Sustainable Utilization of Bio-Resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products - ICSUMAP'23

March 27 -28, 2023. Virtual online meeting, Sofia, Bulgaria

Program and Book of Abstracts

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Scientific Committee

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Program

Time Zone: Eastern European Summer Time

Monday, 27.03. 2023.

	MORNING SESSION I, 27.03 https://teams.microsoft.com/l/meetup-join/19:meeting_NWE5MzhiYzEtOTE5MS00NjQxLWJmYmQtY2Y1NmFiYWExOTc3@thread.v2/0?context=%7B%22Tid%22:%222672d32a-be75-49eb-88c3-3d3f482a3205%22,%22Oid%22:%220ed1e078-0a1f-4230-8ff6-ff851cf18b72%22%7D
9:00	Opening
9: 05	Welcome address – the Director of the Hosting Institution IOCCP, Prof. Vanya Kurteva
9:10	**Plenary Lecture**, Prof. V. Dimitrov Sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products
	Session **Characterization of the genetic and metabolite diversity of MAP for their cultivation and further utilization** Chair: Dr. Krasimir Rusanov
9:50	Invited lecture, Dr. Sylvie Baudino-Caissard Secretion and evolution of the perfume in roses - Biochemistry, Molecular and Cell Biology
10:20 – 10:35	Oral presentation, Dr. Anna Zaharieva Metabolic Profile of In Vitro Cytokinin-Treated and Wild-Grown *Nepeta Nuda*
10:35 – 10:45	*Coffee break*
	MORNING SESSION II, 27.03 https://teams.microsoft.com/l/meetup-join/19:meeting_MTYwNzFjNDAtYjZiZS00MDljLWI3N2MtMWI4YTY2NWUwZTRk@thread.v2/0?context=%7B%22Tid%22:%222672d32a-be75-49eb-88c3-3d3f482a3205%22,%22Oid%22:%220ed1e078-0a1f-4230-8ff6-ff851cf18b72%22%7D
	Session **Polymer-based formulations of medicinal and aromatic plants** Chair Dr. Emi Haladjova
10:45	Invited lecture, Dr Natalia Oleszko-Torbus Copolymers 2-oxazoline for applications in biology and medicine
11:15	Invited lecture, Assoc. Prof. Dr. Natalia Toncheva-Moncheva, Original Synthesis of new assemblies by initiator-free thiol–ene “click” coupling reactions performed by LED UV illumination
11:45	Invited lecture, Assoc. Prof. Mariya Spasova

	Novel fibrous materials based on polylactide and *Portulaca oleracea* plant extract: Preparation, physico-chemical and biological properties
12:15	Oral presentation, Assoc Prof. Emi Haladjova Drug loaded Mixed poliymeric micelles as antibiofilm agents
12:30 – 13:00	Discussion
12:00 – 14:30	*Lunch break*
	AFTERNOON SESSION, 27.03 https://teams.microsoft.com/l/meetup-join/19:meeting_OGI4YTljYmItY2Y5Zi00MTEzLTk0ZjEtYWFhM2Y3YzlkNmRm@thread.v2/0?context=%7B%22Tid%22:%222672d32a-be75-49eb-88c3-3d3f482a3205%22,%22Oid%22:%220ed1e078-0a1f-4230-8ff6-ff851cf18b72%22%7D
	Session **Formulation, characterization and quality control of MAP-based products** Chair Dr. Zahari Vinarov
14:30	Invited lecture, Prof. Dr. Ioanna Chinou, Basic principles of final herbal products quality, according their regulatory frame of registration- Herbal medicines vs food supplements
15:00	Invited lecture, Prof. Dr. Krassimir Velikov Advanced formulation approaches for delivery of water insoluble plant bioactive molecules
15:30	Invited Lecture, Javier Sainz *Stevia*: Why this ancient plant has a key role in the sustainable and healthy substitution of sugar at a global scale?
16:00	Discussion
16:30 – 17:30	Poster session (**List of posters at the end of the Program**)

Tuesday, 28.03. 2023.

Time Zone: Eastern European Summer Time

	MORNING SESSION AND CLOSING, 28.03. https://teams.microsoft.com/l/meetup-join/19:meeting_ZDFiMzM3YzUtMjBhZS00MWJmLTk0ODktN2MzOWM0MjI4YWZj@thread.v2/0?context=%7B%22Tid%22:%222672d32a-be75-49eb-88c3-3d3f482a3205%22,%22Oid%22:%220ed1e078-0a1f-4230-8ff6-ff851cf18b72%22%7D
	Session **Obtaining and chemical characterization of biologically active natural and synthetic compounds** Chair: Dr. Kalina Danova
9:30	Invited lecture, Dr. Evelyn Wolfram Phytochemical and Effect-directed analysis as the basis of evidenced based natural products
10:00	Invited Lecture, Prof. Dr. Ulrike Lindequist Mushrooms - their potential for medicine and a healthy nutrition
	Session **Biological activity of natural and synthetic compounds** Chair: Dr. Iliana Rasheva
10:30	Invited lecture, Dr. Dejan Stojković, Plant industrial by-products as sources of bioactive compounds with potential food and cosmeceutical applications
11:00 – 11:15	*Coffee break*
11:15	Invited lecture, Dr. Danijela Mišić, Catmints (*Nepeta* sp.): biological activities and applicative potential
	Session **Medicinal and aromatic plants by-products - extraction, composition and biological activities** Chair: Dr. Krasimir Rusanov
11:45	Invited lecture, Dr. B. B. Basak, Exploration of value addition potential of waste biomass from medicinal and aromatic plants
12:15 – 12:45	General discussion
12:45 – 13:00	Closing

Link to Virtual Conference Platformhttps://registration.sdewes.org/ICSUMAP23/virtcon/

LIST OF POSTERS

TITLE	AUTHORS	SESSION
Polymeric Micelles of Mixed Composition as Effective Carriers for Biomolecules	Rumena Stancheva*, Emi Haladjova, Maria Petrova, Iva Ugrinova, Ivaylo Dimitrov, Stanislav Rangelov	*Polymer-based formulations of medicinal and aromatic plants*
Role of RAGE Receptors in the Obestatin Effect	Bilyana Ilieva, Milena Keremidarska-Markova, Hristo Gagov, Iliyana Sazdova*	*Biological activity of natural and synthetic compounds*
Effect of Graphene Oxide Nanoparticles on Rat Mitochondrial ATPase And Diamino Oxidase Activity	Milena Keremidarska-Markova*, Milena Mishonova, Milena Shkodrova, Neli Raikova, Dilyana Doncheva-Stoimenova, Natalia Krasteva, Mariela Chichova	
Antiviral Potential of Plants from *Stachys* Genus Against Herpes Viruses	Daniel Todorov*, Anton Hinkov, Kalina Shishkova, Desislava Mantovska, Zhenya Yordanova, Stoyan Shishkov	
Biological Activity of Ferrite Nanoparticles	Iliana Ivanova*, Elena Nenova, Lilia Petrova, Desislava Boteva	
Biological Effects of *Rosa damascena* Extracts on Cultured Human Fibroblasts	Veselina Moskova Doumanova*, Ralitsa Veleva, Antoaneta Trendafilova, Tanya Topouzova-Hristova	
Antifungal Activity of Bulgarian Plant Populations of *Vaccinium vitis-idaea* L.	Rossalina Uzunova, Iliyana Rasheva*, Ivaila Dincheva, Trayana Nedeva, Svetla Angelova	
***In Vitro* and *in Vivo* Antioxidant Capacity of Ethanolic Extracts of *Cuscuta* spp.**	Lyuben Zagorchev*, Vanina Lozanova, Denitsa Teofanova, Yana Evstatieva	
***In Vitro* Cultivation of *Stevia rebaudiana* by Adding Silver Salts of Amino Acid-Based Nanofibers**	Kamelia Miladinova-Georgieva*, Maria Geneva, Maria Petrova, Daniela Tsekova, Elisaveta Kirova, Mariana Sichanova	*Characterization of the genetic and metabolite diversity of medicinal and aromatic plants (MAP) for their cultivation and further utilization*
Construction of Genetic Linkage Map of Lavender (*Lavandula angustifolia* Mill.) Based on SSR Markers	Pavlina Vassileva*	
Targeting of Secondary Metabolite Productivity through Conventional Tissue Culture Techniques. Experience in the *Hypericum, Artemisia and Sideritis* species	Kalina Danova*, Antoaneta Trendafilova, Kalina Alipieva, Jasmina Petreska Stanoeva, Viktoria Ivanova, Ina Aneva, Marina Stefova, Milka Todorova	
Development of a Strategy for Obtaining Highly Productive *Nepeta nuda In Vitro*	Desislava Raykova*, Ana-Maria Nedelcheva, Victoria Vladimirova, Anna Zaharieva, Adriana Krasteva, Zhenya Yordanova, Miroslava Zhiponova	
Assessment of State of Cenopopulations of the	Akerke Serbayeva* N.M. Muhitdinov, Zh. Zhunusbaeva	

