BOOK OF ABSTRACTS

THE 3RD ANNUAL CONFERENCE OF THE PAN-BALKAN ALLIANCE OF NATURAL PRODUCTS AND DRUG DISCOVERY ASSOCIATIONS (PANDA)

1st November, 2021
Belgrade, Serbia

Institute for Biological Research "Siniša Stanković"
National institute of Republic of Serbia
University of Belgrade, Serbia

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ORGANIZER

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CO ORGANIZER

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МИНИСТАРСТВО ПРОСВЕТЕ,
НАУКЕ И ТЕХНОЛОШКОГ РАЗВОЈА

ABOUT PANDA

Pan-Balkan Alliance of Natural Products and Drug Discovery Associations

Being inspired by the Belt and Road Initiative, during the 2nd Balkans-China Mini-symposium on Natural Products and Drug Discovery (11-13 April 2019 in Belgrade, Serbia) with the already existing cooperation, the Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia (IBISS), University of Belgrade and the Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences initiated the establishment of the Pan-Balkan Alliance of Natural Products and Drug Discovery Associations (PANDA).

To promote the mutual development and benefit, PANDA was formally established at the "Belt and Road" International Symposium of Science and Technology Innovation on September 26th 2019 in Shanghai, China. Today, alliance counts various members from the People's Republic of China and from 14 European countries, with two PANDA Secretariats established in Shanghai (opened 2019) and Belgrade (opened 2020).

PANDA represents a non-governmental, non-profit, open international scientific alliance, with the goal to promote:

- Various academic exchange activities among the members of the alliance;
- The mutual development of natural products, drug discovery based on natural resources in these countries, and food safety;
- Cooperative nurturing programs for talents among various organizations within the alliance;
- Innovation in science and technology through joint applications for research funding.

PROGRAMME

Monday 1st November

Belgrade (GMT+1) Beijing (GMT+8)	**Session 1**: 3rd PANDA Council Meeting **Chair**: Yang Ye	
08:00-08:05 15:00-15:05	*Opening Remarks*	**Marina Soković** Ministry of education, science and technological development
08:05-08:30 15:05-15:30	*Approval of New Memberships*	
08:30-08:55 15:30-15:55	*Work plan Discussion*	All council members
08:55-09:00 15:55-16:00	Break	

Session 2: Scientific Webinar on Natural Products and Drug Discovery
Chair: Marina Soković, Jasmina Glamočlija and Ana Ćirić

Belgrade (GMT+1) Beijing (GMT+8)	Lecture title	Speaker	Country
09:00-09:20 16:00-16:20	Directed genome evolution driven by structural variation	**Yingjin Yuan** Vice President, Tianjin University	
09:20-09:40 16:20-16:40	A metabolomic perspective on the impact of Chios mastic gum administration in NAFLD/NASH patients	**Eleni V. Mikropoulou** Department of Pharmacognosy and Chemistry of Natural Products, National & Kapodistrian University of Athens	
09:40-09:55 16:40-16:55	The emerging aspect of antimicrobial concept – exploring the antibiofilm activities of natural products	**Marija Ivanov** Institute for Biological Research "Siniša Stanković", University of Belgrade, Faculty of Biology, Serbia	
09:55-10:15 16:55-17:15	Antioxidant and antidiabetic activity of selected medicinal plants	**Tanja Trifković** Faculty of Natural Science and Mathematics, University of Banja Luka	
10:15-10:30 17:15-17:30	*In vitro* propagation of *Cannabis sativa* L.	**Ana Trajkovska** Ss. Cyril and Methodius University in Skopje	
10:30-10:50 17:30-17:50	Anti-inflammatory potential of Croatian indigenous *Micromeria* species rich in rosmarinic acid	**Sanda Vladimir-Knežević** Faculty of Pharmacy and Biochemistry, University of Zagreb	
10:50-11:10 17:50-18:10	Structure-based discovery of flavonoids as novel inhibitors of SARS-CoV-2 3CL protease	**Yechun Xu** Shanghai Institute of Materia Medica, CAS	

