

**BOOK OF ABSTRACTS**  
**THE 2<sup>ND</sup> BALKANS - CHINA**  
**MINI-SYMPOSIUM ON NATURAL**  
**PRODUCTS AND DRUG DISCOVERY**



МИНИСТАРСТВО ПРОСВЕТЕ,  
НАУКЕ И ТЕХНОЛОШКОГ РАЗВОЈА



**11-13 April, 2019**  
**Belgrade, Serbia**

**Institute for Biological Research "Siniša Stanković",  
University of Belgrade, Belgrade, Serbia**

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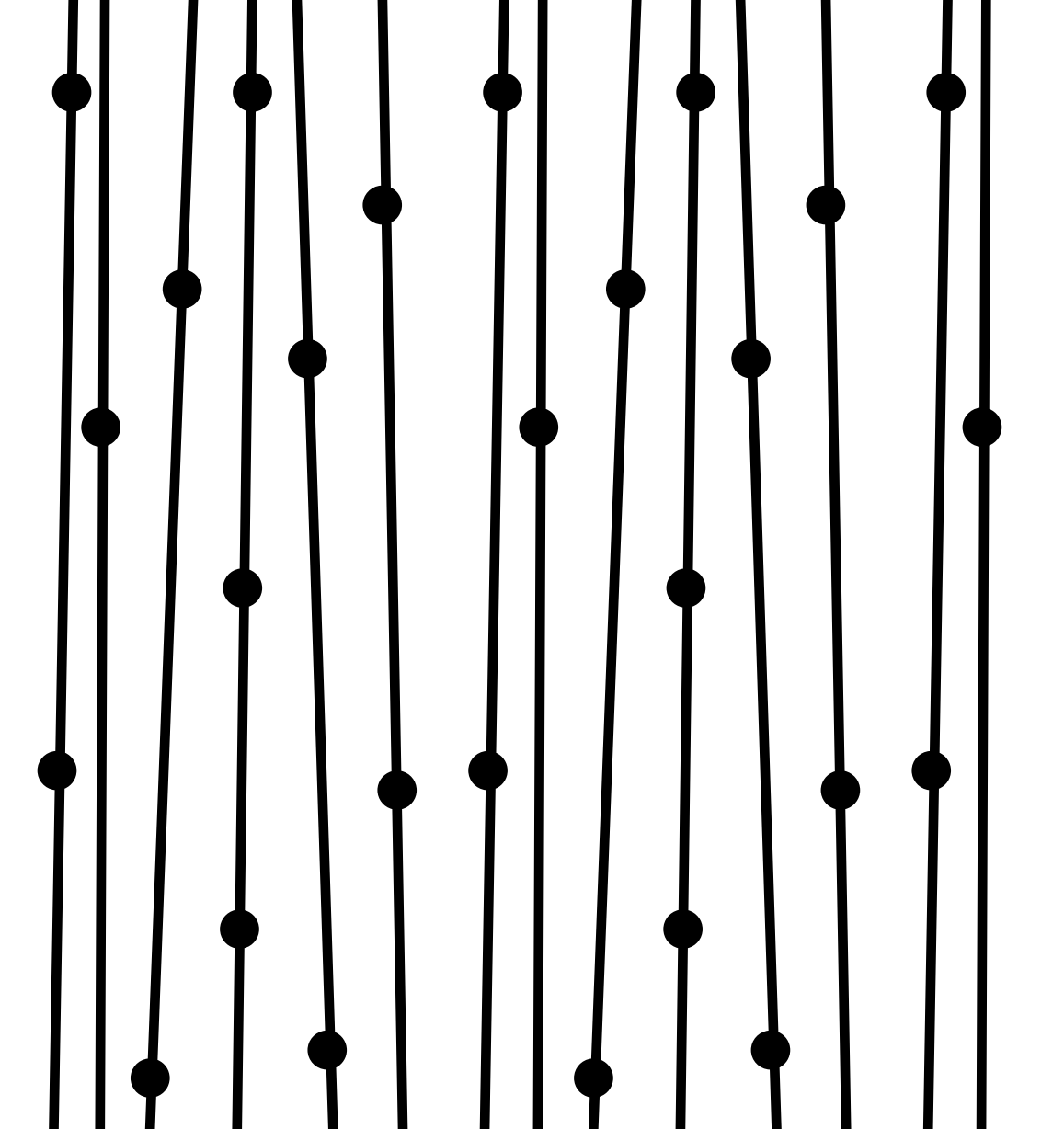
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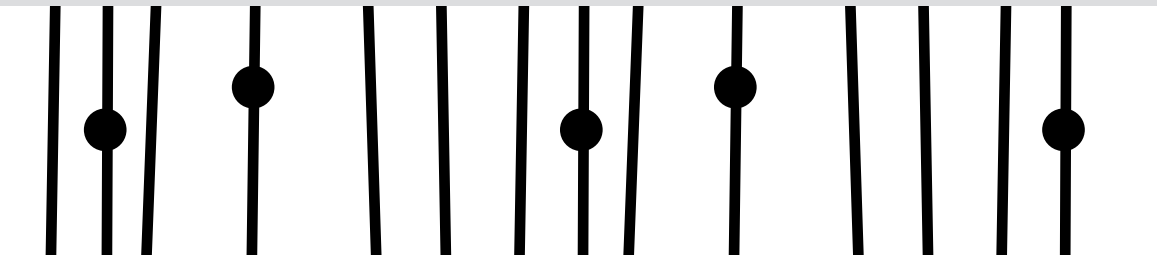
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**PROGRAMME**



Thursday 11<sup>th</sup> April

08:00 - 09:00	<i>Registration</i>	Rectory building University of Belgrade
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*Chairs:* Yang Ye & Marina Soković

09:00 - 09:15	<p>Welcome Remarks  <b>Ivanka POPOVIĆ</b>                  Rector                  University of Belgrade, Serbia</p>
09:15 - 09:25	<p>Opening speech  <b>Mladen ŠARČEVIĆ</b>                  Minister                  Ministry of Education, Science and Technological Development,                  Serbia</p>
09:25 - 09:35	<p>Keynote Speech  <b>Wang JING</b>                  Full-time Deputy Director                  Office for advancing Shanghai's Science and Innovation                  Center Development, China</p>
09:35 - 09:45	<p>Keynote Speech  <b>Vladimir POPOVIĆ</b>                  State Secretary                  Ministry of Education, Science and Technological Development,                  Serbia</p>
09:45 - 10:00	<p>An introduction of Chinese Academy of Sciences (CAS)                  and CAS Shanghai Branch  <b>Ronghui QU</b>                  Vice President                  Chinese Academy of Sciences, Shanghai Branch, China</p>
10:00 - 10:10	<p>Opening speech  <b>Marina SOKOVIĆ</b>                  Principal Research Fellow, IBISS, Serbia</p>
10:10 - 10:20	<i>Signing Ceremony</i>
10:20 - 10:30	<i>Group photography</i>
10:30 - 11:00	<i>Coffee break</i>

## Section 1 Natural products in food quality

*Moderators:* Lijiang Xuan & Marina Soković

11:00 - 11:15	Exploitation of essential oils and hydrosol for the preservation of fresh produce: Quality and Safety issues <b>Nikos TZORTZAKIS</b> , Cyprus
11:15 - 11:30	Polyphenolic profile of honeys from Serbia <b>Živoslav TEŠIĆ</b> , Serbia
11:30 - 11:45	The Role of Mushrooms as Functional Food and Dietary supplements <b>Miomir NIKŠIĆ</b> , Serbia
11:45 - 12:00	Bio-based products from lactic acid bacteria <b>Aleksandra ĐUKIĆ-VUKOVIĆ</b> , Serbia
12:00 - 12:15	Slaughterhouse blood: from wasted by-product of meat industry to added-value products <b>Branko BUGARSKI</b> , Serbia
12:15 - 12:25	Hypericin in traditional herbal medicine Hyperici oleum? <b>Veronika STOILKOVSKA</b> , R. North Macedonia
12:25 - 12:35	Polysaccharides from <i>Inonotus obliquus</i> cultivated mycelium and sterile conk and mycelial cultivation with sea buckthorn press cake <b>Gabriele BELTRAME</b> , Finland
12:35 - 12:50	A viewpoint of encapsulation technologies for value - added food <b>Viktor NEDOVIĆ</b> , Serbia
12:50 - 14:00	<b>Lunch</b> Garden, Rectory building



Section 2 Medicinal plants and fungi

*Chairs:* Ana Ćirić & Yang Ye

14:00 - 14:15	Research on Health Effects of Sea Buckthorn at University of Turku <b>Baoru YANG</b> , Finland
14:15 - 14:30	Investigations of selected groups from the Balkan flora - fundamental and applicative aspects <b>Petar MARIN</b> , Serbia
14:30 - 14:45	Pharmacognostic overview of some <i>Gentiana</i> , <i>Gentianella</i> and <i>Swertia</i> species from Central Balkan <b>Katarina ŠAVIKIN</b> , Serbia
14:45 - 15:00	Drought and salinity stress due to Climate Change alter the physiology and biochemistry of Medicinal and Aromatic plants <b>Antonios CHRYSARGYRIS</b> , Cyprus
15:00 - 15:10	Ethnobotanical study of medicinal plants traditionally used in South-East Serbia (Pčinja district) <b>Jelena ŽIVKOVIĆ</b> , Serbia
15:10 - 16:00	<i>Coffee break</i>
16:00 - 16:15	Organic mushroom growing technology – challenge for the science and practice <b>Ivanka MILENKOVIĆ</b> , Serbia
16:15 - 16:30	Cultivation of arnica ( <i>Arnica montana</i> L.) in various fertilization and propagation models <b>Dejan PLJEVLJAKUŠIĆ</b> , Serbia
16:30 - 16:45	Valuable sources of natural compounds in the Mediterranean: the case of Greek flora <b>Spyridon PETROPOULOS</b> , Greece
16:45 - 17:00	Legalization of Medical Cannabis in Macedonia - how far we have reached <b>Gjoshe STEFKOV</b> , R. North Macedonia
17:00 - 19:00	<i>Dinner</i> Garden, Rectory building

Friday 12<sup>th</sup> April

## Section 3 Bioactive natural products

**Chairs:** Katarina Šavikin & Gjoshe Stefkov

09:00 - 09:15	Identification of bioactive diterpenoids from traditional Chinese medicines <b>Yang YE</b> , China
09:15 - 09:30	Nutritive and therapeutic properties of selected wild growing mushrooms from Serbia <b>Jovana PETROVIĆ</b> , Serbia
09:30 - 09:45	Utilizing the indigenous biosynthetic capacity of medicinal and aromatic plants through optimization of tissue culture conditions <b>Kalina DANOVA</b> , Bulgaria
09:45 - 10:00	Edible and medicinal fungi inhibit enzymes linked to diabetes type-2 therapy <b>Dejan STOJKOVIĆ</b> , Serbia
10:00 - 10:15	Comparative study of antiproliferative potential of three different plant species <b>Danijela DRAKULIĆ</b> , Serbia
10:15 - 10:30	Iridoids of <i>Centaurium</i> and <i>Nepeta</i> species: exploring their diversity, metabolism, ecophysiological roles and bioactivities <b>Danijela MIŠIĆ</b> , Serbia
10:30 - 11:30	<b>Coffee break</b>
11:30 - 11:45	Plant and mushroom extracts as potential intervening supplements in diabetes and diabetic complications <b>Jelena ARAMBAŠIĆ JOVANOVIĆ</b> , Serbia
11:45 - 12:00	Phytochemical investigations of <i>Salvia transsylvanica</i> , <i>Salvia glutinosa</i> , and <i>Salvia officinalis</i> from Romania and their bioactivities <b>Andrei MOCAN</b> , Romania
12:00 - 12:15	Freeze dried <i>Salvia officinalis</i> methanolic extract incorporated into nanostructured lipid carriers for Alzheimer's disease treatment <b>Elena MARKOVA</b> , R. North Macedonia
12:15 - 12:30	The pygidial gland secretions of ground beetles (Insecta: Coleoptera: Carabidae): antimicrobial and antitumour activity of the natural products <b>Srećko ĆURČIĆ</b> , Serbia
12:30 - 14:00	<b>Lunch</b> Garden, Rectory building

