



University of Belgrade - Faculty of Agriculture

1st European Symposium on
Phytochemicals in Medicine and Food
(1-EuSPMF)

Book of abstracts

Belgrade, Serbia
7-9 September 2022

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Medicine and Food

1-EuSPMF



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Univerzitet u Beogradu - Poljoprivredni fakultet**

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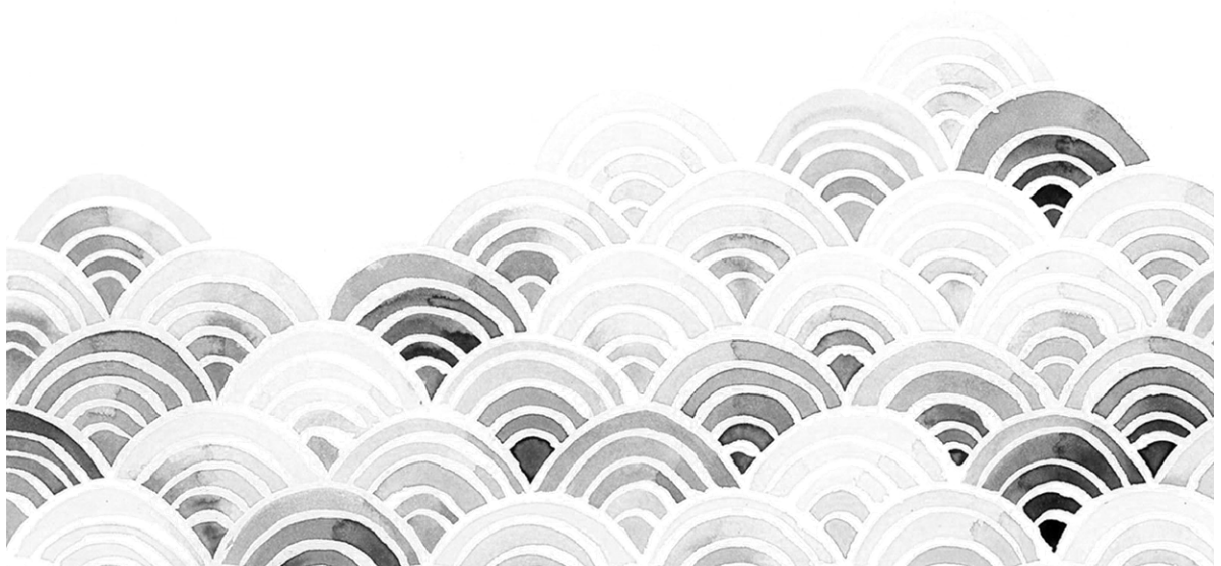
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Welcome note

We are delighted to welcome you to the 1st European Symposium on Phytochemicals in Medicine and Food (1-EuSPMF) to be held in Belgrade, Serbia on September 7-9, 2022.

The first EuSPMF conference is organized by the International Association of Dietetic Nutrition and Safety (IADNS) and the Institute of Food Technology and Biochemistry of the Faculty of Agriculture, University of Belgrade.

The aim of 1-EuSPMF is to introduce recent achievements in the area of phytochemicals and their implementation in medicine and food. Lately, we have witnessed a growing interest of researchers in natural products and their health benefits.

The conference is supported by the Serbian Chemical Society (SCS), and the Phytochemical Society of Europe (PSE), as well as by the international journals Food Chemistry, Phytochemistry Reviews, Journal of the Serbian Chemical Society, Antioxidants, Biomolecules, Horticulturae, eFood and Food Frontiers. A multidisciplinary Topic "Antioxidant Activity of Natural Products" is introduced by Antioxidants BioChem, Biomolecules, International Journal of Molecular Sciences, Marine Drugs and Molecules.

The Institute of Food Technology and Biochemistry of the Faculty of Agriculture, is honored to host scientists from 18 countries which are presenting their work within nine topics. The program consists of 9 plenary lectures, 17 invited lectures, 9 short oral presentations and 53 poster presentations, providing an excellent platform for exchanging ideas, discussing challenges, and setting up new research collaborations.

We hope that the first 1-EuSPMF conference will meet the expectations of the participants and that its organization will be even more successful in the coming years.

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Miloš B. Rajković

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TOPICS

I - Natural resources of bioactive compounds

II - Bioactive compounds and human health

III - Medicinal herbs and spices

IV - Herbal medicines in disease prevention and treatment

V - Novel perspectives of phytochemical use in contemporary medicine

VI - Farm to fork

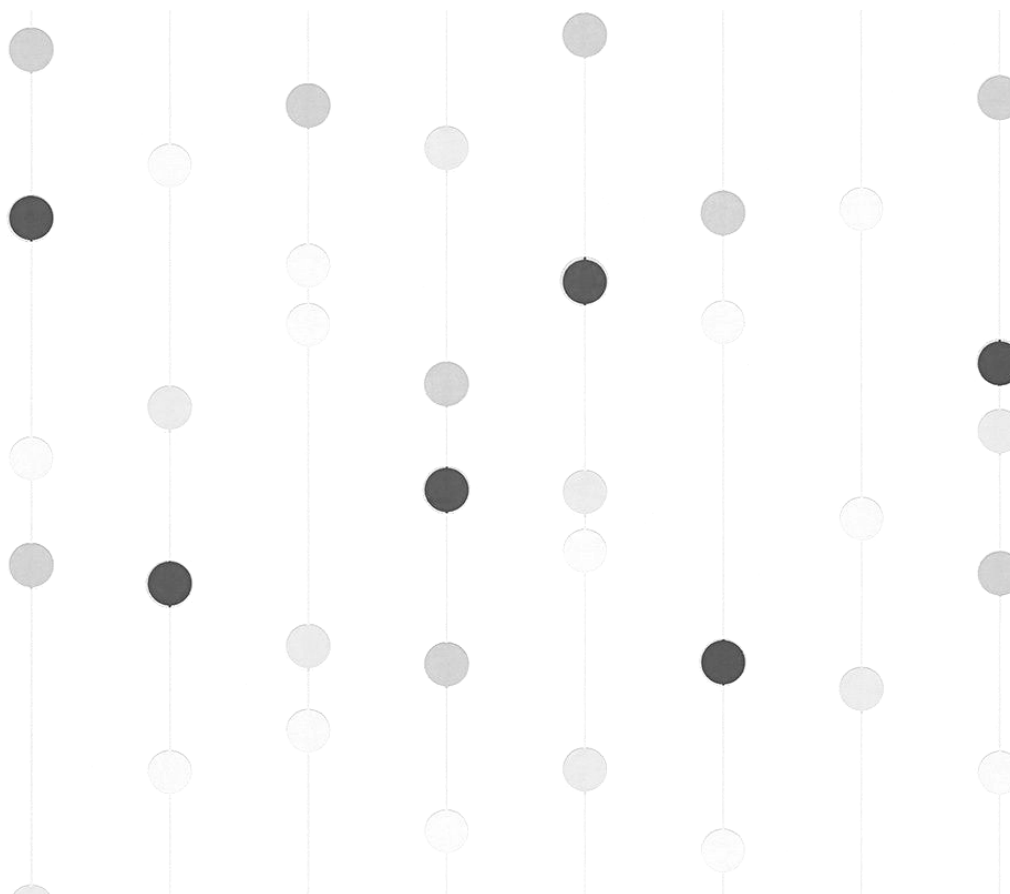
VII - Natural resources in food industry

VIII - Food and agro waste recovery

IX - Analytical methods

Abbreviations:

PL - Plenary lecture **IL** - Invited lecture **OP** - Oral presentation **PP** - Poster presentation



LIST OF PRESENTATION

PLENARY LECTURES		Page
I_PL	<u>Robert Verpoorte</u> , Y.H. Choi Food for health, food for thought	1
II_PL	Li Yang, <u>Jianbo Xiao</u> Myricetin ameliorated prediabetes via immunomodulation and gut microbiota interaction	2
III_PL	Hesham R. El-Seedi Plants mentioned in the Islamic Scriptures and 5700 years ago: Traditional uses and medicinal importance in contemporary time	3
IV_PL	María G. Campos Drug-Herb Interactions as a cause of therapeutic failure or toxic events: Oncology examples of Case Reports	4
V_PL	Maurizio Battino Unraveling the molecular mechanisms underlying the health effects of dietary bioactive compounds	5
VI_PL	Rachel Levi, Or Shapira, Inbal Hanuka Katz, Zoya Okun, <u>Avi Shpigelman</u> Stability of anthocyanins during processing and shelf life: potential implication to processed products	6
VII_PL	A.E.Giannakas, C.E. Salmas, E.Kollia, A. Kopsacheili, C. Birlia, <u>C. Proestos</u> Use of essential oil from plants to produce nanostructures as active packaging materials for the food industry	7
VIII_PL	<u>Esra Capanoglu</u> , Elifsu Nemli, Francisco Tomas-Barberan Valorization of agricultural wastes: Novel approaches and applications	8
IX_PL	Jesus Simal-Gandara Untargeted and targeted metabolomics	9
INVITED LECTURES		
I_IL1	Marina Soković Bioactivity from bioworld	10
I_IL2	Filip Andrić Towards the most desirable natural products and their bioactivity features using multicriteria decision making methods and linear regression/classification algorithms	11
II_IL1	Goran N. Kaluđerović Natural compounds immobilized into mesoporous silica	12
II_IL2	<u>Olgica Nedić</u> , Nikola Gligorijević, Ana Penezić, Simeon Minić, Mirjana Radomirović, Milan Nikolić, Tanja Ćirković Veličković Food antioxidants and their interaction with human proteins	13
III_IL1	Gordana Stojanović Medicinal plants - Traditional application in South-Eastern Serbia	14
IV_IL1	<u>Maria Daglia</u> , Hammad Ullah, Alessandra Baldi, Alessandro Di Minno Brewer's spent grain extract rich in dietary fibers and postprandial glycemia and insulin response in healthy subjects: a monocentric, randomized, cross-over, double-blind, placebo-controlled clinical trial	15
IV_IL2	A. Gulcin Sagdicoglu Celep The role of polyphenols in preventive nutrition	16

V_IL1	Isidora Protić-Rosić, Zorana Lopandić, Milena Zlatanova, Andrijana Nešić, Radmila Miljković, Ivana Lukić, Marina Atanasković-Marković, Marijana Stojanović, <u>Marija Gavrović-Jankulović</u> Immunomodulatory potential of banana lectin in allergen-specific immunotherapy	17
V_IL2	<u>Irena Brčić Karačonji</u> , Nevenka Kopjar, Suzana Žunec, Andreja Jurič, Anja Katić, Ljerka Prester, Vedran Micek, Nino Fuchs, Marijana Neuberg, Goran Kozina, Ana Lucić Vrdoljak Potentially harmful interactions between Δ^9-tetrahydrocannabinol (THC) and irinotecan	18
VI_IL1	Luiz Fernando Cappa de Oliveira New Strategies for Identifying Phytochemicals in Food: Nondestructive Raman Spectroscopy Analysis	19
VI_IL2	<u>Nebojša Nedić</u> , Živoslav Tešić, Milica Nešović, Kazimir Matović, Stevan Blagojević Risks in honey production and its quality shown at the example of honey produced in a national park	20
VII_IL1	<u>Bojana Vidović</u> , Danijel D. Milinčić, Mirjana Marčetić, Jelena Djuriš, Tijana Ilić, Aleksandar Ž. Kostić, Mirjana B. Pešić Goji berries as sources of functional food ingredients	21
VII_IL2	<u>Sandrina A. Heleno</u> , Márcio Carochó, Lillian Barros Plants as a source of natural preservatives for food application	22
VIII_IL1	Biljana Dojnov Recycling of agro waste by fungi for obtaining enzymes and prebiotics	23
IX_IL1	Morlock Gertrud Prioritization strategy to find the important phytochemicals in a complex natural sample	24
IX_IL2	<u>Irena Vovk</u> , Vesna Glavnik, Breda Simonovska, Maja Bensa Challenges in chromatographic analyses of phytonutrients in medicinal plants and food	25
IX_IL3	Vibor Roje Analysis of metals and metalloids in plant materials - a short overview	26
ORAL PRESENTATIONS		
I_OP1	<u>Jelena S. Katanić Stanković</u> , Jelena Đorović Jovanović, Danijela Mišić, Uroš Gašić, Rudolf Bauer Phytochemical profiling and insight into bioactivity of <i>Stellaria holostea</i> L. (Greater Stitchwort) extract	27
II_OP	<u>Niranjan Koirala</u> , Amit Kumar Shrivastava Phytochemical screening and the effect of <i>Trichosanthes dioica</i> in high-fat diet induced atherosclerosis in Wistar rats	28
III_OP	<u>Hammad Ullah</u> , Rita di Matteo, Alessandro Di Minno, Antonietta Rossi, Maria Daglia <i>In vitro</i> anti-inflammatory effect of <i>Allium cepa</i> L. extract rich in quercetin optimized by design of experiments	29
IV_OP	<u>Niranjan Koirala</u> , Roshani Gurung, Sundar Adhikari <i>Reinwardtia indica</i>: phytochemical screening and evaluation of wound healing activity of the extracts in experimental model rats	30

V_OP	Adma N.F. Melo, Tiago B. Afonso, Marta Carvalho, Cláudia Rodrigues, Tânia Ribeiro, <u>Márcio Caroch</u> , Miguel Marques Pinto, Freni Tavaría, Paula Teixeira, J. Pedro Simas, Lillian Barros, Manuela Pintado Chemical characterization, cytotoxic evaluation and anti-SARS-CoV2 activity of plant extracts rich in hydrolysable tannins	31
VI_OP	<u>Vesna Dragičević</u> , Milan Brankov, Milovan Stoilković, Milena Šenk, Željko Dolijanović, Miodrag Tolimir, Milena Simić Sustainable fertilization systems as a prerequisite for improved quality of agricultural products	32
VII_OP	<u>Aleksandra Milenković</u> , Jelena Stanojević, Ljiljana Stanojević, Zoran Ilić, Lidija Milenković Chemical composition and antioxidant activity of black pepper (<i>Piper nigrum</i> L.) fructus essential oil hydrodistillation fractions	33
VIII_OP	Latifeh Pourakbar, Sina Siavash Moghaddam, <u>Jelena Popović-Djordjević</u> Interactive effects of ultraviolet-B and ZnO nanoparticles on biochemical and antioxidant traits of saffron (<i>Crocus sativus</i> L.)	34
IX_OP	<u>Maria Antonietta Carrera</u> , Esther Miguel Gómez, Amadeo Rodríguez Fernández-Alba, María Dolores Hernando Guil Characterization of phenolic content in bee pollen and bee bread originating from different climatic areas	35

POSTER PRESENTATIONS

I_PP1	<u>Sofija Kilibarda</u> , Marina P. Mačukanović-Jocić, Danijel D. Milinčić, Sandra Vuković, Snežana Jarić, Aleksandar Ž. Kostić Phytochemical analysis of <i>Cicerbita pancicii</i> (Vis.) Beauverd-the Balkan's endemic plant	36
I_PP2	<u>Aleksandra Milenković</u> , Ljiljana Stanojević, Zoran Ilić, Lidija Milenković, Ljubomir Šunić, Jelena Stanojević, Dragan Cvetković Essential oil composition and antioxidant activity from different plant part of wild fennel (<i>Foeniculum vulgare</i> Mill.) growing in Montenegro sea-side	37
I_PP3	Nadezhda Petkova, Tatyana Bileva, Manol Ognyanov, Magdalena Stoyanova, <u>Dasha Mihaylova</u> , Ekaterina Valcheva, Neli Grozeva, Vladislav Popov Nutritional characteristics and physico-chemical quality of Florina apple fruits	38
I_PP4	<u>Dasha Mihaylova</u> , Aneta Popova, Ivayla Dincheva Pattern recognition of varieties of peach fruit and pulp from their volatile components using HS-SPME-GC/MS combined with multivariable statistical analysis	39
I_PP5	<u>Mina Janković</u> , Sanja Berežni, Dejan Orčić Isolation and identification of lignan and phenylpropanoid esters from wild chervil (<i>Anthriscus sylvestris</i> (L.) Hoffm.)	40
I_PP6	Maria Inês Dias, Carla Pereira, Carly Albiston, <u>José Pinela</u> , Lillian Barros HPLC-DAD-ESI/MS profiling of phytochemicals in <i>Aesculus hippocastanum</i> fruit	41
I_PP7	<u>Maria Inês Dias</u> , José Pinela, Carla Pereira, Patrícia Ferreira, Maria de Fátima Oliveira, Anabela Martins, Andreia Afonso, Lillian Barros Mycorrhization and micropropagation of chestnut (<i>Castanea sativa</i> Mill.) seedlings as tools to obtain high added-value phenolic compounds	42

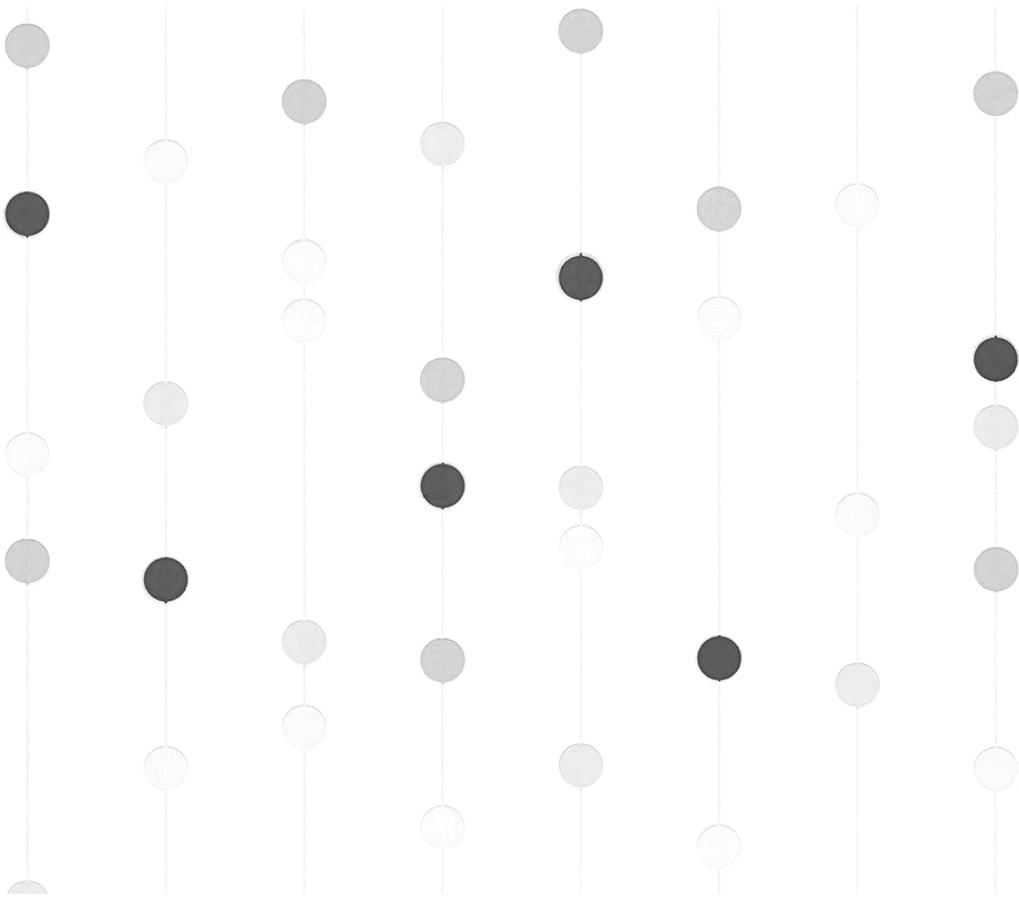
I_PP8	<u>Sladana Žilić</u> , Valentina Nikolić, Nada Ćujić Nikolić, Marijana Simić, Katarina Šavikin, Jelena Živković, Marko Vasić Microencapsulation of anthocyanins from blue maize and their fate during <i>in vitro</i> gastrointestinal digestion	43
I_PP9	<u>Tijana Ilić</u> , Stevan Samardžić, Gordana Zdunić, Dragana Božić, Mirjana Marčetić, Bojana Vidović Screening of the phenolic composition and <i>in vitro</i> biological activities of the fruit of <i>Lycium ruthenicum</i> Murray	44
I_PP10	<u>Nemanja Živanović</u> , Biljana Božanić Tanjga, Dejan Orčić, Mirjana Ljubojević, Marija Lesjak, Nataša Simin Chemical characterization and antioxidant activity of new rose genotypes (<i>Rosa hybrida</i>)	45
I_PP11	<u>Nemanja Živanović</u> , Dejan Orčić, Marija Lesjak, Nataša Simin, Neda Mimica-Dukić Chemical characterization and antioxidant activity of hawthorn (<i>Crataegus</i> spp.)	46
II_PP1	<u>Ana Valenta Šobot</u> , Jelena Filipović Tričković, Dunja Drakulić, Andreja Leskovac, Sandra Petrović, Tatjana Momić Sweroside displays a cytotoxic effect by activating apoptosis in human peripheral blood mononuclear cells	47
II_PP2	Nikolina Lisov, Danka Mitrović, <u>Ivana Sredović Ignjatović</u> , Aleksandar Petrović Total phenolic content and antioxidant properties of fermented grape pomace of Cabernet Sauvignon grape variety	48
II_PP3	<u>Katarina Mitić</u> , Bogdana Simonović, Irena Nikolić, Nebojša Đ. Pantelić Total polyphenols and flavonoids content and antioxidant potential of methanolic extracts of different types of berries	49
II_PP4	<u>Andrea Pirković</u> , Aleksandra Vilotić, Zanka Bojić Trbojević, Dragana Dekanski, Milica Jovanović Krivokuća Effect of oleuropein on the expression of invasive markers and cell adhesion of HTR-8/SVneo extravillous trophoblast cells	50
II_PP5	<u>Andrea Pirković</u> , Sunčica Borožan, Marija Bruić, Milica Jovanović Krivokuća, Biljana Spremo-Potparević Taxifolin protects HTR-8/SVneo extravillous trophoblast cells against H₂O₂-induced oxidative damage and improves antioxidant function	51
II_PP6	<u>Svetlana Bogdanović</u> , Dobrila Randjelović, Ivana Zlatković, Dušica Cirković, Dragana Stanisavljević Antimicrobial activity of acacia honey, with and without additives, against selected pathogens	52
II_PP7	<u>Marija Ivanov</u> , Tamara Carević, Marina Kostić, Ana Ćirić, Jovana Petrović, Jasmina Glamočlija, Marina Soković Selected terpenoids as obstructers of <i>Candida</i> virulence	53
II_PP8	Dragana Božić, <u>Tijana Ilić</u> , Mirjana Marčetić, Stevan Samardžić, Gordana Zdunić, Bojana Vidović <i>In vitro</i> assessment of the prebiotic potential of selected plant extracts	54
II_PP9	Imad Ahmad, Abdul Saboor Pirzada, Khalaf F Alsharif, Maria Daglia, <u>Haroon Khan</u> <i>In silico</i> evaluation of 4-hydroxy isoleucine via ADMETLab 2.0: Therapeutic potential for clinical uses	55