11:10-12:00 18:10-19:00	Lunch Break

Belgrade (GMT+1) Beijing (GMT+8)	Lecture title	Speaker	Country
12:00-12:20 19:00-19:20	Encapsulation of bioactives for value-added food	**Viktor Nedović** Dept of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade	
12:20-12:40 19:20-19:40	Improving foods with natural hypocholesterolemic agents	**Sandrina Heleno** Instituto Politécnico de Bragança: Braganca, Bragança	
12:40-13:00 19:40-20:00	Multi-step domino reactions for the preparation of antiproliferative *O,N*-heterocycles	**Sándor Balázs Király** University of Debrecen, Hungary	
13:00-13:20 20:00-20:20	Isolation and chemical modification of metabolites of *Vitex agnus-castus* extracted with subcritical CO2	**Dorisa Cela** Noval Laboratory, 2030 Durres, Albania	
13:20-13:35 20:20-20:35	Assessment of phytocannabinoids content in Cannabis and its preparations by FTIR spectroscopy	**Olga Gigopulu** Ss. Cyril and Methodius University in Skopje	
13:35-13:55 20:35-20:55	Antimicrobial potential of subtropcal species from Montenegro	**Biljana Lazović** Biotechnical Faculty, University of Montenegro	
13:55-14:15 20:55-21:15	Natural and synthetic approaches in antifungal therapy	**Konstantinos Liaras** Panhellenic Pharmaceutical Association & Pharmaceutical Association of Kilkis	
14:15-14:35 21:15-21:35	A comparative study of essential oil composition of *Thymus* species from Bulgaria	**Antoaneta Trendafilova** Bulgarain Academy of Sciences, Institute of Organic Chemistry with Centre of Phytochemistry	

14:35-14:40 21:35-21:40	Concluding Remarks	**Yang Ye** Shanghai Institute of Materia Medica, CAS

ABSTRACTS

Directed genome evolution driven by structural variation

Ying-Jin Yuan[1,2,*], Sijie Zhou[1,2], Yi Wu[1,2], Bin Jia[1,2], Ze-Xiong Xie[1,2]

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Physical, chemical, and transposon mutagenesis techniques have been widely used to mutagenize target genomes and are usually coupled to a screen or selection for an altered phenotype. Recently, researchers have developed various mature and efficient directed genome evolution strategies to introduce small-scale variations such as single nucleotide polymorphisms (SNPs) and Indels throughout the genome.

Structural variation in the genome is also one of the important triggers for cell phenotypic evolution. *Saccharomyces cerevisiae*, a powerful model organism for eukaryotic genome evolution has been subjected to comparative genomic studies, which provided mechanistic insights underlying genome evolution. *De novo* design and synthesis of yeast genomes provides an approach for directed genome evolution at structural variation level. Synthetic genomics aims to *de novo* synthesize a functional genome redesigned from natural sequences with custom features. Designed genomes provide new toolkits for better understanding organisms, evolution, and the construction of cellular factories. Synthetic Chromosome Rearrangement and Modification by LoxPsymmediated Evolution (SCRaMbLE) is an evolutionary system designed in the synthetic yeast genome that enables rapid evolution of strain traits and provides a fast and efficient method for studying the relationship between genomic structural variation and phenotypic changes.

Here, we introduced the design and synthesis of the synthetic yeast chromosome 5 and 10 according to Sc2.0 principles, on the basis of the complete nucleotide sequence of native chromosomes. We also summarized the technical progresses of SCRaMbLE technology, and highlight several studies where yeast genome rearrangement has successfully improved industrially-relevant phenotypes, including the production of novel medicine, nutrition supplements, anti-tumor molecules, and the tolerance of environmental stress and drug resistance. Finally, we discussed the challenges and future perspectives of directed genome evolution by SCRaMbLE.

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A metabolomic perspective on the impact of Chios mastic gum administration in NAFLD/NASH patients

Eleni V. Mikropoulou[1], Petros Gkiouvetidis[1], Charalampia Amerikanou[2], Andriana C. Kaliora[2], George V. Dedoussis[2], Maria Halabalaki[1]

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Chios mastic gum (CMG) is the resinous secretion obtained from the wounds of the trunk of *Pistacia lentiscus var. Chia*, an endemic evergreen shrub cultivated exclusively in the southern part of the Greek island of Chios. CMG has a rich ethnopharmacological tradition and it has been employed as a phytotherapeutic product since ancient times, mainly for the treatment of gastrointestinal disorders and as a skin-caring agent. It possesses a diverse phytochemical profile characterized by the presence of the insoluble polymer, poly-β-myrcene, different classes of terpenoids and the essential oil. CMG and its components have exhibited a broad spectrum of pharmacological properties, such as antibacterial, anti-inflammatory, antioxidant, anti-diabetic and cardioprotective. What is more, in 2015, CMG was officially recognized by EMA as a traditional herbal medicinal drug by the issuing of a monograph with two therapeutic indications [1].