***Erysimum croceum* of the Trans-Ili Alatau**		
Phenolic Compounds in Waste after Distillation of *Geranium macrorrhizum* L.	Milena Nikolova*, Ana Dobreva	*Medicinal and aromatic plants by-products - extraction, composition and biological activities*
Assessment of Biological Activity of Rose Oil Distillation Waste Water (RODW) Using Different *in Vitro* Cell Culture Lines	Boyka Andonova-Lilova*, Marina Alekseeva, Mila Rusanova, Krasimir Rusanov, Tzvetelina Zagorcheva, Ivan Atanassov	
***Echinacea* Species Introduced in Bulgaria as a Source of Caftaric and Chicoric Acids**	Mariya Vlasheva*, Mariana Katsarova, Ana Dobreva, Stela Dimitrova, Anatoli Dzhurmanski	*Obtaining and chemical characterization of biologically active natural and synthetic compounds*
Solketal Reactions with Ibuprofen - Formation and Characterization of Novel Products	Viktorija Jakimovska Pokupec*, Marina Stefova, Milena Popova, Vassya Bankova	
Fatty Acid Composition of Purslane (*Portulaca oleracea* L.) Leaves	Sabina Taneva*, Svetlana Momchilova	
Green Extraction of Rosmarinic Acid from Lemon Balm (*Melissa officinalis* L.) with subcritical Water.	Ana Atanasova, Ani Petrova, Desislava Teneva, Manol Ognyanov, Yordan Georgiev, Nenko Nenkov, Petko Denev*	
Comparison of Different Techniques for Extraction of Sesquiterpene Lactones from *Inula helenium* Roots	Fahriye Şeyma Özcan, Nihat Özcan, Hilal Dikmen, Özlem Çetin, Mustafa Çelik, Antoaneta Trendafilova*	

Plenary lecture

Sustainable utilization of bio-resources made profitable

Vladimir Dimitrov*

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgaria

The lecture presents the project for establishment of Centre of Competence “Sustainable Utilization of Bio-Resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products”. The financial support is realized by the Operational Program Science and Education for Smart Growth, co-financed by the European Structural Funds. The main task of the project is to establish and organize the infrastructure and human resources within the Centre to support business companies for utilization of Bulgarian medicinal and aromatic plants as source for innovative products. The infrastructure of the Centre is designed to cover the whole process from a plant to a prototype of a final product. The planned scientific activities are demonstrated through the expertise and the corresponding function of the partnering organizations building the Centre.

Invited presentations

Genomics and Rose Fragrance

Sylvie Baudino*

Université Jean Monnet de Saint-Etienne, France

Roses (*Rosa* sp.) are one of the most important horticultural plants, highly appreciated for their ornamental and olfactive values since ancient times. Originating from about ten rose species, more than 30,000 cultivars have been obtained by breeders. The selection of new rose varieties is based on numerous criteria, such as visual traits, resistance to diseases, as well as fragrance. Despite of its importance, fragrance is a complex trait which is difficult to work with since little is known about its heritability.

The fragrance of rose comes from a complex blend of volatile organic compounds (VOCs) of different biochemical families, such as terpenoids, phenylpropanoids and fatty acid derivatives. Hundreds of VOCs were identified by performing solid-liquid extraction of rose petals and chemical analysis using gas chromatography-mass spectrometry. Among these VOCs, geraniol and its derivatives, 2-phenylethanol, and 3,5-dimethoxytoluene are one of the major compounds found in perfumed roses. Biosynthetic pathways that lead to the production of such compounds are starting to be deciphered. In the presentation, the methods which make it possible to elucidate the biosynthetic pathways of VOCs will be presented, emphasizing the recent contributions of genomics. Further understanding of these biosynthetic pathways may lead to the improvement of the selection of new rose varieties.

Copolymers of 2-oxazolines for applications in biology and medicine

Natalia Oleszko-Torbus*, Wojciech Wałach, Agnieszka Kowalczuk, Alicja Utrata-Wesołek, Barbara Mendrek, Marcelina Bochenek
Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabrze, Poland

Poly(2-oxazolines), also known as biocompatible pseudopeptides, are obtained by cationic ring-opening polymerization of five-membered cyclic iminoethers. The type of substituents of the oxazoline ring determines the solubility of poly(2-oxazoline) formed and its thermosensitivity in aqueous solutions, as well as its thermal and crystalline properties in bulk. Many 2-oxazoline copolymers (in water solution) show thermosensitivity around human body temperature. Easy control of their properties opens the way to the potential applications in biology and medicine. An important tool to control the properties of (co)poly(2-oxazolines), is the possibility to functionalize their end groups and substituents on the way of post-polymerization modifications.

The lecture presents an overview of our studies on the design of poly(2-oxazolines) both by appropriate selection of comonomers during copolymerization and by post-polymerization modifications. A series of 2-substituted-2-oxazolines copolymers were synthesized, and the possibility to control mainly thermal and crystalline properties in bulk and solution, but also their hydrophilic-lipophilic balance in aqueous solutions, were carefully examined. Firstly, selected 2-isopropyl- and 2-n-propyl-2-oxazoline copolymers (iPrOx/nPrOx) were discussed. The processing of iPrOx/nPrOx copolymers by electrospinning and rapid prototyping techniques to obtain polymer matrices with specific properties was optimized in order to obtain effective matrices for *in vitro* cell culture. Secondly, the control of thermal and crystalline properties of copolymers based on 2-methyl- and 2-isopropyl-2-oxazoline (MOx/iPrOx) was shown. These copolymers were subjected to selective hydrolysis which allowed to explore their potential use in gene therapy. The potential of other 2-oxazoline copolymers, containing amino groups in the side chains, to form complexes with DNA was also pointed out. Finally, the control of the properties of poly(2-oxazolines) by incorporating an additional substituent at position 4 of the oxazoline ring was investigated. The resulting poly(2,4-substituted-2-oxazoline) was shown as an alternative to poly(2-isopropyl-2-oxazoline) with potential for bio-applications. At the end, the prospective use of poly(2-oxazolines) modified with chelating compounds was mentioned.

Acknowledgements: This work was supported by the National Science Centre, project 2021/43/B/ST4/01493

ЕВРОПЕЙСКИ СЪЮЗ
ЕВРОПЕЙСКИ ФОНД ЗА РЕГИОНАЛНО РАЗВИТИЕ
ОПЕРАТИВНА ПРОГРАМА НАУКА И ОБРАЗОВАНИЕ ЗА ИНТЕЛИГЕНТЕН РАСТЕЖ

Original synthesis of new polymer amphiphiles and hybrid biomacromolecules by initiator-free thiol–ene “click” coupling reactions performed by LED UV illumination

Natalia Toncheva-Moncheva*
Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria

Initiator-free thiol-ene “click” coupling reactions were employed to obtain original polymer amphiphiles and hybrid biomacromolecules. The reactions were performed by a custom-made device supplied by LEDs as UV light source illuminated at a fixed wavelength of 365 nm. The polymer amphiphiles were obtained by reacting poly(allyl glycidyl ether) (PAGE) with various mono- and di-oligo polyethylene glycol (PEG) thiols. On the other hand, appropriately functionalized lipid-mimetic residue and single stranded DNA oligonucleotide were covalently linked to produce hybrid biomacromolecule. The novel amphiphiles were found to self-assemble and co-assembled into variety of structures and fully characterized by means of light scattering (dynamic, static, and electrophoretic) and cryogenic electron microscopy (cryo-TEM). The loading capability of the structures with respect to hydrophilic and hydrophobic substances is examined as well.

Novel fibrous materials based on polylactide and *Portulaca oleracea* plant extract: Preparation, physico-chemical and biological properties

Mariya Spasova*[1], Nikoleta Stoyanova[1], Nevena Manolova[1], Iliya Rashkov[1], Sabina Taneva[2], Svetlana Momchilova[2], Ani Georgieva[3]
[1]Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

Fibrous polylactide (PLA) materials containing plant extract of *Portulaca oleracea* obtained using supercritical carbon dioxide were successfully prepared by electrospinning. The effect of the extract concentration on the morphology and properties of the obtained materials was studied. *P. oleracea* extracts of 0–7.5 wt% in 10 wt% PLA solutions were electrospun. The optimal process conditions for the preparation of defect-free fibers were found. The newly obtained fibrous materials were fully characterized using scanning electron microscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry, TGA analysis, X-ray diffraction and water contact angle measurements. It was found that the incorporation of the crude extract had a significant effect on the average fiber diameter, thermal characteristics and structure of the obtained materials. Moreover, it was found that the electrospun PLA/*P. oleracea* materials showed high antioxidant activity. The cytocompatibility and biocompatibility of fibrous mats was assessed and it was found that the materials loaded with *P. oleracea* enhanced the cell viability of mouse BALB/c-3T3 fibroblasts. Thus, the obtained novel materials could be a potential candidate for tissue regeneration and wound healing applications.