III_PP1	<u>Sandra Vuković</u> , Đorđe Moravčević, Dejana Marinković, Slavica Jelačić, Ana Vujošević, Sofija Kilibarda, Danijel D. Milinčić, Aleksandar Ž. Kostić Antioxidant properties of cinnamon spice	56
III_PP2	<u>Jugoslav Trajković</u> , Dragana Stanisavljević, Dušica Ćirković, Svetlana Bogdanović, Dobrila Randjelović, Dragan Veličković, Milić Vojinović Chemical composition of the essential oil of <i>Thymus serpyllum</i> L.	57
III_PP3	<u>Ilija Cvijetić</u> , Irena Brčić Karačonji, Andrea Jurič, Karlo Jurica, Dušanka Milojković-Opsenica <i>In silico</i> study of the tyrosinase inhibitory potential of arbutin and other bioactive compounds from strawberry tree	58
III_PP4	Milica Jankov, <u>Ilija Cvijetić</u> , Dušanka Milojković-Opsenica DFT study of the antioxidant activity of polyhydroxyflavones identified in houseleek leaf extracts	59
III_PP5	<u>Latifeh Pourakbar</u> , Sina Siavash Moghaddam, Somayeh Maleki Kakelar The antioxidant and antifungal activity of the extract, essential oil and AgNPs green synthesized of aqueous and methanolic extracts of <i>Origanum vulgare</i> L.	60
IV_PP1	<u>Mohammad Omar Faruque</u> , Uddin Shaikh Bokhtear, Hu Xuebo Demystifying Bangladeshi ethnomedicines with modern approaches: <i>Congea tomentosa</i> as a source of potential anti-microbial and anti-cancer agents	61
IV_PP2	<u>Nouredine Djebli</u> , Hadjer Chenini-Bendiab Neuroprotection of date (<i>Phoenix dactylifera</i> L.) extract from Algeria against Neurodegenerative disease - "Alzheimer's case"	62
IV_PP3	<u>Marija Ivanov</u> , Jelena Božunović, Maria Inês Dias, Ricardo C. Calhelha Lillian Barros, Isabel C.F.R. Ferreira, Dejan Stojković <i>Tripleurospermum inodorum</i> – mayweed without scent but with range of bioactivities	63
V_PP1	<u>Ana Valenta Šobot</u> , Jelena Filipović Tričković, Andreja Leskovac, Sandra Petrović, Tatjana Momić Nanotechnology approach for diminishing quercetin toxicity toward peripheral blood mononuclear cells	64
VI_PP1	Masudulla Khan Elucidating the role of mangiferin compound and silver nanoparticles (AgNPs) against plant pathogenic fungi <i>Phomopsis vexans</i>	65
VI_PP2	Svetlana Lakićević, <u>Dušica Ćirković</u> , Jelena Popović-Đorđević, Ivana Mošić, Miodrag Lazić Microbiological characteristics of 'Prokupac' wine enriched with medicinal herbs	66
VI_PP3	<u>Monalisa Sahoo</u> , S.P.L. Balasubramaniam, Balunkeswar Nayak, Vivek Kumar, SN Naik Effect of processing on the antinutrients, antioxidant and techno-functional characteristics of five-leaf yam (<i>Dioscorea pentaphylla</i>)	67
VI_PP4	<u>Svetlana Bogdanović</u> , Dobrila Randjelović, Ivana Zlatković, Zvonko Zlatanović, Milica Stojanović Microbiological quality control of cheeses produced on farms in the territory of the city of Prokuplje	68
VI_PP5	<u>Dušica Ćirković</u> , Svetlana Lakićević Zoran Jovanović, Ivana Zlatković, Bratislav Ćirković, Dobrila Randjelović, Svetlana Bogdanović	69

	Agrobiological traits of grape cultivar 'Tamjanika white' grown in Župa vine district	
VI_PP6	<u>Monalisa Sahoo</u> , Sushree Titikshya, Vivek Kumar, SN Naik Antioxidant activities and structural functional characterization of bitter yam flour (<i>Dioscorea bulbifera</i>) as influenced by different drying techniques	70
VI_PP7	<u>Milica Fotirić Akšić</u> , Biljana Rabrenović, Uroš Gašić, Tomislav Tosti, Dragana Dabić Zagorac, Maja Natić, Mekjell Meland Is there a difference in the profile of the health promoting compounds in plum fruits and kernels grown under the organic and conventional production system?	71
VII_PP1	<u>Jovana Marković</u> , Dragana Mihajlović, Pavle Mašković, Nebojša Banjac, Jelena Mašković, Evica Ivanović Effect of different heat treatments on antioxidative activity in pumpkin (<i>Cucurbita maxima</i>)	72
VII_PP2	<u>Katarina Delić</u> , Danijel Milinčić, Ana Salević, Mirjana Pešić, Viktor Nedović Micro and nano-encapsulation of anthocyanins and its application in food technology	73
VII_PP3	<u>Aleksandar Ž. Kostić</u> , Nebojša Nedić, Danijel D. Milinčić, Slađana P. Stanojević, Živoslav Lj. Tešić, Mirjana B. Pešić Antioxidant properties of monofloral poppy, and bastard indigobush bee-collected pollen as potential functional food ingredient	74
VII_PP4	<u>Dobriła Randjelović</u> , Svetlana Bogdanović, Ivana Zlatković, Zvonko Zlatanović, Dušica Ćirković, Dragana Stanisavljević Microbiological quality control in fresh and dried apple fruit and testing of hydroxymethylfurfural (HMF) content	75
VII_PP5	<u>Dobriła Randjelović</u> , Svetlana Bogdanović, Ivana Zlatković, Zvonko Zlatanović, Sanja Perić, Sladjana Golubović Analysis of anthocyanins content and microbiological quality control in fresh and frozen raspberry fruit	76
VII_PP6	<u>Valentina Nikolić</u> , Slađana Žilić, Marijana Simić, Vesna Kandić, Dejan Dodig, Marko Vasić Oat varieties with different hull colours as potential sources of phytochemicals for the food and feed industry	77
VII_PP7	<u>Marijana Simić</u> , Valentina Nikolić, Vesna Kandić, Slađana Žilić, Marko Vasić, Dejan Dodig Small cereal grains as a source of phytochemicals	78
VII_PP8	<u>Suzana Skeledžija</u> , Miloš B. Rajković, Aleksandar Ž. Kostić, Jelena Popović-Djordjević Content of fatty acids and theobromine in organic cocoa powder	79
VII_PP9	Kosana Šobot, Ljiljana Popović, Alena Stupar, Vladimir Filipović, Milica Nićetin, <u>Jovanka Laličić-Petronijević</u> The influence of the drying method on the preservation of bioactive compounds in the wild garlic (<i>Allium ursinum</i> L.) leaves as a functional ingredient incorporated in cookies	80
VIII_PP1	Custódio Lobo Roriz, Sandrina Heleno, <u>Márcio Carochó</u> , Patricia Morales, Isabel C.F.R. Ferreira, Lillian Barros Two new alternative sources of betacyanin colouring compounds for the food industry	81

VIII_PP2	Milan Kumar Lal, Rahul Kumar Tiwari, Jelena Popović-Djordjević, <u>Nemanja Gršić</u> , Awadhesh Kumar, Ravinder Kumar, Arvind Jaiswal, Dharmendra Kumar, Sushil Sudhakar Changan, Som Dutt, Brajesh Singh Interactive effect of potato peel on <i>in vitro</i> glyceemic response and starch digestibility with potato	82
VIII_PP3	Mekjell Meland, Tomislav Tosti, Biljana Rabrenović, Sandra Šegan, Dragana Dabić Zagorac, Maja Natić, <u>Milica Fotirić Akšić</u> Chemical fingerprint of plum (<i>Prunus domestica</i> L.) kernels grown in Norway	83
IX_PP1	<u>Milica Lučić</u> , Ivana Sredović Ignjatović, Steva Lević, Ivan Zlatanović, Antonije Onjia Effect of ultrasound and chemical pretreatments on L-ascorbic acid of dried bell pepper (<i>Capsicum annuum</i>) studied by factorial design	84
IX_PP2	<u>Simona Jaćimović</u> , Biljana Dojčinović, Biljana Kiprovska, Nebojša Đ. Pantelić Determination of selected macro- and microelements in black chokeberries (<i>Aronia melanocarpa</i> L.)	85
IX_PP3	<u>Milka Jadranin</u> , Gordana Krstić, Miroslav Novaković, Boban Anđelković, Vele Tešević, Slobodan Milosavljević Analysis of diterpenoids from the latex of <i>E. seguieriana</i> Neck. subsp. <i>seguieriana</i> by liquid chromatography–electrospray ionisation mass spectrometry	86
IX_PP4	Nebojša Banjac, Jelena Ladjarević, <u>Dušan Vasić</u> , Dragan Milatović, Djordje Boškov, Jelena Popović-Djordjević Insight into chemical composition of sweet cherry (<i>Prunus avium</i> L.) fruits - application of ATR-FTIR spectroscopy	87
IX_PP5	<u>Nenad Mićanović</u> , Jelena Lađarević, Nebojša Banjac, Jelena Popović-Djordjević Insight into chemical composition of wild growing fruits from Serbia - application of ATR-FTIR spectroscopy	88

ABSTRACTS





I_PL_Food for health, food for thought

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Plants are the source of our food, medicines, cosmetics, agrochemicals, clothes, building materials and fuel. Globally, some 30 species are used as staple food, some 100 species as vegetable and some 100 species for fruits. For medicinal uses the estimations run from 40.000 to 70.000 species. The importance of plants for mankind is obvious. Our ancestors have been very successful in finding edible plants, and eventually learned to cultivate these on large scale. The same happened for medicinal plants, though collection in the wild yielded in most cases sufficient material. In the past years food industry became interested in proving the health effects of food, as proper evidence may lead to higher profits on food with proven pharmacological effects. However, only very few examples have reached the point that the evidence is such that the regulatory authorities accept the health claims. So what is the problem? We learned from the studies of medicinal plants that sometimes single compounds can be found that have a desired pharmacological effect. But for many medicinal plants such evidence is missing. An explanation could be that there is more than one active compound, and that these compounds work in synergy. A reductionist approach to find an active compound will thus fail. Also prodrugs might be present, in that connection the GI-tract microbiome is presently an important area for food research, as the various microorganisms may convert non-active plant metabolites into active compounds. Hydrolysis of glycosides is a well-known example, e.g. salicylic acid. Alan Roses (GSK) said "The vast majority of drugs - more than 90 per cent - only work in 30 to 50 per cent of the people". That means activity may be observed in large clinical trials, but to apply that approach to food is quite costly. Moreover if the active compound(s) are not known, standardized preparations are difficult to make. If no chemical quality control is possible, one needs to standardize the activity in a bioassay. The high variability of the metabolome of plants is a major hurdle for a number of applications. For most diseases a constant level of the medicine in the body is required, e.g. in case of cardiovascular diseases and diabetes. That means that every day the same health food should be eaten in the same quantities, several times per day. The goal of medication, to restore the natural homeostasis with food might thus be difficult to achieve. On the other hand, in prevention vitamins are a clear example of compounds that have a quite irregular daily intake depending on the food eaten every day. To develop food for health we need a systems biology approach. That means to observe, in all possible ways, the effect of complex mixtures of compounds on a biological system. In such an approach the differences between samples are an advantage for finding markers for activity. These markers are needed for the quality control of health food. Plant breeders and plant growers have to develop varieties and plant growth protocols to enable reproducible production. The separation between health food and medicinal plants is vague; it might be on the level of the activity, and the sort of the activity. Traditionally the personalized medicine played an important role in optimizing the pharmacotherapy by evaluating a patient's response to a given treatment. Different levels of active compounds were probably recognized by the healer, who adjusted the prescription on the basis of the patient's response to the treatment. Still much to learn from nature and our ancestors!



II_PL_Myricetin ameliorated prediabetes via immunomodulation and gut microbiota interaction

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This study aimed to evaluate the effects of myricetin on prediabetes on RAW 264.7 cells, Jurkat cells and mice. The cell viability, endocytosis and phagocytosis of macrophages were inhibited in RAW 264.7 cells under high glucose (HG) levels, concurrent with an increase in ROS level. Administration of myricetin at a dose of 10 μ M, restored the immunosuppressive effects mediated by HG. Moreover, the viability of Jurkat cells was also inhibited concurrent with inhibition of IL-2 and IFN- γ expression levels versus increase PD-1 after with treated with myricetin at the dose of 10 μ M. In a prediabetic mouse model fed with high fat diet, increase in body weight, fat mass, fasting blood glucose, TG, TC and LDL-C were observed. Flow cytometry analysis revealed a significant decrease in CD8⁺ T cells in the spleen and blood, whereas the expression of PD-1 on CD3⁺, CD4⁺ and CD8⁺ T cells in the spleen and lymph was significantly elevated. Administration of myricetin showed a potential hypoglycemic and lipid-lowering effect in both prevention and treatment, in addition to restoration of innate and adaptive immune immunity in mice and confirmed the *in vitro* immunomodulation effect. In addition, 16S rRNA showed that myricetin ameliorated prediabetes may be related to decrease in the relative abundance of Acetatifactor, Blautia, Intestinimonas, Anaerotruncus and Peptococcus. Myricetin could alleviate dyslipidaemia, as well as the immunosuppressive effects induced by persistent hyperglycaemia *in vitro* and *in vivo* experiments.

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III_PL_Plants mentioned in the Islamic Scriptures and 5700 years ago: Traditional uses and medicinal importance in contemporary time

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Since thousands of years ago, studies have been carried out globally especially in the Middle East to verify the efficacy of foods and medicinal herbs. Some of the research findings have led to the production of natural products sources-based medicines. In this talk, some potential natural products with beneficial health effects will be presented. First, the importance of traditional plants i.e., *Allium sativum* (garlic) and *A. cepa* (onion) (Quranic Plants); that are used against diabetes type 2, coronary heart disease, obesity, hypercholesterolemia, and hypertension [1]. Moreover, the importance of Cactaceae plants was proved since ancient times; for example, our mescaline discovery from peyote cactus *Lophophora williamsii* shown that the compound displayed psychotropic properties and peyote samples appear to be the oldest plant drug ever i.e., as long as 5700 years ago [2]. Another plant that is traditionally used to reduce blood pressure, regulate body temperature, enhance urination, and cause depletion of antioxidants is Karkadeh (*Hibiscus sabdariffa* (roselle)). Its anti-hypertensive culinary properties as well as therapeutic effects were proved by metabolic tools to be due to the presence of anthocyanins [2], and similarly, the large-scale metabolite profiling of *Ficus sycomorus* (Sycamore Fig) extract, were found to inhibit intestinal coccidiosis in experimentally infected rabbits (*in vivo* studies) [4]. On the other hand, the talk will tackle the green tea, as a non-fermented tea (*Camellia sinensis*), and its bioactive constituents and antioxidant capability were determined by NIR spectroscopy coupled with swarm intelligence algorithm [5]. Finally, bee products are often sold as nutritional supplements, cosmetics and/or health products, as per their anticancer, antimicrobial, antioxidant, anti-nociceptive, and anti-inflammatory activities. Interesting results from bee products will be also discussed (Fig 1).

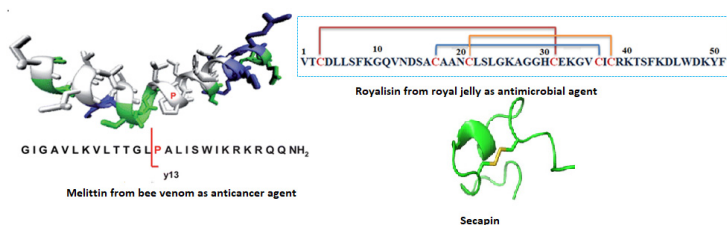


Figure 1. Bioactive compounds from bee products

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IV_PL_Drug-Herb Interactions as a cause of the therapeutic failure or toxic events: Oncology Case Reports

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Drug-drug, Nutrient and/or Herb Interactions are a very hot issue these days. The scientific proves are more and more reliable which help to promote information to patients and clinicians. Both need to be aware of this possibility when a therapy is started. The right communication about the possible interactions among the drugs, food and/or other natural products, including medicinal plants is the best way to get the best for efficacy and the safety, and do not compromise the treatment.

To a better understanding of this question we suggest a deep look in some examples of real clinical cases involving drug-food and drug-herb interactions to highlight the most frequent, for instance in Portugal, but also some from the literature available with Case Reports.

However, depending of the pathology and the therapy apply, these clinical emergencies can be very complex and imply the hospitalization. We will present and discuss some examples from real situations which were follow in our research group (www.oipm.uc.pt) what will give a more broad scenario about this issues [1,2].

In the majority of the cases, the decrease of efficacy which causes therapy failure could be more problematic in oncologic patients. However, situations involving drug-herb interactions and also toxic contaminants, for instance, from dietetic supplements, will increase the risk².

Plants as Aloe vera, Citrus, Curcuma, Ginger, Menthe, Milk Thistle, Ginseng and many others are responsible for many of the accidents cited above, and that we will need to learn to avoid to the security of the patients [3].

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To all our patients and clinicians which work to solve these clinical problems.

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V_PL_Unraveling the molecular mechanisms underlying the health effects of dietary bioactive compounds

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Epidemiological studies have already established a close association between a diet enriched in fruits and vegetables and a lower risk for the onset and development of several non-communicable diseases, including types 2 diabetes, cardiovascular diseases and some types of cancer. Bioactive phytochemicals are of increasing interest for their roles both in preventive strategies and as adjuvants in the treatment of different pathologies. During the past decade, our research group has been widely involved in the evaluation of the protective effects of natural compounds present in different food matrices against several types of stressors, by using diverse *in vitro* and *in vivo* experimental models [1-3]. In this presentation the ability of common dietary polyphenols to modulate the expression of several genes and proteins involved in different biological processes, including apoptosis, inflammation, antioxidant defence, lipid metabolism, proliferation, metastatization, cell cycle and mitochondrial biogenesis, will be presented and deeply discussed.

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VI_PL_Stability of anthocyanins during processing and shelf life: potential implication to processed products

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Polyphenols are secondary metabolites widely distributed in vegetables and fruits. In addition to sensorial aspects, they are known for their antioxidant, anti-inflammatory, and even direct action on cellular activities, therefore often perceived and considered as health-promoting compounds. While numerous studies focus on the identification of the range of structures in different matrixes less attention was given to the understanding of their stability during processing and shelf life as a function of their diverse chemical structure. Yet, this topic is of major importance in the many processed products we consume. Anthocyanins, like other polyphenols, can undergo enzymatic and non-enzymatic deterioration, and also present interactions changing their solubilities. In this talk, an overview of the understanding regarding the stability of anthocyanins will be presented with some examples of recent findings reached in our group regarding the non-enzymatic degradation of anthocyanins. We will discuss the importance of conjugated sugar to both the physical and chemical stability of cyanidins [1] with a special focus on physical stability, as a potential limiting factor to bioactivity. We will also discuss two common external factors, temperature and pressure and how their impact is also co-affected, in a structure-dependent manner, by co-solutes [2-3]. Despite major differences in the reaction rate (k) of anthocyanins observed at different temperatures and pressures the energy of activation (E_a) and activation volume (ΔV^\ddagger) were quite similar. Lastly, we will discuss how all those changes affect the in-vitro antioxidant capacity [4].

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VII_PL_Use of essential oil from plants to produce nanostructures as active packaging materials for the food industry

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In this work, a Thyme oil adsorbed in commercial organo-modified Na-montmorillonite (TO@OrgMt) nanostructure and a ZnO growth on Na-montmorillonite (ZnO@OrgMt) nanostructure were dispersed in chitosan(CS)/poly-vinyl-alcohol(PVOH) matrix to give novel CS/PVOH/TO@OrgMt and CS/PVOH/ZnO@NaMt edible active coatings. Both CS/PVOH/TO@OrgMt and CS/PVOH/ZnO@NaMt were applied as active coatings to extend tofu sausages self-life. Pure CS and CS/PVOH coatings were also used as blank samples. Sausages after coating were stored at 7 °C and microbiological analysis was performed for the total count of mesophilic, psychrotrophic, and lactic acid bacteria as well for 30 days. Coated samples were compared to uncoated sausages (UC) and vacuum-packed sausages (VP). There is no specific European legislation outlining the maximum allowable microbial counts in tofu. However according to the «Soyfoods Association of America's Tofu», tofu products are considered marginally acceptable for consumption when the microbial count is lower than 10^6 - $10^{6.7}$ CFU/g. Most of the tofu sausage samples used on day 0 was above the acceptable limit 10^5 - 10^6 CFU/g but within the marginally acceptable limit. After coating, a decrease in the mesophilic bacteria population was observed in all cases ($<10^5$ CFU/g). Moreover, psychrotrophic and lactic acid bacteria growth was also delayed for all the coating treatments in comparison to UC and UC/ VP sausages. Based on microbiological quality, the shelf life of the tofu sausages was extended, since 12 days before the commercial expiry date, the microbial count was still low ($<10^5$ CFU/g). Finally, no negative impact on the odor of the treated samples was noticed. However, changes in the color and the tenderness (dehydration) were observed.

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VIII_PL_ValORIZATION of agricultural wastes: Novel approaches and applications

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Food processing sustainability, as well as waste minimization, is key concerns for the modern food industry. Significant amount of waste is generated worldwide each year. In addition to the economic losses caused by the removal of these wastes, its impact on the environment is undeniable. The best option is to minimize food waste/by-products since it triggers many global environmental problems, the economy, and society. Different waste management methods are available to use food wastes in different industries. Additionally, novel and green approaches may also be used to valorize food wastes and improve their stability and applicability. In this work, novel methods for waste valorization including their applications for different purposes are covered together with the limitations and future perspectives.



IX_PL_ Untargeted and targeted metabolomics

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Metabolomics is a young field of knowledge that arises linked to other omics such as genomics, transcriptomics, and proteomics. This discipline seeks to understand the performance of metabolites, identifying, quantifying them, and thus understanding its mechanism of action. This new branch of omics science shows high potential, due to its noninvasive character and its close relation with phenotype. Several techniques have been developed to study the metabolome of biological samples, fundamentally nuclear magnetic resonance (NMR), and mass spectrometry (MS), or a combination of several techniques. These techniques are focused to separate, detect, characterize, and quantify metabolites, as well as elucidate their structures and their function on the metabolic pathways they are involved. However, due to the complexity of the metabolome, in most cases it is necessary to apply several of these techniques to understand completely the whole scenery. This contribution is aimed to offer a summary of the current knowledge of these analytical techniques for metabolomics and their application to different fields as food or health sciences. Each technique shows different advantages and drawbacks depending on their technical characteristics and limitations, some factors, such as the aim of the study or the nature of the biological sample will condition the choice. Regarding their applications, NMR has been employed specially to identify new compounds and elucidate structures. The use of MS has gained popularity because of its versatility, easiness to be coupled to separation techniques and its high sensitivity. Metabolomics applications in different science fields are growing each year, due to advances in analytical techniques and combination with other omics that allow increasing the comprehension of metabolic processes. Further development of analytical tools is necessary to continue exploiting all the possibilities of metabolomics [1].

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I_IL1_Bioactivity from bioworld

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Nature is untapped resource of species with medicinal potential, food potential. In recent time different natural sources have been increasingly utilized in pharmaceutical and food industry, as well in medicine. With new concept of using natural products as curative for different kind of diseases and to apply it as a food, research has been strongly focused on this area.