In the framework of the Mast4Health EU programme [2], a metabolomics strategy was adopted in order to study the effect of CMG administration in patients suffering from NAFLD/NASH over a 6-month period. On the one hand, plasma and stool samples were analyzed with an untargeted approach employing a UPLC-ESI-Orbitrap-HRMS platform. On the other hand, a targeted approach was employed for the quantification of 45 amino acids in plasma samples using the Sciex atraq protocol combined with a UPLC-ESI-TripleTOF-SWATH-MS platform. The generated data was subjected to statistical analysis using UVA and MVA methods with different softwares and statistical tools for data visualization and classification, as well as the discovery of related biomarkers. Finally, through this process, several triterpenic acid metabolites, products of phase I and II metabolic reactions of CMG's constituents, were detected and identified for the first time, both in plasma and stool samples. These findings might provide valuable information on the subject of natural product biotransformation within complex biological systems.

References:

1. Pachi, V. K. et al. Traditional uses, phytochemistry and pharmacology of Chios mastic gum (Pistacia lentiscus var. Chia, Anacardiaceae): A review. J. Ethnopharmacol. 254, 112485 (2020).

2. Amerikanou, C. et al. Effect of Mastiha supplementation on NAFLD: The MAST4HEALTH Randomised, Controlled Trial. Mol. Nutr. & Food Res. 2001178 (2021).

The emerging aspect of antimicrobial concept – exploring the antibiofilm activities of natural products

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Until some years ago, the antimicrobial activities of different products have been studied mainly on the planktonic microbial cells. On the other hand, we have now witnessed that when microorganisms cause infections they do not do it solely but are able to communicate and group into the structures called the biofilms. Biofilms are communities of microbial cells packed inside the extracellular matrix composed mainly of polysaccharides and extracellular DNA. By establishing the biofilm structure, microorganisms increase their ability to defy antibiotic treatment and though gain antibiotic resistance. The biofilm structures require few times higher concentrations of antimicrobial agents in order to get diminished, compared to planktonic cells studied in the antimicrobial assays. Though, in order to claim that some product has antimicrobial properties observing its effect on the microorganisms in the biofilms should be a must. Range of natural products has been studied up to date as antibiofilm agents. Some of them could act as agents to prevent infections by reducing the microorganism's ability to group into the biofilms. Others act on the already established biofilms and break down their resistant structures. Just some of the natural products studied so far for their antibiofilm effects are plants *Nepeta nuda* and *Artemisia absinthium*, different polyphenols and terpenes. These agents have displayed wide antibiofilm potential and though provide the step forward towards the development of efficient antibiofilm therapeutics.

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Antioxidant and antidiabetic activity of selected medicinal plants

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The flora of Republika Srpska is characterized by a considerable number of medicinal plants whith medical properties greatly dependent on phenolics content and their antioxidant capacity. The aim of this study was to determine qualitative and quantitative phenolic content by HPLC/MS^2 analysis, antioxidant capacity using ABTS and DPPH tests, antidiabetic activity by measuring inhibition of α-amylase activity, as well as capacity of chelating heavy metals. The investigation was performed on dry leaves ethanolic extracts of five plants: *Salvia officinalis* L., *Trifolium pratense* L., *Agrimonia eupatoria* L., *Cichorium intybus* L. and *Vinca minor* L. The results have shown that extract of A. *eupatoria* have the highest total phenolic content (3.53 mg GAE/mL), the lowest IC_{50} for ABTS (0.33 µg PhOH/mL extract) and higher ability of removing ABTS radical compared to standard Trolox (IC_{50} 0.372 µg/mL). The highest ability of removing DPPH radical was measured for *S. officinalis* (IC_{50} 1.29 µg PhOH/mL extract), which also has a high content of total phenols (3.31 mg GAE/mL). Antidiabetic activity of plant extracts was in the following order: *A. eupatoria>C. intybus>T. pretense>V. minor>Acarbose (standard)>S. officinalis*. The highest capacity to chelate Fe, and lowest total phenolic content was measured in *V. minor*, while *A. eupatoria* has the highest capacity to chelate Cu. The major phenolic compounds in analyzed plants were: rosmarinic acid (814.49 mg/kg) in *S. officinalis*, isoquercetin (494.67 mg/kg) in *A. eupatoria*, ferulic acid (352.8 mg/kg) in *T. pretense*, chlorogenic acid (557.25 mg/kg) in *C. intybus*, while *V. minor* was rich in iridoid loganic acid and organic acid- formic acid (965.7 mg/kg). The phenolic and flavonoid contents were in high correlation with removing ABTS and DPPH radicals and Cu chelating ability, while the correlation between phenolic content and antidiabetic activity was not statistically significant. The paper discusses the connection between the content of phenolic components and their structural characteristics with antioxidant and antidiabetic activity of the studied plants.

