

9th Workshop

FOOD AND DRUG SAFETY AND QUALITY

*September 23rd 2026, Vinča Institute of Nuclear Sciences - National Institute of the Republic of Serbia,
University of Belgrade, Belgrade, Serbia*

PROCEEDINGS

FOOD AND DRUG SAFETY AND QUALITY

9th WORKSHOP: FOOD AND DRUG SAFETY AND QUALITY

September 23rd, 2026, Belgrade, Serbia

is an international *online satellite event* of

PHYSICAL CHEMISTRY 2026

*18th International Conference on Fundamental
and Applied Aspects of Physical Chemistry*

Organized by

VINČA INSTITUTE OF NUCLEAR SCIENCES-NATIONAL INSTITUTE OF THE
REPUBLIC OF SERBIA,
UNIVERSITY OF BELGRADE
Vinča – Belgrade, Serbia



in co-operation with

THE SOCIETY OF PHYSICAL CHEMISTS OF SERBIA



Held under the auspices of the

MINISTRY OF SCIENCE, TECHNOLOGICAL DEVELOPMENT AND
INNOVATION



Organizing Committee

Chairman

Branislav Nastasijević

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Members

Tamara Lazarević-Pašti

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Nevena Todorović Vukotić

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Mirjana Čolović

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Aleksandra Bondžić

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Andreja Leskovic

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Vedran Milanković

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Sandra Petrović

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Ivana Guševac Stojanović

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

Neda Đorđević

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

International Scientific Committee

Chairman

Tamara Terzić

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade,
Serbia

Members

Bhoirob Gogoi

Assam Agricultural University,
Jorhat, Assam,
India

Christoph Unterweger

Wood K Plus—Kompetenzzentrum Holz GmbH, Linz,
Austria

Sanjin Gutić

Faculty of Science, University of Sarajevo, Sarajevo,
Bosnia and Herzegovina

Jana Klopčevska

Faculty of Technology and Metallurgy, Ss. Cyril and
Methodius University in Skopje, Skopje,
North Macedonia

Saddam Weheabby

Chemnitz University of Technology, Measurement and
Sensor Technology, Chemnitz,
Germany

Fatjon Hoxa

Faculty of Biotechnology and Food, Agricultural
University of Tirana, Tirana,
Albania

Bulent Kabak

Faculty of Engineering and Natural Sciences, Hitit
University, Corum,
Türkiye

Ludmila Čechová

Central European Institute of Technology (CEITEC),
Brno University of Technology, Brno,
Czech Republic

Vojislav Stanić

Vinča Institute of Nuclear Sciences-National Institute of
the Republic of Serbia, University of Belgrade, Belgrade,
Serbia

Marija Nišavić

Vinča Institute of Nuclear Sciences-National Institute of the
Republic of Serbia, University of Belgrade, Belgrade,
Serbia

Nataša Sarap

Vinča Institute of Nuclear Sciences-National Institute of the
Republic of Serbia, University of Belgrade, Belgrade,
Serbia

Vladana Petković

Vinča Institute of Nuclear Sciences-National Institute of the
Republic of Serbia, University of Belgrade, Belgrade,
Serbia

Stefana Spasović

University of Belgrade, Faculty of Biology, Belgrade,
Serbia

Marijana Živković

University of Belgrade, Institute of Chemistry, Technology
and Metallurgy, Belgrade,
Serbia

Milan Obradović

Vinča Institute of Nuclear Sciences-National Institute of the
Republic of Serbia, University of Belgrade, Belgrade,
Serbia

Vesna Mandušić

Vinča Institute of Nuclear Sciences-National Institute of the
Republic of Serbia, University of Belgrade, Belgrade,
Serbia

Bojana Laban

Faculty of Sciences and Mathematics, University of Priština
in Kosovska Mitrovica, Kosovska Mitrovica,
Serbia

PHYSICAL CHEMISTRY 2026

*18th International Conference on Fundamental
and Applied Aspects of Physical Chemistry*

9th Workshop

FOOD AND DRUG SAFETY AND QUALITY

September 23rd, 2026, Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Republic of Serbia

PROCEEDINGS

BELGRADE, SERBIA 2026

9th WORKSHOP: FOOD AND DRUG SAFETY AND QUALITY

PROCEEDINGS

Publisher

VINČA INSTITUTE OF NUCLEAR SCIENCES-
NATIONAL INSTITUTE OF THE REPUBLIC OF SERBIA,
UNIVERSITY OF BELGRADE
Vinča - Belgrade, Serbia

Editors

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia,
University of Belgrade, Belgrade, Serbia

Dr. Andreja Leskovac
Dr. Sandra Petrović
Dr. Tamara Lazarević-Pašti

Reviewers

Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia,
University of Belgrade, Belgrade, Serbia

Dr. Sandra Petrović
Dr. Tamara Lazarević-Pašti
Dr. Andreja Leskovac
Dr. Mirjana Čolović
Dr. Nevena Todorović Vukotić
Dr. Neda Đorđević
Dr. Branislav Nastasijević

University of Belgrade, Institute of Chemistry, Technology and Metallurgy,
Belgrade, Serbia

Dr. Marijana Živković

Design

Dr. Andreja Leskovac

Printed by

Apollo Plus d.o.o., Beograd

Print run

20 copies

ISBN

978-86-7306-184-9

https://doi.org/10.68725/FDSQ_WORKSHOP.2026.9

Proceedings of the 9th Workshop: FOOD AND DRUG SAFETY AND QUALITY



©2026 by Andreja Leskovac, Sandra Petrović, Tamara Lazarević-Pašti (eds.). Licensed under Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International Licence (CC BY-NC-ND). <https://creativecommons.org/licenses/by-nc-nd/4.0/>

BELGRADE, SERBIA 2026

CONTENTS

PL 1	PHARMACEUTICAL SOLUBILITY AS A CRITICAL DETERMINANT OF DRUG QUALITY AND PERFORMANCE <u>T. Ž. Verbić</u> , O. S. Marković, M. P. Pešić and M. M. Đurđević	1
PL 2	RAPID METHODS FOR THE DETECTION/QUANTIFICATION OF SELECTED ARTIFICIAL RADIONUCLIDES IN DRINKING WATER SAMPLES <u>N. Sarap</u> , A. Lazić, A. Rikalo, K. Pavićević, J. Nikolov, M. Janković and N. Todorović	5
IL 1	BUILDING A TALENT ECOSYSTEM FOR PERSONALISED PREVENTIVE HEALTHCARE: THE ATTRACTADRIA APPROACH <u>T. Begović</u> , E. Omeragić, A. Elezović, D. Pravdić, D. Lasić, L. Spahić, K. Balić, I. Gulišija, M. Štefanović, S. Džafić, A. Njuhović, H. Rizvanbegović and R. Gelo	13
IL 2	PLASMA ACTIVATED WATER FOR SUSTAINABLE AGRICULTURE: PLANT STRESS MITIGATION AND FOOD SAFETY <u>L. Čechová</u> , T. Vozár, M. Dostál, J. Višinková, Z. Kozáková, P. Pořízka and J. Kaiser	16
IL 3	A ONE HEALTH PERSPECTIVE ON MICROPLASTICS AND NITRO-PAHS CONTAMINATION ACROSS THE SOIL–PLANT–FOOD CONTINUUM: IMPLICATIONS FOR SOIL HEALTH, CROP QUALITY, FOOD SAFETY, AND HUMAN HEALTH <u>B. Gogoi</u>	18
IL 4	FROM PLANT BIOACTIVES TO SUSTAINABLE ACTIVE PACKAGING: NANOEMULSION-BASED STRATEGIES AND APPLICATIONS A. Aykut, M. M. Tosif and <u>G. Goksen</u>	22
IL 5	FROM MOLECULES TO MARKET: PHYSICAL CHEMISTRY TOOLS FOR FOOD INTEGRITY AND CONSUMER TRUST <u>F. Hoxha</u>	28
IL 6	BLACKCURRANT POMACE AS A SOURCE OF BIOACTIVE COMPOUNDS FOR BIOACTIVE AND SKIN-COMPATIBLE COTTON <u>A. Ivanovska</u>	30
IL 7	FOOD SAFETY IN CIRCULAR BIOECONOMY: BIOTECHNOLOGICAL INNOVATIONS FOR FUNCTIONAL AND SUSTAINABLE FOODS <u>J. Klopchevska</u>	31
OP 1	EVALUATING PHYTOTOXICITY AND GENOTOXICITY OF IBUPROFEN ON <i>Allium cepa</i> L. <u>A.-M. Domijan</u> , M. Hamplová and H. Zlámalová Gargošová	32

OP 2	PHYTOCHEMICAL ANALYSIS, DETERMINATION OF ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF LICHEN SPECIES, <i>Heterodermia diademata</i> and <i>Parmotrema tsavoense</i> <u>P. Islary</u> and B. Gogoi	37
P 1	NUTRITIONAL AND BIOACTIVE POTENTIAL OF FRUIT WINES <u>U. Čakar</u> , T. Ilić, M. Pagnacco, J. Maksimović, J. Senčanski, M. Čebela and B. Dorđević	39
P 2	WELLS-DAWSON CONTRAST AGENT CANDIDATE: EFFECT ON OXIDATIVE PROTEIN MODIFICATION M. B. Čolović, G. Gajski, M. Gerić, N. Savić, T. Parac-Vogt, D. Z. Krstić and <u>A.-M. Domijan</u>	45
P 3	HOW DO AGROECOLOGICAL PRACTICES ALTER NON-ENZYMATIC ANTIOXIDANTS IN MAIZE GRAIN? <u>V. Dragičević</u> , J. Vukadinović, M. M. Šenk, M. Simić and M. Brankov	49
P 4	COMPARATIVE ANALYSIS OF PROGESTERONE EFFECTS ON NITRO-OXIDATIVE STRESS AND PROTEIN DAMAGE IN THE RAT PREFRONTAL CORTEX AND HIPPOCAMPUS DURING CEREBRAL HYPOPERFUSION <u>I. Guševac Stojanović</u> , A. Todorović, Z. Stojanović, V. Petković, T. Zečević and D. Drakulić	53
P 5	REMOVAL OF MALATHION FROM WATER USING CARBON-BASED ADSORBENTS PREPARED FROM RABBIT LITTER WASTE T. Terzić, V. Milanković, N. Radivojević, J. Krstić, A. Jocić, <u>S. Gutić</u> , V. Aničijević, S. Brković, I. Pašti and T. Lazarević-Pašti	57
P 6	COMPARATIVE ANALYSIS OF THE MINERAL COMPOSITION OF ORGANICALLY AND CONVENTIONALLY CULTIVATED <i>Lycium barbarum</i> L. FRUITS FROM SERBIA <u>T. Ilić</u> , I. Kodranov and U. Čakar	61
P 7	NATURAL DEEP EUTECTIC SOLVENT (NADES) EXTRACTION IMPROVES POLYPHENOL YIELD AND ANTIOXIDANT ACTIVITY OF SAGE EXTRACTS <u>M. Janić</u> , A. Valenta Šobot, K. Radošević, M. Kostić, S. Brković, T. Momić and J. Filipović Tričković	65
P 8	AUTOMATED MATLAB ANALYSIS OF PORE MORPHOLOGY IN A CLAY-BASED FILTER FOR CIPROFLOXACIN REMOVAL M. Kokunešoski, J. Ružić, Z. Gojković and <u>M. Ječmenica Dučić</u>	69
P 9	EDIBLE CHITOSAN FILMS CONTAINING PROPOLIS AND <i>Fumaria officinalis</i> BIOACTIVES R. Ahmoda, M. Milošević, A. Marinković and <u>A. A. Jovanović</u>	73

P 10	THE EFFECT OF CYCLODEXTRIN ON THE STABILITY OF <i>Rosa canina</i> L. NATURAL DEEP EUTECTIC SOLVENT EXTRACT <u>A. Jovanović</u> , N. Krgović, M. Jovanović, R. Pjanović, J. Živković and K. Šavikin	77
P 11	BIOACTIVITY OF <i>Agaricus bisporus</i> AND <i>Hericium erinaceus</i> MUSHROOMS BY-PRODUCTS D. Tenji, N. Tomić, I. Vulin and <u>M. Jovanović</u>	81
P 12	DETERMINATION AND ASSESSMENT OF PESTICIDE RESIDUES IN FRUITS FROM THE SERBIAN MARKET <u>M. Kalaba</u> , A. Tasić, Ž. Nikolić, M. Čalić, Ž. Tešić and S. Blagojević	85
P 13	ASSESSMENT OF THE OXIDATIVE POTENTIAL OF COMBINED FOOD ADDITIVES UNDER ACIDIC CONDITIONS <u>M. Kostić</u> , A. Valenta Šobot, J. Filipović Tričković, M. Tošić, M. Janić and K. Radošević	89
P 14	CYTOTOXICITY OF NEEM (<i>Azadirachta indica</i>) SEED OIL AND ITS RELEVANCE FOR INHALATION EXPOSURE A. Bošković, S. Petrović, O. Medić, T. Ganić, B. Nikolić and <u>A. Leskovac</u>	93
P 15	COMPARATIVE ANALYSIS OF ANTIOXIDANT STATUS AND GROWTH OF MICROGREENS CULTIVATED UNDER HYDROPONIC CONDITIONS D. Dimitrijević, A. Bučić, D. Stanković, V. Beškoski and <u>N. Lugonja</u>	97
P 16	EFFECTS OF SELENIUM SUPPLEMENTATION ON GROWTH, NDVI, AND ANTIOXIDANT CAPACITY OF <i>Vigna radiata</i> (L.) WILCZEK MICROGREENS A. Bučić, D. Dimitrijević, S. Lekić, V. Beškoski and <u>N. Lugonja</u>	101
P 17	ASSESSMENT OF OXIDATIVE STRESS BIOMARKERS IN RAT LIVER AND SERUM FOLLOWING ZALEPLON ADMINISTRATION <u>J. Martinović</u> , D. Drakulić, A. Todorović, Z. Stojanović, T. Zečević, V. Petković and I. Guševac Stojanović	105
P 18	CONSEQUENCES OF ZALEPLON DISCONTINUATION: EVIDENCE FROM THE FORCED SWIM TEST IN RATS <u>J. Martinović</u> , T. Radukić and M. Zarić Kontić	109
P 19	ASSESSMENT OF ANTIBACTERIAL PROPERTIES OF <i>Azadirachta indica</i> SEED OIL AGAINST SELECTED PHYTOPATHOGENIC BACTERIA A. Bošković, T. Ganić, A. Leskovac, B. Nikolić, S. Petrović and <u>O. Medić</u>	113
P 20	REMOVAL OF METHYLENE BLUE FROM AQUEOUS SOLUTION USING H ₂ O ₂ -MODIFIED HYDROCHAR <u>M. Milenković</u> , M. Simić, S. Porobić-Katnić, O. Todorović and M. Marinović-Cincović	117

P 21	INTERACTION OF QUERCETIN-CAPPED NANOPARTICLES WITH TRYPTOPHAN <u>T. Momić</u>	121
P 22	DESIGN OF SALICYLATE-BASED IONIC LIQUID AQUEOUS BIPHASIC SYSTEMS FOR THE EXTRACTION OF A SYNTHETIC FOOD COLORANT E133 <u>J. Mušović, D. Tekić, A. Jocić, S. Marić and A. Dimitrijević</u>	125
P 23	COMPUTATIONAL BIOMIMETIC PROFILING OF MELATONIN'S EFFECTS IN WINE AGING <u>D. Obradović, B. Lončarević, T. Mitrović, N. Todorović Vukotić, I. Perić, A. Stanisavljević Ilić and N. Đorđević</u>	129
P 24	OBESITY-INDUCED HYPERLEPTINEMIA CONTRIBUTES TO METABOLIC AND INFLAMMATORY ALTERATIONS IN RAT SERUM <u>M. Obradović, K. Banjac, S. Zafirović and E.R. Isenović</u>	133
P 25	PHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF SUB-CHRONIC LONG-CHAIN PFAS EXPOSURE IN MALE WISTAR RATS <u>I. Perić, A. Stanisavljević Ilić, N. Todorović Vukotić, N. Đorđević, S. Pejić, M. Pantelić and V. Beškoski</u>	137
P 26	PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF FRUCTANS ISOLATED FROM SHATAVARI AND SUMA BY MICROWAVE-ASSISTED EXTRACTION <u>N. Petkova, D. Yaneva and D. Vassilev</u>	142
P 27	GENOTOXIC EFFECTS OF NEEM SEED OIL ON HUMAN LYMPHOCYTES A. Bošković, A. Leskovac, O. Medić, T. Ganić, B. Nikolić and <u>S. Petrović</u>	146
P 28	COMPARATIVE <i>IN SILICO</i> ASSESSMENT OF DRUG-LIKENESS AND ANTIOXIDANT POTENTIAL OF SELECTED DIETARY PHENOLIC ACIDS <u>N. Potkonjak, R. Karkalić and V. Anićijević</u>	150
P 29	CHOLINE CHLORIDE-ASCORBIC ACID DEEP EUTECTIC SOLVENT (DES) ALLEVIATES HYDROXYUREA-INDUCED OXIDATIVE DAMAGE IN TK6 CELLS <u>K. Radošević, M. Kostić, M. Janić, A. Valenta Šobot, S. Brković and J. Filipović Tričković</u>	154
P 30	FLUORIDE SUBACUTE SPLEEN TOXICITY IN WISTAR RATS: BENCHMARK DOSE ANALYSIS FOR DNA DAMAGE AND ESSENTIAL ELEMENTS <u>J. Radovanović, S. Kolarević, B. Antonijević, K. Baralić, Z. Bulat, E. Sudar-Milovanović and Z. Mandinić</u>	158

P 31	PREFRONTAL CORTEX NEUROMODULATOR DYSREGULATION DURING BENZODIAZEPINE WITHDRAWAL IN ADULT FEMALE RATS <u>T. Radukić</u> , J. Martinović, M. Dragić, N. Jasnić, A. Stekić and M. Zarić Kontić	162
P 32	PROCESS OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF POLYPHENOLS FROM COCOA BEAN SHELLS AND THEIR ANTIOXIDANT POTENTIAL <u>S. Rakin</u> , M. Petrović, M. Rakin, D. Zarić, D. Bezbradica, and A. A. Jovanović	166
P 33	TARTRAZINE CALIBRATION BY BRIGGS RAUSCHER REACTION AND UV/VIS METHOD: COMPARATIVE STUDY <u>J. Senčanski</u> , J. Maksimović, N. Potkonjak and M. Pagnacco	170
P 34	THE FIRST REPORT ON THE MICROSCOPIC EXAMINATION OF THE BRIGGS RAUSCHER REACTION AND ITS MODIFICATION WITH TARTRAZINE <u>J. Senčanski</u> , J. Maksimović, A. Radulović, M. Kalaba, U. Čakar and M. Pagnacco	174
P 35	FORMULATION OF FUNCTIONAL INGREDIENT BASED ON ENRICHED SKIMMED GOAT'S MILK WITH GRAPE POMACE EXTRACT USING CENTRAL COMPOSITE DESIGN <u>I. D. Sredović Ignjatović</u> , D. D. Milinčić, S. P. Stanojević, A. Ž. Kostić and M. B. Pešić	178
P 36	THE IMPACT OF HIGH-FAT DIET ON LIVER PARAMETERS AND ANTIOXIDANT SIGNALLING <u>J. Stanimirović</u> , K. Banjac, M. Obradović and E. R. Isenović	184
P 37	CHOLINIUM SALICYLATE AS A HYDROTROPIC MEDIUM FOR IMPROVED RESVERATROL SOLUBILIZATION IN WATER AND PRESERVATION OF ANTIOXIDANT ACTIVITY <u>D. Tekić</u> , J. Mušović, A. Jocić, S. Marić and A. Dimitrijević	188
P 38	PRELIMINARY <i>IN SILICO</i> ADMET EVALUATION OF A NOVEL 1,4-DIHYDROPYRIDINE DERIVATIVE: A COMPARATIVE STUDY WITH BENIDIPINE <u>S. J. Tufegđić</u> , M. B. Živković, I. T. Novaković and D. M. Sladić	192
P 39	VALORIZATION OF SPENT COFFEE GROUNDS INTO CARBON-BASED ADSORBENTS FOR CRYSTAL VIOLET REMOVAL FROM WATER T. Terzić, V. Milanković, N. Radivojević, I. Pašti, V. Anićijević, T. Lazarević-Pašti and <u>C. Unterweger</u>	196
P 40	ELEMENTAL PROFILE AND NUTRITIONAL ASSESSMENT OF DIFFERENT AVOCADO CULTIVARS <u>M. Vukomanović</u> , B. Simonović, I. Grbić, A. Krstić and N. Pantelić	200

P 41	SAFETY EVALUATION AND ANTIVIRULENCE ACTIVITY OF <i>Symphytum officinale</i> ETHANOL EXTRACT <u>S. Vuletić</u> , J. Jocković, N. Borjan, A. Ignjatijević, T. Anđić and S. Spasović	204
P 42	PORTABLE COLORIMETRIC DETECTION OF Hg ²⁺ IN WATER USING ECO-FRIENDLY PAPER-BASED SENSOR N. Radivojević, T. Terzić, S. Knežević Graovac, S. Graovac, V. Rajić, M. Milanković, N. Potkonjak, <u>S. Weheabby</u> , I. Pašti, T. Lazarević-Pašti and V. Milanković	208
P 43	ALPRAZOLAM WITHDRAWAL ALTERS DOPAMINE AND SEROTONIN LEVELS IN THE CAUDATE-PUTAMEN OF FEMALE WISTAR RATS T. Radukić, M. Dragić, N. Jasnić, A. Stekić and <u>M. Zarić Kontić</u>	212
P 44	EVALUATION OF URINARY AND SERUM RENAL BIOMARKERS FOLLOWING ACUTE MICROPLASTIC EXPOSURE IN MALE RATS <u>T. Zečević</u> , Z. Stojanović, D. Drakulić, A. Todorović, F. Veljković, V. Petković and I. Guševac Stojanović	216
P 45	STEROIDAL SEMICARBAZONES VERSUS ETHYL- CARBAZATES: DRUG-LIKENESS AND ADMET PROFILE <u>M. B. Živković</u> , S. J. Tufegdžić, N. O. Đorđević and I. T. Novaković	220
P 46	STRUCTURE-PROPERTY RELATIONSHIP IN STEROIDAL HYDANTOINS: <i>IN SILICO</i> EVALUATION OF DRUG-LIKENESS AND ADMET PROFILES <u>M. B. Živković</u> , R. Z. Pavlović, P. B. Stanić and B. M. Šmit	224

PHARMACEUTICAL SOLUBILITY AS A CRITICAL DETERMINANT OF DRUG QUALITY AND PERFORMANCE

T. Ž. Verbić¹, O. S. Marković², M. P. Pešić¹ and M. M. Đurđević²

¹*University of Belgrade – Faculty of Chemistry, Studentski trg 12–16, 11000 Belgrade, Serbia. (tatjanad@chem.bg.ac.rs)*

²*University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.1

ABSTRACT

In pharmaceutical research, solubility plays a key part in the assessment of pharmacokinetic risks. Poor drug absorption, reduced efficacy, excessive metabolism, and adverse reactions are frequently related to issues of drug solubility. During early discovery research at pharmaceutical companies, many thousands of molecules are considered. Most are rejected due to perceived unfavorable properties. Pharmaceutical solubility along with permeability link drug quality with its bioavailability and overall performance. For ionizable drugs, solubility depends on pH, ionic strength, ionization, self-association, complex formation, and solid-phase transformations. Studies demonstrate that apparently simple solubility measurements may conceal multiple aqueous and solid-state equilibria. Our research findings obtained within the physicochemical framework developed by Serajuddin and Avdeef, demonstrate that rigorous solubility measurement and mechanistic analysis are essential for salt selection, formulation design, dissolution testing, and reliable assessment of drug quality and performance.

INTRODUCTION

Solubility is often introduced in pharmaceutical development as a single numerical property: the amount of drug that dissolves in a specified medium at a specified temperature [1]. This description is useful, but for many drug substances it is incomplete. A measured solubility is the observable result of several coupled processes, including dissolution of a particular solid form, ionization, association of dissolved species, complex formation with components of the medium, and possible precipitation or conversion to another solid phase. Consequently, the reported value can depend strongly on experimental conditions and on whether equilibrium has actually been established.

The importance of this issue increases as drug discovery produces a larger proportion of molecules with low aqueous solubility. Poor solubility may limit

dissolution in the gastrointestinal tract and thereby reduce or make variable the fraction of an orally administered dose that becomes available for absorption. Solubility is therefore connected not only with formulation development but also with the reproducibility of drug performance. A compound can have favorable pharmacological potency while remaining difficult to develop as a reliable medicine because its physical form and aqueous behavior are inadequately understood.

The objective of this contribution is to illustrate, through selected studies, why pharmaceutical solubility should be considered a mechanistic property rather than a single number. The examples will demonstrate how careful solubility science can contribute to rational drug quality assessment and formulation design.

DISCUSSION

A majority of the new chemical entities (NCE) that emerged as potential drug candidates in pharmaceutical development during the past 2-3 decades are practically insoluble solids consisting of ionizable molecules [2, 3]. Interpreting their solution properties in the course of measuring solubility as a function of pH is of crucial importance as the pH range within human body goes from 1-3 (gastric pH) to 5-8 (intestinal pH). Although solubility determination seems simple and straightforward, optimal experimental design to measure the aqueous equilibrium solubility of an ionizable substance requires a number of critical considerations [4, 5]. The factors affecting the quality of the obtained results are numerous: temperature, purity, and the composition of the starting compound, type and composition of buffer in use, ionic strength, amount of solid phase in excess, amount of substance to total solution volume ratio, carbon dioxide percentage, mixing and sedimentation time, phase separation method, chemical composition and properties of the solid phase in equilibrium with the solution, etc. A multitude of methods can be found in the literature, but today's druglike molecules can pose special challenges, sometimes not adequately addressed in practice. The published literature is replete with incomplete/ambiguous and potentially misleading examples of solubility reports, frequently documented inconsistently.

Based on above considerations, detailed and precise study of compound solubility and the dissolution rate is at the core of the development of bioavailable drug dosage forms and clinically effective pharmaceutical products that would dissolve in gastric and intestinal fluids after oral administration or not precipitate in the blood after intravenous dosing. Furthermore, high quality experimental data can have a major impact on the quality of statistical methods to predict solubility of druglike molecules in an effort to anticipate solubility requirements and risks during structural design in the early stages of drug discovery [1, 6].

A *white paper* on consensus recommendations for improving data quality in equilibrium solubility measurement of ionizable drugs was published [5]

emphasizing the importance of precise solubility measurements. Optimal experimental design requires a number of critical considerations. Briefly, the aqueous medium to which the substance is added ideally contains a buffer to help attenuate changes in pH due to sample addition. To this medium, the substance may be added in a number of ways: (a) as a highly supersaturated aqueous solution, (b) as a saturated hot solution, (c) as a concentrated solution in a water-miscible organic solvent, or (d) as a solid – which may or may not be in a thermodynamically stable crystalline form. The first three approaches are precipitation routes, while the fourth involves dissolution and is most commonly used and best understood. With sufficient added solid and enough time, chemical equilibrium is established, where the crystalline solid substance is in dynamic equilibrium with its solution form. As the dissolution proceeds, the solid phase can re-precipitate as another form with another structure (polymorphic transitions) and even another composition (salt disproportionation, formation of crystal solvates, new salt forms, or loss of solvate from the starting solid). In solution, the substance may undergo ionization, self-aggregation (forming water-soluble dimers, trimers, etc., or micelles in the case of amphiphilic substances), or complexation with some of the buffer components. Both phases of the solubility process are important to consider, as measured solubility depends both on the solid and the solution forms.

Since, as mentioned earlier, most compounds currently synthesized in drug discovery laboratories have poor aqueous solubility, a variety of methods to increase solubility and/or dissolution rate, and thereby increase their bioavailability, have been developed. Still, most of them, like particle size reduction, salt formation, conversion to amorphous form, solid dispersion, and solubilization in lipids or lipid-surfactant mixtures have their own limitations. To mitigate some of the above limitations, a novel method of drug solubilization in aqueous media by acid–base interaction has been developed in the research group of Prof. Serajuddin, [3, 7-11]. This novel approach of greatly increasing the solubility is based on interaction of a model low-soluble basic drug in an aqueous medium with acidic species that would not normally form salts with it.

CONCLUSION

Solubility is one of the most consequential physicochemical properties of a drug substance, but it is also one of the easiest to oversimplify. Our studies [10-14] demonstrate that pH-dependent solubility may involve several simultaneous aqueous and solid-state equilibria, including ionization, complexation, self-association, common-ion effects, and salt interconversion.

For pharmaceutical development, the key message is straightforward: a solubility value is meaningful only when the chemical system that produced it is understood. Rigorous measurement, appropriate equilibrium modeling, and solid-state characterization transform solubility from a descriptive number into a

mechanistic tool. Such an approach can improve salt selection, formulation design, dissolution testing, and interpretation of drug quality and performance.