Since therapeutic, nutritive, and curative properties of plants, mushrooms, algae and other natural sources were focus both ethnomedicine and scientific/clinical research, these characteristics will be presented as to pointed out novel approaches and applications of these targets.

Mushrooms and plants have great potential in pharmaceutical and food industries as a source of highly appreciated compounds (flavonoids, essential oils, polysaccharides, lectins, terpenoids, peptides, proteins etc.).

Many of biological activities including antiinflammatory, antibacterial, antifungal, antiviral, antineurodegenerative, immunomodulatory, antihypercholesterol, antihyperglycemic potential which are most often highlighted. The immense potential as prophylactic agents of these compounds in diseases associated with bacterial and fungal infections (antimicrobial potential), oxidative stress (antioxidant potential), and tumor cell development (antitumor activity), cancer diseases is tested and presented.

Research in the field of natural products is of great importance for pharmaceutical and food industry, medicine, veterinary, agriculture, and other scientific disciplines are very valuable for possible application and replacement of synthetic drugs, but also contribute to new knowledge which could be a base for new practice of diseases.

New results in this area could be of great significance for pharmaceutical industry which should to goes to a new direction, and that is for sure nature.



I_IL2_Towards the most desirable natural products and their bioactivity features using multicriteria decision making methods and linear regression/classification algorithms

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The chemical complexity of natural products (NPs) often results in their pharmacological polypotency, *i.e.* exertion of multiple activity facets such as antibacterial, antifungal, or antioxidative [1]. However, selecting a NP with desirable properties is not a straightforward task, especially if optimization of one feature results in the deterioration of others [1]. Therefore, the use of multicriteria decision-making (MCDM) algorithms can be a crucial step in finding the best and the least desirable NPs.

In this work the three MCDM algorithms were compared: Derringer's desirability approach [2], the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) [3] and the Sum of Ranking Differences (SRD) [4].

The algorithms were applied to the NPs extraction-bioactivity/chemical-profiling data and compared for their ability to select the best extraction conditions as well as starting plant materials. Furthermore, for the first time the methods were combined with linear regression and classification algorithms, such as Partial Least Square Regression (PLS) and PLS-Discriminant Analysis (PLS-DA), in order to identify the crucial bioactivity features. Additionally, the models were able to successfully predict the desirability of the unknown samples with predetermined bioactivity properties, *i.e.*, to classify them as the best, intermediate or the worst NP candidates.

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II_IL1_Natural compounds immobilized into mesoporous silica

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Cisplatin, the gold standard in cancer treatment, is in use against different types of cancers [1]. The main disadvantage of cisplatin is unacceptable side effects, especially toxicity [1]. Those disadvantages can be avoided by using drug carrier systems such as mesoporous silica nanoparticles (MSNs) [1]. Recent works point out mesoporous silica as carriers for delivery of drug on appropriate location, hinder side effects and suppress drug decomposition [1]. These nontoxic materials may be loaded with a variety of molecules. Loading of highly active anticancer compounds into particles may prevent hydrolysis of the drug and accumulation in the healthy tissues/organs. Different functionalized SBA-15, but also SBA-15 alone, influence on the mechanism of action of natural occurring compounds (e.g. emodin - EO, betulinic acid - BA, xanthohumol - XN) [2-5]. The release of EO from the spherical silica nanomaterials within the HT-29 colon human tumor cells triggered a different response than EO applied alone, whereby the effective concentration for activity remains similar [2]. SBA-15 amplifies the activity of EO *in vitro* against human melanoma A375, as well as mouse melanoma B16 and B16F10 cells and protects the active compound from spontaneous, light promoted inactivation or degradation driven by extremely acidic conditions corresponding to gastric pH [3]. The loading XN into SBA-15 carrier resulted not only in a quantitative but as well as in a qualitative change of its antitumor action [5].

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II_IL2_Food antioxidants and their interaction with human proteins

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Health, disease and ageing are tightly connected with a redox balance. Oxidative stress underlines diabetes, cardiovascular, neurological and other diseases. Edible plants contain an array of compounds which express antioxidative activity, directly correlated with their structure capable of accommodating or releasing electrons. These compounds are often not freely soluble in an aqueous medium, such as human plasma, and interact with host molecules. Ligand-protein (L-P) complexes enable solubilization of ligands at higher concentrations and transport, prolonging their half-life and utility.

Our research work was focused on interactions between resveratrol (R) and fibrinogen [1], (dihydro)alpha-lipoic acid (ALA) and fibrinogen or albumin [2,3], and phycocyanobilin (PCB) and catalase [4]. Resveratrol is found in grapes and berries, leafy greens are a source of ALA and alga Spirulina is a source of PCB. L-P interactions were investigated by following-up structural changes of proteins and/or ligands using spectrometric methods (spectrofluorimetry, CD, FTIR) and by examining the primary role of individual proteins upon ligand binding. Common to all complexes were stable secondary and tertiary structures of proteins accompanied by thermal stabilization in the case of albumin-dihydroALA. L-P interactions were non-covalent and of the order of magnitude 10^4 M^{-1} . A mutually protective effect against both resveratrol and fibrinogen oxidation was found when they formed a complex, while the solubility of resveratrol greatly increased in an aqueous environment. No effect was seen on the coagulation process. Fibrinogen with bound DHLA was also protected from oxidation and formed fibrin with thicker fibres, which may be beneficial for persons with an increased risk of thrombotic complications. PCB-catalase complex protected the pigment from oxidation, enabling prolongation of its half-life and bioactivity. Since only some proteins bind ligands and specific ligands bind only to some proteins, the distribution of plant antioxidants between human proteins under physiological conditions is an intriguing task for investigation.

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III_IL1 Medicinal plants - Traditional application in South-Eastern Serbia

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One can assume that for every illness, which the nature has put in front of us, there is a possible remedy in nature which is waiting to be discovered. A great number of chemists, biologists, pharmacists, and other scientists believe in this prospect, and make great endeavour to determine the exact composition, and pinpoint the principals of biological activity, as well as to prepare derivatives of the existing isolated compounds of natural origin with the hope of arriving at a molecule with enhanced activity. [1] This presentation aims to provide insight into some data published in numerous papers on ethnopharmacological use of medicinal plants in the South-Eastern Serbia. [2-5]. Overcoming the problem of standardization of phytopreparations will also be considered.

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IV_IL1_Brewer's spent grain extract rich in dietary fibers and postprandial glycemia and insulin response in healthy subjects: a monocentric, randomized, cross-over, double-blind, placebo-controlled clinical trial

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Dietary fiber exerts many beneficial effects on human health among which the modulation of glucose metabolism [1-3]. This study was conducted to demonstrate the efficacy on postprandial glycemia and insulin response of a new chemically characterized brewer's spent grain (BSG) extract-based food supplement (BSG-FS), in the form of soluble granules in single dose stick packs (5 g), containing 4 g/stick pack of BSG extract, in a monocentric, randomised, double-blind, placebo-controlled, cross-over clinical trial. Healthy subjects were randomly allocated in two groups ($n = 20$ each group) to consumed a standard meal consisting of breadsticks, oligomineral water, and the BSG-FS (group 1) or placebo (group 2). After the five-days of wash-out period, cross-over of treatments were followed. Random intercept linear mixed model (LMM) was used as the most suitable tool for statistical analysis. BSG extract showed the presence of high molecular weight (HMW) soluble fibers (2.12 g/100g), HMW AXs (5.33 g/100g), low MW AXs (2.17 g/100g), LMW β -glucans (1.92 g/100g), and bioavailable free ferulic acid (6.48 g/100g of digested BSG extract). The results of the clinical trial showed that glycemia measured after BSG extract intake was significantly lower than the corresponding value in placebo group in the descent phase of the glucose peak, (after 90 and 120 minutes), while at the baseline and in the first 60 min the two glycemic curves were substantially overlapped. Otherwise, the insulinemia values of the group 1 were significantly lower from the first 15 min with a growing difference until reaching the maximum difference at 120 min. None of the subjects reported any adverse event with the supplementation. In conclusion, the new BSG-FS can reduce post-prandial glycemia in a statistically significant way as compared to placebo, producing a lower insulinemic response.

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IV_IL2_ The role of polyphenols in preventive nutrition

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Among the biggest causes of death including cancer, stroke and metabolic diseases, a significant number of cases can be prevented through a healthy diet. The beneficial effects of the Mediterranean diet, the most widely followed healthy diet in the world, are principally due to the presence of secondary metabolites, mainly polyphenols whose healthy characteristics are widely recognized [1]. The biological activities of polyphenols are mainly attributed to their antioxidant activities and in maintaining cellular redox state homeostasis. Green tea, cocoa, fruits, and vegetables are very rich in polyphenols and are usually associated with a reduced risk of cardiovascular diseases, diabetes, as well as improvements in blood pressure, insulin resistance, and systemic inflammation [2]. A high-polyphenol diet, including green tea and walnuts is reported to be potentially protective for age-related diseases. Polyphenols can also cross the blood–brain barrier and reduce neuroinflammation. The mechanisms of action of polyphenols go far beyond the modulation of oxidative stress [3]. Several different molecular mechanisms have been described for the beneficial effects of polyphenols including the modulation of cellular signaling pathways such as the activation of the antioxidant enzymes, the regulation of lipid metabolism, modulation of transcription factors and inflammatory pathways, or key cancer related mechanisms such as proliferation, apoptosis, and differentiation. Greatest interest has been focused on the flavonoid subgroup which is widely distributed. Total flavonoids and specific subclasses of polyphenols have been reported to be strongly associated with a low risk of diabetes, cardiovascular events and all-cause mortality [4]. Preventive nutrition focuses on the nutritional aspects while considering the qualitative and quantitative presence of the natural bioactive compounds such as polyphenols and their potential beneficial health effects as part of a proper diet [5] therefore it plays a key role in the treatment, regression and prevention of chronic diseases.

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V_IL1_Immunomodulatory potential of banana lectin in allergen-specific immunotherapy

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Allergen-specific immunotherapy (AIT) is a mainstay treatment that corrects the underlined pathological immune response to allergens – causative agents of allergy, contributing to the management of allergic rhinitis, asthma and venom allergy [1]. Achieved allergen tolerance contributes to the improvement of allergic symptoms, reduced medical requirements, and better quality of life. However, long treatment, costs, reduced patient compliance, and risk of side effects are the major obstacles for AIT. The development of highly immunogenic proteins devoid of IgE reactivity, in combination with potent adjuvant molecules, may shorten the treatment duration and improve the efficacy.

Lectin from *Musa acuminata* (banana) belongs to the family of jacalin-related lectins, which contain a strong mannose-binding domain. Banana lectin (BanLec) exerts immunomodulatory potential in murine macrophages via binding to oligosaccharide structures of TLR2 and CD14 and blocking downstream receptors signaling [2]. Immunomodulatory potential of BanLec explored on murine peritoneal macrophages with a chimera composed of a major birch pollen allergen Bet v 1 and BanLec (Bet v1-BanLec), shows the production of IL10 cytokine implying that the BanLec as an adjuvant can exert a beneficial effect in the allergen-specific immunotherapy in birch pollen allergy [3].

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V IL2_Potentially harmful interactions between Δ^9 -tetrahydrocannabinol (THC) and irinotecan

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Irinotecan (IRI) is one of the most important cytostatics used in the treatment of advanced colon cancer. Some of its side effects such as acute and delayed cholinergic syndrome deteriorate the quality of life in patients. To improve their overall wellbeing, patients often use various supplements, including illegal Δ^9 -tetrahydrocannabinol (THC) preparations, sometimes containing more than 80% THC [1].

Our study investigated the interactions of IRI and THC at the cellular and organ level of Wistar rats by integrating biochemical, molecular biology and analytical methods. The obtained results provide evidence of a synergistic increase in the acute toxicity of IRI tested at a single *intraperitoneal* dose of 100 mg/kg under the influence of THC taken *per os* at a daily dose of 7 mg/kg for one, three and seven days. Concomitant administration of IRI and THC enhances the degree of oxidative stress and genotoxic effects of IRI in liver cells [2]. In rats given the combined treatment, a lowering of leukocyte counts was observed compared with the group treated with single IRI [3]. Concomitant administration of THC with irinotecan enhanced the urinary excretion of THC compared with the group of rats treated with single THC, also suggesting a possible interaction of the tested compounds [4]. From the results obtained, we concluded that concomitant administration of IRI with high doses of THC leads to adverse pharmacokinetic and pharmacodynamic effects. Although the results obtained cannot be directly extrapolated to humans, they indicate a number of undesirable and potentially dangerous side effects as well as the need for extreme caution in the administration of THC in oncology patients.

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VI_IL1_New Strategies for Identifying Phytochemicals in Food: Nondestructive Raman Spectroscopy Analysis

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In the present the chemical composition of biologically relevant compounds has been investigated by chromatographic methods and mainly characterized by mass spectrometry, nuclear magnetic resonance, infrared and ultraviolet spectroscopy [1]. A new alternative is to combine Raman spectroscopy, which is a light scattering technique that provides information of about molecular vibrations, with the other traditional techniques. It is a non-destructive method of analysis suitable for use in studies *in situ* of biomaterials as well as chemical compounds. The technique also offers the advantage of short measurement times requires low amounts of material and is of low sensitivity to water content present in biological samples.

It has been extensively used in analyzing molecules ranging from proteins to small molecules as well as some secondary metabolites [2]. Raman spectroscopy allows *in situ* analysis of inorganic and organic samples as well as *in vivo* analysis of live tissues. The technique has been successfully applied to medical diagnoses, identification of chemical composition of pathogenic and non-pathogenic microorganisms, plant tissues, quality control of food, investigation of textiles, works of arts and gemstones [2].

In this presentation will be discussed the main theoretical approaches for the Raman scattering technique, as well several examples of applications using Raman spectroscopy as the main analytical tool in the chemical analysis of plants; as an example, Figure 1 illustrates the Raman spectra of green coffee beans from different Arabica genotypes [3].

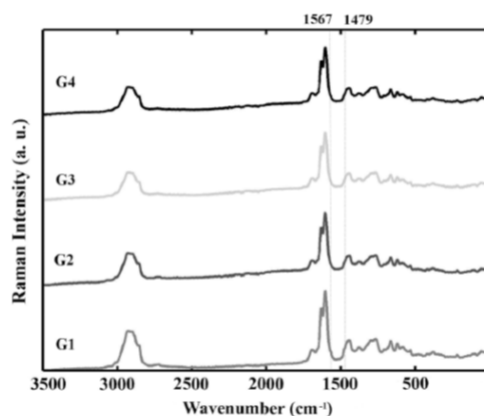


Figure 1. Raman spectra of green coffee beans from different Arabica genotypes

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VI_IL2_Risks in honey production and its quality shown at the example of honey produced in a national park

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Honey is a natural, sweet substance produced by honeybees (*Apis mellifera*) by processing nectar from plants, or from juices from living parts of plants, or by collecting the excretions of insects that feed by suckling the juices in the living parts of plants which bees then collect, process, enrich with their own specific substances, dehydrate and store into honeycombs up to a honey maturation [1]. Honey represents the food which customers associate with nature and consider it useful for their health. During the production process honey is exposed to various risks of contamination which can be a consequence of a environment or procedures applied during the process of beekeeping. The use of a great range of chemical substances in agriculture, together with the presence of heavy metals and microorganisms in a environment can cause both their transfer into hive and presence in the honey [2, 3]. The application of a set of measures and procedures used in a good beekeeping practice removes alike risks in honey production. Determination of honey physico-chemical parameters is a good indicator of its quality [4]. By taking as an example the honey produced in a region of a national park where man lives in harmony with nature with the aim of preserving the already existing nature values and resources, the values of physico-chemical parameters of 27 honey samples were taken for analysis. In addition, a complete melissopalynological analysis was performed, along with antioxidant tests, antimicrobial activity as well as the presence of polyphenol. A good correlation between quantified polyphenols and biological activity was observed [5]. The research showed that the honey produced in a protected region is of a high quality.

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VII_IL1_Goji berries as sources of functional food ingredients

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The fruits of *Lycium barbarum* L. and other species of *Lycium*, known as goji berries, are traditional medicinal foods in China and other Asian countries [1]. Thanks to growing evidence from modern medical research that supports the benefits of the inclusion of goji berries in healthy dietary patterns, these fruits have been advertised on the global market as a superfood and have begun to cultivate in different geographic areas. Besides essential fatty and amino acids, dietary fibers, vitamin C and minerals, goji berries contain a wide range of non-nutritive compounds, such as polysaccharides, carotenoids, and phenolics, including phenolic acids and flavonoids [2]. These compounds contribute to antioxidant, anti-inflammatory, antimicrobial, immuno-stimulating, anti-diabetic, neuroprotective, anti-cancer, prebiotic, and many other health-promoting activities. Therefore, goji berries have the potential to be used as functional foods or sources for obtaining bioactive compounds for food, cosmetics, and pharmaceutical applications. So far, there is evidence that goji berries and their extracts have been successfully used in producing innovative beverages and various food products, such as bakery, confectionary, meat, and milk products, enhancing their nutritional value and sensory qualities [3]. Understanding how genetic, environmental conditions, and many other pre-harvest and post-harvest factors affect the chemical composition and bioactivity of goji berries, along with advances in characterization and in processing methods and techniques to maximize the content and bioavailability of bioactive compounds, provides a basis to support future researches and applications of goji berries [4]. However, the comprehensive evaluation of the safety of goji berries and goji berry-based products should not be neglected.

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VII_IL2_Plants as a source of natural preservatives for food application

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One of the main challenges of the food industry has been the substitution of artificial additives for natural ones. In a world increasingly demanding for healthy foods, functional products, clean labels etc., it is urgent to develop safer, healthier alternatives to preserve foods. Nature has been a fascinating source of distinct classes of compounds (e.g. phenolics, organic acids), recognized for their bioactive properties, namely antioxidants and antimicrobials, two essential attributes to be considered a natural preservative, in addition of having low/no toxicity effects.

To promote the circular economy and sustainability in the agri-food sector, these molecules have been obtained from industrial bioresidues that represent a significant amount of non-valuable raw material, often disposed in landfills, causing a significant negative environmental effect. Given their richness in high added-value compounds, natural matrices such as plants/fruits have been under exhaustive exploitation to obtain added value molecules with significant antioxidant and antimicrobial properties to act as natural preservatives. The focus has been dedicated to the extraction, stabilization, and viability of application in the food sector. Therefore, extracts rich in phenolic compounds (e.g. catechin, quercetin and luteolin derivatives), phenolic acids (e.g. rosmarinic, chicoric, lithospermic, caffeic, caffeoylquinic acids), and hydrolysable tannins (e.g. trigalloyl-HHDP-glucoside) obtained from basil, rosemary, sweet chestnut flowers, sage, oregano; as also in organic acids (citric acid) obtained from citrics, have been subjected to extraction optimization, bioactive evaluation for incorporation in food matrices such as cheese, muffins, yogurts, wine, beer and nutraceuticals, to act as natural preservatives. The main achievements were the development of innovative products in which the shelf-life was extended, or the artificial preservatives were substituted as in the case of yogurts, cheese, muffins and nutraceuticals, highlighting the ChestWine and SpraySafe formulations. ChestWine is a preservative natural extract that is able to substitute the addition of sulphites in wines, and SpraySafe is an edible biofilm that can be applied in foods such as cheese, mushrooms or fruits to substitute the use of plastic wrap. These findings are pertinent and absolutely needed because they provide real alternatives to the use of artificial additives.

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VIII_IL1_ Recycling of agro waste by fungi for obtaining enzymes and prebiotics

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Agro waste materials are agricultural residues (stem, stalks, husks...) and food industrial residues (potato, orange, apple and *etc* peels and soyabean, coconut *etc* cakes). Since each region of the world has different agricultural sectors, specific residues are generating and at the same time a wide range of alternative novel sources of nutrients emerge, such as carbohydrates, proteins and minerals. Proper waste biomass management is one of priority in EU and in world. Filamentous fungi are most potent microorganisms for bioprocessing of waste materials in purpose to obtain value added products such as enzymes, biofuel, bioactive compounds, prebiotics, chemicals, antibiotics... Genus *Aspergillus* and *Trichoderma* are well studied and recognized as potent enzyme producers. Enzyme produced by filamentous fungi such as cellulase, amylase, xylanase, glucosidase, are widely used in industry. Environment-friendly and cost effect solution for their obtaining is utilization of agro waste material as solid substrate for fungal growth [1]. Prebiotics are oligosaccharides with 2-6 units defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" [2]. Epidemiologic studies have significantly recognized prebiotics as an essential constituent of a healthy diet. Prebiotics, *e.g.* FOS are prepared by effective fungal enzymes [3]. Usage of biomass residues as start material for prebiotics production is preferable from the standpoint of ecology and as cheap production process. There are number of agro waste materials that could be used for this propose [4]. Corn cob can be considered as the main source for XOS production [5].

Fungi are saprophytic eukaryotic organisms, habitat lignocellulosic material in nature and they are capable to induce enzymes depending on growth substrate, which actually enables their usage for *in situ* prebiotics production, which is new and trending research in this field. This approach unites enzyme and prebiotics obtaining from agro waste in only one process.

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IX_IL1_Prioritization strategy to find the important phytochemicals in a complex natural sample

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A disruptive prioritization strategy [1] is introduced combining two disciplines, *i.e.* chemistry and biology, to gain understanding of true mechanisms and important compounds in a complex natural mixture that should be known. Multiplex bioassays are integrated in a generic non-target 12D-hyphenation, coupling chromatography/detection dimensions as needed [2,3] and record high-resolution mass spectra fully automated and straight forwardly from the bioautogram [2-4]. The versatile hyphenation dimensions provide useful information for zone identification. Multiplex bioassays allow the detection of opposing effects in mixtures. The differentiation of agonistic, antagonistic, false-positive and synergistic effects [2,3] clearly show that the ubiquitously applied *in vitro* assays may fail in providing true results for mixtures. Beneficial or harmful compounds with antioxidative, enzyme inhibiting, genotoxic, cytotoxic, neurotoxic, and hormonal activity are detectable non-targetedly. Simulated metabolism reactions are also demonstrated on the same surface, termed nanoGIT^{+active} system [5], which point to activity conversions during digestion or hepatic de-/toxication. Such hyphenations were miniaturized to a portable open-source all-in-one 2LabsToGo system.

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IX_IL2_ Challenges in chromatographic analyses of phytonutrients in medicinal plants and food

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People have been consuming phytonutrients in edible plants and plant preparations for thousands of years. However, many phytonutrients daily consumed in unprocessed food (e.g. fruits or vegetables) have not yet been identified. Phytonutrients are a big group of compounds with different bioactivities (antioxidant activity, enhancement of immune response or cell-to-cell communication, lowering blood pressure and/or cholesterol level, etc). More research is needed to connect the mode of action with specific phytonutrient in medicinal plants and foods and to study their possible toxicity and interaction with medicines. Several phytonutrients are nowadays marketed as active ingredients of food supplements (globally considered as food) or functional foods, although many of them have not yet been properly scientifically investigated. Therefore, new analytical methods are needed to control food quality and safety and to provide more information about food composition, as well as to gain knowledge about new possible phytonutrient ingredients for functional food and food supplement products. Chromatographic techniques, especially their combined use and hyphenation to mass spectrometry, are indispensable in the research of phytonutrients.

