Serbian Biochemical Society Thirteenth Conference

"Amplifying Biochemistry Concepts"

Proceedings

Serbian Biochemical Society

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Serbian Biochemical Society
Thirteenth Conference

International Scientific Meeting

September 19-20, 2024, Kragujevac, Serbia

“Amplifying Biochemistry Concepts”

FOREWORD

Dear Colleagues,

Welcome to the 13th Conference of the Serbian Biochemical Society, entitled “Amplifying Biochemistry Concepts”.

I would like to express my gratitude to all the participants who chose to present their valuable work at the conference and share their results with auditorium.

On behalf of the Organizing Committee, I would like to dedicate this year conference to our colleague and friend, and above all a great biochemist and scientist, proffessor Mihajlo Spasić.

On behalf of the Organizing Committee
Editor of the Proceedings
Dragana Robajac

IN MEMORIAM: MIHAJLO SPASIĆ (1952–2024)

Professor Mihajlo Spasić, or Spasa how his pupils and friends have called him, left us suddenly on August the 21st 2024. He was truly and widely loved and respected by his colleagues and students because of his kindness and willingness to advise and help, fundamental understanding of biochemistry and capacity to explain complex processes (which comes only with deep knowledge of the subject), and his wit and vivid spirit. Not many professors at the University of Belgrade have been rated by their students with an average mark of 10.

Professor Spasić spent his research professional career (1978-2019) at the Institute for Biological Research "Siniša Stanković" and Faculty of Chemistry, University of Belgrade. Spasa's favorite field was metabolism of free radicals, which he pioneered in Serbia. After obtaining PhD in Chemistry at former Faculty of Science Belgrade (in 1986), he passed his postdoctoral training (1987-1988) at the Department of Physical Biochemistry, Institute de Biologie Physico-Chimique (Paris, France), set up by Prof A. M. Michelson. This visit was the key event and the beginning of his long-term journey of free radical research in Serbia. Initial collaborative work with a few of his colleagues blossomed into the network of nearly a hundred researchers examining redox processes in biochemistry, physiology, molecular biology, food science, and different fields of medicine. Spasa was the key figure behind the development of this network. His philosophy was to help the experts in all life science fields to recognize, appreciate, and tackle the prooxidative/redox component of their scientific problems. His reference list consists of about 200 papers, cited more than 2400 times, and most support the concept of free radical adaptive homeostasis, the creation of which they significantly contributed.

Spasa finalized the transformation of Yugoslav Biochemical Society into Serbian Biochemical Society (SBS), reinforced SBS presence within the FEBS, and acted as the first president of SBS. He shaped SBS in what it is now, a viable regional hub for biochemists and alike with regular annual conference that promotes young leaders and students and an active participation in FEBS activities.

Our dear Professor was a multifaceted researcher and a highly respected figure in the international biochemical community. He was also a very warm person, a friend with a lot of life experience and stories from which he tried to extract laws that go beyond biochemistry and apply to a bit more complex systems such as general or Serbian society. He came from a well-off family and spent his childhood in the Aleksandar Stamboliski Street, which lays in the elite part of Belgrade. However, his neighbors and friends came from all societal classes, a common setup in the post-war Yugoslavia. Probably this made him both a proud and a relaxed persona, and it was the basis of his sense of humor and irony. We laughed and learned a lot with Spasa. He will be truly missed.

Ivan Spasojević and Duško Blagojević

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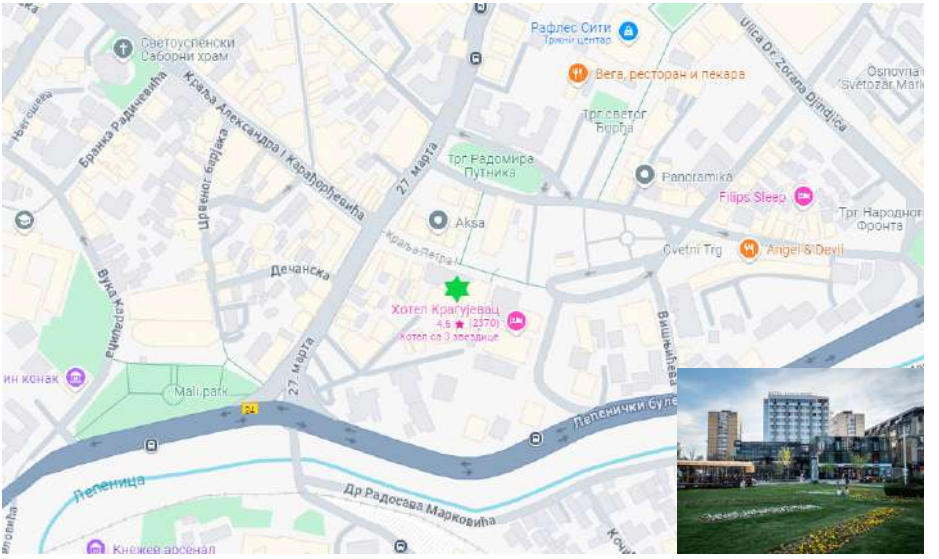
“Amplifying Biochemistry Concepts”

Map of events

The conference venue is located at the “Hotel Kragujevac 4*”.



As we gather to explore the frontiers of biochemistry and celebrate the spirit of discovery, let us also take a moment to appreciate the beauty and allure of Kragujevac. Here’s to an enlightening conference filled with insights, collaboration, and unforgettable experiences in this remarkable city. Welcome to Kragujevac – where science meets serenity, and knowledge knows no bounds! We are committed to ensure you an unforgettable experience.

The Organizing Committee

PROGRAMME

Day 1 – Thursday, September 19th, 2024

08:30 – 10:00 **Participants registration and posters mounting**

10:00 – 10:15 Opening ceremony

10:15 – 11:00 PL1 **Béla Gyurcsik**
University of Szeged, Department of Molecular and Analytical Chemistry, Szeged, Hungary
Environmental effects on enzyme efficiency involved in bacterial defence systems
Plenary/FEBS3+ Lecture

11:00 – 11:30 IL1 **Jelica Milošević**
University of Belgrade - Faculty of Chemistry, Belgrade, Serbia
Peptide inhibitors of amyloid aggregation
Invited lecture

11:30 – 12:15 ***Coffee Break***

12:15 – 12:45 IL2 **Ana Obradović**
University of Kragujevac Faculty of Science, Department of Biolog and Ecology, Kragujevac, Serbia
Hydantoin derivatives: Harnessing antitumor potential and immunomodulation
Invited lecture

12:45 – 13:15 IL3 **Suzana Stanisavljević**
Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia
Transcription factor NRF2 as a key modulator of immune response
Invited lecture

13:15 – 13:30 OP1 **Maja Krstić Ristivojević**
University of Belgrade - Faculty of Chemistry, Department of Biochemistry, Centre of Excellence for Molecular Food Sciences, Belgrade, Serbia
Interaction of beef meat extract proteins and microplastics in simulated gastrointestinal conditions
Oral presentation

13:30 – 15:00 ***Poster Session 1 and 2 & Lunch break***

15:00 – 15:30 IL4 **Nikola Stojanović**
University of Niš, Faculty of Medicine, Niš, Serbia
Neuroinflammation in schizophrenia and other psychotic disorders
Invited lecture

15:30 – 15:45 OP2 **Milica Markelić**
University of Belgrade - Faculty of Biology, Belgrade, Serbia
Protective effects of H_2S donors against injury of endocrine pancreas in diabetic mice include antiferroptotic action
Oral presentation

15:45 – 16:00 OP3 **Olgica Stefanović**
University of Kragujevac Faculty of Science, Department of Biolog and Ecology, Kragujevac, Serbia
Plant-derived metabolites affect different stages of biofilm formation of pathogenic bacteria
Oral presentation

16:00 – 16:15 OP4 **Nemanja Živanović**
University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia
Rose oil distillation wastewater as a new source of pharmacologically active compounds
Oral presentation

19:00 – 24:00 ***Conference dinner***

Day 2 – Friday, September 20th, 2024

08:30 – 10:00 **Participants registration and posters mounting**

10:00 – 10:45 PL2 **Manfred Jung**
University of Freiburg, Faculty of Chemistry and Pharmacy, Institute of Pharmaceutical Sciences, Freiburg, Germany
Chemical epigenetics - modulators of reversible lysine acetylation and methylation
Plenary Lecture

10:45 – 11:15 IL5 **Jelena Munjas**
University of Belgrade, Faculty of Pharmacy, Department of Medical Biochemistry, Belgrade, Serbia
Gene expression of lipid transporters in peripheral blood mononuclear cells in pregnant women: A longitudinal study
Invited lecture

11:15 – 12:00 ***Coffee Break***

12:00 – 12:30 IL6 **Dragana Robajac**
University of Belgrade - Institute for the Application of Nuclear Energy, Belgrade, Serbia
Glycans in health and disease
Invited lecture

12:30 – 13:00 IL7 **Marija Lesjak**
University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia
The influence of plant-based food on the bioavailability of iron
Invited lecture

13:00 – 13:15 OP5 **Edhem Hasković**
University of Sarajevo, Faculty of Sciences, Department of Biology, Sarajevo, Bosnia and Herzegovina
Effect of trioxohydroxytetrafluorotriborate on haematological parameters *in vivo*
Oral presentation

13:15 – 14:45 ***Poster Session 3 and 4 & Lunch break***

14:45 – 15:15 IL8 **Marko Stojanović**
University of Belgrade, Faculty of Medicine, Department of Pharmacology, Belgrade, Serbia
Unlocking the potential: Harnessing biomarkers commonly used in clinical practice to predict complications and outcomes in febrile neutropenia
Invited lecture

15:15 – 15:30 OP6 **Darko Mihaljica**
University of Belgrade, Institute for Medical Research, National Institute of Republic of Serbia, Belgrade, Serbia
Preliminary characterization of putative tick cement protein PA107 – implications for possible applications in biomedicine
Oral presentation

15:30 – 15:45 OP7 **Nenad M. Zlatić**
University of Kragujevac, Faculty of Science, Department of Biolog and Ecology, Kragujevac, Serbia
Secondary metabolites and biological activity of *Teucrium montanum* L. (Lamiaceae)
Oral presentation

16:00 – 16:15 ***Poster and oral presentation awards and closing ceremony***

Posters

(abstracts are enumerated for referencing purposes)

P101 Aleksandar Ivanov
The potential of R-phycocyanin as an albumin substitute for cell growth in meat cultivation

P102 Ana Budimirović
Optimization of spectrofluorimetric assay for screening of opine dehydrogenase mutant libraries

P103 Ana Stanojević
Isolation of Ara h 1 from raw and roasted peanuts, evaluation of immunoreactivity, and monitoring allergen binding to THP-1 macrophages

P104 Anđelo Beletić
The agreement between spectrophotometric and ELISA assay for haptoglobin concentration in cow milk

P105 Anja Stošić
Effect of crosslinking cations on the activity of *T. versicolor* laccase immobilized within alginate hydrogel beads

P106 Anka Todosijević
Antimicrobial activity of 2-(2-ferrocenyl-4-oxothiazolidin-3-yl)acetic acid and its ethyl derivative

P107 Azra Guzonjić
Evaluation of GSH/GSSG and CyS/CySS as oxidative stress markers in small cell lung cancer patients undergoing chemotherapy in Serbia

P108 Budimir S. Ilić
Crystallography-driven drug design: Unlocking potent VMAT2 inhibitors for Huntington's disease

P109 Daria Maria Monti
Assessment of caspase modulation in human epidermoid carcinoma cells (A431) treated with silver nanoparticles synthesized using plant extract

P110 David Fekete
High-affinity binding of concanavalin A to B-phycoerythrin

P111 David Pirić
Global research trends in diffuse large B-cell lymphoma

P112 Faiza Zafar
Peanut allergens affected intestinal barrier function in human intestinal epithelial cell lines Caco-2 and T84

P113 Gligorije Gligorić
A novel approach to extracellular vesicles staining facilitates their tracking during cellular uptake

P114 Iva Uzelac
Effect of prolonged heat stress on the activity of four important metabolic enzymes during diapause of the European corn borer *Ostrinia nubilalis* (Hbn.)

P115 Ivana Beara
Bermet wine polyphenolics as modulators of lipase activity *in vitro*

P216 Ivana Ivelja
Hepatotoxic effects of perfluorooctanoic acid on female Swiss mice

Poster Session 2

P201 Jelena Purać
Molecular insights into aging in honey bees: RNA-Seq analysis of winter worker bees across different age groups

P202 Jelena Radović
Investigation of the effect of algal chromopeptides on the activity of the SARS-CoV-2 main protease Mpro

P203 Jelena Spremo
Evaluation of vitellogenin content in worker honey bees (*Apis mellifera* L.) during ageing

P204 Jovana Jagodić
Exploring trace elements in adrenal tissues: New insights into Cushing's syndrome

P205 Jovana Matić
Interactions of 2-thiohydantoin derivatives with DNA and human serum albumin as a part of their cytotoxic activity against human breast adenocarcinoma

P206 Jovana Stevanović
Diagnostic significance of miR-146a, miR-21 and miR-155 from PBMCs in gestational diabetes

P217 Ljiljana Milovanović
Bioactivity profiling of wine from Vojvodina: Antioxidant potential of Bermet wine

P208 Marija Marin
Potential biological activity of the aqueous extract of *Fumana bonapartei* Maire et Petitm. from the serpentine substrate of the Rogozna mountain

P209 Marija Novaković
Antioxidative potential of *Quercus pericarp*

P210 Marija Opačić
Long-term low-level exposure to perfluorooctanoic acid affects the survival of human endothelial cells in vitro

P211 Marija Paunović
Impact of aromatase inhibitor therapy on plasma phospholipid profiles in women with breast cancer

P212 Marija Stanišić
Immobilization of chemically modified glucose-oxidase by biomineralization in ZIF-8

P213 Marija Stojadinović
Assessing the FBS-substituting potential of algal and cyanobacterial extracts for cell-based meat cultivation

P214 Marija Tanović
Application of ^{31}P NMR spectroscopy in the study of the interaction of Mn(II) with polyphosphate compunds in the green microalgae *Chlorella sorokiniana*

P215 Marijana Janić
Comparison of chemical composition and antioxidant activity of *Salvia officinalis* L. ethanolic leaves extracts

Poster Session 3

P301 Marijana Kovačić
The expression of terminal monosaccharides on immune complexes IgG glycans in rheumatoid arthritis

P302 Marijana Stojanović
PARP inhibitors increase NSCLC sensitivity to cisplatin via modulation of TET activity

P303 Marina Crnković
Changes in CAT and PPX activity induced by sodium deoxycholate in sunflower seedlings infected with *P. brasiliense* and *S. sclerotiorum*

P304 Maša Marković
Phenethyl ester of rosmarinic acid ameliorates acute respiratory distress syndrome

P305 Milena Milutinović
Potential of isorhamnetin to inhibit expression of membrane transporters associated with cancer cell resistance development

P306 Milica Crnoglavac Popović
***In situ* biomimetic mineralization of Laccase@ZIF**

P307 Milica Spasojević Savković
"Green" chemistry approach for preparation of hydrogels used for controlled drug delivery

P308 Miloš Filipović
Gaining insight into disordered and aggregated protein structure via ATR-FTIR spectroscopy

P309 Miloš Matić
Antitumor potential of ethanol *Lycium ruthenicum* extract on various human cancer cell lines

P310 Miloš Šunderić
Interaction of commonly used antioxidants with major circulating proteins: spectroscopic and *in silico* study

P311 Mina Janković
Our favorite spices: Is turmeric always only turmeric?

P312 Minja Derikonjić
Examination of biochemical indicators of cardiometabolic risk in patients with hypothyroidism

P313 Nevena Tomašević
Computational approaches to rational design isocoumarins as warheads for PROTACs against HDAC4 to treat SMA

P314 Nikola Radenković
Isorhamnetin activates apoptotic signaling pathways in colon cancer cells

P315 Olgica Stefanović
Macroalga *Cladophora glomerata* as a potential source of bioactive compounds

P316 Petar Jovanović
Effect of ferulic acid, hyperoside, and rutin on reactivity of human serum albumin Cys-34 thiol group

Poster Session 4

P401 Samo Kreft
Exploring the potential of *Calendula officinalis* L. extract to improve benzyl alcohol's effectiveness in cosmetic products

P402 Sanja Erceg
Adiponectin's potential as a predictor of non-alcoholic fatty liver diseases

P403 Sanja Krstić
Inhibitory effects of *Salvia verticillata* L. extracts on COX-1 and COX-2 enzymes: The influence of extraction methods

P404 Sara Milojević
Cytotoxic, proapoptotic, and antiferroptotic effects of *Amanita muscaria* on choriocarcinoma cells

P405 Sara Protić
Biomineralization of two glucose oxidase mutants in ZIF-8

P406 Srđan Sokanović
Effects of long-term caloric restriction on lipid profile status and testosterone production in aged Wistar rats

P407 Stefan Blagojević
Dysregulation in expression of miRNA machinery genes *DICER1* and *AGO2* in endometrial adenocarcinoma

P408 Stefanela Kvrgić
Effect of calcium nanofertilizer on chemical composition and antioxidant activity of strawberry fruits

P409 Strahinja Pešić
Antioxidant activity of hexane and methanol acorn extracts of four *Quercus* species

P410 Tamara Lujić
Impact of chymotrypsin on physico-chemical properties of PET MNPs

P411 Tamara Todorović
Synthesis, characterization, and antitumor activity of alizarine derivatives

P412 Teodora Knežić
Insect larvae as alternative protein sources – expression of storage protein genes in non-diapausing larvae of the European corn borer *Ostrinia nubilalis* (Hbn.)