Basic principles of final herbal products quality, according their regulatory frame of registration- Herbal medicines vs food supplements

Ioanna Chinou*
Faculty of Pharmacy, National and Kapodistrian University of Athens (NKUA), Greece

Through centuries several herbals have been used widely for their therapeutic properties, leading, after appropriate pharmacological studies have been conducted, to a big number of mostly pure molecules (isolated, or after synthesis) of developed well-known human medicines. Since last decades, public interest towards herbals, has increased dramatically all over the world.

Plants can be found as final products according to the direction to which have been marketed as: herbal medicines, food supplements (named botanicals in that case), cosmetics and/or medical devices. The legal requests for each one of them are dependable and their quality-control scheme differentiates accordingly

European Union has considered medicinal use of herbal products through mainly the Traditional Herbal Medicinal Products (THMPs) Directive (Directive 2004/24EC amending Directive 2001/83/EC as regards THMPs). The Herbal Medicinal Products Committee (HMPC) at the European Medicines Agency (EMA, Amsterdam NL) has adopted guidelines which are intended to support assessment of THMPs or WEUMPs (Well Establish Use Medicinal Products) considering their particular characteristics, while has established community monographs of herbal substances, available at EMA's website. All quality controls for herbal medicines, as also for all kind of medicines, are directed by Eur Pharmacopoeia (EDQM) while all other containing herbals final products' quality is slightly different according the kind of market's law they follow

In this framework, the quality of herbal medicines will be explained in details, while selected examples will be further presented and discussed, especially focused on Herbal medicines vs food supplements.

Advanced formulation approaches for delivery of water insoluble plant-derived bioactive molecules

Krassimir Velikov*
Unilever, Netherlands

The formulation of plant-derived bioactive molecules, phytochemicals, in the design of food and beverages brings enormous technological challenges and can compromise the product functionality. Water insoluble phytochemicals in particular are difficult to formulate and can create Issues related the product stability, appearance, texture, and bioavailability. This paper intends to present the general strategies in using colloidal dispersions as delivery systems for the formulation and delivery of water insoluble phytochemicals. Some illustrative examples on controlling solubility, particle shape and bioaccessibility will be discussed.

Stevia: Why this ancient plant has a key role in the sustainable and healthy substitution of sugar at a global scale?

Javier Sainz*
SWT Stevia, United Kingdom

Reducing the amount of sugar in our diets has been called the most important nutritional challenge of our time. Despite the critical impact such a change would have on our health, progress in developing healthy alternatives of natural origin is lagging behind in terms of developing economically efficient crops capable of providing the sweetness needed to replace relevant amounts of cane and beet sugars. In this presentation, we analyze the competitive advantage of stevia as a crop, the thorough development behind its production, compare its potential with available alternatives, and develop a case study to understand the importance of the entire supply chain to make sugar replacement a natural and healthy reality.

Phytochemical and Effect-directed analysis (EDA) as the basis of evidenced based natural products

Evelyn Wolfram*[1, 2]
[1] Natural Products and Phytopharmacy, Institute of Chemistry and Biotechnology, Zürich University of applied Sciences (ZHAW), Wädenswil, Switzerland
[2] planar4 GmbH, Staefa, Switzerland

Aim: The presentation gives an overview on application of HPTLC based Effect-directed Analysis for herbal products in different categories.
Methodology: Combination of High-Performance Thin Layer Chromatography (HPTLC) with Bioassays delivers chemical and activity information on separated natural multicompound mixtures. Bioassays applied are: DPPH radical scavenging over inhibition of *Candida albicans*, SOS-umuC genotoxicity screening to Yeast estrogen screen (planar YES) to identify endocrine disruptors.
Results: EDA-HPTLC fingerprint comparison delivers a combination of phytochemical fingerprints and bioactivity information. The plenary lecture shows examples of application for the natural product value chain in process and quality controls as well as pre-screening possibilities complementary to sophisticated and validated analytical (e.g. LC-MS) and in vitro bioassay methodologies. The focus will be laid on the challenges of false positive results.
Conclusions: Effect-directed Analysis allows for pre-screening of herbal, plant material and environmental samples and delivers information of quality and safety in one analysis step.
Acknowledgements: The work was supported by ZHAW and planar4 GmbH, Special acknowledgement to the ZHAW students Philippe Schläpfer, Ramona Schlicht and Stefan Weiss, Andreas Schönborn from planar4 GmbH

Mushrooms - their potential for medicine and a healthy nutrition

Ulrike Lindequist*
University Greifswald, Institute for Pharmacy, Greifswald, Germany

Since beginning of mankind nature is the most important source for medicine. Whereas herbal medicines constitute an important part of traditional and of evidence-based medicine worldwide the broad medicinal use of mushrooms was restricted to East Asian countries for a long time. Meanwhile the medicinal use of mushrooms, so called „medicinal mushrooms“, is increasing in many countries.

Medicinal mushrooms can be defined as macroscopic fungi, mostly higher Basidiomycetes, which are used in the form of extracts or powder for prevention, alleviation, or healing of diseases and/or for completion of our nutrition. Most important species are *Ganoderma lucidum*, *Coriolus versicolor* (L.:Fr.) Quél. (syn. *Trametes versicolor* [L.:Fr.] Pilát), *Lentinula edodes* (Berk.) Pegler, *Agaricus subrufescens* Peck, *Ophiocordyceps sinensis* (Berk.) G.H.Sung. J-M-Sung, Hywel-Jones & Spatafora., *Grifola frondosa* (Dicks.:Fr.) Gray, *Hericium erinaceus* (Bull.:Fr.)Pers., *Pleurotus* sp. and some others. For these mushrooms a lot of knowledge about *in vitro* activities, effects in animal assays, traditional experience and mode of action is available. We have also knowledge about several chemical constituents, e.g. polysaccharides, terpenes and nucleosides, responsible for pharmacological effects. Main fields of medicinal application, mostly as complementary therapy, are tumor diseases, neurological diseases, infections or gastrointestinal complaints. Unfortunately, the number of good clinical studies is yet limited.

Besides it is to note that edible mushrooms have a high nutritional value. They are rich in proteins and dietary fiber, possess all essential amino acids and with ergosterol a precursor of Vitamin D. Their content in energy is low.

The lecture gives an overview about the most important medicinal mushrooms, their chemical constituents, their pharmacological activities and their potential for medicine and healthy nutrition. The challenges on the way to high-quality products for medicine and nutrition will be addressed.

Plant Industrial By-Products as Sources of Bioactive Compounds with Potential Food and Cosmeceutical Applications

Dejan Stojković*
Institute for Biological Research “Siniša Stanković” — National Institute of Republic of Serbia, University of Belgrade, Serbia

In the food sector, 30% of the food that is produced annually is wasted globally. 1300 million tons of food, $1 billion in economic costs, $700 billion in environmental costs, and almost $900 billion in social costs total this. In addition, the fruits and vegetables' peels, leaves, roots, tubers, and seeds that are thrown away every year contribute to between 25% and 30% of the waste produced by the food sector. In Europe, one of the industries that produce a significant volume of bio-waste is the food industry. Food waste in Europe totals over 1.3 billion tons a year, of which 700 million tons come from agriculture. These waste materials, which largely come from the processing of cereals, fruits, and vegetables, have a significant potential for recycling.

Using increased conversion techniques, these leftovers can be converted into resources that could lead to the production of bio-products that are theoretically sustainable, including energy, fertilizers, materials, and molecules. In this regard, the valorization of residues and agri-food by-products is currently presented not only as a requirement, but also as a chance to gain new products of high value and of great impact on the economy of the industrial sector.

The use of kiwano peel extracts to obtain bioactive ingredients, eggplant fruit bio-residues, kiwi peels and pineapple peels and crown leaves is studied and presented. Findings reveal that all of these bio residual extracts from industrial bio-waste are good source of phenolic compounds with bioactive properties that could be effectively incorporated into food matrices.

Catmints (*Nepeta* sp.): Biological activities and applicative potential

Danijela Mišić*, Uroš Gašić, Branislav Šiler, Slavica Dmitrović, Marijana Skorić, Dragana Matekalo, Jasmina Nestorović Živković, Tijana Banjanac, Biljana Filipović, Milica Milutinović, Jelena Božunović, Neda Aničić, Luka Petrović, Miloš Todorović, Tamara Lukić
Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia, University of Belgrade, Serbia

The genus *Nepeta*, one of the largest genera of the Lamiaceae family (subfamily Nepetoidae, tribe Nepeteae) comprises around 250 herbaceous, perennial, rarely annual species, native to temperate Europe, Asia, North Africa, and the mountainous region of tropical Africa. Phytochemical investigations have revealed monoterpenes (iridoids), diterpenes, triterpenes, as well as phenolics, including flavonoids, to be the major specialized metabolites in these remarkable plants. The chemical richness of terpenoids and phenolics in catmints plays functional roles in nature, facilitating complex interactions with insects, phytopathogens, and other co-occurring plants. On the other hand, *Nepeta* species are used widely by humans as medicines, flavor additives, fragrances, and toys for cats, and their extensively studied bioactivities are mainly assigned to iridoid monoterpenoids nepetalactones. These compounds are proven repellents against insects (e.g. mosquitos, cockroaches, ticks, flies, mites, termites), and promising bioherbicides against a vast array of weeds (e.g. amaranth, ragweed, goosefoot, dandelion).