This lecture will present methods based on high-performance thin-layer chromatography (HPTLC-densitometry, HPTLC-image analysis, HPTLC-MS/(MS) and high-performance liquid chromatography (HPLC-UV/Vis, (U)HPLC-MS/(MS)) for analyses and characterization of phytonutrients from different groups present in medicinal plants and food – including food supplements. Challenges related to stability of the analytes, lack of chromophores, isomeric structures and lack of commercial standards will be discussed. The examples will include targeted and non-targeted analyses of phytochemicals in medicinal plants and food - including food supplements.

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IX_IL3_Analysis of metals and metalloids in plant materials – a short overview

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Quantification of major and trace metals and metalloids in various plant materials is of a great interest of researchers of various science branches worldwide, because the roles that such chemical elements (can) have in the context of a food chain and, consequently, human's metabolism [1].

A procedure of analysis of toxic metals consists of the steps as follows: (1) sample collection, (2) storage, (3) sample preparation for measurement, (4) quantification of the selected analytes ('measurement'), (5) calculation and expression of the results, and (6) interpretation of the results (Fig. 1). Only accurate and appropriate approach to each of the analytical steps can yield accurate and precise results of an analysis. Thus, a procedure of collecting of samples should be designed in such a manner to preserve that an aliquot reflects the properties of the population. Also, the samples should be handled and stored appropriately to avoid their contamination or possible losses of the analytes [2]. Analytical techniques that are dominantly used for quantification of metals and metalloids in the samples of environmental interest nowadays require a transfer of the analytes into the water solution state, therefore a selection of a solubilization method (reagent and conditions) is an important step that is related to the samples characteristics but also to the research goals [1,2-4]. The last part of a laboratory work in such a research is a quantification of the analytes, and in this context various atomic spectrometric techniques are widespread in use (e.g. ICP-AES(OES), ICP-MS) [1,5].

In the expose a brief presentation of the dominant practice as well as a discussion of challenging aspects and requirements of a procedure will be given.



Figure1. Scheme of a metals and metalloids analysis procedure

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I_OP1_Phytochemical profiling and insight into bioactivity of *Stellaria holostea* L. (Greater Stitchwort) extract

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There are about 120 species in the genus *Stellaria* (family Caryophyllaceae). Most of them have not been sufficiently investigated and therefore have not been used to a large extent in everyday life. There are only certain literature data for the species *Stellaria media* Vill. (Chickweed) that showed excellent antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anti-obesity, anxiolytic, and anti-leishmanial activities [1]. On the other hand, *Stellaria holostea* L. was scarcely examined. Therefore, our study aimed to evaluate the phytochemical composition and biological potential of methanolic extract of *Stellaria holostea* L. aerial parts. Phytochemical profiling was done by UHPLC-MS analysis focusing on phenolic compounds and quantifying the most dominant polyphenolics [2]. Bioactivity was tested using *in vitro* antioxidant assays, antimicrobial tests, and confirmed anti-inflammatory effects were evaluated *in silico* using molecular docking simulations.

S. holostea extract contained a variety of phenolic acids, flavonoids, and their glycosides. In total, 40 compounds were identified. The most dominant phenolic acids in *S. holostea* extract were *p*-coumaric, chlorogenic, and ferulic acids while the flavonoids with the highest content were chrysoeriol, rutin, and naringin. The extract showed moderate antioxidant and antimicrobial potential. Nevertheless, the inhibitory activity towards cyclooxygenases COX-1 and -2 was notable (71.24 and 72.83%, respectively) indicating a significant anti-inflammatory potential. That led us to investigate the *in silico* inhibitory effects on COX-1 and -2 enzymes of the most abundant abovementioned phenolic compounds. The results undoubtedly showed that chlorogenic acid (CA) was the compound with the highest inhibitory potency against COX-1 and COX-2. It was noticed that calculated values of ΔG_{bind} and K_i for CA were lower than values obtained for referent inhibitors. Namely, the molecular docking simulations of COX-1-CA protein-ligand complex obtained lower values of the ΔG_{bind} and K_i than for examined protein-ligand complexes with indomethacin. In the case of COX-2, the difference in calculated values of thermodynamic parameters for COX-2-CA was even more pronounced in comparison with protein-ligand complexes COX-2-NS398.

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II_OP- Phytochemical screening and the effect of *Trichosanthes dioica* in high-fat diet induced atherosclerosis in Wistar rats

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Trichosanthes dioica fruit pericarp has a powerful hypolipidemic and antioxidant properties. Wistar rats were divided into five groups (n = 30). The normal control group received vehicle and standard diet throughout the experimental period. Groups II, III, IV, and V were received a high-fat diet and free access of water for 21 days. Experimental groups IV and V were fed with high-fat diet along with *T. dioica* extract 400 and 800 mg/kg, respectively. Blood was collected at 21 days, and lipid profile estimation was performed. The phytochemical screening was performed by different phytochemical screening methods. Lipid peroxidation and catalase activity were done by taking a liver tissue sample. The phytochemical screening of extract showed good results and indicated the presence of phytoconstituents in extract. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of *T. dioica* was found to be 65.79% at a concentration of 100 µg/ml extract, whereas that of the control was 94.06%. The Wistar rats fed with a high-fat diet with *T. dioica* showed a significant decrease in total cholesterol (TC), triglycerides (TG), very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) levels and an increase in high-density lipoprotein (HDL) level. The lipid peroxidation and catalase activity of methanol extract of *T. dioica* were significantly reduced with individual comparison with all groups. The methanol extract of *T. dioica* has the hypolipidemic effect, which showed a decrease in TC, TG, VLDL, and LDL levels and an increase in HDL level in Wistar rats. It also has antioxidant properties. In this study, we have evaluated the antioxidant and the antihyperlipidemic activities of *T. dioica* in high-fat diet induced hyperlipidemia in Wistar rats.

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III_OP *In vitro* anti-inflammatory effect of *Allium cepa* L. extract rich in quercetin optimized by design of experiments

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Allium cepa L. is an edible plant with the potential to fight against cardiovascular, respiratory, metabolic, disorders, infections, and cancer, owing to the presence of phenolic and organosulfur compounds [1,2]. This study aimed to assess the anti-inflammatory effect of the chemically characterized *Allium cepa* L. rich in quercetin optimized by design of experiments (DoE). Different Italian cultivars of *A. cepa* were selected, and the cultivar with the highest total phenolic contents (TPC) was subjected to the determination of quercetin and its derivatives using LC-MS analysis. *A. cepa* cultivar called “Golden Voghera” contained high TPC (i.e., 44.03 mg GAE/100 g) as compared to the other cultivars such as “Yellow Elenka” (35.77), “White Cenol” (13.80), and “White Orizaba” (17.36). “Golden Voghera” cultivar extracts (obtained from whole bulb, tunic and bulb) were analyzed using RP-HPLC-PDA-ESI-MSn, yielding five identified compounds including quercetin 3 4'-*O*-diglucoside quercetin 3-*O*-glucoside, quercetin 4-*O*-glucoside, 3-*O*-methyl quercetin, and quercetin aglycone. The tunic extract showed a highest amount of total quercetin contents (3.349 ± 0.53 mg/g). A full factorial design technique showed the best extraction conditions (40 % of ethanol, 1g tunic/20 L solvent ratio) to obtain a tunic *A. cepa* extract with the highest quercetin contents (26.6 ± 2.484 mg/g of quercetin aglycone equivalents). HPLC analysis of this extract identified 14 compounds (quercetin + *O*-quercetin dihexoside, quercetin hexoside, quercetin hexoside dimer, quinone methide derivative, quinone methide, and quinone methide). Moreover, as regards the anti-inflammatory activity, *A. cepa* tunic extract significantly inhibited the production of PGE₂ in J774 cell lines stimulated with LPS in concentration-dependent manner (0.1 - 1 mg/mL) while did not significantly influence the release of IL-1 β and TNF- α , and the production of nitrites induced by LPS. The study revealed that *A. cepa* tunic extract rich in quercetin showed significant anti-inflammatory effects against PGE₂.

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IV_OP_Reinwardtia indica: phytochemical screening and evaluation of wound healing activity of the extracts in experimental model rats

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Reinwardtia indica is traditionally used for wound healing. The main aim of this study was to evaluate the wound healing activity of leaves extracts of *R. indica* using the excision wound model in rats. The leaves of *R. indica* were collected from Gondrang, Chitwan, Nepal. Leaves were shade dried, extracted by double maceration and subjected to phytochemical screening. Then, the fusion method was used for the formulation of ointment and evaluated. Rats (n= 24) were divided into four groups with 6 in each. Excision wound model was used, 2 cm diameter (314 mm²), 2 mm depth wound was created. The treatment was given daily topically to all groups and the % mean wound contraction rate was calculated on days 4, 8, 12 and 16. The result was analyzed statistically using Graph pad prism version 5. Phytochemical test revealed the presence of alkaloids, flavonoids, tannins, phenolics, terpenoids, carbohydrates, etc. All the evaluation parameters showed satisfactory results. The extract of *R. indica* ointment (2% w/w and 5% w/w) increased the wound contraction rate day by day. The % means wound contraction rate, on day 12, (80% and 88%), and on day 16, (97% and 100%) and statistically significant difference was at p< 0.0001. The *R. indica* extract ointment showed an increased wound contraction rate. So, in further *R. indica* could be used for commercial production of wound healing ointment.

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V_OP_Chemical characterization, cytotoxic evaluation and anti-SARS-CoV2 activity of plant extracts rich in hydrolysable tannins

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Acorn husks, chestnut flowers, and leaves of eucalyptus, rockrose and laurel, extracted with water or water/ethanol were screened for their polyphenolic profile, antioxidant, antimicrobial and anti-SARS-CoV2 activity, as well cytotoxicity and mutagenicity assays. The highest amounts of hydrolysable tannins, analysed by HPLD-DAD/MS were found in the eucalyptus extracts, followed by rockrose, while laurel only showed condensed tannins. In terms of their antioxidant analysis, the most antioxidant extract depended on the assay, still, the highest total phenols and DPPH values were found for acorn husks, while rockrose and the chestnut flower showed highest ABTS values. Considering ORAC, laurel showed the highest values (3703±87 µMol trolox/mg extract). Considering the antimicrobial activity, the extract that had highest activity against the tested 43 bacterial strains were rockrose and acorn husks. The antifungal activity was quite low, with the extracts only showing mild inhibition in three of the 12 tested species. For the anti-SARS-CoV2 activity, all extracts showed strong inhibition (over 90%) of this coronavirus, although acorn husk and eucalyptus achieved a growth inhibition of 99 and 97%, respectively. To prove the lack of toxicity of these extracts, extracts were screened for cytotoxic and mutagenic properties. In terms of prevention and degradation of DNA, most of the extracts showed preventive action with eucalyptus, rockrose and laurel protecting 100% of the DNA. In the mutagenic evaluation using *Salmonella typhimurium*, none of the extracts showed mutagens that could cause frameshift mutations. Finally, in the human keratinocyte cell line, none of the extracts showed inhibition values, since no inhibition was higher the 30% threshold. Overall, the extracts proved to have interesting antioxidant activity while only average antibacterial effects and very low antifungal capacity. Still, the very high inactivation of SARS-CoV2 activity makes them have promising applicability as antivirals, both in surface cleaning agents and woven into linen for health care workers. Acorn husks and the eucalyptus leaves seem to be the most promising of all extracts.

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VI_OP_Sustainable fertilization systems as a prerequisite for improved quality of agricultural products

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Human health is dependent not just on diet, but mainly on quality of agricultural products as a part of diet. If crops were grown on poorly fertile soils, or they are exposed to severe stresses, lesser amount of mineral elements, particularly essential elements, such as zinc, copper, manganese, magnesium, calcium, iron, and even sulphur, will be absorbed and accumulated, resulting in their deficiency in diets and increased incidence of various chronic diseases.

Together with naturally low soil fertility, climate change, intensive agriculture is one of the main contributors of soil depletion. Thus, various long-term strategies, which are sustainable for agricultural plants and soils, at the same time, must be developed. It is of particular importance to increase a level of organic matter, as a source of mineral nutrients from the soil. The application through soil, as well as via plant foliage of various complex and organic fertilizers, containing macro- and micro-elements, and many stimulating compounds, enables better absorption and metabolisation of nutrients required for plants and nutrients essential for humans. Besides, bio-fertilizers, containing beneficial microorganisms have an important role in nutrients mobilization in soils, particularly from poorly accessible forms. Many bio-fertilizers contain microorganisms that are able to absorb atmospheric nitrogen, thus enriching soil, delivering it to the plants, enabling reduction in amount and costs of nitrogen addition into the soil. Promoting microorganisms are also able to enhance plants ability to absorb water and nutrients by their synergy with roots, resulting in stable and better growth performances of agricultural plants, thus increasing yield and its quality. Some other cropping practices, such as crop rotation, intercropping and use of cover crops, enriches soil with organic matter, reduces losses of nutrients through recycling of harvest residues, therefore increasing soil fertility, as well as quantity and quality of crop yield, at the same time.

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VII_OP_Chemical composition and antioxidant activity of black pepper (*Piper nigrum* L.) fructus essential oil hydrodistillation fractions

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Black pepper (*Piper nigrum* L.) is historically used not only in human diets but also in traditional medicines and home remedies. The aim of this study was to examine the effect of hydrodistillation time on the chemical composition and antioxidant activity of collected essential oils' fractions from black pepper fructus for five hydrodistillation periods: I (0-15 min), II (15-30 min), III (30-60 min), IV(60-90 min) and V (90-120 min). The essential oil was obtained by Clevenger-type hydrodistillation (CHD). The qualitative and quantitative composition of collected essential oil fractions was determined by a combination of gas chromatography with mass spectrometry (GC/MS) and flame ionization detection (GC/FID). The efficiency of different fractions of essential oil to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated using a spectrophotometry method. Essential oil yield increased with the length of the CHD time and reached a maximum at 120 min (3.33%). The content of monoterpenes and aromatic compounds decreased in the range I-V: 64.6-33.6% and I-V: 2.3-0.3%, respectively, while the content of oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes increased (I-V: 0.9-1.9%, I-V: 31.5-52.3%, and I-V: 0.7-12.1%, respectively) with the time of CHD, with small irregular deviations. The most abundant compound of essential oils' fractions was (*E*)-caryophyllene, which content increased with the time of CHD (I-V: 24.9-36.6%). Results indicated that all collected essential oils' fractions exhibited remarkable antioxidant activity (EC_{50} values were in the range I-V: $81.27 \pm 0.327 - 8.02 \pm 0.044$ mg/ml), where the fraction V showed clearly the highest activity. This study proved that CHD time affects the composition and antioxidant activity of black pepper essential oil. Future research may be extended to examine other biological activities of black pepper essential oil fractions, as well as synergistic effects of other identified active components of the essential oil, in addition to (*E*)-caryophyllene, that would affect biological activities.

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VIII_OP_Interactive effects of ultraviolet-B and ZnO nanoparticles on biochemical and antioxidant traits of saffron (*Crocus sativus* L.)

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Ultraviolet B (UV-B) radiation alters cell metabolism in plants through production of free oxygen radicals, but large variability may exist among plant species in their sensitivity to this stress, while detailed information for specific crops is lacking [1]. The aim of this study was to examine the effect of UV-B stress on saffron plants and the role of foliar application of ZnO nanoparticles in alleviating the impact of UV-B. Saffron plants were sprayed with ZnO nanoparticles at a concentration of 50 mg/L. The plants were then exposed to UV-B radiation for 30 min per day for 1 month. At harvest, growth and biochemical variables were determined. UV-B radiation reduced length of shoots and roots, fresh and dry weight of the aerial and underground organs, and number of leaves. Furthermore, UV-B radiation reduced chlorophyll content (chl-a, chl-b) and carotenoids, as well as Hill reaction. Foliar application of ZnO nanoparticles improved belowground fresh weight, belowground dry weight, and leaf number of saffron plants and could alleviate the negative effect of UV-B radiation on aboveground fresh weight, aboveground dry weight, belowground dry weight, shoot length, chlorophyll pigments, Hill reaction and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging of saffron plant leaves. On the other hand, UV-B radiation increased malondialdehyde (MDA) content, absorbent compounds, total phenol and total flavonoid content in leaves of saffron. Overall, UV-B radiation caused major physiological and biochemical changes in leaves of saffron by diminishing growth, reducing biomass production, reducing the amount of pigments, and decreasing Hill reaction. Increasing absorbent compounds, such as flavonoids and phenols and promoting cell death functioned as a defense mechanism against UV-B radiation. These results are in line with our previous findings [2]. Foliar application of ZnO nanoparticles could compensate some of the physiological and biochemical damage caused by UV-B radiation through protecting photosynthetic pigments and improving plant productivity.

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IX_OP_Characterization of phenolic content in bee pollen and bee bread originating from different climatic areas

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The use of bee pollen and bee bread as functional foods has received increasing attention due to their composition, characterized by a 70% of biologically active substances [1,2], and their therapeutic potential [3,4]. At present, some studies showed that bee pollen and bee bread phenolic composition can be deeply influenced by several factors [5]. However, an exhaustive characterization of the polyphenols found in these beekeeping products is currently missing, and the limited amount of available data is not registered in the labeling or prospectus for their use by consumers, health professionals or nutritionists.

The aim of this research work was hence to characterize bee pollen and bee bread based on their polyphenols content and their antioxidant activity. This research dealt with the study of 80 samples with different characteristics in terms of origin, from different climate zones, multi/mono-floral composition and different storage conditions, in dry or fresh form. Total phenolic content (TPC, by Folin-Ciocalteu) and antioxidant activity (AOA, by ABTS, FRAP, DPPH) were evaluated. Moreover, an HPLC-MS/MS method was optimized for the simultaneous quantification of 22 polyphenols of interest. Samples coming from different climatic areas, according to the Koppen classification, showed significant differences in their TPC and AOA. Samples were characterized by a wide range of TPC values (3.50-26.08 mg GAE/g) which were positively correlated with the AOA. Fresh and mono-floral bee pollen samples had higher TPC than dried and multi-floral ones respectively. The targeted MRM analysis revealed a great variability of polyphenols concentration in bee pollen and bread (\approx 20-2100 ng/g). Among the polyphenols studied, rutin, known for its anti-inflammatory potential, was the one detected more frequently and at a higher concentration. Interestingly, bee bread samples showed significantly higher concentrations of four phenolic acids, isorhamnetin and kaempferol than bee pollen, probably resulting from bee pollen's fermentation in the honeycombs.

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I_PP1_Phytochemical analysis of *Cicerbita pancicii* (Vis.) Beauverd- the Balkan's endemic plant

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The Balkan Peninsula is characterized by the great biodiversity, and it is native to extensive number of plant families and species. Moreover, some plants are endemic to certain region such as *Cicerbita pancicii* (Vis.) Beauverd (syn. *Lactuca pancicii*) from the Asteraceae family. This perennial plant can be found in mountainous areas, growing among tall-herb vegetation, as it prefers humid mild limestone slopes along streams [1]. During spring months, *C. pancicii* develops upright stem with lyrate leaves which are composed of broad terminal lobe and several smaller lateral ones. Pollination is occurring as insect are attracted to the decorative lilac-purple flowers, arranged in capitula.

The aim of this study was to perform phytochemical analysis of three distinct plant parts of this endemic species: stem, leaf and inflorescence. For this purpose, plants are collected from Rudinice village, nearby Komarnica canyon in the Montenegro. Furthermore, extraction of plant material was performed in 80% acetone as solvent and content of chlorophyll *a* and *b*, as well as total carotenoid (TCC), total phenolic (TPC) and total flavonoid (TFC) content were determined using spectrophotometric methods. The leaves possessed the highest amount of both chlorophylls *a* (611.52 µg/g d.w.) and *b* (993.38 µg/g d.w.), whereas the lowest values were registered in stem (113.27 µg/g i.e. 45.92 µg/g d.w.). Moreover, TCC was the most abundant in inflorescence (65.71 µg/g d.w.), while in leaves carotenoids were not detected. Analysis of TPC and TFC exhibited the highest distribution of phenolic and flavonoid compounds in leaves of *C. pancicii* (12.51 mg/g GAE and 18.19 mg/g CE d.w.), followed by content in inflorescence (8.23 mg/g GAE and 10.42 mg/g CE d.w.). As the result of this study, the dispersal of mentioned bioactive compounds within plant organs has been recognized and determined.

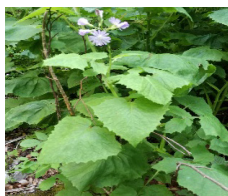


Figure 1. *Cicerbita pancicii* (Vis.) Beauverd

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I_PP2_Essential oil composition and antioxidant activity from different plant part of wild fennel (*Foeniculum vulgare* Mill.) growing in Montenegro sea-side

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The aim of this work was to compare the chemical composition and antioxidant activity of essential oil from fennel leaves and stems. The essential oil was obtained by Clevenger-type hydrodistillation (CHD). The chemical composition of isolated essential oils was determined by gas chromatography with mass spectrometry (GC/MS) and flame ionization detection (GC/FID). The efficiency of isolated essential oils to scavenge 2,2-diphenyl-1-picrylhydrazyl radicals was evaluated using a spectrophotometry method. The yield of essential oil from the fennel leaves (0.83%) was four times higher than the yield of essential oil from the fennel stems (0.21%), during CHD time of 120 min. Forty-six compounds were identified from fennel leaves essential oil, mainly aromatic compounds (68.5%), monoterpenes (17.8%), oxygen-containing monoterpenes (11.8%), and others, where the most abundant compounds were (*E*)-anethole (51.4%) and methyl chavicol (9.3%). Forty-seven compounds were identified from fennel stems essential oil, mainly aromatic compounds (69.7%), oxygen-containing monoterpenes (14.9%), monoterpenes (6.3%), and others, where the most abundant compounds were also (*E*)-anethole and methyl chavicol, with different percentage of representation than previous (55.7% and 7.8%, respectively). The essential oil of fennel stems showed higher antioxidant activity with EC₅₀ value of 2.58 mg/ml, compared to the essential oil of fennel leaves with EC₅₀ value of 6.91 mg/ml. According to the obtained results, the antioxidant potential of isolated essential oils should be addressed to phenolic oil constituents, first of all to (*E*)-anethole and methyl chavicol, which make up more than 60% of both essential oils. Isolated essential oils could be used as a safer alternative to synthetic additives.

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I_PP3_Nutritional characteristics and physico-chemical quality of Florina apple fruits

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Apples occupied the third place worldwide as the most consumed fruits [1], while in Bulgaria their production exceed 22% and the increase with 12% (50 298 tone) yearly [2]. Apples constitute an important part of the human diet, as they are a source of monosaccharides, minerals, dietary fibre, and various biologically active compounds [3]. The aim of the current study was to evaluate the nutritional features and physico-chemical quality of apple fruit cultivar “Florina” grown under organic and conventional agriculture management practices. Total Soluble Solids (TSS) and titrable acidity varied from 11.2 to 12.3 °Brix and 0.16 to 0.20%, respectively. Color characteristics were also determined. Lightness (L*) of the skin ranged 55.37±11.37 to 67.45±1.48 and L* of the flesh was between 77.46±2.10 and 78.82±2.73. The hue angle ranged from 48.06±21.78 to 94.84±11.48. The skin had higher hue values indicating a yellowish color. The Florina apple collected from conventional and organic sward orchard demonstrated higher firmness - 8.98 to 9.60 kg/cm². The total lipid content did not exceed 1 g/100 g, while protein was in the range of 4.0-4.5 g/100 g. Histidine, arginine, asparagine and glutamic acid were the principal amino acids in all apple samples, as their content were higher in the organic sward orchard. The total carbohydrate content was in the range from 67.8 to 63.6 g/100 g dry weight. Sucrose, fructose, glucose, and sorbitol were detected in all apple samples, as their content was the highest in the organically grown fruits. In general, organically grown apples contained the highest level of polysaccharides as pectin. The results of the current research revealed the nutritional potential of Florina apple fruits as a source of essential and semi-essential amino acids, as well as dietary fibers.