Keywords: total phenolic content, α-amylase inhibition, chelation of heavy metals, ABTS, DPPH, HPLC analysis

In vitro propagation of *Cannabis sativa* L.

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Cannabis sativa L. (Cannabaceae) is an important medicinal plant well known for its pharmacological and therapeutic activity. High commercial interest, limited natural resources and variable phytochemical composition of wild growing *C. sativa* plants are the main reasons for cultivation of this plant in controlled conditions. A quite challenging task is obtaining steady supply of generative material for cultivation of *Cannabis*. Growing from seeds not always gives expected results, germinating into plants with desired and specified characteristics. Cloning is another way for obtaining more consistent plants with predictable cannabinoid profile. Micropropagation *in vitro* as an aseptic technique has a high potential in *Cannabis* reproduction and breeding. It allows the rapid propagation of disease-free uniform genetic materials of elite chemotypes in less space despite of the seasonal and geographic factors.

The objective of this study was to investigate *in vitro* regenerative potential of *C. sativa* on MS/B5 medium with various combinations of plant growth regulators (PGRs).

For this purpose, seeds from wild-growing plants were collected on different locations in North Macedonia. The seeds were surface sterilized with 70% ethanol for 1 min. and 1% Ca(OCl)2 for 20 min., then rinsed three times with sterile deionized water. Surface sterilized seeds were inoculated on basal MS/B5 medium with 0.7 % agar and 3 % sucrose. The pH of the medium was adjusted to 5.8 before autoclaving. Two-weeks-old seedlings were used for isolation of apical segments as primary explants for further multiplication on media supplemented with different cytokinins such as benzyladenine (BA), kinetin (KIN) and thidiazuron (TDZ).

The preliminary results showed that 1 mg/L BA was noticed as the most effective cytokinin for axillary shoot induction. After 30 days, *in vitro* shoots were subcultured on MS/B5 media with 1 mg/L BA, 0.2 mg/L gibberellic acid (GA3) and 200 mg/L active charcoal for further elongation. Shoot cultures showed vigorous growth with elongation from 3 to 5 cm. Current experiments are focused on the evaluation of the effects of various auxins such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) on the capacity for rooting of multiplied shoots.

The results from this study will significantly contribute to the further evaluation on regenerative capacity of both, wild-growing and commercial varieties of *Cannabis* in *in vitro* conditions.

Anti-inflammatory potential of Croatian indigenous *Micromeria* species rich in rosmarinic acid

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Micromeria species are rich sources of essential oils and polyphenols. They are traditionally used against colds, wounds, skin infections, headache, cardiac disorders, and digestive system problems. The important representatives of genus *Micromeria* in Croatian flora are *M. croatica* (Pers.) Schott, *M. thymifolia* (Scop.) Fritsch and *M. juliana* (L.) Bentham ex Reichenb. The first two are endemic species in Croatia and in some neighbouring countries, while *M. juliana* is more widely distributed in Mediterranean region. The aim of the study was to evaluate polyphenolic profile and anti-inflammatory potential of these Croatian indigenous plants. Total phenolic acid and flavonoid contents (5.26-6.84% and 0.01-0.09%, respectively) in dried aerial parts were determined spectrophotometrically. Furthermore, eleven hydroxycinnamic derivatives and fifteen flavonoids were identified in the ethanolic extracts by LC-ESI-MS/MS. Most of them were detected in the studied plant species for the first time, such as cryptochlorogenic and salvianolic acids. Rosmarinic acid (13.50-26.1 mg/g) was found to be the predominant constituent (RP-HPLC-DAD). The *Micromeria* ethanolic extracts inhibited Src tyrosine kinase activity in the time-resolved fluorescence resonance energy transfer (TR-FRET) assay. Modulation of interleukin-6 (IL-6) in LPS-stimulated mouse splenocytes by tested extract was determined using ELISA assay. The plant extracts reduced the production of proinflammatory cytokine IL-6, with a substantial contribution of rosmarinic acid contained. Our results indicated the multi-target mechanism of polyphenolic constituents of *Micromeria* species and highlighted their potential in the prevention and treatment of inflammatory-related diseases.