In this sense, pharmaceutical solubility is not merely a preformulation parameter; it is a central link between the physicochemical identity of a drug and its behavior as a medicine.

Acknowledgement

The authors gratefully acknowledge the scientific collaboration with Prof. Dr. Abu T. M. Serajuddin (Dept. of Pharmaceutical Sciences, St. John's University, Queens, NY, USA), Dr. Alex Avdeef (In-ADME Research, Walnut Creek, CA, USA) and Prof. Dr. Martin Kuentz (School of Life Sciences, University of Applied Sciences and Arts, Northwestern Switzerland, Muttenz, Switzerland, and the contributions of other colleagues from their institutions to the studies discussed in this plenary contribution. This work was minimally financially supported by the Ministry of Science, Technological Development, and Innovation of Republic of Serbia (Contract numbers: 451-03-33/2026-03/200168, 451-03-33/2026-03/ 200026).

REFERENCES

- [1] A. Avdeef, Predicting Solubility of New Drugs, Handbook of Critically Curated Data for Pharmaceutical Research, 1st Ed., CRC Press, 2024.
- [2] C. A. Lipinski, *J. Pharmacol. Toxicol. Methods*. 2000, 44, 235 – 249.
- [3] T. Parikh, A. T. M. Serajuddin, *Pharm. Res.* 2018, 35, 127 – 136.
- [4] A. Avdeef, Absorption and Drug Development, 2nd Ed., Wiley-Interscience, Hoboken NJ. 2012.
- [5] A. Avdeef, E. Fuguet, A. Llinàs, C. Ràfols, E. Bosch, G. Völgyi, T. Verbić, E. Boldyreva, K. Takács-Novák, White paper, *ADMET&DMPK*, 2016, 4, 117 – 178.
- [6] A. Llinas, A. Avdeef, *J. Chem. Inf. Model.* 2019, 59, 3036 – 3040.
- [7] S. Singh, T. Parikh, H. K. Sandhu, N. H. Shah, A. W. Malick, D. Singhal, A. T. M. Serajuddin, *Pharm. Res.* 2013, 30, 1561 – 1573.
- [8] A. Shah, A. T. M. Serajuddin, *Int. J. Pharm.* 2015, 484, 172 – 180.
- [9] T. Parikh, H. K. Sandhu, T. T. Talele, A. T. M. Serajuddin, *Pharm. Res.* 2016, 33, 1456 – 1471.
- [10] I. A. Topalović, O. S. Marković, M. P. Pešić, M. H. Kathawala, M. Kuentz, A. Avdeef, A. T. M. Serajuddin, T. Ž. Verbić, *Pharmaceutics* 2024, 16(12), 1545.
- [11] M. H. Kathawala, H. P. Kandagatla, A. Avdeef, M. Kuentz, T. Ž. Verbić, A. T. M. Serajuddin, *Int. J. Pharm.* 2026, 700, 127063.
- [12] O. S. Marković, M. P. Pešić, A. Avdeef, A. T. M. Serajuddin, T. Verbić, *ADMET&DMPK*, 2025, 3128.
- [13] O. S. Marković, N. G. Patel, A. T. M. Serajuddin, A. Avdeef, T. Ž. Verbić, *Mol. Pharm.* 2022, 19(2), 710 – 719.
- [14] O. S. Marković, M. P. Pešić, A. V. Shah, A. T. M. Serajuddin, T. Ž. Verbić, A. Avdeef, *Eur. J. Pharm. Sci.* 2019, 133, 264 – 274.

RAPID METHODS FOR THE DETECTION/QUANTIFICATION OF SELECTED ARTIFICIAL RADIONUCLIDES IN DRINKING WATER SAMPLES

N. Sarap¹, A. Lazić², A. Rikalo³, K. Pavićević¹, J. Nikolov⁴, M. Janković¹ and
N. Todorović⁴

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of
Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade,
Serbia (natasas@vin.bg.ac.rs)*

²*University of Belgrade, Vinča Institute of Nuclear Sciences – National Institute
of the Republic of Serbia, Department of Physical Chemistry, Mike Petrovića
Alasa 12-14, 11351 Belgrade, Serbia*

³*University of Novi Sad, Faculty of Technical Sciences, Department of
Fundamental Disciplines in Engineering, Trg Dositeja Obradovića 6, 21000
Novi Sad, Serbia*

⁴*University of Novi Sad, Faculty of Sciences, Department of Physics, Trg
Dositeja Obradovića 4, 21000 Novi Sad, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.2

ABSTRACT

In the context of the expanding nuclear energy industry and the increasing need for emergency preparedness, the development of rapid, reliable, and efficient methods for environmental radioactivity monitoring is of great importance. This study evaluates rapid methods for the determination of the beta-emitting fission products ^{90}Sr and ^3H in drinking water samples collected from different cities across the Republic of Serbia. Measurements were performed using an ultra-low-background liquid scintillation counter. The activity concentrations of ^{90}Sr and ^3H in all analyzed samples were below the minimum detectable activities (MDA) of 0.55 Bq/L and 2.4 Bq/L, respectively. In addition, the pH and electrical conductivity of the water samples were determined to characterize their basic physicochemical properties. The study also systematically evaluates the detector counting efficiency and MDA, demonstrating the applicability of the proposed methods for fast radiological emergency response.

INTRODUCTION

Systematic monitoring of environmental radioactivity is mandated by the Law on Radiation and Nuclear Safety and Security [1]. Continuous monitoring provides essential information on changes in environmental radioactivity levels and enables the early detection of radiological contamination resulting from

natural or anthropogenic sources. The obtained data serve as the basis for assessing the impact of ionizing radiation and evaluating the associated radiation exposure of the population.

In the event of a radiological emergency, such as a nuclear reactor accident or the use of nuclear weapons, decision-makers require rapid and reliable analytical data to support timely actions aimed at minimizing both immediate and long-term consequences. Among the radionuclides of greatest concern is the long-lived fission product ^{90}Sr , owing to its relatively long half-life (28.8 years), chemical similarity to calcium, and high radiotoxicity resulting from its daughter radionuclide ^{90}Y [2]. Following its release into the environment, ^{90}Sr can be transferred through various environmental pathways into the food chain and ultimately accumulate in the human body, particularly in bone tissue [3].

Another radionuclide of particular importance is tritium (^3H), a radioactive isotope of hydrogen with a half-life of 12.3 years [4]. Tritium is produced naturally in the upper atmosphere through interactions between cosmic rays and atmospheric nitrogen [5], while anthropogenic sources include atmospheric nuclear weapons testing, nuclear power plants, and nuclear fuel reprocessing facilities [6]. In the environment, tritium is predominantly present as tritiated water (HTO), which behaves chemically like ordinary water. Consequently, it is readily incorporated into living organisms and distributed throughout body fluids. Although tritium emits low-energy beta particles and is generally considered to have relatively low radiotoxicity, its biological significance arises from its ability to replace stable hydrogen in water and organic molecules, thereby contributing to internal radiation exposure [7].

Considering the importance of radionuclide determination during emergency situations, there is a need for methods that combine short analysis time with sufficient sensitivity and reliability. Therefore, the objective of this study was to evaluate two rapid liquid scintillation counting methods for the determination of ^{90}Sr and ^3H in drinking water and to assess their applicability for emergency preparedness. The methods were jointly developed and applied by the Department of Radiation and Environmental Protection, Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade (VINS) and the Department of Physics, Faculty of Sciences, University of Novi Sad (DPFS).

EXPERIMENTAL

This study included 10 drinking water samples taken from the following cities in the Republic of Serbia: Subotica (W1), Novi Sad (W2), Ruma (W3), Zrenjanin (W4), Belgrade (W5), Kragujevac (W6), Zlatibor (W7), Zaječar (W8), Niš (W9), and Vranje (W10). The tap water samples (about 1.5 L) were collected during June 2026.

Prior to radionuclide analysis, all water samples were characterized by measuring their pH and electrical conductivity. The pH was measured using an InoLab pH meter equipped with a SenTix-81 glass electrode (WTW, Germany), while electrical conductivity was determined with a Cond 7110 conductometer fitted with a TetraCon 325-x conductivity sensor (WTW, Germany).

The applied methods for determination of ^{90}Sr and ^3H require minimal sample preparation. For ^{90}Sr analysis, 20 mL of water sample was transferred directly into a 20 mL polyethylene vial and measured by the Čerenkov counting technique. For ^3H determination, 8 mL of the water sample was mixed with 12 mL of Ultima Gold LLT scintillation cocktail (PerkinElmer, Finland) in a 20 mL polyethylene vial. The prepared samples were stored in the sample holder of the liquid scintillation counter for 6 h prior to measurement to allow complete decay of chemiluminescence and photoluminescence.

The determination of ^{90}Sr activity by the Čerenkov counting method was performed according to the procedure described in Ref. [8], while ^3H activity was determined using the direct method reported in Ref. [9]. Distilled water was used as the background sample for ^{90}Sr measurements, whereas deep-well water (distilled raw water) served as the background for ^3H analysis. The counting efficiencies were determined using certified ^{90}Sr and ^3H standard solutions supplied by the Czech Metrology Institute (Prague, Czech Republic). The ^{90}Sr standard solution (Serial No. 060117-654097) had a certified activity of 1.015 kBq on 30 January 2017, while the ^3H standard solution (Serial No. 060117-713448) had a certified activity of 1.068 kBq on the same reference date. Background samples and calibration standards were prepared following the same procedure as the analyzed samples to ensure identical measurement conditions.

Measurements were carried out using a QuantulusTM 1220 ultra-low-background liquid scintillation counter (PerkinElmer, Finland). Each sample was measured for a total counting time of 300 min, divided into three consecutive counting cycles. Background samples were measured immediately before each measurement series. Spectral acquisition and data processing were performed using the WinQ and EASY View software packages.

The activity concentrations of ^{90}Sr and ^3H were calculated according to the procedures described in Refs. [4,8]. The minimum detectable activity (MDA) was evaluated using the Currie equation [10]. According to Currie's criterion, the MDA represents the lowest activity concentration that can be detected with a 95% confidence level under the specified measurement conditions.

RESULTS AND DISCUSSION

The activity concentrations of ^{90}Sr and ^3H , as well as the pH value and electrical conductivity (EC) for the investigated drinking water samples are presented in Table 1. Comparative results of determining the activity concentrations for the

mentioned artificial radionuclides in the VINS and DPFS are also given in Table 1.

Table 1. The activity concentrations of ^{90}Sr and ^3H , pH values and electrical conductivity in investigated tap waters.

Sample	VINS/ DPFS	^{90}Sr (Bq/L)	^3H (Bq/L)	pH	EC at 25°C ($\mu\text{S}/\text{cm}$)
W1	VINS	< 0.55	< 2.4	7.4	547
	DPFS	< 0.32	< 2.2		
W2	VINS	< 0.55	< 2.4	7.6	591
	DPFS	< 0.32	< 2.2		
W3	VINS	< 0.55	< 2.4	7.9	691
	DPFS	< 0.32	< 2.2		
W4	VINS	< 0.55	< 2.4	7.8	688
	DPFS	< 0.32	< 2.2		
W5	VINS	< 0.55	< 2.4	7.8	483
	DPFS	< 0.32	< 2.2		
W6	VINS	< 0.55	< 2.4	6.9	408
	DPFS	< 0.32	< 2.2		
W7	VINS	< 0.55	< 2.4	7.1	185
	DPFS	< 0.32	< 2.2		
W8	VINS	< 0.55	< 2.4	7.3	553
	DPFS	< 0.32	< 2.2		
W9	VINS	< 0.55	< 2.4	7.2	441
	DPFS	< 0.32	< 2.2		
W10	VINS	< 0.55	< 2.4	7.1	145
	DPFS	< 0.32	< 2.2		

As shown in Table 1, the electrical conductivity of the analyzed drinking water samples ranged from 145 to 691 $\mu\text{S}/\text{cm}$. These values are well below the maximum recommended limit of 2500 $\mu\text{S}/\text{cm}$ specified by the Regulation on the Quality and Other Requirements for Natural Mineral Water, Spring Water, and Bottled Drinking Water [11]. The measured pH values ranged from 6.9 to 7.9, corresponding to neutral and slightly alkaline waters, which are typical characteristics of drinking water.

The activity concentrations of ^{90}Sr and ^3H in all investigated samples were below their respective minimum detectable activities. Consequently, these radionuclides were not detected in the investigated drinking water samples, indicating the absence of measurable anthropogenic contamination by ^{90}Sr and ^3H at the time of sampling.

The derived activity concentrations for ^{90}Sr and ^3H in drinking water are 4.9 Bq/L and 100 Bq/L, respectively [12]. The MDAs achieved in this study (0.55

Bq/L for ^{90}Sr and 2.4 Bq/L for ^3H) are considerably lower than these guidance levels, demonstrating that the proposed methods provide sufficient sensitivity for both routine environmental monitoring and rapid radiological emergency assessment.

The quality assurance of the procedures was verified through the analysis of certified reference materials (water matrix) supplied by the International Atomic Energy Agency (IAEA). The results independently obtained by VINS and DPFS agreed with the certified values within the 95% confidence interval, confirming the accuracy, reliability, and reproducibility of the applied methods.

The determination of counting efficiency and the dependence of the MDA on counting time are presented in Figures 1 and 2 for ^{90}Sr (VINS) and in Figures 3 and 4 for ^3H (DPFS). The obtained efficiency values and low MDAs demonstrate that the proposed methods are suitable for screening of drinking water samples, providing adequate sensitivity for emergency response as well as routine environmental radioactivity monitoring.

As shown in Figs. 1 and 2, in the VINS, the detection efficiency for ^{90}Sr was 34.2(3)%, while achieving an MDA of 0.55 Bq/L for a distilled water sample measured for 300 min using the Čerenkov counting technique. For ^3H (in the DPFS, Figs. 3 and 4), the detection efficiency was 36.33(15)%, yielding an MDA of 2.2 Bq/L for a 20 mL sample prepared with an 8:12 sample-to-scintillation cocktail volume ratio and measured under identical counting conditions.

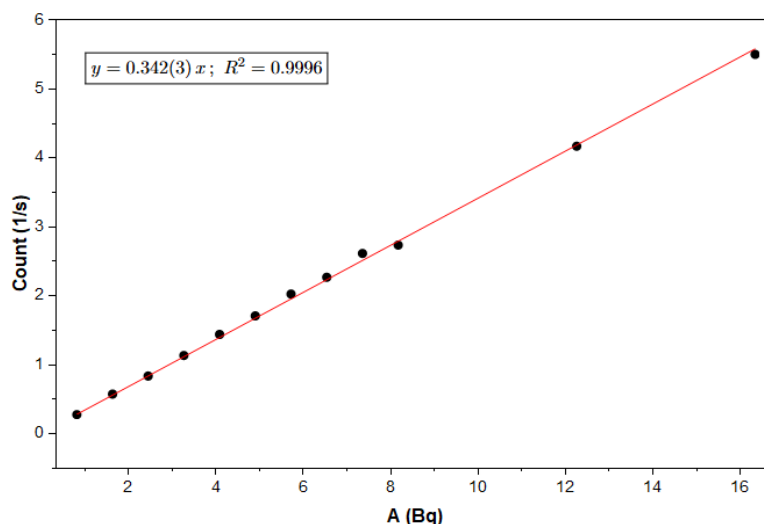


Figure 1. Determination of the detection efficiency for ^{90}Sr .

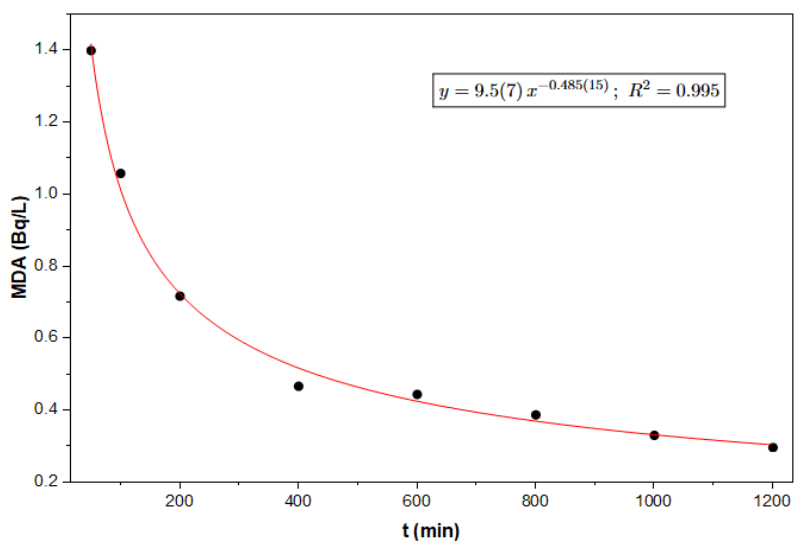


Figure 2. The MDA dependence on measurement time for 20 mL distilled water in a vial.

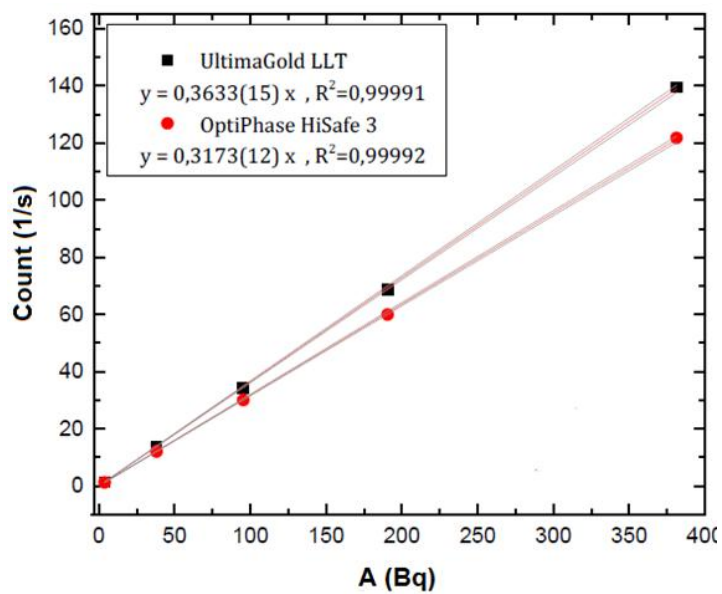


Figure 3. Determination of the detection efficiency for ^3H .

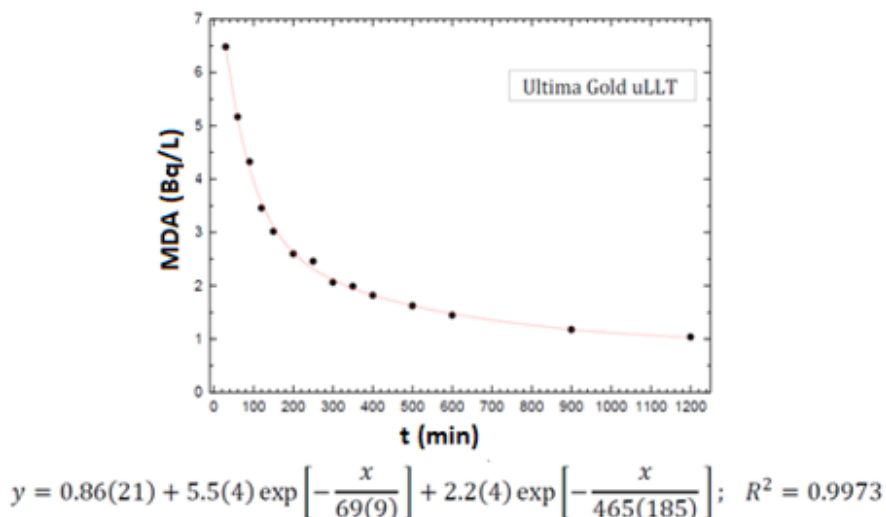


Figure 4. The MDA dependence on measurement time for 8:12 (distilled raw water: cocktail) volume ratio in a 20 mL vial.

CONCLUSION

Rapid screening methods based on liquid scintillation counting were successfully evaluated for the determination of ^{90}Sr and ^3H in drinking water samples. The applied procedures require minimal sample preparation, allowing considerably faster analysis than conventional radiochemical methods. None of the analyzed drinking water samples contained detectable concentrations of ^{90}Sr or ^3H , confirming their radiological safety and compliance with current regulatory recommendations. The determined maximum MDAs of 0.55 Bq/L for ^{90}Sr and 2.4 Bq/L for ^3H are well below the corresponding guidance levels for drinking water, demonstrating the suitability of the proposed methods for rapid screening applications. The results also confirmed the reliability of the applied methodologies through quality assurance procedures based on certified reference materials. The established protocols provide a practical strategy for the best response in eventual radiological incidents and can be easily incorporated into national programs for assessing the radiological safety of drinking waters.

Acknowledgement

The authors are grateful for the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-33/2026-03/200017, 451-03-33/2026-03/200125 and 451-03-34/2026-03/200125).

REFERENCES

- [1] Official Gazette of the Republic of Serbia, Number 95/2018 and 10/2019, 2019.
- [2] S. Holmgren Rondahl, H. Ramebäck, *Applied Radiation and Isotopes*, 2018, 140, 87-95.
- [3] Z. Zhou, H. Ren, L. Zhou, P. Wang, X. Lou, H. Zou, Y. Cao, *Molecules*, 2023, 28, 90.
- [4] J. Nikolov, N. Todorovic, M. Jankovic, M. Vostinar, I. Bikit, M. Veskovic, *Applied Radiation and Isotopes*, 2013, 71, 51-56.
- [5] M. J. Madruga, M. M. Sequeria, A. R. Gomes, *Journal of Research of the National Institute of Standards and Technology*, 2000, 105, 541.
- [6] L. J. Pujol, J. A. Sanchez-Cabeza, *Journal of Radioanalytical and Nuclear Chemistry*, 1999, 242, 391-398.
- [7] C. Varlam, I. Stefanescu, I. Faurescu, I. Vagner, D. Faurescu, D. Bogdan, *Romanian Journal of Physics*, 2011, 56, 233-239.
- [8] N. Todorović, I. Stojković, J. Nikolov, B. Tenjović, *Journal of Environmental Radioactivity*, 2017, 169-170, 197-202.
- [9] I. Jakonić, N. Todorović, J. Nikolov, I. Krajcar Bronić, B. Tenjović, M. Vesković, *Radiation Physics and Chemistry*, 2014, 98, 69-76.
- [10] L. L. A. Currie, *Analytical Chemistry*, 1968, 40, 586-593.
- [11] Official Gazette of Serbia and Montenegro, Number 53/05, 2005.
- [12] Official Gazette of the Republic of Serbia, Number 36/2018

BUILDING A TALENT ECOSYSTEM FOR PERSONALISED PREVENTIVE HEALTHCARE: THE ATTRACTADRIA APPROACH

T. Bego¹, E. Omeragić¹, A. Elezović¹, D. Pravdić², D. Lasić³, L. Spahić⁴, K. Balić⁵, I. Gulišija⁶, M. Štefanović⁷, S. Džafić⁸, A. Njuhović⁹, H. Rizvanbegović¹⁰ and R. Gelo¹¹

¹University of Sarajevo - Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina (tamer.bego@ffsa.unsa.ba)

²School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

³Ministry of Education, Science, Culture and Sports of Herzegovina-Neretva Canton, Mostar, Bosnia and Herzegovina

⁴Verlab Institute, Sarajevo, Bosnia and Herzegovina

⁵Bloomteq, Sarajevo, Bosnia and Herzegovina

⁶Genos / GlycanAge, Zagreb, Croatia

⁷Sestre milosrdnice University Hospital Center, Zagreb, Croatia

⁸Ministry of Civil Affairs of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina

⁹Ministry for Science, Higher Education and Youth of Sarajevo Canton, Sarajevo, Bosnia and Herzegovina

¹⁰SERDA, Sarajevo, Bosnia and Herzegovina

¹¹Croatian Science Foundation, Zagreb, Croatia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.3

ABSTRACT

AttractAdria is a Horizon Europe initiative that strengthens research and innovation ecosystems in the Adriatic region by improving research careers and capacity in personalised preventive healthcare. Its ecosystem-based approach combines institutional reform, research-support hubs, mentorship, training, mobility and academia-industry collaboration. Transferable skills are developed alongside competencies in biomarkers, genomics, glycomics, digital health, artificial intelligence, and clinical and translational research. By linking widening-region institutions with international partners, healthcare providers, industry, policymakers and funders, the project aims to improve career development, talent circulation and the region's ability to translate multidisciplinary research into responsible preventive-health innovation.

Keywords: personalised preventive healthcare, research careers, talent ecosystem, capacity building, AttractAdria

INTRODUCTION

Personalised preventive healthcare requires institutions able to support researchers across disciplines and sectors. In widening countries of the Adriatic region, fragmented career support, uneven access to training and mobility, and weak links among academia, healthcare, industry and policy can restrict translation of research into prevention-oriented solutions.

AttractAdria addresses these constraints as an ecosystem challenge rather than a series of isolated training activities.

PROJECT FRAMEWORK

AttractAdria is a Horizon Europe Coordination and Support Action coordinated by the University of Sarajevo and implemented by partners from Bosnia and Herzegovina, Croatia, Ireland and Norway. Its four components are institutional reform aligned with the European Charter for Researchers and the Human Resources Strategy for Researchers, research-support hubs and mentorship, transferable and specialised training, and mobility, networking and academia-industry exchange.

Training connects project management, proposal writing, science communication, intellectual property, open science, FAIR data and digital tools with artificial intelligence in healthcare, genomics, glycomics and translational research. Two-way knowledge exchange couples international expertise with regional talent and healthcare contexts. These capacities are also relevant to food and drug safety and quality research.

IMPLEMENTATION AND DISCUSSION

Initial implementation includes mapping and gap analysis, capacity-building and stakeholder dialogue. Intended outputs are stronger career-support structures, mentored early-career researchers, mobility mechanisms, cross-sector collaborations and greater readiness for international proposals. Open science, gender equality, public engagement and responsible data practices are cross-cutting requirements.

Pairing skill development with institutional reform is a strength: training cannot compensate for unclear career pathways or weak support services, while policy documents have little effect without staff, researcher uptake and resources. Since long-term career and health outcomes are not yet established, evaluation should separate activity counts from outcomes such as institutional adoption, new collaborations, proposal participation and researchers' perceived career support. Sustainability beyond project funding is therefore a key test.

CONCLUSION

AttractAdria aligns institutional policy, support services, advanced training, mobility and cross-sector partnerships. If implemented and sustained, this model can improve talent retention and the translation of multidisciplinary research into prevention-oriented health innovation.

Acknowledgement. This work is supported by the European Union's Horizon Europe research and innovation programme under Grant Agreement No. 101216942 (AttractAdria). Views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

PLASMA ACTIVATED WATER FOR SUSTAINABLE AGRICULTURE: PLANT STRESS MITIGATION AND FOOD SAFETY

L. Čechová¹, T. Vozár¹, M. Dostál¹, J. Višinková¹, Z. Kozáková², P. Pořízka¹
and J. Kaiser¹

¹*Central European Institute of Technology, Brno University of Technology,
Purkyňova 123, 60200 Brno, Czech Republic (Ludmila.Cechova@vut.cz)*

²*Faculty of Chemistry, Brno University of Technology, Purkyňova 118/464, 612
00 Brno, Czech Republic*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.4

ABSTRACT

Plasma Activated Water (PAW) has attracted increasing interest across scientific disciplines due to its potential to promote plant growth, mitigate environmental stress, and inactivate microorganisms. During plasma–liquid interactions, reactive oxygen and nitrogen species (RONS) generated by plasma are transferred into the aqueous phase, resulting in changes in physicochemical properties, including decreased pH and increased conductivity, as well as enrichment with reactive species such as hydrogen peroxide (H₂O₂), nitrite (NO₂[−]), and nitrate (NO₃[−]). Nitrogen species can provide nutrients that support plant growth, while H₂O₂ and acidification contribute to the antimicrobial properties of PAW [1]. Depending on the intended application, PAW can be produced from different water sources, including distilled water, tap water, and wastewater [2].

This work investigates the effects of PAW on plant growth and elemental uptake under different stress conditions, including heavy-metal contamination, salinity, or drought. Plants were cultivated under controlled conditions and exposed to selected abiotic stressors. Plant growth and stress-related responses were evaluated, with particular emphasis on elemental uptake and redistribution within plant tissues. The spatial distributions of selected elements were determined using Laser-Induced Breakdown Spectroscopy (LIBS). LIBS is an optical emission spectroscopy technique based on laser-induced plasma and enables rapid multi-element analysis with spatially resolved detection [3].

The study contributes to understanding the interactions among PAW, abiotic stress, and elemental uptake in plants and evaluates the potential of plasma-based water treatment as a tool for improving plant resilience and supporting safer and more sustainable agricultural production.

Acknowledgments:

This work was supported by the project 8X25016 (Ministry of Education, Youth and Sports, Czech Republic). JK and PP acknowledge the support of grant no. FSI-S-26-9044 (Brno University of Technology).