In spite of the plenitude of fundamental research that has documented the vast bioactive potential of *Nepeta* species, their commercial exploitation is restrained. There are several important factors that make the commercialization of *Nepeta* sp. most challenging, including limited bioresources, limited availability of bioactive compounds in purified forms, challenges to optimize the cultivation and standardize the quality of plant material, low number of studies focusing on the effects on non-target organisms and on the mutual relationships of individual compounds, instability and relatively low retainability of volatile nepetalactones, and others.

The challenge ahead is to provide sustainable sources of bioactive compounds of *Nepeta* sp. and thus establish a background for their commercialization. One of the solutions might be the creation of alternative sources of these biomolecules through synthetic biology approaches, thereby developing microbial or plant systems producing bioactive compounds of interest. Essential prerequisites to achieve this is the comprehensive and simultaneous acquiring, analyzing and interpreting the overall diversity of specialized metabolites within the genus *Nepeta*, reconstruction of the molecular background of the chemical diversity and elucidation of the biosynthetic pathways, as well as adopting the biotechnology tools to scale-up the production of the compounds of interest.

Acknowledgments: This research is supported by the Science Fund of the Republic of Serbia, within the project "Omics-guided disentangling of the iridoid diversity in the genus *Nepeta* L. for *in planta* metabolic engineering towards sustainable exploitation- NEPETOME" (2022-2025), Grant No. 7749433. The work is further supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, within the Grant No. 451-03-47/2023-01/ 200007

Exploration of value addition potential of waste biomass from medicinal and aromatic plants

Biraj Basak*, Rohan Sarkar
Indian Council of Agricultural Research (ICAR), Dept. of Agricultural Research and Education (DARE), Govt. of India

Agricultural and industrial sector based on medicinal and aromatic plants (MAPs) generates large quantity of residual biomass including both distillation residue as well as non-utilized part. The non-utilized part of medicinal plants and the distillation waste of aromatic plants are quite promising for extracting phytochemicals. Further use of residual biomass as raw material for producing animal feed, biogas, biochar, compost and biopesticides provides additional economic benefits to the MAPs stakeholders. Though lots of studies regarding dual utilization of residual biomass have been carried out, most of them indicated that the technologies were developed at the laboratory scale and therefore these need to be standardized for commercial exploitation by the industry. So, in the future, up-scaling of laboratory-based technology into industrial scale should be given prime importance. However, some areas still need to be addressed such as 1) development of more economically viable routes of biomass valorization like bio-coal, biofuel and commercial cellulose 2) up-scaling of potential laboratory-based technology into industrial scale, and 3) assessment of economic benefit of MAPs residual biomass valorization based on the integration of cost of valued products and cost involved in mitigating the environmental impact as a result of dumping or burning of residual biomass. The main focus of this presentation is the application of biorefinery model to harness the potential of MAPs residual biomass.

Oral presentations

Metabolic profile of *in vitro* cytokinin-treated and wild-grown *Nepeta nuda*

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Nepeta nuda L. also known as "catmint" is a perennial medicinal plant, widespread on mountain ranges and forest biomes in Bulgaria. The phytochemical composition of *N. nuda* includes a variety of active compounds, such as phenolic antioxidants, volatiles and other metabolites with potential role for the established antibacterial, antiviral, antioxidant, and antitumor activity.
In this study, we aimed to clarify how different *ex situ* conservation strategies impact the metabolic activity of *N. nuda* by comparison of wild-growing plants (flowers and leaves) and *in vitro* cultivated ones. In parallel, we monitored the effect of the phytohormone cytokinin 6-benzylaminopurine (BAP) as an elicitor of metabolic biosynthesis in *in vitro* conditions.
Comparison between the four variants showed an overall increase of phenolic antioxidants in flowers of wild-grown plants compared to *in vitro* control samples, and the cytokinin addition up scaled twice the phenolic content and DPPH radical-scavenging activity in comparison to flowers. Quantification by UHPLC/qqqMS2 instrument of major phenolics and iridoids revelaed that BAP induces significant increase of phenolic acids (caffeic and rosmarinic acids), flavonols (isoquercetin and astragalin), the iridoid epideoxyloganic acid, and the quinic acid. Further studies adopting GC-MS analysis revealed volatiles characteristic for *N. nuda*, such as germacrene D, caryophyllene and 1,8-cineole/eucalyptol, which were more abundant in samples from nature. On the other hand, the concentration of the main volatile compound in catmint`s essential oil, 4a-α,7-β,7a-α-nepetalactone, was increased under *in vitro* cultivation. In addition, the content of non-polar (fatty acids, alkanes, sterols) and polar (organic acids, amino acids, alcohols, sugars, phenolics) compounds was estimated. Enrichment in fatty acids, alkanes, certain sterols, amino acids and alcohols was observed in *in vitro* plants. The content of organic acids was significantly higher in plants from nature. Similarly to wild-grown plants, BAP induced the content of sugars and phenolic compounds.
In conclusion, the optimal metabolic potential of the species can be achieved in natural habitats, although the content of bioactive chemicals of *N. nuda* can be enhanced in *in vitro* conditions by the addition of the phytohormone BAP. Further ongoing research is focused on detailed investigation of biological activities and their correlation with specific compounds.

Drug loaded mixed polymeric micelles as antibiofilm agents

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In this work polymeric micelles from cationic poly(2-(dimethylamino)ethyl methacry-late)-b-poly(ε-caprolactone)-b-poly(2-(dimethylamino)ethyl methacrylate) and non-ionic poly(ethylene oxide)–b-poly(propylene oxide)–b-poly(ethylene oxide) triblock copolymers, as well as from their mixtures at different molar ratios were formed. The resulting mixed micelles were characterized by dynamic and electrophoretic light scattering. They were in nanoscale range with hydrodynamic diameter around 35 nm and the ζ-potential value strongly dependent on their composition. A model antibacterial agent was loaded into the micelles as the encapsulation efficiency and drug loading content of the polymeric carriers were determined by two independent methods. Both HPLC and UV spectrophotometry showed drug loading above 90% depending on micellar composition. A delayed drug release profile for all compositions over the period of 24 h was observed. Cytotoxicity of the resulting drug delivery systems was evaluated and a composition depended cell viability enhancement was observed without cell destruction or morphological signs of cell death. All micellar systems were capable to detach pre-formed bacterial biofilms and significantly reduced their biomass. The metabolic activity of the biofilm was strongly suppressed by the drug loaded mixed micelles indicating the successful drug delivery and release.

Poster presentations

Polymeric micelles of mixed composition as effective carriers for biomolecules

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Cationic polymer micelles have shown great potential as carriers of biomolecules. These are nanosized polymeric particles, consisting of a non-ionic hydrophobic core surrounded by a positively charged hydrophilic shell. Both, the core and the shell can be loaded with biologically active substances of different nature, composition, and properties. The cationic shell of the micelles, however, is typically associated with enhanced cytotoxicity. Furthermore, the strong electrostatic interactions between the positively charged shell segments and negatively charged cargo molecules frequently hinder the effective release of the latter. In this work, aiming at controlling the release of cargo molecules and reducing the cytotoxicity, we report on development of systems of mixed polymeric micelles. They were prepared by co-assembly of two distinctly different amphiphilic block copolymers that do not possess chemically identical moieties – a cationic diblock copolymer, poly(4-methyl-piperazin-1-yl)-propenone-b-polylactide, and a non-ionic triblock copolymer, poly(ethylene oxide)–b-poly(propylene oxide)–b-poly(ethylene oxide). Micelles, composed of a segregated hydrophobic core and a mixed shell of cationic poly(4-methyl-piperazin-1-yl)-propenone and non-ionic poly(ethylene oxide) segments, were spontaneously formed in aqueous solution. The resulting structures were characterized with size in the 60 – 120 nm range and moderately positive values of the ζ potential (around 16 mV), depending on the composition. They were successfully loaded with anionic biomolecules by electrostatic interactions with the cationic moieties in the shell. The hybrid copolymer-copolymer micelles exhibited high loading capacity and controllable release kinetics dependent on the nonionic block copolymer content. The cytotoxicity of the carriers as well as their ability to transfer biomolecules to target sites in cells were evaluated.