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I_PP4_Pattern recognition of varieties of peach fruit and pulp from their volatile components using HS-SPME-GC/MS combined with multivariable statistical analysis

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The fruit's aroma profile, composed of a complex mixture of volatile organic compounds, is among the core attributes related to the overall taste and consumer preference. *Prunus persica* L. is a preferred summer fruit with a distinct favorable olfactory characteristic.

The volatile composition of both peach fruit and fruit pulp from eight peach cultivars (4 native and 4 introduced) was investigated to compare their composition and assess flavor contributing compounds. 65 compounds were profiled after a HS-SPME-GC-MS analysis, including 16 esters, 14 aldehydes, 5 alcohols, 7 hydrocarbons, 7 ketones, 8 acids and 8 terpenes. The most common compounds were esters, acids and aldehydes. Although the same compounds were identified in both fruit and pulp, their quantities differed in favor of the whole fruit.

Furthermore, according to the provided principal component analysis (PCA) and hierarchical cluster analysis (HCA), the relative quantities of the identified volatile compounds fluctuated depending on the studied cultivar. The obtained results could successfully be applied for the metabolic chemotaxonomy of peaches and differentiation of the metabolites present in different parts of the peach.

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I_PP5_Isolation and identification of lignan and phenylpropanoid esters from wild chervil (*Anthriscus sylvestris* (L.) Hoffm.)

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Anthriscus sylvestris (L.) Hoffm., known as wild chervil, is a widespread wild-growing Apiaceae species used in traditional medicine as antipyretic, analgesic, antitussive, diuretic, antihypertensive etc. It is known [1-4] to be rich in lignans, phenylpropanoids and polyacetylenes with antiproliferative, anti-inflammatory, insecticidal and antiviral activity. As a part of our on-going comprehensive study of *A. sylvestris*, we have employed flash chromatography, CPC, preparative HPLC, LC-MS/MS and NMR to isolate and identify six acylated lignans and phenylpropanoids from root extract (Figure 1): β -angeloyloxylatifolone (**1**), β -(2-methylbutanoyloxy)latifolone (**2**), β -(3-methylbutanoyloxy)latifolone (**3**), acetylpodophyllotoxin (**4**), angeloylpodophyllotoxin (**5**) and isovaleroyloxypodophyllotoxin (**6**). Only **4** and **5** were previously reported in *A. sylvestris* [2-4], while **2**, **3** and **6** were never reported in nature.

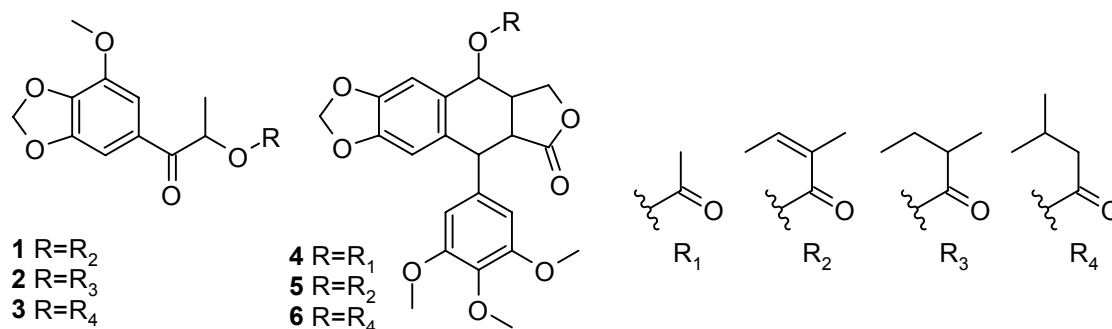


Figure1. Structures of identified compounds

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I_PP6_HPLC-DAD-ESI/MS profiling of phytochemicals in *Aesculus hippocastanum* fruit

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Aesculus hippocastanum L. (Sapindaceae) is a large, broad-crowned deciduous tree cultivated as an ornamental in urban areas in the Northern hemisphere. Among the scientific community, it is known mainly for steroidal glycosides, among which aescin is the main active constituent and responsible for most of its medicinal properties [1,2]; but other bioactive compounds such as flavonol glycosides have also been described in this plant [3]. In folk medicine, while the bark has been used to treat dermatitis, the leaves and seeds are used for their anti-inflammatory effect [2]. In Europe, seed extracts and supplements have been used to treat varicose veins among other venous disorders [4]. Therefore, this study aimed to characterize the phenolic and saponin profiles of the kernel, shell, and husk of the horse chestnut fruit. After separating, lyophilizing, and grinding the three fruit parts, hydroethanolic extracts were prepared by sonoextraction at 400 W (20 kHz) for 40 min. The chromatographic separation and identification of phenolic compounds and saponins were achieved by high-performance liquid chromatography coupled with photodiode array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI/MS), using different elution gradients [5]. It was possible to identify 31 phenolic compounds in the hydroethanolic extracts, including phenolic acids (caffeic acid and *p*-coumaroyl derivatives), flavan-3-ols ((+)-catechin and (-)-epicatechin derivatives), and flavonols (*O*-glycosylated quercetin, isorhamnetin derivatives, and kaempferol). While flavonols and flavan-3-ols predominated in the seed kernel and shell, respectively, the husk had comparable amounts of both phenolic constituents. The four main aescin saponins were consistently detected in the seed kernel extracts, namely aescin Ia and Ib (β -aescin) and isoescin Ia and Ib (α -aescin). Overall, the different parts of the inedible horse chestnut fruit have a different phytochemical profile in qualitative and quantitative terms. Further studies will be important to evaluate biological activities of the different seed extracts.

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I_PP7_Mycorrhization and micropropagation of chestnut (*Castanea sativa* Mill.) seedlings as tools to obtain high added-value phenolic compounds

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MiChestnut3 is a project of the company DEIFIL whose main objective is to produce more resistant and productive hybrid chestnut (*Castanea sativa* Mill.) seedlings through micropropagation and mycorrhization techniques. In this work, in addition to the agronomic traits of the micropropagated mycorrhizal chestnut seedlings, it was also important to evaluate the changes induced by mycorrhization in the phenolic profile of these plants. Phenolic compounds are plant secondary metabolites involved in plant-microbe interactions/symbiosis and act as signaling molecules in the establishment of arbuscular mycorrhizal symbioses, as well as in plant defense mechanisms [1]. According to the literature, considerable increases in phenolic compounds in host plants as a result of arbuscular mycorrhizal fungus inoculation have been reported during the progression of the infection [1]. Therefore, this work was carried out to study the impact of the type of fungal inoculum and the period of mycorrhization (before or after potting) on the qualitative and quantitative profile of phenolic compounds of the roots and leaves of the chestnut seedlings produced by DEIFIL. After collection and lyophilization of the plant material, hydroethanolic extracts were prepared and the phenolic compounds were characterized by HPLC-DAD-ESI/MS [2]. Ellagic acid derivatives and *O*-glycosylated flavonoids were the major phenolic compounds in both plant roots and leaves, which agreed with previous reports [2,3]. A statistical analysis showed that the type of inoculum and period of mycorrhization significantly ($p < 0.05$) affected the phenolic profile of the chestnut hybrids. In general, the mycorrhizal seedlings with the fungi *Amanita caesarea* and *Boletus edulis* were those that presented the highest levels of phenolic compounds. Relationships between the levels of these signaling compounds and the agronomic performance of chestnut seedlings were also found. The results bring new perspectives into the future production of a hybrid chestnut tree resistant to ink disease in the main traditional Portuguese varieties.

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I_PP8_Microencapsulation of anthocyanins from blue maize and their fate during *in vitro* gastrointestinal digestion

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Although the results provided the evidence of high levels of health beneficial phytochemicals of colored maize [1], it is currently being produced only in small amounts for making specialty foods or for use in ornamentation [2]. The aim of the present research was to develop and examine microencapsulation systems of anthocyanins from waste product of blue maize processing using conventional wall material as maltodextrin (MD), in combination with novel one, hydroxypropyl- β -cyclodextrin (HPCD). Liquid blue maize extract was spray dried with and without adding carrier agents: MD (30%), HPCD (30 %) and combination of both carriers (15% MD and 15% HPCD). The obtained samples were analyzed for the powder property parameters, for the content of phenolic compounds, antioxidant capacity and for the fate of anthocyanins (ACNs) in the digestive system. The content of total free phenolic compounds in microencapsulates was 31380 mg CE/kg on average. A smaller percentage consists of phenolic acids. Seven of them were detected. The ACNs content in the starting raw material was 1426 mg CGE/kg with acylated Cy-3-(6'-Mal-Glu) than Cy-3-Glu as dominant. In microencapsulates the content was 10677 mg CGE/kg on average with a variation of about 4%. Digestion fluids of microencapsulates with 30% of HPCD after each *in vitro* phase had the highest residue of ACNs (54-69%). On the other hand, the microencapsulation system with 15% MD and 15% HPCD showed the lowest stability. Therefore, the digestibility of these microencapsulates was the highest and amounted to 73.63%. Considering the results, it can be concluded that waste product of blue maize processing in form of microencapsulates can be used in the food and pharmaceutical industry and can also contribute significantly to the daily intake of ACNs, especially acylated forms whose daily intake has been estimated at 23% compared to 77% of non-acylated ones [3].

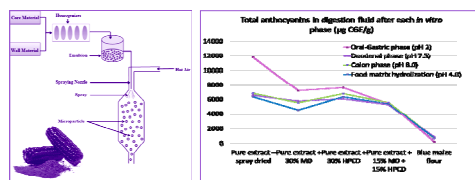


Figure 1. Total anthocyanins in digestion fluids after each *in vitro* phase

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I_PP9_Screening of the phenolic composition and *in vitro* biological activities of the fruit of *Lycium ruthenicum* Murray

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Lycium ruthenicum Murray (Solanaceae), also known as black goji or black wolfberry, has been used for centuries in traditional medicine and nutrition in Asian countries [1]. In recent years, black goji berries have been gaining attention as one of the most valuable sources of anthocyanins with many health-promoting effects [2]. Therefore, this study aimed to investigate the phytochemical composition and *in vitro* biological properties of black goji berry cultivated in southeastern Serbia. The total phenolics, flavonoids, anthocyanins, and tannins content were determined spectrophotometrically. HPLC-DAD-ESI-MS analysis was used to identify the phenolic compounds. Methanol extract from black goji berry was screened for *in vitro* antioxidant (DPPH[•], ABTS^{•+}, FRAP, CUPRAC, and β -carotene bleaching methods), antimicrobial, and hypoglycemic (α -amylase, α -glucosidase) potential. Phytochemical screening of the extract confirmed the dominant presence of anthocyanins and hydroxycinnamic acid derivatives, with petunidin 3-*p*-cumaroylrutinoside-5 glucoside as the main phenolic compound [3]. The total phenolic content was 18.13 ± 0.15 mg gallic acid equivalents/g freeze-dried sample. A high correlation is found between the total phenolic content and the antioxidant activities using different *in vitro* antioxidant assays. The antimicrobial activity of the black goji berry extract against eight laboratory control strains was not pronounced (MIC > 2 mg/ml). The IC₅₀ values were 6.55 ± 0.38 mg/mL and 7.37 ± 0.02 mg/ml for α -amylase, and α -glucosidase inhibition activity, respectively. Overall, obtained results suggest that the black goji berry cultivated in Serbia should be considered a valuable source of bioactive compounds for further use in the food, nutraceutical, and cosmetic industries.

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I_PP10_Chemical characterization and antioxidant activity of new rose genotypes (*Rosa hybrida*)

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Roses belong to family Rosaceae, genus *Rosa*, which is made of about 200 species and 30000 cultivars. Most important products of roses are essential oil, rose water, rose concrete and rose concentrate. Rose flowers are rich in phenolics, flavonoids, anthocyanins and carotenoids. These compounds can act as hydrogen donors and reducing agents which results in potent antioxidant activity. Thanks to that, there is a growing interest in research of new rose genotypes as sources of natural antioxidants [1].

In this study, 80% methanol extracts of flowers from 10 new rose genotypes were prepared. Chemical composition was evaluated by determining total phenolic and flavonoid content, as well as by LC-MS-MS analysis of selected compounds. Antioxidant activity was evaluated by DPPH[•] and FRAP assays [2].

Results of LC-MS-MS analysis revealed that these roses are rich in flavonoid (quercetin and kaempferol) glycosides and gallic and quinic acids. Total phenolic content varied in the range of 124.5-248.9 µg galic acid eq./mg d.w., while total flavonoid content was in the range of 19.9-59.7 µg quercetin eq./mg d.w. Compared to total phenolic and flavonoid content (165 µg galic acid eq./mg d.w and 81 µg quercetin/mg d.w, respectively) of *Rosa damascene* extract, new genotypes examined in this study have been shown as richer sources of total phenolics [1]. In DPPH[•] assay IC₅₀ values were in the range of 0.0177-0.0449 mg/mL, while in FRAP assay they ranged from 80.45 to 209.2 µg ascorbic acid eq./mg d.w., which shows that they have high antioxidant activity.

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I_PP11_Chemical characterization and antioxidant activity of hawthorn (*Crataegus* spp.)

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Hawthorn is a member of rose family, Hawthorn genus, with 280 varieties. In Europe most common are *Crataegus monogyna* and *Crataegus laevigata*. Hawthorn is rich in phenolic compounds like phenolic acids and flavonoids. It exhibits many biological activities, such as antioxidant, anti-inflammatory, cardioprotective, anti-diabetic, neuroprotective, and others. These activities are directly connected with phenolic composition [1].

The aim of this study was to compare chemical composition and antioxidant activity of hawthorn aerial parts and fruit methanol extracts, as well as to compare them with commercial extracts of hawthorn. Chemical composition was evaluated by determining total phenolic and flavonoid contents, as well as by LC-MS-MS analysis of selected phenolic compounds. Antioxidant activity was evaluated by DPPH[•] and FRAP assays [2].

LC-MS-MS analysis showed that the content of chlorogenic, quinic acid, vitexin, kaempferol-3-*O*-glucoside, quercetine-3-*O*-glucoside and galactoside was higher in extract of aerial parts while fruit extracts contained more epicatechin. Both extracts of aerial parts had higher content of total phenolics and flavonoids as well as higher antioxidant potential, compared to extract of fruit. Prepared extract of aerial parts showed higher antioxidant potential than the commercial one, while the commercial extract of fruit was more potent than the prepared one. Based on the results of this study it can be concluded that aerial parts of hawthorn are valuable sources of phenolic compounds and natural antioxidants.

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II_PP1_Sweroside displays a cytotoxic effect by activating apoptosis in human peripheral blood mononuclear cells

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Sweroside (Sw) is a secondary metabolite commonly found in plants belonging to the Gentianaceae family [1]. This iridoid compound is well-known for its anti-inflammatory [2], antidiabetic [3] and antitumor properties [4], which have been studied in pathological model systems. In transformed cell lines, Sw displays an antitumor effect by apoptosis activation [5]. Since healthy cells are also exposed to cancer therapy applied *in vivo*, our goal was to determine Sw's cytotoxic concentration in primary human peripheral blood mononuclear cells (PBMCs) after 48 h of treatment with a range of concentration from 20 μM to 130 μM Sw *in vitro*, and to analyze whether the cytotoxic effect was due to activated apoptosis. According to the obtained results of the trypan blue dye exclusion test, 48 h of treatment with 50 μM Sw and higher concentrations led to a significant decrease in cell number. The DNA fragmentation assay indicated that following 50 μM Sw treatment, cells are dying in an apoptosis-like manner since the level of DNA fragments was 3.5 times higher than in the untreated control. The type of cell death was confirmed by immunoblot analysis of apoptosis-specific protein markers, which revealed the elevation of cleaved caspase-3 and PARP1 89 kDa fragments. Our findings showed that like in transformed cell lines, Sw in healthy cells can also activate apoptosis. A potential difference in sensitivity to Sw treatment between healthy and transformed cells could justify Sw treatment in anticancer therapy.

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II_PP2_ Total phenolic content and antioxidant properties of fermented grape pomace of Cabernet Sauvignon grape variety

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Phenolic compounds are secondary plant metabolites with potential beneficial effects on human health because of their antioxidant activity and antimicrobial, antiviral, and anti-inflammatory properties. Grape pomace has high content of phenolics due to an incomplete extraction during the winemaking process. It has been shown that approximately 60–65% of phenolic compounds remain in the grape pomace after red wine production [1,2]. In this study, pomaces from Cabernet Sauvignon grape variety were analyzed in order to determine total phenolic content and antioxidant capacity. Alcohol fermentation with maceration was carried out by microvinification method at temperature of 25°C using the “pigeage” system. Free sulfur dioxide 5 g/hL was added to the grape pomace. Yeast *Saccharomyces cerevisiae* (FX10, Laffort, France) in the amount of 20 g/hL were used. Maceration lasted 3, 5, 7, 14, 21 day, respectively and after that liquid parts were separated. On the other hand, remaining samples of pomace were frozen (-80°C) and after that lyophilized. Total phenolic compound content and antioxidant capacity were analyzed in extracts obtained after extraction of pomace with water/methanol solution (50:50, v/v). Total phenolic content was evaluated by Folin-Ciocalteu method [3] and antioxidant capacity by FRAP method [4]. The highest content of total phenolic compounds was determined in sample which maceration lasted three days and it was 2822.19 mg/kg GAE of fermented pomace. For the same sample antioxidant capacity was 26.35 mmol Fe²⁺/kg of fermented pomace. Total phenolic content and antioxidant capacity were well correlated. The coefficient of correlation was 0.8276. Accordingly, grape pomace represents an important source of phenolic compounds, which can be useful from technological, pharmacological and environmental points of view.

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II_PP3_Total polyphenols and flavonoids content and antioxidant potential of methanolic extracts of different types of berries

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Berries are rich dietary sources of phenolic compounds, including phenolic acids, tannins, and flavonoids which are known to have various health benefits [1]. These bioactive compounds are believed to be potent antioxidants that can remove free radicals in cells which may trigger multiple pathological conditions such as the formation of cancer cells, cardiovascular disorders, neurodegenerative diseases, and inflammation [2,3].

In this respect, this study aimed to determine the content of total polyphenols and flavonoids of chokeberry, blueberry, and strawberry methanolic extracts and their antioxidant potential. The content of polyphenols and flavonoids was determined using standard Folin-Ciocalteu and aluminum chloride methods, respectively [4]. Antioxidant capacity was evaluated by the ability to remove 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals [5]. The obtained results indicate that the chokeberry sample has the highest total polyphenols content, 703.35 ± 48.02 μg of gallic acid equivalents (GAE)/g of fresh weight (FW) of the sample, followed by strawberry, 107.31 ± 7.02 μg GAE/g FW, and blueberry 82.69 ± 3.61 μg GAE/g FW. Moreover, the content of total flavonoids was the most abundant in the chokeberry sample, 690 μg of catechin equivalents (CE)/g FW, followed by blueberry, 82.21 ± 0.00 μg CE/g FW, and strawberry 77.44 ± 8.02 μg CE/g FW. The chokeberry sample has shown the ability of DPPH inhibition of $69.77\% \pm 5.64$ at the sample concentration of 1.2 mg/mL, while the strawberry sample exhibited a higher ability to scavenge DPPH free radicals than the blueberry sample ($86.35\% \pm 0.81$, $75.11\% \pm 2.12$, respectively) at the same applied sample concentration of 13.2 mg/mL. Based on the content of selected bioactive compounds and antioxidant activity, it can be considered that the consumption of analyzed fruits may have a beneficial effect on human health.

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II_PP4_Effect of oleuropein on the expression of invasive markers and cell adhesion of HTR-8/SVneo extravillous trophoblast cells

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Trophoblast invasion is one of the crucial steps during early pregnancy when extravillous trophoblast cells (EVTs) invade into the maternal decidua at the fetal-maternal interface. Disturbance in these tightly regulated processes can lead to placental dysfunction and pregnancy loss. Adhesion molecules integrins ($\alpha 1\beta 1$ and $\alpha 5\beta 1$), as well as proteolytic enzymes - matrix metalloproteinases (MMP) -2 and -9 are essential for the EVT's invasive phenotype. Plant-derived polyphenols such as oleuropein (OLE) present in Mediterranean diet, are powerful antioxidants known for their health benefits but there is a lack of data on their direct effects and safety in placental cells [1,2]. Although OLE treatment improved gestational outcomes in animal models its role in human pregnancy remains inconclusive [3]. Thus, the aim of this research was to examine the influence of OLE on the cell adhesion in EVT cells HTR-8/SVneo and to evaluate the expression of invasive markers- integrin subunits and MMPs by using RT-qPCR. The crystal violet cell adhesion assay was used to evaluate HTR-8/SVneo adhesion to plastic after 24h of OLE treatment (10 and 100 μM).

The results showed that OLE did not significantly alter cell adhesion to plastic. However, lower concentration (10 μM) of OLE significantly increased the expression of mRNA for all investigated integrin subunits $\alpha 1$, $\alpha 5$ and $\beta 1$ ($p < 0.01$) while 100 μM OLE did not show significant effects. Also, 10 μM OLE treatment significantly stimulated the expression of both MMP-2 and MMP-9 ($p < 0.01$) after 24 h incubation.

The obtained results demonstrate that OLE could have influence on the invasive phenotype of EVT cells and that it might have impact on placentation process. These results provide foundation for further mechanistic studies in primary trophoblast cells that could help understand the exact molecular mechanism of OLE effects and to determine its impact on placentation processes in early pregnancy.

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II_PP5_Taxifolin protects HTR-8/SVneo extravillous trophoblast cells against H₂O₂-induced oxidative damage and improves antioxidant function

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An increase of oxidative stress in the placenta has been recognized as a significant factor contributing to adverse pregnancies outcomes [1,2]. Considering the link between pregnancy disorders and increased placental oxidative stress, inhibition of oxidative damage through the use of antioxidants could be significant in preventing these disturbances. Taxifolin, a dietary flavonoid, plays an important role in human health by exerting antioxidant function [3]. However, its protective effects have not been examined in relation to human placenta. Thus, the aim was to investigate the protective potential of taxifolin against H₂O₂ induced oxidative damage in extravillous trophoblast cells *in vitro*. The levels of lipid peroxidation through lactate dehydrogenase (LDH) and malondialdehyde (MDA) quantification, protein damage (nitrite and carbonyl levels) and reduced glutathione (GSH) were analyzed, as well as catalase (CAT) activity. Oxidative stress parameters were measured by spectrophotometric analysis.