REFERENCES

- [1] T. Vozár, et al. *J. Phys. D: Appl. Phys.* 58.18, (2025), 185206.
- [2] L. Čechová, et al. *J. Phys. D: Appl. Phys.* 58, (2025), 115204.
- [3] T. Brennecke, L. Čechová, et al. *Spectrochim. Acta Part B: At. Spectroscopy*, 205, (2023), 106684.

A ONE HEALTH PERSPECTIVE ON MICROPLASTICS AND NITRO-PAHS CONTAMINATION ACROSS THE SOIL–PLANT–FOOD CONTINUUM: IMPLICATIONS FOR SOIL HEALTH, CROP QUALITY, FOOD SAFETY, AND HUMAN HEALTH

B. Gogoi

Bioremediation Technology Research Group, Department of Botany, Bodoland University, Assam, 783370, India

*DBT-NECAB, Assam Agricultural University, Assam, 785013, India
(bhoirobgogoi1998@gmail.com)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.5

ABSTRACT

In the era of the “Plasticene” the extensive production, use, and disposal of plastic materials have emerged as major environmental challenges, particularly because of the progressive fragmentation of discarded plastics into microplastics (MPs). MPs are generally described as plastic particles whose sizes ranging from 0.1- 5 mm and are formed by the breakdown of larger plastic materials through abiotic and biotic processes, such as ultraviolet radiation, temperature variations, mechanical abrasion, and microbial activity and enzyme degradation. As a result, the ubiquitous presence and increasing accumulation of MPs in soils-particularly agricultural soils-has emerged as a major global environmental problem. The annual input of MPs into agricultural soils in the United States has been estimated to be over 70 kilotons, highlighting the significant scale of plastic buildup in terrestrial ecosystems. Similarly, approximately 110-730 kilotons of MPs are added annually to agricultural soils across Europe and North America. Elevated MP concentrations have also been reported in several Asian countries, including Japan, South Korea, and China, further demonstrating the global concern of this emerging contaminant. Due to their small size, high surface-area-to-volume ratio, and hydrophobic surface properties, MPs can interact with and transport a wide range of contaminants, including phthalates, heavy metals, persistent organic pollutants (POPs), microorganisms, antibiotic-resistance genes, and pharmaceuticals. Such contaminant-carrying behavior is often described by the “Trojan Horse” mechanism, whereby MPs can facilitate the transport and potential redistribution of associated contaminants within environmental compartments. Among these co-contaminants, nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are an important and potentially harmful group of organic pollutants. Nitro-PAHs are PAH derivatives formed due to the nitration

of PAHs, either during combustion or via atmospheric and environmental transformation reactions. They are formed mainly from combustion-related sources, including burning fossil fuels, coal, petroleum-derived materials, and other carbonaceous substances. Their environmental importance arises from their persistence, toxicity, and capacity to generate reactive intermediates and oxidative stress, which may contribute to adverse biological effects. The co-occurrence of MPs and nitro-PAHs in agricultural environments may create complex contaminant interactions and severe effects. Because of their hydrophobic surfaces, MPs may adsorb nitro-PAHs and act as mobile carriers or environmental reservoirs, influencing their transport, persistence, partitioning, and bioavailability. In contrast, environmental aging and surface modification of MPs can alter their adsorption ability and interactions with soil elements. As a result, the presence of MPs can change the environmental fate and bioavailability of nitro-PAHs, potentially influencing how they interact with soil microbes, plant roots, and crops. MPs can alter soil structure, porosity, water-holding capacity, nutrient availability, and microbial community composition, whereas nitro-PAHs can provide extra toxicological stress to soil microorganisms. As a result, co-exposure may impair microbial activities and biochemical processes that are critical for maintaining soil fertility and resilience. Changes in microbial community structure and activity can subsequently influence the degradation and transformation of both contaminants, creating a dynamic feedback relationship between pollutant exposure and soil biological functioning.

The consequences of these changes extend beyond soil and directly affect plant health and crop quality. Plant roots are continuously exposed to contaminated soil and interact with MPs and nitro-PAHs via adsorption, absorption, and rhizosphere-mediated mechanisms. MPs and associated nitro-PAHs may have an impact on root development, nutrient absorption, photosynthesis, cellular redox equilibrium, and overall plant growth. Exposure can stimulate the development of reactive oxygen species (ROS), leading to oxidative stress and the activation of antioxidant defense systems. Furthermore, interactions between MPs and nitro-PAHs may modify contaminant bioavailability and influence their accumulation and mobility throughout plants. This raises particular concern when contaminants reach edible tissues. Ultimately, the soil-root interface can affect crop quality, productivity, and nutritional safety. Furthermore, the accumulation of MPs and nitro-PAHs in edible parts of plants presents a possible pathway for dietary exposure in people and animals, hence connecting environmental pollution and food safety. Following exposure, these pollutants may interact with biological tissues, accumulating or remaining in adipose tissue. Growing data suggest that exposure to MPs and nitro-PAHs can cause serious health consequences, including oxidative stress, inflammation, endocrine disruption, reproductive toxicity, gut microbiota dysbiosis, DNA damage, genotoxicity, and neurotoxicity. These effects may arise through complex interactions involving cellular oxidative imbalance, inflammatory signaling,

mitochondrial dysfunction, and disruption of normal metabolic and physiological processes.

These possible consequences highlight the need to assess MPs and nitro-PAHs contamination from both an environmental standpoint and an integrated food-safety and One Health framework. Addressing this emerging challenge requires the development of integrated, sustainable, and evidence-based mitigation strategies that can simultaneously reduce contaminant burden and restore ecosystem health. In addition to improving soil biological functioning and encouraging plant growth, biological interventions-particularly microbial bioremediation and phytoremediation approaches offer sustainable and eco-friendly ways to lower the bioavailability and remediation of MPs and nitro-PAHs. Similarly, biochar, nanomaterials, and other cutting-edge soil amendments also enhance the immobilization of contaminants, alter their bioavailability, and aid in the restoration of deteriorated soil qualities. Future studies should integrate advanced omics tools-such as metagenomics, transcriptomics, proteomics, and metabolomics with phytoremediation and microbial bioremediation approaches in order to improve these strategies' efficacy and mechanistic understanding. Together, these methods can deliver a broad view of shifts in microbial community composition, functional genes, gene expression patterns, protein responses, and metabolic pathways linked to contaminant degradation and plant-microbe interactions. Furthermore, coupling multi-omics datasets with artificial intelligence (AI) and machine-learning methods may help identify the key microbial taxa, metabolic pathways, biomarkers, and environmental drivers that control contaminant transformation and removal. Technology-enabled remediation frameworks could support the design of more targeted, efficient, and predictive strategies to mitigate the adverse effects of MP and nitro-PAH contamination. These technical measures should be reinforced by public education, responsible plastic management, sustainable agricultural practices, and effective environmental policies that reduce contaminant inputs at their source. However, the success of these mitigation strategies should not be assessed solely on the basis of reductions in total contaminant concentrations. A comprehensive evaluation should also consider changes in contaminant bioavailability, soil microbial diversity and functionality, plant uptake and translocation, crop quality, residual toxicity, ecosystem recovery, and potential transfer of contaminants into the food chain. Such a holistic assessment will be essential for ensuring that remediation strategies are not only effective in reducing contamination but also contribute to long-term soil health, food safety, environmental sustainability, and human health within a One Health framework.

REFERENCES

- [1] X. Kong, J. Zhang, Q. Ji, C. Li, X. Chen, X. Cao, F. Zhu, S. Yang, S. Li and H. He, *Chemosphere*, 2023, 320, 138050.
- [2] C. He, X. Cao, Z. Tu, Y. Lyu, K. Tang, J. Lin, H. Su, S. Hu, X. Zhang, Z. Liu and Z. Zhou, *Environ. Chem. Ecotoxicol.*, 2026, 8, 497–510.
- [3] Z. Shafi, I. Bose, S. Husain, S. Sharma and R. Singh, *Food Biosci.*, 2026, 81, 109113.
- [4] M. Rasta, N. S. Lashkaryan, X. Shi, M. S. Taleshi, A. H. Vayghan, A. Ahmadi, M. A. Kakakhel, J. Manke, L. Liu and Y. Wu, *Food Chem.*, 2026, 509, 148610.
- [5] Y.H. Lai, T.Y. Song, Y.H. S. Wu, C.C. Liao, J.-T. Lin and D.-J. Yang, *Food Res. Int.*, 2026, 232, 118877.
- [6] W. Zhu, Y. Chen, X. Hong, Y. Chen, W. Chen, Z. Ouyang, S. Gui and R. Fan, *Toxicol. Environ. Health Sci.*, 2026, 18, 303–318.

FROM PLANT BIOACTIVES TO SUSTAINABLE ACTIVE PACKAGING: NANOEMULSION-BASED STRATEGIES AND APPLICATIONS

A. Aykut, M. M. Tosif and G. Goksen

Department of Food Technology, Vocational School of Technical Sciences at Mersin Tarsus Organized Industrial Zone, Tarsus University, 33100, Mersin, Türkiye (guldengoksen@tarsus.edu.tr)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.6

ABSTRACT

In this study, an active film was prepared using an extract from the fruit of *Arbutus andrachne* L. and pumpkin seed protein loaded into the nanoemulsion. To make the whole plant extracts, *A. andrachne* fruits were extracted in water, methanol, and ethanol with a fruit-to-solvent ratio of 1:15 (w/v) for 10 min by ultrasonication, and the total phenolic content (TPC) of the extracts was determined. Among the tested solvents (water, 4.20 mg/L; methanol, 5.94 mg/L; ethanol, 3.67 mg/L), the extract prepared in methanol was found to have the highest TPC, and this extract was used for further preparation of nanoemulsion. The obtained nanoemulsion-loaded extract was then added to a matrix of pumpkin seed proteins forming films. The morphology of SEM revealed a relatively continuous film with a compact shape and the presence of dispersed particulate domains and no significant cracks or defects in the film, which indicated good incorporation and compatibility of the nanoemulsion in the protein matrix. Overall, based on the results, it is concluded that the active film fabricated using pumpkin seed protein and *A. andrachne* phenolic extract-loaded nanoemulsion has the potential for use as an active ingredient in the development of active films for food packaging applications.

Keywords: Nanoemulsion; Plant-extract; Protein; Active packaging

INTRODUCTION

With the growing amount of petroleum-based plastic packaging waste, there has been great interest in packaging materials that are biodegradable and renewable in food packaging. In this context, protein, polysaccharide, and lipid-based edible and biodegradable films have emerged as promising materials to offer packaging functionality and minimize the reliance on conventional plastics. Among the biopolymer materials, protein films are particularly interesting by the presence of several functional groups in proteins that could provide three-dimensional cohesive networks via hydrogen bonding, hydrophobic interactions,

electrostatic interactions, and disulphide bonding. Furthermore, protein matrices can be used to carry active ingredients such as antioxidants and antimicrobial agents, thereby providing, in addition to simple food containment, other active-packaging functions [1,2].

Increasingly, plants proteins are the focus of study for the sustainable development of films because of their renewable origin as well as the possibility of valorisation of agricultural resources. Pumpkin (*Cucurbita* spp.) seeds are an underused agro-industrial resource with high protein, lipids, minerals and other bioactive compounds contents. The pumpkin seed protein exhibited desirable functional properties including emulsifying, foaming, water and oil binding and film forming properties. Based on these properties, pumpkin seed protein can be considered as a good source of biodegradable packaging materials. A previous study has shown the feasibility of using pumpkin seed protein in the preparation of edible films. Pumpkin seed protein has additionally been studied for its applications in packaging for foods in recent years. The hydrophilic nature and limited functional performances of protein films in high moisture environments are still challenges, which can be addressed by the addition of suitable functional additives and delivery systems at the nanoscale [3,4].

The incorporation of plant-derived phenolic compounds into active films is an attractive approach for developing active packaging because phenolics possess antioxidant and, in many cases, antimicrobial properties. *Arbutus andrachne* L., commonly known as the Greek strawberry tree, produces edible fruits that are recognized as a rich source of phenolic compounds and antioxidants. Serçe et al. [5] reported substantial variation in the total phenolic content of *A. andrachne* fruits collected from different regions of Turkey, confirming their potential as a natural source of bioactive compounds. Nevertheless, the effective incorporation of plant extracts into protein-based films can be challenging because phenolic compounds may exhibit limited stability, poor compatibility with the polymer matrix, and uncontrolled release.

Nanoemulsion-based delivery systems offer a promising strategy to overcome some of these limitations. Nanoemulsions provide finely dispersed droplets with a high interfacial area and can facilitate the incorporation, stabilization, and controlled delivery of bioactive compounds in food systems. Their application as carriers for active compounds has received considerable attention because small droplet dimensions can improve physical stability and facilitate interactions between encapsulated compounds and surrounding food matrices [6]. Incorporation of such systems into protein-based films may therefore improve the dispersion of bioactive extracts within the film-forming network while providing a potential route for active-compound delivery.

Therefore, the present study aimed to develop a pumpkin seed protein-based active film incorporating an *A. andrachne* fruit extract-loaded nanoemulsion. Water, methanol, and ethanol were initially evaluated as extraction solvents based on total phenolic content, and the most effective solvent was selected for

subsequent nanoemulsion preparation. The developed active film was subsequently characterized by scanning electron microscopy to evaluate its surface morphology and the incorporation of the extract-loaded nanoemulsion within the pumpkin seed protein matrix.

EXPERIMENTAL

Arbutus andrachne L. extract was prepared using different solvents, including water, methanol, and ethanol. Briefly, 1:15 (w/v) fruits and solvent were blended with a laboratory blender. The slurry was subjected to ultrasonication for 10 min, followed by filtration [7]. Total phenolic content (TPC) of each solvent was determined, and based on the results, a suitable solvent was used for further processing. A plant extract was incorporated into the nanoemulsion system at 5 % (w/v), and a plant-extract-loaded nanoemulsion was formed. The nanoemulsion was homogenized under the optimum conditions to achieve a stable and uniform dispersion. The pumpkin seed protein (3 % w/v) was added to 100 mL of distilled water and stirred continuously until a uniform dispersion of protein was achieved. The plant-extract-loaded nanoemulsion was mixed with pumpkin seed protein dispersion in a ratio of 1:0.25 (v/v) protein dispersion to the nanoemulsion and was dispersed in the protein dispersion with continuous stirring for uniform dispersion. Glycerol was used as a plasticiser at 30% (w/w) of the protein content, and its mixing was carried out for 1 h. The sonication process was performed for 10 min at 37 °C to achieve better dispersion of the nanoemulsion and minimize aggregation. This was then transferred to glass Petri dishes and allowed to dry for 24 hours at 45 °C. The films were dried and then carefully removed from the casting surface and stored in controlled conditions for subsequent characterization. The resulting films were named plant-extract-loaded pumpkin seed protein nanoemulsion films (PE-PPF). Scanning electron microscopy of the film was conducted to study the surface morphology of film [8].

RESULTS AND DISCUSSION

The total phenolic content (TPC) of *Arbutus andrachne* L. fruit extracts obtained using different extraction solvents was evaluated to select the most suitable solvent for subsequent nanoemulsion preparation. The TPC varied considerably depending on the extraction solvent. Among the tested solvents, the methanolic extract exhibited the highest TPC (5.94 mg/L), followed by the aqueous extract (4.20 mg/L) and ethanolic extract (3.67 mg/L), as shown in Figure 1. The higher TPC observed in the methanolic extract indicates that methanol was more effective in extracting phenolic compounds from *A. andrachne* L. fruits under the applied extraction conditions. Therefore, methanol was selected as the extraction solvent for further preparation of the extract-loaded nanoemulsion and its incorporation into potato protein-based films. The higher TPC obtained with methanol can be primarily attributed to its polarity and its ability to solubilize a

broad range of low- and medium-molecular-weight phenolic compounds. Phenolic acids, flavonoids, and their glycosides possess different degrees of polarity, and consequently, solvent polarity strongly influences their extraction and recovery. Previous studies have demonstrated that methanol can provide high recovery of polar and moderately polar phenolic compounds because of its favorable interactions with hydroxyl-containing phenolics. Differences in solvent polarity can therefore result in substantial differences in both the quantity and composition of extracted phenolics [5].

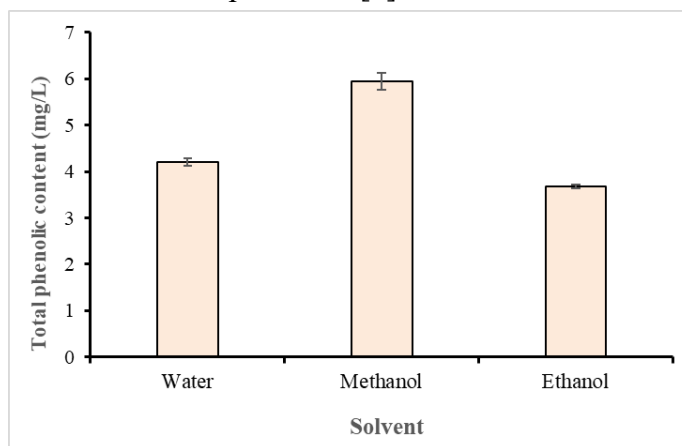


Figure 1. Total phenolic content of *A. andrachne* L. fruits determined under different extraction conditions.

The surface morphology of the protein-based film incorporated with *Arbutus andrachne* L. extract-loaded nanoemulsion was examined using scanning electron microscopy (SEM) at 2000× magnification (Figure 2). The SEM micrograph revealed a relatively continuous and compact film matrix with a predominantly homogeneous surface morphology. Small dispersed particulate features were observed throughout the matrix, which may be associated with the presence of the nanoemulsion droplets and/or interactions between the nanoemulsion components and the protein matrix. Importantly, no extensive cracks, large pores, or pronounced structural discontinuities were evident, suggesting that the nanoemulsion was reasonably well distributed within the protein-based film-forming network.

The relatively compact morphology may be attributed to the formation of intermolecular interactions between protein chains and the components of the extract-loaded nanoemulsion. Protein-based films are generally formed through the development of hydrogen bonding, hydrophobic interactions, electrostatic interactions, and other non-covalent associations among protein molecules during drying. Incorporation of nanoemulsions can further modify these interactions because the dispersed oil phase and emulsifier provide additional interfaces within the protein matrix. When the nanoemulsion droplets are

sufficiently stabilized and uniformly dispersed, they can become embedded within the continuous protein network without producing large-scale phase separation, resulting in a relatively continuous film structure. The absence of prominent cracks and large voids is particularly relevant because major structural discontinuities can indicate poor compatibility between the dispersed phase and the film-forming matrix and may negatively affect the mechanical and barrier properties of the resulting film. In the present micrograph, the relatively smooth and compact morphology indicates good compatibility between the protein matrix and the extract-loaded nanoemulsion under the conditions used for film preparation. The small dispersed features observed on the surface may represent nanoemulsion-derived domains [8].



Figure 2. Scanning electron microscopy (SEM) of nano-emulsion-loaded pumpkin seed protein film.

CONCLUSION

The present study demonstrated the feasibility of developing an active biodegradable film based on pumpkin seed protein incorporating an *Arbutus andrachne* L. fruit extract-loaded nanoemulsion. Among the solvents evaluated for extraction, methanol resulted in the highest total phenolic content (5.94 mg/L), compared with water (4.20 mg/L) and ethanol (3.67 mg/L), and was therefore selected for subsequent nanoemulsion preparation. Incorporation of the extract-loaded nanoemulsion into the pumpkin seed protein matrix resulted in a relatively continuous and compact film morphology, as observed by SEM, without prominent cracks or large structural defects. The presence of dispersed domains indicated successful incorporation of the nanoemulsion within the protein matrix and suggested satisfactory compatibility between the active delivery system and film-forming network. Overall, the findings indicate that *A. andrachne* extract-loaded nanoemulsion can be effectively incorporated into pumpkin seed protein films, providing a promising approach for the development of biodegradable and potentially active food-packaging materials. Further studies evaluating mechanical, barrier, antioxidant, antimicrobial, release, and food-preservation properties are recommended to establish the practical application of the developed film.

Acknowledgement

The development of the INTACTBIOPACK project (PRIMA/2023/Section 2 Call) is funded that the Partnership for Research and Innovation in the Mediterranean Area (PRIMA) supported by the European Union H2020 programme. This study was supported by Scientific and Technological Research Council of Turkey (TUBITAK) under the Grant Number 124N037. The authors thank to TUBITAK for their supports. This paper was produced from the master's thesis of Abdurrahman Aykut, under the supervision of Dr. Gulden Goksen.

REFERENCES

- [1] Liu, M., Zhang, X., Wei, A., Li, H., Zhang, H., Zheng, L., ... & Wang, J. (2024). Protein-based active films: Raw materials, functions, and food applications. *Comprehensive Reviews in Food Science and Food Safety*, 23(2), e13302.
- [2] Zubair, M., Mujahid, M., Shahzad, S., Rauf, Z., Hussain, A., Ayyash, M., & Ullah, A. (2025). Exploring protein-based films and coatings for active food packaging applications: A comprehensive review. *International Journal of Biological Macromolecules*, 146070.
- [3] Lalnunthari, C., Devi, L. M., & Badwaik, L. S. (2020). Extraction of protein and pectin from pumpkin industry by-products and their utilization for developing edible film. *Journal of Food Science and Technology*, 57(5), 1807-1816.
- [4] Xie, Y., Wang, Y., Jin, X., Zhang, X., & Yang, R. (2025). Pumpkin seed proteins: The potentially alternative protein supplements for food applications. *Foods*, 14(22), 3969.
- [5] Serçe, S., Özgen, M., Torun, A. A., & Ercişli, S. (2010). Chemical composition, antioxidant activities and total phenolic content of *Arbutus andrachne* L.(Fam. Ericaceae)(the Greek strawberry tree) fruits from Turkey. *Journal of Food Composition and Analysis*, 23(6), 619-623.
- [6] Salvia-Trujillo, L., Soliva-Fortuny, R., Rojas-Graü, M. A., McClements, D. J., & Martín-Belloso, O. (2017). Edible nanoemulsions as carriers of active ingredients: A review. *Annual review of food science and technology*, 8, 439-466.
- [7] Zannou, O., Tosif, M. M., Eliuz, E. A. E., Goksen, G., & Tahergorabi, R. (2026). Enhanced extraction and functional properties of 3-deoxyanthocyanidins from red sorghum leaf sheaths via ultrasound-assisted extraction with natural deep eutectic solvent. *Ultrasonics Sonochemistry*, 107881.
- [8] Tosif, M. M., Dhull, S. B., Ali, N., Iqbal, M., Alasmari, A. F., & Chawla, P. (2024). *Cordia dichotoma* fruit mucilage and flaxseed oil nanoemulsion loaded bioactive film: Synergistic approach to enhance antimicrobial properties and bread shelf-life. *Food Bioscience*, 61, 104996.

FROM MOLECULES TO MARKET: PHYSICAL CHEMISTRY TOOLS FOR FOOD INTEGRITY AND CONSUMER TRUST

F. Hoxha

*Department of Agri – Food Technology, Faculty of Biotechnology and Food, Agricultural University of Tirana. Street Paisi Vodica, 1029, Tirana, Albania
(fhoxha@ubt.edu.al)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.7

ABSTRACT

Food systems are becoming increasingly complex due to globalization, extended supply chains, emerging food fraud practices, and growing consumer demand for transparency and authenticity. In this context, physical chemistry plays a crucial role in understanding the molecular and physicochemical properties of foods and transforming analytical information into practical tools for food integrity, safety, and quality assurance.

This presentation explores the contribution of modern physical chemistry approaches to the evaluation of food authenticity and consumer trust, with particular emphasis on non-destructive analytical techniques and digital innovation. Spectroscopic methods such as Near-Infrared Spectroscopy (NIR) and Hyperspectral Imaging (HSI), combined with chemometric and artificial intelligence-based data analysis, are increasingly being applied for rapid characterization of food matrices, detection of adulteration, assessment of quality parameters, and traceability studies.

Special attention will be given to applications in honey and olive oil value chains, where molecular fingerprinting and physicochemical profiling can support the identification of botanical/variety and geographical origin, detection of fraudulent practices, and development of more transparent supply chains. The presentation will further discuss the integration of portable analytical devices, digital spectral databases, and data-driven approaches as emerging tools for real-time food authentication and quality monitoring.

Beyond analytical performance, the presentation highlights the broader societal and regulatory relevance of physical chemistry tools in strengthening food integrity systems, supporting evidence-based policies, enhancing market transparency, and increasing consumer confidence. The transition “from molecules to market” illustrates how molecular-level information can be translated into practical solutions that contribute to safer, more sustainable, and more trustworthy food systems.

Keywords: Physical chemistry; food integrity; food authenticity; spectroscopy; hyperspectral imaging; chemometrics; honey; olive oil; food safety; consumer trust.

BLACKCURRANT POMACE AS A SOURCE OF BIOACTIVE COMPOUNDS FOR BIOACTIVE AND SKIN-COMPATIBLE COTTON

A. Ivanovska

*University of Belgrade, Innovation Center of the Faculty of Technology and Metallurgy in Belgrade, Karnegijeva 4, 11000 Belgrade, Republic of Serbia
(aivanovska@tmf.bg.ac.rs)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.8

ABSTRACT

This study investigates the valorization of blackcurrant pomace extract as a source of bioactive compounds for the functionalization of cotton fabrics with skin-compatible properties. Blackcurrant pomace was subjected to water/ethanol extraction using response surface methodology, resulting in a total phenolic content of 36.04 mg GAE/g DW, together with flavonoid and anthocyanin contents of 5.28 mg QE/g DW and 5.18 mg/g DW, respectively. Cotton fabric was subsequently functionalized via layer-by-layer (LbL) protocol, in which the extract was incorporated into chitosan/pectin multilayers comprising four, eight, or twelve bilayers. The resulting cotton fabrics showed no detectable cytotoxicity and exhibited almost maximal antioxidant and antibacterial efficacy against *E. coli* and *S. aureus*. The multilayer functionalization architecture also allowed the amount of extract deposited on the cotton surface to be controlled within the range of 0.09–2.70%, while promoting the retention and stabilization of its bioactive constituents. This controlled functionalization was accompanied by changes in fabric coloration and moisture management behavior. Molecular docking simulations were further employed to characterize the interactions of HPLC-identified anthocyanins, namely cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside, and delphinidin-3-O-rutinoside, with the polysaccharide components (cellulose, chitosan, and pectin). The simulations indicated progressively stronger binding interactions when moving from cellulose toward chitosan and pectin. Among the investigated fabrics, cotton coated with eight chitosan/pectin bilayers containing blackcurrant pomace extract provided a favorable combination of bioactivity and moisture-management performance, demonstrating the potential of this approach for the development of medical, protective, and skin-compatible textile materials.

Funding: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant numbers (451-03-33/2026-03/200287).

FOOD SAFETY IN CIRCULAR BIOECONOMY: BIOTECHNOLOGICAL INNOVATIONS FOR FUNCTIONAL AND SUSTAINABLE FOODS

J. Klopchevska

*Department of Food Technology and Biotechnology, Faculty of Technology
and Metallurgy, Ss. Cyril and Methodius University in Skopje, Rugjer
Boshkovikj 16, 1000 Skopje, North Macedonia (jana@tmf.ukim.edu.mk)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.9

ABSTRACT

The circular bioeconomy can transform food-chain side streams into functional and sustainable foods. This presentation examines fermentation, enzyme-assisted extraction, and cascading biomass valorization as strategies for recovering proteins, dietary fiber, phenolics, pigments, and other valuable compounds. Attention is given to food-safety-by-design, from feedstock selection and traceability through processing, formulation, and regulatory assessment. Circular systems may introduce or redistribute microbiological, chemical, physical, and allergen hazards. A practical framework is proposed to map material flows, prioritize hazards according to their occurrence, persistence, transfer, and health relevance, and implement validated controls, monitoring, and final-product verification. The presentation also distinguishes technological functionality and compositional improvement from demonstrated human health benefits. Circular biotechnology can support resource efficiency and food innovation, but safety, sustainability, and health claims must be substantiated for each product and process.

Keywords: Circular bioeconomy; food safety; food-safety-by-design; biotechnological innovation; functional foods; sustainable food systems; fermentation; biomass valorization.

EVALUATING PHYTOTOXICITY AND GENOTOXICITY OF IBUPROFEN ON *Allium cepa* L.

A.-M. Domijan¹, M. Hamplová² and H. Zlámlová Gargošová²

¹ University of Zagreb Faculty of Pharmacy and Biochemistry, Kovacicева 1, 10000 Zagreb, Croatia (adomijan@pharma.hr)

² Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, Brno University of Technology, 61200 Brno, Czech Republic

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.10

ABSTRACT

The aim of this study was to test the phytotoxicity and genotoxicity of ibuprofen (IBU) using the *Allium* test, a well-established bioassay for monitoring environmental pollutants. Onions' roots (n=10 bulbs per treatment) were exposed to IBU (1-100 mg/L) or to dH₂O (control) for 72 h in a controlled environment (dark, 20 °C, 75 % humidity). Following exposure, root length and fresh weight were recorded, and in meristem cells of root tips the frequency of chromosomal aberrations (CAs) was estimated. For statistical analysis, one-way ANOVA followed by Dunnett's test (p<0.05) was used. The decrease in root length and fresh weight was observed at higher IBU concentrations (50 and 100 mg/L). An increased frequency of CAs was recorded already at the lowest IBU concentration (1 mg/L). The most frequent CAs were C-mitosis, anaphase bridges, and sticky chromosomes, indicating aneugenic as well as clastogenic effects of IBU. These results indicate the importance of monitoring the presence of IBU in the environment.