Role of RAGE receptors in the obestatin effect

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The aim of this research is to study the obestatin signaling on *in vitro* heart preparations of *Pelophylax ridibundus* via RAGE receptors. It is known that, the myocardial β-adrenoreceptors and cAMP-dependent protein kinase targets downstream are responsible for the observed positive inotropic effect of obestatin. The application of increasing obestatin concentration (1 nmol/l - 1000 nmol/l) significantly enhances the force of contraction of excised and cannulated frog hearts. *In vitro* preparations of excised frog hearts consist mainly of cardiac muscle tissue, endothelium and autonomic neurons, whose axon projections are forming long chains of varicosities placed closely to the muscle cells. Our data reveal the involvement of neuronal MAP kinase pathway in obestatin signaling in the heart. Activation of the intracellular signaling chain leads to an increase in cAMP and activation of PK A → MEK1/2 → ERK1/2 pathway. Administration of MEK1/2 inhibitor U0126 completely abolished the effect of obestatin. The activated ERK1/2 phosphorylate the key enzyme for adrenaline synthesis - tyrosine hydroxylase. This leads to the synthesis of new adrenaline molecules and their release in the synaptic terminal. This is confirmed by the lack of effect of obestatin in the presence of the administered tyrosine hydroxylase (3-IT).

The receptor for advanced glycation end products (RAGE) is a multi-ligand receptor that is able to bind several different ligands. Interaction between RAGE and its ligands activates various cellular processes, including inflammation, proliferation, apoptosis, autophagy, and migration. Expression of RAGE and its ligands is kept at a low level in a wide range of cell types, including endothelium, smooth muscle cells, mononuclear phagocytes, neurons, and cardiac myocytes. In addition to local actions in peripheral tissues and distant effects at the central level, obestatin exhibits various cellular effects. Evidence suggests that it increases proliferation and inhibits apoptosis and inflammation in various cell types. The use of RAP (RAGE antagonist peptide) in 10 μmol/l concentration did not abolish the inotropic effect of obestatin in frog heart at any used concentrations.

It is concluded that, the activation of the MAP-kinase signaling pathway by obestatin is not as a result of coupling to RAGE receptors and the obestatin is not a ligand for this receptor respectively.

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Effect of graphene oxide nanoparticles on rat mitochondrial ATPase and diamino oxidase activity

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Due to its superior physicochemical properties the oxidized derivative of graphene – graphene oxide (GO) is one of the most widely used carbon-based materials in electronic devices, biosensors, for energy storage and others. Recently in the biomedical field GO nanoparticles are considered as a promising drug delivery system in targeted cancer treatment. Prior to its clinical application nanomaterial's physicochemical characteristics and mechanisms of interactions with target cells should be precisely studied. Mitochondria appear to be one of the most sensitive targets for nanomaterials. A number of studies have focused on the cytotoxicity of nanoparticles with respect to changes in cell morphology, cell viability, metabolic activity, and oxidative stress. Limited studies have demonstrated nanoparticles-induced impairment of the mitochondrial function. There are no reports on direct or indirect effects of GO nanoparticles on the mitochondrial ATPase activity and diamine oxidase (DAO) activity.

Therefore, the aim of this work was to investigate the effects of GO nanoparticles in different concentrations (4, 10, 25, 50 and 100 μg/ml) on the ATPase activity of rat liver mitochondria and DAO activity.

The ATPase activity of intact mitochondria was not affected by GO when applied at a concentration of 100 μg/ml, which suggests that these nanoparticles do not possess a quick uncoupling effect on intact liver mitochondria. GO slightly reduced ATPase activity of DNP-uncoupled mitochondria. However, these data are not convincing enough to conclude that GO nanoparticles are able to pass readily through the inner mitochondrial membrane to affect the ATPase activity. GO nanoparticles at all tested concentrations significantly inhibited ATPase activity of freeze-thawed mitochondria in a dose-dependent manner (to 46.97 ± 6.59% of the control at the highest concentration of 100 μg/ml). All applied concentrations of GO induced a concentration-dependent increase in DAO activity compared to the control.

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Antiviral potential of plants from *Stachys* genus against Herpes viruses

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Human alphaherpesviruses are a group of viruses that can cause a number of infections in humans, including cold sores, genital herpes, and encephalitis. These viruses are widespread, with approximately two-thirds of the world's population under the age of 50 infected with herpes simplex virus type 1 (HSV-1) and approximately 491 million people aged 15-49 years infected with herpes simplex virus type 2 (HSV-2). Antiviral resistance to acyclovir and the increasing prevalence of these viral infections highlight the need to develop new antiviral drugs. Plants and their extracts offer a promising avenue for the discovery of new antiviral agents. In this study, we investigated the antiviral potential of two *Stachys* species, *Stachys scardica* and *Stachys bulgarica*, against human alphaherpes virus type 1 (HSV-1) and type 2 (HSV-2). *Stachys* is a genus of flowering plants in the mint family (Lamiaceae), which includes over 300 species. Many species of *Stachys* have been used in traditional medicine to treat various diseases, including infectious ones. *Stachys scardica* and *Stachys bulgarica* are two species that are native to the Balkan Peninsula and have been used in Balkan traditional medicine for their medicinal properties. Methanolic extracts from *in situ*, *in vitro* cultivated and *ex vitro* acclimated plants of *S. scardica* and *S. bulgarica* were tested for antiviral activity against HSV-1 and HSV-2.

Our results suggest that both species may have some antiviral potential against the extracellular form of the viral models, despite the weak (less than 99%) inactivation observed. Extracts from *in situ* plants showed slightly higher activity against HSV-2 than the other extracts. Although the observed activity is weak, it provides a basis for further research to identify the active compounds and optimize the extraction methods in order to improve the antiviral activity of these plants.

Natural sources of antiviral compounds have advantages over synthetic drugs. They demonstrate lower toxicity, higher bioavailability and lower risk of developing resistance. Therefore, the identification and characterization of antiviral compounds from the genus *Stachys* can be a basis for the development of new antiviral drugs. Our findings provide a basis for further research to investigate the antiviral potential of these plants and their active compounds, which may lead to the development of new and effective antiviral drugs.

ЕВРОПЕЙСКИ СЪЮЗ
ЕВРОПЕЙСКИ ФОНД ЗА РЕГИОНАЛНО РАЗВИТИЕ
ОПЕРАТИВНА ПРОГРАМА НАУКА И ОБРАЗОВАНИЕ ЗА ИНТЕЛИГЕНТЕН РАСТЕЖ

Biological activity of ferrite nanoparticles

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The two minerals Fe_2O_3 and Fe_3O_4 are naturally occurring iron oxides, but the difference lies in their chemical and physical properties and uses. The natural form of Fe_2O_3 is called hematite and that of Fe_3O_4 is called magnetite. Both are colored oxides, but of different colors, which are used as pigments and possess ferromagnetic properties. There are many different methods for obtaining magnetic nanoparticles from iron oxides, and most often this happens through physical, chemical and biological ways. Green synthesis is more advantageous than traditional chemical synthesis since it is economical, reduces pollution, and enhances environmental and human health safety.

In the 1970s, magnetic nanoparticles found their application as carriers. Nowadays, these kinds of magnetic nanomaterials are also used for cell therapy, sensors for various molecules and metabolites, magnetic separation and resonance, as antibacterial and antifungal agents, and as drug carriers. In addition to their antimicrobial action, they can be used to treat cancer because they have been shown not to harm human cells.

Magnetic nanoparticles are characterized by an increased surface area, which improves their chemical reactivity and mobility, due to their small size. Adhesion of nanoparticles to microbial cells, formation of reactive oxygen species and their penetration into cells are recognized as the most important features of antimicrobial action. Plant extracts from medicinal plants increase their stability and antimicrobial activity. Ferrite nanoparticles are interesting for their property to heat up in a magnetic field and lose their magnetic properties upon reaching 45°C. This quality is important in the detection and treatment of tumors, since the loss of magnetic properties at elevated temperature protects the surrounding healthy tissue from burning.

The nanoparticles entry into the environment must be monitored, as they can cause toxic effects on some aquatic inhabitants. Our experiments with *Daphnia magna* showed a 100% toxic effect of Fe_2O_3 at 48 h at a concentration of 0.005 mg/mL.