Structure-based discovery of flavonoids as novel inhibitors of SARS-CoV-2 3CL protease

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3C-like protease ($3CL^{pro}$) is a highly conserved cysteine proteinase that is indispensable for coronavirus replication, providing an attractive target for developing broad-spectrum antiviral drugs. Traditional Chinese medicines (TCMs) have evolved over thousands of years and are invaluable sources for drug discovery and development. We investigated the anti-SARS-CoV-2 potential of Shuanghuanglian preparation, a TCM with a long history for treating respiratory tract infection in China. Baicalin and baicalein, two flavonoids from Shuanghuanglian, were characterized as the first noncovalent inhibitors of SARS-CoV-2 $3CL^{pro}$ and exhibited potent antiviral activities in a cell-based system. Remarkably, the binding mode of baicalein with SARS-CoV-2 $3CL^{pro}$ determined by X-ray protein crystallography was distinctly different from those of known $3CL^{pro}$ inhibitors. Baicalein was productively ensconced in the core of the substrate-binding pocket, acting as a "shield" in front of the catalytic dyad to effectively prevent substrate access to the catalytic dyad within the active site. The simple chemical scaffold, unique mode of action and encouraging antiviral activity *in vitro*, coupled with the favorable safety data from previous clinical trials studies, suggest that baicalein is a good candidate for the development of critically needed anticoronaviral drugs (*Acta Pharmacol. Sin.* 2020, 41, 1167).

Inspired by this finding, a series of flavonoids were further tested using the enzymatic assay. As a result, myricetin, a flavonoid found in many food sources, showed good inhibitory activity against the protease and the replication of SARS-CoV-2 in cells. The crystal structure of the SARS-CoV-2 $3CL^{pro}$ bound with myricetin reveals an unexpected covalent binding mode that the pyrogallol moiety of myricetin covalently links to the catalytic cysteine, which is distinct to the non-covalent binding mode of baicalein. This discovery not only establishes the molecular mechanism of action of myricetin, but also illuminates the pyrogallol as a warhead suited for engaging the catalytic cysteine of $3CL^{pro}$ for the first time. Insights from our mechanistic studies have led us to rationally design myricetin derivatives as well as prodrugs with improved antiviral activities and the potential of oral administration. Therefore, our study describes the state-of-the-art of the cysteine-directed chemical modification by the natural product and reports non-peptidomimetic covalent inhibitors of $3CL^{pro}$s (*Nat. Commun.* 2021, 12, 3623).

Encapsulation of bioactives for value-added food

Viktor Nedović[1], Ana Salević[1], Steva Lević[1], Verica Đorđević[2], Bojana Balanč[2], Branko Bugarski[2]

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The latest trends in contemporary industrial production of foods involve the inclusion of bioactive compounds with potential health benefits, such as vitamins, probiotics, minerals, polyphenols, omega-3-fatty acids, and phytosterols into the foodstuff.

Most of them are sensitive to surrounding conditions and influences that might cause the loss of functionality or degradation of an ingredient before it has time to act. Encapsulation is widely used for the protection and preservation of the stability of an active compound during processing, storage, and consumption. It provides a physical barrier between active compounds and the environment. There is a multitude of possible benefits of encapsulated bioactives in the food industry. Encapsulation has to provide an adequate concentration and uniform dispersion of actives. The interest for encapsulated active compounds relies also on the possibility to overcome solubility incompatibilities between ingredients, e.g., active compounds and the food matrices. Another goal of employing encapsulation is to modify the physical characteristics of the original material in order to allow easier handling, to help the separation of the components of the mixture that would otherwise react with one another. In addition, encapsulation can be applied to prevent reaction with other components in food products such as oxygen or water.

Furthermore, encapsulation is used to mask unpleasant feelings during eating, such as bitter taste and astringency of polyphenols. It is also a useful tool to provide delivery of bioactive molecules (e.g., antioxidants, minerals, vitamins, phytosterols) and living cells (e.g., probiotics) at the desired place or within an appropriate time.

The paper describes several examples of different encapsulation techniques/carrier materials/bioactive molecules developed in our laboratories for the production of value-added food.

Improving foods with natural hypocholesterolemic agents

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The increasing awareness of consumers regarding what they eat and what effects the foods will cause in their health, has boosted the food industry to invest in the development of healthier foods that bring health benefits. Therefore, huge efforts have been made by the scientific community in finding natural ingredients that could bring extra benefits to foodstuff. In this perspective, hundreds of natural matrices have been exploited as sources of bioactive molecules with the objective of incorporating them in foods, thus providing the food industry with functional foods.