INTRODUCTION

Increasing use of pharmaceuticals in human and veterinary medicine has led to their accumulation in the environment. Nowadays, pharmaceuticals are considered the most critical emerging contaminants detected in water supplies worldwide [1]. Special concerns are non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen (IBU), since they are available without a prescription [2]. According to the Agency for Medicinal Products and Medicinal Devices of Croatia, in Croatia, IBU is in 14th place on the list of the most utilized pharmaceuticals in 2024 [3]. NSAIDs together with antibiotics are the most frequently found pharmaceuticals in wastewater treatment plant effluents and aquatic environments worldwide [1].

IBU can reach the environment from several sources: (1) directly as a result of waste disposal by the pharmaceutical industry, (2) from inappropriate disposal leaching from landfills, or (3) via wastewater after excretion [2, 4]. The removal

of IBU in wastewater treatment plants is incomplete; its chemical stability makes conventional treatments inefficient in removing IBU [1]. Additionally, due to chemical stability, IBU has a relatively slow rate of degradation. As a result, IBU is detected in surface waters, drinking waters, and soil. The concentrations of IBU in aquatic environmental samples are at $\mu\text{g/L}$; in wastewater samples, the detected IBU concentration was up to $1673 \mu\text{g/L}$ [5]. In soil samples, IBU concentrations ranged from 321 to $610 \mu\text{g/kg}$ [5]. It is shown that IBU can bioaccumulate in a wide range of living organisms [2, 4]. Consequently, IBU can reach the food chain and affect human health. Due to its ubiquitous presence in the environment, IBU represents a threat to non-target organisms. Therefore, the aim of this study was to test the phytotoxicity and genotoxicity of IBU using the *Allium* test, a well-established test for biomonitoring environmental pollutants.

EXPERIMENTAL

Stock solution of IBU (Sigma Aldrich, St. Louis, MO, USA, $\geq 98\%$ purity) at a concentration of 0.1 g/mL was prepared in ethanol. IBU test solutions in the concentration range $1\text{--}100 \text{ mg/L}$ were prepared by diluting the IBU stock solution in dH_2O .

The biological experiment was performed as described previously [6-8]. Firstly, healthy onion bulbs (*Allium cepa* L., var. Stuttgarter riesen) were selected, and their roots' growth were started in dH_2O (onions were kept in a controlled environment: dark, $20 \pm 2^\circ\text{C}$, humidity 75% for 24 h). Afterwards, bulbs were divided into 5 groups (each group having 10 bulbs) and their roots were exposed to IBU in concentrations of 1, 10, 50, and 100 mg/L or to dH_2O (control) for 72 h. Throughout the experiment, the onions were kept in a controlled environment (dark, $20 \pm 2^\circ\text{C}$, humidity 75%). At the end of exposure, roots were examined for morphological changes, and root length and fresh weight were recorded. Afterwards, two roots of each bulb were placed in fixative (ethanol:glacial acetic acid; 3:1; v/v) and stored at 4°C . Microscopic slides were prepared by staining the root tips with aceto-orcein (1% orcein in 45% glacial acetic acid) for 15 min. The meristem cells of root tips were examined for chromosomal aberrations (CAs), such as C-mitosis, anaphase bridges, and sticky chromosomes using a microscope (CX22 LED, Olympus, Tokyo, Japan). The frequency of CAs was estimated on approximately 100 divisions per slide. For statistical analysis, one-way ANOVA followed by Dunnett's test ($p < 0.05$) was used.

RESULTS AND DISCUSSION

Allium test is considered a standard for testing environmental pollutants [6, 9]. There are several advantages of the *Allium* test, such as: onions are easy to handle, their cells have relatively large chromosomes, and, importantly, the results of the *Allium* test have good correlation to mammalian-derived cell lines [6-10]. Furthermore, stable chromosome number and rare occurrence of

spontaneous chromosomal damage make onion suitable for genotoxic studies [10].

In this study, onion roots were exposed to IBU in concentrations of 1-100 mg/L for 72 h. Results on roots' length and fresh weight are presented in Table 1. Exposure to higher IBU concentrations significantly reduced root length and fresh weight compared to controls exposed to dH₂O.

Table 1. Root length and fresh weight after 72 h exposure to IBU in the concentration range 1-100 mg/L or dH₂O (control).

Treatment	Root length (cm)	Fresh weight (g)
Control (dH ₂ O)	4.32 ± 0.6 ^a	0.24 ± 0.05 ^{a,b}
1 mg/L	4.75 ± 0.6 ^a	0.33 ± 0.07 ^a
10 mg/L	4.75 ± 0.7 ^a	0.32 ± 0.07 ^a
50 mg/L	2.28 ± 0.3 ^c	0.16 ± 0.04 ^c
100 mg/L	1.95 ± 0.3 ^c	0.18 ± 0.05 ^c

Different letters indicate significantly different values at p<0.05 according to ANOVA.

Previously, IBU phytotoxicity was tested on several plant models. Rede et al. [4] tested the impact of IBU on germination of lettuce seeds (*Lactuca sativa* L.). In that study, IBU, even at the highest concentration applied (1 mg/L, 5 days exposure), had no impact on germination or leaf length. However, in the study of de Silva et al. [11], in which lettuce seeds were exposed to a much higher IBU concentration (600 mg/L, 72 h exposure), a reduction in germination of 62% was observed, while IBU at lower concentrations (20 and 200 µg/L, 72 h) had no effect on lettuce germination. Menicagli et al. [12] in seagrass *Cymodocea nodosa* (Ucria) Asch. exposed to IBU in the concentration range 0.25-25 µg/L for 12 days observed no change in growth, although a change at the cellular level (in photosynthetic efficiency) was recorded. A concentration-dependent reduction in fresh weight was observed in *Lemna minor* exposed to IBU (5-400 mg/L) for 15 days (higher concentrations) or 21 days (lower concentrations) [2]. Mentioned studies indicate that phytotoxicity of IBU depends on plant model, concentration, and duration of exposure. Kornacka et al. [2] observed that *Lemna minor* may adapt to lower IBU concentrations. This could be the case in this study, since an increase in root length and fresh weight at lower IBU concentrations, although not statistically significant, was observed.

There are limited studies on IBU genotoxicity. De Silva et al. [11] observed an increase in the frequency of micronuclei in lettuce exposed to IBU (at a concentration of 600 mg/L for 72 h), while lower IBU concentrations (20 and 200 µg/L, 72 h) did not induce the formation of micronuclei. A micronucleus is the result of chromosome breaks (and formation of chromosome fragments) or chromosome losses that cannot be incorporated into the main nucleus during the

cell cycle; thus, a micronucleus mainly indicates a mutagen, i.e., a tested compound that acts directly on DNA [9]. In this study, following IBU exposure, root meristem cells were examined for CAs as C-mitosis, anaphase bridges, and sticky chromosomes (Fig. 1). Even the lowest applied IBU concentration (1 mg/L) increased the frequency of CAs in comparison to controls (Table 2).

Chromosomal aberrations are results of changes in chromosomal structure or in the total number of chromosomes. CAs, such as chromosome bridges and breaks, are indicators of clastogenic action (affecting the DNA molecule), while chromosome losses, delays, adherence, and C-metaphase result from aneugenic effect (affecting the mitotic spindle) of the tested compound [6, 9]. Results of this study indicate that IBU has clastogenic as well as aneugenic effects in onion root cells that are recorded even at the lowest applied IBU concentration (1 mg/L).

Table 2. Frequency of chromosomal aberrations after 72 h exposure to IBU in the concentration range (1-100 mg/L) or dH₂O (control).

Treatment	Chromosomal aberrations frequency (%)
Control (dH ₂ O)	6.6 ± 0.9 ^e
1 mg/L	18.0 ± 6.5 ^d
10 mg/L	29.5 ± 4.0 ^{b,c}
50 mg/L	46.6 ± 9.9 ^a
100 mg/L	51.3 ± 7.1 ^a

Different letters indicate significantly different values at $p < 0.05$ according to ANOVA.

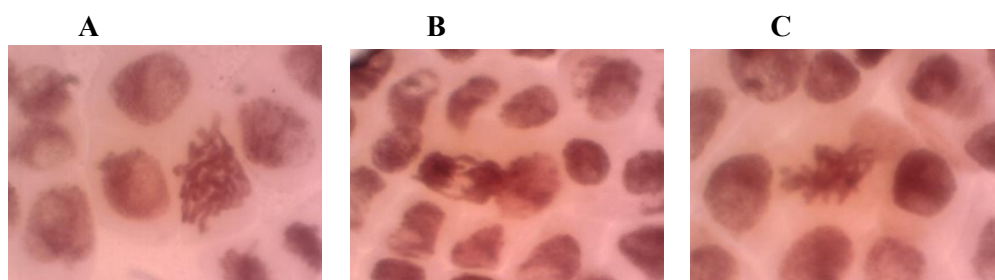


Figure 1. Chromosomal aberrations after 72 h exposure to IBU (50 mg/L). (A) C-mitosis; (B) bridges; (C) sticky chromosomes. Light microscope, magnification 400x.

CONCLUSION

Results of this study indicate that IBU phytotoxicity is linked to its genotoxic potential. Its genotoxic action includes both direct action on the DNA molecule and on the mitotic spindle. Therefore, the presence of IBU in the environment

should be monitored, and precautions should be taken to prevent its entrance in the food chain in order to protect the environment as well as human health.

Acknowledgment

This study has been funded by the European Union (NextGenerationEU) under the National Recovery and Resilience Plan 2021–2026 and supported by the project FarmInova (KK.01.1.1.02.0021) funded by the European Regional Development Fund.

REFERENCES

- [1] R. Hernandez-Tenorio, E. Gonzalez-Juarez, J. L. Guzman-Mar, L. Hinojosa-Reyes, A. Hernandez-Ramirez, *J. Hazardous Materials Adv.*, 2022, 8, 100172.
- [2] H. Kornacka, M. Sitarska, M. Wolf-Baca, *Int. J. Environ. Sci. Tech.*, 2026, 23, 229.
- [3] Halmed. <https://www.halmed.hr/en/Promet-proizvodnja-i-inspekcija/Promet/Potrosnja-lijekova/Izvjesca-o-prometu-lijekova/>
- [4] D. Rede, L. H. M. L. M. Santos, S. Ramos, F. Oliva-Teles, C. Antao, S. R. Sousa, C. Delerue-Matos, *Sci. Total Environ.*, 2019, 673, 102-109.
- [5] J. Jan-Boblero, J. A. Cruz-Maya, *Molecules*, 2023, 28, 2097.
- [6] G. Fiskesjö, *Hereditas*, 1985, 102, 99-112.
- [7] G. Fiskesjö, in: W. Wang, J.W. Gorsuch, J.S. Hughes (Eds), Lewis Publishers, New York, 1997, pp. 308–333.
- [8] V. Vujčić Bok, M. Gerić, Gajski G, S. Gagić, A.-M. Domijan, *Molecules*, 2023, 28, 2046.
- [9] D. M. Leme, M. A. Marin-Morales, *Mutat. Research*, 2009, 682, 71–81.
- [10] P. Firbas, T. Amon, *Caryologia*, 2014, 67, 25-35.
- [11] L. M. da Silva, V. S. V. Dutra, G. V. A. Nascimento, P. N. C. R. de Paula, F. J. da Silva, *Saude Meio Ambiente*, 2023, 12, 245-258.
- [12] V. Menicagli, M. R. Castiglione, E. Cioni, C. Spano, E. Balestri, M. De Leo, S. Bottega, C. Sorce, C. Lardicci, *J. Hazardous Materials*, 2024, 476, 135188.

PHYTOCHEMICAL ANALYSIS, DETERMINATION OF ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF LICHEN SPECIES, *Heterodermia diademata* and *Parmotrema tsavoense*

P. Islary¹ and B. Gogoi^{2,3}

¹Department of Botany, Kokrajhar University, Kokrajhar, Assam, India
(pungbilib@gmail.com)

²Bioremediation Technology Research Group, Department of Botany,
Bodoland University, Assam, 783370, India

³DBT-NECAB, Assam Agricultural University, Assam, 785013, India

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.11

ABSTRACT

Lichens, symbiotic associations between fungi and photosynthetic partners, are increasingly recognized as prolific sources of diverse secondary metabolites with significant pharmaceutical, agricultural, and biotechnological applications. This study delves into the phytochemical composition, antioxidant properties, and antimicrobial effectiveness of two lichen species, *Heterodermia diademata* and *Parmotrema tsavoense*, particularly against phytopathogens *Xanthomonas oryzae*, *Colletotrichum gloeosporioides*, *Pyricularia oryzae*, and *Sclerotinia sclerotiorum*. Qualitative phytochemical screening revealed the presence of a variety of bioactive compounds, including alkaloids, flavonoids, phenolic compounds, tannins, and triterpenoids, indicating their rich metabolite profile and potential therapeutic value. Quantitative estimation of total phenolic content and total flavonoid content was performed using the Folin-Ciocalteu and aluminum trichloride methods, respectively, demonstrating substantial levels of antioxidant-associated compounds in both lichen extracts. The antioxidant activity of the extracts was evaluated using in vitro assays, i.e., phosphomolybdenum total antioxidant capacity, ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. The results revealed notable antioxidant potential, which can be attributed to the abundance of phenolic and flavonoid constituents capable of neutralizing reactive oxygen species and reducing oxidative stress. Furthermore, the antimicrobial efficacy of the lichen extracts was assessed against four economically important plant pathogens, including *Xanthomonas oryzae*, *Colletotrichum gloeosporioides*, *Pyricularia oryzae*, and *Sclerotinia sclerotiorum*, using the disc diffusion method. Among them, *Parmotrema tsavoense* exhibited comparatively stronger antimicrobial activity, producing larger zones of inhibition against the targeted pathogens. The observed

antioxidant and antimicrobial activities suggest that these lichens possess significant potential as natural sources of bioactive compounds for the development of eco-friendly plant disease management strategies and novel antimicrobial formulations. Overall, the findings highlight the value of *H. diademata* and *P. tsavoense* as promising reservoirs of biologically active metabolites and provide a scientific basis for their further exploration in agricultural, pharmaceutical, and biotechnological applications. Future studies focusing on the isolation, characterization, and mode of action of individual bioactive compounds are warranted to fully exploit their practical potential.

Keywords: Antimicrobial, crop plants, lichen, plant pathogens

NUTRITIONAL AND BIOACTIVE POTENTIAL OF FRUIT WINES

U. Čakar¹, T. Ilić¹, M. Pagnacco², J. Maksimović³, J. Senćanski⁴, M. Čebela⁵
and B. Đorđević³

¹*University of Belgrade, Department of Bromatology, Faculty of Pharmacy,
Vojvode Stepe 450, 11000 Belgrade, Serbia. (urosc@pharmacy.bg.ac.rs)*

²*University of Belgrade, Institute of Chemistry, Technology and Metallurgy,
National Institute of the Republic of Serbia, Njegoševa 12, 11000 Belgrade,
Serbia*

³*University of Belgrade, Faculty of Physical Chemistry, Studentski trg 12-16,
Belgrade, Serbia*

⁴*University of Belgrade - Institute for Multidisciplinary Research, National
Institute of the Republic of Serbia, Kneza Višeslava 1, Belgrade, 11030, Serbia*

⁵*Vinča Institute of Nuclear Sciences – National Institute of the Republic of
Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade,
Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.12

ABSTRACT

Sour cherry can be used for wine production. For this process, an innovative microvinification procedure was applied. In this procedure, half of the samples were produced without pits, and under conditions with or without added sugar and/or the enzymatic preparation glycosidase (EPG). Selected phenolic compounds were identified and quantified using UPLC/MS-MS analysis. The inhibitory activity of sour cherry wine samples against α -glucosidase was also investigated, and the contribution of specific compounds to this activity was estimated by a molecular docking study. Antioxidant activity was determined by the Briggs-Rauscher method. Wines produced with pits and with the addition of sugar and EPG showed higher phenolic content, better inhibitory activity against α -glucosidase, and improved antioxidant properties. In summary, the phenolic profile and biological activity of sour cherry wines depended on the microvinification procedure used in their production.

INTRODUCTION

Intake of fresh or processed fruits and vegetables has a positive effect on human health, so their consumption should be essential and regular in a well-balanced diet. It is important to note that regular consumption implies an intake of 400 g of fruits and vegetables, as well as two glasses of red wine daily [1]. The development of chronic non-communicable diseases such as heart disease and

diabetes mellitus could be prevented by consuming foods rich in antioxidants [2]. Numerous naturally occurring compounds such as phenolic acids, flavonoids, and anthocyanins originate from fruits and their derived products. Among them, hydroxybenzoic and hydroxycinnamic acid derivatives are particularly notable [3,4]. Fruit wine is one product obtained from fruit processing. During this process, active principles are retained in the final product [5].

Drupe fruit wines, such as those made from sour cherry, are especially known as rich sources of phenolic compounds with good antioxidant properties [6]. It is also important to highlight the natural active principles from fruit that display α -glucosidase inhibitory activity. The aforementioned enzyme is involved in the final phase of carbohydrate digestion. Natural active principles with α -glucosidase inhibitory activity have much fewer side effects than synthetic ones and can be used in the control of postprandial hyperglycemia [7,8]. Additionally, the antioxidant activity of fruit can be determined by various methods, and one of them is the Briggs-Rauscher (BR) oscillating reaction. The BR reaction generates reactive oxygen and iodine radicals present in living organisms. As a result of these reactions, the quenching of oscillatory dynamics may occur, which is used to determine the antioxidant activity of different samples [9].

The production of high-quality sour cherries in Serbia is a good precondition for producing wine from this fruit. This study aimed to investigate how an innovative microvinification procedure influences the content of selected phenolic compounds and antioxidant activity. In addition, the α -glucosidase inhibitory activity of lyophilized fruit wine samples was evaluated, as well as the contribution of phenolic compounds to this activity.

EXPERIMENTAL

The sour cherry (*Prunus cerasus*) was obtained from Grocka, Serbia. Shortly after picking from the orchard, sour cherries were processed into wine. In half of the fruit, pits were removed. An innovative microvinification procedure was conducted in two sets, and the first step in both sets was fruit disintegration. In the first set, before fermentation began, sugar and the enzymatic preparation glycosidase (EPG) were added to the fruit must, while the second set was conducted without addition. In both subsets, controlled alcoholic fermentation was conducted using a selected pure commercial wine yeast strain (Lalvin 71B, Lallemend, Canada yeast strain *Saccharomyces cerevisiae*) at the amounts of 20 g per 100 kg, respectively. Microvinification was conducted in barrels with a pigeage system, while alcohol fermentation was conducted at 20°C over the next 7 to 10 days. After fermentation, sour cherry wine was separated from pomace by sedimentation and kept at 12°C for the next six months, until further studies.

Physicochemical properties of fruit wines such as total soluble solids (TSS, expressed in °Brix), pH, total titratable acidity (TTA) and alcohol concentration (expressed as volume (vol. %)) were evaluated.

Solid-phase extraction (SPE) on Oasis HLB 6CC 200 mg cartridges (Waters, Milford, MA, USA) was applied to decrease the influence of the matrix during phenolics identification. UPLC/MS-MS analysis was used for quantification of phenolic compounds, performed using a Waters Acquity Ultra Performance H-Class System (Waters, Milford, MA, USA).

Lyophilized wine samples were tested for α -glucosidase inhibitory activity using the modified method described previously [10]. The α -glucosidase from *Saccharomyces cerevisiae* dissolved in phosphate buffer (pH = 6.8) was used, while the substrate solution was p-nitrophenyl α -D-glucopyranoside (PNPG in phosphate buffer 1.5 mg/mL). Absorbance was measured on ELISA reader, Multiskan EX, Thermo Scientific (Waltham, Massachusetts, USA) before and after incubation at 37°C for 5 min. Acarbose was used as a positive control. The α -glucosidase inhibitory activity standards were actually determined in the same way as for the lyophilized fruit wine samples. The results were expressed as IC₅₀ (μ g/mL).

Molecular docking study was conducted on crystal structures of α -glucosidase (PDB ID: 2ZE0) which was retrieved from Protein Data Bank [11]. Prepared 2D coordinates of phenolic compound standards were converted to 3D and optimized with Chem3D 7 (CambridgeSoft, Cambridge, Massachusetts, USA). PDBQT files of the enzyme and ligands were prepared in Autodock Tools, while docking calculations were carried out in the Autodock Vina software. Blind docking with acarbose over the whole protein was carried out for finding of the binding sites. Finally, the binding energies (kcal/mol) were used in the interpretation of the obtained results.

Determination of antioxidant activity of fruit wines was conducted by Briggs-Rauscher reaction [9]. The experiments were performed in a stationary well-stirring (σ = 900 rpm) reactor. The total volume of BR reaction solution was 25 ml while the temperature of the reaction mixture was maintained on 37.2 °C. When the temperature of 37.2 was reached, hydrogen peroxide was added. After initiating the reaction, 50 μ l of wine sample was added. The time evolution of the BR reaction was potentiometrically monitored using a Pt electrode as a measuring electrode, and Hg/HgSO₄ as a reference electrode.

RESULTS AND DISCUSSION

Physicochemical parameters such as the TSS content of the must and pH are important for wine quality. The first parameter can predict the alcohol content (Vol. %) in the wine, while the second directly affects its flavor and aroma. The results obtained after UPLC/MS-MS analysis of sour cherry wine samples prepared without pits and without sugar/EPG addition were compared with those prepared with pits and with sugar/EPG (Table 1). The presence of sugar and EPG significantly affected the content of selected phenolic compounds ($p < 0.05$). Higher sugar content before fermentation leads to higher alcohol content in the

final product, which improves the extraction of phenolic compounds ($p < 0.05$). EPG releases phenolics from their glycoside form. It is important to note that the microvinification procedure significantly affects the content of phenolic compounds. The highest content was found in sweet cherry wine samples prepared with sugar and EPG. The presence of pits did not lead to a significant increase in the content of phenolic compounds ($p > 0.05$). The two major phenolic compounds in sweet cherry wines were chlorogenic and protocatechuic acids (Table 1).

Table 1. Content of selected phenolic compounds in the sour cherry wines ($\mu\text{g/mL}$)

Type of Vinification	Chlorogenic acid	Gallic acid	Protocatechuic acid	Caffeic acid	<i>p</i> -Coumaric acid
Sour cherry -pit	675 $\pm$ 15.3	0.67 $\pm$ 0.03	153 $\pm$ 14.2	95 $\pm$ 11.3	1.97 $\pm$ 0.02
Sour cherry-pit, Sugar+enzyme+	731 $\pm$ 16.4	1.31 $\pm$ 0.02	185 $\pm$ 16.4	118 $\pm$ 14.4	5.37 $\pm$ 0.03
Sour cherry +pit	681 $\pm$ 17.5	0.72 $\pm$ 0.04	157 $\pm$ 15.3	97 $\pm$ 10.2	2.11 $\pm$ 0.01
Sour cherry+pit, Sugar+enzyme+	735 $\pm$ 25.7	1.42 $\pm$ 0.03	187 $\pm$ 20.5	121 $\pm$ 12.3	5.77 $\pm$ 0.03

Hydroxycinnamic acid derivatives such as caffeic and *p*-coumaric acids were also identified. The content of gallic acid was the lowest in the analyzed samples. The obtained results are in good agreement with the literature, which reports that sour cherries are a rich source of chlorogenic acid [5]. The α -glucosidase inhibitory activity showed the same trend as the UPLC/MS-MS analysis ($p < 0.05$) (Table 2). IC₅₀ values for fruit wines and phenolic standards were obtained from sigmoidal inhibition curves. The results indicated that the analyzed samples exhibited good α -glucosidase inhibitory activity (Table 2). These findings are supported by studies on different sour cherry products, which have shown significant α -glucosidase inhibitory activity. Accordingly, it is possible to include sour cherries in the diet as part of therapeutic postprandial hyperglycemia control [12].

Table 2. Activity of black chokeberry wines against α -glucosidase

Type of Vinification	IC ₅₀ (μ g/ml)
Sour cherry -pit	61 $\pm$ 1.23
Sour cherry-pit, Sugar+enzyme+	49 $\pm$ 1.11
Sour cherry +pit	58 $\pm$ 2.12
Sour cherry+pit, Sugar+enzyme+	47 $\pm$ 1.15

The highest contribution to the α -glucosidase inhibitory activity of the samples was observed for chlorogenic and protocatechuic acids. Molecular docking studies revealed the binding energies of the quantified phenolic compounds, which ranged between -6.1 and 8.7 kcal/mol. Compounds with binding energies lower than -6 kcal/mol are considered to have higher binding affinity. The binding energy for acarbose was -9.5 kcal/mol. The results obtained for some derivatives of hydroxycinnamic acids (caffeic and p-coumaric acids) and hydroxybenzoic acids (gallic and protocatechuic acids) are in good agreement with the literature data [13].

The results of the BR method revealed good antioxidant potential of the samples, which was in the range from 37s to 147s. It is important to underline that the experimental conditions for the BR method are similar to physiological conditions. Specifically, the temperature was about 37°C, and the pH of the BR reaction solution was about 2, which corresponds to the pH of gastric fluid (stomach acid). This makes the reaction suitable for investigating the antioxidant capacity of biologically active compounds, which could indicate their potential activity in the human organism [9]. The highest antioxidant activity was observed for the sample made with pit and sugar/EPG, while the lowest activity was observed in the sample produced without pit.

CONCLUSION

In summary, sour cherry wine can be highlighted as an important source of naturally occurring antioxidants with health-promoting properties. The findings also indicate that sour cherry wine inhibits α -glucosidase and may potentially be used as a functional (medicinal) food in the prevention of diabetes mellitus and other chronic diseases. Phenolic acid derivatives, together with other active principles – both phenolic and non-phenolic – are responsible for the beneficial health effects of fruit wine. Moderate consumption of sour cherry wine may be recommended as part of a healthy, well-balanced diet.

Acknowledgement

This work was supported by the Ministry of Science Technological Development, and Innovation of Serbia, Grants no. 451-03-33/2026-03/ 200161, 451-03-34/2026-03/20016, 451-03-33/2026-03/200146, 451-03-33/2026-03/200026, 451-03-33/2026-03/200053.

REFERENCES

- [1] K.J. Joshipura, F.B. Hu, J.E. Manson, M.J. Stampfer, E.B. Rimm, F.E. Speizer, G. Colditz, A. Ascherio, D. Spiegelman, W.C. Willett, B. Rosner, *Ann. Intern. Med.*, 2001, 132, 1106-1114.
- [2] World Health Organization (WHO), Global report on diabetes, Geneva, 2016. http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf.
- [3] S.Y. Wang, *Acta Hort.*, 2003, 620, 461-473.
- [4] K. Robards, P.D. Prenzler, G. Tucker, P. Swatsitang, W. Glover, *Food Chem.*, 1999, 66, 401-436.
- [5] A. Czyzowska, E. Pogorzelski, *Eur. Food Res. Technol.*, 2002, 214, 148-154.
- [6] M. Johnson, A. Lucius, T. Meyer, E. Gonzalez de Mejia, *J. Agric. Food Chem.*, 2011, 59, 8923-8930.
- [7] J.B. Xiao, P. Hogger, *Curr. Med. Chem.*, 2015, **22**, 23-38.
- [8] G.J. McDougall, F. Shpiro, P. Dobson, P. Smith, A. Blake, D. Stewart, *J. Agric. Food Chem.*, 2005, 53, 2760-2766.
- [9] S. Ullah, G. Hu, M.U. Rahman, A. Hayat, A.U. Rehman, I. Ullah, A. Ahmad, W. Uddin, W. Ma, Z. Fang, M. Y. Nawabi, R. Ullah, J. Song, *Int. J. Electrochem. Sci.* 2019, 14, 10176-10186.
- [10] P. McCue, Y.I. Kwon, K. Shetty, *J. Food Biochem.*, 2005, 29, 278-294.
- [11] K.F. Huang, Y.R. Wang, E.C. Chang, T.L. Chou, A.H. Wang, *Biochem. J.*, 2008, 411, 181-190.
- [12] P. Nowicka, A. Wojdyło, J. Samoticha, *J. Funct. Foods*, 2016, 25, 397-407.
- [13] H. Tae Kyung, E. Seung Hee, K. Ju-Sung, *Plant Omics*, 2014, 7, 166- 170.

WELLS-DAWSON CONTRAST AGENT CANDIDATE: EFFECT ON OXIDATIVE PROTEIN MODIFICATION

M. B. Čolović¹, G. Gajski², M. Gerić², N. Savić³, T. Parac-Vogt⁴, D. Z. Krstić⁵
and A.-M. Domijan⁶

¹University of Belgrade, "Vinča" Institute of Nuclear Sciences-National
Institute of the Republic of Serbia, P.O. Box 522, 11000 Belgrade, Serbia.