P413 Tijana Mićović
Antimicrobial activity of *Hyssopus officinalis* L. essential oils from Montenegro and Serbia

P414 Vanja Tatić
Tissue-specific expression of peptidoglycan recognition peptides in a non-model insect species

P415 Višnja Kosić
Characterization of free and immobilized pullulanase on modified bentonite: Sustainable enzyme immobilization

P416 Yaraslau Dzichenka
Identification of novel ligands of human cytochromes P450 among steroidal 1,2,4,5-tetraoxanes

FEBS3+ lecture

Environmental effects on enzyme efficiency involved in bacterial defence systems

Béla Gyurcsik[1*]

[1]*Department of Molecular and Analytical Chemistry, University of Szeged, Szeged, Hungary*

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Two types of bacterial defence systems are discussed. **(1) TEM-1 β-lactamase**, protecting bacteria from β-lactam antibiotics, is not a metalloenzyme but it offers potential sites for metal ion binding. Recently, we have studied the effect of Ni(II), Cd(II), and Hg(II) on the structure and catalytic activity of the enzyme.[1,2] Interaction of TEM-1 β-lactamase surface histidines with Ni(II) was proven by immobilized Ni(II) ion affinity chromatography and mass spectrometry. The sulphur-containing methionine residues may coordinate soft metal ions. However, only a slight change was observed in the circular dichroism spectra upon interaction with metal ions. Mass spectrometry and ^{119m}Hg perturbed angular correlation spectroscopy revealed weak binding of Hg(II). During the catalysed hydrolytic process of ampicillin, slow conversion of the primary reaction product to a secondary product was detected. Ni(II) and Cd(II) promoted the catalytic activity of the enzyme, while Hg(II) had an inhibitory effect. Hg(II) and Ni(II) inhibited the growth of *E. coli*, but addition of ampicillin could neutralize this toxic effect by complexing the metal ions. **(2) NColE7** is the nuclease domain of the colicin E7 bacterial toxin. It is a metalloenzyme purified together with Zn(II). Surprisingly, the apo form is more active than the Zn(II)-bound form. Addition 1 eq of Cu(II) ions to the apo-enzyme has similar effect, while 1 eq of Cd(II) ions did not decrease the catalytic to the same extent due to the lower affinity towards the catalytic site than Zn(II) supported by mass spectrometry. The enzyme is extremely active with 1 eq of Ni(II) ion, and could not be significantly inhibited by the excess of the Ni(II) ions.[3] Our findings suggest that the non-native metal ions may modify the catalytic mechanism, providing a chance to design more efficient antibiotic compounds.

References

1. Nafaee ZH, Hunyadi-Gulyás É, Gyurcsik B. Temoneira-1 β-lactamase is not a metalloenzyme, but its native metal ion binding sites allow for purification by immobilized metal ion affinity chromatography. Protein Express Purif 2023;201:106169.
2. Nafaee ZH et al. Revisiting the hydrolysis of ampicillin catalyzed by Temoneira-1 β-lactamase, and the effect of Ni(II), Cd(II) and Hg(II). Protein Sci 2023;32:e4809.
3. Nafaee ZH, Hajdu B, Hunyadi-Gulyás É, Gyurcsik B. Hydrolytic mechanism of a metalloenzyme is modified by the nature of the coordinated metal ion. Molecules 2023;28:5511.

Plenary lecture

Chemical epigenetics – modulators of reversible lysine acetylation and methylation

Manfred Jung[1*]

[1]*Institute of Pharmaceutical Sciences, University of Freiburg, Freiburg, Germany*

[*]*e-mail: manfred.jung@pharmazie.uni-freiburg.de*

Our research focus is `Chemical Epigenetics´ and deals with the development and application of chemical and biochemical tools to dissect biological pathways, to validate therapeutic targets and to discover and optimize potential drugs addressing a wide range of epigenetic targets. This includes bifunctional Proteolysis targeting chimeras (PROTACs)[1] for chemically induces protein degradation as well as target engagement assays like NanoBRET.[2]

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (DFG, CRC992 Medical Epigenetics).

References

1. Schiedel M et al. Chemically induced degradation of sirtuin 2 (Sirt2) by a proteolysis targeting chimera (PROTAC) based on sirtuin rearranging ligands (SirReals). J Med Chem 2018;61:482-491.
2. Vogelmann A, et al. Development of a NanoBRET assay to validate inhibitors of Sirt2-mediated lysine deacetylation and defatty-acylation that block prostate cancer cell migration. RSC Chem Biol 2022;3:468-485.

Invited lectures

Peptide inhibitors of amyloid aggregation

Jelica Milošević[1]

[1]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia*

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Amyloid aggregation is involved in the pathogenesis of various human diseases, primarily neurodegenerative, but also metabolic and other systemic diseases. Current therapies are mostly symptomatic treatments that do not address the underlying cause of the condition. To develop effective therapeutics, it is crucial to understand the mechanism of fibril formation. The amyloid fibrillation cascade is complex and involves three processes: primary nucleation, secondary nucleation, and elongation (with fragmentation). This process is characterized by a repertoire of intermediate states, which are potential targets for therapeutics. Special emphasis is placed on oligomeric and fibrillar forms, as they cause the most harm within the organism due to their toxicity. Studying the mechanism of amyloid formation common to various disease-related intrinsically disordered proteins is thus fundamental for therapeutic development and identifying novel diagnostic markers among these intermediate states. Consequently, biochemical studies of amyloid aggregation, particularly those monitoring kinetics and structure of aggregating species, are extensively conducted. Some of the most promising therapeutics for Alzheimer's disease and similar amyloid-related conditions appear to be peptide-based, either antibodies designed to target specific states in the aggregation process, chaperones with broad substrate specificity and specific thermodynamic properties, or small peptides that facilitate drug delivery. In addition to novel therapeutic strategies, early diagnosis is crucial for addressing amyloid diseases. Therefore, many studies aim to find fast screening methods for potential therapeutics and to specifically detect intermediate species that could revolutionize chemical diagnostics, surpassing current imaging techniques.

Acknowledgements

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Hydantoin derivatives: Harnessing antitumor potential and immunomodulation

Ana Obradović[1*], Miloš Matić[1], Milica Paunović[1], Branka Ognjanović[1], Bojan Božić[2], Biljana Božić Nedeljković[2]

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[2]Institute of Physiology and Biochemistry "Ivan Đaja", Faculty of Biology, University of Belgrade, Belgrade, Serbia

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Hydantoin and its newly synthesized derivatives are the focus of research due to their numerous biological activities and emerging beneficial effects in various pathological conditions, including cancer. Their primary clinical use is in the treatment of epilepsy and cardiac arrhythmias, but hydantoin derivatives have also shown significant anti-inflammatory and antitumor potential. One of the most prominent antitumor properties is its high antiproliferative potential in various cancer cell lines. Through various studies over the past decades, different series of hydantoin derivatives have shown antiproliferative activity with varying degrees of apoptosis in cancer cells. Different series of derivatives significantly decreased cell survival and caused a reduction in oxidative stress parameters in treated cells, indicating their significant antioxidant effect. The cell migration index was significantly decreased after treatment with the different hydantoin derivatives, suggesting their inhibitory role in various processes of cancer cell motility and invasion, which are crucial for tumor invasion. An important feature of tumor progression is chronic inflammatory response propagated by immune cells that activates pro-inflammatory genes and cytokines. A certain hydantoin derivatives reduce the expression of pro-inflammatory genes and pro-inflammatory cytokines related to carcinogenesis and tumor growth, suggesting a significant anti-inflammatory effect of some hydantoin compounds, which could be an additional mechanism of their antitumor effect. The results of various studies show that some of the investigated compounds from different series of synthesis have the potential to be used as new chemotherapeutic agents against the growth and progression of cancer in different tumor types.

Acknowledgements

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Transcription factor NRF2 as a key modulator of immune response

Suzana Stanisavljević[1*]

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Nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor that plays a crucial role in immune regulation by promoting antioxidant defenses, modulating inflammation, supporting immune cell function, and influencing autoimmune responses. Dysregulation of NRF2 signaling is implicated in the pathogenesis of autoimmune diseases and chronic inflammatory conditions. NRF2 activators, through their anti-inflammatory and antioxidant properties, have shown potential therapeutic benefits in preclinical and clinical studies for diseases characterized by dysregulated inflammation and oxidative stress such as multiple sclerosis (MS). MS is an autoimmune, neurodegenerative, and inflammatory disease of the central nervous system (CNS). In our model of MS, experimental autoimmune encephalomyelitis (EAE), we have investigated the role of NRF2 activators on several immune cell types important for the pathology of CNS autoimmunity. Ethyl pyruvate (EP), a redox analog of MS drug dimethyl fumarate, has been proven to have beneficial effects in EAE, by suppressing encephalitogenic T cells and macrophages and inducing tolerogenic properties in dendritic cells (DCs). In a recent study, we have shown that EP achieves its effects in DCs by increasing the expression of several NRF2 downstream genes and by decreasing the expression of NF-kB genes, suggesting that the tolerogenic effect of EP is achieved through the activation of NRF2 signaling pathway. Another tested substance, named SB140, is a derivative of cholic bile acid. This compound was tested in immune cells implicated in the pathogenesis of EAE: microglia and encephalitogenic T cells, and it was shown to have anti-inflammatory effects. These results demonstrate that both agents have potent immunomodulatory effects and suggest that they act as NRF2 activators. Both EP and SB140 are indicated as promising therapeutics in MS, but also in other diseases mediated by inflammation and oxidative stress, and thus, their role should be further investigated.

Acknowledgements

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Neuroinflammation in schizophrenia and other psychotic disorders

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Following the concept that inflammation of the central nervous system is in the foundation of some mental health disorders this review aimed to give a better insight into inflammatory events associated with schizophrenia and psychosis in general. Cascade of events and the change in secreted molecules by microglia occurring during neuroinflammation might predispose or provoke psychotic episode. Such molecules include cytokines, chemokines, products of arachidonic acid metabolism, reactive oxygen species (ROS) and bacterial products or proteins generated by host to fight microorganisms. In this review the role of the major known inflammatory molecules in the disruption of tissue function will be discussed. Among the cytokines frequently examined in Schizophrenia such as IL-6, a trait marker, and IFN-γ, a state marker, together with some smaller non-protein molecules were overviewed. Among the discussed markers it appears that there is no strong evidence supporting a single marker, or a panel of markers for that matter, that would have direct implications for the prevention, diagnosis and treatment. However, the collected data might point to some pathophysiological events associated with Schizophrenia and psychosis in general.

Acknowledgements

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Gene expression of lipid transporters in peripheral blood mononuclear cells of pregnant women: A longitudinal study

Jelena Munjas[1*], Marko Stanković[2], Minja Derikonjić[1], Marija Sarić Matutinović[1], Sandra Vladimirov Sopić[1], Tamara Antonić[1], Aleksandra Stefanović[1], Jelena Vekić[1], Daniela Ardalić[2], Milica Miljković Trailović[1], Tamara Gojković[1], Jasmina Ivanišević[1], Snežana Jovičić[1], Željko Miković[2,3] and Aleksandra Zeljković[1]

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PLTP, SRB1, ABCA1 and ABCG1 transporters are localized on the placenta where they mediate the uptake of maternal HDL-cholesterol and its transfer to the fetal circulation[1]. Herein we investigated longitudinal changes the *PLTP*, *SRB1*, *ABCA1* and *ABCG1* expression in maternal peripheral blood mononuclear cells (PBMC) during heathy pregnancy and their relation to the maternal smoking status before pregnancy. *PLTP*, *SRB1*, *ABCA1* and *ABCG1* expression was quantified using qPCR. In T2, *PLTP* expression was upregulated compared to the T1, while in T3 it was downregulated compared to T1 and T2. *SRB1*, *ABCA1* and *ABCG1* expression did not change significantly. A significant negative correlation was observed between the triglyceride concentration and the *ABCA1* and *ABCG1* in the T1 and T2. ABCA1 mRNA corelated negatively with total cholesterol during whole pregnancy, and in T2 it correlated negatively with LDL-cholesterol. In T2, PLTP mRNA correlated negatively with HDL-cholesterol. In women who had smoked before pregnancy *SRB1* expression was significantly upregulated in T1 and T2, whereas *ABCA1* and *ABCG1* expression was significantly upregulated during T1. *PLTP* expression changes through healthy pregnancy, which is in line with changes in maternal lipid status. Maternal smoking status before pregnancy significantly affected *ABCA1*, *ABCG1* and *SRB1* expression, possibly affecting cholesterol homeostasis.

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References

1. Wild R, Feingold KR. Effect of pregnancy on lipid metabolism and lipoprotein levels. In: Feingold KR, Anawalt B, Blackman MR, et al., eds. Endotext. South Dartmouth (MA): MDText.com, Inc.;2023.

Glycans in health and disease

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More than a century passed from the moment of first discovery of nucleic acids until the finalization of the process of human genome docoding. Deciphering the human glycome, consisting of far less complex carbohydrates, will take more due to an initial misinterpretation of their potential function in living systems. It took decades for them to metamorphose from an ordinary and uninteresting protein/cell jewlary into an inevitable component of cell function, that reflects it's (and hence the organisms') environmental conditions and surroundings. Their versatility, complexity and different supplementing techniques needed to employ for an adequate and complete investigation, as well as understanding the context of the cell/organism in which they are found will help us understand normal/healthy biological processes. The obtained knowledge is slowly being translated into understanding of pathological processes, and finally, into broadening the field of glycotherapy, giving new or additional tools for treating or reverting cellular processes. Much more is expected in years to come.

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The influence of plant-based food on bioavailability of iron

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Maintaining proper iron levels is crucial for metabolic functions. Inadequate iron intake depletes reserves, leading to iron deficiency anemia (IDA). The World Health Organization aims to reduce IDA in women of reproductive age by 50% by 2030, though no country is currently on track to meet this goal. IDA causes fatigue, impaired concentration, and, in pregnancy, can lead to premature birth and low infant birth weight. It also affects children's physical and cognitive development. Conversely, excessive iron, particularly in its free form, can cause oxidative damage and contribute to conditions like hemochromatosis and infections. Plant-based foods contain non-heme iron, which is less readily absorbed. Plant-based foods often contain compounds that inhibit iron absorption, which can be beneficial or detrimental depending on an individual's iron status. Compounds like phytic acid and flavonoids can inhibit iron absorption, while vitamin C and carotenoids can enhance it. Those with iron deficiency should avoid high-phytate and flavonoid foods, while those with iron overload may benefit from their consumption to help manage iron levels.

Unlocking the potential: Harnessing biomarkers commonly used in clinical practice to predict complications and outcomes in febrile neutropenia

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Febrile neutropenia (FN) is a frequent and serious complication in cancer patients undergoing standard therapy. Its incidence ranges widely, from 10% to 50% in patients with solid tumors and up to 80% in those with hematological malignancies. FN significantly impacts patient outcomes, often necessitating dose reductions or delays in cancer treatment, which can adversely affect overall survival. The mortality rate associated with FN is substantial, between 10% and 30%. Given its profound impact, early identification of patients at high risk for adverse outcomes is crucial. The Multinational Association for Supportive Care in Cancer (MASCC) risk index is a useful tool for stratifying FN risk but has limitations, including subjectivity and difficulty in emergency settings. Biomarkers such as procalcitonin and C-reactive protein (CRP) have demonstrated potential in predicting FN complications. Procalcitonin, in particular, has shown superior performance over the MASCC score in predicting bacteremia and mortality in FN patients. CRP, while useful, is less effective than procalcitonin in certain contexts. Moreover, malnutrition, common in cancer patients, may also influence FN outcomes. Albumin, a marker for nutritional status, correlates with inflammation and malnutrition and may hold promise for FN prediction. Emerging ratios, such as the procalcitonin to albumin ratio (PAR) and the CRP to albumin ratio (CAR), have shown efficacy in predicting complications in various conditions but have not yet been explored in FN. This review emphasizes the urgent need for further research to validate these biomarkers and their ratios in FN, potentially leading to better risk stratification and management strategies, thereby improving patient outcomes.

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Speed talks

Interaction of beef meat extract proteins and microplastics in simulated gastrointestinal conditions

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The growing problem of microplastic pollution threatens ecosystems and human health. Given that much meat is packaged in plastic, it's essential to study how meat proteins interact with plastic.[1] Microplastics can linger in the gastrointestinal tract (GIT) and may bind with dietary proteins, including those in red meat, which is significant for the alpha-Gal syndrome (AGS), linked to the α-Gal epitope in red meat.[2] This study examines how red meat proteins interact with polypropylene microplastics (PP) in simulated gastrointestinal conditions. Meat proteins were extracted using simulated saliva fluid and incubated with PP in simulated gastric and intestinal fluid at 37°C. After incubation, soft and hard corona proteins bound to microplastics were analyzed *via* SDS-PAGE. The α-Gal epitope on these proteins was tested using monoclonal anti-α-Gal antibodies. Protein bands of interest were analyzed *via* mass spectrometry. Results showed significant formation of both soft and hard protein coronas around microplastics, especially in SIF at 37°C, with α-Gal epitope present on multiple protein bands.

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References

1. Guerreiro TM, de Oliveira DN, et al. Migration from plastic packaging into meat. Food Res Int 2018;109:320-324.
2. Commins SP. & Platts-Mills TA. Anaphylaxis syndromes related to a new mammalian cross-reactive carbohydrate determinant. J Allergy Clin Immunol 2009;124:652-657.

Protective effects of H_2S donors against injury of endocrine pancreas in diabetic mice include antiferroptotic action

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In addition to apoptosis and necrosis, the involvement of ferroptosis, a newly defined form of iron-dependent cell death, in diabetes (DM)-induced β-cell failure has recently been described by our team.[1] In addition, ferroptosis has emerged as a new promising target of H_2S and related reactive sulfur species (RSS) in other pathologies. The protective role of H_2S in DM has also been demonstrated. However, it is still unclear whether targeting ferroptosis via the H_2S signaling pathways could have antidiabetic effects. H_2S, originally considered a toxic gas, is now a recognized gasotransmitter and its metabolites, such as persulfides, polysulfides and other RSS, as redox signaling molecules that control important (patho)physiological processes. Therefore, we aimed to investigate the potential of different H_2S/RSS donors in preventing the development of DM type 1 (T1D) in mice, focusing on their antiferroptotic effect. DM was induced in C57BL/6 mice (streptozotocin, 65 mg/kg i.p., from D1-3). Synthetic H_2S/RSS donors GYY4137, sodium sulfide (Na_2S_4) and cysteine-3-sulfide (Cys-S3) were administered i.p. from T1-T21. Our biochemical, histological and PCR results showed the strongest antidiabetic effect of Cys-S3 among the donors tested, as it improved both glycemia, glycosuria, islet size and insulin positivity. It also improved parameters involved in ferroptosis protection of the endocrine pancreas: increased GSH, decreased lipid peroxidation (evidenced by decreased 4-HNE positivity), increased expression of Nrf2 and GPX4, confirming its potential in preventing the development and progression of T1D.

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References

1. Stancic A, Saksida T, Markelic M, et al. Ferroptosis as a novel determinant of β-cell death in diabetic conditions. Oxid Med Cell Longev 2022;14:3873420.