Among the bioactive molecules found in the nature, mycosterols, that due to their high chemical similarity with phytosterols, can also exert a strong hypocholesterolemic potential. According to the literature, *Agaricus bisporus* L. is the richest mushroom in mycosterols, specially ergosterol. Also important is that *A. bisporus* is the most produced mushroom worldwide, generating tons of non-commercial raw material (e.g. damaged mushrooms, subtracts, pieces of mushrooms) that have no economic value, but, are rich sources of mycosterols. Bearing this in mind, *A. bisporus* residues were fully exploited: i) extraction optimization through different extraction technologies; ii) characterization of the obtained extracts through HPLC-UV, in terms of mycosterols; iv) evaluation of the hypocholesterolemic potential using CaCo2 cells; v) toxicity of the obtaining extracts in normal cells; vi) stability evaluation of the obtained extracts to light exposure, temperature, solubility; vii) incorporation in dairy products in the free form and also after encapsulation processes (yogurts, cheese); viii) effects on the developed foods in terms of nutritional, physical and chemical profiles; ix) evaluation of the hypocholesterolemic potential along the shelf-life.

All the developed yogurts and cheeses was made in strong collaboration with enterprises dedicated to the production and commercialization of dairy products and also involved in the project ValorNatural, a project dedicated to the development of natural additives and bioactives.

The accomplishment of all the previous steps allowed the establishment of the most promising extraction technique and the ideal extraction conditions, as also the to determine the active and nontoxic dose of the obtained mycosterol-enriched extract to be incorporated in the products. Also, the main goal of developing a product with hypocholesterolemic potential was also achieved; a decrease in the cholesterol absorption in about 30% was observed. These promising results originated a national Portuguese patent.

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Multi-step domino reactions for the preparation of antiproliferative *O,N*-heterocycles

Sándor Balázs Király, Attila Mándi, Tibor Kovács, Mihály Kajtár, Tibor Kurtán

Domino reactions represent an excellent method for the preparation of complex condensed heterocycles[1]. In our group, the domino Knoevenagel - ring closure reactions of 2H-chromene derivatives are studied. From previous results, both the structure of the starting material (substitution of the aldehyde and the 2*H*-chromene subunits) and the reagent determines the mechanism of the cyclization step. In the reactions with nitroamides, -esters and -ketones, a new four-step (Knoevenagel - intramolecular hetero Diels-Alder - ring opening - aromatic substitution) reaction cascade, affording hydroxyindole derivatives, was identified. The mechanism of the reaction is supported by quantum chemical calculations of the individual steps. Based on the activity of analogue hydroxiindoles[2-3], the antiproliferative activities of the products were tested, and they showed promising results, and a moderate dependence on the C-2 indole substituent.

In reactions with aniline derivatives (Povarov reaction), a number of chromano condensed [1,6]-naphthyridine derivatives were prepared in good to excellent yields. The antiproliferative effects were tested on a human glioblastoma cell line (U87). The best candidate showed an IC_{50} value of 2-5 μM, which is two magnitudes lower, than the currently used agent, temozolomide (150-200 μM). The structure-activity relationship from the obtained results was studied, in order to further improve the activity.

[1] Lutz F. Tietze, Domino Reactions, 2014, Wiley-VCH.

[2] Filippo, M. *et. al*. Indole derivatives inhibitors of enzyme lactate dehydrogenase (LDH). U.S. Patent US20140343001A1, November 20, 2014.

[3] Bonnefoy-Berard, N. *et. al*. Compounds targeting the BFL-1 anti-apoptotic protein and uses there of for the treatment of cancer. International Patent, WO2015086593A1, June 18, 2015.

Isolation and chemical modification of metabolites of *Vitex agnus-castus* extracted with subcritical CO_2

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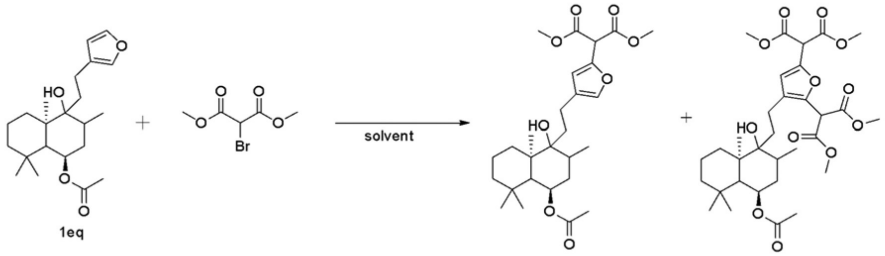
The use of supercritical fluids allows isolation of a wide range of secondary metabolites from aromatic and medicinal plants. Subcritical CO_2, near its critical point, appears to have some very special solvent features, which allow a high selectivity towards diterpenes, lipids and carotenoids. These type of compounds are known for their biological effects. For instance, rotundifurane has been reported to induce apoptosis in human myeloid leukaemia.