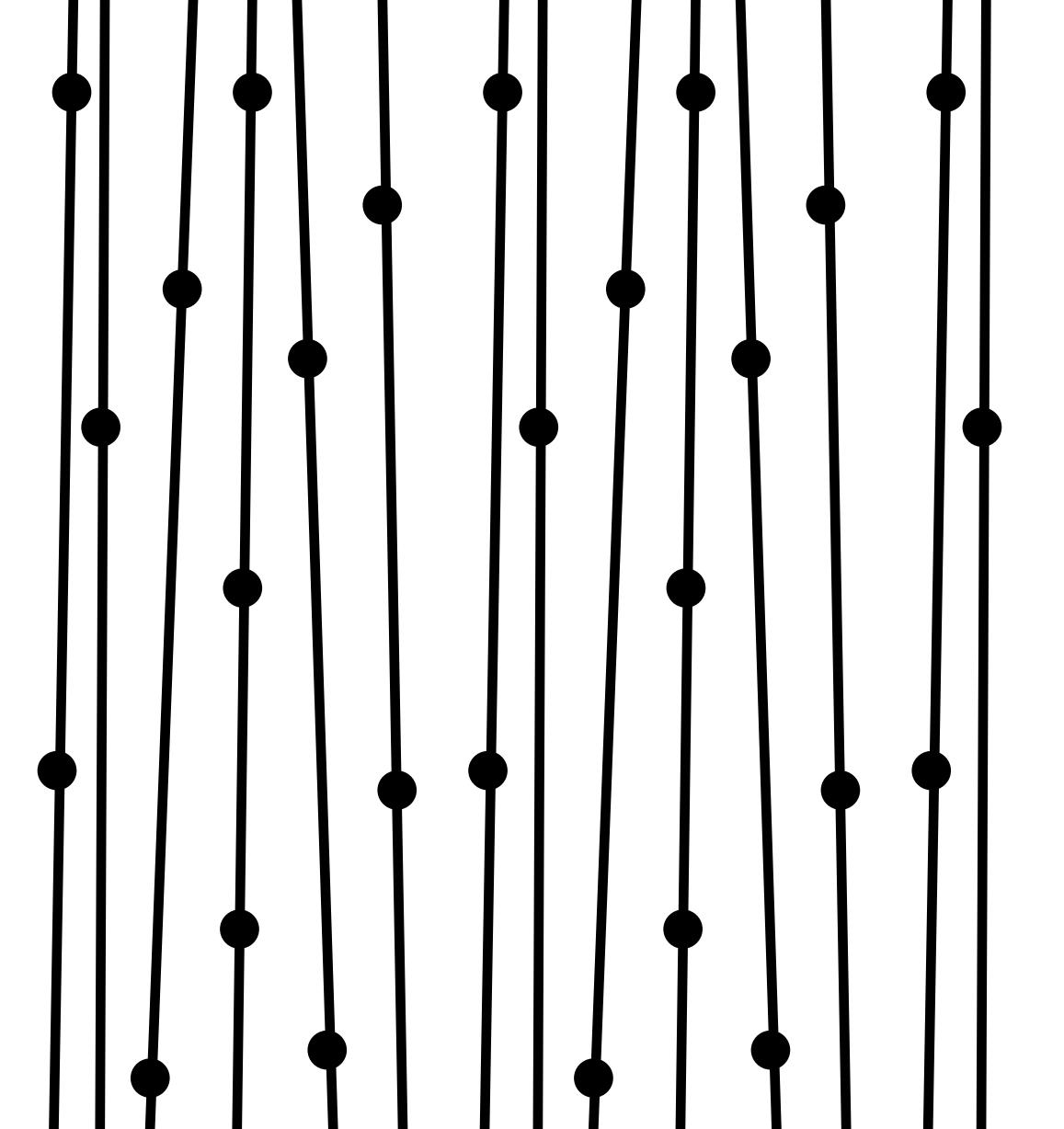
Section 4 Drug discovery

*Chairs:* Viktor Nedović & Jasmina Glamočlija

14:00 - 14:15	13-year-R&D and 13-year-postmarketing, a long and risky road to be a blockbuster in clinic <b>Lijiang XUAN</b> , China
14:15 - 14:30	C–H Activation: a Late Stage Functionalization Tool for Drug Discovery <b>Huixiong DAI</b> , China
14:30 - 14:45	Quantifying randomness in real interaction networks and examples in biology <b>Marija MITROVIĆ DANKULOV</b> , Serbia
14:45 - 15:00	High throughout antimycobacterial drug screening platform and study of mechanisms of action of antimycobacterial drugs <b>Tianyu ZHANG</b> , China
15:00 - 15:15	GPCR–targeted Drug Discovery <b>Xin XIE</b> , China
15:15 - 16:00	<i>Coffee break</i>
16:00 - 16:15	Bavachinin, a novel natural pan-PPAR agonist, exhibits unique synergism with synthetic PPAR- $\alpha$ -and - $\gamma$ activators <b>Yiming LI</b> , China
16:15 - 16:30	Discovery of hits/lead compounds based on natural product-like libraries generated from chromones <b>Chunhao YANG</b> , China
16:30 - 16:45	Spin textures in molecular magnets and designed polypeptides <b>Dimitrije STEPANENKO</b> , Serbia
16:45 - 17:00	Concluding remarks <b>Yang YE, Marina SOKOVIĆ</b>
18:00	<i>Closing ceremony</i> Gala dinner, Restaurant Vizantija

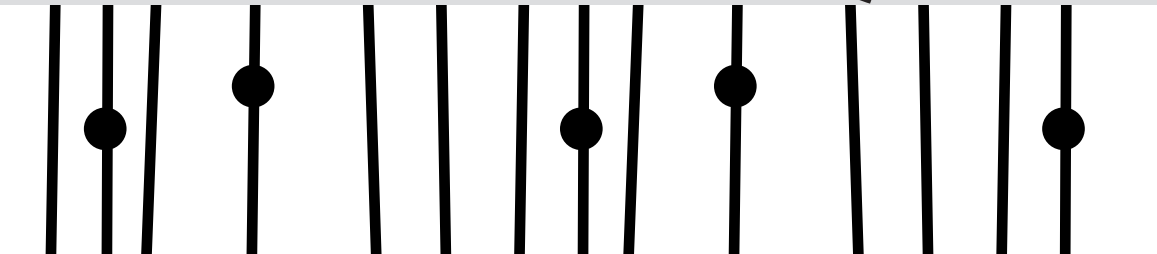
Saturday 13<sup>th</sup> April

10:00	<i>Excursion</i>	<i>Roman city and legionary fort Viminacium, Smederevo</i>
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# SECTION 1

NATURAL PRODUCTS IN FOOD QUALITY



## Exploitation of essential oils and hydrosol for the preservation of fresh produce: Quality and Safety issues

Nikos Tzortzakis, Panayiota Xylia, Antonios Chrysargyris

Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3036, Limassol, Cyprus.

Postharvest losses of fruit and vegetables are considerable, with chemical applications to be of great consumer concerns regarding food safety. Alternative sanitizers are under investigation, with natural compounds such as essential oils (EOs) and hydrosol (Hyd) to achieve scientific and consumer's interest for the preservation of fresh produce. Both EOs and Hyd pose antimicrobial and antioxidant properties while EO application has been stated as GRAS. Dittany (*Origanum dictamnus*) essential oil (50 ppm) suppressed disease (*Botrytis cinerea*) development by reducing lesion growth and fungal sporulation in pepper, tomato and eggplant fruits stored at 12 °C and 95% RH. *In vitro*, fungal development was completely inhibited by the application of 100 or 250 ppm of EO volatiles. Moreover, different washing treatments with aqueous solutions of marjoram (*Origanum majorana*) EO (1:1500 v/v), marjoram Hyd (1:15 v/v), ascorbic acid (AA) (1%) and their respective combinations maintained the quality of shredded carrot under storage at 4 °C for 9 days. Marjoram Hyd resulted in darker carrot colour and increased respiration at the 6<sup>th</sup> and 9<sup>th</sup> days of storage. Decay incidents as observed by the total viable counts and yeast and filamentous fungi counts were decreased by single or combined treatment during storage. Similarly, mint (*Metha piperita*) EO (1:1000 v/v) and mint Hyd (1:10 v/v) application on shredded carrot decreased the microbial load of *Escherichia coli* and *Listeria monocytogenes* on the 6<sup>th</sup> day of storage. Mint Hyd increased the total phenolics and antioxidants of carrots while quality attributes were maintained in general. Overall, the results suggest that EO and Hyd may be considered as an alternative food preservative treatment, significantly reducing or eliminating decay/pathogens infection during fresh produce storage.

**Keywords:** vegetables; essential oil; fungal growth; natural products; quality-related attributes; volatiles; food borne pathogens

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## Polyphenolic profile of honeys from Serbia

Živoslav Tešić

*Faculty of Chemistry, University of Belgrade*

Polyphenols represent an important group of secondary plant metabolites. Although they are found in honey in small quantities, they can provide important information on botanical origin. Their presence in honey originates primarily from nectar and also from pollen and propolis. Serbia has a long tradition in the production of honey. The most important kinds of honey from this area are lime, acacia, sunflower as well as polyphloral honey. That was the reason to perform a study on this kind of honey with much detail. Polyphenolic profiles of unifloral Serbian honeys, propolis, bee pollens, and nectar were analyzed using Ultra-High-Performance Liquid Chromatography (UHPLC) coupled with a hybrid mass spectrometer which combines the Linear Trap Quadrupole (LTQ) and OrbiTrap mass analyzer. Rapid UHPLC method was developed in combination with a high sensitivity accurate mass scan and a simultaneous data-dependent scan. The detection was performed using heated electrospray ionization (H-ESI) in negative mode. Polyphenol profiles nectar, propolis, bee pollen, and honey can provide important information on the botanical origin of honey (1). Glycosides are significantly present in the nectar and pollen, unlike the propolis and honey. Glycosyl profile provides important information on the botanical origin of honey. Flavonol rhamnosides are proposed as markers of acacia honey since they were not detected in any other type of honey. Chlorogenic acid has been proposed as a potential marker for acacia honey, and ellagic acid as a possible marker for rapeseed honey. Eriodictyol together with quercetin could be suggested as floral markers for sunflower honey in Serbia (2).

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2. Gašić, U., Kečkeš, S., Dabić, D., Trifković, J., MilojkovićOpsenica, D., Natić, M., & Tešić, Ž. (2014) *Food Chem.* 145, 599–607.

## The Role of Mushrooms as Functional Food and Dietary Supplements

Anita Klaus, Milena Savić, Jovana Vunduk, Miomir Nikšić

*Institute for Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, Belgrade 11080, Serbia, Email: miomir.niksic@gmail.com*

Mushrooms were prized for centuries for their taste and texture, but also as high value foods that are rich source of proteins, fibers, vitamins and minerals. Many edible and certain non-edible mushrooms have valuable health benefits contain biologically active compounds like polysaccharides but also phenolic compounds. More than 2000 mushrooms are thought to possess approximately 130 medicinal functions, including antioxidant, anti-hypercholesterolemia, antimicrobial, antioxidative, antitumor, immunomodulatory and other activities.

On the market, there are already many products based on the fungi extracts. Based on the chosen extraction solvent, the type and concentration of bioactive compounds varies in the final extract, which consequently, reflects on the spectrum of their pharmacological activities. But mushrooms are undervalued in spite that they have huge potential for providing health value. The main reason is likely that the active ingredients of mushrooms are too complex for synthesis or is it that we did not more plunge into the molecular structure. Dietary supplements are flooding the market but consumers are becoming increasingly mistrustful and seeking proof for health claims made and to identify the active ingredients. Also regulatory requirements when making health claims with respect to dietary supplements became more and more complex and stricter in Europe but also in China. For these reasons, it is important to pay more attention of extension of the use of mushrooms as the food and beverage mass products.

We discussed the use of mushrooms in mass production of food and beverages and showed our results with plum, grape, grain and herb brandies, their influence on aging, colors and other sensory characteristics and also biological activities. The possible use of Ganoderma in brewing as a raw material for the production of beer with improved functional properties was discussed.

Research in other food products are also explored like coffee, mushrooms enriched with selenium, soft drinks; products like cookies, bars etc., all with some additional nutritional properties.

**Keywords:** mushrooms, functional food, biological activity, dietary supplements

## Bio-based products from lactic acid bacteria

**Aleksandra Djukić-Vuković<sup>1\*</sup>, Dragana Mladenović<sup>1</sup>, Ljiljana Mojović<sup>1</sup>**

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Lactic acid bacteria (LAB) are a diverse group of microorganisms with lactic acid production as a common characteristic. LAB are part of the human diet from the first days of life, as part of breast milk microbiota, throughout life. Since ancient times, LAB ferment dairy and meat products, beverages or cereals contributing to preservation, flavor and texture of food, but also to a health status of the host, since many are probiotics. Their capability to interact with host immunity and colonize gut makes them particularly interesting as vectors for oral vaccine, bacteriophage therapy, synthetic biology and for a personalized approach to treatment.

Production of lactic acid by LAB from renewable substrates is another extremely active field of research today. Cheaper and more sustainable production of lactic acid is a goal. Lactic acid as a platform chemical can be valorized in different ways, but currently the highest expansion is in the field of poly-lactic acid polymer production. Poly-lactic acid polymers are the most represented biocompatible and biodegradable plastic used for various applications, including biomedicine.

Other compounds like exopolysaccharides are naturally excreted by LAB, with the aim to protect cells and to help retention on surfaces. These characteristics are promising for their application as excipients for pharmaceutical formulations. LAB are also producing bacteriocines, antimicrobial peptides which enable colonization of the gut. Live LAB biomass or some of these products or even attenuated LAB biomass has proven successful in altering response of the host to the stressful environmental condition.

LAB participate in the symbiosis of the human body and its microbiota interacting through metabolism, immunity, bioavailability and biotransformation of the food and drugs we take. Additionally, microbial communities are dynamic and different in every person. This leads to the untapped potential for their exploitation and necessity to better understand their role both inside and outside the human body.

**Keywords:** lactic acid, probiotics, biomass, exopolysaccharides, bacteriocines, personalized approach to treatment



## Slaughterhouse blood: from wasted by-product of meat industry to added-value products

Branko Bugarski<sup>1</sup>, Ivana Drvenica<sup>2</sup>, Ana Stančić<sup>2</sup>, Vesna Ilić<sup>2</sup>

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Blood is the first by-product of meat industry, which is usually treated as waste and discarded. High chemical oxygen demand (500,000mg O<sub>2</sub>/l) of the disposal of large quantities of slaughterhouse blood creates enormous environmental issues. On the other hand, slaughterhouse blood has the potential to be collected and processed to generate high-added-value pharmaceutical and food ingredients, based on its exceptional nutritive value and excellent functional properties (Lynch *et al.* 2017). Therefore, in line with a growing global pressure on the food industry to try to improve sustainability in recent years, great attention is directed towards new technologies for recovery and optimum utilization of slaughterhouse blood.