²Institute for Medical Research and Occupational Health, Ksaverska cesta 2,
10000 Zagreb, Croatia

³Université Paris-Saclay, Institut Lavoisier de Versailles, CNRS, UVSQ, 45
Avenue des Etats-Unis, 78035 Versailles, France

⁴KU Leuven, Department of Chemistry, Celestijnenlaan 200F, 3001 Leuven,
Belgium

⁵University of Belgrade, Faculty of Medicine, Institute of Medical Chemistry,
Višegradska 26, 11000 Belgrade, Serbia

⁶University of Zagreb, Faculty of Pharmacy and Biochemistry, Ante Kovačića
1, 10000 Zagreb, Croatia (adomijan@pharma.hr)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.13

ABSTRACT

Prooxidative properties of hafnium(IV)-substituted Wells-Dawson polyoxometalate, $K_{16}[Hf(\alpha_2-P_2W_{17}O_{61})_2] \cdot 19H_2O$ (Hf-WD 1:2), a promising contrast agent for micro-computed tomography (microCT) and biological transmission electron microscopy (TEM) imaging, were evaluated *in vitro*. The effect of Hf-WD 1:2 on carbonylated protein level, as a biomarker of severe oxidative stress, was determined in human plasma after 4-hour exposure to 1-100 μM Hf-WD 1:2 at 37 °C. The studied contrast agent candidate did not result in increased oxidative protein modification compared to control sample, that indicates its favourable toxicity profile and safe biomedical application. Additionally, 100 μM Hf-WD 1:2 induced a remarkable decrease of carbonylated protein concentration. This result forms a basis for further research on the antioxidant potential of Hf-WD 1:2.

INTRODUCTION

Polyoxometalates (POMs) are inorganic anionic cage complexes consisting of transition metals in their high oxidation states (typically W^{6+} and V^{5+}) bridged by oxygen [1]. Recent studies revealed a new field of POM utility as promising contrast agents which are able to enhance the visualization of cell structures in imaging techniques for ultrastructural analysis such as transmission electron microscopy (TEM) and micro-computed tomography (microCT) [2, 3].

Our most recent findings [4] revealed the potential of hafnium(IV)-substituted Wells-Dawson POM, $K_{16}[Hf(\alpha_2-P_2W_{17}O_{61})_2] \cdot 19H_2O$ (Hf-WD 1:2) (Figure 1) as a promising non-radioactive contrast agent for biological TEM imaging. The WD POM demonstrated superior contrast properties compared to conventional radioactive uranyl-based reagents, enhancing the visualization of subcellular structures. As the potential application of novel agents for biomedical purposes requires a favorable toxicological profile, the aim of this study was to evaluate *in vitro* prooxidative properties of Hf-WD 1:2 as a promising contrast agent with potential clinical application. For this purpose, the effect of Hf-WD 1:2 on carbonylated protein level, as a key biomarker of oxidative stress, cell injury, and biological decay associated with aging, was studied using human blood as a model system for *in vitro* toxicity evaluation.

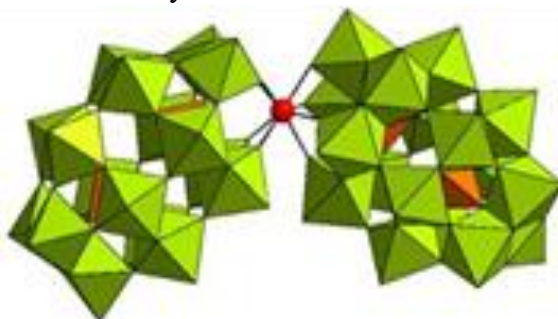


Figure 1. Structure of hafnium(IV)-substituted WD POM, $K_{16}[Hf(\alpha_2-P_2W_{17}O_{61})_2] \cdot 19H_2O$ (Hf-WD 1:2).

EXPERIMENTAL

The studied Hf-WD 1:2 was synthesized according to the published procedure [5].

Blood samples were taken from a healthy male and female individual, who agreed to participate in this investigation, which was approved by the Institutional Ethics Committee and observed the ethical principles of the Declaration of Helsinki. For the treatment, 100 μ L of the stock/working solutions of Hf-WD 1:2 were added to 900 μ L of human whole blood samples to reach studied final concentrations (Figure 2) and incubated at 37 °C for 4 h. Carbonylated proteins were determined in human plasma samples [6]. All measurements were done in triplicates.

RESULTS AND DISCUSSION

The concentrations of carbonylated proteins, as an indicator of excessive free radical production, were measured in the samples of human plasma after 4-hour exposure *in vitro* to Hf-WD 1:2 within the concentration range of 1-100 μ M. Untreated human plasma samples were taken as controls, and the highest POM concentration was limited by its solubility in water. Human blood samples were

taken from a male and female individual, and the results were expressed as a percentage of the corresponding control values.

The obtained results (Figure 2) show a dose-dependent effect of Hf-WD 1:2 on carbonylated protein levels. After 4-hour treatment, Hf-WD 1:2 resulted in a decrease in carbonylated protein concentrations related to the control for all followed treating doses. The most significant effect was noticed for the POM concentration of 100 μ M, which reduced carbonylated protein level by 28% compared to the control value.

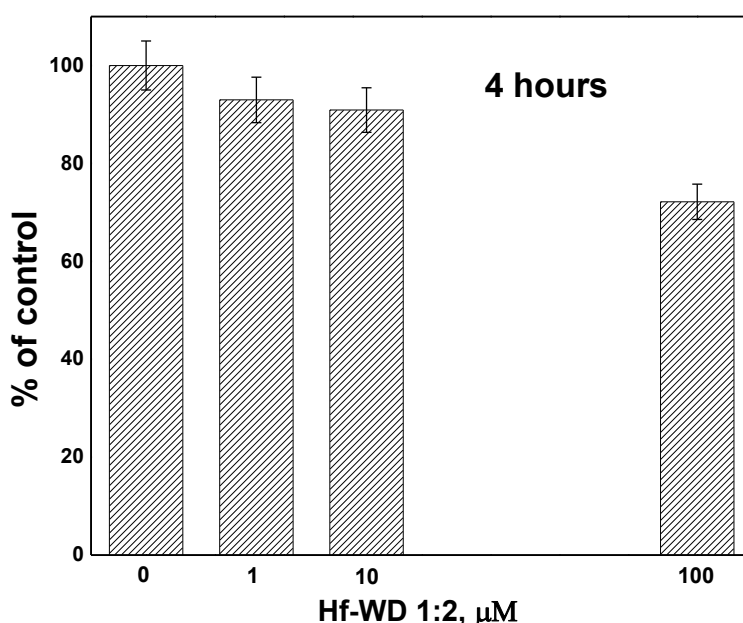


Figure 2. The effect of **Hf-WD 1:2** on carbonylated protein level (% of control) in human plasma after 4-hour treatment *in vitro* at 37 °C. The results are expressed as mean values $\pm$ S.E.M.

The obtained decrease of carbonylated protein levels in human plasma indicates that Hf-WD 1:2 did not induce generating free radicals resulting in oxidative stress and consequently oxidative protein modification. This effect might be explained possible antioxidant properties of Hf-WD 1:2 acting as a constant “scavenger,” immediately removing even minimal amounts of reactive oxygen species as soon as they appear. In addition, Hf-WD 1:2 may have stimulated or supported the natural antioxidant enzymes already present in the blood, such as superoxide dismutase and catalase, making them more effective at eliminating basal oxidative stress. Surely, these assumptions should be confirmed using appropriate experimental methods.

CONCLUSION

Hafnium(IV)-substituted WD POM, a promising contrast agent candidate for TEM and microCT, did not generate oxidative protein modification as the result of prooxidative action, which suggests its favourable toxicity profile and safe biomedical application.

Acknowledgement

This study was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (contracts no. 451-03-33/2026-03/200017 and 451-03-34/2026-03/200110) and Science Fund of the Republic of Serbia (POMCACT project no. 6526393). The authors acknowledge the bilateral project Serbia-Croatia (no. 337-00-205/2019-09/19) and Erasmus+ KA103 European Mobility Program (author M. Č.).

REFERENCES

- [1] D. E. Marcano, N. D. Savić, K. Declerck, S. A. Abdelhameed, T. N. Parac-Vogt, *Chemical Society Reviews*, 2024, 53, 84–136.
- [2] S. de Bournonville, S. Vangrunderbeeck, G. Kerckhofs, *Contrast Media & Molecular Imaging*, 2019, 2019, 8617406.
- [3] M. Nakakoshi, H. Nishioka, E. Katayama, *Journal of Electron Microscopy*, 2011, 60, 401–407.
- [4] A. Milosavljević, M. Četković, T. Kravić-Stevović, M. Stojanović, M. B. Čolović, N. D. Savić, T. N. Parac-Vogt, D. Z. Krstić, *International Journal of Molecular Sciences*, 2026, 27, 5523.
- [5] A. P. Ginsberg, *Inorganic Syntheses*, Wiley, New York, 1990.
- [6] R. L. Levine, J. Williams, E. R. Stadtman, E. Shacter, *Methods in Enzymology*, 1994, 233, 346–357.

HOW DO AGROECOLOGICAL PRACTICES ALTER NON-ENZYMATIC ANTIOXIDANTS IN MAIZE GRAIN?

V. Dragičević, J. Vukadinović, M. M. Šenk, M. Simić and M. Brankov

*Maize Research Institute Zemun Polje, Slobodana Bajića 1, 11185 Zemun Polje
– Belgrade, Serbia (vdragicevic@mrizp.rs)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.14

ABSTRACT

Agroecology improves sustainability and biodiversity, preserving ecological balance. The objective of this study was to examine the impact of cover crops (oat and rye), combined with weed control measures (mechanical weed control, bio-herbicide, full and reduced herbicide rates, compared to the weedy check), on the status of non-enzymatic antioxidants. While rye, as a cover crop, promoted accumulation of phenolics and glutathione, thereby increasing the DPPH radical-scavenging capacity, fallow was associated with greater phytate accumulation, which may play a presumable role in grain yield development. It was shown that a combination of various agroecological practices could have a great impact on antioxidants in maize grain.

INTRODUCTION

Owing to the growing trend in the EU to reduce the use of pesticides by 50% by 2030 [1], more sustainable farming practices were promoted as a way to restore agroecosystems and biodiversity. Serbia is in the process of aligning agricultural policies with the EU Common Agricultural Policy, so it is important to reduce pesticide application.

Cover crops provide many benefits, from soil moisture preservation, reduction of soil erosion and nutrient runoff to weed suppression [2,3]. Due to the fact that there are no documented data on nutrient status in grain of agroecologically grown maize, the objective of this study was to examine the impact of cover crops (oat and rye), together with various weed control measures (mechanical weed control, bio-based herbicide, full and reduced herbicide rates, compared to the weedy check) on the status of non-enzymatic antioxidants.

EXPERIMENTAL

The research started in 2023, at the experimental field of the Maize Research Institute “Zemun Polje”, Belgrade, Serbia (44°52' N 20°20' E). The experiment included three replications, where the main plot was cover crop (CC), sown in November 2023: rye (*Secale cereale* L.), oat (*Avena sativa* L.), and fallow (NoCC). Sub-plots were weed control measures: herbicides nicosulfuron + tembotrione, applied either at the recommended rate (FLR) or at ½FLR,

bioherbicide (BH) – pelargonic acid; mechanical control (MC), done using an inter-row cultivator, and the weedy check (WC). After CCs termination (April 2024), maize (ZP388) was sown.

After harvest, maize grains were dried at 60°C and milled. After extraction with 5% trichloroacetic acid, phytic P (P_{phy}) and glutathione (GSH) were determined spectrophotometrically [4,5]. Soluble phenolics (Phen) were determined after extraction with double-distilled water [6] and expressed as μg of *3-hydroxy-4-methoxycinnamic acid* g^{-1} dry matter. DPPH radical scavenging activity was performed using the method by Brand-Williams et al. and Wong et al. [7,8]. Results were presented as mean $\pm$ standard deviation (SD). Correlation analysis between maize grain yield (GY) and analysed antioxidants (Pearson's correlation coefficients) was also performed.

RESULTS AND DISCUSSION

Irrespective of the greater variability in the concentration of antioxidants, the rye variant had the highest average values of phenolics, GSH, and DPPH radical reduction capacity (Table 1). Comparing weed control treatments, it is obvious that higher P_{phy} and DPPH radical reduction capacity were in FLR, followed by $\frac{1}{2}$ FLR, indicating that herbicides, by removing weeds, are able to increase the antioxidant capacity of the maize grain [9]. It is interesting that phytate mainly contributed to the reduction capacity of DPPH radical (Table 2), which could be tied to the greater resistance to oxidative stress [10].

The highest P_{phy} was in NoCC+FLR and oat+FLR (Table 1), while the highest reduction capacity of DPPH radical was in NoCC+FLR, again evidencing that the variant with fallow supports accumulation of non-enzymatic antioxidants in maize grain, particularly phytate, which could be potentially tied to greater phosphorus availability and certain microbial activity [11]. BH contributed mainly to the accumulation of phenolics and GSH in maize grain, with the highest value obtained in combination with rye, evidencing the distinctive impact of CCs and weed control measures on antioxidants in maize grain [12]. This could be one of the reasons for the significant positive correlation between maize grain yield and the reduction capacity of the DPPH radical and phytate (Table 2), supporting the hypothesis that grain yield and further grain performance closely rely on antioxidants, particularly phytate [10,13].

Table 1. The concentration of non-enzymatic antioxidants in maize grain (mean±SD).

	Pphy			Phen.			GSH			DPPH		
	NoCC											
WC	2.59	±	0.01	734.3	±	13.09	345.2	±	12.81	59.59	±	0.78
FLR	3.02	±	0.12	595.6	±	4.32	383.3	±	2.30	68.30	±	1.02
½FLR	2.99	±	0.08	567.2	±	22.94	475.8	±	15.21	65.12	±	1.02
BH	2.66	±	0.07	738.8	±	36.58	599.2	±	6.57	54.65	±	0.49
MC	2.99	±	0.11	514.7	±	1.63	473.0	±	5.81	58.74	±	0.61
Mean	2.85	±	0.08	630.1	±	15.71	455.3	±	8.54	61.28	±	0.78
	Oat											
WC	2.60	±	0.05	635.4	±	10.24	386.7	±	4.63	53.38	±	0.49
FLR	3.03	±	0.09	626.4	±	18.20	465.3	±	11.72	65.12	±	0.29
½FLR	2.87	±	0.06	612.9	±	15.85	471.7	±	13.21	55.82	±	0.25
BH	2.59	±	0.06	642.9	±	21.05	530.0	±	0.43	51.20	±	0.45
MC	2.71	±	0.06	615.9	±	12.14	501.9	±	10.22	55.19	±	1.06
Mean	2.76	±	0.06	626.7	±	15.50	471.1	±	8.04	56.14	±	0.51
	Rye											
WC	2.53	±	0.06	810.8	±	21.43	342.6	±	0.43	64.37	±	0.20
FLR	2.72	±	0.19	795.8	±	8.22	485.2	±	8.27	68.25	±	0.49
½FLR	2.87	±	0.08	684.1	±	24.29	521.5	±	12.49	67.45	±	0.86
BH	2.55	±	0.04	895.5	±	28.03	601.4	±	9.29	58.27	±	1.06
MC	2.66	±	0.10	637.6	±	17.27	549.0	±	5.24	64.27	±	0.78
Mean	2.66	±	0.09	764.7	±	19.85	499.9	±	7.15	64.52	±	0.68

Table 2. Correlation between analyzed non-enzymatic antioxidants and maize grain yield (GY), Pearson's correlation coefficients (r)

	GY	Pphy	Phen	GSH
Pphy	0.52*			
Phen	-0.09	-0.69*		
GSH	0.04	-0.07	0.15	
DPPH	0.55*	0.45*	0.07	-0.22

* Significant at $p < 0.05$

CONCLUSION

The combination of various agroecological practices that serve as weed control could contribute significantly to the maize grain yield quality, from the perspective of non-enzymatic antioxidants. While rye, as CC, promoted accumulation of phenolics and GSH, consequently increasing the reduction capacity of DPPH radical, it was shown that fallow was responsible for phytate accumulation, which plays a presumed role in grain yield development.

Acknowledgement

The author(s) declared that financial support was received for this work and/or its publication, funded by the European Union under Grant Agreement No. 101083589. Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or REA. Neither the European Union nor the granting authority can be held responsible for them. This work was partially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-33/2026-03/200040; Sustainable Development Goals - Zero Hunger).

REFERENCES

- [1] A. Tataridas, P. Kanatas, A. Chatzigeorgiou, S. Zannopoulos, I. Travlos, *Agronomy*, 12, 2022, 589.
- [2] O.A. Osipitan, J.A. Dille, Y. Assefa, Radicetti E., et al., *Crop Sci.*, 59 2019, 833-842.
- [3] J.S. Bergtold, S. Ramsey, L. Maddy, J.R. Williams, *Renew. Agric. Food Systems*. 34, 2019, 62-76.
- [4] V. Dragicevic, S. Sredojević, V. Perić, A. Nišavić, et al. *Acta Period. Technol.*, 42, 2011, 11–21.
- [5] M. Sari-Gorla, S. Ferrario, L. Rossini, C. Frova, et al. *Euphytica*, 67, 1993. 221–230.
- [6] A. Simić, S. Sredojević, M. Todorović, L. Đukanović, et al. *Seed Sci. Technol.*, 32, 2004, 213–218.
- [7] W. Brand-Williams, M.E. Cuvelier, C. Berset, *LWT - Food Scie. Technol.* 28, 1995, 25–30,
- [8] S.P. Wong, L.P. Leong, J.H. William Koh,. *Food Chem.* 99, 2006, 775–783.
- [9] J. Mesarović, J. Srdić, S. Mladenović-Drinić, V. Dragičević, et al. *J. Cereal Sci.*, 87, 2019, 132-137.
- [10] E. Doria, L. Galleschi, L. Calucci, C. Pinzino, et al, *J. Exp. Bot.*, 60, 2009, 967–978.
- [11] M. Hallama, C. Pekrun, S. Pilz, et al. *Plant Soil* 463, 2021, 307–328.
- [12] V. Dragicevic, Ž. Dolijanović, B. Janosevic, M. Brankov, et al. *Agronomy*, 11, 2021, 981.
- [13] A. Z. Oo, H. Asai, K.Songyikhangsuthor, B. Vongphouthone, et al., *Front. Agron.*, 2026, 1743691.

COMPARATIVE ANALYSIS OF PROGESTERONE EFFECTS ON NITRO-OXIDATIVE STRESS AND PROTEIN DAMAGE IN THE RAT PREFRONTAL CORTEX AND HIPPOCAMPUS DURING CEREBRAL HYPOPERFUSION

I. Guševac Stojanović¹, A. Todorović¹, Z. Stojanović², V. Petković¹, T. Zečević¹ and D. Drakulić¹

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (igusevac@vin.bg.ac.rs)*

²*Institute of Technical Sciences of SASA, University of Belgrade, Knez Mihailova Street 35/4, 11000, Belgrade, Republic of Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.15

ABSTRACT

Cerebral hypoperfusion (CH), a reduced cerebral blood flow, contributes to the development and progression of various neurodegenerative diseases for which adequate treatments are lacking. To investigate the neuroprotective effects of the hormone progesterone (P₄), male Wistar rats were divided into three groups: a control group treated with vehicle (flaxseed oil, 1 mg/kg/day) and groups subjected to permanent bilateral common carotid artery occlusion (2VO) to induce CH and that were treated with vehicle or P₄ (1.7 mg/kg/day) for 7 days. The prefrontal cortex (PFC) and hippocampus (HIPP) were isolated to perform a comparative analysis of P₄ effects on nitric oxide (NO) and advanced oxidation protein products (AOPP) levels. Obtained results suggest that P₄ differentially modulates regional nitro-oxidative pathways and protects HIPP from protein damage. This study sheds light on the potential neuroprotective role of the P₄ mechanism against CH.

INTRODUCTION

Cerebral hypoperfusion (CH) represents a significant pathological factor in the development and progression of neurodegenerative diseases such as vascular dementia, leading to region-specific damage in vulnerable central nervous system (CNS) structures [1]. The prefrontal cortex (PFC) and the hippocampus (HIPP) are particularly sensitive to decreased blood supply [2].

At the cellular level, CH deprives tissue of oxygen and nutrients, increasing the overproduction of reactive oxygen species (ROS) and inducing nitro-oxidative stress. Under these conditions, changes in nitric oxide (NO) homeostasis promote its reaction with superoxide anions to generate peroxynitrite (ONOO⁻). This aggressive oxidant directly targets functional

protein structures, modifying amino acid side chains and cross-linking the protein backbone, which finally results in the accumulation of advanced oxidation protein products (AOPP).

Progesterone (P₄) is a female steroid hormone that can exert potent neuroprotective actions [3]. However, its specific impact on 2VO-induced nitro-oxidative imbalance and macromolecular damage remains to be fully evaluated. To address this, the present study investigates the comparative effects of P₄ on NO and AOPP levels in the PFC and HIPP of rats subjected to permanent bilateral common carotid artery occlusion (2VO), an established experimental model widely used to mimic CH.

EXPERIMENTAL

All animal procedures were approved by the Ethical Committee for the Use of Laboratory Animals of VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, Belgrade (authorization numbers 02/11 and 323-07-04253/2016-05). Adult male Wistar rats were randomly divided into three experimental groups (n=6 per group): (I) Sham-operated controls receiving vehicle (commercial flaxseed oil, 1 mg/kg/day, s.c., Sham + V group); (II) group subjected to 2VO and treated with vehicle (commercial flaxseed oil, 1 mg/kg/day, s.c., 2VO + V group) and (III) 2VO group treated with P₄ (1.7 mg/kg/day, s.c., 2VO + P₄ group) dissolved in vehicle. For that purpose, rats were anesthetized with chloral hydrate and subjected to a ventral midline neck incision and the common carotid arteries were carefully exposed and isolated from the carotid sheaths and vagus nerve. In the 2VO groups, both arteries were permanently double-ligated using 5-0 silk sutures, whereas sham-operated controls underwent the identical surgical procedure without actual occlusion. Treatments were administered once daily starting immediately post-surgery. Following decapitation, the PFC and HIPP were rapidly dissected on ice and stored at -70°C. Tissue samples were homogenized in ice-cold buffers followed by differential centrifugation [4]. All assays were performed in duplicate in 96-well plates using a Tecan Sunrise microplate reader (Tecan Group Ltd., Männedorf, Switzerland). Total NO production was assessed by measuring stable nitrite (NO₂⁻) levels using the Griess reagent protocol [4]. Advanced oxidation protein products (AOPP) were quantified at 340 nm in the presence of potassium iodide against chloramine-T standards [5]. Statistical evaluation was performed via one-way ANOVA followed by Tukey's post-hoc test (p < 0.05).

RESULTS AND DISCUSSION

Permanent bilateral common carotid artery occlusion induced region-specific changes in nitro-oxidative profiles in both CNS structures. Regional differences were evident in the regulation of total NO levels. In the PFC, CH caused a significant reduction in NO levels compared to the control group (*p < 0.05, 2VO + V vs. Sham + V, Fig. 1A), indicating impaired local vascular dynamics.

Progesterone treatment completely reversed this deficit, restoring cortical NO to control levels, making it elevated compared to 2VO + V group ($^{\#}p < 0.05$, 2VO + P₄ vs. 2VO + V) (Fig. 1A). In contrast, the HIPP showed the opposite response, 2VO led to a significant accumulation of NO ($*p < 0.05$, 2VO + V vs. Sham + V, Fig. 1B). Administration of P₄ did not produce a statistically significant effect compared to the 2VO + V group, although values returned toward control levels (Fig. 1B).

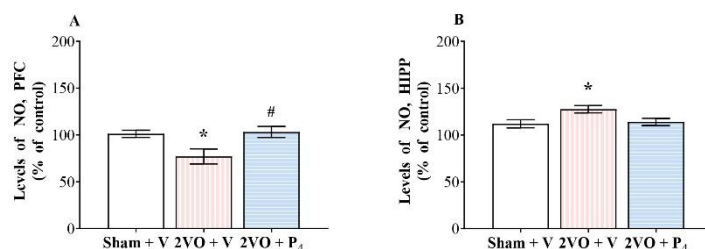


Figure 1. Comparative changes in total nitric oxide (NO) levels in the prefrontal cortex (PFC, A) and hippocampus (HIPP, B). All data are presented as % of control (Sham + V). $*p < 0.05$, 2VO + V vs. Sham + V; $^{\#}p < 0.05$, 2VO + P₄ vs. 2VO + V.

The downstream impact was reflected in protein oxidation. In the PFC, AOPP remained unchanged in all experimental groups (Fig. 2A). However, the HIPP tissue demonstrated a significant increase in AOPP levels in 2VO + V group compared to the control group ($*p < 0.05$, 2VO + V vs. Sham + V, Fig. 2B), confirming that localized HIPP nitro-oxidative stress induced protein modification. Importantly, treatment with P₄ prevented 2VO-induced AOPP accumulation in the HIPP, maintaining protein oxidation at control levels (Fig. 2B). This indicates that P₄ effectively counteracts regional nitro-oxidative stress and protects HIPP proteins from oxidative degradation during CH.

Altogether, these findings highlight the translational potential of P₄ as a targeted neuroprotective strategy against CH-related neurodegeneration.

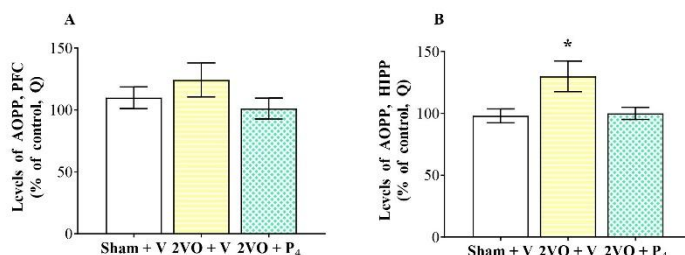


Figure 2. Comparative changes in advanced oxidation protein products (AOPP) levels in the prefrontal cortex (PFC, A) and hippocampus (HIPP, B). All data are presented as % of control (Sham + V). $*p < 0.05$, 2VO + V vs. Sham + V.

CONCLUSION

This comparative study demonstrates that CH activates structurally distinct pathophysiological pathways in the rat brain, characterized by ischemic NO deficiency in the PFC and NO accumulation in the HIPPO. Progesterone provides region-specific neuroprotection by normalizing cortical and hippocampal NO homeostasis and suppressing AOPP accumulation, thereby protecting vulnerable CNS structures from irreversible macromolecular damage.

Acknowledgement

This work was partially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-33/2026-03/200017 and 451-03-33/2026-03/200175).

REFERENCES

- [1] Y. Zhao, C. X. Gong, *Behav. Brain Res.*, 2016, *291*, 307–314.
- [2] F. Cechetti, A. S. Pagnussat, P. V. Worm, V. R. Elsner, J. Ben, M. S. da Costa, R. Mestriner, S. N. Weis, C. A. Netto, *Brain Res.*, 2012, *1485*, 109–116.
- [3] J. M. Azeez, T. R. Susmi, V. Remadevi, V. Ravindran, A. S. Sujatha, R. S. Ayswarya, S. Sreeja, *Am. J. Cancer Res.*, 2021, *11*, 5214–5232.
- [4] M. Stanojlović, I. Guševac Stojanović, M. Zarić, J. Martinović, N. Mitrović, I. Grković, D. Drakulić, *Cell. Mol. Neurobiol.*, 2020, *40*, 829–843.
- [5] Z. Stojanović, D. Drakulić, J. Martinović, A. Todorović, F. Veljković, N. Filipović, I. Guševac Stojanović, *Microplastics*, 2026, *5*, 54.

REMOVAL OF MALATHION FROM WATER USING CARBON-BASED ADSORBENTS PREPARED FROM RABBIT LITTER WASTE

T. Terzić¹, V. Milanković¹, N. Radivojević¹, J. Krstić², A. Jocić¹, S. Gutić³, V. Anićijević⁴, S. Brković¹, I. Pašti⁵ and T. Lazarević-Pašti¹

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia*

²*University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, 11000 Belgrade, Serbia*

³*Faculty of Science - University of Sarajevo, Zmaja od Bosne 33-35, Sarajevo 71000, Bosnia and Herzegovina (sgutic@pmf.unsa.ba)*

⁴*Military Academy, University of Defence in Belgrade, Veljka Lukica Kurjaka 33, 11042 Belgrade, Serbia*

⁵*University of Belgrade – Faculty of Physical Chemistry, Studentski Trg 12–16, 11158 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.16

ABSTRACT

The presence of organophosphate pesticides in water represents an important environmental concern due to their toxicity and possible transfer through the food chain. This study investigates carbon-based adsorbents prepared from rabbit litter waste for the removal of malathion (MLT) from water. The waste biomass was chemically impregnated and carbonized at 900 °C. The prepared adsorbents were characterized by SEM and BET analysis, while MLT adsorption was evaluated through batch experiments and quantified by UPLC. Adsorption equilibrium was reached within 30 min, and the maximum adsorption capacities were 17.66 and 27.52 mg g⁻¹, depending on the chemical treatment applied. The results suggest that rabbit litter waste can be valorized into efficient adsorbents for the treatment of pesticide-contaminated water.