Plant-derived metabolites affect different stages of biofilm formation of pathogenic bacteria

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Bacterial biofilm is a complex community of bacterial cells enclosed in an extracellular polymer matrix and attached to a biotic or abiotic substrate. Biofilm is a common cause of chronic infections in humans, so due to the growing resistance to antibiotics, alternative methods for controlling bacterial biofilm using natural agents have been proposed. In this study, antibiofilm activity of plant's metabolites (caffeic acid, gallic acid, rutin, hesperidin, carvacrol and thymol) against five clinical isolates of human pathogenic bacteria, *Staphylococcus aureus*, *Proteus* sp. and *Pseudomonas aeruginosa*, was examined. Inhibitory effects on biofilm adsorption, biofilm development and on mature biofilm were evaluated using crystal violet test and effect on metabolic activity was confirmed by resazurin dye test. Bacterial motility, hydrophobicity, and auto-aggregation were also examined. Minimum inhibitory concentrations (MIC), determined by broth microdilution method, were 2048-4096 μg/ml for phenolic acids; >1024 μg/ml for flavonoids; 128-512 μg/ml for terpenes regarding the strain. The most active metabolites were the terpenes, followed by the phenolic acids and flavonoids. Their activity varied on the stage of biofilm formation. Accordingly, inhibition of biofilm adsorption (after 4h of incubation) was ≥50% at all tested concentrations (MIC–1/32MIC). The tested phenolic acids and terpenes inhibited biofilm development better than the flavonoids, especially against *S. aureus* biofilms. Moreover, eradication of mature biofilms was noticed at higher tested concentrations (MIC–1/4MIC). Besides disturbing biofilm formation, caffeic acid, gallic acid, carvacrol and thymol have caused biofilm metabolic inactivity. In addition, bacterial motility and auto-aggregation were affected by tested phenolic acids. Fluorescence micrographs have confirmed the antibiofilm activity of tested compounds. The overall results indicate a great potential of the tested plant-derived metabolites in the treatment of infections caused by biofilm-producing bacteria.

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Rose oil distillation wastewater as a new source of pharmacologically active compounds

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Hydrodistillation of rose oil, widely used in perfumery and cosmetics, generates significant amounts of wastewater rich in bioactive compounds, which can pose environmental challenges. This study analyzed the chemical profile and biological activities of wastewater from hydrodistillation of rose petals from 10 new genotypes of *Rosa* × *hybrida* L. to explore potential uses. Chemical profile was determined by measuring the total phenolic content (TPC) and total flavonoid content (TFC), and quantitative LC-MS/MS analysis of 45 selected compounds. The results showed that the wastewaters have high TPC (79.4–181 mg galic acid equivalents per g of dry extract) and TFC (1.92–25.9 mg quercetin equivalents per g of dry weight), and are rich in quinic, protocatechuic and gallic acid, kaempferol, quercetin, kaempferol–3–O–glucoside, quercitrin, hyperoside, isoquercetin, and rutin. The wastewaters exhibited strong antioxidant activity in both DPPH and FRAP assays. They strongly inhibited α-glucosidase, whereas the potential to inhibit acetylcholine esterase and α-amylase was very low. The results suggest that rose oil distillation wastewaters have the potential to be utilized in the pharmaceutical industry, thereby contributing to the circular economy.

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Effect of trioxohydroxytetrafluorotriborate on hematological parameters *in vivo*

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In this paper, the goal was to monitor the influence of boroxine (trioxohydroxytetrafluorotriborate) on the hematological parameters of *Rattus norvegicus* (Berkenhout 1769) *in vivo*. Halogenated boric acid derivatives - boroxines are currently being investigated as potential enzyme modifiers and therapeutics. Boroxine is particularly important as an enzyme inhibitor that can be used for therapy in cancer patients.[1,2] It has recently been discovered that $K_2[B_3O_3F_4OH]$ can be used for the prevention and treatment of benign and malignant skin changes, manifesting in the form of neuroma or tumours. In our study, the control group numbered 6 individuals, the group where boroxine was administered intraperitoneally (IPG) and the group where boroxine was administered orally (OG) each had nine individuals. Among the hematological parameters, we monitored the number of leukocytes and DKS, the number of erythrocytes, hemoglobin concentration, hematocrit value, hematological indexes (MCV, MCH and MCHC) and the number of platelets. After the statistical analysis (ANOVA and student's T test) of the examined parameters between the control and IP groups, a statistically significant difference was shown for the number of leukocytes ($p=0.04$), number of lymphocytes ($p=0.02$), number of neutrophils ($p=0.01$), number of erythrocytes ($p=0.01$), hemoglobin ($p=0.02$), hematocrit ($p=0.005$), MCH ($p=0.05$) and MCHC ($p=0.006$), where the values of all parameters were significantly lower in the IP group compared to the control, except for MCH and MCHC where the values of these hematological indices were significantly higher in the IP group. When comparing the values of tested parameters of the control and OG groups, we found significant differences for the number of leukocytes ($p=0.04$), number of lymphocytes ($p=0.005$), number of basophils ($p<0.001$), hematocrit ($p=0.04$), MCH ($p=0.04$), MCHC ($p=0.001$) and platelet count ($p=0.05$) The obtained values for the tested parameters between the IP and OG groups after the statistical analysis showed a significant difference for eosinophils ($p=0.03$) and basophils ($p<0.001$), the other tested parameters showed no significant difference. The biggest difference in the tested parameters was noted between the control group and individuals from the group where boroxine was administered intraperitoneally, which may be the result of a higher concentration of boroxine, which is more easily and quickly resorbed when administered intraperitoneally, whereas the oral method of application goes through the digestive tract, where a smaller dose of boroxine is absorbed.

References

1. Adams J. & Kauffman M. Development of the proteasome inhibitor Velcade (Bortezomib). Cancer Invest 2004;22:304-311.
2. Paramore A. & Frantz S. Bortezomib. Nat Rev Drug Discov 2003;2:611-612.

Preliminary characterization of putative tick cement protein PA107 – implications for possible applications in biomedicine

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In addition to enabling blood meal uptake, by inhibiting blood coagulation, immune response activation and inflammation, tick salivary proteins (TSPs) are also the key components of tick cement. It forms a plug that surrounds tick mouth parts and protects them from host defense molecules, keeps a feeding wound free of microbes, and provides a firm attachment to host skin during feeding. A tick-unique protein present across tick genera - PA107 is a putative cement protein, transcribed in tick salivary glands at the start of a feeding. Herein, a detailed *in silico* analysis of its primary and tertiary structure was performed, along with the immunogenicity assessment by using the recombinant protein derived from *Ixodes ricinus* species, medically the most important tick in Europe. The screening of the primary structure placed it to the glycine-rich protein family, revealing in parallel an overlapping 15mer at the C-terminus and borderline homology to non-tick proteins with antimicrobial activity. The analysis of tertiary structure revealed a high degree of intrinsic disorder for monomeric PA107, in contrast to highly ordered structures for different oligomeric states that might correlate with the putative role in the tick cement formation process. *In vitro* findings showed the lack of humoral response induction in experimentally infested rats and persons bitten by the *I. ricinus* ticks. Controllable (de)polymerization, strength, and non-immunogenic features of tick cement and its components (i.e. PA107) could be used for development of new tissue glues, with better performances and lower toxicity compared with currently used.

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Secondary metabolites and biological activity of *Teucrium montanum* L. (Lamiaceae)

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Teucrium montanum has been the subject of extensive scientific research due to its wide-ranging applications, particularly in the areas of biological activity and phytochemical analysis.[1] This plant exhibits significant adaptability, thriving on substrates like basic carbonates, ultrabasic serpentinites, and acidic silicates. The chemical composition of these substrates influences the concentration and diversity of secondary metabolites in the plant's organs, which are crucial for its pharmaceutical potential. The secondary metabolites in *T. montanum* leaves, flowers, and stems have been studied, revealing that metabolite concentration varies depending on the plant part and the site of biosynthesis. *T. montanum* produces a wide range of compounds, especially phenolics, iridoid glycosides, and volatile terpenoids. Using the UHPLC/DAD/(−)HESI−MS^2 method, various phenolic acids and flavonoids were identified, including chlorogenic acid, syringic acid, quercetin, and catechin. The study showed that *T. montanum* from calcareous habitats has higher concentrations of chlorogenic and syringic acid, while samples from serpentinite habitats contain more catechin, quercetin, and isoquercitrin. The geological substrates play a crucial role in shaping the chemical profiles of *T. montanum*, demonstrating its adaptive strategies to different environments. Additionally, a comparative analysis using GC×GC-MS revealed that serpentinite habitats yield three times more essential oils than calcareous habitats, with sesquiterpenes being the dominant compounds. Unique compounds were found in each habitat type, emphasizing the plant's chemical diversity. Due to its rich secondary metabolite content, *T. montanum* exhibits various biological activities, including antioxidant, antiviral, antimicrobial, and anticancer properties. This highlights the plant's potential in pharmaceutical applications.

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References

1. Stanković M. Teucrium Species: Biology and applications. Springer, Cham, Switzerland, 2020, 435 pp.

Poster Session 1

The potential of R-phycocyanin as an albumin substitute for cell growth in meat cultivation

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Cultured meat is a promising solution to reduce land and water usage and limit pollution, but it is still costly. The main obstacle is finding alternatives to fetal bovine serum (FBS) as growth supplements. Among numerous molecules present in FBS, albumin is the most abundant component, and significant efforts are being invested in finding albumin alternatives that should provide sustainable meat cultivation. Algal proteins are promising alternatives to albumin in cell cultivation because they are cheaper and cleaner production process. R-phycocyanin (R-PC) is a photosynthetic protein from *Porphyra*, a red macroalgae with substantial annual production (several million tons annually). Covalently attached chromophores give R-PC substantial antioxidant potential and metal-binding activities. This makes R-PC a good candidate for albumin replacement in cell media. Given the crucial role of albumin's fatty acid (FA) binding in cell cultivation, it is essential to validate R-PC as a potential albumin replacement by confirming its ability to bind FA. In this study, we probe the potential of R-PC to replace albumin to cultivate the QM7 cell line (myoblasts from Japanese quail). First, we characterised the binding of selected fatty acids to R-PC from the commercial *Porphyra* preparation using the R-PC fluorescence quenching approach. We tested the binding of saturated fatty acids (palmitic and stearic), monounsaturated fatty acids (oleic), and polyunsaturated fatty acids (linoleic and linolenic). Fluorescent spectroscopy showed the stronger binding of unsaturated fatty acids compared to the saturated ones, while the linolenic acid exhibits the highest affinity. MTT and Neutral Red assays and direct cell counting revealed a significant boost in cell growth (viability and number of cells) in the presence of R-PC compared to the control. Overall, this work demonstrated the significant potential of R-PC to bind FA and replace albumin in the cell growth medium, an essential prerequisite for the sustainable production of cultured meat.

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Optimization of spectrofluorimetric assay for screening of opine dehydrogenase mutant libraries

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Opine dehydrogenase from *Desulfohalobium retbaense* was identified through a sequence-based screening of a metagenomic library.[1] The gene-encoding enzyme was subsequently heterologously expressed in *Escherichia coli* to facilitate its functional characterization. To enhance the enzyme's catalytic efficiency, a mutant library was generated via error-prone PCR, to identify variants exhibiting improved activity. A fluorescent assay was meticulously developed and optimized to screen these variants, employing L-alanine, pyruvate, and NADH as substrates in a 50mM sodium phosphate buffer at pH 7.5.[2] Initial screening results indicated the presence of several promising mutants with potential for enhanced specific enzymatic activity. These candidates are undergoing further validation to confirm the mutations and assess their stability and efficiency. Future work will focus on a detailed biochemical characterization of these mutants, which may provide insights into the structure-function relationships governing opine dehydrogenase activity. These optimized enzymes could also hold potential for industrial applications where enhanced catalytic properties are desirable.

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References

1. Kaličanin N. et al. Heterologous expression and partial characterization of a putative opine dehydrogenase from a metagenomic sequence of desulfohalobium retbaense. Chem Bio Chem 2023;24:e202300414.
2. Standley M, Allen J, Cervantes L, Lilly J, Camps M. Fluorescence-based reporters for detection of mutagenesis in E. coli. Methods Enzymol 2017;591:159-186.

Isolation of Ara h 1 from raw and roasted peanuts, evaluation of immunoreactivity, and monitoring allergen binding to THP-1 macrophages

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Peanut allergy is a food allergy with increasing prevalence worldwide.[1] Ara h 1 is one of the main peanut allergens besides Ara h 2 and Ara h 3.[1] Antigen-presenting cells such as macrophages have a crucial role in inducing inflammation, phagocytosis, processing and subsequent presentation of antigens in various diseases, including allergies.[2] The objectives of this research include isolating and purifying Ara h 1 from both raw and roasted peanuts, verifying its immunoreactivity, and investigating its binding/uptake by THP-1 macrophages. The research methodology includes the purification of Ara h 1 using a combination of precipitation and chromatographic methods. The purity and immunoreactivity of Ara h 1 were confirmed in SDS-PAGE and Western blot. Ara h 1 was labeled with FITC to monitor its binding to macrophages. THP-1 monocytes were differentiated into macrophages using PMA, and their ability to bind/uptake Ara h 1 was analyzed by flow cytometry. To verify allergen binding, fluorescence in the supernatant was measured following macrophage treatment. The results show that Ara h 1 was successfully purified from both raw and roasted peanuts, revealing two isoforms with molecular masses of 63.5 and 54 kDa. Both isoforms of Ara h 1 demonstrated immunoreactivity with peanut allergic patient's sera. THP-1 macrophages showed the ability to bind/uptake Ara h 1 from raw and roasted peanuts, with this process being dependent on the concentration and treatment time in a similar manner in the uptake of both Ara h 1 from raw and roasted peanuts. Macrophages play an active role in the uptake of allergens, even after thermal processing of peanuts, and allergens such as Ara h 1 can still exhibit similar allergenic properties even after harsh thermal processing.

Acknowledgements

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References

1. Palladino C & Breiteneder H. Peanut allergens. Mol Immunol 2018;100:58-70.
2. Lendeckel U, Venz S, Wolke C. Macrophages: shapes and functions. ChemTexts 2022;8:12.

The agreement between spectrophotometric and ELISA assay for haptoglobin concentration in cow milk

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Increased haptoglobin (HP) concentration in milk can be a biomarker of subclinical mastitis (SCM), the biggest issue associated with dairy cows' health and productivity.[1] An automated spectrophotometric (SPF) assay for HP in serum, employing hemoglobin (Hb)-HP complex pseudo-peroxidase activity, can positively interfere with lactoperoxidase activity in milk. We modified SPF to calculate the HP concentration as the difference between measurements with and without added Hb. To test the agreement with the bovine-specific ELISA (AssayGenie, Ireland), we parallelly analyzed 59 milk samples: 23 mastitis-free (MF), 28 with mild (MSCM), and 8 with advanced SCM (ASCM). In all samples, median (lowest-highest) results were 22.4 (0.05–101.6) μg/mL and 10.5 (0.55–83.2) μg/mL for SPF and ELISA, respectively. The Bland-Altman plot showed satisfactory concordance. The Passing-Bablok regression detected no systematic difference, while the proportional difference was 6.22%. SPF results in MF were lower than in MSCM only. ELISA showed the same difference, plus ASCM results were higher than both other groups. For differentiating MSCM from MF, ROC analysis yielded an AUC of 0.749 for SPF, which was lower ($P<0.001$) than the value for ELISA (0.995). The corresponding cut-offs were 20 μg/mL (sensitivity 79%, specificity 69%) for SPF and 3.8 μg/mL (with sensitivity 93% and specificity 100%) ELISA. The results indicated that notwithstanding the acceptable analytical agreement, SPF diagnostic performance remained below ELISA, thus meriting additional modification, optimization, and evaluation efforts.

Acknowledgements

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References

1. Farkaš V et al. Biomarkers for subclinical bovine mastitis: a high throughput TMT-based proteomic investigation. Vet Res Commun 2024;48:2069-2082.
2. Ljubić B, Mrljak V, Turk R, Brady N, Eckersall D. Assay of haptoglobin, the plasma acute-phase protein, by hemoglobin binding: Optimization of a novel chromogen. Vet Clin Pathol 2021;50:104.

Effect of crosslinking cations on the activity of *T. versicolor* laccase immobilized within alginate hydrogel beads

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Alginate hydrogels have been widely used as enzyme immobilization carriers due to their simple preparation, low toxicity and biodegradability. The ability of alginate to form hydrogels through covalent interactions with divalent cations reduces to risk of enzyme leaching.[1] Using two types of cations crosslinking agents (Zn^{2+} and Ca^{2+}) at varying concentrations, *T. versicolor* laccase was imobilized withing alginate hydrogel beads via encapsulation.[2] The specific enzyme activity of laccase immobilized in beads with Zn^{2+} as the crosslinking agent was 2-5 times higher, depending on the ion concentration, compared to the enzyme in beads crosslinked with calcium ions. At the optimal concentration of each crosslinking ion, laccase within zinc-alginate beads showed better thermal stability, retaining 70% of its acitivity after incubation at 60°C, comapred to 50% retention for laccase in calcium-alginate beads. As a result of increased extent of interactions[3], the alginate beads with zinc displayed signifcantly better mechical stability after incubating the beads at optimum pH for 4 days, resulting in the enzyme keeping 60% of its original activity, 6-8 times more than the enzyme imobillized in calcium-alginate beads.

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References

1. Meyer J, Meyer LE, Kara S. Enzyme immobilization in hydrogels: A perfect liaison for efficient and sustainable biocatalysis. Eng Life Sci2022;22:165-177.
2. Reig-Vano B, Huck-Iriart C, de la Flor S, Trojanowska A, Tylkowski B, Giamberini M. Structural and mechanical analysis on mannuronate-rich alginate gels and xerogels beads based on calcium, copper and zinc as crosslinkers. Int J Biol Macromol 2023;246:125659.
3. Chan LW, Jin Y, Heng PWS. Cross-linking mechanisms of calcium and zinc in production of alginate microspheres. Int J Pharm 2002;242:255-258.

Antimicrobial activity of 2-(2-ferrocenyl-4-oxothiazolidin-3-yl)acetic acid and its ethyl derivative

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Thiazolidine and its derivatives are common pharmacophores present in numerous natural products and other bioactive molecules. These heterocycles are one of the most frequently used compounds in drug development due to their wide spectrum of biological activities such as antimicrobial, antidiabetic, anticancer, anticonvulsant, anti-HIV, and antioxidant activities.[1] Several years ago we showed that the ferrocene-containing derivatives of these heterocycles possess high biological potential.[2] As a part of our ongoing research in the development of new antimicrobials, herein, we report the synthesis, electrochemical, and spectral characterization of two compounds that combine three molecules: 4-thiazolidinone, amino acids, and ferrocene. The antimicrobial potential of these two compounds was determined using four different bacterial and fungal strains. Compounds showed slightly better antifungal activity with lower MIC values compared with their antibacterial effects. Both newly synthesized compounds possessed MIC values of 1 mg/mL for most tested fungal species, while MIC values for the most studied bacterial species were 2 mg/mL.