We are pointing out the technology for usage of slaughterhouse blood erythrocytes as an inexpensive starting material in production of membranes of erythrocytes (i.e. ghosts) as drug delivery systems and hemoglobin (Hb) as heme-iron supplement. Developed process of gradual hypotonic hemolysis is based on the specific osmotic properties of erythrocytes from bovine and porcine slaughterhouse blood (Bugarski and Dovezenski 2000, Kostić *et al.* 2014). This process was evaluated as an encapsulation procedure for dexamethasone sodium phosphate and sodium diclofenac into the erythrocyte ghosts. Due to the possibility of scaling up the process, it is designed in such a way that only low-priced buffer systems are used. Obtained formulations have shown strong potential for biomedical application in terms of prolonged drug delivery, by demonstrating sustained drugs release over 3 days *in vitro* (Drvenica *et al.* 2016, Bukara *et al.* 2016). Providing high hemolysis extent (>90%), the same process allows isolation of Hb molecules free of membrane contaminant (Stojanović *et al.* 2012). Hb additionally purified by tangential ultrafiltration modulated proliferation/differentiation of committed hematopoietic stem cells *in vitro*, indicating its potential in prevention/treatment of anemia. It also modulated proliferation, migration and differentiation of mesenchymal stem cells (Stančić *et al.* 2018). These data reveal Hb as a good candidate for stem cell culture media component as well.

Besides obtained added-value products by developed cost effective process, these investigations gave new fundamental insights in less known biological functions of erythrocyte membranes and extracellular hemoglobin.

**Keywords:** gradual hemolysis; erythrocytes; hemoglobin; erythrocyte membranes; drug delivery systems, stem cell cultures; heme-iron

**References:**

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## Hypericin in traditional herbal medicine Hyperici oleum?

Veronika Stoilkovska<sup>1</sup>, Jelena Acevska<sup>2</sup>, Gjose Stefkov<sup>1</sup>

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Quality control of herbal medicines is based on selection of one or more chemical markers in the herbal preparations or herbal drugs. Selection criteria must be made considering the roles and physicochemical factors which may affect the effective use of chemical markers. According to the German Commission E and Swiss Pharmacopoeia St. John's Wort Oil (Hyperici Oleum) is official herbal preparation and spectrophotometric determination is suggested as quality control parameter where hypericin content is expressed as sum of all derivatives of hypericin like hypericin, pseudohypericin, protohypericin and isohypericin (Anyževska *et al.*, 2010, EMEA/HPMC/101303/2008). The aim of this study was to develop HPLC method for quantitative determination of hypericin in St. John's Wort oil using solid phase extraction as sample preparation method and compare it to the official spectrophotometric method. SPE procedure using aminopropylsilica extraction cartridges was successfully applied for separating hypericin and other phytocomponents from vegetable oil solution. For HPLC analysis Ph.Eur. analytical method for quantitative determination of total hypericins (hypericin and pseudohypericin) given in Hyperici herbae extractum siccum, quantificatum monograph was chosen and appropriately modified. Hypericin was not detected in St. John's Wort Oil by the proposed HPLC method, although the content of hypericin derivatives, determined by spectrophotometric method, was in compliance with the Ph. Helv. 11.0. Analysis of the obtained results suggests possibility that the target component hypericin may undergo chemical modification as unidentified peaks were detected in the chromatogram of St. John's Wort Oil. Further analysis of different preparation methods for St. John's Wort Oil and their affection to hypericin content, stability testing of hypericin in vegetable oil, as well as quantitative and qualitative determination of the possible degradation products of hypericin are required.

## Polysaccharides from *Inonotus obliquus* cultivated mycelium and sterile conk and mycelial cultivation with sea buckthorn press cake

G. Beltrame<sup>1</sup>, Z. Han<sup>2</sup>, B. Yang<sup>1</sup>

<sup>1</sup>Food Chemistry and Food Development, Department of Biochemistry, University of Turku, Finland

<sup>2</sup>Institute of Microbiology, Heilongjiang Academy of Sciences, Harbin, China

*Inonotus obliquus* (Fr.) Pilat is a Basidiomycete, obligate parasite of birch trees. It is distributed in the northern hemisphere, in particular in subarctic areas. It is a common species of Finnish forests (1).

The fungus produces a conk eruption from the bark, called Chaga, which is a renowned folk remedy. Polysaccharides possess a prominent role among its bioactive compounds (2).

The exploitation of Chaga is hampered by conk slow growth. A promising cheaper alternative for Chaga polysaccharides production is mycelium submerged cultivation. For future nutraceutical implementation, and due to the different production method, polysaccharides obtained from submerged cultivation require throughout investigation and comparison with conk extract.

In our study, a Chinese strain of *I. obliquus* was cultivated and polysaccharides were extracted with hot water. Polysaccharides were extracted from Chinese wild sterile conk, with the same procedure, and from Finnish sterile conk, as comparison. After purification, sugar content, monomer composition, and molecular weight of polysaccharides were determined.

Our results showed that conk extracts contained low molecular weight polysaccharides, mainly composed of xylose. In contrast, mycelium polysaccharides were both of low and high molecular weight and consisted mainly of glucose. Differences between Finnish and Chinese conk extracts were noted as well.

Aiming to increase the mycelium yield for polysaccharide extraction, sea buckthorn press cake was added to the medium. Its influence on fungal macromolecular composition will be the subject of future studies.

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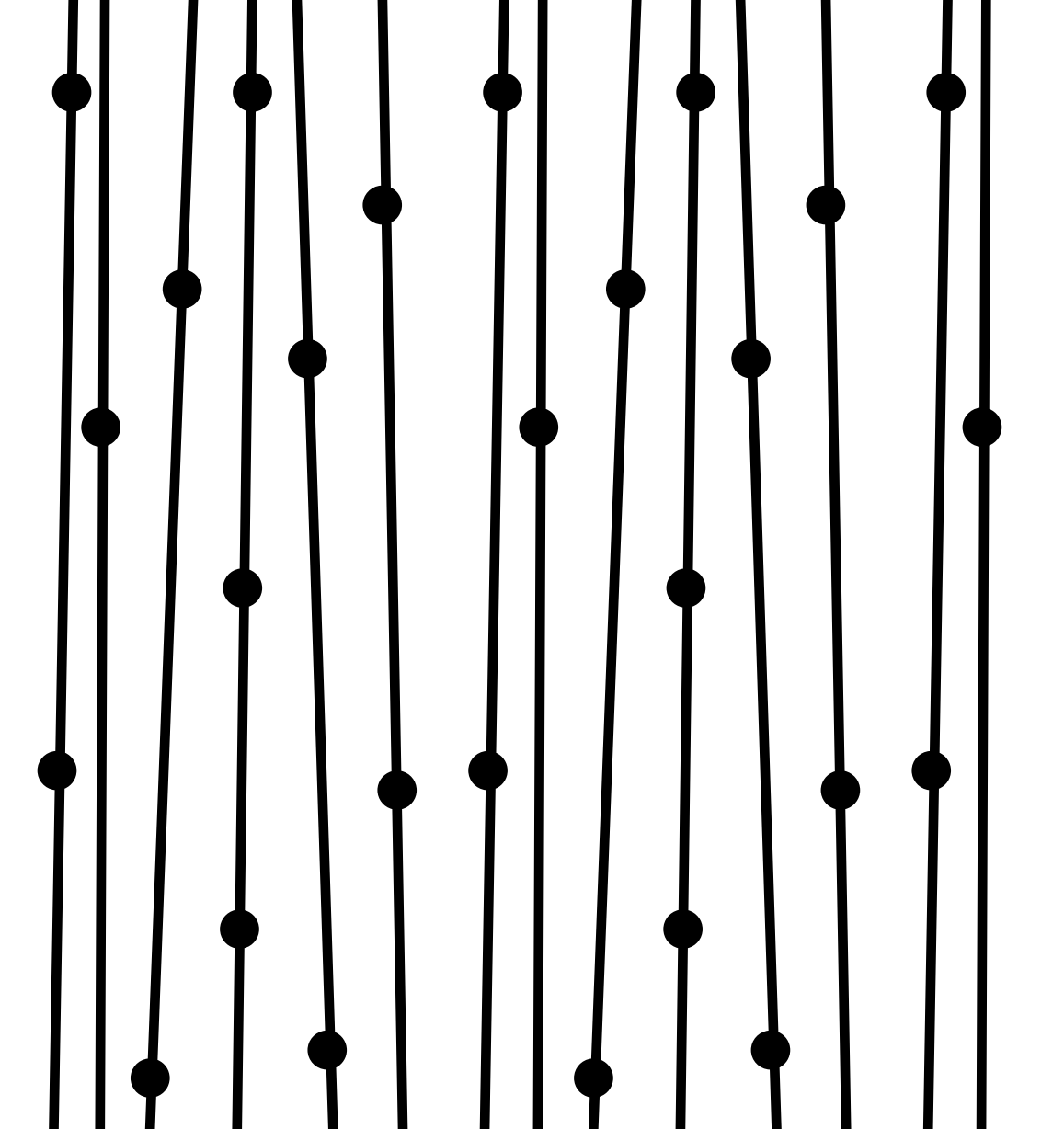
## **A viewpoint of encapsulation technologies for food with added value**

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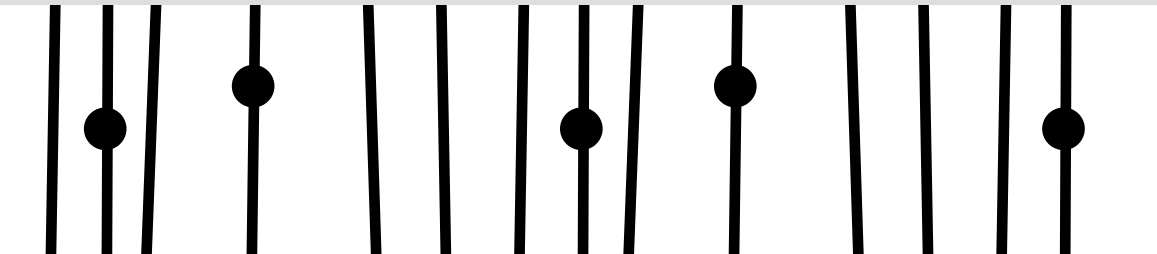
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Food industry is faced with challenges towards production of high quality food with enhanced safety, improvement of process efficiency and reduction of environmental pollution. In this sense, encapsulation, a process to entrap active agents within a carrier material, presents a promising approach for many applications. For example, in development of functional food products, encapsulation of bioactive compounds, such as vitamins, minerals, polyphenols and omega-3-fatty acids, has a great interest as a way to overcome their poor stability and susceptibility to adverse external factors and detrimental food processing conditions. Also, it promotes higher bioavailability and controlled release of these compounds. In addition, encapsulation protects viability and functionality of probiotic cells. Additional benefits of encapsulation are less evaporation of volatile compounds, such as aroma and taste masking, such as astringency and bitterness of polyphenols. Encapsulation may also be used to immobilize cells or enzymes in processing applications, such as fermentation and metabolite production. In addition, incorporation of active agents with antioxidant and/or antimicrobial properties, such as plant extracts and essential oils, within a biodegradable material set off innovations in food packaging. In this context, concept of active food packaging has been developed with the aim to enhance quality and safety of a food product and reduce environmental pollution related to plastic packaging deposition. For encapsulation of active compounds, a wide variety of carrier materials and techniques may be used depending on the type of actives of interest, functionality and specific application. The selection criteria of a carrier material is based on those certified as generally recognized as safe (GRAS) for food applications. In that term, biopolymers are mainly used. Principles, advantageous and limitations of many encapsulation techniques for food applications, such as spray drying, spray cooling/chilling, fluidized bed coating, freeze drying, extrusion techniques, encapsulation in liposomes and cyclodextrins, are known. Future research aspects should be focused on modeling of encapsulation technologies to industrial requirements.



## SECTION 2

### MEDICINAL PLANTS AND FUNGI



## Research on Health Effects of Sea Buckthorn at University of Turku

### Baoru Yang

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University of Turku (UTU) has a long history of sea buckthorn research of close to half a century. During the past three decades, sea buckthorn research at UTU has covered multiple areas of basic and applied sciences in food chemistry, food development and technology, bioactivities and health effects, plant biochemistry and physiology of sea buckthorn. The cross-disciplinary research has resulted in significant new findings that have been published as large number of scientific publications in high quality international journals in food sciences and nutrition. The research and industrial cooperation has enhanced utilization and commercialization of sea buckthorn products in the international market.

An important focus of the research of the Food Chemistry and Food Development group at UTU has been the health promoting properties of sea buckthorn. The research methods have included *in vitro* models and animal experiment, but most importantly human clinical studies including both long-term intervention studies and post-prandial research. The research of UTU has demonstrated potential of sea buckthorn berries and sea buckthorn products in management of chronic health problems such as allergy, diabetes and metabolic syndrome, and cardiovascular diseases by regulating immune function and sugar and lipid metabolism as well as by combating oxidative stress.

Application of the state-of-art methods of metabolomics has made it possible to identify new biochemical markers of physiological impact of sea buckthorn.