In our work, we have investigated some chemical modifications of rotundifurane's structure in order to improve its biological properties.



Different reactions, based on radical chemistry, have been performed, different precursor and reaction conditions have been used and different new structures have been isolated. The ratio of mono and di substituted products has been also investigated. The overall yields are moderated and, depending on the reaction conditions, we could obtain a total control of mono or di substituted products.

Keywords: Subcritical CO_2, secondary metabolites, Vitex, rotundifurane

Assessment of phytocannabinoids content in Cannabis and its preparations by FTIR spectroscopy

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Phytocannabinoids, the family of plant-derived C21 terpenophenolic compounds, are the leading Cannabis constituents responsible for its pharmacological activity. Among them tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most abundant ones, naturally occurring as acidic precursors. Consequently, their qualitative and quantitative determination is considered as critical quality attribute in the uprising Medical Cannabis industry. However, quality control of Cannabis final products can be extremely challenging, considering the variability of phytocannabinoid content in the plant material. Thus, there is apparent need for continuous monitoring of the quality parameters throughout the entire production process. Infrared spectroscopy has been successfully used for quantitative analysis in various fields including pharmaceutical, food industry, agriculture, and biological evaluation. Therefore, our main goal was development of FTIR spectroscopic method for quantification of THC and CBD in Cannabis flowers and extracts, which can be used as process analytical technology (PAT) in Cannabis quality control. MIR spectra were collected on ATR FTIR spectrometer and a detailed characterization of the peaks of the molecules of interest (THC and CBD), as well as identification of the spectral characteristics of the decarboxylation process of Cannabis flowers, was performed. Further, the obtained data was used for multivariate analysis to develop and optimize calibration models which were tested on prediction set in order to estimate the prediction capability of each MIR model against the referent analytical technique (HPLC). Partial least squares (PLS) models using second derivatives of the raw MIR spectra (1800–400 cm−1) with Savitsky-Golay smoothing were constructed. The PLS models presented satisfactory R2Y and RMSEP of 0.95 and 3.79% for THC, 0.99 and 1.44% for CBD in the Cannabis extract samples, respectively. Similar statistical indicators were noted for the PLS models for THC and CBD prediction of decarboxylated Cannabis flowers (R2Y and RMSEP were 0.99 and 2.32% for THC, 0.99 and 1.33% for CBD respectively). The VIP plots of all models demonstrated that the THC and CBD distinctive band regions bared the highest importance for predicting the content of the molecules of interest in the respected PLS models. In summary, ATR MIR in combination with chemometrics should be considered as potential PAT tool to be used for continuous and rapid THC and CBD content estimation in ensuring quality of the Medical Cannabis products.

Antimicrobial potential of subtropcal species from Montenegro

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Within the project of bilateral cooperation between Montenegro and Serbia, a project called 'Biocontrol of phytopathogenic fungus by natural products from subtropical plants order Rosales' is being implemented. Natural plant extracts are an alternative to synthetic fungicides or as an additional way to reduce their use. The choice and application of plant extracts depends on their functional characteristics, availability, cost-effectiveness and their impact on phyto-pathogens.

The project is focused on researching the biological properties of extracts of two subtropical plants from Montenegro: *Celtis australis and Ziziphus jujuba*, that previously were not recognised for this purpose.

Four extracts of *Z. jujuba* obtained from dried fruits (using various solvents: methanol, water, dichloromethane and n-butanol), and methanolic extracts prepared from leaves and unripe mesocarps of *C. australis*, were evaluated for their phenolic compounds composition as well as antimicrobial and cytotoxic properties. Both species extracts were rich in phenolic compounds.

Results showed that all of *Z. jujuba* extracts possess antibacterial/antifungal activity against seven tested bacterial species (belonging to human infectious agents and food contaminants) and fungi (four clinical isolates of dermatomycetes and six phytopathogens). The extracts showed very high antimicrobial potential, slightly weaker in the aqueous extract. No difference in antifungal potential according to the origin of the extracts was shown.

Extracts of *Celtis australis* contain various bioactive constituents and possess higher antifungal potential than 'Previcur', which is a commercial systemic fungicide used in controling production of vegetable seedling.