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References

1. Jain A K, Vaidya A, Ravichandran V, Kashaw S K, Agrawal R K. Resent developments and biological activities of thiazolidinone derivatives: A review. Bioorg Med Chem 2012;20:3378-3395.
2. Pejović A et al. Discovery of anxiolytic 2-ferrocenyl-1,3-thiazolidin-4-ones exerting GABAA receptor interaction via the benzodiazepine-binding site. Eur J Med Chem 2014;83:57-73.

Evaluation of GSH/GSSG and CyS/CySS as oxidative stress markers in small cell lung cancer patients undergoing chemotherapy in Serbia

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Small cell lung cancer (SCLC), which accounts for around 15% of lung cancer cases worldwide, is characterized by rapid growth and early metastasis and represents a major health challenge worldwide. Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, is associated with the progression of SCLC and contributes to both tumor development and resistance to chemotherapy. The aim of this study was to investigate the markers of oxidative stress in SCLC patients undergoing chemotherapy. Plasma samples were collected from 60 SCLC patients at three different time points: before chemotherapy, after 2 cycles, and after 4 cycles of chemotherapy. The concentrations of reduced glutathione, oxidized glutathione, cysteine, and cystine were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and expressed as the ratio of reduced and oxidized forms (GSH/GSSG and CyS/CySS). The results of the Friedman analysis showed a statistically significant difference in the CyS/CySS ratio during the treatment period ($p=0.041$), with the GSH/GSSG ratio approaching significance ($p=0.056$). A post-hoc Wilcoxon analysis revealed that the CyS/CySS ratio was lowest after 2 cycles of chemotherapy, indicating a peak in oxidative stress during this treatment phase. CyS/CySS and GSH/GSSG ratios are potential biomarkers for monitoring oxidative stress in SCLC patients undergoing chemotherapy. These markers could be a valuable tool for better patient stratification, which would allow a targeted approach to the treatment of SCLC.

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Crystallography-driven drug design: Unlocking potent VMAT2 inhibitors for Huntington's disease

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Huntington's disease (HD) is a devastating neurodegenerative disorder, characterized by severe motor and cognitive deterioration. The vesicular monoamine transporter 2 (VMAT2) is a key target for therapeutic intervention, with tetrabenazine being the primary inhibitor used to mitigate symptoms.[1] Utilizing the crystal structure of VMAT2 in complex with tetrabenazine[1,2], a Fragment-Based Drug Design approach combined with Medicinal Chemistry Transformations[3] led to the discovery of novel tetrabenazine derivatives. These derivatives exhibited significantly higher binding affinities to VMAT2, suggesting the potential for greater therapeutic efficacy. This research demonstrates the power of merging crystallographic data with sophisticated medicinal chemistry to enhance drug design, ultimately contributing to the development of more effective treatments for Huntington's disease. The identification of these high-affinity tetrabenazine derivatives represents a substantial step forward in the pursuit of improved therapeutic options for HD.

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References

1. Dalton MP, Cheng MH, Bahar I, Coleman JA. Structural mechanisms for VMAT2 inhibition by tetrabenazine. Elife 2024;12:RP91973.
2. Wu D, et al. Transport and inhibition mechanisms of human VMAT2. Nature 2024;626:427-434.
3. Segall M, Champness E, Leeding C, Lilien R, Mettu R, Stevens B. Applying medicinal chemistry transformations and multiparameter optimization to guide the search for high-quality leads and candidates. J Chem Inf Model 2011;51:2967-2976.

Assessment of caspase modulation in human epidermoid carcinoma cells (A431) treated with silver nanoparticles synthesized using plant extract

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In this study, the effect of synthesized silver nanoparticles using *Lythrum salicaria* L. root extract (LSAgNPs) on apoptosis-related caspases in human epidermoid carcinoma cells (A431) was estimated. Previous results showed that LSAgNPs exhibit significant cytotoxicity against A431 cells[1]. To elucidate the role of LSAgNPs in apoptotic pathways, A431 cells were treated with LSAgNPs at a concentration of 100 μg/mL. The subsequent effects on pro-caspase-3, pro-caspase-7, and pro-caspase-9, inactive precursor forms of caspases, which are crucial enzymes in the process of apoptosis, were assessed using Western blot analysis. The results demonstrate that LSAgNPs treatment led to a significant reduction in pro-caspase levels: pro-caspase-3 decreased by approximately 15%, pro-caspase-7 by 25%, and pro-caspase-9 by 40%, indicating the activation of apoptosis. A reduction in the intensity of these pro-caspase bands obtained using Western blotting analysis suggests their activation into functional caspases: caspase-3 and caspase-7 which are critical executioner caspases involved in the execution phase of apoptosis, and caspase-9 which is an initiator caspase playing a key role in the intrinsic apoptotic pathway. The more pronounced influence on caspase-9 suggests that LSAgNPs predominantly cause the intrinsic apoptotic pathway activated by cellular stress and realized mitochondrial cytochrome c into the cytosol. These results indicate that the possible mechanism of LSAgNPs cytotoxicity may be the generation of free radicals in cells and oxidative damage of cellular components, that further leads to the activation of apoptosis.

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References

1. Srećković NZ et al. Application potential of biogenically synthesized silver nanoparticles using *Lythrum salicaria* L. extracts as pharmaceuticals and catalysts for organic pollutant degradation. RSC Adv 2021;11:35585-35599.

High-affinity binding of Concanavalin A to B-Phycoerythrin

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Pigments are an important food additives that play a significant role in the sensory properties of food. Nowadays, the focus has shifted to more natural, healthy, and non-toxic edible pigments. One of the primary sources of natural pigments is algae. They represent an abundant supply of different pigments, such as chlorophyll, phycobiliproteins (PBPs), carotene, etc. B-phycoerythrin (B-PE), a major phycobiliprotein of microalgae *Porphyridium purpureum*, has a vivid pink colour, giving it a promising potential for application in the food industry. However, their limited stability is the major challenge in applying PBPs in the food industry. In order to replace synthetic pigments with PBPs globally, it is necessary to keep them stable during industrial processing. This study aims to stabilise B-PE by probing its binding to concanavalin A (ConA), lectin from jack-bean. We utilised fluorescence spectroscopy to characterise interactions between B-PE and different oligomeric states of ConA at pH 5.5 (dimer) and pH 7.2 (tetramer). ConA-induced enhancement of fluorescence of B-PE chromophore revealed a stronger affinity of B-PE to dimeric ConA (Ka of 1.70×10^7 M^{-1}) compared to the tetramer form (Ka of 0.94×10^7 M^{-1}). We demonstrated that the presence of sugars (glucose and mannose) did not inhibit the binding at pH 7.2; however, the binding was partially inhibited at pH 5.5, indicating that the oligomerisation form of ConA influences the mode of ConA: B-PE interactions. VIS-CD spectroscopy revealed that ConA binding influences the chiral environment of the B-PE chromophore. Thermal stability study demonstrated higher stability of B-PE chromophore in the presence of ConA at both pH values. Overall, these results indicate that stabilising B-PE by lectins is a promising approach to strengthening the application potential of PBPs in the food industry.

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Global research trends in diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the predominant type of non-Hodgkin lymphoma, the most common haematological malignancy.[1] Herein we present a bibliometric study of published literature on DLBCL retrieved from PubMed and Scopus databases. Over the past three decades, an exponential growth of the number of publications on DLBCL has been noted, with a significant citation impact. The country with the most articles in this field has changed; first it was Netherlands (until 2000), then the USA (2001–2020), and currently it is China. The number of scientific collaborations in the field among different institutions and countries has also grown. The most prominent institutions and their collaborations can be extracted from the literature by means of a specific density map. The co-occurrence density map of keywords reveals the research directions over a chosen timeframe. For example, the term 'machine learning' appeared in the period from January 2021. to July 2024, while it was absent earlier, indicating that the significant application of machine learning to studies of DLBCL has only recently begun.

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References

1. Mafra A et al. Global patterns of non-Hodgkin lymphoma in 2020. Int J Cancer 2022;151(9):1474-1481.

Peanut allergens affected intestinal barrier function in human intestinal epithelial cell lines Caco-2 and T84

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Food allergies, particularly peanut allergy, have become a major health issue, affecting up to 2% of the overall population in developed countries.[1] Defining the effects of interaction between peanut allergens and the intestinal epithelium is critical for determining the mechanisms underlying allergic responses. Caco-2 and T84 are often used models for investigating such interactions. Therefore, this study aimed to characterize these two cell lines and assess their response towards peanut allergens. Caco-2 and T84 cells were seeded and harvested at days 0, 7, 14, and 21 post-seeding. qRT-PCR was performed to determine the expression of Toll-like receptors (TLR2, TLR4, TLR5), tight junction proteins (E-cadherin, ZO-1), mucins (MUC2, MUC5B), and alkaline phosphatase (ALP). The findings revealed that T84 cells are more like colonocytes and Caco-2 cells resemble enterocytes. Cell lines were subsequently treated with recombinant peanut allergens (Ara h 2, Ara h 8, Ara h 9) as well as whole peanut extracts derived from raw and roasted peanuts, with lipopolysaccharide (LPS) serving as a positive control. Cell viability and LDH assays were performed to ensure non-cytotoxicity of allergens. TEER measurements before and after treatment demonstrated a significant reduction in barrier function after using raw and roasted peanut extracts but not pure peanut proteins. These outcomes suggest that the complex composition of whole peanut extracts contributes significantly to the disruption of intestinal barrier function.

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References

1. Rao H et al. The effect of the food matrix on the in vitro bio-accessibility and IgE reactivity of peanut allergens. Molecular nutrition & food research 2020;64(14):e1901093.

A novel approach to extracellular vesicles staining facilitates their tracking during cellular uptake

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Most cell types secrete extracellular vesicles (EVs), membrane-derived structures that carry and exchange heterogeneous cargo among cells, thereby allowing them to communicate. Regarding this, EVs are emerging as drug delivery systems and several strategies have been developed to facilitate their tracking *in vitro* or *in vivo*.[1] In this study, we established a novel approach to EVs staining using Oil Red O (ORO), a lipophilic dye. Due to higher solubility in fats than in aqueous solution, ORO inserts into the lipid-bilayer of EVs, staining them, without changing their zeta potential. ORO fluorescence intensity changes after binding to EVs helping us to estimate the optimal EVs/ORO ratio required for efficient staining. However, upon staining EVs we are left with a mixture of stained EVs and an excess of unbound dye requiring additional purification steps. Standardly used methods for EVs isolation are shown to be insufficient for dye excess removal, often resulting in EVs loss or aggregation.[1] To achieve complete removal of excess dye, we used a previously reported methacrylate-based copolymer column functionalized with nanobodies for purifying EVs from serum samples.[2] We adapted this chromatography method for simultaneous EVs purification and staining. Uptake of EVs obtained in such a manner was verified on SKBR3 cells. Treated cells were analysed by flow cytometry and a shift in the red fluorescence indicated that ORO stained EVs are uptaken by cells. We have demonstrated that a straightforward, rapid and a cost-effective chromatography technique can be adopted for simultaneous purification and staining of EVs allowing for efficient visualisation of their uptake and simple evaluation of their biological activity.

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References

1. Rautaniemi K et al. Adressing challenges in the removal of unbound dye from passively labelled extracellular vesicles. Nanoscale Adv 2022;4:226-240.
2. Filipović L et al. Affinity-based isolation of extracellular vesicles by means of single-domain antibodies bound to macroporous methacrylate-based copolymer. N Biotechnol 2022;69:36-48.

Effect of prolonged heat stress on the activity of four important metabolic enzymes during diapause of the European corn borer *Ostrinia nubilalis* (Hbn.)

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Global climate changes and warming have brought unusually high temperatures during winter, which may negatively affect the ability of overwintering insects to enter diapause, a type of dormancy during which they do not feed, suppress their metabolism, conserve energy stores, and gradually acquire cold hardiness. The European corn borer *Ostrinia nubilalis* is a common corn pest whose 5th instar larvae adopt facultative diapause and must experience low winter temperatures to fully develop cryoprotective mechanisms necessary to survive unfavourable environmental conditions. Here we investigated the simultaneous effects of prolonged heat stress and diapause program on the metabolic profile of larvae and pupae of *O. nubilalis*. The activity of four important metabolic enzymes: citrate synthase (CS), lactate dehydrogenase (LDH), alanin aminotransferase (ALT) and aspartate aminotransferase (AST) were determined spectrophotometrically in whole-body homogenates of non-diapausing larvae and pupae, as well as diapausing larvae held in either field or warm acclimation conditions from November to April. The results showed that long-term exposure to above-average winter temperatures during diapause caused a generally higher metabolic rate in warm-acclimated larvae compared to those in field conditions, which increased the level of oxidative stress, caused a faster depletion of energy reserves and negatively affected the ability of larvae to complete diapause and metamorphose into pupae. Our results provide a solid background for studies on ecophysiological modelling of insect populations in both agroecosystems and natural habitats. Moreover, it can provide valuable information for conservation biology to predict responses of other cold-adapted animal species to climate warming and model their future population trends in temperate and subpolar regions.

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Bermet wine polyphenolics as modulators of lipase activity *in vitro*

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Bermet is a specially flavored wine, which is made from grapes from vineyards on the slopes of Fruška Gora (Vojvodina, Serbia) with the addition of up to 26 different aromatic medicinal plants. These wines have been produced since the 15th century according to the traditional procedure, but, until now, data on their chemical composition and biological activities are scarce. Presence of bioactive compounds originating from herbs could have a beneficial effect on health during moderate consumption of Bermet wine, so there is a great interest to investigate their composition and biological activity.[1] The aim of this study was to evaluate *in vitro* effect of 8 red and 4 white Bermet wine on lipase activity, digestive enzyme involved in lipid metabolism. It is well known that polyphenols have certain biological activity and therefore can contribute to the health benefits of medicinal plants and wine. Therefore, quantitative analysis of 30 polyphenols by HPLC-UV/VIS techniqe was applied to elucidate differences in samples phenolic profile. Also, the content of total polyphenols, tanins, flavonoids and monomeric anthocyanins were evaluated by spectrophotometric methods. Chemical analysis showed that all wines had significant amounts of total polyphenols, flavonoids, tannins and monomeric anthocyanins. The most abundant polyphenol compounds in examined samples were gallic acid and catechin. On the other hand, all analysed wines showed modest lipase inhibition activity except for one. The activity range varied from 0.63 to 2.18 ng orlistat eq/mL of wine. The findings of this study may enhance the international recognition of Bermet wines from Vojvodina.

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References

1. Gorjanović S et al. Serbian aromatized wine “Bermet”: Electrochemical, chemiluminescent and spectrophotometric determinationof antioxidant activity. J Serb Chem Soc 2019;84:1–13.

Hepatotoxic effects of perfluorooctanoic acid on female *Swiss* mice

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Perfluorooctanoic acid (PFOA) is a synthetic fluorinated compound found in non-stick and stain-resistant consumer products. As a persistent organic pollutant, PFOA resists degradation and accumulates in biological systems, raising concerns about its effects on human and ecological health. The aim of our study was to investigate whether PFOA can induce liver damage by affecting relative liver weight and the activity of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in blood. In this 14-days study, twenty-four female *Swiss* mice were divided into 4 groups (6 per group): one control group and three experimental groups exposed to 0.06, 1.15 and 22 mg PFOA/kg B.W./day through drinking water. Relative liver weight was calculated as the absolute liver weight divided by final body weight, multiplied by 100 for each animal. Activities of ALT, AST and ALP in plasma were determined by Dialab Autolyser. Statistical analysis of obtained data was performed using STATISTICA® version 13.0 (StatSoft, Inc). Data from control and treated mice were compared using One-way analysis of variance (ANOVA) for multiple comparisons, followed by a Dunnet post-hoc test. Our study demonstrated a dose-dependent increase in relative liver weight in mice, with significant elevations observed in groups treated with 1.15 mg and 22 mg PFOA/kg B.W./day. Additionally, a dose-dependent increase in the activity of all investigated liver enzymes was observed. Detected increase was statistically significant in the group treated with 22 mg PFOA/kg B.W./day. Given that elevated liver enzyme levels are widely recognized as biomarkers for hepatotoxicity, and the significant increase in relative liver weight as an indicator of hepatomegaly, these findings underscore the significant liver damage induced by PFOA, following a 14-day exposure period.

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Poster Session 2

Molecular insights into aging in honey bees: RNA-Seq analysis of winter worker bees across different age groups

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Honey bees (Apis mellifera L.) are essential pollinators known for their remarkable lifespan plasticity, particularly the difference between summer and winter bees. Winter bees can live several months longer than their summer counterparts, making them an ideal model for aging studies. This research focuses on the molecular mechanisms of aging in winter worker bees through RNA-Seq analysis of three age groups: newly emerged (W0), two months old (W2), and four months old (W4) bees. RNA was extracted from the abdomens and sequenced using the DNBSEQ platform, followed by comprehensive bioinformatics analysis. The comparison between W2 and W0 revealed the highest number of DEGs (775), indicating significant transcriptomic shifts during this transition. A total of 344 genes were differentially expressed in at least two comparisons (W2/W0, W4/W2, and W4/W0). Hierarchical clustering revealed distinct gene expression patterns associated with aging. Key driver gene analysis highlighted critical genes related to cuticle formation, venom activity, and polyamine metabolism, such as CPR6, melittin, and spermine synthase, suggesting adaptations in structural and metabolic processes with age. KEGG pathway analysis identified enrichments in pathways linked to peroxisome function, lipid metabolism, glycan degradation, and lysosomal activity, highlighting shifts in cellular maintenance and energy regulation during aging. These findings offer valuable insights into the molecular mechanisms of aging in honey bees and provide a foundation for understanding longevity across species. Further research is needed to explore the specific roles of these genes and pathways in maintaining bee health and resilience during aging.

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Investigation of the effect of algal chromopeptides on the activity of the SARS-CoV-2 main protease M^{pro}

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This study aimed to investigate whether and to what extent enzymatic digests of phycobiliproteins (PBPs) from algae inhibit the activity of M^{pro} from the SARS-CoV-2 virus. Phycobiliproteins are light-harvesting proteins from cyanobacteria and red algae. Phycobiliprotein chromopeptides are released by the action of proteases and possess various biological activities. C-Phycocyanin (C-PC) and B-Phycoerythrin (B-PE), the major PBPs in *Arthrospira platensis* and *Porphyridium purpureum,* respectively, were digested with pepsin and pancreatin. Monitoring M^{pro} activity by fluorescence measurements showed that only the pancreatin digest of B-phycoerythrin (B-PE) could inhibit M^{pro} activity by 74% compared to the control reaction. Chromopeptides from B-PE digest were separated using high-performance liquid chromatography (HPLC) into two main fractions. Further monitoring of enzyme kinetics demonstrated that both fractions inhibit M^{pro} activity but less effectively than the complete B-PE digest. Estimated IC_{50} values, expressed by chromophore equivalents, were 1.3μM for initial digest, 4.3μM for Fraction 1 and 3.7μM for Fraction 2. Tandem mass spectrometry identified 12 chromopeptide sequences potentially responsible for inhibiting M^{pro}, originating from α- and β-subunits of B-PE. These results show that chromopeptides are primary components of the B-PE digest responsible for inhibiting M^{pro} activity and could potentially be used in treating coronavirus-induced diseases in conjunction with other standard treatments.