INTRODUCTION

Malathion is a widely used organophosphate pesticide, and its extensive use in crop protection raises environmental and public health concerns due to its toxicity, mobility, and potential to persist in soil and water systems [1]. Among available methods for pesticide removal, adsorption is an efficient and environmentally friendly strategy. Carbon-based adsorbents are particularly

promising due to their porous structure, high surface area, and favorable surface chemistry [2]. In this study, rabbit litter waste was converted into carbon-based adsorbents and evaluated as a sustainable material for MLT removal from water, thereby supporting waste valorization and contributing to the remediation of pesticide-contaminated water in the context of food and environmental safety.

EXPERIMENTAL

Rabbit litter waste (rabbit sawdust containing urine) was used as a precursor for carbon-based adsorbents. The biomass was impregnated with either H_3PO_4 or NaOH , yielding CRL_H and CRL_N , respectively, and carbonized in a tube furnace under nitrogen flow (100 L h^{-1}) at 900°C for 60 min, with a heating rate of 5°C min^{-1} . The prepared materials were characterized by SEM. Nitrogen adsorption measurements were performed at 77 K using a Sorptomatic 1990 analyzer after degassing at 200°C for 24 h, and the specific surface area was calculated using the BET method. MLT adsorption experiments were performed as previously described [2].

RESULTS AND DISCUSSION

The SEM micrographs (Figure 1) reveal an irregular, layered carbonaceous morphology with visible surface roughness and cracks, suggesting structural changes induced by carbonization and chemical treatment. Such a morphology may contribute to the accessibility of adsorption sites involved in MLT removal.

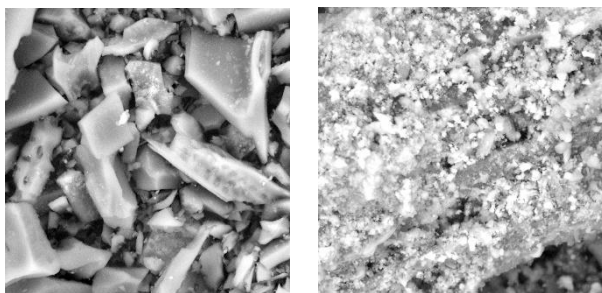


Figure 1. SEM micrographs of CRL_H (left) and CRL_N (right) materials

The specific surface area of the prepared materials, determined by the BET method, was significantly higher for CRL_H ($419 \text{ m}^2 \text{ g}^{-1}$) than for CRL_N ($109 \text{ m}^2 \text{ g}^{-1}$), indicating that H_3PO_4 impregnation was far more effective in developing the material's surface area.

The equilibrium contact time was determined by monitoring the residual MLT concentration in solution after contact with the adsorbent materials. The results, presented in Figure 2, showed that the MLT concentration reached a nearly constant value after 30 min, which was selected as the equilibrium contact time for further adsorption experiments.

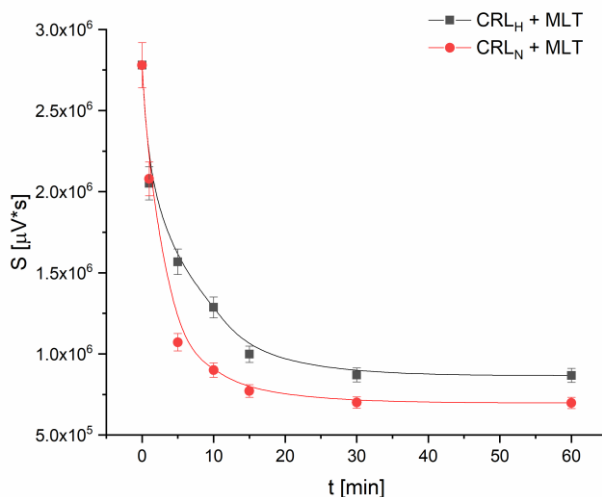


Figure 2. Determination of equilibrium contact time.

Adsorption isotherms were analyzed using the non-linear Freundlich and Langmuir models to evaluate MLT adsorption behavior and adsorbent affinity (Table 1). The Freundlich model provided a very good fit to the experimental data for both materials, with n values higher than 1, indicating favorable adsorption on energetically heterogeneous surfaces. The higher K_F value obtained for CRL_N suggests a stronger affinity of MLT toward this material than toward CRL_H . Although the Langmuir model also provided an acceptable fit, its assumption of monolayer adsorption on a homogeneous surface should be used with caution. Therefore, the Langmuir q_{max} values are primarily used for comparison between the two materials, rather than as absolute maximum adsorption capacities. The higher q_{max} value obtained for CRL_N (27.52 mg g⁻¹) compared to CRL_H (17.66 mg g⁻¹) follows the same trend as the Freundlich model and indicates better adsorption performance of CRL_N . This improvement may be attributed to the favorable influence of NaOH impregnation on surface chemistry and the accessibility of adsorption sites. However, the results also suggest that a developed specific surface area alone is not necessarily sufficient for efficient MLT adsorption, most likely due to incomplete surface accessibility and the specific interaction requirements of MLT molecules.

Table 1. Parameters of the Freundlich and Langmuir isotherm models for MLT adsorption at an adsorbent dose of 1 mg mL⁻¹

Material	Freundlich isotherm			
	$K_F ((\text{dm}^3 \text{ mg}^{-1})^{1/n})$	n	χ^2	R^2
CRL _H	0.165	1.321	0.173	0.980
CRL _N	0.264	1.321	0.050	0.998
	Langmuir isotherm			
	$K_L (\text{dm}^3 \text{ mg}^{-1})$	$q_{\text{max}} (\text{mg g}^{-1})$	χ^2	R^2
CRL _H	0.005	17.661	0.225	0.975
CRL _N	0.005	27.519	0.145	0.993

CONCLUSION

Rabbit litter waste was successfully converted into carbon-based adsorbents for the removal of MLT from water. Although CRL_H showed a higher specific surface area, CRL_N exhibited better adsorption performance, indicating the important role of surface chemistry and accessible adsorption sites. The results highlight rabbit litter waste as a promising precursor for sustainable adsorbents in pesticide-contaminated water treatment.

Acknowledgement

This work was funded by the Serbian Ministry of Science, Technological Development, and Innovations (grant numbers 451-03-34/2026-03/200146, 451-03-33/2026-03/200017, and 451-03-33/2026-03/200026). The authors also acknowledge support from the Serbian Academy of Sciences and Arts (project no. F-49). The authors also acknowledge the support provided by the Serbian Ministry of Defence, Republic of Serbia (project code: VA-TT/1/22-24).

REFERENCES

- [1] T. Lazarević-Pašti, T. Terzić, A. Leskovic, S. Petrović, V. Milanković, N. Radivojević, J. Krstić, K. Kokanov Stanković, A. Jocić, S. Brković, I. Pašti, J. Xenobiot., 2026, 16, 75.
- [2] T. Tasić, V. Milanković, K. Batalović, S. Breitenbach, C. Unterweger, C. Fürst, I. Pašti, T. Lazarević-Pašti, Foods, 2023, 12, 2362.

COMPARATIVE ANALYSIS OF THE MINERAL COMPOSITION OF ORGANICALLY AND CONVENTIONALLY CULTIVATED *Lycium barbarum* L. FRUITS FROM SERBIA

T. Ilić¹, I. Kodranov² and U. Čakar¹

¹University of Belgrade, Faculty of Pharmacy, Department of Bromatology, Vojvode Stepe 450, 11000 Belgrade, Serbia (tilic@pharmacy.bg.ac.rs)

²University of Belgrade, Faculty of Chemistry, Department of Analytical Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.17

ABSTRACT

Goji berry (*Lycium barbarum* L.) is recognised as a valuable source of essential minerals. This study compared the mineral composition of organically and conventionally grown goji berries cultivated in Serbia. Fresh fruits were collected from an organic plantation in Subotica and a conventional plantation in Smederevo. Mineral composition was determined by ICP-OES and ICP-MS following microwave-assisted acid digestion. Potassium was the predominant mineral in both samples. Organically grown fruits contained significantly higher levels of K, Na, Mg, P, S, Cu, and Mn ($p < 0.05$), whereas conventionally grown fruits showed a higher calcium content. No significant differences were observed for Fe and Zn. These findings indicate that cultivation system influences mineral accumulation and suggest that organically produced goji berries possess a more favourable mineral profile.

INTRODUCTION

Goji berry (*Lycium barbarum* L., Solanaceae) is widely recognised as a functional food due to its high nutritional value and abundance of bioactive compounds, including minerals, vitamins, polysaccharides, carotenoids and phenolic compounds [1]. Owing to its nutritional and health-promoting properties, goji cultivation has expanded beyond its traditional growing regions in Asia and has become increasingly important in many European countries, including Serbia [2].

Minerals are essential nutrients involved in numerous physiological processes, and their concentration in fruits is influenced by genotype, environmental conditions, soil properties and cultivation practices [3].

Organic and conventional production systems differ in fertilisation and crop management, which may affect mineral accumulation in plant tissues. However, studies comparing the mineral composition of organically and conventionally

grown goji berries remain limited, particularly for fruits cultivated in Serbia. Therefore, the aim of this study was to compare the mineral composition of organically and conventionally grown *Lycium barbarum* fruits produced under Serbian growing conditions.

EXPERIMENTAL

Fresh, ripe *Lycium barbarum* fruits were harvested from an organic plantation in Subotica (northern Serbia) and a conventional plantation in Smederevo (central Serbia). Samples were washed, homogenised and subjected to microwave-assisted acid digestion using HNO₃/H₂O₂ according to a previously described procedure [2]. Macroelements (K, Na, Ca, Mg, P and S) were determined using ICP-OES (iCAP 6300 Duo, Thermo Fisher Scientific, USA), while microelements (Fe, Zn, Cu, Mn) were analysed by ICP-MS (iCAP Qc, Thermo Fisher Scientific, UK). Results are expressed as mean $\pm$ SD (n = 3). Statistical differences between cultivation systems were evaluated using Student's *t*-test (*p* < 0.05).

RESULTS AND DISCUSSION

The mineral composition of organically and conventionally grown *Lycium barbarum* fruits is presented in Table 1.

Potassium was the predominant mineral in both cultivation systems, followed by phosphorus and sodium. Organically grown fruits contained significantly higher concentrations of K, Na, Mg, P, S, Cu, and Mn, whereas conventionally grown fruits exhibited a significantly higher calcium content. No significant differences were observed for Fe and Zn.

The obtained mineral distribution agrees with previous reports describing potassium as the dominant mineral in goji berries cultivated in different geographical regions [4,5]. Likewise, the mineral concentrations observed in the present study are comparable with those previously reported for Italian *Lycium barbarum* fruits [6]. The observed differences between cultivation systems may be attributed to variations in fertilization practices and nutrient availability. Organic fertilizers enrich the soil with essential nutrients and improve its physicochemical and biological properties, thereby enhancing mineral uptake and accumulation in plants [3]. This may explain the higher concentrations of most minerals observed in organically cultivated goji berries compared with conventionally grown fruits. Conversely, the higher calcium concentration in conventionally grown fruits may reflect differences in fertilization strategy and soil composition. Overall, both cultivation systems produced mineral-rich fruits, highlighting the nutritional value of Serbian goji berries.

Table 1. Mineral composition (mg/kg) of organically and conventionally cultivated *Lycium barbarum* fruits.

Mineral (mg/kg)	Organic goji	Conventional goji
K	4367 ± 21.50 ^a	2951 ± 11.00 ^b
Na	740.3 ± 9.38 ^a	625.1 ± 10.20 ^b
Ca	149.1 ± 6.00 ^a	224.2 ± 5.01 ^b
Mg	255.3 ± 5.51 ^a	146.7 ± 3.51 ^b
P	1571 ± 20.50 ^a	1008 ± 13.20 ^b
S	527.1 ± 8.10 ^a	333.3 ± 7.51 ^b
Fe	5.76 ± 0.81 ^a	7.92 ± 1.13 ^a
Zn	6.10 ± 0.49 ^a	5.36 ± 0.64 ^a
Cu	2.94 ± 0.16 ^a	2.48 ± 0.11 ^b
Mn	1.42 ± 0.02 ^a	0.65 ± 0.03 ^b

Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate statistically significant differences between cultivation systems (Student's t-test, $p < 0.05$)

CONCLUSION

The cultivation system significantly influenced the mineral composition of Serbian goji fruits. Organically grown berries contained higher concentrations of most essential macro- and microelements, whereas calcium was higher in conventionally grown fruits. These findings confirm the high nutritional quality of Serbian goji berries and indicate that organic cultivation may improve their mineral profile.

Acknowledgement

The research presented in this article is part of a study financially supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, through two Grant Agreements with the University of Belgrade – Faculty of Pharmacy (No. 451-03-136/2025-03/200161 and No. 451-03-137/2025-03/200161).

REFERENCES

- [1] A. D. Tudor, C. Bolohan, V. Tudor, I. R. Teodorescu. Main active components of goji berry and their nutritional importance - A review. *JALSE*. 2023, 55(2(190)), 111–132.
- [2] T. Ilić, I. Đuričić, I. Kodranov, Lj. Ušjak, S. Kolašinac, M. Milenković, M. Marčetić, D. Božić, B. Vidović. Nutritional value, phytochemical composition and biological activities of *Lycium barbarum* L. fruits from Serbia. *Plant Foods Hum Nutr.* 2024, 79(3), 662–668.

- [3] A. C. Pedro, M. C. Sánchez-Mata, M. L. Pérez-Rodríguez, M. Cámara, J. L. López-Colón, F. Bach, et al. Qualitative and nutritional comparison of goji berry fruits produced in organic and conventional systems. *Scientia Horticulturae*. 2019, 257, 108660.
- [4] D. Montesano, G. Rocchetti, L. Cossignani, L. Lucini, M. S. Simonetti, F. Blasia F. Italian *Lycium barbarum* L. berry: Chemical characterization and nutraceutical value. *Natural Product Communications*. 2018, 13(9), 1934578X1801300.
- [5] E. Covaci, M. Senila, L. F. Leopold, N. K. Olah, C. Cobzac, V. Ivanova-Petropulos et al. Characterization of *Lycium barbarum* L. berry cultivated in North Macedonia: A chemometric approach. *JBR*. 2020, 10(2), 223–241.
- [6] A. Niro S, Fratianni, G. Panfili, L. Falasca, L. Cinquanta, M. R. Alam. Nutritional evaluation of fresh and dried goji berries cultivated in Italy. *Italian Journal of Food Science*. 2017, 29(3), 398-408.

NATURAL DEEP EUTECTIC SOLVENT (NADES) EXTRACTION IMPROVES POLYPHENOL YIELD AND ANTIOXIDANT ACTIVITY OF SAGE EXTRACTS

M. Janić, A. Valenta Šobot, K. Radošević, M. Kostić, S. Brković, T. Momić
and J. Filipović Tričković

*Vinča Institute of Nuclear Sciences – National Institute of the Republic of
Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade,
Serbia (marijana.janic@vin.bg.ac.rs)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.18

ABSTRACT

In traditional medicine, sage is frequently utilized for disease prevention and treatment. This study compared the polyphenol content and radical scavenging capacity of sage leaf extracts prepared with choline chloride:urea mixed with 10% water (ChCl:U) and sage water extract (dH₂O) made under the identical experimental conditions. Total phenolic content (TPC), total tannin content (TTC), and total flavonoid content (TFC) in both extracts were measured. Antioxidant activity of sage extracts was estimated using the DPPH and ABTS assays. The results showed that solvent polarity affected polyphenol extraction from sage. Higher polyphenol content (TPC and TTC) and radical scavenging properties against ABTS^{•+} were observed in sage extract made with ChCl:U. TFC and neutralization towards DPPH[•] were negligibly higher in the water extract. The findings showed that NADES based on choline chloride is an effective solvent for sage leaf extraction.

INTRODUCTION

Sage (*Salvia officinalis* L.) has a complex secondary metabolite profile, abundant in rosmarinic acid (a caffeic acid dimer), carnosic acid and carnosol (phenolic diterpenes), and flavonoids (luteolin and apigenin glycosides), making it an excellent source of natural antioxidants [1]. These phenolic compounds are essential for the neutralization of reactive oxygen species (ROS), which in turn reduces the risk of chronic diseases [2]. Aqueous extraction, although environmentally friendly, exhibits high surface tension and low affinity for semi-polar and hydrophobic compounds (e.g., carnosic acid), resulting in poor mass transfer, low target yields, and rapid thermal degradation of the extracted polyphenols [3]. Natural deep eutectic solvents (NADES) provide a highly adaptable and environmentally friendly solution to address these restrictions. These systems consist of a quaternary ammonium salt that acts as a hydrogen-bond acceptor (HBA) and an organic molecule that acts as a hydrogen-bond

donor (HBD). NADES have good biodegradability and biocompatibility, low cost, and simple preparation. The drawbacks of using NADES for extract preparation include their low volatility and high viscosity [1, 3, 4, 5].

NADES like choline chloride: urea (ChCl:U) offer a sustainable, non-toxic, and highly efficient alternative [1, 3]. This study investigates the efficacy of ChCl:U in recovering phenolic compounds from sage leaves and evaluates the resulting antioxidant capacity.

EXPERIMENTAL

Sage extract preparation

ChCl:U NADES was prepared by combining choline chloride (ChCl) and urea (U) in a 1:2 molar ratio, according to the previously reported procedure [5]. The final solvent for sage extraction was prepared by mixing 90% ChCl:U with 10% water to achieve slightly better solubility and lower viscosity. It was stated that NADES diluted up to 50% with water is stable and retains intact hydrogen bonds [6]. 100 mg of pulverized sage leaves were extracted with 2 mL of solvent. Extraction in an ultrasonic bath (Elma, Schmidbauer GmbH, Singen, Germany) lasted 90 minutes at 50°C, similar to previously described [3, 5]. A pure water extract of sage (dH₂O) was prepared under the same conditions as the control for comparison purposes.

Chemical characterization of sage extracts

The total phenolic content (TPC) and total tannin content (TTC) were determined using the capacity of phenolics to interact with the Folin–Ciocalteu (FC) reagent, forming a blue-colored complex [2, 5]. The total flavonoid content (TFC) was evaluated by using the ability of flavonoids to bind Al³⁺, allowing them to form yellow Al-complexes [2, 5]. Chemical characterization of selected extracts was determined spectrophotometrically (Sunrise, Tecan Group Ltd, Männedorf, Switzerland). TPC and TTC were expressed as mg of gallic acid equivalents (GAE) per g of dried weight (d.w.), while TFC was expressed as mg of quercetin equivalents (QE) per g of dried weight (d.w.).

Antioxidative capacity of sage extracts

The radical scavenging activity of sage extracts towards DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation was evaluated using previously described methods [2, 5]. Measurements were performed using spectrophotometric assays (Sunrise, Tecan Group Ltd, Männedorf, Switzerland), resulting in a color change from the purple-colored stable DPPH[•] to the yellow-colored reduced form DPPH-H, as well as decolorization of the blue-green ABTS^{•+}. The results were expressed as IC₅₀ values (the concentration of the sample required to neutralize 50% of the selected radicals).

RESULTS AND DISCUSSION

According to the results of our research, a better extraction yield of polyphenols was achieved using ChCl:U with 10% water addition, compared to water (Fig. 1A). TPC in the ChCl:U extract was 36% higher, while TTC was 18% higher compared to the dH₂O sage extract. On the other hand, TFC in the dH₂O sage extract was merely 5% higher.

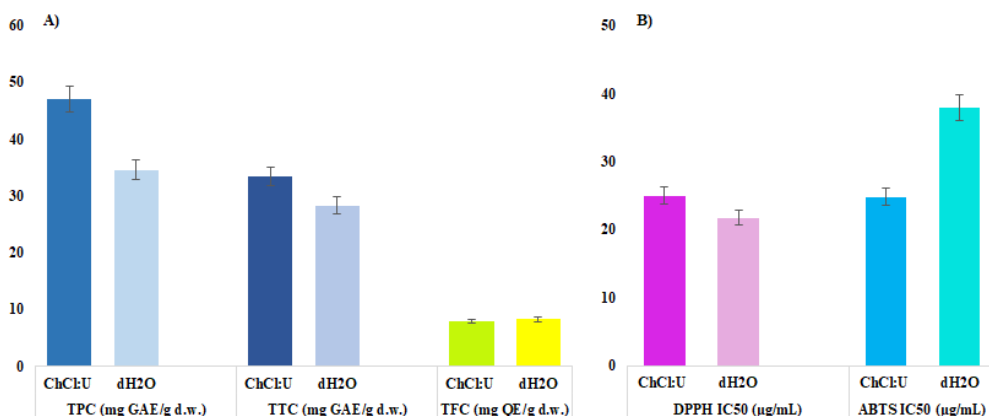


Figure 1. A) Results for chemical characterization of sage extracts; B) Results for DPPH[•] and ABTS^{•+} scavenging of sage extracts.

It has previously been observed that ChCl:U can effectively extract phenolic compounds from plant material, particularly sage, when compared to conventional solvents like water and ethanol [3 - 5]. Nevertheless, information on TTC for NADES extraction of sage has not previously been reported in the literature.

The IC₅₀ value was 15% higher for ChCl:U sage extract in scavenging DPPH[•], indicating lower antioxidant activity than the water extract, as shown in Fig. 1B. On the contrary, the ability of ChCl:U extract to neutralize ABTS^{•+} was better, with 53% higher inhibitory potency compared to the water sage extract. When comparing antioxidant profiles, an extract showing higher ABTS^{•+} and lower DPPH[•] scavenging capability reflects a solvent-dependent extraction and is assumed to be due to different mechanisms of action [5]. The results of the DPPH[•] neutralization of NADES sage extract are consistent with the literature reports for various types of sage extracts [1, 2, 4, 5]. The literature on the radical scavenging of ABTS^{•+} by NADES sage extracts remains limited. Results obtained in this research for extracts neutralizing ABTS^{•+} are in correspondence with previously published results for different types of sage extracts [2, 5, 7].

CONCLUSION

The application of the same extraction method using ChCl:U and water indicated that NADES exhibited superior efficacy for extracting polyphenols from sage leaves. The sage extract derived from ChCl:U exhibited elevated total phenolic and total tannin contents, although the total flavonoid content was somewhat greater in the water extract, along with modest radical scavenging properties against DPPH[•]. Conversely, the antioxidant activity against ABTS⁺⁺ was much superior in the ChCl:U sage extract. Our findings suggest that ChCl:U NADES may serve as an efficient solvent for the extraction of polyphenols from sage leaves.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant no. 451-03-33/2026-03/ 200017 and 451-03-3627/2025-03/3456).

REFERENCES

- [1] T. Jurić, R. Ždero Pavlović, D. Uka, I. Beara, T. Majkić, S. Savić, M. Žekić, B.M. Popović, *Ind. Crops Prod.*, 2024, 214, 118559.
- [2] A. Valenta Šobot, M. Janić, I. Popović, T. Lazarević-Pašti, T. Momić, A. Krstić, J. Filipović Tričković, *Arch. Ind. Hyg. Toxicol.*, 2024, 75, 137 – 146.
- [3] M. Jakovljević, S. Jokić, M. Molnar, I. Jerković, *Plants*, 2021, 10, 80.
- [4] M. Jakovljević, J. Vladić, S. Vidović, K. Pastor, S. Jokić, M. Molnar, I. Jerković, *Plants*, 2020, 9, 153.
- [5] M. Janić, N. Zdolšek, J. Marinković, T. Lazarević-Pašti, T. Momić, S. Brković, A. Valenta Šobot, J. Filipović Tričković, *J. Mol. Liq.*, 2025, 434, 127995.
- [6] E. Chevé-Kools, Y. H. Choi, C. Roullier, G. Ruprich-Robert, R. Grougnet, F. Chapeland-Leclerc, F. Hollmann, *Green Chem.*, 2025, 27, 8360 – 8365.
- [7] K. Kozics, V. Klusová, A. Srančíková, P. Mučaji, D. Slameňová, Ľ. Hunáková, B. Kusznierewicz, E. Horváthová, *Food Chem.*, 2013, 141, 2198 – 2206.

AUTOMATED MATLAB ANALYSIS OF PORE MORPHOLOGY IN A CLAY-BASED FILTER FOR CIPROFLOXACIN REMOVAL

M. Kokunešoski, J. Ružić, Z. Gojković and M. Ječmenica Dučić

Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (marija.jecmenica@vin.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.19

ABSTRACT

The presence of antibiotics such as ciprofloxacin in the environment poses a significant threat to living organisms, including adverse effects on human health. Municipal wastewater originating from households, hospitals, and industrial sources is the primary pathway by which this pharmaceutical enters natural waterways. Ciprofloxacin is a synthetic fluoroquinolone antibiotic that continually accumulates in the environment and is difficult to degrade naturally. A clay-based filter under development removed over 80% of this compound from water at various concentrations. To further improve its removal, the filter surface was investigated using automated, MATLAB-based image analysis.

INTRODUCTION

The presence of antibiotics in the environment poses a major challenge for ecosystem protection and public health [1]. Ciprofloxacin, a synthetic fluoroquinolone, is particularly concerning due to its chemical stability and continuous accumulation in aquatic ecosystems [2,3]. This paper explores the use of Kolubara clay-based filters for removing organic pollutants from water. Previous work demonstrated ciprofloxacin removal efficiencies exceeding 80% from aqueous solutions (6 μM and 9 μM in phosphate buffer, pH = 5), with rates of ~82% and ~85%, respectively [4]. While pore size distribution is fundamental for filter characterization, pore shape has emerged as an equally important factor influencing filtration performance [5], affecting hydrodynamic characteristics, specific surface area, tortuosity, and overall removal efficiency [6]. Automated digital image processing in MATLAB [7] enables fast, objective quantification of both pore size and shape parameters (circularity, eccentricity), essential for comprehensive filter characterization and synthesis optimization.

EXPERIMENTAL

The chemical composition (wt%) of clay is: SiO₂ 88%, Al₂O₃ 6.05%, Fe₂O₃ 2.06%, K₂O 1.76%, Na₂O 1.05%, TiO₂ 0.48%, MgO 0.35%, CaO 0.18% [8]. The raw clay was thermally and chemically treated to remove organic impurities and iron oxide content, respectively. The powder mixture, prepared by blending purified clay with 0.5 wt% aqueous H₃BO₃, was uniaxially cold-pressed at 40 MPa in a Ø40 mm mould and sintered at 1300 °C for 4 h. Full details are given in [4].

An optical micrograph of the sintered sample cross-section was acquired using a stereo microscope (Zeiss Stemi 508) at 10x magnification and saved in JPEG format (1920 × 1080 pixels, 24-bit RGB). A single representative micrograph was analyzed (Figure 1a).

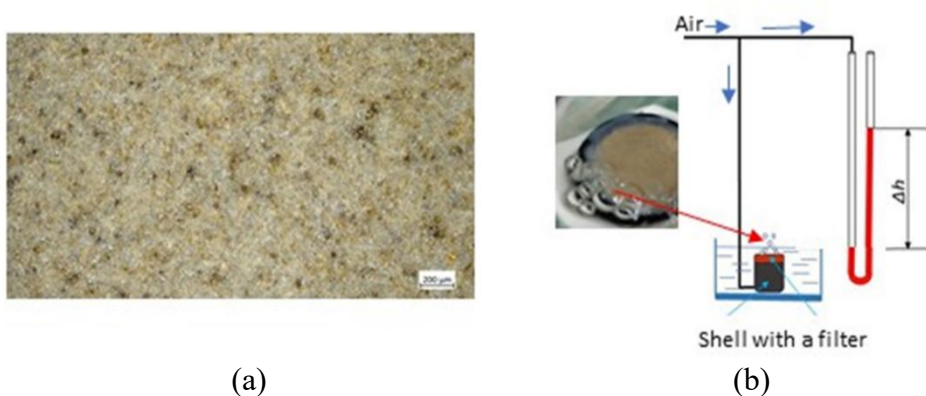


Figure 1. (a) Optical microscopy image of the tested sample; **(b)** in-house built setup and position of the largest pores on the tested filter

All image processing was performed in MATLAB R2023a [7]. The automated pipeline comprised: 1) spatial calibration using the embedded 200 µm scale bar; 2) RGB to grayscale conversion (*rgb2gray*), uneven illumination correction (*imflatfield*, kernel 40 pixels), and contrast enhancement (*imadjust*); 3) adaptive thresholding for pore segmentation (*imbinarize*, ForegroundPolarity = bright, sensitivity = 0.40); 4) hole-filling (*imfill*) and noise removal (*bwareaopen*, 15 pixels); 5) measurement of pore properties via regionprops. Derived parameters were: equivalent circular diameter, circularity (1 = perfect circle, approaching 0 = irregular shape), and eccentricity (0 = circle, 1 = line segment).