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## Investigations of selected groups from the Balkan flora – fundamental and applicative aspects

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Balkan Peninsula, as a part of Mediterranean region - one of important world centers of plant diversity, is very rich from floristic aspect, as a result of influences of various ecological and historical factors. High diversity of plants in this region, including a number of endemic and relict species, represents valuable basis for taxonomic and phylogenetic investigations and also natural source of new biologically active compounds.

From the fundamental aspect the main goal was to find new additional characters that could solve phylogenetic relationships and taxonomy of taxa wild-growing in Balkan region, using micromorphological, phytochemical, molecular and other methods. From the applicative aspect the goal was the extraction, isolation and structure elucidation of new biologically active compounds from selected species, which could be applied in pharmacy, medicine, cosmetics and nutrition.

The species are selected according to the several criteria: unresolved taxonomic and phylogenetic position, economic importance, presence of desirable specialized metabolites, possible biological activity of different extracts and components. Special attention is paid to the investigation of representatives of Lamiaceae (*Thymus*, *Satureja*, *Salvia*), Apiaceae (*Cachrys*, *Opopanax*, *Tordylium*), Asteraceae (*Centaurea*, *Xeranthemum*, *Amphoricarpos*), Rosaceae (*Rubus*), since these families include numerous edible, spicing, aromatic and medicinal plants with pharmacological properties. Also, representatives of some nonflowering seed plants such as Pinaceae (*Picea*, *Pinus*), Cupressaceae (*Juniperus*), as well as some nonvascular plants such as liverworts (*Scapania*, *Marchantia*, *Porella*, *Ptilidium*) were examined.

Results of fundamental and applicative approaches obtained during last decade are very promising in understanding of Balkan flora and its potential in discovering of new metabolites which could be used in medicine and related disciplines. Collaborative work and joint projects with scientists from international institutions is recommended. This conference should be good platform for further investigations, with the focus on work on autochthonous floras of China and Serbia as a natural sources of new drugs.

## Pharmacognostic overview of some *Gentiana*, *Gentianella* and *Swertia* species from Central Balkan

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In the flora of Central Balkan there are about 50% of the *Gentiana* and *Gentianella* species recorded for the flora of Europe. In our studies, different *Gentiana*, *Gentianella* and one *Swertia* species was investigated. Main differences among aerial parts and roots of *Gentiana lutea* was that roots contains biphenyl derivatives such amarogentin located in bark of the roots, as well as the closely related amaropandin and amaroswerin, do not contain flavonoids isoorientin and isovitexin nor xanthone mangiferin. The leaves of *G. lutea* showed to be valuable source of bioactive compounds. Methanolic extracts of flowers and leaves of *G. lutea*, together with the isolated compounds mangiferin, isogentisin and gentiopicrin, showed antimicrobial activity. Orally consumed *G. lutea* root extract showed the potential to reduce the cytotoxic effect of x-ray irradiation on normal human immunocompetent cells PBMC of healthy people, without changing the susceptibility of malignant cells to be destroyed by irradiation. New phenolic compound, norswertianin-8-O-primveroside was isolated from the aerial parts of *G. dinarica*. The induction and establishment of hairy root cultures of *G. dinarica* using two strains of *Agrobacterium rhizogenes* (A4M70GUS and 15834/PI) was reported for the first time. HPLC analysis of methanol extracts of hairy root clones showed the presence of xanthone compounds with norswertianin-1-O-primveroside as the one of the most abundant. Xanthones were the major constituents in investigated *Gentianella* species represented with demethylbellidifolin, demethylbellidifolin-8-O-glucoside and bellidifolin-8-O-glucoside as the main compounds. The radioprotective effects of ether and methanolic fractions as well as xanthones demethylbellidifolin, demethylbellidifolin 8-O-glucoside, bellidifolin 8-O-glucoside and flavonoid swertisin against chromosomal damage induced by gamma-rays were determined using the micronucleus test.

From roots of *Swertia punctata* two new xanthone compounds were isolated ie. isobellidifolin-1-O-primveroside and methylisogentiakochianin-1-O-gentiobioside. Since the population of *S. punctata* is scarce and endangered, an alternative way for biomass production must be found.

## Drought and salinity stress due to Climate Change alter the physiology and biochemistry of Medicinal and Aromatic plants

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The effect of Climate Change (CC) on plants is of great concern and attracting great scientific interest. Among environmental stresses, salinity and drought stress are present and decrease crop production and usually affecting negatively the produce quality. The CC effects on Medicinal and Aromatic Plants (MAP) is also a fact, and various means including deficit irrigation or strategies to alleviate salinity stress are under research. Lavender (*Lavandula angustifolia*) and Greek sage (*Salvia fruticosa*) plants grown in soil were subjected to adequate irrigation (AI), moderate (MWD) and severe water deficit (SWD). Plants under water stress exhibited reduced growth and altered chemical composition than plants under regular irrigation. In addition, water stressed plants benefited in essential oil (EO) yield and quality, with higher concentrations of antioxidants (phenolic) level. Interestingly, EOs vapours from lavender plants under MWD caused significantly higher mortality to females of the two-spotted spider mite (*Tetranychus urticae*) than EOs from plants under AI, providing oils insecticidal activity. In another study, lavender (*Lavandula angustifolia*) was grown hydroponically under salinity (0-25-50-100 mM NaCl) conditions. High (100 mM NaCl) salinity decreased plant growth, content of phenolics, antioxidant status and EO yield, while low-moderate salinity levels had maintained the volatile oil profile of lavender. Foliar application of K and Zn lighten the presumable detrimental effects of salinity, in terms of fresh biomass, EO yield and maintained higher antioxidant capacity. It seems that moderate salinity stress along with balanced levels of K foliar application changed the primary metabolites pathways in favor of major volatile oil components biosynthesis and that lavender plant has the potential to be cultivated under prevalent semi-saline conditions. Therefore, the performance of crops under CC is important to be studied and mechanisms for alteration of induced stress are of great significance considering the importance of the oil composition as well.

**Keywords:** climate change; drought stress; salinity stress; antioxidants; insecticidal activity; foliar application

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## Ethnobotanical study of medicinal plants traditionally used in South-East Serbia (Pčinja district)

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Balkan Peninsula is one of the most important biodiversity centers in Europe. Despite that, the usage of plant species in traditional medicine of some Balkan regions remained largely unexplored in the past. This study aimed to collect and document data on the traditional use of medicinal plants in Pčinja district in South-Eastern Serbia, which is among the least developed regions in Serbia. The survey was carried out using semi-structured interviews and 113 informants were interviewed. Quantitative ethnobotany factors were calculated, allowing us discussing the results.

The informants reported data on 86 medicinal plants belonging to 43 families. The most dominant botanical families were Lamiaceae (30% of species), Asteraceae (26% of species) and Rosaceae (19% of species). Species with the highest number of use reports were *Mentha piperita*, *Hypericum perforatum*, *Matricaria chamomilla*, *Salvia officinalis*, *Urtica dioica*, *Thymus serpyllum* and *Tilia cordata*. The majority of the plants were reported to have 1-3 different usages and the species with most diverse uses were *Hypericum perforatum* and *Urtica dioica*. In Pčinja district folk medicine is primarily used for healing minor diseases with some exceptions. The most frequent medicinal uses were for treating diseases of the digestive system, respiratory system and diseases of the skin and subcutaneous tissue, followed by general and unspecified diseases (such as pain, fever and health prevention). Usually, the administration was primarily oral followed by topical applications. Leaves were dominantly exploited plant parts and the most frequent preparation form was infusion. The current study represents a useful documentation which can contribute to preserving ethnobotanical knowledge in South-Eastern Serbia. For some well-known plant species new applications have been recorded. This can present a good starting point for new investigations.

## Organic mushroom growing technology – challenge for the science and practice

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Production of cultivated mushrooms in modern times represents an increasingly engineered venture, a set of complex, technical solutions in complicated, huge plants. It is forgotten that the success of mushroom technology is exclusively conditioned by its ability to meet their biological demands. Regardless of the production capacity, the biological and physiological requirements of the organism we grow remain the same. Ekofunji's production technology is based on the principle of biology monitoring not only of cultivated mushrooms, but also of their competitors for space and food, as well as pathogens. Thanks to the monitoring of life cycles and the knowledge of the biological demands of those types of mushrooms we grow, and on the other hand, those whose presence is not desirable in the growing units, we managed to implement the principles of organic production in our own production. Yearly, based on such conceptual technological solutions, we produce about 100t of certified organic *A. bisporus* (champignon) and about 30t of the organic certified *P. ostreatus* (oyster).

## **Cultivation of arnica (*Arnica montana* L.) in various fertilization and propagation models**

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A three-year cultivation field trial on *Arnica montana* has been conducted at mountain Tara in a three-factorial manner. Induced factors were planting date (spring and autumn), fertilization type (manure and NPK) and mode of reproduction (generative and clonal). Morphological parameters have been observed and correlated with flower heads yield. Qualitative analysis has included total sesquiterpene lactones and targeted flavonoid compounds. Spring planting of clonally propagated plants showed to be the least successful model due to the low percentage of rooted plants and weak habitus development. Other models yielded from 59.8 - 143.6 kg/ha and 116.3 - 258.7 kg/ha flower heads in the second and third year, respectively. Total sesquiterpene lactones content in flower heads ranged from 4.6 – 13.9 mg/g, while the amounts of two dominant flavonoid compounds quercetin-3-O-glucoside and kaempferol-3-O-glucoside ranged from 7.8 – 13.9 mg/g and from 2.1 – 4.7 mg/g, respectively. All induced factors in the field trial significantly influenced variation in both morphological and chemical parameters, where fertilized variants from autumn planting have shown to be superior in flower heads yield with satisfying levels of secondary metabolites.

## Valuable sources of natural compounds in the Mediterranean: the case of Greek flora

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The flora of the Mediterranean basin includes many wild edible species, which are known since ancient times for their pharmaceutical and nutritional properties and have been used by humans as food and/or as practical 'medicines' for ailments throughout the centuries. Many of these species are used as leafy greens and are the basic ingredients in many traditional dishes and local recipes of regional interest. In particular, due to terrain morphology Greek flora hosts a very rich patrimony of wild edible species ecotypes and local landraces of horticultural crops that have always been an important food source for the rural communities of the country, especially in time periods of food shortage; several studies have demonstrated the valuable role of these sources in the so-called "Mediterranean diet" which is in the epicenter of scientific research during the last decades due to its health promoting effects and contribution against several chronic diseases that afflict modern societies, such as diabetes, cardiovascular diseases and cancer. Our research team has conducted research on several such species in order to evaluate their nutritional, chemical composition, antimicrobial properties, bioactivities and cytotoxic effects under variable conditions. The species of interest include *Cichorium spinosum* and other wild edible species of the Asteraceae family, globe and wild artichoke, okra, garlic and onion landraces, purslane cultivars and ecotypes which proved to be rich sources of bioactive compounds while our research efforts focused on environmental factors (abiotic stressors) and cultivation practices that could affect bioactive compounds profile and increase bioactive properties. Overall, Greek flora is abundant with species which are valuable sources of bioactive compounds that could be used as complementary food sources and local biodiversity and agro-ecosystems could be valorized in the pharmaceutical and nutraceutical industry.



## **Legalisation of Medical Cannabis in Macedonia - how far we have reached**

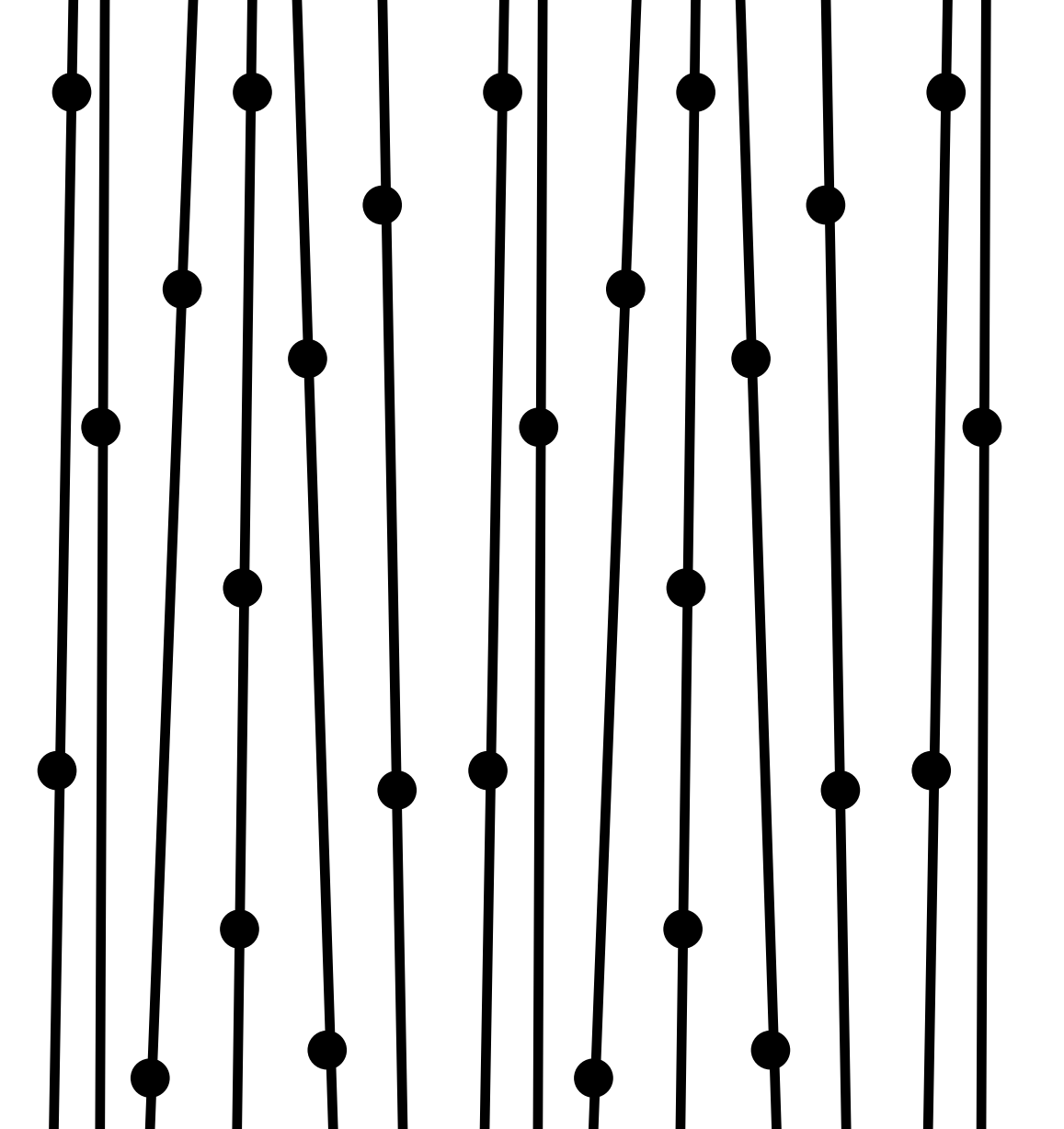
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Cannabis is a well-known drug and a controlled substance, which possession and use was illegal in most countries of the world. Nowadays, the usage of cannabis and its legalization for medical use has become a worldwide trend. In many countries there have been initiatives to amend the existing laws in order to produce drugs based on natural ingredients of cannabis, as well as other related products, synthetically produced cannabinoids, available to patients.