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Evaluation of vitellogenin content in worker honey bees (*Apis mellifera* L.) during ageing

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Worker bees are characterized by seasonal lifespan variation (summer *vs.* winter bees). To assess the changes that occur with age, the content of vitellogenin (Vg) was determined in both generations of bees. This glycolipoprotein has previously been correlated with longevity and increased winter survival.[1] In this study, summer bees were sampled biweekly, while winter bees were sampled bimonthly in 2022. The content of Vg and its relative gene expression was assessed using an ELISA kit and qPCR in the abdomen and head of bees from three summer (S0, S2 and S4) and three winter (W0, W2 and W4) groups. These groups represented the beginning, midpoint, and end of the ageing process. Groups S0 and W0 were comprised of newly emerged bees. The results obtained from the abdomen showed an age-dependent increase in Vg content in both generations of bees, which is in contrast to previous findings.[2] Changes in Vg content were not recorded in the head, possibly due to the localization of Vg-producing cells.[3] In contrast, an upregulation of the Vg gene was observed in the heads of summer bees, suggesting an influence of additional regulatory mechanisms.

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References

1. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. Predictive markers of honey bee colony collapse. PLoS One 2012;7(2):e32151.
2. Amdam GV, Ihle KE, Page RE. Regulation of honey bee (Apis mellifera) life histories by vitellogenin. Elsevier Academic Press, San Diego. 2009.
3. Corona M et al. Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. Proc Natl Acad Sci 2007;104:7128-7133.

Exploring trace elements in adrenal tissues: New insights into Cushing's syndrome

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Cushing's syndrome, a complex disorder characterized by excessive cortisol production, can arise from adrenal cortex dysfunction or the prolonged use of synthetic glucocorticoids for therapeutic purposes. The incidence of Cushing's syndrome is 2-4 diagnosed cases per million each year.1 The elemental status of adrenal tissues affected by Cushing's syndrome is not elucidated thus far. Our research aims to provide initial insight by investigating the elemental composition of adrenal tissues affected by Cushing's syndrome (CST) and comparing these findings with those from healthy adrenal tissues (HAT). Obtained results revealed striking differences in elemental status between CST and HAT samples. Notably, CST tissues exhibit significantly higher levels of zinc (Zn), selenium (Se), cadmium (Cd), thorium (Th), and magnesium (Mg). Specifically, Zn concentrations are twice as high in CST tissues compared to HATs. Furthermore, CSTs Cd levels are four times higher, and Th levels were an astonishing 18 times higher in CSTs compared to HATs. Conversely, HAT samples show increased levels of nickel (Ni), sodium (Na), and potassium (K) compared to CSTs. These findings underscore the potential significance of essential trace elements such as Zn and the toxic elements Cd and Th in the pathogenesis of Cushing's syndrome. The results of this analysis shed a light into the concentrations of selected elements in the CST samples. By identifying these distinct elemental contents, this research opens new avenues for understanding the possible role of trace elements in this disorder. This initial insight into the elemental profile of adrenal tissues in Cushing's syndrome could contribute to an in-depth understanding of the pathogenesis of disease.

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References

1. Raff H, Carroll T. Cushing's syndrome: from physiological principles to diagnosis and clinical care. J Physiol 2015;593(3):493-506.

Interactions of 2-thiohydantoin derivatives with DNA and human serum albumin as a part of their cytotoxic activity against human breast adenocarcinoma

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Hydantoins are a big class of heterocyclic ureides which exhibit a wide variety of biological activities, while some of them are also commercially available drugs known for their use in medicine.[1-2] Hydantoins are known to exhibit antitumor activity in various ways and in our previous work, we have presented cytotoxic activity of zingerone 2-thiohydantoin derivatives.[3] In this continuation of our previous work, possible mechanisms of their cytotoxicity are considered by examining their interactions with DNA, as well as with human serum albumin. Using UV-Vis spectroscopy, moderate binding to CT DNA of the most active compound in the series was observed. Furthermore, significant displacement of ethidium bromide was noted, indicating an intercalation mechanism. Binding with human serum albumin was studied using fluorescence spectroscopy and the results show a strong binding affinity of the most active compound towards HSA and that the strenght of the interaction allows transfer of the compound and its release upon arrival at the target.

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References

1. Konnert L, Lamaty F, Martinez J, Colacino E. Recent advances in the synthesis of hydantoins: The state of the art of a valuable scaffold. Chem Rev 2017;117:13757-13809.
2. Cho SH, Kim S-H, Shin D. Recent applications of hydantoin and thiohydantoin in medicinal chemistry. Eur J Med Chem 2019;164:517-545.
3. Virijević K D, Stanić P B, Muškinja J M, Katanić-Stanković J S, Srećković N, Živanović M N, Šmit B M. Synthesis and biological activity of novel zingerone-thiohydantoin hybrids. J Serb Chem Soc 2022;87:1349-1358.

Diagnostic significance of miR-146a, miR-21 and miR-155 from PBMCs in gestational diabetes

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MicroRNA-based mechanisms are heavily dysregulated in disorders of glucose metabolism, including gestational diabetes (GDM) and its common successor, type 2 diabetes mellitus. Since oxidative stress (OS) and the interconnected low-level inflammation (IFM) accompany GDM and the associated pregnancy complications, we selected known OS/IFM-related microRNAs as candidates for a study on biomarker properties in GDM. The aim of the conducted research was to evaluate the diagnostic and prognostic significance of miR-146a-5p, miR-155-5p and miR-21-5p from peripheral blood mononuclear cells (PBMCs), as well as their potential as indicators of glucose and lipid status in the second/early third pregnancy trimester. PBMCs were extracted from peripheral blood samples obtained from 45 women diagnosed with GDM and 45 healthy normoglycaemic pregnant controls (pregnancy weeks 24-30). Relative quantification of mir-146a-5p, miR-155 and miR-21-5p was conducted by quantitative real-time PCR after a reverse transcription step that utilized stem-loop primers. A significant increase in the level of expression of miR-146a-5p and miR-21-5p was observed in GDM patients, compared to normoglycaemic controls ($p=0.009$ and $p=0.003$, respectively). Expression of both miR-155-5p and miR-21-5p demonstrated positive correlation with the values of anthropometric characteristics of the newborn of GDM patients, while for miR-146a an opposite direction of correlation was found in controls. However, the expression of these microRNAs did not associate with the later pregnancy or neonatal complications. MiR-146a-5p further proved indicative of the insulin resistance and iron status in GDM patients. The presented results illustrate the potential of OS/IFM-related microRNAs from PBMCs to serve as novel biomarkers in GDM.

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Bioactivity profiling of wine from Vojvodina: antioxidant potential of Bermet wine

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Bermet is a sweet wine enriched with various medicinal plants and spices, originally intended for medicinal purposes, but later produced as a regular dessert wine. This wine is a signature of Vojvodina, wine region in northern Serbia. It is well known that moderate wine consumption can have some health benefits due to the polyphenolics present in wine. The aim of this study was to compare the polyphenolic profile (HPLC analysis), content of total anthocyanins, polyphenols, flavonoids and tannins (spectrophotometric methods) in 12 Bermet wines from Vojvodina. In addition, the study aimed to evaluate their potential to inhibit lipid peroxidation (spectrophotometric method) and oxidative stress (fluorimetric method) in *in vitro* model systems. The most abundant polyphenol compounds in examined samples were gallic acid (2.3–51.3 mg/L) and catechin (to 19.5 mg/L). Stilben piceid was detected in some samples in higher content (0.3–9.9 mg/L) then resveratol (0.1–9.9 mg/L). Unexpectedly, anthocyanins were detected in only four samples. Regarding atioxidant potential, the activity range varied from 3.1 to 38.3 μg trolox equivalent/mL of wine (lipid peroxidation) and 0.75–3.65 mg trolox equivalent/mL of wine (oxidative stress). The obtained results showed that analysed wines present a valuable source of polyphenols, as well as that they have significant antioxidant potential. The presented results support further investigation of the biological activity of poorly investigated Bermet wine from Vojvodina.

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References

1. Miljić U, Puškaš V. Medicinal Plants in Bermet, Serbian Aromatic Wine. Acta Agric Serb 2012;17:83-92.

Potential biological activity of the aqueous extract of *Fumana bonapartei* Maire et Petitm. from the serpentine substrate of the Rogozna mountain

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Fumana bonapartei (Maire et Petitm.), a perennial plant from the rockrose family (*Cistaceae*) grows mainly on dry rocky or sandy sites in maquis, gardens and rock gardens[1] is widespread and endemic in the Balkans. It is known that the type of location and the time of harvest have a relative influence on the chemical composition of the plant. The aim of our research was to analyze the chemical composition of the water extract of *F. bonapartei* on the serpentine substrate of the Rogozna mountains (Kosovo and Metohija). The aerial parts were collected in June, the leaves were ground very finely to obtain the powder. The dry powder was extracted with water, the aqueous extract was frozen and lyophilized to obtain the crude dry extract. Chemical analysis of the extract revealed that *F. bonapartei* contains (tR mass): quinic acid- 1.09; gallic acid- 2.19; floramannoside (digaloylquinic acid)- 5.00; β-glucoside para-coumarin- 5.50; benzylprimeveroside- 6.04; caffeic acid glucoside- 6.58; myrizetin-3-galactoside- 6.83; quercetin-diglucoside- 7.13; rutin- 7.30; quercetin-3-galactoside-7.44 and luteolin-7-O-glucopyranoside- 8.33. The results showed that *F. bonapartei* contains biologically active components such as quercetin, rutin and quinic acid, which are involved in protecting plants against UV radiation and pathogens. The presence of a significant amount of quercetin and rutin, proven antioxidant and anticander agents[2,3] indicates the important biological activity of this species and the possibility of its potential application in pharmacy and medicine.

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References

1. Bogdanović S, Boršić I, Rešetnik I, Šegedin T. Taxonomic revision of the genus *Fumana* (Cistaceae) in Crotia. Plant Biosyst 2012;1:1-17.
2. Thi Lan Pham, et al. Antioxidant activity of an inclusion complex between rutin and β-cyclodextrin: experimental and quantum chemical studies. RSC Advances 2024;14:18330-18342.
3. Liao Y et al. Quercetin exerts an anti-tumor immune mechanism by regulating IL-6/JAK2/STAT3 signaling pathway to deplete Treg cells. Toxicon 2004;243:107747.

Antioxidative potential of *Quercus* pericarp

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The pericarp of *Quercus* species serves as a protective barrier for acorns against physical damage, pests, and diseases and prevents water loss. The aim of this study was to investigate methanol (ME) and hexane (HE) extracts from the pericarps of four different *Quercus* species (*Q. cerris*, *Q. frainetto*, *Q. pubescens*, *Q. pedunculiflora*) for their antioxidant potential using different assays. In most tests, ME had higher antioxidant potential. The highest Total Phenolic Content (TPC) among all tested samples was determined in *Q. pedunculiflora* ME (16.05 ± 0.15 mgGAE/g) while *Q. pubescens* HE had the highest TPC (1.03 ± 0.08 mgGAE/g) among HE. The Total Flavonoid Content (TFC) was higher in HE with the highest value measured in *Q. pubescens* HE (5.48 ± 0.21 mgQE/g), while among ME, the highest value was in *Q. frainetto* ME (4.37 ± 0.11 mgQE/g). The total antioxidant capacity and FRAP value, determined by phosphomolybdenum and FRAP assays were higher in ME, with *Q. pedunculiflora* ME showing the highest total antioxidant capacity (179.42 ± 6.17 mgAA/g) and the highest FRAP value (241.83 ± 2.23 µM $FeSO_4$). ME also showed better metal chelating activity compared to HE with *Q. frainetto* ME having the highest metal chelating activity (IC_{50} 253 ± 31.14 µg/ml). *Q. cerris* ME exhibited the highest scavenging capacity towards the DPPH and $OH^{\bullet}$ radicals (IC_{50} 6.54 ± 0.35 µg/ml and IC_{50} 249.43 ± 3.46 µg/ml) while *Q. frainetto* ME showed the best ability to scavenge $O_2^{\bullet-}$ and $^{\bullet}NO$ (IC_{50} 19.67 ± 1.31 µg/ml and IC_{50} 12.03 ± 0.74 µg/ml). In conclusion, *Q. pedunculiflora* and *Q. frainetto* exhibited the highest antioxidant potential in most assays.

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Long-term low-level exposure to perfluorooctanoic acid affects the survival of human endothelial cells *in vitro*

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Perfluorooctanoic acid (PFOA), a synthetic chemical known for its persistence in the environment and often referred to as a "forever chemical," is commonly used in various industrial and consumer products. PFOA is recognized for its toxic effects on the nervous, endocrine, and reproductive systems, while emerging research suggests it may also impact vascular function, potentially contributing to cardiovascular diseases. However, the mechanisms underlying PFOA-induced vascular dysfunction remain unclear. Notably, there is a lack of *in vitro* studies that accurately replicate real-life exposure scenarios. In this study, we investigated whether long-term, low-level exposure to PFOA influences the survival of human endothelial cells *in vitro*. EA.hy926 cells originating from three different cryopreserved stock vials (biological replicates) were exposed to either control conditions (0.05% DMSO) or three concentrations of PFOA relevant to human exposure (1, 10, and 100nM) and cultured independently in cell culture flasks for 12 weeks. After 6 and 12 weeks of exposure, we assessed metabolic activity using the alamarBlue™ assay, cell viability with the Trypan blue dye-exclusion test, cell proliferation through cell counting, and apoptosis/necrosis via flow cytometry for annexin V and propidium iodide. Our results showed no significant impact on metabolic activity and cell viability after 6 and 12 weeks of exposure to any of the investigated concentrations of PFOA. However, a decrease in cell proliferation was observed after 12 weeks of exposure to 100nM PFOA. In addition, a slight decrease in the number of live cells and an increase in necrotic cells were noted after 6 weeks of exposure to 1nM PFOA, though apoptosis rates remained unchanged. After 12 weeks of exposure, all concentrations of PFOA led to a reduction in live cells and an increase in necrotic cells. Furthermore, there was an increase in the number of early and late apoptotic cells, particularly noticeable in the group of cells exposed to 1nM PFOA. In summary, our findings indicate that prolonged exposure to low-level PFOA adversely affects EA.hy926 cell proliferation and negatively impacts cell survival by promoting apoptosis/necrosis.

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Impact of aromatase inhibitor therapy on plasma phospholipid profiles in women with breast cancer

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Breast cancer represents about 30% of all new cancer cases in women annually, making it the second most common cancer globally and a key priority for public health and treatment efforts. Aromatase inhibitors (AIs) are considered the standard of care for adjuvant treatment of postmenopausal women with hormone receptor-positive breast cancer (HR+), after surgery and chemotherapy. There is evidence that both tumor cells and AIs significantly affect lipid metabolism. We investigated how AI therapy affects plasma phospholipid (PL) profiles in postmenopausal women with HR+ breast cancer history. The targeted PL profiles of 30 women who had been on AI treatment for at least two years (AI group) were compared with those of 30 post-surgery breast cancer patients before initiating AI therapy (BI group). Using targeted high-throughput LC/MS lipidomic analysis, 79 PLs were analysed, including phosphatidylcholines (PC), phosphatidylserines (PS), phosphatidylethanolamines (PE) phosphatidylinositols (PI) and glycerophospholipids (PG). Significant differences were observed in 32 PLs, all with higher level in the AI group. Most upregulated PLs contained palmitic, stearic, oleic, and linoleic acids, with increased concentrations found in these PLs: PG 16:0/18:1, PG 16:0/18:2, PG 18:0/18:2, PG 18:2/18:2, PI 16:0/16:0, PI 16:0/16:1, PI 16:0/18:0, PI 16:0/18:1, PI 18:0/16:1, PI 16:0/18:2, PI 18:1/16:1, PI 18:0/18:1, PI 16:0/20:3, PI 18:0/20:2, PI 18:0/20:3, PI 16:0/20:4, PI 18:0/20:2, PI 18:1/20:3 and PI 18:0/22:4. The remaining upregulated PLs were not detected as distinct molecules with two specific fatty acid chains but as their sum. These findings suggest that AI therapy is associated with significant alterations in plasma PL metabolism, particularly involving these four fatty acids, in women with breast cancer hystory. Additional research is needed to explore the clinical implications of these lipid metabolism changes and their potential impact on treatment outcomes.

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Immobilization of chemically modified glucose-oxidase by biomineralization in ZIF-8

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Previous works showed that the characteristics of biomineralized enzymes can be improved by periodate oxidation.[1,2] Furthermore, functional groups such as histidine and aspartate that can interact with zinc and 2-methylimidazole could significantly influence the process of biomineralization and maybe increase the specific activity and stability of biocomposites. In this work, we have optimized the reaction conditions, such as the concentration of zinc and 2-methylimidazole, and determined that the best results are obtained with the lowest concentrations of zinc and 2-methylimidazole. Also, we have shown that by introducing new functional groups within the enzyme molecule, especially histidine vs aspartate, the specific activity of the biocomposite is twice as high compared to the native enzyme due to the presence of coordinative bonds. The biocomposites synthesized in this way also showed very high stability towards detergents like sodium dodecyl sulfate, which indicates that the enzyme is immobilized inside ZIF-8.

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References

1. Albayati SH, Nezhad NG, Taki AG. Rahman R. Efficient and feasible biocatalysts: Strategies for enzyme improvement. A review. Int J Biol Macromol 2024;276:133978.
2. Stanišić MD et al. Chemical modification of glycoproteins' carbohydrate moiety as a general strategy for the synthesis of efficient biocatalysts by biomimetic mineralization: The case of glucose oxidase. Polymers 2021;13:3875.