In the cells exposed to H₂O₂ only, all markers of oxidative damage were increased, as well as the CAT activity, while GSH content was reduced. The preincubation of HTR-8/SVneo cells with 10 μM and 100 μM taxifolin for 24 h prior to H₂O₂ exposure, produced significant decrease of both markers of lipid peroxidation (LDH and MDA), and reduced the nitrite as well as protein carbonyl levels, indicating inhibition of protein oxidation. Also, it decreased the high levels of CAT activity, followed with a significant increase in GSH content. Although both concentrations of taxifolin cause significant protection against H₂O₂ oxidative damage, the 100 μM concentration was more effective. The results show that taxifolin ameliorates H₂O₂ induced lipid and protein oxidation and improves the cellular antioxidant status. These results suggest that taxifolin might have beneficial impact on human trophoblast cells, and provide first step towards future studies on the use of taxifolin as effective natural antioxidant for prevention and therapeutic use during pregnancy.

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II_PP6_Antimicrobial activity of acacia honey, with and without additives, against selected pathogens

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Honey is a product of nature that has been used for nutritional and medicinal purposes for more than 5,500 years [1]. Honey contains around 180 chemical compounds with multiple functions derived from plant nectar, pollen and the bees themselves [2]. Many studies have shown the antimicrobial, anticancer and anti-inflammatory effects of honey [3]. The aim of this scientific work is to examine the existence of differences in the antimicrobial activities of acacia honey without additives, acacia honey with the addition of cinnamon and acacia honey with the addition of cumin, according to different pathogenic microorganisms. For this purpose, the agar disk diffusion method was used. It has been tested 100 µl each of acacia honey without additives, acacia honey with cinnamon, and acacia honey with cumin were tested and applied to sterile discs with a diameter of 9 mm. The most significant antimicrobial activity against all tested pathogens was shown by acacia honey with the addition of cinnamon, applied against *Staphylococcus aureus* (inhibition zone was 20 mm), slightly weaker against *Pseudomonas aeruginosa* and *Proteus mirabilis* (inhibition zone was 15 mm), while the weakest activity was recorded against *Escherichia coli* and *Salmonella enteritidis* (zone of inhibition was 12 mm). Acacia honey with the addition of cumin showed slightly weaker antimicrobial activity, and according to *S. aureus* the zone of inhibition was 15 mm, *P. aeruginosa* 14 mm, *P. mirabilis* 12 mm, while it did not show antimicrobial activity against *E. coli* and *S. enteritidis*. Acacia honey without additions showed the weakest antimicrobial activity compared to the others, the inhibition zone for *S. aureus* was 15 mm, *P. mirabilis* 10 mm, while no antimicrobial activity was observed for *P. aeruginosa*, *E. coli* and *S. enteritidis* species. Based on the results, we can come to the conclusion that the addition of honey, like cinnamon and cumin, enhances the antimicrobial effect of honey.

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II_PP7_Selected terpenoids as obstructers of *Candida* virulence

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Terpenoids are the most complex class of natural products with over 80,000 identified representatives found in plants, microorganisms, insects, and marine invertebrates. Their anticandidal potential, especially impact on fungal virulence is, however, not fully explored yet. We aimed to investigate anticandidal, antibiofilm, and antihyphal properties of selected terpenoids (borneol, thymol, geraniol, and carvacrol). The strongest antifungal potential towards *Candida albicans* strains could be noticed for borneol (lowest minimal inhibitory concentration, MIC, 0.06 mg/mL) followed by thymol (lowest MIC 0.12 mg/mL). On the other hand, MIC of geraniol was up to 8 mg/mL. Non-*albicans Candida* strains were most susceptible to thymol (MIC 0.12 mg/mL towards *C. krusei* H1/16) and borneol (MIC 0.12 mg/mL towards *C. parapsilosis* ATCC 22019). When the terpenoids were applied in their previously determined minimal inhibitory concentrations the strongest reducing impact on *C. albicans* ATCC 10231 biofilm forming ability was observed for carvacrol (60% inhibition), followed by geraniol (56% inhibition). Lower concentration of terpenoids (0.5 MIC) led to similar impairment of biofilm formation – the strongest impact was noticed for geraniol (54% inhibition), followed by carvacrol (47% inhibition) as determined by crystal violet assay. MTT antibiofilm assay highlighted great potential of carvacrol and geraniol to reduce viability of *Candida* cells in biofilms. Inhibition of hyphal formation could be noticed only upon application of carvacrol (14.29% of hyphal cells compared to the untreated control with 50.67% hyphae) suggesting wide antivirulence potential exhibited by this molecule. Other compounds explored in this study have displayed high MIC values (geraniol), low antibiofilm potential (thymol, borneol) or lack of antihyphal properties (all except carvacrol). Potential of carvacrol to reduce fungal virulence should be further studied on molecular level and in *in vivo* assays.

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II_PP8_ *In vitro* assessment of the prebiotic potential of selected plant extracts

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In recent years, many preclinical studies have provided evidence that polyphenol compounds and their metabolites exert prebiotic-like effects by selectively stimulating intestinal bacteria growth and/or activity [1]. In this study, we investigated the chemical composition, antioxidant capacity, and prebiotic potential of methanol extracts obtained from *Prunus spinosa* L. (blackthorn) and *Lycium ruthenicum* Murray (black goji) fruits. Polyphenolic profiles were characterized by using HPLC-DAD-ESI-MS and antioxidant capacity was estimated with DPPH•, ABTS•+, and FRAP assays. The influence of plant extracts (0.312-5 mg/L) on the growth of three probiotic lactobacilli, probiotic yeast (*Saccharomyces boulardii*), and two probiotic mixtures was examined. Studied plant extracts were characterized by different phenolic compound patterns. Hydroxycinnamic acid derivatives, quercetin glycosides and anthocyanins were detected in blackthorn extract, while black goji extract was characterized by the presence of hydroxycinnamic acid derivatives and acylated anthocyanins. The blackthorn fruit extract showed higher antiradical and reduction activity than those obtained from black goji fruits. Both extracts have influenced the growth of all tested probiotics in a concentration-dependent manner, especially of yeast, *S. boulardii*. The black goji extract had more stimulatory effects, possibly due to higher anthocyanins content. When the black goji extract was added to the growth medium at the concentration of 5 mg/mL, about 2-fold stimulation of the growth of *S. boulardii* was observed. In conclusion, extracts of the black goji and blackthorn fruits promote the growth of probiotics *in vitro*. Further studies are still needed to assess the prebiotic potential of these plant extracts.

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II_PP9_In silico evaluation of 4-hydroxy isoleucine via ADMETLab 2.0: Therapeutic potential for clinical uses

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Computer aided prediction of drug-like properties along with pharmacokinetic studies has made it more amenable to decide or predict a potential drug candidate. For this study, ADMETLab 2.0 was employed to determine important drug-related properties. 4-hydroxy isoleucine is compliant with drug-like physicochemical properties, some outliers presenting slight variations from the normal range. A rejection was of concern raised by QSAR based 'medicinal chemistry' (QED score, MCE-18, and golden triangle). But being an effective natural product and compliant with giants' drug-ability rules (Lipinski's, Pfizer, GSK), the rejection is not significant. Pharmacokinetically, it has a suitable profile, except that Caco-2 permeability had identified low absorption, yet sufficient MDCK permeability was predicted. Blood Brain Barrier (BBB) permeation may add CNS side effects, while a very slight probability of being CYP2C9 substrate exists. None of the well-known toxicities were predicted in silico, being congruent with wet lab results, except for a 'very slight risk' for respiratory toxicity predicted. The molecule is non-ecotoxic as analyzed with common indicators such as bioconcentration, IGC₅₀ for *Tetrahymena pyriformis*, LC₅₀ for fathead minnow and *daphnia magna*. The toxicity parameters identified 4-hydroxy isoleucine as nontoxic to androgen receptors, PPAR- γ , mitochondrial membrane receptor, heat shock element, and p53. However, out of seven parameters, not even a single toxicophore was detected. Hence, it is a very logical approach to proceed further with a detailed pharmacokinetics and drug development process for 4-hydroxy isoleucine. Moreover, some of the alarming factors can be chased for correction following SAR approach from a clinical perspective.

III_PP1_Antioxidant properties of cinnamon spice

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Cinnamomum zeylanicum (syn. *C. verum*), known as true, Ceylon or Mexican cinnamon is the evergreen tree, which have many applications: spice, in perfumery, flavoring and pharmaceutical industries. Cinnamon bark is the most often used part, usually as a powdered spice or as a component for the extraction of essential oil [1]. Many studies are reported that cinnamon spice contains bioactive compounds which show antioxidant, antimicrobial and antifungal properties. The antioxidant properties of cinnamon are attributed to the high content of phenolic compounds, especially cinnamaldehyde and eugenols, which stand out as the main antioxidants [2].

In this study, the antioxidant properties of cinnamon spice were investigated using the following spectrophotometrically assays: CUPRAC, FRAP, TAC and DPPH[•]. For these purposes, extracts of dry, powdered cinnamon spice (obtained from local market) were prepared by solvent extraction (SE) and ultrasound assisted extraction (USE) in warm water (50 °C) and 80% acetone (Fig. 1). The obtained results indicated similar antioxidant activity in acetone and water extracts in FRAP assay, while in other assays, acetone extracts exhibited higher antioxidant activity compared to water extracts. Acetone extracts prepared by SE and UAE showed the highest antioxidant activity in DPPH[•] assay, 85.50±0.38 % of inhibition and 85.31±0.10% of inhibition, respectively, compared to other assays. The lowest antioxidant activity was observed in water extract prepared by UAE 1.63±0.06 mg/g AAE, in CUPRAC assay. Based on the obtained results which are in accordance with the available scientific data, cinnamon is recognized as a spice with an expressed antioxidant activity.

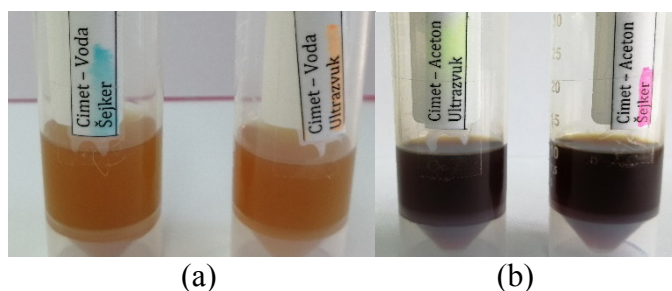


Figure 1. (a) Water extracts; (b) Acetone extracts of cinnamon spice

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III_PP2_Chemical composition of the essential oil of *Thymus serpyllum* L.

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The purpose of this study was to examine the chemical composition of the essential oil of *Thymus serpyllum* L. collected on the slopes of the Kopaonik Mountain (Serbia). *Thymus serpyllum* L. (wild thyme) belongs to the genus *Thymus*, family Lamiaceae [1]. Species of the family Lamiaceae have a long and a rich tradition of use for flavouring, food preservation, and medicinal purposes, due to their curative and preventive properties [2]. Wild thyme (*Thymus serpyllum* L., Lamiaceae) is a valuable medicinal and aromatic plant. The isolation of the essential oil was carried out using hydrodistillation in the Clevenger type apparatus under Ph. Eur. [3], while the chromatographic analysis of essential oil was carried out using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The number of registered and identified components in essential oil was twenty three. Sesquiterpenes are the dominant class of compounds of tested essential oils with the principal component *trans*-caryophyllene (29.3 %). Generally, besides *trans*-caryophyllene, γ -muurolene and α -humulene as dominant constituents, other main ones were α -pinene (7.2%), myrcene (6.8%), thymol (5.9%), and camphor (3.8%). The content of isolates is important in the contest of their use in medicinal purposes, in industry as raw material, and in cookery where the quality of products highly depends on chemical content of isolates. Terpenoid profile can be significant for standard production of juices and beverages, e.g., vermouth in which is essential that chemical content of used herbs is regular, which depends on climate, way of growing, picking, preparing and keeping [4].

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III_PP3 *In silico* study of the tyrosinase inhibitory potential of arbutin and other bioactive compounds from strawberry tree

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Tyrosinase is an enzyme involved in the generation of dopamine quinones, which has an important role in oxidative stress associated with different health disorders. It is also a common molecular target for the design of anti-melanogenic agents. Strawberry tree (*Arbutus unedo* L.) leaves are used in Mediterranean folk medicine to treat urinary tract infections, where arbutin is recognized as the main bioactive compound [1]. The inhibition of tyrosinase might be responsible for the intracellular antioxidant activity of arbutin. Moreover, other compounds from strawberry tree leaves, fruits and honey such as hyperoside and homogentisic acid [2] may inhibit tyrosinase owing to the presence of phenolic group(s) as pharmacophore. Herein, we performed virtual screening of 25 compounds including β -arbutin (naturally occurring compound), α -arbutin (synthetic substance), 13 synthesizable arbutin derivatives designed using DrugSpaceX [3], other compounds from strawberry tree [4], and kojic acid as a positive control. 3D structures were optimized on the PM7 level, and then were docked into the active site of *Bacillus megaterium* tyrosinase (pdb 3NQ1) using AutoDock Vina 1.1 [5]. The binding mode of β -arbutin is stabilized via hydrogen bond donor interactions with His231, His42, His60, Arg209, Gly216, and Asn205, hydrogen bond acceptor interaction with Arg209, and hydrophobic interactions with His208, Val218 and Ala221 (Figure 1). The binding energy (BE) of β -arbutin is -5.9 kcal/mol. Hyperoside and two arbutin derivatives (DA8 and DA14, Figure 1) interact more favorably with tyrosinase with the BE of -6.7, -7.2 and -7.3 kcal/mol, respectively.

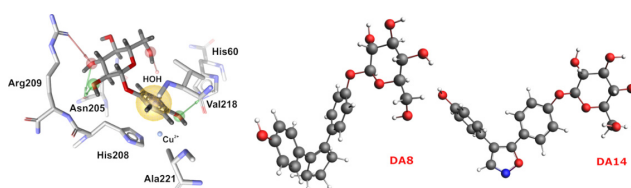


Figure 1. Binding mode of β -arbutin into tyrosinase (left), and 3D structures of two improved arbutin derivatives

The results of this study provide valuable structural insights into the molecular mechanisms of biological action of arbutin and other bioactives from strawberry tree and might be exploited to develop more potent and drug-like analogues.

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III_PP4_DFT study of the antioxidant activity of polyhydroxyflavones identified in houseleek leaf extracts

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Previously, we identified kaempferol, quercetin and their 3-O-glycosides as potent antioxidants in methanolic extract of houseleek (*Sempervivum tectorum*) leaves using the High-Performance Thin-Layer Chromatography (HPTLC) and DPPH· (2,2-diphenyl-1-picrylhydrazyl) derivatization. Herein, we report bond dissociation enthalpies (BDEs) and ionization potentials (IPs) of these compounds calculated using Density Functional Theory (DFT) with M06-2X functional and 6-31+G(d,p) basis set in the gas phase and SMD models of water (physiological medium) and pentyl ethanoate (simulated lipid environment). BDEs and IPs map the thermodynamic feasibility of hydrogen atom transfer (HAT) and single electron transfer followed by proton transfer (SET-PT), two common mechanisms how small molecules quench reactive free radicals.

The HAT is a favorable antioxidant mechanism for all studied compounds in both solvents. The lowest BDE of 76.64 kcal/mol was found for 3'-OH group of quercetin, which is comparable with BDE for resveratrol. Glycosylation in the 3'-position of flavone ring blocks this antioxidant hotspot, increases the IP by 3-7 kcal/mol in all media, and negligibly influences the activity of -OH groups on A and B rings. This result corroborates previous results on the antioxidant activity of *S. tectorum* leaf juice obtained by EPR spectroscopy [1].

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III_PP5_The antioxidant and antifungal activity of the extract, essential oil and AgNPs green synthesized of aqueous and methanolic extracts of *Origanum vulgare* L.

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Plant-mediated green synthesis of nanomaterials has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness [1]. Green silver nanoparticles (AgNPs) were synthesized by using water and methanol extracts of *Origanum vulgare* L. Ultraviolet-visible spectroscopy and SEM analysis showed that spherical aqueous and methanolic AgNPs had 19.43 and 18.35 nm, respectively. Aqueous extract showed the highest total phenolic content (50.28±1.00 mg gallic acid/g) and methanolic AgNPs exhibited the strongest activity as a DPPH scavenger with 89.68±0.25% and IC₅₀=19.63±0.34 µg/ml. Comparison of the antifungal activity of *Origanum vulgare* L. essential oil, AgNPs and extracts was done against the fungi *Aspergillus niger*, *Botrytis cinerea* and *Penicillium expansum*. The most sensitive pathogen to antifungal agents was *Penicillium expansum*. Essential oil at 1000 ppm showed complete antifungal activity against all three microorganisms tested. Among AgNPs and extracts the best antifungal activity was recorded by methanolic AgNPs in the following order *Penicillium expansum* > *Botrytis cinerea* > *Aspergillus niger*. Carvacrol (56.53%), thymol (21.18%), *p*-cymene (6.22%), and γ-terpinene (5.67%) were determined as the main compounds of essential oil by GC-MS analysis. The results suggest that the *Origanum vulgare* L. can be a rich source of different unique antimicrobial activity, which may have various applications in agriculture and the control of plant disease in the future. These results are in line with our previous findings [2].

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IV_PP1_Demystifying Bangladeshi ethnomedicines with modern approaches: *Congea tomentosa* as a source of potential anti-microbial and anti-cancer agents

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The indigenous communities of Bangladesh use several plants/parts, with unknown ingredients, for the treatment of cancers and microbial diseases, often with promising anecdotal results, although few if any have been formally screened. To investigate such treatments, we documented the traditional use of 276 plant species from 292 informants of Bangladesh following established techniques. Notably, 28 species were reported with new therapeutic uses and 13 species described have never been studied phytochemically or pharmacologically. Of these, stem of *Congea tomentosa* was selected for *in-vitro* studies. Bioassay-guided screening led us to identify 10 compounds using GC-MS analysis from the petroleum ether extract while two compounds (β -amyirin and stigmasterol) were isolated and identified from the ethyl acetate fraction using mass spectrometry and NMR spectroscopy. Compound-2 (stigmasterol) displayed promising antimicrobial activity against tested microorganisms. On the other hand, among all fractions tested for anticancer activity, the aqueous fraction was dominant in activity against U-251 cell lines. The aqueous fraction was further purified by HPLC (high performance liquid chromatography), and within it we identified three compounds by NMR and Mass spectral analysis. Among the isolated compounds, compound-2 concentration-dependently inhibited U-251 cell proliferation without inducing cytotoxic effects in a normal cell line and had the potential to induce apoptosis in U-251 cells by activation of the intrinsic/mitochondrial pathway. In addition, compound-2 also suppressed the activity of Stat3 (signal transducer and activator of transcription 3) and Akt (protein kinase B), postulating that compound-2 induced apoptosis might be triggered by the inhibition of Stat3 and Akt expression. Despite the ethnobotanical importance of *Congea tomentosa*, no chemical or biological studies have been published to date which support its traditional applications in Bangladesh. However, this study demonstrates the possible use of this plant in the treatment of cancers/microbial diseases, and supports its traditional use.



IV_PP2_Neuroprotection of date (*Phoenix dactylifera* L.) extract from Algeria against Neurodegenerative disease - "Alzheimer's case"

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Alzheimer's disease is a progressive neurodegenerative disease characterized by cognitive and functional decline, leading to behavioural disturbances and progressive and irreversible loss of cognitive functions, including memory, with the installation of a lethal course of dementia. To date, few pharmacological solutions are available in the treatment of Alzheimer's disease. The date fruit (*Phoenix dactylifera* L.) represents a national heritage in Algeria, of its gustatory and nutritional qualities, but not only, owing to its richness in active phytochemical compounds, but it could also be considered a new therapeutic potential. Firstly, a determination of the phytochemical composition of date pulps and seeds extracts was carried out by determining the content of phenolic compounds such as total phenolics, flavonoids, condensed tannins and hydrolysable tannins by colorimetric methods. A chromatographic analysis was also performed of the phenolic acids and flavonoids profile by HPLC-UV. The present research aimed to investigate the *in vivo* neuroprotection activity of Algerian date pulp extract. Our work was focused to characterize the effect of a hypothetical treatment of a model of Alzheimer's disease in adult mice after eleven weeks of chronic treatment with ingestion of AlCl₃ (10 mg/kg) and D-galactose by intraperitoneal (120 mg/kg) grass dates on memory by assessing the effects of this fruit on spatial memory performance, the tests of behaviour and memory for the anti-Alzheimer's activity, indicated prophylactic and/or curative effects of the aqueous date pulps and seeds extracts. This was confirmed by histological examinations established on the target brain.

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IV_PP3_ *Tripleurospermum inodorum* – mayweed without scent but with range of bioactivities

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Tripleurospermum inodorum (L.) Sch. Bip. (Asteraceae), scentless mayweed, is a common weed species, but despite its abundance it is still underexplored in the terms of its bioactivities. The aim of the study was to explore the bioactivities of scentless mayweed methanolic extract including antimicrobial, antibiofilm, antioxidant, cytotoxic, and anti-inflammatory activity and to provide deeper insight into its chemical composition. It was shown that *T. inodorum* inhibits growth of human pathogenic microorganisms with minimal inhibitory concentration (MIC) in range 3-12 mg/mL, with the lowest MIC noted for the species *Candida albicans* 475/15, *C. albicans* 17/15, *C. parapsilosis* ATCC 22019, *Pseudomonas aeruginosa* (IBRS P001), *Yersinia enterocolitica* (ATCC 23715), and *Klebsiella pneumoniae* (ATCC 13883). Its biofilm potential was promising - application of plant extract (1.5 and 3 mg/mL) reduced *C. albicans* 475/15 biofilm biomass and biofilm cell viability for more than 50%. Antioxidant assays suggested the plants antioxidant potential at 442.2 mmol GAE (gallic acid equivalent)/100 mg DW (dry weight) as in ABTS[®] assay, 277.8 mmol GAE/100 mg DW in DPPH, and 204.4 mmol GAE/100 mg DW as recorded in the FRAP assay. Wide cytotoxic potential of the extract was noticed for the range of cells: AGS (gastric adenocarcinoma, GI₅₀ 198.7 µg/mL), CaCo2 (colorectal adenocarcinoma, GI₅₀ 278.2 µg/mL), MCF-7 (breast adenocarcinoma, GI₅₀ 265.7 µg/mL), NCI-H460 (lung carcinoma, GI₅₀ 62.9 µg/mL), and VERO (non-tumour cell line, GI₅₀ >400 µg/mL). Likewise, low IC₅₀ (8.4 µg/mL) in the RAW 246.7 anti-inflammatory assay suggests the strong anti-inflammatory potential of this weed. The dominant polyphenols in the extract were two apigenin derivatives: apigenin-*O*-pentoside and apigenin-*O*-acetylhexoside (5.234 mg/g and 4.929 mg/g extract, respectively). *T. inodorum* is a rich source of different bioactive polyphenols and its methanolic extract displays a range of bioactive features that should be further examined in order to develop novel herbal medicines.

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V_PP1_Nanotechnology approach for diminishing quercetin toxicity toward peripheral blood mononuclear cells

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Quercetin (Q) is one of the most common and well researched antioxidant flavonoids, which usually occurs in plant-based foods, and medicinal plants. It was shown that quercetin exerts many beneficial effects on human health, including prevention of cancer and heart diseases. Quercetin was found to be toxic toward various types of cancer cell, still, at higher concentrations it was also shown toxic toward normal human cells [1,2].