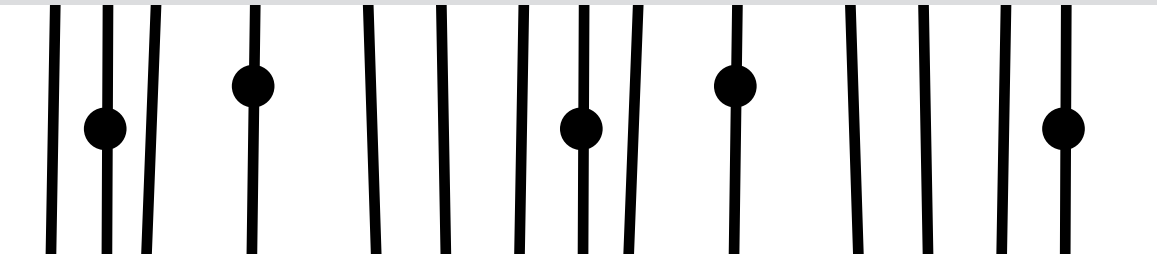
Cannabis is known since ancient times as a source of fiber, food, oil, medicine. Research studies have clearly shown that cannabinoids are highly efficient, primarily in the treatment of nausea and vomiting and the management of chronic pain. For patients who suffer from severe, chronic diseases, such as cancer and AIDS, cannabis has been shown to relieve several symptoms at the same time in more efficient way than some registered medicines. Besides, there is a great potential of cannabinoids to be applied for the treatment of many other pathological conditions, such as multiple sclerosis, Alzheimer's disease, spinal cord injuries, Tourette's syndrome, epilepsy, hypertension, glaucoma, although it is necessary to complete the last phases of clinical trials in order to register these compounds as drugs, and to assure safe therapy for these diseases (Ben Amar, 2006).

Therefore, due to the increased global necessity of cannabis based products and for the sake of reinforcing the ability of R. of Macedonia to respond to the challenges arising from this issue, changes of the Law for narcotic substances in R. Macedonia has been done. Thus, an opportunity, for starting a business in cultivation of high potency cannabis, extraction, production and marketing authorization of cannabis based products for medicinal use for humans and scientific-research work, has appeared. Present situation and legal framework are reported.



## SECTION 3

BIOACTIVE NATURAL PRODUCTS



## Identification of bioactive diterpenoids from traditional Chinese medicines

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Natural products play an important role in new drug discovery. As one of the most important types of natural compounds, diterpenoids have attracted considerable attentions due to their diversified structures and a spectrum of bioactivities such as anti-bacterial, anti-malarial, anti-inflammation, anti-cancer and so on. Searching for bioactive diterpenoids is still a hot topic in the field of natural products.

A systematic investigation of unique diterpenoids from a medicinal herb *Podocarpus nagi* has been carried out, resulting in the isolation of 44 nor-diterpenoid lactones with 16 of them being new, especially a novel binor-diterpenoid with dehydroxylation at C-7. Notably, most of isolated diterpenoid lactones displayed remarkable potency to increase LDL uptake in HepG2 cells at a concentration of 5  $\mu$ M. *In vivo* study further showed that nagilactone B, an abundant diterpene existing in this plant, dose-dependently decreased TC, TG, and LDL levels in high fat diet induced dyslipidemic hamsters. Mechanism study indicated nagilactone B exerted lipid-lowering effect by elevating LDLR mRNA and protein level. It is the first time to report anti-hyperlipidemic activity for naturally occurring diterpene lactones *in vitro* and *in vivo*. Nagilactone B represent a new type of compounds with promising lipid-lowering activity.

## Nutritive and therapeutic properties of selected wild growing mushrooms from Serbia

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Selected species from the genera: *Agrocybe*, *Laetiporus*, *Pleurotus* and *Polyporus* harvested in Serbia have been chemically characterized and their biological potential was evaluated. Results of macronutrient profile suggest that tested macromycetes are a rich source of carbohydrates and proteins, with low fat content which makes them excellent candidates for low-calorie dietary regimens. Selected metabolites analysis revealed presence of: free sugars (rhamnose, fructose, mannitol, glucose and trehalose), four isoforms of tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ - tocopherol) and total of 27 fatty acids with nutritionally desirable linoleic acid as the most abundant. Samples turned out to be rich in organic (oxalic, quinic, malic, citric and fumaric acid) and phenolic acids (protocatechuic, *p*-hydroxybenzoic, *p*-coumaric and cinnamic acid) as well, which was later on associated with multiple biological activities. Results of antioxidant activity (evaluated via reducing power, DPPH· scavenging activity,  $\beta$ -caroten/linoleic acid and TBARS test) indicated that selected macromycetes are potent antioxidant agents with ability to neutralize free radicals, inhibit lipid peroxidation and reducing power ( $EC_{50}/EC_{0.5}$  is in range of 0,25 – 23,78 mg/mL). Potent antimicrobial potential of ethanolic and methanolic extracts prepared from fruiting bodies was demonstrated against several clinically relevant pathogenic microorganisms, and given their ability to eradicate pathogenic microorganisms, tested mushrooms may have role in the formulation of new antimicrobial agents. Methanolic extracts were not that promising in their cytotoxic properties towards HeLa, NCI-H460 and MCF-7 carcinogenic cell lines, but preliminary analysis also showed that tested samples were non-toxic for the porcine primary cells, which may provide opportunity for safe human consumption of preparations derived from these mushrooms.

Thanks to the production of biologically active compounds which have beneficial health effects alongside favorable nutrient profile, the selected macromycetes have potential as functional foods. Future prospects open up new avenues for improving their status to proper medicinal products with significant potential in maintaining and improving health, longevity and quality of life.

## Utilizing the indigenous biosynthetic capacity of medicinal and aromatic plants through optimization of tissue culture conditions

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Secondary metabolite production is one of the adaptation mechanisms of plant individuals to cope with the fluctuations of their indigenous environment. Therefore secondary metabolites productivity of medicinal and aromatic plants in nature is determined not only by the genetic predisposition of the species but also by of by the combined effects of climatic and geographic factors, as well as plant phenology.

By providing the tools for standardized environment maintenance, plant biotechnology is a flexible approach for targeting valuable phytopharmaceuticals through modification of growth conditions.

*Inula* and *Artemisia* species, known for the variability of volatile and extractable secondary metabolites production in their natural conditions were studied. The experimental model included modification of culture conditions such as application of plant growth regulators and utilizing either agar solidified or liquid media.

As a result, *in vitro* lines of the species were selected, based on the morphotypes obtained – such as root suppressed, normally root developing shoots, genetically non-modified root lines, as well as cell culture aggregates. The obtained lines were characterized by the selective stimulation of secondary metabolites characteristic for the species – such as terpenoids, sesquiterpene lactones and polyphenolics.

The understanding of key factors related to the production of target secondary metabolites makes it possible to optimize culture conditions in biotechnologically cultivated plants and utilize the indigenous biosynthetic capacity of the wild genotype without performing genetic modifications.

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## Edible and medicinal fungi inhibit enzymes linked to diabetes type-2 therapy

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*Diabetes mellitus* is a chronic disorder of metabolism followed by abnormal rise in plasma glucose levels, as a consequence of unequilibrated insulin production and/or insensitivity to the effect of this hormone in signal transduction of cellular receptors. One of the effective strategies for diabetes type-2 management is the inhibition of complex polysaccharide hydrolysis by pancreatic  $\alpha$ -amylase and absorption limitation of glucose by inhibiting intestinal  $\alpha$ -glucosidase enzyme.

*Agaricus blazei* Murrill, *Coprinus comatus* (O.F.Müll.) Pers., *Cordyceps militaris* (L.) Fr., *Inonotus obliquus* (Ach. ex Pers.) Pilát, *Morchella conica* Pers. and *Phellinus linteus* Berk. & M.A. Curtis were investigated for their antidiabetic properties. *In vitro* assays on  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibition were performed with methanolic extracts of the selected mushrooms. Furthermore, we calculated the necessary daily intake of mushroom extracts and dry mushroom powders based on the equivalent doses of therapeutic drug acarbose given to diabetic patients per day.

Our comparative study on enzyme inhibition showed that the most promising potential is ascribed to *I. obliquus* extract, while no inhibition of  $\alpha$ -amylase was recorded with *M. conica* and *C. militaris* methanolic extract at the tested concentration. The lowest daily intake of mushroom powder was suggested for *I. obliquus* with the dose of  $3 \times 1.148$  g/day, while the highest was predicted for *P. linteus*  $3 \times 2.215$  g/day.

Although majority of previous studies showed *in vivo* antidiabetic potential of water and polysaccharidic mushroom extracts by different experimental approaches, our study is the first highlighting *in vitro* antidiabetic potential by inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase with methanolic extracts; which makes the investigated species more promising for the diabetes type-2 treatment by another additional and different mechanism of action.

**Keywords:** Diabetes mellitus,  $\alpha$ -Amylase,  $\alpha$ -Glucosidase, Mushroom, Edible and medicinal.

**Acknowledgment:** The authors thank to Serbian Ministry of Education, Science and Technological Development for the financial support (grant number 173032).

## Comparative study of antiproliferative potential of three different plant species

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Cancer represents a heterogeneous group of diseases characterized by uncontrolled proliferation of cells, tissue invasion and metastasis. Despite current therapeutic strategies which include a combination of surgery, radiation and chemotherapy, cancer is second leading cause of death worldwide next to cardiovascular diseases. Therefore, the identification of new drugs with high sensitivity to cancer cells is warranted. The anticancer properties of plants and their compounds have been shown since ancient times and nowadays, there is a growing interest for identification of new plant-derived anticancer agents.

This study was designed to analyze antiproliferative potential of three different plant species, namely *Ononis spinosa* L. (Fabaceae), *Anthriscus cerefolium* (L.) Hoffm. (Apiaceae) and *Phlomis fruticosa* L. (Lamiaceae). The aerial parts of plants were collected, dried and the plant material was subjected to successive extraction with methanol and then evaporated to dryness on a rotary vacuum evaporator. The obtained methanolic extracts were used for further analysis. Cytotoxic effect of methanolic extracts on HaCaT (spontaneously immortalized human keratinocyte cell line), MCF-7 (breast cancer cell line), SiHa (cervical cancer cell line), A172 (glioblastoma cell line) and HepG2 (human hepatocellular carcinoma cell line) cells was determined by crystal violet colorimetric assay. Antiproliferative activity of methanolic extracts on glioblastoma cells was evaluated by Ki67 immunostaining.

The results showed that plant extracts reduced viability of all tested cell lines. The most active extract was the one from *O. spinosa*, indicating by the lowest IC<sub>50</sub> value. Ki67 immunostaining indicates antiproliferative activity of methanolic extracts on glioblastoma cells.

**Keywords:** cancer; plants; methanolic extracts; antiproliferative activity; KI-67.

**Acknowledgment:** The authors thank to Serbian Ministry of Education, Science and Technological Development for the financial support (grant numbers 173051, 173032).

## Iridoids of *Centaurium* and *Nepeta* species: exploring their diversity, metabolism, ecophysiological roles and bioactivities

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Iridoids are oxidized cyclic derivatives of monoterpenoid geraniol, with a fused cyclopentane and pyran ring system potentially decorated at various positions and glycosylated at position C-1. A great diversity of iridoids in *Centaurium* (fam. Gentianaceae) and *Nepeta* species (fam. Lamiaceae) offers a platform to study their metabolism, ecophysiological roles, and bioactivities. Many iridoids and iridoid-derived compounds of these genera are well known for their antibacterial, anti-inflammatory, anti-tumor, chemo-preventive, hepatoprotective, anti-diabetic, phytotoxic and various others activities.