Biological effects of *Rosa damascena* extracts on cultured human fibroblasts

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Aim: In the present study, we investigated the biological effects on cultured human HSF fibroblasts of two probes: a total extracts of Rosa damascena after etheric oil production as well as one obtained by ethanol acetate and enriched in flavonoid glycosides.
Methods: In determining the effect on cell survival, treatment concentrations ranging from 0 to 1000 µg/ml for total and 0-300 µg/ml for enriched flavonoid glycosides were used. The treatment was carried out for 24 and 72 hours. Crystal violet staining, Tripan blue staining and flow cytometry was performed to evaluate biological effects on human skin fibroblasts. Changes in cell morphology were observed with treatment up to 2000 µg/ml.
Results: We found that the short-term treatment showed minimal cytotoxic effect for both extracts, with survival at the highest concentrations applied being 85% relative to control. Longer treatment with the total extract showed a dose-dependent suppression of survival or proliferation of HSF cells. To check the possibility of cell-cycle arrest, we perform flow-cytometry of cells threated with low dose (10 µg/ml) and the first effective dose of 100 µg/ml and found insignificant changes in cell cycle distribution of cells. The observed effect could be due to irreversible membrane damage and subsequent cell death. Concentrations up to 1000 µg/ml did not affect significantly cellular morphology, where doses over 1100 caused cellular shrinking and loss of typical fibroblast-like morphology of cells.
Conclusions: Extracts of *Rosa damascena* showed low cytotoxicity and insignificant effect on cell cycle, but affect cellular membrane of some cells and this leads to cell death after 72 h treatment.

Antifungal activity of Bulgarian plant populations of *Vaccinium vitis-idaea* L.

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The cranberry belongs to the group of functional foods and experimental studies prove its beneficial effect on individual functions in the body. Cranberry fruits are rich natural source of antioxidants - flavonoids, vitamins, minerals, essential fatty acids, carotenoids and phytosterols. The object of the present study were the Bulgarian plant populations of *Vaccinium vitis-idaea* L. from the natural high-mountain locations of wild cranberries in different regions of Bulgaria. There are 4 types of wild cranberries in Bulgaria: Caucasian (*Vaccinium arctostaphylos*), blue (*Vaccinium uliginosum*), red (*Vaccinium vitis-idaea* L.) and black (*Vaccinium myrtillus*). We tested antimicrobial activity of plant material - "Stara planina - Beklemeto" and "Rhodope - Perelik" against two yeast species. From the data obtained, fraction of flavonoids demonstrated highly pronounced antimicrobial activity against the two yeast test cultures - *Candida lusitaniae* and *Rhodotorula sp.* A wide variety was observed among the MIC values concerning the yeast test cultures: from no inhibition against *Rhodotorula* sp. to MIC 4.5 mg/ml of phenolic acids fraction. In general this fraction showed better performance compared to fraction flavonoids. Compared to the positive control - commercial preparation nystatin (156 µg/ml), it exceeds it by 53.8% - 66.7%. The analysis of samples of plant material "Rhodope - Perelik" showed a similar trend of highest activity of flavonoid fraction, followed by phenolic acid fraction and base extract. Quantitatively, the highest values (for flavonoids) were on the order of 20%–85% higher than those of the positive control.

In vitro and *in vivo* antioxidant capacity of ethanolic extracts of *Cuscuta* spp.

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Members of the genus *Cuscuta* (Convolvulaceae) are prominent holoparasitic plants with substantial agricultural and ecological effect. They are also known in the traditional medicine as valuable medicinal plants due to their relatively high content of various flavonoids and other compounds with biological activity. However, not all species are equally potent in their activity. For example the Chinese dodder *C. chinensis,* known as Tu Si Zi is often mistaken with the similar in appearance, but less valuable *C. australis*. The present study aims to evaluate the antioxidant activity of ethanolic extracts of three native and one introduced Bulgarian species, differing in their distribution and host range. First, the antioxidant activity was tested with the $ABTS^{\cdot+}$ assay, showing that all four extracts are potent radical scavengers with *C. epithymum* being the most active and *C. campestris* (the introduced species) - the less active. They were further tested *in vivo* on *Saccharomyces cereviseae* NBIMCC 537 experimental system, based on their ability to recover growth under H_2O_2 treatment. Although all tested extracts exhibited a certain degree of recovery of the yeasts' growth, although not corresponding to the ABTS assay results. Therefore, we suggested that along with the antioxidant properties, the extracts may also possess a different degree of antimicrobial activity. Although further studies are needed to establish the chemical constituents, and most importantly the influence of host species on the parasite antioxidative properties, this pilot study demonstrates the high potential of Bulgarian *Cuscuta* species as a source of compounds with biological activity.

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In vitro cultivation of *Stevia rebaudiana* by adding silver salts of amino acid-based nanofibers

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Stevia rebaudiana Bertoni is a valuable medicinal plant with wide application in the pharmaceutical and food industry. It is a source of sweet components (steviol glycosides), vitamins, minerals, amino acids, fatty acids, flavonoids, phenolic compounds, etc.

The increasing need for obtaining high biomass and secondary metabolites necessitates the use of biotechnological approaches for mass propagation of the plant. The present study describes the effect of Ag salts of two types of amino acid-based nanofibers on some biometric characteristics and accumulation of phenols, flavonoids and soluble sugars in *Stevia* grown *in vitro*.

Ag+ ions are well known as an antimicrobial agent, an ethylene inhibitor, and a factor with a stimulatory effect on some morphological and biochemical parameters in plant tissue culture. In this research, the Ag+ ions were bound to amino acid nanofibers forming salts: Ag salt of decanoyl-L-Aspartic acid-N-hexylamide (NF1-Ag salt) and Ag salt of N,N′-Bis (N-decanoyl-L-Asparagine) diamino hexane (NF2-Ag salt). This implies the gradual release of Ag+ ions in the medium.

Construction of genetic linkage map of lavender (*Lavandula angustifolia* Mill.) based on SSR markers

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Lavender (*Lavandula angustifolia* Mill.) is an essential oil-bearing and medicinal plant of great economic and social importance to Bulgaria. Despite the growing industrial cultivation and interest of using new lavender varieties, no genetic map of the lavender was reported so far. This largely hampered the QTL analysis and application of marker assisted selection. Here we present the first genetic linkage map of lavender based on microsatellite (SSR) markers. The poster presents the results of development of SSR markers based on next generation sequencing of DNA derived from Bulgaria most popular lavender varieties, var. Hemus, as well application of selected SSR marker set for genotyping of segregating population derived from self-pollinated var. Hemus plants. The genetic linkage of the tested SSR markers and span of the identified linkage groups are reported. The next increase of map density by including SRAP markers is discussed.

Targeting of secondary metabolite productivity through conventional tissue culture techniques. Experience in the *Hypericum, Artemisia* and *Sideritis* species

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The Balkan Peninsula is considered one of the most important centers of European plant biodiversity, expressed in rich flora and high endemism percentage. This phenomenon is determined by numerous factors such as the role of the region as a glacial refugium, leading to the survival of many relict taxa, its topographic complexity and richness of habitat types, diversity, as well as the stability of environmental conditions.

An *in vitro* collection of medicinal and aromatic plants derived from the richness of the Balkan flora was established. A wide spectrum of conventional plant cell tissue and organ techniques have been experimented on representatives of the *Hypericum* genus, the Balkan endemic *Sideritis scardica* Griseb. and essential oil bearing *Artemisia alba* Turra. This work summarizes the approaches established for the targeted delivery of biologically active compounds of diverse chemical type such as hypericins, phenolics, flavonoids, phenylethanoid glycosides, as well as essential oils from different types of *in vitro* lines of the studied species.

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Development of a strategy for obtaining highly productive *Nepeta nuda in vitro* cultures

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The *in vitro* cultivation of plants and their subsequent *ex vitro* adaption back to nature allow rapid propagation of plants with identical genome. This technique could be applied for conservation of endangered species, as well as for assuring material for analyses of plants with medical potential and respective extraction of valuable phytochemicals. Furthermore, the *in vitro* growth gives the opportunity to study plants under controlled environment deprived of stress factors. In these conditions, the bioactive potential is strongly reduced, and phytoeffectors with different nature could be investigated. The developmental and environmental signals (growth and differentiation, light, temperature, nutrients, etc.) are transduced in the cell by plant hormones. The aim of this study was to elaborate a strategy for obtaining highly productive *in vitro* cultures of the catmint *Nepeta nuda* (Lamiaceae) by screening the effect of various factors, such as combinations of plant hormones, type of explant, illumination conditions, and the number of passages in *in vitro* culture. Among the hormones, series of concentrations of four auxins (2.4-D, Picloram, IAA, NAA) and three cytokinins (BAP, Kinetin, TDZ) have been tested for biomass production, accumulation of phenolic compounds and related DPPH radical-scavenging activity. The presence of Picloram and TDZ induced fine callus of undifferentiated cells suitable for cell cultures, however, the phenolic antioxidants were reduced. On the other hand, combinations between IAA, NAA, and BAP, TDZ, showed significant increase in biomass and phenolics. Petiole and stem explants demonstrated much higher proliferation ability than leaves. The optimal established procedure was to perform the first explant cultivation in dark where good callus formation occurred. These dark-grown explants were again transferred to a new medium and split into light or dark conditions, as this time the ones grown in light gave improved results. In comparison to untreated *in vitro* plants, the applied biotechnological strategy led to nearly 10 folds increase in total content of phenolics. Further studies on the corresponding biological activities, e.g. antiviral, antibacterial, would assist the selection of best variants and the identification of specific bioactive compounds. In conclusion, the strategy of using elicitors for increased metabolic rate of cell cultures has the perspective for up scaled accumulation of molecules of pharmacological interest.