Assessing the FBS-substituting potential of algal and cyanobacterial extracts for cell-based meat cultivation

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Cultured meat technology is a form of cellular agriculture where instead of raising and slaughtering animals, meat is produced from animal cells grown in a lab. The technology currently relies heavily on the use of fetal bovine serum (FBS) in cell media; hence the production is extremely expensive and contributes greatly to ammonia and greenhouse gas emissions. Achieving successful commercialization of cell-cultured food requires the critical resolution of manufacturing costs and safety concerns. Our research is focused on identifying commercially viable and ecologically sustainable alternatives to FBS. In this study, we evaluated the potential of water-based algal and cyanobacterial extract to stimulate cell growth for meat cultivation under 90% reduced serum conditions. Extracts were compared in viability, proliferation and Trypan blue exclusion assays. In the first phase of the screening, extracts were evaluated in ZEM2S (zebra fish) cell culture in 1% FBS supplemented media. Based on their ability to either exhibit protein tolerance or to promote cell proliferation, ten extracts were selected and further assayed in QM7 cell culture. QM7 cell line (myoblasts from Japanese quail), is highly relevant for meat cultivation, because of their ability to differentiate into muscle fibers. Extracts derived from two microalgae species, *Arthrospira platensis* and *Dunaliella tertiolecta*, demonstrated the highest tolerance in cell culture, above 10 μg/ml (expressed in total protein concentration). Tolerance at a concentration of 100 μg/ml was demonstrated exclusively using a purified extract of *Arthrospira platensis*, commonly known as blue spirulina, which supported cell growth through multiple passages.

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Application of ^{31}P NMR spectroscopy in the study of the interaction of Mn(II) with polyphosphate compunds in the green microalgae *Chlorella sorokiniana*

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Metabolism of metals in microalgae and their adaptation to metal excess is of high environmental importance. Microalga *Chlorella sorokiniana* shows a capacity to adapt to high levels of Mn by accumulating it intracellularly[1]. Phosphate compounds take part in this process, but the dynamics, form and potential changes in their levels remain unclear. We applied ^{31}P NMR spectroscopy to analyse the interactions of Mn(II) excess with phosphate metabolites in *C. sorokiniana*. Microalgal cultures were incubated for 1, 24 and 72h with 1mM Mn(II), washed with Tris buffer to remove extracellular phosphates, and ^{31}P NMR spectra collected. The signals were assigned to polyphosphates (polyP; shift at -22.5ppm), pyrophosphates (PPi; shift at -4.3ppm), orthophosphates (Pi; shift at 2.4ppm), and phosphorylated sugars (PS; shifts at 4.5, 4.2, and 3.7ppm). The strongest interaction was with polyP, leading to broadening of the PolyP signal due to strong paramagnetism of Mn(II). The linewidth of polyP signal increased with the incubation time ranging from 252Hz in control to 290Hz at 1h, and 394Hz at 24h. However, after 72h the line width of the polyP signal was close to the 1h values (283Hz). These results imply that polyP acts as a transitory ligand for Mn(II) ions storage in *C. sorokiniana*, which is consistent with our previous findings[2]. Pi and PS signals did not show significant changes in width, indicating that no stable bond is formed between these compounds and Mn(II). This also confirms that alterations in the width of polyP signal did not come from the Mn-induced change in general magnetic susceptibility.

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References

1. Liu J et al. Effect of limitation of iron and manganese on microalgae growth in fresh water. Microbiology 2018;164:1514-1521.
2. Vojvodic S et al. A three-step process of manganese acquisition and storage in the microalga *Chlorella sorokiniana*. J Exp Botany 2023;74:1107-1122.

Comparison of chemical composition and antioxidant activity of *Salvia officinalis* L. ethanolic leaves extracts

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Sage (*Salvia officinalis* L.) from the Salvia genera is widely distributed all over the world and it is used in traditional medicine. Sage possesses anticancer, anti-inflammatory, anti-nociceptive, antioxidant, antimicrobial, and antimutagenic activities. Sage leaves contain different phytochemical compounds including polyphenols, tannins, flavonoids, and terpenoids1. In this study, we aimed to examine the chemical composition and antioxidant activity of sage leaf extracts obtained with 50% ethanol (50ESE) and 80% ethanol (80ESE), to evaluate the influence of extragent composition on the extract characteristics. Chemical composition was analysed by employing GC–MS analysis, and determining total phenolic content (TPC), total tannin content (TTC), and total flavonoid content (TFC)2. Antioxidant capacity was examined by DPPH assay2. Based on our results, both extracts are a rich source of polyphenols and terpenoids and have good radical scavenging activity. However, TPC and TFC were higher in 80ESE, and TTC was higher in 50ESE. GC–MS analysis showed that the content of α-thujone and L-camphor were low in both extracts, with almost equal content. DPPH assay indicated that a slightly lower IC50 value and higher antioxidant potential had 80ESE. The results indicated that the polarity of the solvent and different water content had different effects on the extraction of polyphenols from sage leaves.

Acknowledgments

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References

1. Ghorbani A, Esmaeilizadeh M. Pharmacological properties of Salvia officinalis and its components. J Tradit Complement Med 2017;7:433-444.
2. Valenta Šobot A et al. Aqueous sage leave extract attenuates inflammation and oxidant-induced genotoxicity in human peripheral blood mononuclear cells. Arh Hig Rada Toksikol 2024;75:137-146.

Poster Session 3

The expression of terminal monosaccharides on immune complexes IgG glycans in rheumatoid arthritis

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Rheumatoid arthritis (RA) is characterised by the presence of rheumatoid factors (RFs; autoantibodies against Fc region of IgG molecules) and autoantibodies against citrullinated peptides (ACPAs), mostly of IgG isotype. Both RFs and ACPAs form immune complexes (IC). IgG glycans are important for IgG proinflammatory effects and in this study, we analysed expression of terminal monosaccharides of circulating immune complexes (CIC) IgG glycans in RA. IgG from polyethylene glycol (PEG) precipitable CICs of healthy individuals and RA patients, were isolated by Protein G chromatography. The expression of IgG heavy and light chains' terminal galactose (Gal), sialic acid, N-acetyl galactosamine (GalNAc), fucose (Fuc), and bisecting GalNAc were performed with reducing SDS-PAGE followed by blotting with RCA I, SNA, GS II, UEA and succ. WGA lectins. We detected a decreased expression of Gal (39%) and increased expression of sialic acid (31%) on CIC IgG heavy chains. Due to difference in IgG light chains glycans expression, in both control and RA, two light chain bands (L1 and L2), slightly different in molecular weight were detected. CIC IgG L1 (higher molecular weight) expressed increased level of Gal (35%) and decreased level of GalNAc expression (21%) on RA, comparing to control. On the IgG L2 light chains, an increased Fuc expression was detected (24%). The difference between RA and control in the expression of other CIC IgG heavy and light chains' monosaccharides was not observed. The precise location (variable vs. constant domains) and glycan monosaccharide composition on IgG light and heavy chains in RA need further investigation using additional enzymatic and high-performance methods (HPLC/MS).

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PARP inhibitors increase NSCLC sensitivity to cisplatin via modulation of TET activity

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Non-small cell lung cancer (NSCLC) is one of the deadliest cancers worldwide. Cisplatin (CisPt) is the most used chemotherapeutic for NSCLC treatment. However, primary or acquired resistance of NSCLC to CisPt often hampers beneficial clinical effects. The usage of poly (ADP-ribose) polymerases (PARP) inhibitors for sensitizing NSCLC to CisPt has been extensively explored, but its underlying mechanisms are not completely deciphered. Knowing that decreased activity of ten-eleven translocation enzymes (TETs) is associated with poor CisPt sensitivity in lung cancer, and since TETs could be inhibited by PARP-dependent posttranslational modifications, we hypothesized that PARP inhibitors might improve NSCLC sensitivity to CisPt via enhancement of TETs' activity. Research has been conducted on human NSCLC cell line A549. Cells were cultivated for 72h in the presence of PARP inhibitor niraparib (Nir; 1.5μM), CisPt (5μM) or their combination (CisPt/Nir), while cells cultivated in the medium were used as a control. Western blot analyses revealed that Nir and CisPt, applied alone or in combination, promoted expression of TETs (TET1 and TET2). A decrease in 5-methylcytosine DNA content in CisPt/Nir-treated cells comparing to CisPt-treated cells, point to the enhancement of TETs' activity due to PARP inhibition. Finally, annexin V (AnnV) / propidium iodide (PI) staining revealed that Nir enhanced susceptibility of A549 cells to CisPt-induced apoptosis (CisPt: 69.7% AnnV+PI-, 5.7% AnnV+PI+; CisPt/Nir: 73.8% AnnV+PI-, 10.4% AnnV+PI+). Obtained results support our hypothesis on beneficial TET-mediated effects of PARP inhibitors in NSCLC treatment and imply on the importance of assessment of epigenome profile for the estimation of NSCLC resistance to platinum agents.

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Changes in CAT and PPX activity induced by sodium deoxycholate in sunflower seedlings infected with *P. brasiliense* and *S. sclerotiorum*

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The activity of two antioxidant enzymes, catalase – CAT[1] and pyrogallol peroxidase – PPX[2], was measured in leaves and roots of sunflower seedlings, as parameters of oxidative stress. Seedlings were treated first with sodium deoxycholate, and after 24 h were inoculated[3] with *Pectobacterium brasiliense* or *Sclerotinia sclerotiorum*. The aim of this research was to explore whether sodium deoxycholate acts as an elicitor in sunflower seedlings and whether applied before the attack of the mentioned patoghens can contribute to reduction of oxidative stress in sunflower seedlings. The results showed that in the seedlings infected with *P. brasiliense*, 5 mg/L deoxycholate significantly decreased the activity of CAT in roots and PPX in both leaves and roots, compared to the inoculated control (without deoxycholate treatment), while 10 mg/L deoxycholate caused a decrease only in PPX activity, in both leaves and roots. In contrast, in the seedlings infected with *S. sclerotiorum* treatment with both concentrations of deoxycholate increased the activity of these enzymes in the leaves.

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References

1. Elevarthi S, Martin B. Spectrophotometric assays for antioxidant enzymes in plants. In: Sunkar R (ed) Plant stress tolerance, Humana Press, Stillwater, Oklahoma, 2010, pp 273-280.
2. Morkunas I, Gmerek J. The posible involvement of peroxidase in defense of yellow lupine embryo axes against Fusarium oxysporum. J Plant Physiol 2007;164:185-194.
3. Duarte V, De Boer S, Ward L, De Oliviera A. Characterization of atypical Erwinia carotovora strains causing blackleg of potato in Brazil. J Appl Microbiol 2004;96(3):535-545.

Phenethyl ester of rosmarinic acid ameliorates acute respiratory distress syndrome

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Acute respiratory distress syndrome (ARDS) became a focus of intensive research due to its death toll during the Covid-19 pandemic. It is an uncontrolled and excessive inflammatory response mediated by proinflammatory molecules such as IL-6 and TNF, secreted by the cells of the immune system as a response to infection. In this study, phenethyl ester of rosmarinic acid (PERA), was tested in the model of murine ARDS induced in C57BL/6 mice by intranasal administration of polyinosinic:polycytidylic (poly(I:C)). Intraperitoneal administration of PERA ameliorated the ARDS-related histopathological changes in the lungs of poly(I:C)-induced ARDS and decreased numbers of immune cells in the lungs and draining lymph nodes (DLN). Specifically, fewer CD4+ T cells and less activated CD8+ T cells were observed in DLN. Consequently, the lungs of PERA-treated animals had fewer damage-inflicting NKT cells and NK cells. In addition, the expression and production of proinflammatory cytokines, TNF and IL-6 were downregulated in the lungs. Also, the levels of NO, determined with DAF-FM fluorocytometricaly, was reduced in PERA treated aninmals. Our results imply that PERA should be further evaluated as a potential candidate for ARDS therapy.

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Potential of isorhamnetin to inhibit expression of membrane transporters associated with cancer cell resistance development

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Isorhamnetin (ISO), a flavonoid found in various plants' leaves and fruits, achieves many pharmacological activities *in vitro* and health benefits. It has been reported that ISO inhibited the growth of various cancer cell lines, induced apoptosis, and reduced cancer cell invasion and migration, acting as an antitumor substance. However, its effects on biomarkers related to the emergence of cancer cell resistance have not been examined yet. Thus, this study aimed to investigate the effects of ISO on membrane transporters, as the main responsible for cancer cell resistance development. The mRNA expression of membrane transporters P-glycoprotein (*P-gp*), *BCRP* (*Breast Cancer Resistance Protein*), *MRP* (Multidrug Resistance Protein) *1, 2, 5,* and *8*, as well as *GSTP1* (Glutathione S-transferase P1) was monitored in DLD1 and HCT-116 colon cancer cells by qPCR methods. The level of GSH was observed calorimetrically and P-gp protein expression by immunocytochemistry. Results show that ISO inhibited mRNA expression of all investigated transporters in DLD1 cells. However, the difference in effects on both cell lines originated from the colon was observed. On HCT-116 only the expression of *P-gp* has a decreasing trend, and *MRP1* was significantly decreased. Expression of all other transporters was increased, as well as glutathione content in treated cells compared to control and *GSTP1* on transcriptional level suggesting metabolizing the ISO and ejection with GSH-dependent pumps. Since downregulation of drug transporters expression may prevent or reverse drug resistance in cancer cells the ISO, as a natural medicinal compound, could be used as a chemosensitizer to overcome membrane transporters mediated drug resistance in future experiments.

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In situ biomimetic mineralization of Laccase@ZIF

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The rapid, straightforward, cost-effective synthesis of metal-organic frameworks (MOFs) and their various potential applications and ease of implementation make them highly attractive materials. In this study, we focused on zeolitic imidazolate frameworks (ZIFs), one subgroup of MOFs that could be synthesized under mild conditions, and our goal was to immobilize laccase in ZIF-8 using the biomimetic mineralization method.[1,2] We varied few parameters during biomimetic mineralization to identify the most optimal conditions for best preservation of enzymatic activity. Zinc concentration, zinc imidazole ratio, and source of laccase enzyme were examined. The outcome of our study demonstrates that the most desirable conditions are the ones with the lowest zinc concentration, zinc imidazole ratio, and laccase from *Trametes versicolor* with 0.88 IU/g specific activity. Besides this, we discovered that washing with sodium dodecyl sulfate solution has a destructive effect in the case of laccase, which is not the case with glucose oxidase. This implies that the type of enzyme greatly impacts the formation of biocomposite, and there is an excellent area for future investigation.

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References

1. Stanišić M et al. Chemical modification of glycoproteins' carbohydrate moiety as a general strategy for the synthesis of efficient biocatalysts by biomimetic mineralization: The case of glucose oxidase. Polymers 2021;13(22):3875.
2. Kumari S, Ehrman N. R, Gassensmith J. J. Expanding past ZIF-8: Biomimetic mineralization using other MOFs. Matter 2023;6(8):2570-2573.

“Green” chemistry approach for preparation of hydrogels used for controlled drug delivery

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Thermosensitive hydrogels based on N-isopropyl acrylamide (NIPAM) were synthetized using bio-based initiator and the controlled release of encapsulated water soluble and insoluble active compounds was studied.[1] An effect of the concentration of either cross-linker, initiator and temperature of surrounding on the hydrogels swelling and controlled delivery of active compounds was investigated. With an increase in the amount of the cross-linker the degree of swelling declines resulting in lower quantities of the released drug. The increase in the initiator concentration slightly decreases the swelling degree and thus, the amount of released drug. Rise in the temperature of surrounding leads to lower swelling degrees of NIPAM hydrogels. Poorly water-soluble drug was released in higher amounts than the water-soluble one.

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References

1. Marković M et al. Novel eco-friendly initiation system based on vitamin C for energy efficicent synthesis of PMAA hidrogel used for delivery of phenolic compounds. Building a new conceptual framework for receptor heteromers. Chem Eng J 2023;459:141580.

Gaining insight into disordered and aggregated protein structure via ATR-FTIR spectroscopy

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During the last decades, Fourier-transform infrared spectroscopy (FTIR) with attenuated total reflectance (ATR) has gained a substantial role in monitoring structural changes of proteins. The conformation of the polypeptide backbone, reflecting the pattern of intramolecular hydrogen bonding, is affecting the vibrational energy of carbonyl groups and, consequently, the absorption of infrared light. Specifically sensitive to the conformational state of a polypeptide is the Amide I region (1700-1600 cm^{-1}), whose individual bands correlate with distinct secondary structures. ATR-FTIR thus provides the possibility to determine secondary structure content by deconvolution of the Amide I region, which makes it a valuable tool for investigating structures of novel proteins. Furthermore, the high sensitivity of ATR-FTIR enables discrimination between different β-sheet conformations, including intramolecular and intermolecular β-sheet. Compared to other methods for secondary structure determination, such as circular dichroism, this advantage makes infrared spectroscopy an excellent tool for monitoring aggregation processes. To highlight FTIR contribution as a reliable parameter in protein structural analysis, here we show the data regarding the determination of the secondary structure of disordered, native globular proteins, the monitoring of discrete conformational changes upon destabilizing treatments, and the monitoring of structural changes in the aggregation process.

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Antitumor potential of ethanol *Lycium ruthenicum* extract on various human cancer cell lines

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According to data, cancer is the leading cause of death worldwide, and breast cancer is the most common type of cancer in women. Colorectal carcinoma is the third most commonly diagnosed cancer worldwide with significant death rate despite all the advanced therapeutic and surgical interventions available. Chronic myeloid leukemia is a genetically heterogeneous disease that originates after sequential acquisition of mutations and genomic aberrations resulting in clonal expansion of leukemia-initiating cells. The aim of this study was to investigate the effects of six different concentrations of ethanol extract of *Lycium ruthenicum* (EELR), popularly referred to as Goji berries, on human breast cancer MDA-MB-231, colon cancer HCT-116 and myeloid leukemia K562 cell lines. The parameters of cell proliferation and oxidative/antioxidative status were measured spectrophotometrically after short-term (24h) and long-term treatment (72h). Migration potential was tested for two concentrations after 72h exposure, using Boyden chamber transwell migration assay, as well as Nrf-2 expression level by ELISA. The acquired results suggest that on the highest concentrations EELR exhibited antiproliferative effects, and cell viability was decreased in dose-dependent manner after 24h and 72h treatment. The results showed a decrease in superoxide anion radical, while nitrite concentrations and gluthatione levels were increased compared to non-treated cells. The migration index of MDA-MB-231, HCT-116 and K562 cells was reduced and the expression level of Nrf-2 was downregulated. The results indicate that EELR exerts significant antiproliferative activity. Also, EELR potentiates the sensitivity of cancer cells on deterioration effects of oxidative species making these cells vulnerable to different prooxidants. The extract reduces migratory capacity of the tested cells, suggesting its antimetastatic potential. All these data suggest significant antitumor capacity of the tested extract, making it a convenient candidate for further investiations in the development of more effective chemotherapies.