Iridoids and secoiridoids are highlighted as high resolution chemotaxonomic classifiers for *Nepeta* and *Centaurium* genera, suitable for the evaluation of intra- and inter-population diversity, and for the diversification among species, subspecies and hybrid plants. Chemical diversity observed at the intra-population level is at least partially determined by different expression patterns of secoiridoid biosynthesis-related genes.

By adopting comparative metabolomics and transcriptomics approaches, accompanied by chemometric data processing, it was possible to study organ-specific and developmentally- regulated biosynthesis of major iridoids in *Nepeta* and *Centaurium* species as influenced by various abiotic factors (drought, wounding, etc.), elicitors (methyl jasmonate), or endogenous factors (ploidy level). Furthermore, the isolation and functional characterization of some of the iridoids biosynthetic genes has been performed. Information gathered will help us gain deeper insight into the iridoid metabolism and develop strategies for engineering the production of bioactive iridoids in homologous or heterologous systems, with the aim to establish their sustainable exploitation from renewable sources.



## Plant and mushroom extracts as potential intervening supplements in diabetes and diabetic complications

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Diabetes is a metabolic disorder characterized by impaired pancreatic insulin production and/or insufficient cell response to insulin and is characterized by chronic hyperglycemia which induces metabolic abnormalities in different cells and tissues. High intracellular glucose concentrations induce overproduction of reactive oxygen species and activate the formation of advanced glycation end-products, the polyol pathway, the hexosamine pathway and the protein kinase C pathway, influencing gene expression and activity of different regulatory proteins. These changes lead to diabetic end-organ complications affecting vascular system, kidneys, eyes, peripheral nerves, liver and gastrointestinal system. Diabetes represents one example of a disease that has been treated according to the traditional medicine world-wide and as such represents a good model for investigation of beneficial effect of different plant and mushroom extracts and isolated compounds in the management of diabetes and diabetes-related complications. Plants and mushrooms extracts are the source of metabolites such as polyphenols, polysaccharides, terpenes, alkaloids and antibiotics with pronounced biological activities including antioxidant, antitumor, anti-inflammatory, antidiabetic, antimutagenic, anti-hepatotoxic and immunostimulant properties. We provided an overview of the beneficial effects of the examined extracts obtained from flowering plant (*Centaureum erythraea*), sweet chestnut (*Castanea sativa*), edible mushroom (*Lactarius deterrimus*) and natural products containing  $\beta$ -glucans (from cereal grains) in the treatment of diabetes. Their antioxidant and antidiabetic properties *in vitro* and positive effects on different processes involved in the onset and progression of diabetes and its complications *in vivo* are presented and possible mechanisms that underlie these effects are suggested.

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## Phytochemical investigations of *Salvia transsylvanica*, *Salvia glutinosa*, and *Salvia officinalis* from Romania and their bioactivities

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In the Romanian traditional medicine sage species are used as remedies for coughs, rheumatism, inflammatory and bacterial diseases, as well as antidiabetic remedies.

In this study, an HPLC method was applied for determination of 22 phenolic compounds in extracts of *S. glutinosa*, *S. transsylvanica*, and *S. officinalis*. The enzyme inhibitory potential of the extracts was evaluated using microtiter assays, and the antimicrobial potential was tested using the microdilution assay for eight microorganisms. These extracts were further tested on three different cancer cell lines (A549, HepG2 and MCF-7) at increasing concentrations (1.56-200 µg/mL) for 24h/48h.

The chromatographic fingerprint revealed that among investigated compounds, the dominant compounds of *Salvia* species are rutin (1357.9 - 4070.2 µg g<sup>-1</sup>) and catechin (1112.6 - 1911.1 µg g<sup>-1</sup>). Concerning the enzyme inhibitory assays, both *S. officinalis* and *S. transsylvanica* extracts exhibited an important inhibitory potential against alpha-glucosidase (27.01 mmolACAE/g extract, and 25.62 mmolACAE/g extract, respectively). The most sensitive bacteria to the extracts were *Enterobacter cloacae* (MIC = 0.01 mg/mL, MBC = 0.02 mg/mL for *S. officinalis*) and *Bacillus cereus* (MIC = 0.09 mg/mL, MBC = 0.18 mg/mL), while *Penicillium funiculosum* was the most sensitive fungal strain to *S. officinalis* extract (MIC = 0.06 mg/mL, MFC = 0.12 mg/mL). From the three extracts, the *S. officinalis* extract exhibited the most potent cytotoxic effect. Interestingly, when testing on the estrogenic responsive cell line MCF-7, an increase in the viability was observed for intermediary doses which we hypothesize to be related to the estrogen-like compounds present in *Salvia* species.

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## Freeze dried *Salvia officinalis* methanolic extract incorporated into nanostructured lipid carriers for Alzheimer's disease treatment

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Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disease that accounts for 70% of all patients with dementia with approximately 36 million cases in the world. Having in mind that AD pathophysiology involves more than one aspect and that single target drugs are not always efficient in the desired extent, promoting multi-targeted drug therapy would be a better pathway to achieve efficient treatment. Considering that natural products are multi-target as they are rich reservoir for drug discovery because of their diversity and complexity of structures, it has been suggested, on the basis of traditional medicine, its *in vitro* cholinergic binding properties, antioxidative effect, modulation of mood and cognitive performance in humans that *Salvia officinalis* might potentially provide a novel natural treatment for AD. In order to improve its penetration into the brain and circumvent problems associated with blood brain barrier, incorporation of *Salvia officinalis* extracts into nanostructured lipid carriers (NLC) might be a rational approach into the development of novel dosage form for efficient AD treatment. Three different formulations of NLC loaded with freeze dried methanolic extract of *Salvia officinalis* (FMSE) were prepared using solvent evaporation method. Lipid phase consisted of 0.1g (NLC-FMSE1), 0.15g (NLC-FMSE2) and 0.2g (NLC-FMSE3) phospholipon 90H (kindly donated by Phospholipid, Germany) and 0.065g oleic acid (Sigma-Aldrich, Germany), 4.3g ethanol (Alkaloid, Macedonia) and 0.025g FMSE. Water phase was composed of 0.18g Tween 80 (Merck, Germany), 0.045g poloxamer 407 (BASF, Germany) and 8.8g distilled water. All prepared formulations were characterized with particle size in range of  $132 \pm 0.97$  to  $154 \pm 0.2$  nm, unimodal particle size distribution from  $0.918 \pm 0.1$  to  $1.181 \pm 0.02$  Span, encapsulation efficiency of  $52.74 \pm 1.2$  to  $77.71 \pm 1.8$  % and prolonged drug release (18 to 22% for 24h). Obtained results were in favor of their potential for efficient treatment of Alzheimer's disease.

## The pygidial gland secretions of ground beetles (Insecta: Coleoptera: Carabidae): antimicrobial and antitumour activity of the natural products

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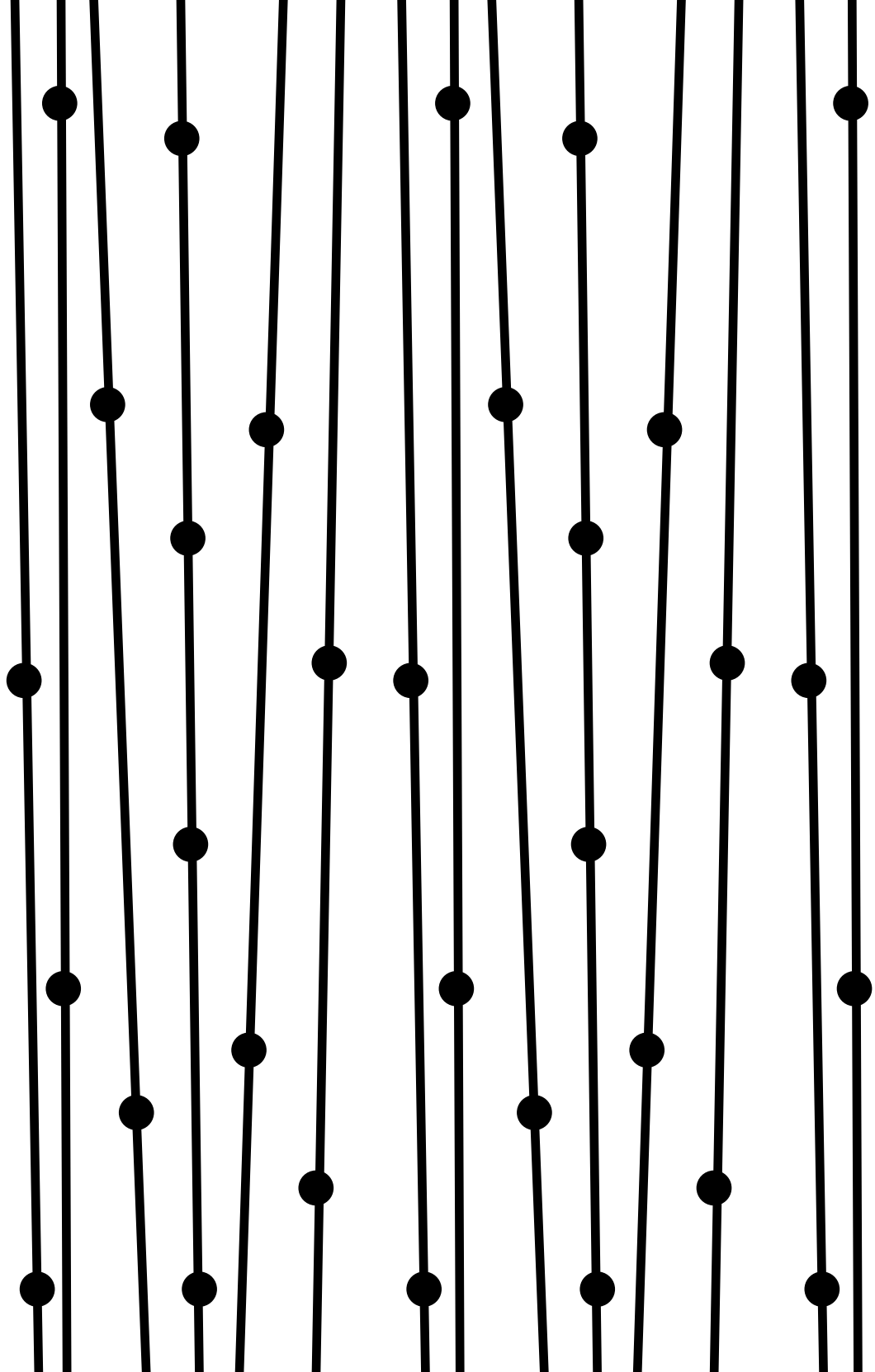
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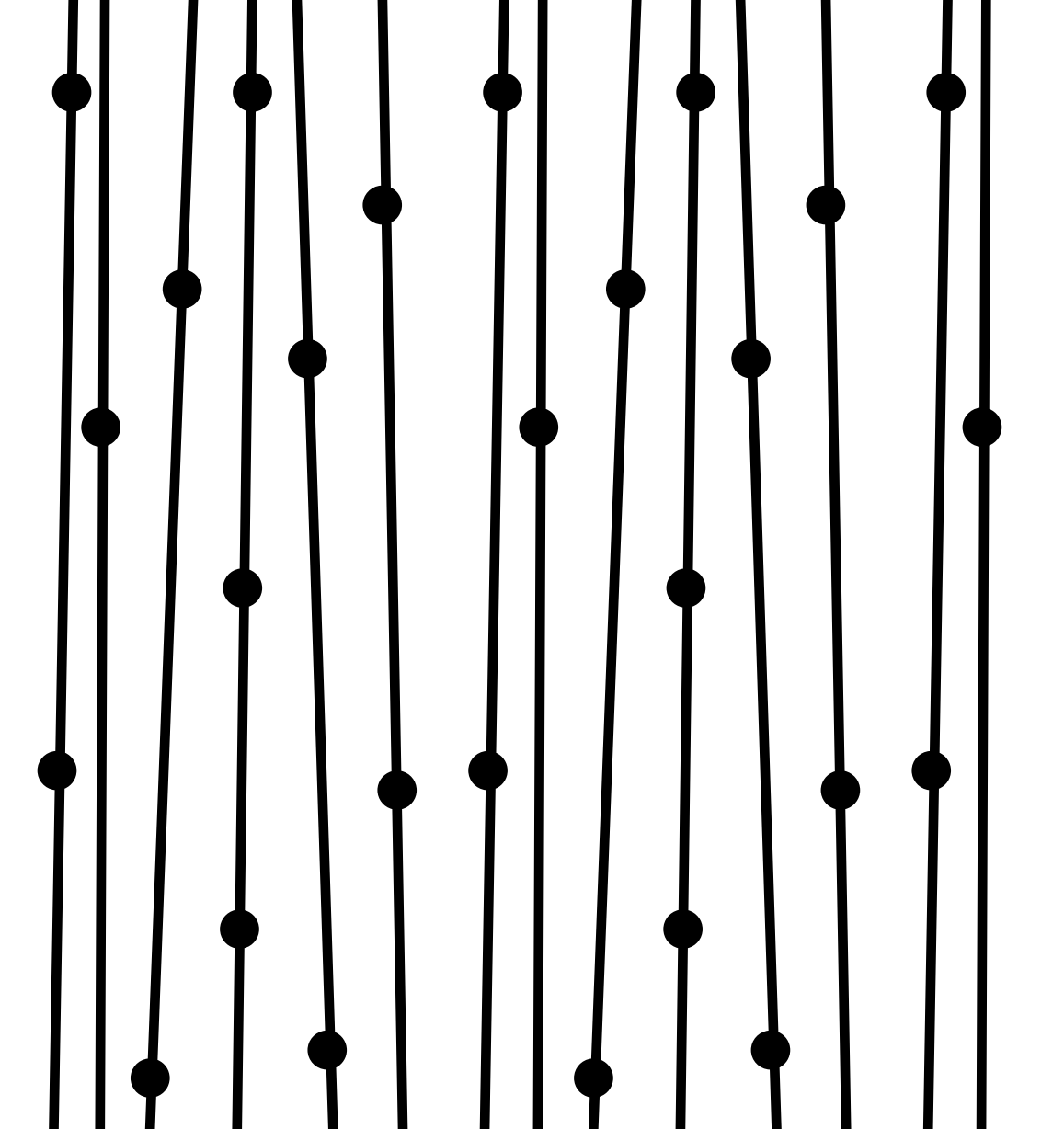
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In beetles, secretions are products of different exocrine glands, of which particular emphasis is on pygidial glands, which are common for the suborder Adephaga. Antimicrobial activity of the pygidial gland secretions of six selected ground beetle species was observed against human pathogens. A microdilution method was applied in order to determine minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs). We tested 16 laboratory and clinical strains of human pathogens (eight bacterial gram-positive and gram-negative species, and eight fungal species). The secretions of tested ground beetles have a certain level of antimicrobial activity, which differed between species, which is caused by the difference in chemical composition of the mixtures. The highest antibacterial effect was observed after treatment of most pathogens with secretion of *Laemostenus punctatus* and *Carabus ullrichii*. The highest resistance of tested pathogenic strains was noticed after treatment of the secretion of *Calosoma sycophanta*, which achieved antibacterial effect against *E. coli* only. The secretions of *L. punctatus* and *C. sycophanta* each showed a strong antifungal effect compared to positive controls.