Therefore, both species extracts represent potential, *Z. jujuba* as source of new compounds with antimicrobial activity, while extracts of *Celtis australiscan* has a potential for application in agriculture as an alternative to the syntethic fungicides.

Worth mentioning is that the examined extracts are natural biodegradable fungicides which enable a more efficient control of pathogenic bacteria and fungi.

Keywords: *Celtis australis*, *Ziziphus jujuba*, plant extracts, antimicrobial activity

Natural and synthetic approaches in antifungal therapy

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Since the ancient times, treatments for all kinds of diseases originated from nature and especially plants. The discovery of several antimicrobial compounds accelerated the use of many types of agents with the production of antifungals lagging behind the faster growth in the production of antibacterials. Inadequate activity and toxicity of existing antifungal drugs, alongside with the advent of resistance, increased the need for developing new drugs and approaches for the treatment of fungal diseases.

Investigation of various sources of natural compounds is one of the possible ways to discover innovative antifungal agents. Extracts of plants, fungi, sponges, but also pure isolated compounds like flavonoids, lactones, essential oils and their constituents are being studied as promising antifungal agents. Moreover, studying the drugs that have already been approved and are being used in clinical practice, alongside with other chemical derivatives which have been synthesized and evaluated for their antifungal activity by various research groups, could aid in the effort for discovering lead compounds for the treatment of fungal infections. The chemical structures and the activity of the potent derivatives should always be taken into consideration and accordingly discussed to show the impact of different chemical groups on the antifungal potential.

Combination of the opportunities that nature gives us, with modern and novel technologies based on proteomics, molecular biology, synthetic chemistry and computational modeling can lead to the discovery of active compounds with all the desirable characteristics of an effective medicine that pathogenic fungi will not easily adapt to and acquire resistance mechanisms. New antifungal compounds could be designed on the basis of natural, semi-synthetic, or synthetic compounds. Future investigation in this field and utilization of *in vitro* and *in vivo* data, clinical studies, synthetic and multidisciplinary approaches remain a high priority.

References:

Sokovic, M. and Liaras, K., 2020. *Antifungal Compounds Discovery: Natural and Synthetic Approaches*. Elsevier.

A comparative study of essential oil composition of *Thymus* species from Bulgaria

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The species of genus *Thymus* are highly valued medicinal and aromatic plants and are used by people since the ancient times to treat diseases of the respiratory and digestive system, as well as of colds, but also just as herbal tea and as spices. *Thymus* species possess diverse biological activities and have, therefore, a significant potential for application in the pharmaceutical, cosmetic and food industries. The taxonomy of this genus is still a challenge because of high population variability, even if there are data on morphology, micromorphology, chemical composition, and products resulting from secondary metabolism.

Bulgarian flora is represented by 21 species belonging to two sections – *Hyphodromi* and *Serpyllum*. Of them, six species are endemic to the Balkan Peninsula. Surprisingly, *Thymus* species in Bulgaria have received only occasional attention from experts mainly on their distribution, and the aspects of their use as medicinal and aromatic plants. As a part of an ongoing project, we have started an in-depth investigation of the distribution, diversity, systematics, and phytochemical properties of the genus *Thymus* species in Bulgaria. The aim of our study was to characterize the essential oil components of 15 *Thymus* species of Bulgarian origin and to bring additional insights about their relationship. GC-MS/FID analysis of the essential oils obtained from the aerial parts led to the identification of 120 compounds in concentration more than 0.1%, accounting for 94.6-99.7% of the total oil. The obtained results showed considerable variation in the chemical composition of the species in this study: *T. pulegioides*, *T. sibthorpii*, *T.vandasii*, *T. moesiacus*, *T. jankae*, and *T. longicaulis* were rich in monoterpenoids, *T. atticus*, *T. leucotrichus*, *T. striatus* and *T. pannonicus* contained sesquiterpenoids predominately, while *T. thracicus* was characterized with almost equal amounts of mono- and sesquiterpenoids. Aromatic compounds dominated in the other 4 species, but differed in their major component: thymol in *T. glaberescens*, *T. zygioides* and *T. perinicus*, and carvacrol in *T. callieri*. The essential oil content of endemic *T. perinicus* and *T. thracicus* is reported for the first time. Principal component analysis (PCA) based on the content of all constituents was also applied to demonstrate the relationship between the studied *Thymus* species. The described essential oil composition contributed to the phytochemistry of these species and confirmed the chemical polymorphism, a widespread phenomenon within the genus *Thymus*.

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