Assessment of state of cenopopulations of the *Erysimum croceum* of the Trans-Ili Alatau

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The population of *Erysimum croceum* in the Trans-Ili Alatau was studied for the first time. This study was carried out in a comprehensive manner using modern geobotanical and floral methods. Age states and density of individuals within the population were determined. The article describes three coenopopulations in two populations of *E. croceum* M. Pop.

The protection of rare, endemic plants is currently given great attention. One of such rare, endemic plants registered in the Red Data Book of Kazakhstan is *E. croceum*. However, to date, there are no special studies devoted to the study of the population of this unique plant. The aim of the work was to find the location of *E. croceum* and study its population using modern methods of geobotanical and floristic research and to assess its current state.

The ecological-cenotic confinement of *E. croceum* cenopopulations was revealed. The floristic composition of plant communities with its participation was also studied.

The first population of *E. croceum* was found at the foot of a high hill in one of the left branches of the Small Almaty Gorge. It is located on the southeastern exposure of the middle part of the hill. The area is small, length - 150-200 m, width - no more than 100-150 m. Within the population, we described two cenopopulations (CP 1 and CP 2).

CP 1. The vegetation cover is represented by a bulb-catchment and mixed herb association (ass. *Hedysarum flavum, Alchemilla sibirica, Silene wallichiana, Cerastium tianschanicum. Aquilegia atrovinosa, Allium atrosanguinenum, A. turkestanicum, A. schoenoprasoides*). The total projective cover is 95-100%. The vegetation cover has a three-tier structure. The first tier is *A. turcestanica*, *A. atrovinosa, H. flavum* 70-90 cm high, the second tier is *C. tianschanicum, P. stepposa, Potentilla evestita, Myosotis palustris* 30-60 cm high, the third tier is *Polygala hybrida, Sedum hybridum, Alchemilla sibirica* 15-25 cm high.CP 2. The vegetation cover is represented by a mixed herb and cereal association (ass. *Alopecurus pratensis, Fastuca supina, P. stepposa, Dactylis glomerata - H. flavum, Alchemilla sibirica, A. atrovinosa, C. tianschanicum*). The total projective cover is 85-90%. The vegetation cover has a four-tier structure. The first tier is *Trisetum sibiricum, Dactylis glomerata, Rheum wittrockii, Rumex acetosa* 100-130 cm high, the second tier is *Chamaenerion angustifolium, A. atrovinosa, Polygonum songoricum, Erysimum croceum, Valeriana turkestanica* 60-90 cm high, the third tier is *Potentilla nervosa, A. atrosanguineum, P. stepposa* 30-55 cm high, fourth tier - *Thymus marschellianus, M. palustris, Potentilla nivea, S. hybridum* 15-25 cm high.

CP 3. The vegetation cover is represented by a mixed herb and cereal association with the participation of *Picea schrenkiana* (ass. *Poa nemoralis, Poa pretense, D. glomerata, C. tianschanicum, Silene pseudotenuis, Solidago virgaurea, Lamium album, Geranium collinum, Chamaenerion angustifolium*). The total projective cover is 95-100%. In the vegetation cover, a five-tier structure is observed. The first layer is *P. schrenkiana* up to 50 m high, the second tree layer is *Sorbus tianschanica* 3-5 m high. The third shrub layer is *Rosa alberti, Lonicera hispida, Spiraea hypericifolia* 100-140 cm high. The fourth layer is *Milium effusum, D. glomerata, A. atrovinosa, Lathyrus gmelinii* 70-90 cm high. Fifth tier – *Solidago virgaurea, Erigeron seravschanicus, G. collinum, H. flavum* 35-65 cm high.

Of the life forms, hemicryptophytes predominate - 62 species (60.2%). In second place are therophytes - 12 species (11.6%). Nanomicrophanerophytes are represented by 9 species (8.7%). Of the macrophanerophytes, there are 2 species - *P. schrenkiana* and *S. tianschanica*. Lianas are represented by one species - *Atragene sibirica*.

Of the ecological types, mesophytes predominate - 90-95% of the floristic composition. Xeromesophytes are represented by single species. There are petrophilous species, these include *S. hybridum*. Xerophytes, hydrophytes absent.

Within population 2, we identified 13 groups of useful plants. The first place among them is occupied by anti-erosion plants. Second place is occupied by fodder plants - more than half of the floristic composition. Of these, 22 species (21.4%) are eaten excellently by livestock, 20 species (19.4%) - quite well, 33 species (32.0%) - satisfactorily. 16 species (15.5%) are badly eaten by livestock (only when there is no food). 12 species (11.6%) of plants are not eaten by livestock. In third place is the group of medicinal and honey plants with 13 species each (25.2% taken together). The fourth place is occupied by groups of weeds and poisonous plants with 12 species in each (together 22.3%). In particular, *P. schrenkiana* is at the same time a technical, tannic, ornamental and anti-erosion plant; *S. tianschanica* - food, vitamin, tanning and ornamental plant; *Rubus caesius* - food, vitamin, medicinal and honey plant; *Arctium tomentosum* - medicinal, food, honey, oil plant; *Taraxacum officinale* is a medicinal, melliferous, food and weed plant.

Phenolic compounds in waste after distillation of *Geranium macrorrhizum* L.

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The genus *Geranium* is represented in the Bulgarian flora by 23 species but only *Geranium macrorrhizum* L. is used for production of aromatic products in Bulgaria. The essential oil is known as “zdravets” oil, different than *Pelargonium ssp.* derived "geranium oil”. Waste after steam and water distillation of *Geranium macrorrhizum* aerial parts were analyzed for bioactive compounds by GC/MS and TLC. Methanol extracts and ethyl acetate fractions after alkaline hydrolyze of the studied materials were obtained. Total phenolic content was determined by Folin-Ciocalteu’s reagent. Extracts and fractions were examined for free radical scavenging activity by DPPH assay.

Flavonoid aglycones (kaempferol 3-methyl ether, kaempferol 3,4'-dimethyl ether, quercetin, quercetin 3-methyl ether, quercetin 3,7,3',4'-tetramethyl ether) and glycosides (hyperoside) were detected by TLC. Gallic acid was found as the major component of the extracts and fraction by GC/MS analysis. The higher total phenolic content was found in the fractions than methanolic extracts. More that phenolic content was determined to be higher in the extracts and fractions obtained after steam than water distillation. Significant free radical scavenging activity was established for extracts and fractions but the last exhibited stronger antiradical activity.

The received data showed that waste after steam and water distillation of Bulgarian “zdravets” is a rich source of flavonoids and gallic acid, compounds with significant antiradical activity.

Assessment of biological activity of rose oil distillation waste water (RODW) using different *in vitro* cell culture lines

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Assessing the biological activity of rose oil distillation wastewater (RODW) using different cell lines, such as BJ (normal human fibroblasts), MRC-5 (human lung fibroblasts), and SaOs-2 (human osteosarcoma cells), can provide a more comprehensive understanding of the potential effects of RODW on various cell types and its future applications. This new data will be useful for clarification of the potential effect in cosmetic products, skincare, understand its biological effects or its role as potential pharmaceutical substance for human and animal application. The experiments were performed with: non-tumor cells BJ (normal human fibroblasts), MRC-5 (human lung fibroblasts) and tumor cell line SaOs-2 (human osteosarcoma cells) cell cultures as model systems for evaluated biological effect of the RODW.
The studies were carried out by methods with different cellular / molecular targets and mechanisms of action, such as: cytotoxicity assays (MTT) for evaluated cell viability and proliferation and JC-10 assay is a widely used technique to assess mitochondrial membrane potential in live cells. The double staining with Acridine orange (AO) and propidium iodide (PI) was used for to assess cell viability and apoptosis and pathomorphological analysis on treated cells with RODW, as well as statistical analysis.
The investigations were performed - the cells were cultured in different concentration of RODW for 24h and 48h intervals of incubation.
Our results revealed that show cell-specific response: 1) A cell-specific response is found - the relatively higher sensitivity the MRC-5 fibroblast are more sensitive than SaOs-2 and BJ cells; 2) Cell viability decreases with time of exposure of RODW; 3) Cell viability of SaOs-2 remains comparable to the control at 48h at the highest concentration of cell treatment.
Examination of the cytopathological changes by double staining with acridine orange and propidium iodide AO/PI showed that no changes were observed in the morphology of the treated cells treated with 20µg/ml; RODW administered at a concentration of 50-100 µg/ml- slightly reduced cell monolayer with cells with preserved cell membrane, agglomeration and cell inclusions were observed.
In conclusion, assessing the biological activity of rose oil distillation wastewater using different cell lines (BJ, MRC-5, and SaOs-2) needs additional investigation for understanding of the potential effects of RODW on various cell types and cells mechanism of action. By evaluating cytotoxicity, cellular morphology, researchers can determine the potential benefits and drawbacks of using RODW.
Acknowledgements: КП-06 ПМ66/7/ BG-175467353-2022-03-0066 „ Model systems of animal cell lines for evaluation of cytotoxicity effect and antimicrobial activity of plant extracts and essential oils with potential for application in pig breeding.