One of the approaches to overcome this shortcoming offers nanotechnology which enables the novel perspective of phytochemical usage in contemporary medicine [3]. The strategy of binding quercetin to the gold nanoparticles during their synthesis was used, which resulted in quercetin capped gold nanoparticles (NPQ) [4]. Trypan blue exclusion test [5] was used to evaluate peripheral blood mononuclear cells (PBMC) viability after their exposure to either NPQ or free Q during 24, 48 and 72 h, at 37 °C, in the range of quercetin concentrations from 5 to 50 µg/mL. A significant reduction in the cell count was observed in PBMC cultures treated with 10, 20, and 50 µg/mL of free Q, for all exposure times. The treatments of increasing concentrations and exposure times lowered the cells viability, resulting in 63% of the viable cells, following 72 h of the treatment with 50 µg/mL of free Q. Although NPQ treatments affected the cells viability in a concentration- and time-dependent manner the treatment with 50 µg/mL of NPQ for 72 h, had a milder effect on PBMC cultures than free Q, resulting in 81% of the viable cells (Figure 1). According to the obtained results, NPQ were shown less toxic toward PBMC than free Q.

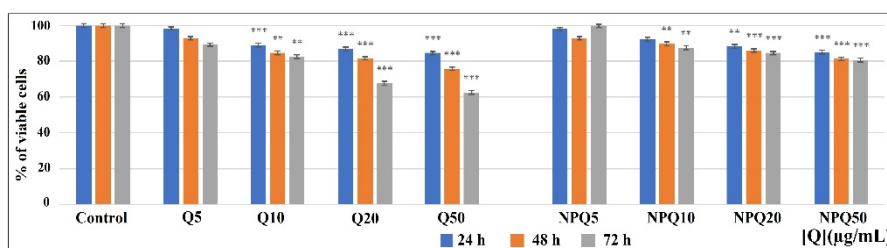


Figure 1. Viability of PBMC treated with different concentrations of free Q, and NPQ, for 24, 48 and 72 h. The control represents untreated cells. Results are presented as average \pm SD of three independent experiments

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VI_PP1_Elucidating the role of mangiferin compound and silver nanoparticles (AgNPs) against plant pathogenic fungi *Phomopsis vexans*

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In this experiment, an attempt has been made to develop safer agrochemicals that could inhibit the attack of pests and pathogens. With this view, silver nanoparticles (AgNPs) were prepared by using *Mangifera indica* leaves and the mangiferin was isolated from the plant and screened the impact on pathogenic fungi. The nanoparticles were characterized by using ultraviolet spectroscopy absorbance [1]. Transmission electron microscopy results showed the distribution of nanoparticles and particle size was 100 nm. Mangiferin compound was also characterized by UV and NMR techniques [2,3]. The results are evidenced that the plant-based AgNPs and mangiferin have the potential to inhibit plant pathogenic fungi *Phomopsis vexans*. From this study, we concluded that synthesized AgNPs with mangiferin have the potential to serve as a safer alternative for plant disease management.

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VI_PP2_Microbiological characteristics of 'Prokupac' wine enriched with medicinal herbs

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The autochthonous variety 'Prokupac' (*Vitis vinifera* L.) is the dominant variety in the vineyards of southern Serbia [1]. The aim of this study was to determine the influence of medicinal herbs on the microbiological activity of the obtained flavored wines. For this study, grapes of the variety 'Prokupac' from the vineyards of Jug-Bogdanovac, located in the Toplica region were used. Dried and ground plant parts were added separately to 'Prokupac' grape juice before fermentation: anise seeds (*Pimpinella anisum*), cinnamon bark (*Cinnamomum verum*, synonym, *C. zeylanicum*), wormwood leaf (*Artemisia absinthium*) and licorice root (*Glycyrrhiza glabra*) in amount of 1% (w/w). The microbiological activity of wine was determined by the disc-diffusion method [2,3]. Testing of the antimicrobial activity of wine was performed with strains of microorganisms: Gram-positive bacteria: *Bacillus subtilis* ATCC, Gram-negative bacteria: *Escherichia coli* ATCC and *Salmonella typhimurium* ATCC and yeast: *Candida albicans* ATCC. The obtained results were compared with the activity of antibiotics: chloramphenicol (30 µg/disc), streptomycin (10 µg/disc) and nystatin (30 µg/disc). For microbiological analysis, the following media were used: Müller-Hinton agar for the cultivation of G(+) bacteria and G(-) bacteria and Sabouraud dextrose agar for the cultivation of *Candida albicans*. Using the disk diffusion method, it was observed that Gram (+) bacteria from the genus *Bacillus subtilis* showed the highest sensitivity, while the wines did not show antimicrobial properties against Gram(-) bacteria and *Candida albicans* yeast. 'Prokupac' wines enriched with anise and licorice showed a bacteriostatic effect on Gram(+) bacteria from the genus *Bacillus subtilis* (inhibition zone 14 mm, i.e. 46.67% of the activity of chloramphenicol, i.e. 40.0% of the activity of streptomycin), whereas 'Prokupac' wine enriched with cinnamon showed a greater bactericidal effect on Gram(+) bacteria *Bacillus subtilis* (inhibition zone 16 mm; 53.33% of chloramphenicol activity, i.e. 45.71% of streptomycin activity). Obtained results are in agreement with our previous work [4]. The addition of medicinal herbs increased the antimicrobial potential of studied 'Prokupac' wine samples.

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VI_PP3_Effect of processing on the antinutrients, antioxidant and techno-functional characteristics of five-leaf yam (*Dioscorea pentaphylla*)

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Global concerns of the 21st century include food, nutrition, and energy security. Around 820 million people are hungry or suffering from food insecurity [1], and the hunger indices of the majority of nations are attainable. Yam is a possible carbohydrates source. Yam is rich in phytonutrients and has excellent functional, nutritional, and physiological advantages (antioxidant activity, hypoglycemic activity, anticancer activity, analgesic activity), making it an attractive emerging commodity for food and medicine [2]. Post-harvest processing of tubers may generate functional foods and nutraceuticals to treat ailments. Anti-nutritional factors (saponin, phytic acid, tannin, calcium oxalate, etc.) restrict their use and commercialization. Recent research has drastically lowered anti-nutrients to avoid this constraint. Prior to drying, Yam slices were treated with different processing methods mainly soaking (4 h, 8 h, 12 h), boiling (5, 15, 25 min) and steaming (at 100°C for 5, 15 and 25 min). Pre-treated slices are dried by hot air oven drying method at 50°C, ground to prepare flour and stored for further study. Results showed more than 50% reduction in tannin and trypsin inhibitor content while oxalate and phytic acid were reduced to 60%. Total phenolics and flavonoids significantly were reduced with increase in time of boiling. Contradicting to this, soaking for longer time increased the bioactive content and antioxidant activity. This may be due to the breaking down of covalent bond for release of compounds for better extraction. SEM micrographs revealed that the size and shape is affected by the processing conditions. Soaking maintained the shape of starch granules without much degradation, but boiling had more damaged cranny uneven surface. Increased boiling diminished the crystallinity, while prolonged soaking increased it. The most effective pretreatment for removing antinutrients was determined to be a 25-minute boil. However, combination of boiling and soaking may be more effective at removing the substance.

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VI_PP4_Microbiological quality control of cheeses produced on farms in the territory of the city of Prokuplje

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Milk and dairy products are widespread in the diet of children and adults [1]. Cheese, as a dairy product produced from thermally unprocessed milk, represents a suitable medium for the development of microorganisms and thus represents a risk to the health of consumers [2]. The aim of this scientific work is to determine the microbiological correctness of cheese produced on agricultural farms in the territory of the city of Prokuplje (Serbia). Microbiological parameters are determined according to the Rulebook on Amendments to the Rulebook on General and Special Conditions of Food Hygiene (Official Gazette of RS, 62/18), at any stage of production, processing and circulation and include coagulase-positive staphylococcus, *Salmonella* sp. and *Listeria monocytogenes*. The total number of coagulase positive bacteria was determined according to the standard SRP EN ISO 6882:2017 method, *Salmonella* sp., according to the standard SRP ISO 6579-1:2017 method and *L. monocytogenes* according to the standard SRP EN ISO 11290-1:2017 method. From a total of 5 households from the territory of the city of Prokuplje, 3 samples of cheese were sampled that were made in the traditional way, from raw milk. The microbiological analysis of the treated samples did not determine the presence of these microorganisms in any sample. Since the results are in accordance with the Rulebook, the samples can be considered microbiologically safe, and therefore it can be concluded that a high level of hygiene and animal health protection is maintained at the agricultural households.

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VI_PP5_Agrobiological traits of grape cultivar 'Tamjanika white' grown in Župa vine district

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'Tamjanika' is considered as a native Serbian grape, grown in Serbia and North Macedonia, and it is mostly used in the production of dry varietal wines. These white wines are typically light, floral, and refreshing. The 'Tamjanika white' variety belongs to the Muscat varieties, and its muscat smell comes primarily from linalol, geraniol and linalool oxides. The Muscat-type cultivars were included within the *Convar orientalis* subconvar *caspica* [1]. The study aimed to observe important agrobiological traits of the cultivar 'Tamjanika white' in the agroecological conditions of Župa vine district, as well as possibility for its spreading in this district. Selected agrobiological traits of were studied: phenological observations, yielding potential for buds and bunches for vine. The experiment has been carried out at a productive vineyard in the period of growing yield at the location Svračak (Aleksandrovac), in the period 2008-2010. Agroecological conditions were favorable for growth and development of grape cultivar 'Tamjanika white'. The vineyard was planted in 2002, with planting distance 2.8 x 0.9 m. Three pruning variants with short and long spurs, and three variants of mixed pruning with canes of eight and ten buds long were applied. The fertile bud load per vine varied from 18-36 buds per vine. This cultivar expressed positive agrobiological characteristics that were dependent on environmental conditions, which is line with our previous findings [2]. Results of the study confirm a possibility of successful growing of 'Tamjanika white' cultivar in the Župa vine district.

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VI_PP6_Antioxidant activities and structural functional characterization of bitter yam flour (*Dioscorea bulbifera*) as influenced by different drying techniques

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More than 820 million people are either starving or agonizing due to fragility and shortage of food, and for most countries, the hunger indexes are still through far unreachable conditions. Food, nutrition and energy security is a primary global concern of 21st century [1]. Yams have potential to address these issues and are abundant sources of carbohydrates and phyto-compounds (dioscin, diosgenin, mucin, choline, allantoin). Moreover, yams exhibit high value of functional, nutritional properties, and health benefits (antioxidant activity, hypoglycemic activity, anticancer activity, analgesic activity) which make them an industrial emerging product in terms of food and medicinal value [2]. Total 550-650 tons of *Dioscorea* species were used by global pharmaceutical industry with market price of \$500 million and in India, 100% of steroid drugs produced from locally available *Dioscorea* species [3,4]. But, high moisture content, restrict the broader use of yams. Drying is a crucial processing step for enhancing the storability of yam slices and powder. The research was undertaken to study the effect of different drying processes (sun drying, shade drying, hot air drying, microwave drying (MWD), infrared drying (IRD) and freeze drying) on the nutritional, functional, structural and bioactive components content of yam flours. The antioxidant activity of the IR dried sample was retained at maximum. The starch content of the dried yam flours ranged between 46 and 59%. Resistance starch content of the freeze drying was maximal (48%) and decreased after thermal treatment. TGA and DSC confirmed the stability of yam flours in high temperatures while XRD, FTIR and SEM micrographs confirmed the gelatinization of starch during MWD and IRD. However, the FD sample showed the retention of original particle shape. IR dried flour had more antioxidants and low moisture content compared to other drying methods.

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VI_PP7_Is there a difference in the profile of the health promoting compounds in plum fruits and kernels grown under the organic and conventional production system?

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Organic fruit production has become very popular due to the 'sustainable intensification' of the production with less pesticide and heavy metal residues, pronounced aroma, better soil health and higher biodiversity. On the other hand, some studies proved no consistent differences between those two growing systems. The aim of this study was to compare the total polyphenolic profile (TPC), antioxidant capacity (RSA), total anthocyanins (TAC), sugar and polyphenolic profile of the fruit's skin and pulp and total oil content and fatty acids of the kernels in two plums (*Prunus domestica* L.) cultivars ('Požegača' and 'Stanley') that were grown organically and conventionally. Both TPC and RSA were much higher in the skin compared to the pulp. The profiles of sugars and polyols were determined using high performance anion exchange chromatography with electrochemical detection on gold electrode. The separation and quantification of the polyphenols were performed using a Dionex Ultimate 3000 UHPLC system connected to TSQ Quantum Access Max triple-quadrupole mass spectrometer. The composition of fatty acids was determined according to the standard method by capillary gas chromatography. Skin of organic 'Požegača' and conventional 'Stanley' gave the highest TPC (7.52 g and 7.10 GAE/kg frozen weight, respectively) and RSA (33.41 and 40.63 mmol TE/kg, respectively). The pulp of the organic 'Požegača' had the highest level of glucose, fructose and sorbitol (42.16, 23.29 and 55.05 g/kg, respectively), while sucrose was the highest in the flesh of the organic 'Stanley'. The most abundant polyphenolic compound in the skin was rutin that was the highest in the organic 'Požegača' (49.034 mg/L), while in the pulp those were chlorogenic acid and caffeic acid whose quantities were the highest in the organic 'Požegača' (1.162 and 1.382 mg/L, respectively) and organic 'Stanley' (0.817 and 1.688 mg/L, respectively). Kernel's total oil content varied too and ranged from 22.13% (conventional 'Stanley') to 25.45% (conventional 'Požegača'). Out of analyzed fatty acids the most abundant were oleic acid and linoleic acid. The highest level of oleic, but the lowest level of linoleic fatty acids had kernels of conventional 'Stanley' (74.76% and 18.34%, respectively), but the lowest oleic and the highest linoleic had organic 'Požegača' (66.58% and 25.39%). Results of this study proved that both cultivars and both production systems gave fruits with high health promoting compounds, but cultivar 'Požegača' gave better results in organic production while 'Stanley' in conventional system of growing.

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VII_PP1_Effect of different heat treatments on antioxidative activity in pumpkin (*Cucurbita maxima*)

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Pumpkin (*Cucurbita maxima*) contains significant amounts of diverse phytochemicals, including polyphenols (flavonoids, tannins) and carotenoids. Some of these compounds are known antioxidants, capable of neutralizing harmful biological free radicals, thus protecting health of living organisms. However, the numerous food-processing technologies decrease the amounts of naturally occurring antioxidants, due to enzymatic and nonenzymatic oxidation processes. The aim of this research was to determine the influence of different heat treatments (cooking, baking in conventional and microwave oven) on the antioxidant activity of phytochemicals present in the pumpkin.

The antioxidant activity was quantified spectrophotometrically, at the specific wavelengths, utilizing standard colorimetric reactions. The total antioxidant capacity of the analyzed samples was determined by phosphomolybdate method using ascorbic acid as a standard [1]. Antioxidant activity was measured by the inhibition of lipid peroxidation (FTC) [2] and free radical scavenging (DPPH[•] [3], hydroxyl [4] and ABTS radical cation [5]) methods.

The highest total antioxidant capacity was found in a raw pumpkin sample (5.58±0.33 mg AAE/g), while the lowest value was found in a sample of pumpkin baked in conventional oven (2.88±0.32 mg AAE/g). Inhibition activity against lipid peroxidation (IC₅₀) was the highest in raw pumpkin (16.72±0.73 µg/ml), and the lowest one was in conventional baked pumpkin (8.49±0.31 µg/ml). Free radical scavenging activity measured by DPPH and hydroxyl radicals (IC₅₀) were the highest in raw sample (35.67±1.99 µg/ml) and (19.46±1.60 µg/ml), respectively, while the lowest values were in conventional baked pumpkin (15.68±1.32 µg/ml) and (9.69±2.01 µg/ml), respectively. Antioxidant activity measured by ABTS radical cation scavenging (IC₅₀) was the highest in raw sample (41.63±0.61 µg/ml) and the lowest value was found in a sample baked in conventional oven (21.32±0.45 µg/ml). The results showed that different heat treatments significantly affected on antioxidant activity, especially baking in conventional way, what makes it the least favorable process in this study.

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VII_PP2_ Micro and nano-encapsulation of anthocyanins and its application in food technology

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Encapsulation is a process of entrapment of one substance within another, producing particles with diameters of a few nm to a few μm [1]. It is used to preserve bioactive compounds such as anthocyanins, improve physicochemical functionalities, and enhance their health-promoting and anti-disease activities [2]. Anthocyanins are water-soluble natural pigments, widely spread in plants, vastly significant for human health. They are sensitive to deteriorative factors such as pH, temperature, and light. Encapsulation of anthocyanins requires further research to develop methods that will increase their stability. Recently investigated methods are coacervation, spray drying, liposomal system, electrospraying, microwave-assisted encapsulation [3], usage of biopolymer and lipid-based transporters [2], co-encapsulation of anthocyanins and α -tocopherol via spray drying [4], usage of polymers and protein associated vesicles [5]. Comparative research gave different results with the tendency to develop new methods that will improve the stability of anthocyanins. Until now, the application of encapsulated anthocyanins in food technology comprises natural food colorants and additives.

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VII_PP3_Antioxidant properties of monofloral poppy and bastard indigobush bee-collected pollen as potential functional food ingredient

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Antioxidants are important part of human healthy diet. In order to obtain food enriched with antioxidants novel diets reach to some natural plant-based resources. Bee-collected pollen can serve as excellent source of different antioxidants (in particular phenolic compounds and carotenoids) [1,2] and can be used as an excellent functional food ingredient [3,4,5]. In order to obtain reach source of antioxidants for functional food preparation two different monofloral (contain more than 80% of one pollen grain types) bee-collected pollen samples originated from poppy (*Papaver somniferum*) and bastard indigobush (*Amorpha fruticosa*) were collected from Belgrade area, and stored adequately for further analyses. Both samples were extracted with 80% methanol (MeOH) and digested with sodium hydroxide and hydrochloric acid in order to obtain antioxidants reach extracts: extractable (EP) and bound phenolic (BP) fractions [1]. Standard spectrophotometric methods were applied in order to determine antioxidant properties of attained samples: *in vitro* phosphomolybdenum total antioxidant capacity (TAC), ferric reducing power (FRP) as well as cupric reducing antioxidant capacity (CUPRAC) assays. All results are expressed based on dry matter (d.w.) as ascorbic acid equivalents (AAE). According to obtained results both samples exhibited high capability for reduction of Cu^{2+} ions (19.02-69.00 mg/g AAE d.w.) while ability for iron ions reduction was significantly lower (0.31-4.33 mg/g AAE d.w.). In addition TAC values for samples were in the range from 0.92 to 28.92 mg/g AAE d.w. In all cases, EP samples expressed significantly higher antioxidant activities compared to BP. Based on given results it can be concluded that bee-collected pollen can be used as an excellent source of antioxidants and functional food ingredient.

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VII_PP4_Microbiological quality control in fresh and dried apple fruit and testing of hydroxymethylfurfural (HMF) content

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Apple (*Malus* sp.) is a genus of woody plants from the Rosaceae family. Until now, more than 10,000 varieties have been developed in the world [1]. Apple fruit is a rich source of vitamin C, vitamin A, potassium, pectin, dietary fiber, as well as a significant amount of different classes of phenolic compounds. Apple fruits are used fresh throughout the year, but also for the production of juices, marmalades, jams, compotes, apple vinegar and dried fruits [2]. During the drying process, there is more or less degradation of colors and the creation of undesirable oxidative products, as well as the creation of colored polymers. Intermediate products such as furfural, hydroxymethylfurfural and decomposition products of various sugars are formed [3,4]. The aim of this scientific work is to examine water activity (a_w), pH, acid content (such as citric), hydroxymethylfurfural (HMF) content and to determine the total number of the Enterobacteriaceae in fresh and dried apple fruit. Water activity was measured with an awmeter (Pawkit). The pH value was measured with a pH meter (InoLab WTW, Germany). The acid content was determined by the volumetric method, by titration with NaOH. Hydroxymethylfurfural (HMF) content was determined spectrophotometrically (Jenway spectrophotometer) [5]. Determination of the total number of the Enterobacteriaceae was carried out according to the SRPS EN ISO 21528-2:2017 standard. All the values of the above mentioned parameters are within the range that can be considered acceptable for use in food, while an increased content of HMF was observed in the dried apple fruit.

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VII_PP5_ Analysis of anthocyanins content and microbiological quality control in fresh and frozen raspberry fruit

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Raspberry (*Rubus idaeus* L.) is a plant from the Rose family and the most important berry fruit in our country. More than 1,200 varieties of red, black and purple raspberries have been selected in the world, but only red varieties (Willamette, Meeker) are grown here [1, 2]. The raspberry fruit is tender and sensitive, beautifully colored, very tasty, with a specific aroma and a very rich chemical composition. The fruit is a source of carbohydrates, organic acids, vitamins, colored substances, enzymes and phytohormones [3,4]. The raspberry fruit is used fresh, but also as a raw material in the food industry for the production of juices, nectars, syrups, concentrates, dried and frozen fruits. The aim of this scientific work is the examination of pH, acid content (like citric), total anthocyanin content and microbiological quality control of fresh and frozen raspberry fruit. The pH value was measured with a pH meter (InoLab WTW, Germany). The content of acids was determined by the volumetric method, by titration with NaOH. Total anthocyanin content was determined spectrophotometrically (Jenway spectrophotometer) [5]. The total number of Enterobacteriaceae was determined according to the SRPS EN ISO 21528-2:2017 standard. The test results indicated that both fresh and frozen raspberry fruits are a good source of anthocyanins, which indicates that their consumption would be beneficial for health and that they could be useful in the production of functional food containing a high dose of anthocyanins. Microbiological methods performed in order to examine the total number of Enterobacteriaceae indicated the presence of Enterobacteriaceae less than 10 cfu/g in fresh and frozen raspberry fruit.

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VII_PP6_Oat varieties with different hull colours as potential sources of phytochemicals for the food and feed industry

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Whole grain cereals are considered essential components of nutrition because of their health-promoting properties. Oats (*Avena sativa* L.) are naturally gluten-free cereals suitable for persons suffering from celiac disease, abundant in dietary fibre, among which particularly β -glucans offer medical advantages like lowering cholesterol levels, reducing glucose uptake, and decreasing plasma insulin levels [1,2]. Nevertheless, oats are abundant in phenolic acids, flavonoids, carotenoids, vitamin E, and phytosterols [3,4]. This study aimed to investigate the antioxidant properties of the dehulled grain and hulls of three oat genotypes with different hull colours: yellow, brown and black. The contents of total phenolic compounds, phenolic acids, β -glucan, and antioxidant capacity of the oat grains and oat hulls were analysed. Significant differences were noticed among the tested samples, especially between parameter values determined in hulls, compared to those in grain. Oat hulls had higher contents of total phenolic compounds (11320.11-24352.48 μg GAE/g d.m.) compared to grain (841.89-982.08 μg GAE/g d.m.); as well as the detected phenolic acids, namely: *p*-coumaric, ferulic, isoferulic, vanillic and syringic acid. Ferulic acid was predominant, in both the grain (395.88-589.14 $\mu\text{g/g}$ d.m.) and hulls (4987.02-13794.82 $\mu\text{g/g}$ d.m.). The antioxidant capacity was higher in oat hulls, also, ranging from 42.31 mmol Trolox/kg d.m., in yellow hulls to 53.16 mmol Trolox/kg d.m. in brown hulls and from 22.61 mmol Trolox/kg d.m. in black grain to 25.06 mmol Trolox/kg d.m. in brown grain. Conversely, the β -glucan content determined in the grain samples was significantly higher, ranging from 4.07% to 5.33% in grain, and only 0.03-0.06% in hulls. The anthocyanins and proanthocyanidins were not detected, which indicates that the colour of the oat varieties did not originate from these pigmented bioactive compounds. The investigated oat genotypes exhibit notable potential for use by the food and feed industries as sources of phytochemicals with potential health-promoting benefits.