The maximum pore diameter obtained by image analysis (127.0 µm) showed 95% agreement with the experimental reference value of 133.5 µm [4], determined by the first bubble-point method using a custom-built apparatus based on ASTM F316 [9] adapted for macroporous ceramics (Figure 1b). Compressed air was introduced at the bottom of the shell, and pressure was

gradually increased until the first air bubble appeared at the upper sample surface. This validation confirms the accuracy of the segmentation and measurement procedures applied in the present pore shape analysis.

RESULTS AND DISCUSSION

The automated MATLAB algorithm detected 5,077 individual pores on the analyzed micrograph. Results of the pore shape analysis are summarized in **Figure 2 (a)** and **(b)**. The mean circularity of 0.48 and wide standard deviation (± 0.26) reveal a highly heterogeneous pore population, with most pores deviating significantly from ideal circular geometry. The eccentricity distribution is shifted toward higher values (median 0.85), confirming pronounced pore elongation, a direct consequence of uniaxial pressing, where particle rearrangement occurs preferentially perpendicular to the pressing direction during synthesis.

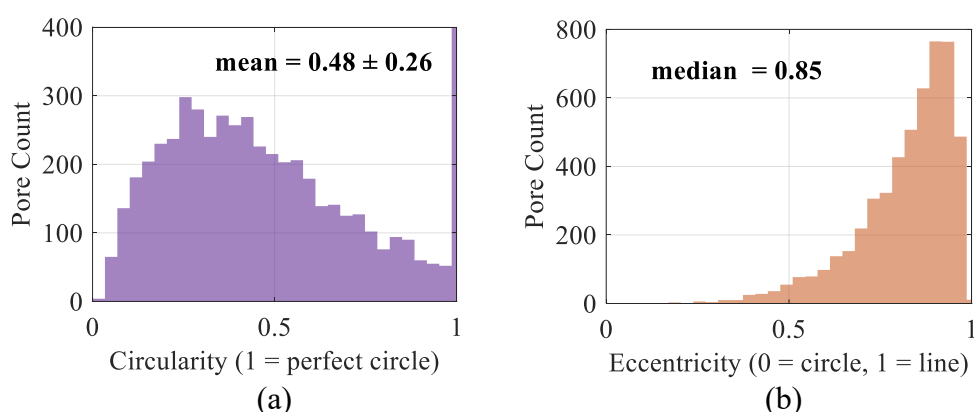


Figure 2. (a) Circularity distribution histogram; **(b)** Eccentricity distribution histogram.

This anisotropic morphology directly benefits filtration performance. Elongated and irregular pores exhibit a higher surface-to-volume ratio than spherical pores of equivalent diameter. The tortuous flow paths extend fluid residence time, enabling ciprofloxacin removal. Furthermore, interconnected elongated pores reduce "dead-end" porosity, maximizing utilization of the filter's internal surface area. Varying preparation pressing pressure or employing isostatic pressing could tune pore shape for optimal performance of the filter.

CONCLUSION

The automated MATLAB pipeline provides rapid feedback on morphological effects, enabling efficient optimization of filter fabrication conditions. A limitation is the single-micrograph analysis; future work should assess multiple regions and replicate specimens. If a clay-based filter that removes pharmaceuticals from water were to become an integral part of the water

treatment system within the food and pharmaceutical industries, the quality of the final products would increase, thereby directly benefiting public health and quality of life.

Acknowledgement

This work was partially supported by The Fund for Innovation Activity, Republic of Serbia, the program of Proof of Concept (Grant no. PoC 5925), and the Serbian Ministry of Education, Science, and Technological Development (contract number: 451-03-33/2026-03/200017).

REFERENCES

- [1] B. M. Al-Howri, S. Ismail, M. Khajavian, *Environ. Monit. Assess.* 2025, 197, 1095.
- [2] K. K. Sodhi, D. K. Singh, *J. Water Process Eng.* 2021, 43, 102218.
- [3] L. Asad, Z. U. H. Khan, F. Ullah, F. *RSC Adv.* 2026, 16(17), 15119–15151.
- [4] M. Kokunešoski, Z. Gojkovic, J. Ružić, *Ceramics* 2026, 9, 66.
- [5] J. Adler, *J. Am. Ceram. Soc.* 2005, 88, 3261–3271.
- [6] I. M. Griffiths, I. Mitevski, I. Vujkovic, M. R. Illingworth, P. S. Stewart, *J. Membr. Sci.* 2020, 598, 117664.
- [7] J. Little, C. Moler, "MATLAB," MathWorks, Inc., Natick, Massachusetts, USA.
- [8] M. Kokunešoski, A. Šaponjić, V. Maksimović, M. Stanković, M. Pavlović, J. Pantić, J. Majstorović, *Ceram. Int.* 2014, 40, 14191–14196.
- [9] ASTM International. ASTM F316-03(2019), Standard Test Methods for Pore Size Characteristics of Membrane Filters by Bubble Point and Mean Flow Pore Test; ASTM International: West Conshohocken, PA, USA, 2019.

EDIBLE CHITOSAN FILMS CONTAINING PROPOLIS AND *Fumaria officinalis* BIOACTIVES

R. Ahmoda¹, M. Milošević², A. Marinković¹ and A. A. Jovanović³

¹University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4,
11000 Belgrade, Serbia

²University of Belgrade, Institute of Chemistry, Technology and Metallurgy -
National Institute of the Republic of Serbia, Njegoševa 12, 11000 Belgrade,
Serbia

³University of Belgrade, Institute for the Application of Nuclear Energy INEP,
Banatska 31b, 11080 Belgrade, Serbia (ajovanovic@inep.co.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.20

ABSTRACT

Chitosan-based edible films plasticized with ethyl lactate and enriched with propolis and *Fumaria officinalis* extract (4 and 8 wt%) were developed and characterized as potential active food packaging materials. Film morphology was analyzed by optical microscopy and SEM, while antioxidant activity was evaluated using DPPH• and ABTS•⁺ assays. The 4 wt% film exhibited a compact and homogeneous structure, indicating good dispersion of bioactive compounds within the chitosan matrix. In contrast, the 8 wt% film showed increased heterogeneity and visible aggregates, suggesting partial extract agglomeration at higher concentrations. Both films demonstrated strong antioxidant activity, which increased with extract content. The 8 wt% film showed significantly higher DPPH• scavenging activity, while ABTS•⁺ inhibition remained above 95% for both samples.

INTRODUCTION

Edible films are biodegradable biopolymer-based layers applied directly to food surfaces to improve quality, extend shelf life, and reduce moisture and oxygen transfer. Among biopolymers, chitosan is widely used due to its excellent film-forming ability, biocompatibility, biodegradability, and suitability for various bioactive compounds that can enhance functional performance [1,2]. Natural additives such as *Fumaria officinalis* extract and propolis are of particular interest, as both are rich in phenolic compounds, responsible for strong antioxidant and antimicrobial properties, contributing to additional protective and bioactive effects in food packaging applications [3,4]. This study aimed to develop chitosan-based edible films plasticized with ethyl lactate and enriched with *F. officinalis* extract and propolis, and to evaluate their functional and structural properties. The films were characterized in terms of antioxidant

activity, surface morphology using optical microscopy and scanning electron microscopy (SEM), and swelling behavior to assess their suitability for potential application as active food packaging materials.

EXPERIMENTAL

The aerial part of *F. officinalis* was extracted with 50% ethanol (ultrasound-assisted, 15 min), followed by solvent removal *via* rotary evaporation and storage of the dried extract at 4°C [5]. Chitosan-based film-forming solutions were prepared in aqueous lactic acid with triglyceryl citrate as a plasticizer. Propolis, pre-emulsified with ethyl lactate and GlucoPON®, was added to the chitosan solution, followed by incorporation of fumitory extract at 4 and 8 wt%. The mixtures were cast and dried to form films. Film morphology was analyzed by optical microscopy and SEM. Antioxidant activity of the films was evaluated using DPPH• and ABTS•⁺ assays by measuring absorbance changes at 517 and 734 nm, respectively. Film samples (3 mg) were dissolved in PBS and reacted with radical solutions prepared according to standard methods [5].

RESULTS AND DISCUSSION

At 4 wt%, the film surface is relatively uniform and compact, indicating good dispersion of the extract in the chitosan matrix, with only minor irregularities likely due to natural heterogeneity of chitosan films (Figure 1a).

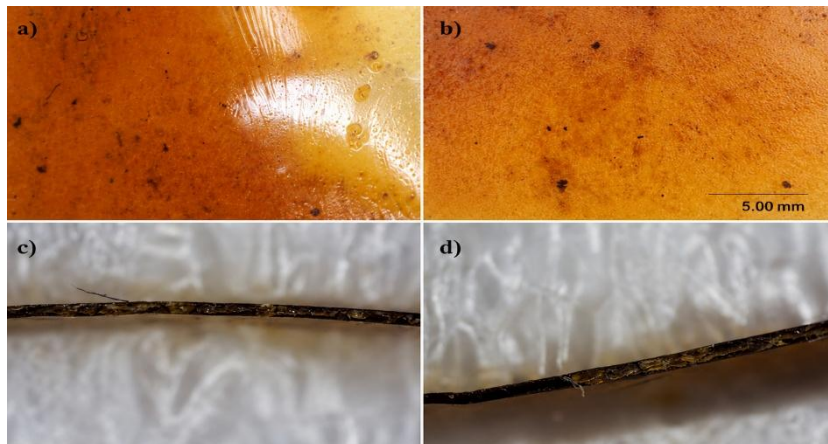


Figure 1. Optical micrographs of films with propolis and *Fumaria officinalis* extract: 4 wt% (a and c) and 8 wt% (b and d).

In contrast, the 8 wt% film shows a more heterogeneous surface with visible aggregates, suggesting partial agglomeration of the extract and increased surface roughness (Figure 1b). Cross-sectional images confirm these findings: the 4 wt% film has a dense, continuous structure and good compatibility between components. The 8 wt% film displays structural irregularities and reduced

uniformity, likely due to saturation of interactions such as hydrogen bonding between chitosan and extract constituents.

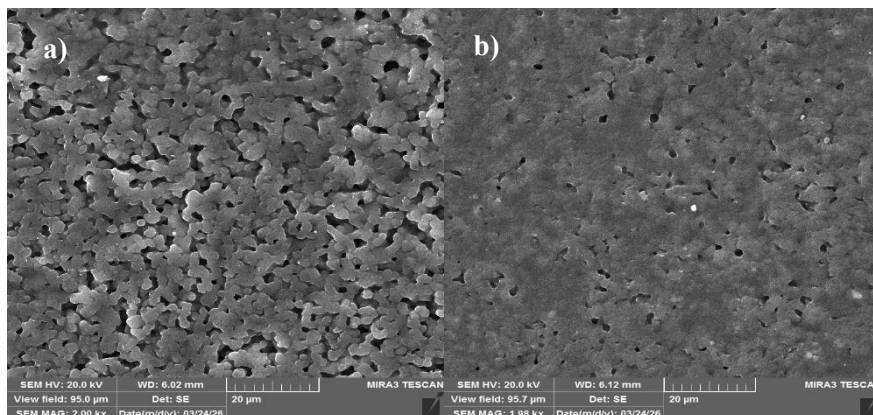


Figure 2. Images of scanning electron microscopy of films with propolis and *Fumaria officinalis* extract: 4 wt% (a) and 8 wt% (b), 2000× magnification.

SEM micrographs revealed differences in the morphology of chitosan films containing 4 and 8 wt% extract (Figure 2). The 4 wt% film exerted a homogeneous, compact surface, indicating good dispersion of the extract within the chitosan matrix. In contrast, the 8 wt% film exhibited a more heterogeneous morphology with visible aggregates, suggesting partial agglomeration and reduced uniformity at higher extract concentration.

Table 1. Antioxidant potential of films with propolis and *Fumaria officinalis* extract (4 and 8 wt%).

Sample	Inhibition of DPPH• (%)	Inhibition of ABTS• ⁺ (%)
Film with 4 wt% extract	53.64±1.15	95.33±2.61
Film with 8 wt% extract	89.06±2.13	98.34±1.99

Both films showed strong antioxidant activity, which increased with higher extract concentration (Table 1). The 8 wt% film exhibited significantly higher DPPH• inhibition compared to the 4 wt% film, while both samples showed very high ABTS•⁺ scavenging activity. The enhanced antioxidant potential is attributed to the higher phenolic compound content from propolis and *F. officinalis* extract [3-5]. ABTS•⁺ inhibition values were higher than DPPH• for both samples, suggesting greater effectiveness of the bioactive compounds toward ABTS radicals, likely due to differences in radical solubility and reaction mechanisms [6,7].

CONCLUSION

Chitosan films containing propolis and *F. officinalis* extract exhibited promising antioxidant properties and successful incorporation of bioactive compounds into the polymer matrix. Increasing the extract concentration enhanced radical scavenging activity, particularly against ABTS•⁺. The results indicate that although the extract can be successfully incorporated into chitosan films, higher loading reduces structural homogeneity and increases phase separation, which may negatively influence key properties such as mechanical strength, flexibility, and barrier performance. Nevertheless, the developed films show potential for application as active edible packaging materials.

Acknowledgement

This research was supported by the Ministry of Science, Technological Development, and Innovation of Serbia (451-03-136/2026-03/200019, 451-03-136/2026-03/200135, and 451-03-136/2026-03/200026), United Nations Sustainable Development Goal 3 (Good Health and Well-Being).

REFERENCES

- [1] M. Z. Elsabee, E. S. Abdou, *Mater. Sci. Eng C.*, 2013, 33, 1819-1841.
- [2] V. Falguera, J. P. Quintero, A. Jiménez, J. A. Muñoz, A. Ibarz, *Trends in Food Sci. Technol.*, 2011, 22, 292-303.
- [3] Y. Prokopenko, I. Surzhykov, O. Golovchenko, V. Mishchenko, V. Georgiyants, *Fitoterapia*, 2025, 186, 106780.
- [4] R. Hossain, C. Quispe, R.A. Khan, A.S.M. Saikat, P. Ray, D. Ongalbek, B. Yeskaliyeva, D. Jain, A. Smeriglio, D. Trombetta, R. Kiani, F. Kobarfard, N. Mojgani, P. Saffarian, S.A. Ayatollahi, C. Sarkar, M.T. Islam, D. Keriman, A. Uçar, M. Martorell, A. Sureda, G. Pintus, M. Butnariu, J. Sharifi-Rad, W.C. Cho, *Chin. Med.*, 2022, 17, 100.
- [5] A. Ahmoda, A. Pirković, V. Milutinović, M. Milošević, A. Marinković, A. A. Jovanović, *Plants*, 2025, 14, 352.
- [6] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free Radic. Biol. Med.*, 1999, 26, 1231-1237.
- [7] R. Wołosiak, B. Drużyńska, D. Derewiaka, M. Piecyk, *Molecules*, 2022, 27, 50.

THE EFFECT OF CYCLODEXTRIN ON THE STABILITY OF *Rosa canina* L. NATURAL DEEP EUTECTIC SOLVENT EXTRACT

A. Jovanović¹, N. Krgović², M. Jovanović³, R. Pjanović⁴, J. Živković² and K. Šavikin²

¹University of Belgrade, Institute for the Application of Nuclear Energy INEP, Banatska 31b, 11080 Belgrade, Serbia (ajovanovic@inep.co.rs)

²Institute for Medicinal Plants Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia

³University of Niš, Faculty of Medicine, Bulevar Dr Zorana Đinđića 81, 18000 Niš, Serbia

⁴University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.21

ABSTRACT

Rosehip is a valuable source of polyphenols with significant antioxidant potential. This study examined the influence of β -cyclodextrin (β -CD) on the stability of total polyphenol content (TPC) in rosehip extracts prepared using a natural deep eutectic solvent. During storage, TPC decreased in all samples, with greater stability observed at 4°C than at 25°C. The addition of β -CD improved polyphenol retention during storage, particularly at 4 wt%, indicating enhanced protection of phenolic compounds through the inclusion complex formation. These findings indicate that the combined use of β -CD and refrigerated storage effectively enhances the stability of rosehip polyphenols.

INTRODUCTION

Rosehip is traditionally used as an anti-inflammatory medicinal plant with pain-relieving properties [1]. Recent studies have investigated its potential in the treatment of various disorders, including inflammatory diseases, arthritis, diabetes, obesity, and cancer, mainly due to its antioxidant phytochemicals such as ascorbic acid, phenolics, and fatty acids [1,2]. Cyclodextrins are promising compounds for improving the solubility and stability of plant bioactives through inclusion complex formation [3]. Cyclodextrin-based extraction is considered an emerging green technology for efficient extraction and improved utilization of polyphenols from plant materials [4-6].

EXPERIMENTAL

Dried and ground rose hips were obtained from the Institute for Medicinal Plants Research "Dr Josif Pančić", Serbia. The rosehip extracts were prepared employing an ultrasound probe Sonopuls (Bandelin, Germany) and natural deep eutectic solvent (betaine and citric acid with 50% water) in the absence or presence of β -cyclodextrin (β -CD, 2 and 4 wt%), the solid-to-solvent ratio of 1:100 g/mL, extraction time of 12 min, and amplitude of 43%. The total polyphenol content (TPC) was determined in all samples using the modified Folin-Ciocalteu assay [7] on the 1st and 30th days after storage at 4°C and 25°C.

RESULTS AND DISCUSSION

Table 1 presents the total polyphenol content (TPC) of rosehip extracts prepared using a natural deep eutectic solvent, with and without β -CD, monitored over 30 days of storage at 4°C and 25°C.

Table 1. Total polyphenol content (TPC) in rosehip extract with a natural deep eutectic solvent in the absence and presence of β -cyclodextrin (β -CD).

Sample	Storage conditions	TPC (mg GAE*/mL)	
		1 st day	30 th day
Extract without β -CD	4°C	1.09±0.07	1.04±0.08
	25°C		0.98±0.05
Extract with 2 wt% β -CD	4°C	1.07±0.10	1.01±0.01
	25°C		0.97±0.05
Extract with 4 wt% β -CD	4°C	0.84±0.09	0.92±0.11
	25°C		0.88±0.15

*GAE, gallic acid equivalent.

The TPC of rosehip extracts prepared using a natural deep eutectic solvent showed only minor variations during storage and in the presence of β -CD. In the absence of β -CD, TPC slightly decreased from 1.09±0.07 mg GAE/mL on the 1st day to 1.04±0.08 mg GAE/mL after 30 days at 4°C, while a more pronounced reduction was observed at 25°C (0.98±0.05 mg GAE/mL). A similar trend was observed for extracts containing 2 wt% β -CD, where TPC remained relatively stable during storage at 4°C (1.07±0.10 to 1.01±0.01 mg GAE/mL), while a decrease was recorded at 25°C (0.97±0.05 mg GAE/mL). In contrast, the addition of 4 wt% β -CD resulted in lower initial TPC values but generally maintained or slightly improved stability over the storage period, particularly at 4°C (0.84±0.09 to 0.92±0.11 mg GAE/mL). This suggests that higher cyclodextrin concentration may enhance the formation of inclusion complexes with phenolic compounds, thereby improving their resistance to degradation and/or oxidation during storage. Namely, storage temperature had a stronger effect on phenolic stability than β -CD concentration, indicating partial degradation or structural

transformations of phenolic compounds under room temperature conditions. Similar findings have been reported for rosehip-derived polyphenols, where storage temperature significantly affected the stability and retention of bioactive compounds [8,9].

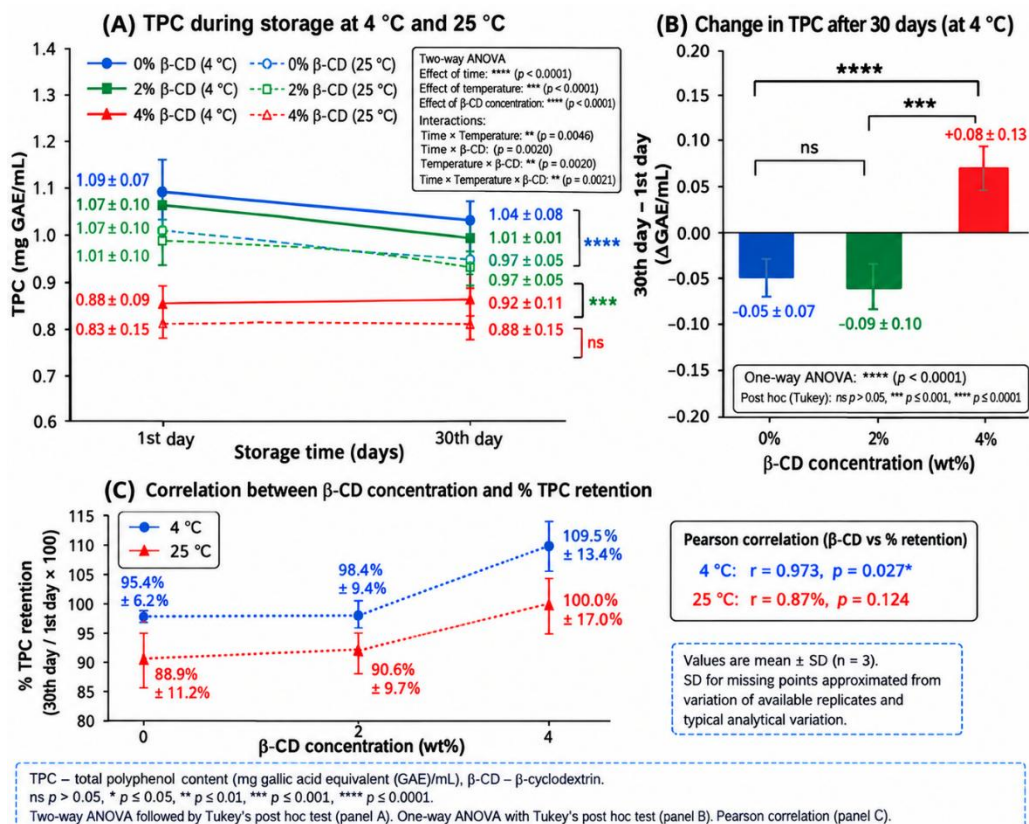


Figure 1. Changes in total polyphenol content (TPC) and correlation analysis as affected by storage temperature and β -cyclodextrin (β -CD) concentration.

Figure 1 shows that storage temperature and β -CD concentration influence TPC during storage. Although TPC decreased within all samples, formulations stored at 4°C showed greater stability than those at 25°C. The addition of β -CD further improved polyphenol retention, with 4 wt% β -CD providing the highest stability, while samples without β -CD showed the greatest losses. Correlation analysis confirmed a significant positive relationship between β -CD concentration and TPC retention at 4°C. Namely, β -CD encapsulation combined with refrigerated storage effectively improved polyphenol stability.

CONCLUSION

This study investigated the effect of β -CD and storage conditions on the stability of polyphenols in rosehip extracts obtained using ultrasound-assisted extraction with a natural deep eutectic solvent. The addition of β -CD improved the stability of polyphenols during storage, while refrigerated conditions (4°C) significantly enhanced total polyphenol content (TPC) retention compared to room temperature. The most pronounced stabilizing effect was observed at 4 wt% β -CD, suggesting efficient inclusion complex formation with phenolic compounds. These findings indicate that combining β -CD encapsulation with low-temperature storage is a promising strategy for preserving bioactive compounds in plant extracts.

Acknowledgement

This research was supported by the Ministry of Science, Technological Development, and Innovation of Serbia (451-03-136/2026-03/200019, 451-03-33/2026-03/200003, 451-03-137/2026-03/200113, and 451-03-136/2026-03/200135), United Nations Sustainable Development Goal 3 (Good Health and Well-Being).

REFERENCES

- [1] J. Gruenwald, R. Uebelhack, M. I. Moré, *Phytomedicine*, 2019, 60, 152958.
- [2] I. Mármol, C. Sánchez-de-Diego, N. Jiménez-Moreno, C. Ancín-Azpilicueta, M. J. Rodríguez-Yoldi, *Int. J. Mol. Sci.* 2017, 18, 1137.
- [3] T. Loftsson, M. E. Brewster, *J. Pharm. Sci.* 1996, 85, 1017-1025.
- [4] N. El Darra, H. N. Rajha, E. Debs, F. Saleh, I. El-Ghazzawi, N. Louka, R. G. Maroun, *Int. J. Food Sci.* 2018, 2018, 1-9.
- [5] R. Cai, Y. Yuan, L. Cui, Z. Wang, T. Yue, *Trends Food Sci. Technol.* 2018, 79, 19-27.
- [6] J. A. Kazlauskaite, L. Ivanauskas, J. Bernatoniene, *Pharmaceutics*, 2021, 13, 620.
- [7] A. Jovanović, V. Djordjević, G. Zdunić, D. Pljevljakušić, K. Šavikin, D. Gođevac, B. Bugarski, *Sep. Purif. Technol.* 2017, 179, 369-380.
- [8] I. Odriozola-Serrano, D. P. Nogueira, I. Esparza, A. A. Vaz, N. Jiménez-Moreno, O. Martín-Belloso, C. Ancín-Azpilicueta, *Antioxidants*, 2023, 12, 1035.
- [9] K. N. Kasapoğlu, J. Kruger, A. Barla-Demirkoz, M. Gültekin-Özgüven, J. Frank, B. Özçelik, *Foods*, 2023, 12, 781.

BIOACTIVITY OF *Agaricus bisporus* AND *Hericium erinaceus* MUSHROOMS BY-PRODUCTS

D. Tenji¹, N. Tomić², I. Vulin¹ and M. Jovanović³

¹Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

²Institute of Technical Sciences of SASA, University of Belgrade, Knez Mihailova 35/IV, Belgrade, Serbia

³Institute of General and Physical Chemistry, Studentski trg 12/V, Belgrade, Serbia (mrajicjovanovic@iofh.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.22

ABSTRACT

Extracts obtained from *Hericium erinaceus* (HeE) and *Agaricus bisporus* (AbE) solid waste were investigated for their capacity to modulate the growth of the probiotic (*E. coli* Nissle 1917) and foodborne pathogen (*E. coli* O157:H7) *Escherichia* strains, and cytotoxic potential against the human hepatocellular carcinoma cell line (HepG2). In selected assays, the tested concentrations did not exceed 1 mg/mL. HeE extract stimulated growth, while AbE extract did not disturb the growth dynamics of both *E. coli* strains. HeE extract exhibited significant cytotoxicity towards HepG2 cells (up to 43.1%). The obtained results confirmed that not only medicinal and edible mushrooms, but also their by-products, could exhibit biological effects.

INTRODUCTION

Mushroom production generates a large amount of by-products such as waste and off-grade mushrooms that lack market value, that ranges between 5% and 20% of production volume [1]. By-products generated during mushroom production and processing are repurposed for energy production and bioremediation, and could find applications as animal feed, fertilizer, bio-based materials, and cosmeceuticals [2]. With this in mind, the discarded material from mushroom extraction could retain bioactives, the levels of which vary according to the specific extraction technique used. To date, components derived from specific medicinal mushrooms have become valuable ingredients of a functional food and dietary supplements [3]. Lion's mane mushroom, *Hericium erinaceus*, based supplements and products exhibit a diverse array of biological activities, including antimicrobial, immunoregulating, antioxidant, anti-inflammatory, hypolipidemic, and antiviral functions. In general, edible and medicinal mushrooms could serve as prebiotics, promoting the formation of the gut microbiota and so providing health benefits to the host [4]. *H. erinaceus* products

are available in various forms, including health wines, beverages, teas, extracts and solid food products [5]. Among the frequently grown varieties is also the button mushroom, *Agaricus bisporus* (J.E. Lange), which is widely cultivated in Europe and comprises about 32% of the world mushroom production [1]. The purpose of this study was to verify whether fungal by-products, much like the fungi they originate from, can be considered as carriers of biological activities. Therefore, this study investigates whether the extracts prepared from *A. bisporus* and *H. erinaceus* solid waste (AbE and HeE) can modulate growth of both probiotic and foodborne pathogen *Escherichia coli* strains and exert *in vitro* cytotoxicity towards hepatocarcinoma cells (HepG2).

EXPERIMENTAL

Mushrooms by-products were collected after commercial preparation of water extracts (Serbia). Here, AbE and HeE solid waste has been subjected to maceration in a 50% water/ethanol solution for 24 h with constant stirring (150 rpm). Then the liquid was separated from the plant material, filtered, and centrifuged (6200×g, 10 min). The solvent was evaporated using a rotary evaporator at 39°C under vacuum. The dried extracts were dissolved in ethanol to a final concentration of 100 mg/mL. Determination of growth curves was based on the methodology of Lehtinen *et al.* [6]. A cytotoxicity assay was carried out against HepG2 cells with AbE and HeE by employing an MTT assay [7]. GraphPad Prism 6.0 software was used for statistical data analysis.