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Interaction of commonly used antioxidants with major circulating proteins: Spectroscopic and *in silico* study

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Antioxidants, used in human nutrition as food supplements, show poor bioavailability and reduced stability, so in order to utilize their beneficial effects their binding to circulatory proteins is desirable. Besides albumin and IgG, other major proteins in the circulation are fibrinogen (Fib), transferrin (Tf) and alpha-2-macroglobulin (α_2M). They are all potential candidates for antioxidant binding. Quercetin, resveratrol and dihydrolipoic acid are among the most commonly used antioxidants in human nutrition. The idea of the present study was to investigate whether these three antioxidants bind to the mentioned plasma proteins. After testing the affinity for ligands, the pair with the highest association constant was α_2M/resveratrol (4.5×10^4 M^{-1}), so this complex was chosen to be spectroscopically characterised, and subjected to docking simulation, in order to elucidate the structure of the binding site(s). The computational simulaitons revealed that α_2M possesses four potential binding sites for resveratrol, and that the formation of hydrogen bonds is crucial for binding. The binding of resveratrol to α_2M leads to mutual protection from oxidative stress and significantly increases hidrosolubility of resveratrol. Both these features serve to increase the bioavailability and bioactivity of resveratrol in the circulation.

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Our favorite spices: Is turmeric always only turmeric?

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Independently or as a key ingredient in curry spice and mustard, turmeric is a widely used spice that is added to dishes to enhance color (bright yellow), aroma and preservation. The primary active component of turmeric, curcumin, is characterized by numerous biological activities including anti-inflammatory, antioxidant, anticancer, antimicrobial and others, thus exhibiting health benefits[1]. However, the benefit could be outweighed by unexpected risk in case of spice contamination with hazards exerting toxic effects. Thus, present study raises the question: is turmeric always only turmeric? To reveal the probable contaminants of turmeric spice, data from the Rapid Alert System for Food and Feed[2], an EU system for the exchange of information on safety risks, were extracted and evaluated. Mainly as a result of official control on the market (43.5%), border control (27.4%) and company's own check (27.4%), a total of 62 notifications related to turmeric products were reported during 2011-2023, with a sharp peak registered in 2021 (19). The main contaminants identified were pesticide residues (19 cases, of which 12 recorded the presence of ethylene oxide, 7 chlorpyrifos and 1 both of them), mycotoxins (17; 15 aflatoxins and 2 ochratoxin A) and pathogenic microorganisms (13; 12 *Salmonella* and 1 co-occurrence of *Salmonella* and *Bacillus cereus*), followed by lead (5) and presence of unauthorized colors (Sudan), indicating product adulteration. As expected, raw materials mostly originated from India (71.0%). Considering that ethylene oxide and aflatoxins are potent carcinogens (IARC Group 1), lead probable (Group 2A) and ochratoxin A possible (Group 2B), while microbiological hazards cause an immediate risk for consumers health, it is not surprising that for more than two-thirds of the notifications (71.0%) the risk was deemed serious, classifying 56.4% of the consignments as warnings, while 25.8% were rejected at the border. These findings, along with the observed increase in turmeric's popularity among European consumers and despite the small amounts of turmeric added to food, point to the need to ensure the absence of health threats hidden in our favorite spices.

References

1. Verma RK, Kumari P, Maurya RK, Kumar V, Verma RB, Singh RK. Medicinal properties of turmeric (*Curcuma longa* L.): A review. Int J Chem Stud 2018;6(4):1354-1357.
2. https://food.ec.europa.eu/safety/rasff_en (Accessed: 03/08/2024).

Examination of biochemical indicators of cardiometabolic risk in patients with hypothyroidism

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Hypothyroidism is an endocrine disorder caused by decreased secretion of thyroid hormones. Studies have shown that there is an association between hypothyroidism, lipid status and coronary heart disease1. The aim of this research was to examine the differences in the parameters of glucose homeostasis and advanced lipid profile in patients with hypothyroidism and healthy individuals, as well as the independent effect of thyroxine (T4) levels on changes in lipid status. The study included 30 subjects with normal thyroid hormone status and 12 diagnosed with hypothyroidism. Blood samples (serum and plasma), taken after overnight fasting, were used for biochemical analyses. Basic biochemical parameters were determined by routine methods, T4 concentration by immunoassay, and glycohemoglobin (HbA1c) by immunoturbidimetric method. The diameter of low-density and high-density lipoprotein particles (LDL and HDL) and the relative proportion of small, dense LDLs were determined by gradient gel electrophoresis. Patients with hypothyroidism were older ($p=0.046$) and had a higher percentage of HbA1c ($p=0.042$). There were no differences in parameters of routine, or advanced lipid profile among groups. The results of multivariate regression analysis showed that T4 independently affects the concentration of total cholesterol ($p=0.025$), LDL-cholesterol ($p=0.024$) and LDL-particle diameter ($p=0.033$). In patients with hypothyroidism, T4 deficiency can independently modify size and concentration of LDL particles, thus contributing to the development of an unfavorable lipid profile, and an increase of cardiometabolic risk.

References

1. Delitala AP, Fanciulli G, Maioli M, Delitala G. Subclinical hypothyroidism, lipid metabolism and cardiovascular disease. Eur J Intern Med 2017;38:17-24.

Computational approaches to rational design isocoumarins as warheads for PROTACs against HDAC4 to treat SMA

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With spinal muscular atrophy (SMA) progression, denervation of skeletal muscles triggers overexpression of histone deacetylase 4 (HDAC4) and upregulation of Atrogin-1 and MuRF1 for structural and functional muscle protein breakdown via the myogenin-dependent pathway. SMA could be palliatively treated by targeted degradation of HDAC4 by means of proteolysis targeting chimeras (PROTACs), bifunctional drugs having warhead-linker-E3 ligase ligand topology. Innovative HDAC4 inhibitors (HDAC4Is, viz. warheads) were designed (1) by aligning 9 co-crystalized and 78 literature-available HDAC4Is through covalent docking with Vina1.2.5.; (2) by exploring their pharmacodynamics profiles through Py-CoMFA[2] 3-D QSAR models; (3) by lead optimization of the most potent co-crystalized HDAC4I with the PDB ID: 5A2S. Lead optimization endowed into several isocoumarins, feasible to be converted into PROTACs.

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Isorhamnetin activates apoptotic signaling pathways in colon cancer cells

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In recent years, colon cancer has become a serious health problem, as confirmed by the increase in its incidence and mortality. The treatment of colon cancer involves a combination of surgical resection, radiotherapy, chemotherapy, and immunomodulatory therapy; however, a large percentage of patients experience recurrence through late metastases. Many commercial cytostatics are plant-derived products, indicating the huge potential of plants in the development of new drugs in colon cancer therapy. An important fact is that plant-derived products mostly trigger apoptosis in cancer cells, which is the desired pathway of their cytotoxic effect. They also have fewer adverse effects than commercial cytostatics. Therefore, our study aimed to investigate whether the plant flavonol isorhamnetin, in addition to its numerous pharmacological properties, has a pro-apoptotic effect on SW-480 and HT-29 colon cancer cells. Expression of apoptotic biomarkers was analyzed by qPCR and immunocytochemistry. The results showed that isorhamnetin significantly increased the *Caspase 8* mRNA levels and Fas receptor protein levels in both colon cancer cell lines compared to controls, as indicators of the extrinsic apoptosis pathway. Furthermore, the increased mRNA and protein expression of Caspase 9 in both colon cancer cell lines under the influence of isorhamnetin suggests the activation of the intrinsic mitochondrial pathway, which is also confirmed by the disturbed ratio of pro- (*Bax*) and anti-apoptotic (*Bcl-2* and *Bcl-xL*) members of the Bcl-2 family. Thus, the described results indicate that isorhamnetin activates both apoptotic signaling pathways in SW-480 and HT-29 cells, making it suitable for further investigations that could contribute to the development of new therapeutic approaches for colon cancer treatment.

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Macroalga *Cladophora glomerata* as a potential source of bioactive compounds

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In this study, freshwater macroalga *Cladophora glomerata* was examined for its potential biological activities. Total phenolic content, pigments' content, antioxidant potential, antimicrobial, antibiofilm activity, and cytotoxicity of ethanol and acetone extract were evaluated. The extracts were also subjected to FT-IR analysis. Total phenolic content and pigments' content were determined spectrophotometrically. Antioxidant potential was examined by DPPH test and reducing power test. Antimicrobial and antibiofilm activities were evaluated by broth microdilution and crystal violet test, respectively, while MTT test was applied for cytotoxicity. The content of total phenolic compounds, chlorophyll *a*, chlorophyll *b*, and carotenoids varied depending on the type of extract. In ethanol extract, total phenolic content was 11.35 mgGAE/g, chlorophyll *a* was 7.18 µg/ml, chlorophyll *b* was 3.77 µg/ml, whereas carotenoids were not detected. In acetone extract, total phenolic content was 16.37 mgGAE/g, chlorophyll *a* was 10.53 µg/ml, chlorophyll *b* was 1.71 µg/ml, and carotenoids were 0.80 µg/ml. The FT-IR analysis showed that lipids, unsaturated fatty acids, proteins, carbohydrates, and phenols were present in studied algal extracts. The extracts had moderate DPPH radical scavenging activity (28.68-42.44%) and low reducing power compared with ascorbic acid. The antimicrobial activity expressed as minimum inhibitory concentrations varied from 0.31 mg/mL to 10 mg/mL. The strains of *Staphylococcus aureus* and *Bacillus cereus* isolated from food samples, as well as *S. aureus* ATCC 25923, were the most sensitive. The antibiofilm activity was tested for the first time indicating noticeable results against *S. aureus* biofilm (up to 98.70% of inhibition). In addition, the extracts showed a cytotoxic effect on choriocarcinoma (JAR) cells but without selectivity on normal fetal lung fibroblast (MRC-5). This is the first report on the biological activities of *C. glomerata* from Serbia.

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Effect of ferulic acid, hyperoside, and rutin on reactivity of human serum albumin Cys-34 thiol group

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Human serum albumin (HSA) has seven fatty acid (FA) binding sites across its three domains, which overlap with its main ligand binding sites.[1,2] FA binding can affect the reactivity of HSA's Cys-34 thiol group, influencing its antioxidant properties.[2] This study investigated how selected polyphenols affect the reactivity of HSA with bound stearic acid (S) in molar ratios S/HSA of 1:1 and 4:1. The binding of S increased the Cys-34 thiol group reactivity from 14.11×10^{-3} s^{-1} for defatted HSA to 17.49×10^{-3} s^{-1} (23.95%) for S/HSA 1:1 and 29.27×10^{-3} s^{-1} (107.41%) for S/HSA 4:1. Polyphenols ferulic acid (F), hyperoside (H), and rutin (R) altered k' values differently: increasing them in S/HSA 1:1 complexes (F for 6.3%; H for 16.18%; R for 8.82%) and decreasing them in 4:1 complexes (F for 6.94%; H for 17.81%; R for 17.13%). According to the results obtained, selected polyphenols, binding to subdomain IIA, influence Cys-34 thiol reactivity in HSA/S complexes, exhibiting both cooperative and competitive effects depending on the S/HSA ratio.

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References

1. Bhattacharya AA, Grüne T, Curry S. Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin. J Mol Biol 2000;303:721-732.
2. Pavićević ID, Jovanović VB, Takić MM, Penezić AZ, Aćimović JM, Mandić LM. Fatty acids binding to human serum albumin: Changes of reactivity and glycation level of cysteine-34 free thiol group with methylglyoxal. Chem Biol Interact 2014;224:42-50.

Poster Session 4

Exploring the potential of *Calendula officinalis* L. extract to improve benzyl alcohol's effectiveness in cosmetic products

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Benzyl alcohol is commonly used in cosmetics and pharmaceuticals but can cause side effects like skin and eye irritation, allergic reactions, and toxicity in newborns. To enhance its effectiveness and minimize its concentration, plant extracts with antimicrobial properties can be utilized. This study aimed to assess the impact of ethanolic extract of *Calendula officinalis* L. on the antimicrobial efficacy of benzyl alcohol against common cosmetic contaminants, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus brasiliensis*, and *Candida albicans*. The checkerboard assay was used to determine the minimum inhibitory concentrations (MICs) of *C. officinalis* extract and benzyl alcohol mixtures, and the fractional inhibitory concentration (FIC) indexes were used to assess synergistic effects. The total phenolics content of *C. officinalis* extract was 18.87 mg GAE/g extract, while the total flavonoid content was 9.59 mg QUE/g extract. *C. officinalis* extract showed moderate antibacterial activity with a more pronounced effect against *S. aureus* (62.5 µg/mL) and *E. coli* (31.25 µg/mL). However, in combination with benzyl alcohol, the extract did not reduce the MIC of benzyl alcohol (0.5% for bacterial strains and 0.03% for fungal strains). Based on the FIC values (1.5) for most examined strains, it can be concluded that an indifferent effect predominates, suggesting no interaction between the tested combinations of extract and preservatives. An antagonistic effect was observed with *C. albicans*. Therefore, the ethanol extract of calendula did not significantly enhance the efficacy of benzyl alcohol. However, considering that *C. officinalis* is rich in non-polar bioactive compounds, the next step would be to examine the influence of marigold oil macerate on the effectiveness of benzyl alcohol.

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Adiponectin's potential as a predictor of non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is globally the most prevalent chronic liver condition, affecting about one in four adults. It is characterized by the accumulation of triglycerides in liver cells (steatosis), which can progress to steatohepatitis, a more severe form of the disease. Adiponectin, an adipokine, is known for its inverse association with insulin resistance, lipid accumulation, and inflammation, all of which are key processes in the development of NAFLD.[1] The aim of this study was to determine whether adiponectin can predict the risk of developing steatosis. The study included 99 participants who underwent ultrasonography at Clinical Hospital Centers Zemun and Zvezdara and were divided into two groups: 50 patients with steatosis and 49 apparently healthy controls (control group, CG). Biochemical markers were determined in serum by spectrophotometry, except for C-reactive protein (CRP) by immunoturbidimetry on biochemical analysers. Adiponectin was measured by ELISA (DuoSet ELISA R&D System Europe Ltd, Abingdon, UK) according to the manufacturer's protocol. Compared to CG, patients had a higher proportion of men ($p=0.003$), higher body mass index (BMI) ($p<0.001$) and CRP ($p<0.001$) and lower high-density lipoprotein (HDL)-cholesterol ($p=0.001$). Adiponectin was lower in the patients than in the CG ($p=0.008$). Univariate binary regression analysis revealed a significant predictive power of adiponectin for NAFLD as evidenced by the following odds ratio, OR (95% Confidence Interval, CI): 0.861 (0.772-0.960) ($p=0.007$). After applying multivariate binary logistic regression analysis (adjustments were made for sex and BMI), adiponectin lost its independent significant predictive power for NAFLD, as shown by the following OR (95% CI): 0.918 (0.808-1.042) ($p=0.186$). In conclusion, adiponectin is negatively associated with the risk of developing NAFLD, but not independently of sex and BMI.

References

1. Shabalala SC et al. The effect of adiponectin in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and the potential role of polyphenols in the modulation of adiponectin signaling. Biomed Pharmacother 2020;131:110785.

Inhibitory effects of *Salvia verticillata* L. extracts on COX-1 and COX-2 enzymes: The influence of extraction methods

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This study aims to evaluate the inhibition of COX-1 and COX-2 enzymes by *Salvia verticillata* L. root extracts obtained using three distinct extraction methods: traditional ethanol extraction (TE) at room temperature for 21 days, short-duration ethanol maceration with triple one-day cycles (ME), and ultrasonic extraction (UE). All extracts were evaluated at a concentration of 50 μg/mL. Notably, all tested extracts demonstrated remarkable COX-1 enzyme inhibition. ME and UE extracts showed similar inhibition rates of 80.2 and 79.8%, respectively, while TE extract exhibited slightly lower inhibition. Rosmarinic acid, a key compound of *Salvia* species, exerted 75.3% of inhibition at a concentration of 10 μM, while the standard indomethacin (1.25 μM) achieved 85.1% of inhibition. All extracts, as well as rosmarinic acid, displayed lower inhibition of COX-2 activity. Among all the extracts, TE extract achieved the highest COX-2 inhibition (56.5%). Obtained results highlight that both, maceration and ultrasonic extraction methods, can be effective in obtaining *Salvia verticillata* extracts with high COX-1 inhibitory potential. However, TE extract demonstrated noticeable higher inhibitory effect on COX-2 activity. According to this study, *S. verticillata* extracts may be a useful source of COX-1 inhibitors, while the selection of extraction methods may be important for the development of anti-inflammatory therapeutics from this plant species.

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Cytotoxic, proapoptotic, and antiferroptotic effects of *Amanita muscaria* on choriocarcinoma cells

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The pharmaceutical industry has long harnessed nature-derived bioactive compounds to address cancer treatment challenges, particularly overcoming cancer cell resistance[2] to programmed cell death, including apoptosis and ferroptosis. The cytotoxic effect of *Amanita muscaria*[1] acetone extract on JAR choriocarcinoma cell line was evaluated using MTT assay. The IC_{50} value obtained after 24 hours of treatment was used to evaluate the potential apoptotic and ferroptotic effects[3] of the extract. The antiferroptotic effect was determined by analyzing key redox parameters ($O^{\cdot-}$ and GSH) and the relative expression of essential antiferroptotic genes (*GPX4*, *SLC7A11*, and *FSP1*). Our results indicate that the IC_{50} value after 24 hours predominantly induced a proapoptotic effect on choriocarcinoma cells, as evidenced by AO/EB staining. Both the proapoptotic and antiferroptotic effects were observed regardless of the induction of oxidative stress. This is the first report demonstrating proapoptotic and antiferroptotic effects of *A. muscaria* acetone extract on choriocarcinoma cells.

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References

1. Dushkov A, Vosahlova Z, Tzintzarov A, Kalikova K, Krizek T, Ugrinova I. Analysis of the ibotenic acid, muscimol, and ergosterol content of an *Amanita muscaria* hydroalcoholic extract with an evaluation of its cytotoxic effect against a panel of lung cell lines *in vitro*. Molecules 2023;28:6824.
2. Figueiredo L, Regis WCB. Medicinal mushrooms in adjuvant cancer therapies: an approach to anticancer effects and presumed mechanisms of action. Nutrire 2017;42:28.
3. Lei G, Zhuang L, Gan B. Targeting ferroptosis as a vulnerability in cancer. Nat Rev Canc 2022;22;381-396.

Biomineralization of two glucose oxidase mutants in ZIF-8

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The efficiency of enzyme immobilization depends on the method of immobilization as well as enzyme activity and stability. We have previously evolved two glucose oxidase (GOx) mutants, M214T and M561S, with enhanced activity and stability and optimized the conditions for their expression in *P. pastoris*.[1] In this work, we have expressed, purified, and immobilized these two GOx mutants (M214T, M561S) within ZIF-8 by biomineralization. After immobilization, biomineralized mutants were characterized, and M214T had three times higher specific activity per gram of biocomposite than the mutant M561S. Thermostability at 65°C of immobilized mutant enzymes was also determined. The obtained result confirmed the influence of the soluble enzyme-specific activity on the activity of the immobilized one.