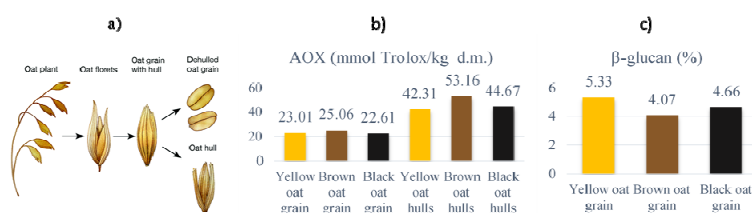


Figure 1. Hulled oat constituents a); antioxidant capacity b); β -glucan content

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VII_PP7_Small cereal grains as a source of phytochemicals

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Among health-promoting phytochemicals residing in whole grains of cereals, phenolic compounds have gained much attention in many scientific research areas, as they have strong antioxidant properties. Their concentrations in cereals are influenced by types, varieties and the part of the grain [1,2]. In this study, the content of total phenolics, flavonoids, proanthocyanidins and phenolic acids, as well as the content of yellow pigments and tocopherols, were determined in whole grains of bread and durum wheat, rye, hull-less barley and hull-less oat, each represented with four genotypes.

Considerable variation in antioxidant capacity and phytochemical contents was observed between the cereals. The total phenolic and flavonoid contents were the highest in hull-less barley, followed by hull-less oat, rye, durum wheat and bread wheat. Proanthocyanidins were detected only in hull-less barley, with high coefficient of variation among genotypes of 30.18%. HPLC analyses indicated the presence of ferulic acid in all the species, in most of samples it was the dominant one. Chlorogenic and caffeic acids were determined in extracts of many species, though in hull-less oat and durum wheat samples chlorogenic and caffeic acid, respectively, were not found. *p*-Coumaric acid was present only in rye and hull-less oat. Except phenolic acids, monomeric phenolic compounds catechin and epicatechin were detected in hull less barley. All cereal grains tested in this study contained yellow pigments with the following descending order: durum wheat > hull-less oat > bread wheat > rye and hull-less barley.

Relatively high variations among genotypes within species concerning total phenolic content, flavonoids, tocopherols and yellow pigments, provide a foundation for those interested in utilizing or improving small grain varieties for their health benefits. Of particular interest is to increase the content of tocopherols and yellow pigments, because bakery industries mainly prefer light than wholegrain flour.

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VII_PP8_Content of fatty acids and theobromine in organic cocoa powder

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Theobroma cacao L. (or cocoa) is a tropical crop of high importance, mainly due to the commercial value of its beans. The cocoa tree is native to South America but it was introduced to some tropical parts of Africa and Asia as well. Cocoa powder is obtained in a complex manufacturing process from dried cocoa beans. It is rich in proteins, carbohydrates, organic acids, fibers, polyphenols, theobromine and nutritional elements. Moreover cocoa is a good source of fatty acids (FAs), predominantly palmitic, stearic and oleic acid, which are considered as major lipid constituents [1,2]. In this regard cocoa is widely applied in food industry; for drinks, manufacturing chocolate, candy, pastry, ice cream and other products. Health benefits of cocoa are mainly attributed to the high content of antioxidants of *Theobroma cacao* beans. On the other hand, theobromine in cocoa is responsible for the liking of the food/beverage due to its psychoactive effect [3]. This work aimed to study fatty acids and theobromine in cocoa powder commercially available in markets in the Republic of Serbia. Fatty acids in the form of methyl esters (FAMES) were analyzed by gas chromatography with a flame ionization detector (GC/FID), whereas theobromine was determined by spectrophotometric method [4,5]. Content of FAs (expressed as percentage on the 100 g of cocoa powder) varied from 11.67-12.77%. Saturated FAs were found in higher % compared to unsaturated FAs, both mono and polyunsaturated FAs. Palmitic and stearic acids were dominant saturated FAs, whereas oleic and linoleic were the most abundant among unsaturated FAs. Content of theobromine was in from 2.26 to 2.67 %, which corroborated with literature data. Obtained results indicated that examined cocoa samples were a good source of palmitic, stearic and oleic acids, as well as theobromine.

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VII_PP9_The influence of the drying method on the preservation of bioactive compounds in the wild garlic (*Allium ursinum* L.) leaves as a functional ingredient incorporated in cookies

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Wild garlic (*Allium ursinum* L.) is a representative of self-sown edible plants, known as a rich source of bioactive components, such as sulfur compounds, which provide flavor, as well as bioactive properties, along with phenolics [1]. The increased interest in wild garlic is caused by studies confirming that antioxidant rich foods play an essential role in the prevention of cardiovascular and neurodegenerative diseases, cancer, as well as many other disorders caused by free radicals [2,3]. However, due to time-limited availability of this plant, the application of the appropriate drying technique and the latter incorporation into food products, i.e. cookies, offers the possibility of its prolonged use, and simultaneously enrichment of the final products. In present research, the content of total thiosulfinates, total phenols and flavonoids in cookies made with the addition of 10% of wild garlic leaves osmotically dehydrated in an aqueous solution of NaCl and sucrose, as well as in sugar beet molasses, was determined spectrophotometrically [4]. The samples dried by dehydration in molasses showed values of total thiosulfinates (0.48 ± 0.00 mg EAC/100 g dm) almost twice as high compared to the samples dried in a clear solution (0.28 ± 0.01 mg EAC/100 g dm). The same trend was observed with total phenols (1.68 ± 0.01 mg EGA/100 g dm for molasses; 1.02 ± 0.01 mg EGA/100 g dm for clear solution), as well as flavonoids (0.42 ± 0.01 mg EC/100 g dm for molasses; 0.25 ± 0.01 mg EC/100 g dm for clear solution), according to the literature the most dominant compounds of wild garlic. Therefore, the method of drying was reflected in the content of bioactive components of wild garlic leaves, and consequently in the cookies, whereby dehydration in sugar beet molasses proved to be the best way to preserve these beneficial components, thus increasing the functionality of final product widely used in everyday diet.

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VIII_PP1_ Two new alternative sources of betacyanin colouring compounds for food industry

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Natural resources have been used to recover natural agents with different functions, such as colourant capacity, which are considered healthier alternatives, safer, and associated with beneficial effects on health. This has occurred in the last decade as a result of consumer demand and awareness of some issues related to artificial food additives. A class of natural colouring compounds known as betalains can be employed in the food business as substitutes for their widely used artificial equivalents, which have lately been labelled as less beneficial substances. *Amaranthus caudatus* L. (Ac) [1] and red-fleshed pitaya *Hylocereus costaricensis* (F.A.C.Weber) Britton & Rose (Hc) [2] few examples of this by-products like ac flowers and hc peels should be utilised to recover a natural colourant component with strong colouring power and known bioactive qualities. The goal of this work was to obtain a betacyanin-rich extract that was then analysed using several HPLC methods to determine its composition in terms of betacyanins, tocopherols, and organic acids. Analyze whether there was any cytotoxicity or antibacterial activity. For the Ac extract it was possible to identify four betacyanin, amaranthine (171 ± 1 mg/g extract), isomaranthine (38 ± 1 mg/g) as the major, and betanin (1.6 ± 0.1 mg/g), isobetanin (1.3 ± 0.1 mg/g) as the minor compounds. Three of the four isoforms of tocopherols were detected, being β -tocopherol de major, for the organic acid, it was possible to detect the oxalic, shiquimic and traces of fumaric acid. For the Hc extract the major betacyanin detected was the 6'-O-malonylbetanin (phylocactin; 14.77 ± 0.26 mg/g of extract), the four isoforms of tocopherols were detected, and oxalic, malic and traces of fumaric acids were detected, being oxalic the major compound. Both of the extracts presented minimum growth inhibitory concentrations (MICs) ranging from 5-20 mg/mL, with no cytotoxicity. After such promising results, this plant can be a viable alternative to obtain natural colorant ingredients.

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VIII_PP2_ Interactive effect of potato peels on *in vitro* glycemc response and starch digestibility with potato

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Potato is a carbohydrate-rich crop that is consumed worldwide as a staple food. It can be consumed directly by boiling, frying and baking process. Moreover, it can be used as an ingredient for the preparation of different processed products. Potato peel is an important constituent that is generally discarded and considered an agricultural waste before processing or cooking [1-4]. The present study attempts to utilize potato peels (PP) in various food combinations that might affect the *in vitro* glycemc index (GI), resistant starch (RS), and related parameters in the processing cultivar (Kufri Chipsona 3) and table cultivar (Kufri Jyoti) respectively. The addition of PP at different concentrations of 5, 10 and 15 %, respectively to potato flesh showed a significant reduction ($P < 0.05$) in starch hydrolysis index (SHI), GI and glycemc load (GL). Similarly, there was also a significant increase in the RS content of potato and PP combination. The maximum change in the predicted glycemc response was observed in the PP with 15%. However, the addition of 10% of PP to potato flesh might be useful due to organoleptic issues. A higher reduction in the *in vitro* glycemc response was observed in the Kufri Chipsona 3 as compared to Kufri Pukhraj. The results of the study suggest that adding PP to potato (also starchy crops) might be an effective strategy for managing the post-prandial rise in blood glucose level.

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VIII_PP3_Chemical fingerprint of plum (*Prunus domestica* L.) kernels grown in Norway

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The production and consumption of fruit products are increasing and causes huge amount of by-products that create disposal and environmental problems. The plum kernels are by-products of industrial process which are not currently used in commercial purpose and are discarded as a waste. The objective of this study was to analyse the total oil content, fatty acids composition, tocopherols, carotenoids and sugars in kernels from 27 plum (*Prunus domestica* L.) grown in Norway. The composition of fatty acids was determined according to the standard method by capillary gas chromatography. Carotenoids and tocopherols analysis was done by the Agilent 1200 HPLC system. The determination of sugars was performed on Dionex ICS 3000 system. The oil content varied from 8.46% ('Herman') to 25.2% ('Opal'). Oleic acid (up to 75.74% in 'Mount Royal') and linoleic acid (up to 44.53% in 'Herman') were the most abundant followed by palmitic acid (up to 7.18% in 'Herman'). Out of carotene, plum kernels stored the highest levels of lutein and beta carotene. Lutein varied from 10.66 ('Edwards') to 19.53 mg/100g ('Avalon'), while beta carotene was from 11.87 ('Rivers early Prolific') to 22.55 mg/100g 'Reine Claude Souffriau'. In the investigated kernels of plum cultivars 'Reeves' had the highest level of δ - and β -tocopherol (9.12 and 29.56 mg/100g, respectively), while cultivar 'Washington' was distinguished by the α -tocopherol (72.98 mg/100g) and cultivar 'Valor' by the γ -tocopherol (18.36 mg/100g). The major sugars, glucose, sucrose and fructose followed by sorbitol, accounted up to almost 95% of all sugars quantified. The variation in analyzed content of the studied chemical compounds in Norwegian plum kernels helped us differentiate examined cultivars and proved that the kernel's composition was attributed to genetic factors.

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IX_PP1_Effect of ultrasound and chemical pretreatments on L-ascorbic acid of dried bell pepper (*Capsicum annuum*) studied by factorial design

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Drying is one of the methods for the preservation of fruits and vegetables [1]. Peppers are still dried in open sunlight, although this method has many shortcomings (long drying time, microbial contamination, depending on weather conditions, final product of poor color) [2]. Deterioration of numerous nutrients, which occurs during drying, can be prevented by applying various pre-treatments and/or different drying methods [2–4]. The aim of this study was to investigate effects of different pretreatments and drying methods on L-ascorbic acid (L-AA) content in dried bell pepper. Effect of following parameters were studied: applied additive (0.25% citric acid, 0.25% potassium metabisulfite or their mixture), temperature, time, pH value of pretreatment solution, ultrasound pretreatment, pepper slices size, pepper mass and drying method (hot air-drying and freeze-drying). For organization of experiments fractional factorial design was used. Results showed that only the drying method significantly affects the content of L-AA after a month of storage. Freeze-drying was a better method for L-AA preservation. The interaction between the drying method and the size of pepper slices was also meaningful, although this interaction wasn't statistically significant. Better preservation of L-AA, for hot air-dried samples, was achieved with a slices size of 4×4 cm compared to smaller (2×2 cm) and larger ones (8×8 cm). In the case of freeze-dried samples, the slice size didn't affect the preservation of L-AA. The medium slices of the pepper fruit provided better penetration of the additives, which resulted in a better protective effect on L-AA during the hot air-drying. On the other hand, smaller pieces (2×2 cm) led to higher losses, probably due to the higher leaching of L-AA. Larger slices (8×8 cm) proved to be the most unfavorable for the preservation of L-AA (maybe due to less diffusion of additive during pretreatment and its later weak effect during drying).

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IX_PP2_Determination of selected macro- and microelements in black chokeberries (*Aronia melanocarpa* L.)

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Black chokeberries (*Aronia melanocarpa* L.), belonging to the Rosaceae family, are considered to be functional food due to the presence and the high content of various bioactive components such as vitamins, minerals and polyphenols [1]. Aronia berries are a good dietary source of essential elements, in addition to their high content of beneficial nutrients for health and well-being [2]. There is little research on the mineral composition of the chokeberries, so the presented study is focused on the determination of twenty-four elements in black chokeberry fruits. Analysed elements were: aluminium (Al), arsenic (As), boron (B), barium (Ba), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), sulphur (S), selenium (Se), strontium (Sr), vanadium (V) and zinc (Zn), using the optical emission spectrometry with inductively coupled plasma (ICP-OES) technique.

The multi-elemental composition and substances performing a beneficial impact on health can vary depending on factors such as cultivar, fertilization, maturation or climate conditions, as well as the date of their harvest [3]. According to the obtained results, concentrations of major elements in the sample were: 281.94 (Ca), 1587.07 (K), 147.19 (Mg), 314.24 (P) and 92.18 (S), expressed as $\mu\text{g g}^{-1}$. Concentrations of the toxic elements As, Cd and Pb were below method quantification limits in the studied sample.

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IX_PP3_Analysis of diterpenoids from the latex of *E. seguieriana* Neck. subsp. *seguieriana* by liquid chromatography–electrospray ionisation mass spectrometry

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The genus *Euphorbia* (Euphorbiaceae) is composed of a latex-bearing species well known for the chemical diversity of their isoprenoid constituents. Over 650 diterpenoids, with diversified macrocyclic and polycyclic skeletons, including ent-abietanes, ent-atisanes, ent-kauranes, ent-pimaranes, ent-isopimaranes, casbanes, jatrophanes, lathyranes, jatrophanes, daphnanes, tiglianones, ingenanes, segetanes, paralianes, pepluanes, myrsinanes and euphoractines, specific to the Thymelaeaceae and Euphorbiaceae families, have been isolated from these plants. These compounds show the wide range of therapeutically relevant biological activities (e.g., antitumor, cytotoxic, multi-drug-resistance-reversing, and anti-viral properties, various vascular effects, and anti-inflammatory activity) [1].

E. seguieriana, a species with several varieties occurring from Europe to Pakistan and north-western China, is a perennial herbaceous flowering plant that reaches a height of up to 60 cm. Its latex is used to treat wounds and warts on the skin [2]. So far, ingenols [3], diterpene lactones of the abietane type, myrsinanes, a tetracarboxylic diterpene related to myrsinane [4], and diterpenes structurally related to 17-hydroxymyrsynol, cyclomyrsinol and lathyranone [5], have been isolated from *E. seguieriana* Neck. subsp. *seguieriana*.

In this study, we analysed the chloroform extract of the latex of *E. seguieriana* Neck. subsp. *seguieriana* collected on Deliblato sand in flowering season by reversed-phase liquid chromatography–electrospray ionisation mass spectrometry (LC-ESI MS). From the data obtained from LC-ESI MS analysis, premyrsinane, myrsinane and lathyranone nicotinoyl esters are the dominant diterpene metabolites of the latex. In the continuation of the research, compounds will be isolated, their structures elucidated, and their biological activities examined.

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IX_PP4_Insight into chemical composition of sweet cherry (*Prunus avium* L.) fruits - application of ATR-FTIR spectroscopy

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Sweet cherry (*Prunus avium* L.) is one of the most appreciated temperate fruit trees, whose fruits are among the first to ripen in the season. Fruits are used mainly for fresh consumption, and very little for processing. Sweet cherry fruits contain large amounts of soluble solids (10-22%) most of which are sugars. Glucose and fructose are dominant, while the amount of sucrose is significantly lower. The content of organic acids is relatively low (0.3-1.2%), and malic acid is dominant. Sweet cherries are also a rich source of bioactive compounds, such as polyphenols, vitamins, minerals, and dietary fibers. The most abundant classes of phenolic compounds are anthocyanins, phenolic acids and flavonols, which contribute to antioxidant potential of the fruits. Anthocyanins, which give the fruits their red color, are especially important. Cyanidin-3-rutinoside followed by cyanidin-3-glucoside is the most abundant anthocyanin in sweet cherry fruit. Moreover, neo-chlorogenic acid, chlorogenic acid, and *p*-coumaroylquinic acid are most represented phenolic acids. High content of bioactive compounds in sweet cherries are mainly responsible for many beneficial health effects: reduced inflammation and symptoms of arthritis, regulation of blood pressure, reduced risk of a stroke, weight loss, prevention of cancer, diabetes and Alzheimer's disease [1].

The aim of the present research was to examine chemical composition of nine cherry cultivars varieties: 'Vega', 'Carmen', 'Grace Star', 'Samba', 'Black Star', 'Olympus', 'Benton', 'Sela', and 'Staccato', using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy. The sweet cherry samples were weighed, and then dissolved in 50:50 ethanol/water (v/v) solutions. Ultrasound-assisted extraction was used to extract the antioxidant compounds. After extraction, the samples were filtered and evaporation was carried out on a vacuum on the 40°C [2]. The cherry extract was recorded in absorbance mode, using a Nicolet™ iS™ 10 ATR-FTIR Spectrometer (Thermo Fisher Scientific). The ATR-FTIR spectra of analyzed samples were recorded in the range 500-4000 cm⁻¹. The obtained absorption maxima indicated presence of esters, monosaccharides, polysaccharides, flavonoids and anthocyanin pigments in studied cherry cultivars varieties. Intensity of specific bands differed between cherry cultivars.

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IX_PP5_Insight into chemical composition of wild growing fruits from Serbia - application of ATR-FTIR spectroscopy

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Medlar (*Mespilus germanica* L.), blackthorn (*Prunus spinosa* L.) and hawthorn (*Crataegus mopsogyna* Jacq. L.) are fruits belonging to the Rosaceae family. Medlar originates from the southeastern part of Europe, Turkey, Iran, and Iraq. It is well known among folks in this part of the world but, due to limited growing area, it is not recognized among the wide scientific community although it has been proven as a good source of phenolic compounds (chlorogenic acid, rutin, *p*-coumaric acid, quercetin, vanillin, protocatechuic acid, gallic acid, caffeic acid, etc.), sugars (glucose, sucrose, fructose) different organic acids (malic, tartaric, oxalic, citric, etc.) and vitamins, especially vitamin C [1,2]. Among fatty acids saturated ones such as hexanoic and hexadecanoic acids were predominant as well as stearic, oleic, linoleic, and behenic acids [3]. Blackthorn is one of the important wild plants with powerful healthpromoting properties. Its chemical composition is characterized by the presence of phenolic compounds which act as antioxidants, specifically flavonol heterosides (quercetin and kaempferol), phenolic acids (especially 3-*O*-caffeoylquinic acid), and coumarin derivatives, anthocyanins (particularly cyanidin-3-*O*-rutonoside and peonidin-3-*O*-rutonoside) which are responsible for its dark blue color [4]. Hawthorn is an endemic member of the *Rosaceae* family which ripens in mid-autumn, is used for different culinary purposes, such as the preparation of jellies, jams, and syrups [5]. The chemical composition of hawthorns is characterized with the predominance of the following compounds: kaempferol and quercitrin (flavonol), apigenin (flavone) and ursolic acid (phenolic acid). Additionally, flavan-3-ol monomers, dimers, trimers, and tetramers were determined too. In addition, both unripe and ripened fruits contained significant quantities of tocopherols, β -carotene as well as vitamin C [5]. Chemical compositions of medlar, blackthorn and hawthorn fruit extracts were analyzed using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy. The spectra were recorded in the the range 500-4000 cm^{-1} , using Nicolet™ iS™ 10 ATR-FTIR Spectrometer (Thermo Fisher Scientific). The obtained absorption maxima indicated presence of monosaccharides, polysaccharides and polyphenols in studied medlar, blackthorn and hawthorn extracts.

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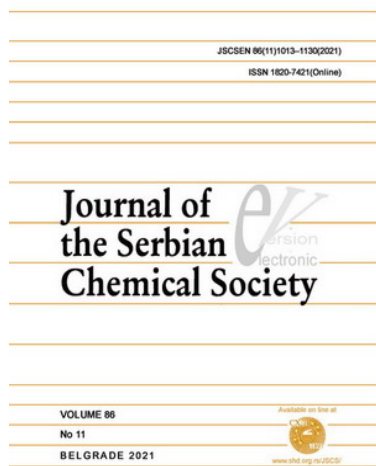
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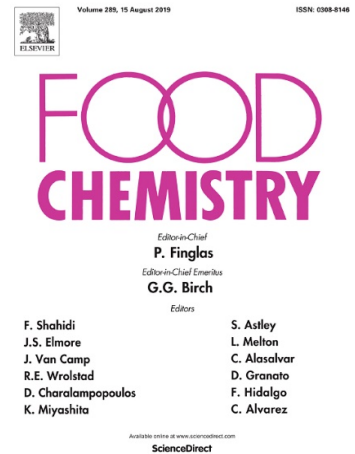
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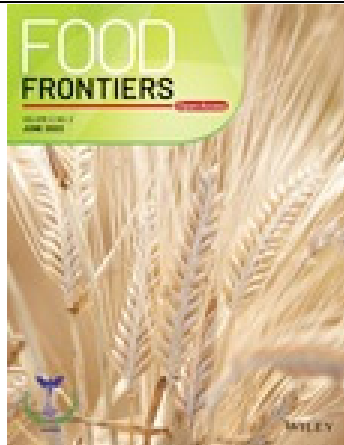
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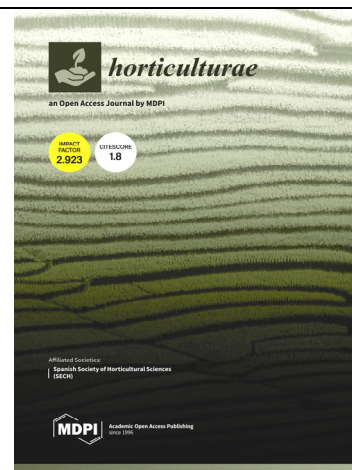
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