Echinacea species introduced in Bulgaria as a source of caftaric and chicoric acids

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A comparative study of the caftaric and chicoric acid content in plant material of *Echinacea species* from the IRAP Genofund, in different phases of vegetation, and in different parts of the plant, was conducted by HPLC. Sample 1 is a two years old plant from a population of *Echiancea purpurea*, Sample 2 is a one-year old plant of *Echiancea purpurea* (origin Germany) and Sample 3 is a four-year old plant of *Echinacea pallida* (origin Germany).

In Sample 1, chicoric and caftaric acids accumulate equally in the aerial parts during all three phases of development, while in the root their amount is the highest in the phase of seed formation. In aerial parts of Sample 2, chicoric acid accumulation peaked in the vegetation phase compared to the mass flowering and seed formation phases. The same tendency was observed in the root accumulation of both chicoric and caftaric acid from Sample 2. The highest amount of caftaric acid was found in the phase of mass flowering. In Sample 3 both acids' concentrations were higher in flowers and leaves compared to stems and roots. Both acids' concentrations are comparable to those found in the stems of Sample 1 and Sample 2 during mass flowering phase. The concentrations of chicoric and caftaric acids in the root of Sample 3, however, remain significantly lower.

The species of *Echinacea* introduced in Bulgaria are standardized based on the amount of caftaric and chicoric acids they contain. According to European Pharmacopoeia 8.0, their amount in the aerial parts should be no less than 0.1%, and 0.5% in the roots respectively. Our experimental results show that the sum of the acids in the aerial parts ranges from 2.5% to 3.7%, and in the roots it varies from 1.8% to 3.9% during the different phases of vegetation.

Solketal reactions with ibuprofen - formation and characterization of novel products

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Using alcohols as solvents is known to increase the solubility of active pharmaceutical ingredients in various pharmaceutical formulations especially ones intended for external use. One such solvent that gained popularity in recent years is the glycerol ketal with acetone that is considered a novel bio-based “green” solvent and a promising potential alternative to some harmful solvents due to being miscible with water and organic solvents in the polarity range from alcohols to hydrocarbons. It is commercially known as solketal and its chemical name is 2,2-dimethyl-1,3-dioxolane-4-methanol or 1,2-isopropylideneglycerol.
Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) which has non-narcotic, analgetic and antipyretic action. Its chemical name is (RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid and it has a carboxylic group that can react with alcohols and form esters.
The possible products of the reaction of ibuprofen, as the acid, with the free hydroxyl group within the solketal structure have been studied in this work. In addition, solketal is known to dissociate in acidic media to its starting materials (glycerol and acetone) that opens the possibility for formation of more than one product in the reaction of solketal with ibuprofen. A mixture of ibuprofen and solketal was prepared in mole ratio 1:1 and subjected to different degradation and extractive procedures, from mild to extensive. The products were first separated by RP-HPLC and characterized by mass spectrometry after atmospheric pressure chemical ionization (APCI). Also, column chromatography on silica was further used for isolation of the products that were then characterized by NMR.
Based on the obtained ^{1}H and ^{13}C NMR data, two products were characterized as: 1-monoglycerol ester of ibuprofen and 2- a solketal ester of ibuprofen (with a 1,3-dioxolane ring), the latter one being reported for the first time as a possible product in forced degradation of topical formulations of ibuprofen containing solketal.

Fatty acids composition of Purslane (*Portulaca oleracea* L.) leaves

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Purslane (*Portulaca oleracea* L.) is a succulent annual plant widespread all over the world, but mostly in tropical and subtropical zones. It has been used in traditional medicine since ancient times as a remedy for skin inflammations, liver, kidney, stomach, respiratory and other problems. Nowadays there are also scientific evidences for its beneficial effects on people with diabetes and high blood cholesterol levels. Besides as a medicinal plant, it is consumed as a fresh salad and ingredient in various dishes and baked goods.

Purslane is known as one of the richest sources of the essential omega-3 C18:3 (alpha linolenic) fatty acid among green leafy plants. This fatty acid is much more abundant in the leaves compared to the stems. Therefore, the aim of our study was to determine and compare the fatty acids composition of purslane leaves extracted by four different methods: two procedures using chloroform-methanol mixtures, hexane extraction in Soxhlette apparatus, and the green method with super-critical carbon dioxide. In the extracts, fourteen fatty acids were identified and measured, the amount of which depended on the respective extraction procedure. High content (30–34%) of alpha linolenic (omega-3 C18:3) fatty acid was found after solvents extraction, whereas supercritical CO_2 ensured only 13%. Similarly, the other main fatty acid C18:2 (linoleic) was measured in levels of 31-33% and 12%, respectively in solvents and supercritical CO_2 extracts. On the other hand, the long-chain saturated fatty acids C20:0, C22:0, C24:0 and C26:0 were extracted by supercritical CO_2 more than twice better than by the other solvents.

Thus, it can be summarized that organic solvents ensure extracts enriched with the essential omega-3 and omega-6 fatty acids (more than 55%), whereas the supercritical CO_2 extraction yields more than 60% long-chain saturated fatty acids, the latter with beneficial effects for decreasing of metabolic syndrome risk.

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Green extraction of rosmarinic acid from lemon balm (*Melissa officinalis* L.) with subcritical water

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Rosmarinic acid (RA), an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid is a potent radical scavenger, chelator of prooxidant ions and inhibitor of lipid peroxidation. RA-containing extracts are widely used natural antioxidants in food products, and many herbal preparations and food supplements, containing RA, are marketed with claims for health effects. The current study investigated the effectiveness of subcritical water extraction (SWE) for the recovery of RA from lemon balm (*Melissa officinalis*), as a “green” alternative to conventional hydro-alcoholic extraction. Different duration (10 min and 20 min) and extraction temperatures (100°C and 150°C) were applied, and the obtained subcritical water extracts were characterized for their content of RA, along with other phenolic constituents, and antioxidant activity by ORAC and HORAC methods. Subcritical water applied at temperature 100°C was equally efficient as ethanol in extracting RA. However, the further elevation of temperature to 150°C decreased RA content by up to 20% due to its thermal degradation. Content of RA in dried extracts was between 2.36% and 5.55% and the higher temperature of SWE increased extract yield by up to 41%. The higher extraction yield resulted from the degradation of plant material from subcritical water as evidenced by the increased extraction and degradation of proteins, pectin and cellulose. These results reveal that SWE is an efficient technology for the extraction of RA and other antioxidants from lemon balm, at reduced extraction time and without the use of toxic organic solvents. Furthermore, by modification of SWE conditions dry extracts with different purity and content of RA could be obtained. These extracts could be used in the food industry as food antioxidants, or in the development of food supplements and functional foods

Comparison of Different Techniques for Extraction of Sesquiterpene Lactones from *Inula helenium* Roots

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Inula helenium L. is a medicinal plant that is officially listed in some European pharmacopoeias. The roots of this plant are rich in eudesmane-type sesquiterpene lactones such as alantolactone (AL) and isoalantolactone (IAL) which displayed various pharmacological properties, such as hepatoprotective, anti-inflammatory, antitumor, antibacterial, antidematophytic, antifungal and anticancer properties. Based on this information, the efficiency of the extraction technique is very important on recovering of bioactive sesquiterpene lactones AL and IAL from *Inula helenium* roots. For this purpose, the current study was focused on the extraction of sesquiterpene lactones using high pressure homogenizer (HPH) and ultrasonic probe (UAE). Maceration was applied as a conventional technique and these techniques were compared in terms of extraction efficiency, time, energy and solvent consumption. Single-factor experiments were conducted for HPH, UAE and maceration. Highest AL and IAL yields (38.2±0.7 mg/g and 34.4±0.2 mg/g) were obtained at HPH at 90 MPa pressure and 4 passes, followed by maceration for 5 days (26.9±0.4 mg/g and 30.7±0.3 mg/g) and UAE (24.3±0.2 mg/g and 23.1±0.1mg/g) with 70% of ultrasound amplitude for 3 min. On the point of energy efficiency HPH was determined as the most efficient method among other methods. UAE afforded good yields of AL and IAL in shortest time, while maceration was determined as the least efficient method due to its long extraction time. On the other hand, the lowest solvent consumption was observed in the maceration, while the highest was in the HPH. Consequently, HPH and UAE demonstrated to be a sustainable alternative to conventional extraction, showing the potential to increase the extraction yield and to decrease the extraction time and energy consumption.

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