there is no registered graminicide for controlling of annual and perennial monocots weeds. Our experiments showed that normal range of graminicides used in the Czech Republic is a highly selective to *Silybum marianum* plants even at dose rate controlling couch grass.

Key words: *Silybum marianum* L., herbicides, selectivity, yield

AUXIN APPLICATION TO ENHANCE ROOTING OF DALMATIAN SAGE, IMMORTELLE AND LAVANDIN

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The Croatian coast and islands are known by its richness of naturally occurring aromatic and medicinal plants. The Dalmatian sage (*Salvia officinalis* L.) and immortelle (*Helichrysum italicum* /Roth/G. Don) are native plants distributed along coast and islands, while lavandin (*Lavandula x intermedia* Emeric ex Loisel.) is successfully grown commercially and in gardens. The aim of this work was to test the efficacy of auxins for rooting of plants collected from natural habitat in Croatia. The sage and immortelle were collected at one location, whereas lavandin was collected at three locations at island Hvar. The cuttings were dipped in solution containing indole-3-acetic acid (IAA) at 1.0 mg L⁻¹, indole-3-butyric acid (IBA) 0.3 mg L⁻¹, 1-naphthaleneacetic acid (NAA) at 0.5 mg L⁻¹, or tap water (control), and grown under conditions favourable for rooting. The rooting percentage of sage cuttings was enhanced by application of auxins (67% to 69%) compared to control (44%), and similar trend was observed for number of roots per cutting. Application of IAA or IBA increased sage root length compared to control or NAA. The rooting percentage of immortelle was under 60% when IAA and control were applied, while NAA application resulted in 85% rooted cuttings. The application of NAA increased number of roots, but no effect of auxins on immortelle cutting root length was observed. There was no difference in rooting percentage of lavandin cuttings considering auxin application compared to control, however, significantly higher percentage of rooted cuttings was observed for landraces from Brusje and Velo grablje compared to landraces from Hvar. The auxin application increased the number and length of the roots per cutting of landraces from Brusje and Velo grablje compared to ones from Hvar. Auxin application can significantly increase rooting percentage of sage and immortelle and ensure fast and true-to-type propagation of genotypes with desirable morphological and biochemical traits.

Key words: propagation, transplants, hormone

BREEDING SYNTHETICS IN CARAWAY (*CARUM CARVI* L.)

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Caraway (*Carum carvi* L.) seeds are primarily used as spice or tea. Due to essential oil content it eases gastrointestinal afflictions. In the past caraway was grown entirely as biennial plants until annual cultivars were developed in the 1990th. However, annual cultivars fail to reach the level of yield of biennial caraway down to the present date. Therefore, we endeavour to try novel ways of breeding for caraway. Caraway breeding usually conducted population breeding to improve cultivars. In addition, line breeding was implemented to gain more homogeneous populations. Besides, DH-technology has been developed. This might facilitate hybrid breeding in future to exploit heterosis. Though, lacking a system for pollination control, breeding of synthetic cultivars is tested as a first approach. For this purpose, a polycross will be performed of 14 inbred lines and 2 annual cultivars as standards. Performance of inbred lines, cultivars and progenies will be tested in subsequent years to measure the general combining ability (GCA) and to get a notion of the actual heterosis effect, which can be exploited by breeding synthetics.

Key words: caraway, *Carum carvi*, breeding synthetics

CULTIVATION PRACTICES OF THE FIRST LARGE SCALE INDOOR FACILITIES FOR PRODUCTION OF HIGH THC CANNABIS IN REPUBLIC OF MACEDONIA

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Cannabis (*Cannabis sativa* L., Cannabaceae) is a well-known drug and a controlled substance, which possession and use are illegal in most countries of the world. Nowadays, the use of cannabis and its legalization for medical use has become a worldwide trend. Laws and attitudes toward cannabis are changing these days. Legalization of cannabis use for medical purposes is a hot topic at the global level and in most countries, there have been initiatives to amend the existing laws in order to make drugs based on natural ingredients of cannabis, as well as other related products, synthetically produced, available to patients. In 2016, changes of the Law for narcotic substances in RM were made, concerning legalization of cannabis for medicinal and scientific purposes, in a way of cultivation, production of cannabis medical preparations as well as import of pharmaceuticals and cosmetics, containing cannabis or cannabinoids. Therefore, due to the increased global necessity of medical cannabis with high THC content, in order to respond to the challenges arising from this issue, NYSK Holding have started a large scale indoor production of different varieties of cannabis, using several cultivation practices. Alongside the production of plant material, from indigenous and foreign origin, cannabis extracts (mainly cannabis oil) and various products are prepared.

Key words: medicinal cannabis, legal growing

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