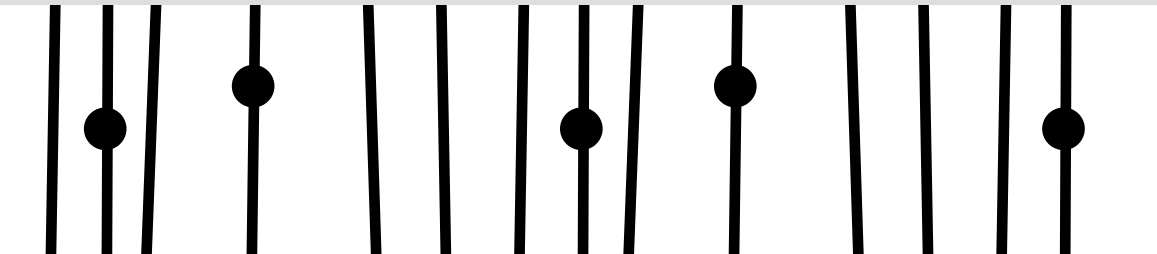
Inhibition of the proliferation of human tumor cell lines and porcine non-tumor cells by the secretion of adults of four ground beetle species was observed as well. The sulphorhodamine B (SRB) assay was applied to establish GI<sub>50</sub> values of the tested secretions. All secretions have shown certain antiproliferative effect on the tested cell lines, but statistically insignificant in most cases, which confirms the absence of cytotoxicity on the non-tumor cell line. Special emphasis is put on the secretion of *L. punctatus*, which reached approximately 50% inhibition of cell proliferation (GI<sub>50</sub> value) of MCF7 (breast adenocarcinoma) cell line, and showed a potential antitumour activity. The antimicrobial, antitumour and antiproliferative potential of the secretions of ground beetles was demonstrated for the first time in this study.





## **SECTION 4**

**DRUG DISCOVERY**



## **13-year-R&D and 13-year-postmarketing, a long and risky road to be a blockbuster in clinic**

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The road of drug discovery and development is tortuous and full of risk. Thanks for its long-history practice as folk medicine, TCM afford us a lot clue to develop a new drug more efficiently and less costly, compared with developing from scratch. *Salvia miltiorrhiza* Bunge, known as “Danshen”, is a typical TCM for promoting blood circulation and removing blood stasis. Its preparations were widely applied clinically for the treatment of cardiovascular and cerebrovascular disease. However, most of them cannot keep consistent efficacy and safety because of its obscure constituents and uncontrollable quality.

Based on the investigation of hydrophilic constituents of *Salvia miltiorrhiza*, magnesium lithospermate B (MLB) and other depsides salts were identified as the most effective components for ameliorating ischemic myocardial injury. Fortunately MLB is also abundant in the herb even though it is mixed with other salt forms and derivatives. This finding inspired us to use MLB as the key quality control marker for innovative drug of *Salvia miltiorrhiza*, differentiating from other traditional preparation. A quality standard including fingerprinting, as well a preparation process was elaborated to enrich MLB and afforded consistent and identified constituents and controllable quality. Mode of action investigation indicated depsides salts can protect cardiovascular system by selectively modulating L-type calcium current in ventricular myocytes, modulating intracellular calcium concentration in vascular smooth muscle cells, inhibiting migration and proliferation of VSMC, antioxidation, anti-platelet aggregation and anti-inflammation, respectively.

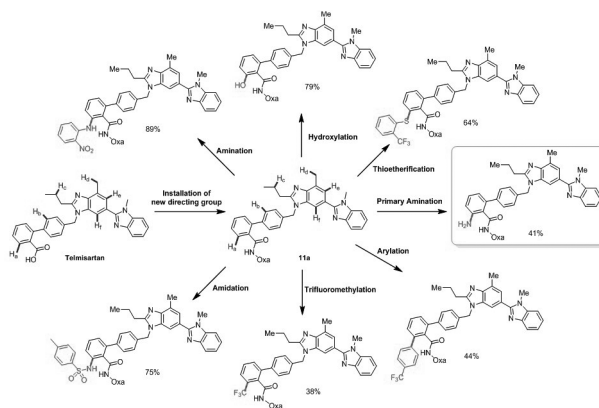
After 13 years R&D, *Salvia Miltiorrhiza* Depsides Salts (SMDS) has been confirmed to be safe and effective for the treatment of patients with coronary artery disease and chronic angina pectoris in multi-centre clinical trials. It was licensed by CFDA in 2005. After launching the market, another 13 years passed. Series of post-marketing investigation including Phase IV clinical trial, efficacy and safety in real world and several RCT study of SMDS were conducted to afford more evidence supporting its application in clinic effectively and safely. Till now, more than 20 million patients were benefited from SMDS. A blockbuster with billions of RMB in annual sales is rising.

## C–H Activation: a Late Stage Functionalization Tool for Drug Discovery

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One of the long-standing issues in directed C–H functionalization is that nitrogen or sulfur present in heterocyclic substrates may outcompete binding of a transition metal catalyst to a desired directing group. This competitive binding—leading to either catalyst poisoning or C–H functionalization at an undesired position—has largely hindered the application of C–H functionalization in late-stage heterocycle drug discovery. Herein we report on the use of an oxazoline-based directing group that is capable of overriding the poisoning effect of a wide range of heterocycle substrates. The potential utility of this directing group in pharmaceutical drug discovery is illustrated by diversification of Telmisartan (an antagonist for the angiotensin II receptor) through Cu-mediated C–H amination, hydroxylation, thiolation, arylation, and trifluoromethylation.



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## Quantifying randomness in real interaction networks and examples in biology

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Biological systems can be represented as a complex network, where network nodes represent units of the system, while links represent interactions between them. These networks are neither of regular or random structure, but rather an intricate combination of order and disorder. Scientists have developed large set of different topological measures for characterization and description of different structural properties of real networks. It turns out that these statistical measures are not independent, i.e., many properties appear as a statistical consequence of relatively small number of fixed topological properties in real network. We explore this dependence in two different biological networks, protein-protein interaction and brain network, using the method of dk-series. We find that many important local and global topological properties of protein-protein interaction network are closely reproduced by dk-random graphs whose degree distributions, degree correlations, and clustering are as in the corresponding real network, while this is only in part true for biological network. These differences are a consequence of different spacial constraints present during the evolution of these networks.

## High throughout antimycobacterial drug screening platform and study of mechanisms of action of antimycobacterial drugs

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Mycobacteria usually grow very slowly, for example, it takes 3 to 6 weeks for *Mycobacterium tuberculosis* and ~3 months for *Mycobacterium ulcerans* to form a visible colony on agar containing enriched media. Traditional methods that depend on enumerating colony forming units (CFU) of bacteria to evaluate efficacy of a drug would significantly prolong the duration of preclinical studies required for development of antimycobacterial compounds/treatments. Autoluminescent reporter mycobacteria were constructed using the *luxCDABE* operon, which can emit light without the addition of exogenous substrates. Relative light units (RLU) counts correlated well with CFUs of mycobacteria. Use of this technology enabled rapid (3 seconds) and serial real-time monitoring of mycobacteria in the same samples *in vitro* and even in the same small live animals for evaluation of drug activity. Therefore, this approach can drastically reduce the time, effort, animals and resources necessary for assessments that require monitoring of mycobacteria growth. This method is highly efficient in active compound discovery both *in vitro* and *in vivo*.

Existing treatments for mycobacterial infections are lengthy or losing effectiveness due to resistance/toxicity. New powerful drugs with new mechanisms of action could address the current need for novel regimens. We have collaborated with tens of labs for discovery of new antimycobacterial compounds. One of them is TB47, which is a new agent with activity against *M. tuberculosis*, the bacteria that causes tuberculosis. Using various *in vitro*, in macrophage and *in vivo* approaches, we found that TB47 is bacteriostatic against *M. tuberculosis*. However, later we found it is highly bactericidal against *M. ulcerans* both *in vitro* and in live mouse model. Later we identified QrcB, a protein of the electron transport chain, is the target of TB47. Another good anti-TB drug candidate is ilamycin E, which was discovered from a streptomyces from deep sea. We have found it has a novel target (unreported). BTW, we have studied the mechanisms of pyrazinamide, Oxazolidinone, sulfamethoxazole+ trimethoprim, and novel quinolones.

## GPCR-targeted Drug Discovery

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G-protein coupled receptors (GPCRs), also known as 7 transmembrane receptors, are the largest family of cell surface receptors. They are involved in a wide variety of physiological and pathological processes and are the largest family of druggable targets. Our group is interested in the biological functions of GPCRs and their roles in major diseases including autoimmune disease, neurodegenerative diseases, metabolic diseases and etc. In addition to the mechanism study, we also screen and develop drugs targeting GPCRs. Natural compounds may exert their effect by targeting GPCRs.

For example, Herbal-based food supplement is widely used to treat obesity. Among them, the *Hoodia gordonii* (Asclepiadaceae) supplements are extremely popular. The African cactiform has been used for thousands of years by Xhmani Bushmen as an anorexant during hunting trips and has been proposed as a new agent for the management of body weight. However, the true active components and molecular targets of Hoodia remain unclear. We have demonstrated that Gordonoside F, a steroid glycoside isolated from *Hoodia gordonii*, but not the widely known P57, activates specifically GPR119, a receptor critically involved in metabolic homeostasis, and leads to increased insulin secretion and reduced food intake. These results not only demonstrate that the activation of GPR119 receptor is an important mechanism underlying *Hoodia gordonii*'s therapeutic effect, but also suggest that Gordonoside F or its congeners could be developed into new drugs in treating metabolic disorders.

## **Bavachinin, a novel natural pan-PPAR agonist, exhibits unique synergism with synthetic PPAR- $\alpha$ and - $\gamma$ activators**

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Pan-peroxisome proliferator-activated receptors (PPAR) agonists have long been desired as therapeutic drugs against metabolic syndrome. However, limited efficacy and undesirable adverse effects have hindered the development of PPAR agonists. In this study, we identified bavachinin as a novel natural pan-PPAR agonist from the traditional anti-diabetic herb malaytea scurfpea fruit. Bavachinin exhibited properties desired for an anti-diabetic PPAR-target agonist without weight gain and hepatotoxicity. Intriguingly, bavachinin synergized with synthetic PPAR- $\alpha$  and - $\gamma$  agonists on PPAR transcriptional activities, and glucose and TG lowering effects *in vivo*. Furthermore, we demonstrated that bavachinin share the same one stronger canonical site as rosiglitazone and had the other alternate binding site via cellular and NMR and docking methods. To our knowledge, it is the first time to find the synergistic effects between PPAR agonists by activating the alternate binding site, which might increase efficiency and decrease toxicity of the market drugs with a lower-dosage.