Acknowledgements

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References

1. Kovačević G, Ostafe R, Fischer R, Prodanović R. Influence of methionine residue position on oxidative stability of glucose oxidase from Aspergillus niger. Biochem Eng J 2019;146:143-149.

Effects of long-term caloric restriction on lipid profile status and testosterone production in aged *Wistar* rats

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Here we analyzed the effect of long-term (18 months) caloric restriction (60% reduction in daily food intake) on lipid homeostasis and testosterone production during male rat aging. As it was expected, serum and testicular testosterone (T) levels were significantly reduced in intact 24-month-old rats compared to 6-month-old controls. Decreased T synthesis and production were accompanied by decreased expression of *Lhr*, steroidogenic genes/protein (*Star/StAR, Cyp11a1, Cyp17a1, Hsd3b1, Hsd17b3*), and *Insl3* expression. Pituitary expression profiles of *Lhb, Fshb Cga, Gnrhr, Prl*, and *Pomc* were not changed during aging while *Prlr* showed a significantly decreased expression level at the age of 24 months. Decrement in testosterone production was accompanied by increased serum levels of total cholesterol, LDL, and triglycerides. Serum levels of HDL and Vitamin D were not changed with aging. Caloric restriction additionally decreased T levels in the serum and testicular extracts of aged animals as well as the expression of the *Star, Cyp11a1*, and *Ldlr* in the testicular tissue without affecting the expression profile of genes responsible for *de novo* synthesis and metabolism of cholesterol (*Npc1, Hmgcr, Cyp46, Screbf1, Soat1, Abca1*). At the same time, we were able to detect elevated expression of *Lhb* and *Fshb* in the pituitary tissue. Dietary treatment decreased levels of total cholesterol, triglycerides, Vitamin D, and elevated HDL. The treatment also decreased expression level of *ApoB* and increased expression of *Hmgcr* and *Ldlr* in the liver. In line with the obtained results, it is likely that primary hypogonadism has been presented in our model, with evidence that long-term caloric restriction has changed lipid status profile and has no beneficial effects on testosterone homeostasis during rat aging.

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Dysregulation in expression of miRNA machinery genes *DICER1* and *AGO2* in endometrial adenocarcinoma

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Disruptions in the function and expression of various components involved in the biogenesis of miRNA molecules are frequently observed in various carcinomas[1]. Alterations in *DICER1* and *AGO2* expression can result in substantial changes in miRNA expression levels[2]. Endometrial cancer (EC) is the fourth most common malignancy among women in Europe, with an estimated annual prevalence of approximately 125,000 cases in 2023. Given that approximately 80% of EC cases are adenocarcinomas (EAC), our research primarily focused on investigating the gene expression of *DICER1* and *AGO2* for the early detection of EAC. To quantify gene expression through real-time qPCR, total RNA was isolated from 34 EAC tissues and 16 benign controls. The expression levels of *DICER1* mRNA were significantly downregulated in EAC tissues compared to control tissues ($p < 0.001$), whereas no difference in *AGO2* mRNA levels was observed ($p > 0.05$). Significant differences in the expression of the tested molecules were not obtained by comparing EAC type, stage, or grade. ROC curve analysis revealed the excellent diagnostic potential of DICER1 in distinguishing EAC from control tissues, with an AUC > 0.9. In contrast, the expression of AGO2 did not demonstrate effective diagnostic potential.

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References

1. Hata A, Kashima R. Dysregulation of microRNA biogenesis machinery in cancer. Crit Rev Biochem Mol 2015;51(3):121-134.
2. Huang JT, Wang J, Srivastava V, Sen S, Liu SM. MicroRNA machinery genes as novel biomarkers for cancer. Front Oncol 2014;4:113.

Effect of calcium nanofertilizer on chemical composition and antioxidant activity of strawberry fruits

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Strawberry (*Fragaria × ananassa*) is one of the most widely cultivated and consumed fruits globally. Their widespread popularity comes from a unique combination of sweet and tangy flavors, an enticing fragrance, and vibrant color. Its sensory attributes make the fruit a favorite among consumers in both fresh and processed forms, including juices, jams, and desserts. They are a rich source of vitamins, minerals, dietary fibers and bioactive compounds, including polyphenols. Previous studies on foliar application of calcium nanofertilizers on plants showed enhanced growth, root, shoot length and higher amount of biomass, but their effect on chemical composition of plants was rarely investigated. In this study, the effect of foliar application of commercial calcium nanofertilizer Fitosmart® on chemical composition and antioxidant activity of strawberry fruits was examined. Fertilizer was applied on one-year-old plants during the growth seasons in year 2023 and 2024 in concentrations of 0.3 and 0.5%. The total soluble solids (TSS), total titratable acids (TTAC) and vitamin C contents, as well as the content of quinic acid and 44 selected phenolic compounds were investigated, while antioxidant activity was evaluated by DPPH assay. Based on the results of this study, it can be concluded that Fitosmart® had no significant effect on TSSC (6.62 °Brix for control vs 5.12 °Brix for 5% Fitosmart®), TTAC (0.79% for control vs 0.69% for 5% Fitosmart®) and content of vitamin C (23.4 mg/100g FW for control vs 19.1 mg/100 g FW for 5% Fitosmart®). LC-MS/MS revealed that the most dominant compounds were ellagic acid, quinic acid, chlorogenic acid, kaempferol and astragalin. The content of these compounds was higher in fruits from strawberries treated with fertilizer compared to fruits from controls. Samples showed good antioxidant activity with IC_{50} values of 0.41 mg/mL for control and 0.22 mg/mL for 0.5% Fitosmart®, suggesting that Fitosmart® caused an increase in antioxidant activity. The overall conclusion of the study is that foliar application of calcium nanofertilizer Fitosmart® demonstrated no adverse effects on strawberry fruits. On the contrary, it enhanced the fruits' protective capacities against free radicals, increasing their resistance to environmental factors.

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Antioxidant activity of hexane and methanol acorn extracts of four *Quercus* species

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In addition to their use as food sources and timber *Quercus* species are also utilised for their medicinal properties against burns and gastrointestinal disorders. The aim of this study was to determine the antioxidant potential of hexane and methanol extracts (HE and ME, respectively) from acorns of four different *Quercus* species (*Q. cerris*, *Q. frainetto*, *Q. pubescens* and *Q. pedunculiflora*). ME showed a higher antioxidant potential than HE in most assays. The highest Total Phenolic Content (TPC) among all tested samples was determined in *Q. cerris* ME – (6.35 ± 0.04 mgGAE/g), while *Q. pedunculiflora* HE - (1.56 ± 0.06 mgGAE/g) showed the highest phenolic content among the HE. To the contrary, Total Flavonoid Content (TFC) was higher in the HE, with the highest value in *Q. frainetto* HE – (10.67 ± 0.57 mgQE/g), while among the ME the highest flavonoid content was found in *Q. pedunculiflora* ME – (2.64 ± 0.05 mgQE/g). *Q. frainetto* ME showed the highest total antioxidant capacity determined by the phosphomolybdenum assay (53.41 ± 4.91 mg AAE/g), while the highest FRAP value was determined in the ME of *Q. cerris* (10.31 ± 0.54 mMFeSO4/g) which also showed the highest metal chelating activity (IC_{50} 6.63 ± 0.33 μg/ml). The scavenging activities of ME against DPPH (*Q. cerris* ME - IC_{50} 11.95 ± 0.01 μg/ml), •NO (*Q. frainetto* ME - IC_{50} 1.79 ± 0.06 μg/ml), OH• (*Q. cerris* ME - IC_{50} 92.54 ± 0.21 μg/ml) and O_2•− radicals (*Q. pubescens* ME - IC_{50} 0.76 ± 0.44 μg/ml) were much higher than among HE of the same species. The exception was the greater ability of *Q. cerris* HE to scavenge hydrogen peroxide (IC_{50} 126.90 ± 15.13 μg/ml) compared to its ME which had the lowest IC_{50} among the ME (158.60 ± 67.79 μg/ml). Among all tested *Quercus* species, *Q. cerris* exhibited the highest antioxidant potential in most assays, followed by *Q. frainetto*, while methanolic extracts generally showed better antioxidant properties than the hexane extracts.

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Impact of chymotrypsin on physico-chemical properties of PET MNPs

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Ingestion of micro- and nanoplastic particles (MNPs) is one of the main routes of exposure for humans and MNPs presence in food and beverages is well documented.[1] To understand the impact of MNPs on human health, it is essential to understand their fate in the gastrointestinal tract (GIT). Gastrointestinal digestion has been shown to change the surface chemistry of PET MPs in vitro.[1] We examined the effect of a digestive enzyme, chymotrypsin (activity 50 U/mL), on PET MNPs in simulated intestinal fluid (SIF) after 24h of incubation at 37°C. The surface chemistry of MPs was determined through Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR), while NPs were characterized with laser diffraction. Chymotrypsin has a minor effect on the surface chemistry of PET MPs. However, there is a notable increase in the specific surface area of NPs treated with chymotrypsin compared to the controls. The increased surface area of NPs indicates changes in their morphology.

Acknowledgements

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References

1. Thodhal Yoganandham S, Hamid N, Junaid M, Duan J, Pei D. Micro(nano)plastics in commercial foods: A review of their characterization and potential hazards to human health. Environ Res 2023;236:116858.
2. Tamargo A et al. PET microplastics affect human gut microbiota communities during simulated gastrointestinal digestion, first evidence of plausible polymer biodegradation during human digestion. Sci Rep 2022;12:528.

Synthesis, characterization, and antitumor activity of alizarine derivatives

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Anthraquinone is a well-known scaffold constituting anthracycline drugs such as daunorubicin, doxorubicin, mitoxantrone, and ametantrone that are widely used for the treatment of cancer.[1] In continuation of our research,[2] we synthesized a small series of novel alizarin derivatives with differently substituted anilines. The novel synthesized compounds were characterized using IR and NMR spectroscopy. In addition to synthesis and characterization, the antitumor activity of previously and novel synthesized compounds was tested. The cytotoxicity of the investigated compounds was evaluated against two human leukemia cancer cell lines (K562 and HL-60), two solid tumor cell lines (HeLa and MCF-7) and normal human lung fibroblasts (MRC-5) using the MTT test. The obtained results show a concentration-dependent inhibitory effect of the compounds tested on the growth of cancer cells. Particularly high activity and selectivity are found for the benzyl derivative of the diacylhydrazine series which, in addition to anthraquinone, also contains a vanillin pharmacophore, and for the *p*-chloro substituted aniline containing derivative from the amide anthraquinone series.

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References

1. Lown JW. Anthracycline and anthracenedione-based anticancer agents. Amsterdam: Elsevier; 1988.
2. Todorović T, Muškinja J, Marković V. Synthesis and spectral caracterization of novel alizarin diacylhydrazines derived from vanillin. 59th Meeting of Serbian Chemical Society, Novi Sad, Serbia, 2023, Book of Abstract OH-10.107.

Insect larvae as alternative protein sources – expression of storage protein genes in non-diapausing larvae of the European corn borer *Ostrinia nubilalis* (Hbn.)

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The European corn borer (ECB), a pest insect species, has the potential to be utilized in cellular agriculture as a beneficial resource due to its high larval protein content. Insect storage proteins, high molecular mass hexamers serving as amino acid depots, could be an alternative to animal-derived proteins used in cultivated meat bioprocesses. To identify the developmental stage of non-diapausing ECB larvae that would yield the highest amount of storage proteins necessary for downstream analyses, we analyzed the relative expression of genes encoding the arylphorin subunit (*aryl*) and two other storage proteins (*store1* and *store2*), normalized to actin (*act*) and ribosomal protein S3 (*rps3*) as housekeeping genes. Total RNA was isolated from whole-body homogenates of non-diapausing 2nd/3rd, 4th, and 5th instar larvae, followed by cDNA synthesis. Finally, the relative gene expression of *store1*, *store2*, and *aryl* genes at different stages of ECB active larval development was analyzed, and the results were subjected to statistical analysis (ANOVA). The expression of *store1* and *store2* genes increases throughout larval development, with peak expression observed in 5th instar larvae. The expression of *aryl*, on the other hand, was consistently high throughout active larval development, particularly during the 4th instar, likely since individual storage protein subunits need to be synthesized in sufficient amounts to successfully arrange themselves into mature proteins. The obtained results are expected considering that non-diapausing larvae grow rapidly, feed intensively, and synthesize ever-increasing amounts of storage proteins, necessary for pupal development.

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Antimicrobial activity of *Hyssopus officinalis* L. essential oils from Montenegro and Serbia

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In this study, antimicrobial activity of essential oils extracted from the aerial flowering parts (herbs) of *Hyssopus officinalis* subsp. *aristatus* (Godr.) Nyman (Lamiaceae) collected from five different locations in Montenegro, or purchased in Serbia, were investigated. Additonally, their antibacterial activity in combination with antibiotics was studied. The antimicrobial activity against selected standard bacterial and yeast strains was investigated using the broth microdilution method. Two standard antibiotics were used for comparison: the aminoglycoside antibiotic amikacin and the cephalosporin antibiotic ceftriaxone. The antimicrobial activity of the essential oil-amikacin combination was investigated using the checkerboard assay. The main components of the essential oils were 1,8-cineole, *cis*-pinocamphone, *β*-pinene and limonene in varying quantities. Most of the essential oils tested showed no significant antimicrobial activity. However, an essential oil rich in *cis*-pinocamphone showed moderate activity against both *Staphylococcus aureus* and *Escherichia coli* (MIC = 400 μg/ml). The overall effect of the essential oils and antibiotic combinations against *E. coli* or *S. aureus* ranged from additive (FICI = 0.625) to indifferent (FICI = 1.5), depending on the source of the essential oil.

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Tissue-specific expression of peptidoglycan recognition peptides in a non-model insect species

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To initiate an immune response to microbial injury, recognition of microbes is essential. Insects, lacking adaptive immunity, identify bacteria non-specifically through pathogen-associated molecular patterns in cell walls. This recognition relies on pattern recognition receptors, including peptidoglycan recognition proteins (PGRPs). Despite their highly conserved nature, the exact functions of many PGRPs remain unclear. We investigated the tissue-specific responses of three PGRPs to bacterial infection in the European corn borer, Ostrinia nubilalis. Total RNA was isolated from non-diapausing 5th instar larvae infected with *Pseudomonas aeruginosa* and *Staphylococcus aureus*, as well as a non-infected group. Tissues involved in immune response (haemolymph, fat body, intestine, and epidermis) were dissected for analysis, with whole-body homogenates used as controls. After cDNA synthesis, the relative expression of the three PGRP genes was determined. All three genes exhibit significantly low expression levels in the haemolymph, which is rich in metabolically active proteins but lacks transcripts. In contrast, the fat body, an insect's liver analogue, shows steady gene expression even in non-infected groups, suggesting that PGRPs may be constitutively expressed. Similarly, expression levels in the epidermis remain mostly unchanged. This allows for a rapid immune response, as tissues in contact with bacteria do not require a time-window for PGRP synthesis. Notably, PGRP-A and -C showed significant expression differences in the intestine, indicating their potential role in gut immunity to prevent immune overactivation after bacteria consumption. Our findings provide a good foundation for research into part of the insect immunity related to recognition of bacteria and subsequent activation of appropriate responses. Moreover, it highlights the intricate mechanisms insects employ to manage microbial challenges effectively.

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Characterization of free and immobilized pullulanase on modified bentonite: Sustainable enzyme immobilization

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The growing demand for sustainable and cost-effective biocatalysis in the food industry has highlighted the importance of using natural materials for enzyme immobilization. Clays, such as bentonite, are naturally occurring, environmentally friendly, chemically inert, thermostable, and inexpensive resources with inherent properties that can be easily tailored to support enzyme immobilization. Despite being considered tailings due to its swelling property, bentonite from the Bogovina mine in Serbia can be repurposed as a valuable enzyme support, offering a sustainable alternative to synthetic materials. Various modifications of bentonite clays were studied, and the most appropriate support was chosen for pullulanase enzyme. Pullulanase is particularly significant in starch modification processes, such as starch saccharification and the formation of resistant starch, due to its ability to specifically hydrolyze α-1,6-glycosidic linkages in starch and oligosaccharides. The enzyme was purified using affinity chromatography (IMAC) and characterized using standard biochemical methods, including the determination of Km, Vmax, temperature and pH optima, and stability profiles. These parameters were also assessed for pullulanase immobilized on pillared bentonite clay that was subsequently activated by acid treatment. The comprehensive characterization of both free and immobilized enzyme underscores the potential of using modified natural clays for enzyme immobilization, offering significant advantages in terms of cost reduction and sustainability.

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Identification of novel ligands of human cytochromes P450 among steroidal 1,2,4,5-tetraoxanes

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Cytochrome P450 enzymes (P450s) are involved in a wide variety of biotransformations including endogenous substrates (e.g. steroids, fatty acids, prostaglandins and leukotrienes) as well as exogenous compounds (xenobiotics, as drugs or environmental toxins). P450s are responsible for most of the important reactions (drug metabolism, hormone synthesis etc.) in tissues such as liver, gastrointestinal tract, brain, lung, kidney and heart. 1,2,4,5-tetraoxanes are specific class of peroxide compounds exhibiting antimalarial and antitumor activity, and very low cytotoxicity against normal cell lines. In the present work we examined binding potency of a group of steroidal mixed 1,2,4,5-tetraoxanes in the active site of four isoforms of human cytochrome P450 (CYP7A1, CYP7B1, CYP2E1 and CYP21A2). UV-VIS spectroscopy, reconstruction of enzymatic activity and SAR analysis were used in this study. A group of compounds with high affinity to the active site of all the enzymes was found, among which 2 new CYP7A1 ligands, 1 new CYP7B1 ligand, 3 ligands for CYP2E1 and 4 for CYP21A2. For a group of molecules K_d values were in the micromolar range: 3.91μM and 4.51μM (CYP7A1), 7.1μM (CYP7B1), 2.1μM and 3.8μM (CYP2E1) and 2.6μM (CYP21A2). This is comparable with the K_d values for natural ligands – cholesterol, DHEA, arachidonic acid and testosterone. SAR analysis of novel ligands allowed to identify pharmacophore features of the molecules that are crucial for compound binding. The data obtained are of great importance for the in-depth understanding of the mechanism of the molecular recognition/interaction of human P450s and their ligands in facilitating the discovery, design and development of novel bioregulators of the enzymes.

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