## **Discovery of hits/lead compounds based on natural product-like libraries generated from chromones**

Chunhao Yang

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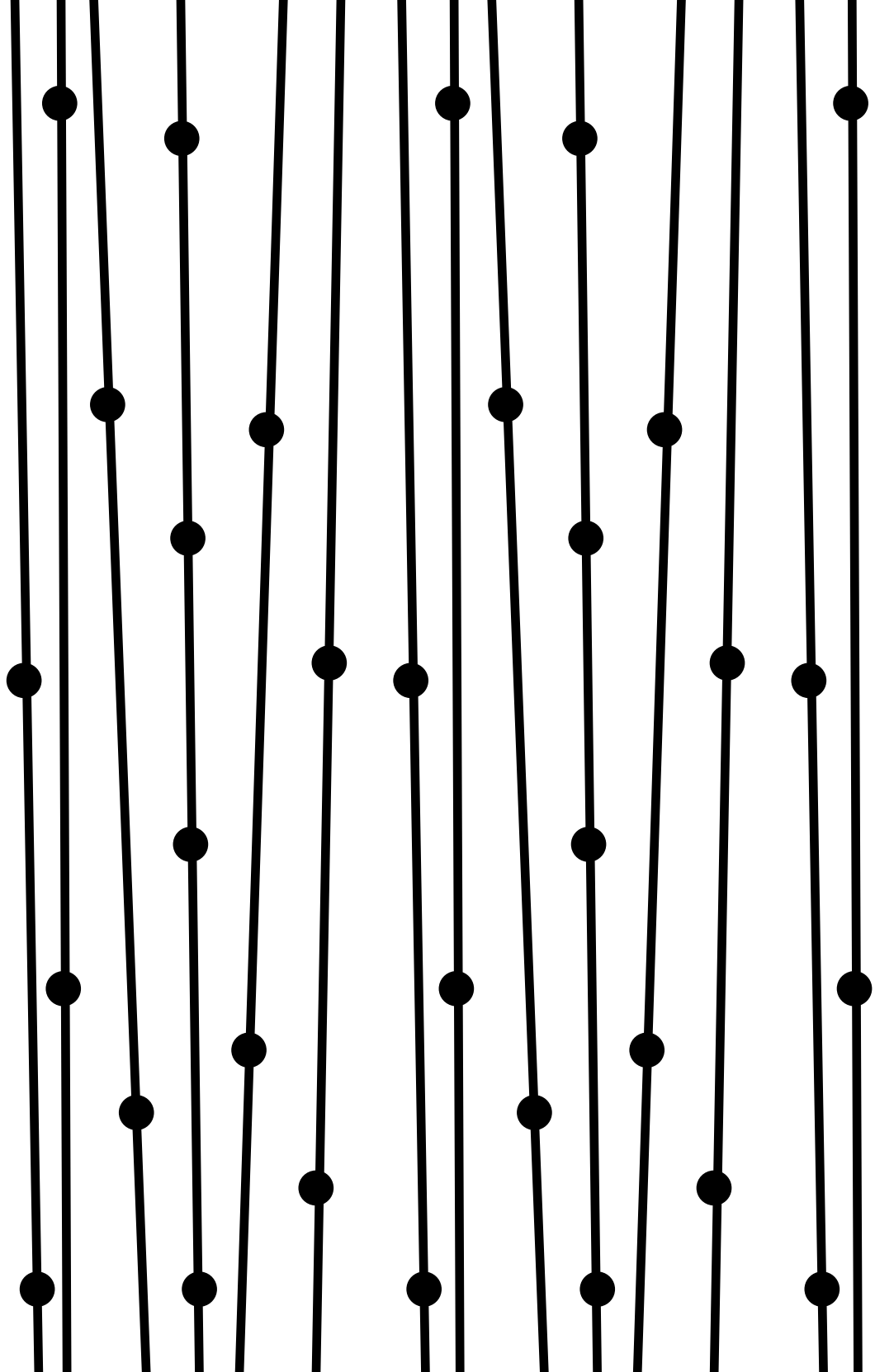
Natural products play important roles in drug discovery and chemical biology. As we know, many approved drugs and drug candidates in clinical trials are derived from natural sources. Additionally, natural products have been widely used to elucidate complex cellular mechanisms, such as cell cycle regulation, signal transduction et al. This may lead to the identification of important drug targets for treatment of disease. Despite the increased need for new natural products with novel skeletons or mechanisms, their isolation and structure elucidation still remain a highly labor intensive process. As an alternative, constructing libraries of natural product analogues and natural product-like compounds is an efficient strategy. It provides more rapid access to larger collections of heterocyclic compounds that possess greater diversity and incorporate optimized physical and pharmacological properties into their structures. My group has been working on developing novel heterocycles with potential bioactivities by one-pot sequential reactions or cascade reactions for many years. Here are several examples to deliberate our thinking processes on constructing natural product-like libraries from chromones for hit/lead discovery.

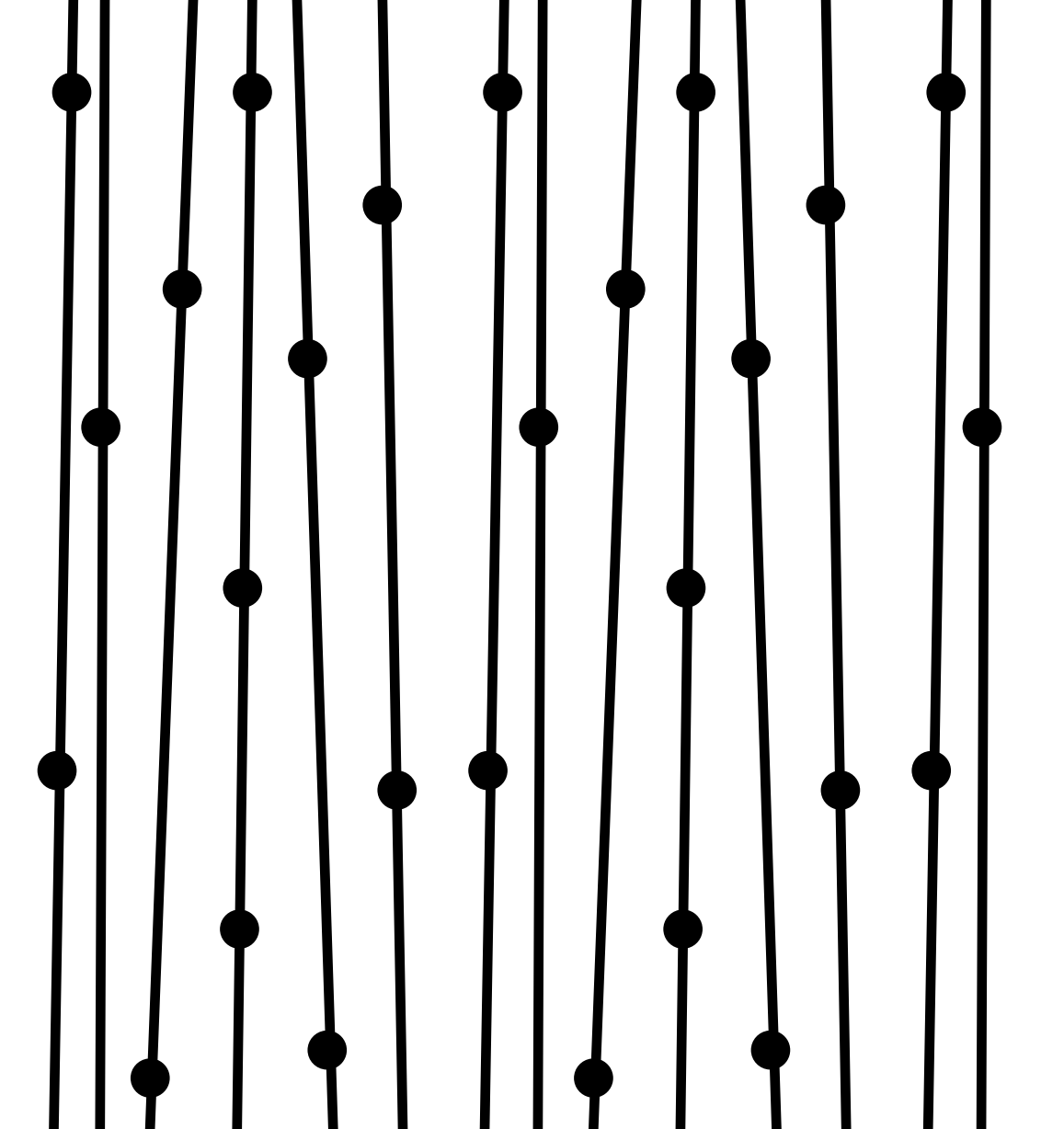
## Spin textures in molecular magnets and designed polypeptides

Dimitrije Stepanenko

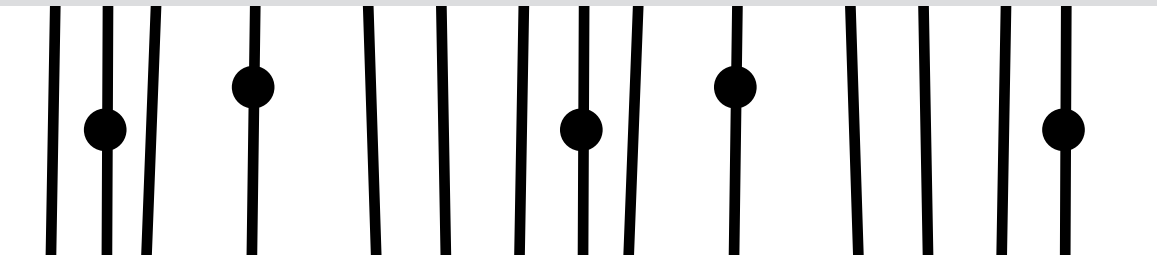
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Molecular magnets behave as large spins at low energies. They show hysteresis controlled by quantum tunneling of magnetization, long spin coherence times, and spin texture in the ground state. Coupling of these spins to an external electric fields would provide a superior mechanism for their control and manipulation. We show that triangular low-spin antiferromagnets with broken inversion symmetry have well defined chirality of spin texture. This degree of freedom behaves as a pseudospin-1/2 and coupled to electric fields. We discuss modified natural polypeptides as a scaffold for the design of molecular magnets with large number of interacting spins. Typical behavior of large polypeptides with embedded f-elements is that of a spin glass.





# AUTHORS INDEX





**A**

Aleksandra Đukić-Vuković.....	15
Aleksandra Uskoković.....	40
Alina Diuzheva.....	41
Ana Ćirić.....	37, 43
Ana Kalušević.....	20
Ana Salević.....	20
Ana Stančić.....	16
Anca Pop.....	41
Andrei Mocan.....	41
Anita Klaus.....	14
Anja Tolić.....	40
Antoaneta Trendafilova.....	36
Antonios Chrysargyris.....	12, 27

**B**

Baoru Yang.....	19, 22
Bojana Balanč.....	20
Branko Bugarski.....	16, 20

**C**

Chunhao Yang.....	52
-------------------	----

**D**

Danijela Drakulić.....	38
Danijela Mišić.....	39
Dejan Pljevljakušić.....	30
Dejan Stojković.....	28, 37, 38
Dijana Krstić-Milošević.....	26
Dimitrije Stepanenko.....	53
Dragana Mladenović.....	15
D. Shalabalija.....	42

**E**

Elena Markova.....	42
--------------------	----

**G**

- Gabriele Beltrame .....19  
Gianina Crişan .....41  
Gjoshe Stefkov .....18, 32, 42  
Gokhan Zengin.....41  
Goran Poznanović .....40

**H**

- Huixiong Dai .....41

**I**

- İonel Fizeşan .....41  
Isabel C.F.R. Ferreira .....60  
Ivana Drvenica .....16  
Ivanka Milenković .....29  
I. Cvetkovikj .....42

**J**

- Jasmina Glamočlija .....35, 37, 43  
Jelena Acevska.....18  
Jelena Arambašić Jovanović .....40  
Jelena Živković.....28  
Jovana Petrović.....35, 37  
Jovana Rajić .....40  
Jovana Vunduk .....14

**K**

- Kalina Danova .....36  
Katarina Šavikin .....26, 28

**L**

- Lijiang Xuan .....46  
Lillian Barros.....35  
L Taneska .....42

**Lj**

- Ljiljana Mojović.....15  
Ljubodrag Vujisić .....43  
Lj. Mihailova .....42

**M**

Marcello Locatelli .....	41
Marija Mitrović Dankulov .....	48
Marija Nenadić.....	43
Marija Sinadinović.....	40
Marija Smiljković.....	37
Marina Kostić .....	37
Marina Soković .....	35, 37, 38, 41, 43
Melita Vidaković .....	40
Menelaos C. Stavrinides .....	27
Milan Ilić.....	28
Milena Savić.....	14
Milena Stevanović .....	38
Milka Todorova .....	36
Miloš Đorđević.....	40
Miomir Nikšić.....	14
Mirjana Mihailović.....	40
M. Glavas Dodov .....	42
M. Kostovska .....	42
M. Simonoska Crcarevska .....	42

**N**

Nebojša Menković.....	26
Nevena Grdović.....	40
Nikola Vesović.....	43
Nikolaos Tzortzakis .....	12, 27

**P**

Panayiota Xylia .....	12
Petar Marin .....	25

**S**

Sandrina Heleno .....	35
Simone Carradori .....	41
Slobodan Milosavljević .....	26
Spyridon Petropoulos .....	31
Srećko Ćurčić .....	43
Steva Lević .....	20
Svetlana Dinić .....	40

**T**

Teodora Janković .....	26
Tianyu Zhang .....	49

**V**

Vassilis D. Litskas .....	27
Vele Tešević .....	43
Verica Đorđević .....	20
Veronika Stoilkovska .....	18
Vesna Ilić .....	16
Viktor Nedović .....	20
Viktoria Ivanova .....	36

**X**

Xin Xie .....	50
---------------	----

**Y**

Yang Ye .....	34
Yiming Li .....	51

**Z**

Z. Han .....	19
--------------	----

**Ž**

Živoslav Tešić .....	13
----------------------	----

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