RESULTS AND DISCUSSION

E. coli is the most dominant gram-negative bacterium of the normal intestinal microbiota. Among *E. coli* strains, probiotic strains, as well as pathogens and pathobionts, were recognized. Our study assessed the growth dynamics of probiotic *E. coli* Nissle 1917 and the foodborne pathogen *E. coli* O157:H7 with and without AbE and HeE extracts (Figure 1). HeE extract stimulated growth of both *E. coli* strains, while AbE extracts did not disturb the growth dynamics of *E. coli* strains. By contrast, *A. bisporus* was capable to stimulate the growth of G-positive probiotic bacteria. El-Maradny [4] demonstrated that polysaccharide extracts from *A. bisporus* significantly stimulated the growth of *L. paracasei*. Contrary to our results, Ramos *et al.* [8] discussed that crude extracts obtained from *A. bisporus* exert anti-*E. coli* activity. It has also been discussed that *H. erinaceus* exert antibacterial effect against G-positive and G-negative bacteria including *E. coli* [9].

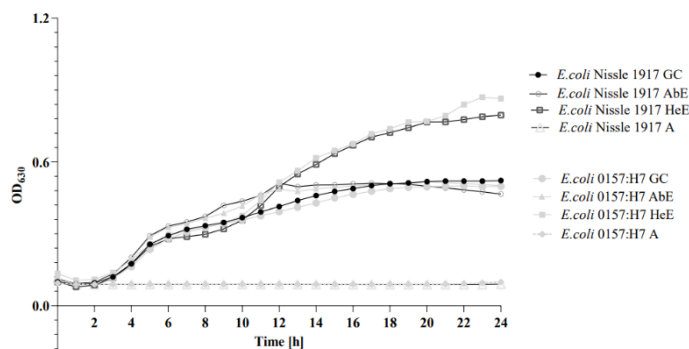


Figure 1. Growth dynamic of the *E. coli* strains during 24 h with AbE and HeE treatment. GC-growth control; A-antibiotic, vancomycin.

Previously, it has been confirmed that compounds isolated from *H. erinaceus*, such as the bioactive peptide Lys-Ser-Pro-Leu-Tyr (KSPLY, Mw 608.38 Da) lack cytotoxic potential. Moreover, it contributed to the proliferation of HepG2 cells [10]. In our study, HeE extracts significantly decreased the viability of HepG2 cells (up to 43.1%). Next, literature data indicated that *A. bisporus* exhibited cytotoxicity against HepG2 [11]. Within this study, we found no evidence of cytotoxicity in the by-products derived from *A. bisporus*.

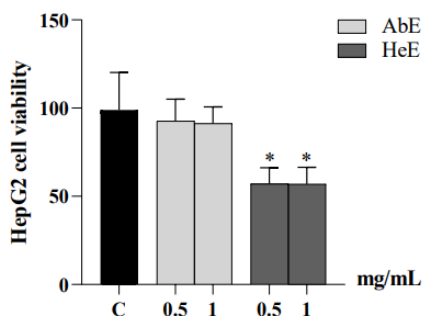


Figure 2. HepG2 cell viability. C-growth control. A significant difference in means between samples and control according to the Dunnett's test ($p < 0.05$).

CONCLUSION

Edible mushrooms are recognized not only for their nutritional value but also for their diverse biological activities. Here, it was confirmed that extract from *Hericium erinaceus* solid waste can also exhibit prominent bioactivity.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (451-03-33/2026-03/200051, 451-03-33/2026-03/200175, 451-03-33/2026-03/200125 & 451-03-34/2026-03/200125). This research supports the United Nations Sustainable Development Goals, SDG3 (Good Health and Well-Being), SDG9 (Industry, Innovation and Infrastructure), and SDG17 (Partnership for the Goals). The authors are grateful to Dr. Jovana Vunduk for providing mushrooms waste.

REFERENCES

- [1] S. M. Fraga, F. M. Nunes, *Applied Sciences*, 2020, 10, 2232.
- [2] F. Antunes, S. Marçal, O. Taofiq, A. M. M. B. Morais, A. C. Freitas, I. C. F. R. Ferreira, M. Pintado, *Molecules*, 2020, 25, 2672.
- [3] J. H. Kim, C. C. Tam, K. L. Chan, N. Mahoney, L. W. Cheng, M. Friedman, K. M. Land, *Applied Sciences*, 2022, 12, 4591.
- [4] Y. A. El-Maradny, A. S. Abouakkada, A. A. G. Abbass, A. F. Abaza, E. M. El-Fakharany, *Scientific Reports*, 2025, 15, 31113.
- [5] F. Ge, Y. Chen, B. Wang, W. Zhou, B. Du, L. Hou, *Molecules*, 2025, 30, 1850.
- [6] J. Lehtinen, S. Järvinen, M. Virta, E. M. Lilius, *Journal of Microbiological Methods*, 2006, 66, 381-389.
- [7] M. Jovanović, J. Vunduk, D. Mitić-Ćulafić, E. Svirčev, P. Vojvodić, N. Tomić, L. N. Ismi, D. Tenji, *Nutrients*, 2024, 16, 3368.
- [8] M. Ramos, N. Burgos, A. Barnard, G. Evans, J. Preece, M. Graz, A. C. Ruthes, A. Jiménez-Quero, A. Martínez-Abad, F. Vilaplana, L. P. Ngoc, *Food Chemistry*, 2019, 292, 176-87.
- [9] R. B Raja-Razali, N. A. Zahia-Azizan, C. S. Yee, M. A. Ushidee-Radzi, Z. Ilham, N. Hamid, A. Klaus, W. A. Wan-Mohtar, *Journal of Food Science*, 2026, 91, e71026.
- [10] Z. Xu, Q. Hu, M. Xie, J. Liu, A. Su, H. Xu, W. Yang, *Food Science and Human Wellness*, 2023, 12, 1893-1904.
- [11] G. V. S. Pabasara, M. D. M. Fernando, W. K. S. M. Abeysekera, L. V. C. Liyanapathirana, *BMC Complementary Medicine and Therapies*, 2025, 25, 233.

DETERMINATION AND ASSESSMENT OF PESTICIDE RESIDUES IN FRUITS FROM THE SERBIAN MARKET

M. Kalaba¹, A. Tasić², Ž. Nikolić¹, M. Čalić¹, Ž. Tešić³ and S. Blagojević¹

¹*Institute of General and Physical Chemistry, Studentska 12-16, 11158
Belgrade, Serbia (milicaffh@yahoo.com)*

²*Chemistry Department, Scientific Institute of Veterinary Medicine of Serbia,
Janisa Janulisa 14, 11000 Belgrade, Serbia*

³*University of Belgrade, Faculty of Chemistry, Studentska 12-16, 11158
Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.23

ABSTRACT

The presence of pesticide residues in fruits represents an important aspect of food quality and consumer safety. This study aimed to evaluate the occurrence and distribution of multiple pesticide residues in conventionally and organically produced fruits available on the Serbian market. Fruit samples from different groups (berries, stone fruits, and citrus fruits) were analyzed using GC-MS. The most frequently detected pesticides were cypermethrin, fludioxonil, boscalid, and tebuconazole. Berries and stone fruits showed the highest diversity of detected residues, while organic fruits generally exhibited lower residue occurrence. The highest individual concentrations were observed for imazalil in sour cherry and pyraclostrobin in raspberry samples. Although most detected concentrations were within regulatory limits, the findings demonstrate that pesticide application during cultivation may result in detectable residues in fruits and highlight the importance of continuous monitoring.

INTRODUCTION

Fruit consumption is strongly associated with beneficial health effects due to the presence of many bioactive compounds. Intensive agricultural production requires the application of plant protection products to prevent losses. Although pesticides play an important role in modern agriculture, their intensive or inappropriate use may result in the accumulation of residues in edible plant parts. The presence of pesticide residues in food represents an important food safety concern due to possible long-term dietary exposure and potential adverse effects on human health. To protect consumers, maximum residue limits (MRLs) have been established by national and international regulatory bodies, while continuous monitoring programs are implemented to verify compliance and assess dietary exposure. Previous studies [1,2] have shown that fruits,

particularly berries and stone fruits, belong to the food groups where multiple pesticide residues are frequently detected. Differences in residue occurrence may be related to agricultural practices, crop susceptibility, environmental conditions, and post-harvest treatments.

The aim of this study was to investigate the occurrence and distribution of pesticide residues in different fruits available on the Serbian market, with emphasis on comparing conventional and organic production systems and evaluating their implications for food safety.

EXPERIMENTAL

Conventionally and organically produced fruit samples (a total of 77 samples), including berries, stone fruits, and citrus fruits, were obtained from the Serbian market over two years. Samples were homogenized and prepared for pesticide residue analysis using a multiresidue extraction procedure based on the QuEChERS method. The determination of pesticide residues was performed by gas chromatography coupled with tandem mass spectrometry (GC-MS).

RESULTS AND DISCUSSION

The summary of detected pesticide residues in different fruit categories from the Serbian market is presented in Table 1. The analysis of 77 fruit samples demonstrated that pesticide residues were detected in most of the samples, while residue occurrence and composition varied among different fruit types. It was shown that various pesticide residues belong mainly to fungicides and insecticides (such as fludioxonil, boscalid, tebuconazole, pyraclostrobin, cypermethrin, pyridaben, imazalil) (Table 1).

Berries, including raspberry, blackberry, strawberry, and blueberry, showed the highest diversity of detected pesticide compounds. Frequently identified residues included cypermethrin, fludioxonil, boscalid, and tebuconazol (Table 1). Several berry samples contained multiple pesticide residues simultaneously, suggesting the intensive use of combined crop protection treatments during production. Among berry samples, the highest concentrations were observed for pyraclostrobin ($0.5522 \text{ mg kg}^{-1}$) and boscalid ($0.3867 \text{ mg kg}^{-1}$) in raspberry samples (Table 1).

Stone fruits, including plum and sour cherry, also demonstrated considerable variability of pesticide residue. Fludioxonil, cypermethrin, tebuconazole, and other fungicides were commonly detected. The highest individual concentration observed in the dataset was recorded for imazalil (1.2842 mg/kg , Table 1) in sour cherry samples, indicating the importance of continuous monitoring of certain fruit commodities with higher susceptibility to fungal contamination or post-harvest treatments. Pyridaben, which is an insecticide in the acaricide group, was important for plum samples, with the highest value of 0.144 mg/kg (Table 1).

Table 1. Summary of detected pesticide residues in different fruit categories from the Serbian market.

Fruit group	No. of samples	Most frequently detected pesticides	Max detected concentration (mg kg⁻¹)	Food safety observation
Berries (raspberry, blackberry, strawberry, blueberry)	39	Cypermethrin, Fludioxonil, Boscalid, Tebuconazole	up to 0.55	Multiple residues frequently detected; levels generally below MRLs
Stone fruits (plum, sour cherry, apricot)	21	Fludioxonil, Cypermethrin, Tebuconazole, Imazalil	up to 1.28	Occasional elevated residues requiring monitoring
Citrus fruits (lemon)	2	Cypermethrin, Imazalil	<0.02	Low residue occurrence
Organic fruits	15	Fludioxonil, Cypermethrin, Boscalid (sporadic)	<0.05	Lower residue frequency compared with conventional fruits

Organic fruits generally exhibited a lower number of detected residues compared with conventional samples. However, occasional detection of pesticide compounds was observed, which may be associated with environmental contamination and spray drift from neighboring agricultural areas.

Overall, the detected concentrations were generally low, and the residue patterns were comparable with those reported by the European Food Safety Authority (EFSA) [3]. The obtained residue profiles indicate that pesticide occurrence is highly dependent on fruit type and production conditions. In addition, the presence of multiple residues in individual samples highlights the necessity of regular surveillance programs. These results contribute to the current knowledge of pesticide residue occurrence in fruit available on the Serbian market and provide useful data for future exposure assessment and food safety evaluation.

CONCLUSION

This study provides an overview of pesticide residue occurrence in different fruit samples available on the Serbian market. The results demonstrated variability in residue profiles among berries, stone fruits, and citrus fruits, with multiple pesticide residues detected in several samples. Fungicides and insecticides represented the predominant groups of detected pesticides, reflecting their

widespread application in fruit production. Among the detected fungicides, pyraclostrobin was identified in several berry samples, with the highest concentration observed in raspberry samples. Organic fruits generally showed a lower frequency of pesticide detection. However, the occasional detection of pesticide residues emphasizes the need for continuous monitoring programs to provide reliable data for dietary exposure assessment, support regulatory actions, and ensure consumer confidence in food safety. The obtained data contribute to a better understanding of pesticide contamination patterns in fruits and provide valuable information for future food safety evaluations and consumer exposure assessments.

Acknowledgement

This work was partially supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia under the contract on the realization and financing of scientific research work of research organizations: Contract Nos: 451-03-33/2026-03/200051 (M.K., Ž.N., M.Ć., S.B.), 451-03-33/2026-03/200030 (A.T.).

REFERENCES

- [1] X. Luo, X. Zeng, D. Wei, C. Ma, J. Li, X. Guo, L. Cheng, Z. Mao, Z. *Food Addit. Contam. Part B Surveill.* 2023, 16, 244–252.
- [2] I. Kecojević, D. Mrkajić, V. Tomović, B. Bajić, M. Lazović, A. Joksimović, M. Tomović, A. Martinović, D. Vujadinović, S. Stefanović, V. Đorđević. *Foods*, 2025, 14(10), 1828.
- [3] European Food Safety Authority (EFSA), P. Medina Pastor, L. Carrasco Cabrera, G. Di Piazza, C. González Ciria. *EFSA Journal*, 2026, 24, e10054.

ASSESSMENT OF THE OXIDATIVE POTENTIAL OF COMBINED FOOD ADDITIVES UNDER ACIDIC CONDITIONS

M. Kostić, A. Valenta Šobot, J. Filipović Tričković, M. Tošić, M. Janić and K. Radošević

Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (kostic.mila@vin.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.24

ABSTRACT

Sodium nitrite (NaNO_2) and butylated hydroxyanisole (BHA) are food additives that may undergo chemical interactions in acidic conditions, influencing oxidative processes. This study assessed their individual and combined effects on ROS and mitochondrial membrane potential in MRC-5 fibroblasts exposed for 3h at pH 7.0 and pH 5.5. ROS were measured using DCFH-DA, and mitochondrial potential using TMRE. No significant alterations in mitochondrial membrane potential were observed following any of the applied treatments, while intracellular ROS production was significantly increased under acidic conditions, particularly after combined NaNO_2 and BHA exposure. Overall, the findings indicate that combined NaNO_2 and BHA treatment significantly altered intracellular ROS levels under acidic conditions, but not at physiological pH, suggesting a synergistic effect of the treatment on ROS induction without accompanying alterations in mitochondrial membrane potential.

INTRODUCTION

Reactive oxygen and nitrogen species (ROS/RNS) regulate cellular signaling and redox homeostasis, whereas their excessive production induces oxidative stress [1]. pH strongly influences intracellular redox balance and the formation of reactive intermediates [2]. Under acidic conditions, sodium nitrite (NaNO_2) generates reactive nitrogen species through nitrous acid formation [3,4]. Although butylated hydroxyanisole (BHA) is a widely used antioxidant, it may also exhibit pro-oxidant effects depending on the redox environment [5]. Since sodium nitrite and BHA may coexist in the acidic gastric environment following dietary exposure, they may undergo chemical interactions leading to the formation of reactive intermediates with potential carcinogenic properties [6]. Therefore, their combined effects under acidic conditions need further attention. Accordingly, we investigated the individual and combined effects of NaNO_2 and

BHA on ROS production and mitochondrial membrane potential in MRC-5 fibroblasts under neutral and acidic (pH 5.5) conditions.

EXPERIMENTAL

Human fetal lung fibroblasts (MRC-5) were cultured under standard conditions (37 °C, 5% CO₂). Cells were exposed for 3 h to NaNO₂ (20 and 50 µM) and BHA (20 and 50 µM), individually or in combination, in HBSS (10% FBS) at neutral pH or pH 5.5 (adjusted with 2 N HCl). The selected concentrations were chosen based on preliminary XTT cytotoxicity experiments to ensure subcytotoxic exposure while allowing the assessment of early oxidative responses [unpublished data]. Untreated cells in HBSS (pH 7.0, and pH 5.5) served as controls. Intracellular ROS levels were measured using 2',7'-dichlorofluorescein diacetate (DCFH-DA) according to a previously described fluorimetric method. Briefly, following treatment, cells were incubated with 20 µM DCFH-DA for 30 min at 37 °C in the dark, and fluorescence intensity was measured at excitation/emission wavelengths of 485/535 nm [7]. Mitochondrial membrane potential ($\Delta\Psi_m$) was assessed using TMRE, a fluorescent dye that accumulates in mitochondria according to the mitochondrial membrane potential. Cells were incubated with 2 µM TMRE for 30 min at 37 °C, washed with PBS, and fluorescence was measured at 549/575 nm [8]. The combined effect of BHA and NaNO₂ treatment was evaluated using the Bliss independence model by comparing experimentally observed and predicted combination effects, with Δ Bliss values used to determine synergistic, additive, or antagonistic interactions [9]. Statistical analysis was performed using one-way ANOVA (IBM SPSS), with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

At physiological pH (7.0), individual treatments increased ROS without statistical significance, apart from treatment with 50 µM BHA ($p < 0.05$). Moderate ROS increases do not necessarily indicate oxidative damage, since low ROS levels also participate in physiological redox signaling [1,10]. Under acidic conditions (pH 5.5), only combined exposure increased ROS-associated fluorescence levels, resulting in strong synergistic effects confirmed by Bliss analysis. The synergistic effect of combined treatments under acidic conditions was more pronounced at higher tested concentrations with 50 µM BHA and 50 µM NaNO₂ (Figure 1, B). Acidification promotes nitrite conversion into reactive nitrogen intermediates, enhancing oxidative and nitrosative stress [4]. Interactions between ROS and RNS may generate highly reactive oxidants, which may contribute to oxidative damage [11]. In addition, BHA may exhibit pro-oxidant activity under specific redox conditions and, together with nitrite, may influence reactive species formation [5].

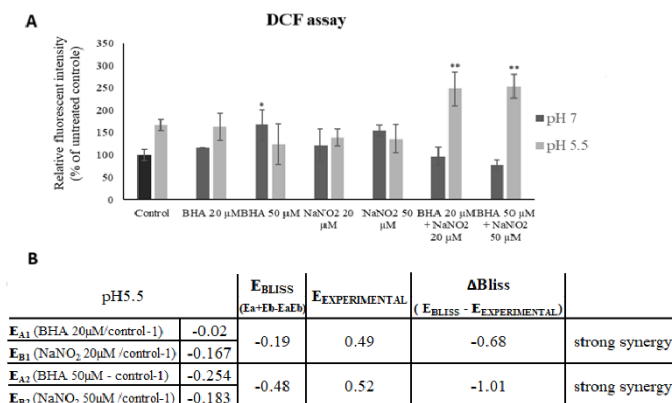


Figure 1. Effects of NaNO₂, BHA, and combined treatments on intracellular ROS levels in MRC-5 cells. **(A)** DCF assay after 3 h exposure at pH 7.0 and 5.5. Results are expressed as % of untreated control. * $p < 0.05$; ** $p < 0.01$. **(B)** Bliss independence analysis of combined NaNO₂ and BHA treatments at pH 5.5 showing predicted (E_{Bliss}), experimental, and ΔBliss values.

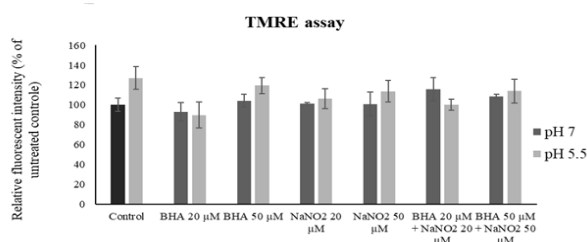


Figure 2. Mitochondrial membrane potential in MRC-5 fibroblasts exposed to NaNO₂, BHA, and combined treatments, at pH 7.0 and pH 5.5, determined by the TMRE assay. The results are presented as a percentage of the untreated control.

TMRE analysis did not reveal significant changes in mitochondrial membrane potential, suggesting that within the 3 h treatment window, ROS alterations were mainly associated with NaNO₂ and BHA exposure rather than mitochondrial dysfunction. Further studies are needed to determine how these early oxidative responses influence subsequent cellular outcomes.

CONCLUSION

Combined sodium nitrite (NaNO₂) and butylated hydroxyanisole (BHA) exposure induced pH-dependent changes in intracellular ROS production in MRC-5 cells, with a pronounced pro-oxidative response under acidic conditions and no significant changes in mitochondrial membrane potential. Bliss independence analysis confirmed synergistic ROS induction under acidic conditions, particularly following treatments with higher food additive

concentrations. Since these additives may coexist in the acidic gastric environment following dietary intake, the findings highlight the importance of evaluating their combined biological effects for food safety assessment.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant no. 451-03-33/2026-03/200017 and 451-03-3627/2025-03/3456).

REFERENCES

- [1] H. Sies, D. P. Jones, *Nat. Rev. Mol. Cell Biol.*, 2020, 21, 363–383.
- [2] H. J. Forman, H. Zhang, *Nat. Rev. Drug Discov.*, 2021, 20, 689–709.
- [3] J. O. Lundberg, E. Weitzberg, *Cell*, 2022, 185, 2853–2878.
- [4] N. S. Bryan, J. Loscalzo (Eds.), *Nitrite and Nitrate in Human Health and Disease*, Humana Press, New York, 2017.
- [5] G. M. Williams, M. J. Iatropoulos, J. Whysner, *Food Chem. Toxicol.*, 1999, 37, 1027–1038.
- [6] B. J. Phillips, A. C. Tee, P. A. Carroll, R. Purchase, D. G. Walters, *Toxicology in Vitro*, 1994, 8, 117–123.
- [7] B. Kalyanaraman, V. Darley-USmar, K. J. A. Davies, P. A. Dennery, H. J. Forman, M. B. Grisham, H. Ischiropoulos, *Free Radic. Biol. Med.*, 2012, 52, 1–6.
- [8] L. C. Crowley, M. E. Christensen, N. J. Waterhouse, *Cold Spring Harb. Protoc.*, 2016, 2016, pdb. Prot 087361.
- [9] Q. Liu, X. Yin, L. R. Languino, D. C. Altieri, *Methods Mol. Biol.*, 2015, 1313, 19–25.
- [10] D. P. Jones, *Antioxid. Redox Signal*, 2006, 8, 1865–1879. EA1
- [11] R. Radi, *J. Biol. Chem.*, 2013, 288, 26464–26472.

CYTOTOXICITY OF NEEM (*Azadirachta indica*) SEED OIL AND ITS RELEVANCE FOR INHALATION EXPOSURE

A. Bošković¹, S. Petrović², O. Medić¹, T. Ganić¹, B. Nikolić¹ and A. Leskovac²

¹University of Belgrade, Faculty of Biology, Studentski trg 12-16
11000 Belgrade, Serbia

²Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (andreja@vin.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.25

ABSTRACT

Widely perceived as a natural and safer alternative to synthetic pesticides, neem (*Azadirachta indica*) seed oil is extensively used as a biopesticide in agriculture; however, its potential effects following inhalation exposure remain insufficiently characterized. This study assessed its cytotoxicity in the human lung epithelial A549 cell line using the MTT assay. A significant concentration-dependent decrease in cell viability was observed, with minimal effects at low concentrations and pronounced cytotoxicity at higher concentrations. These findings indicate that neem seed oil can exert cytotoxic effects relevant to inhalation exposure, emphasizing the need for careful safety evaluation under realistic conditions of use. Further studies are warranted to better characterize the mechanisms of toxicity and to support a more comprehensive evaluation of the safety of neem-based biopesticides.

INTRODUCTION

To date, thousands of plant species have been evaluated for pest and disease control in modern agriculture, leading to the widespread use of numerous horticultural mineral oils, botanical extracts, and essential oil-based formulations. Among these, neem (*Azadirachta indica*) seed oil has gained widespread use owing to its reported insecticidal, antifungal, and antibacterial effects, which are attributed to its complex chemical composition, rich in bioactive compounds such as azadirachtin, nimbin, salannin, and various fatty acids [1].

While neem oil is often considered relatively safe based on traditional use and low acute toxicity in mammals, its complex chemical composition and formulation additives may trigger adverse cellular responses under certain exposure conditions [2]. Neem-based formulations are commonly applied as sprays, generating aerosols and fine droplets that can be inhaled by operators and individuals in the surrounding environment. In this context, its increasing use as

a sprayable biopesticide has raised questions about human exposure under realistic use conditions. This study aims to investigate its cytotoxic potential using the human lung epithelial cell line A549 as a model system. The A549 cell line is commonly used in toxicological research as a model for respiratory exposure and to evaluate the cytotoxic effects of inhaled toxicants [3]. By focusing on inhalation exposure scenarios, the study's findings are intended to contribute to a more comprehensive understanding of the safety profile of neem-based biopesticides and to support their responsible use.

EXPERIMENTAL

Preparation of neem seed oil treatment solutions

Commercially available, cold-pressed neem (*Azadirachta indica*) seed oil (Eterra, TemaCo d.o.o., Novi Sad, Serbia; country of origin: India) was used in the experiments. Stock emulsions of neem oil were prepared by mixing the oil with the surfactant Tween 20 (Sigma-Aldrich, France) and Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific, USA). Working concentrations were prepared by serial dilution.

Cell culture

A549 cells (ATCC CCL-185, Manassas, VA, USA) were cultured in DMEM supplemented with 10% fetal bovine serum (Capricorn Scientific, Germany) and 1% penicillin/streptomycin (Capricorn Scientific, Germany) at 37 °C in 5% CO₂. Cell viability and density were assessed by trypan blue exclusion, as previously described [4]. Cells were seeded in 96-well plates (1×10^4 cells/well), allowed to attach and form a monolayer for 24 h, and then treated with neem oil at final concentrations of 0.01–2 mg/mL for 24 h. Untreated cells served as negative controls. The final concentration of Tween 20 in the cultures did not exceed the toxicity threshold (<0.01%) [5], except at 2 mg/mL neem seed oil, where it reached 0.02%, so a corresponding solvent control (0.02% Tween 20) was included. All experiments were performed in triplicate.

Cytotoxicity assessment (MTT assay)

Cytotoxicity was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The absorbance was measured at 570 nm (reference 630 nm). Cell viability was expressed relative to untreated controls. The IC₅₀ was defined as the concentration that reduced cell viability by 50%.

Statistical analysis

Statistical analysis was performed using the Student's t-test with Statistica 8 and OriginPro 8.5.1 for Microsoft Windows. The p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results of the study demonstrate a concentration-dependent cytotoxic effect of neem oil after 24 h of exposure, with substantial reductions in cell viability at concentrations ≥ 0.5 mg/mL ($p < 0.05$) and near-complete loss of viability at 2

mg/mL ($p < 0.001$) (Fig. 1.). The calculated IC_{50} value (0.89 mg/mL) indicates moderate cytotoxic potency under the tested conditions. While cell viability remained above ~85–90% at concentrations ≤ 0.025 mg/mL, suggesting limited acute toxicity in this range, the progressive decline observed at 0.05 mg/mL and above indicates the onset of cellular stress.

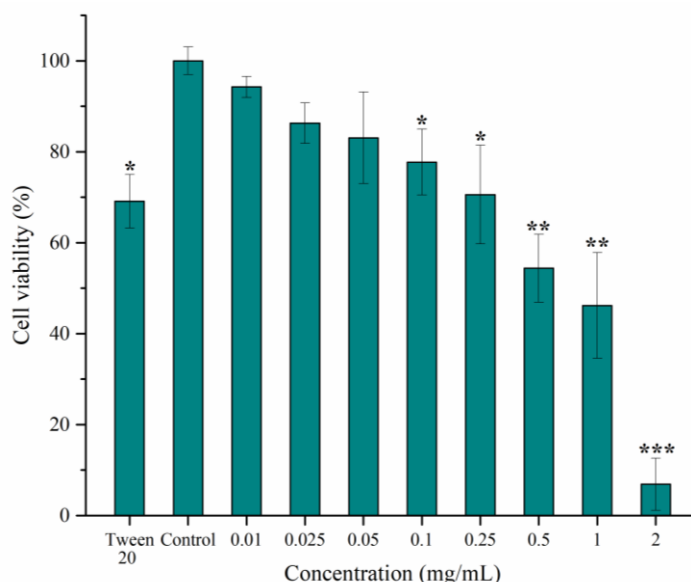


Figure 1. Cell viability (%) (mean $\pm$ SD) following exposure to increasing concentrations of neem seed oil, calculated relative to the untreated control (100%) for all tested concentrations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control.

At the highest concentration, viability decreased to 6.88%, confirming pronounced cytotoxicity. Notably, treatment with Tween 20 (0.02%) alone resulted in approximately 70% viability, indicating that the additional reduction is primarily attributable to the intrinsic toxicity of neem oil rather than the surfactant. This distinction is important, as it highlights that the biological activity of neem-based formulations may not always be attributed solely to the active substance but may instead reflect the combined, potentially additive effects of both active and formulation constituents. The observed cytotoxicity of neem oil may be associated with mitochondrial dysfunction and disruption of membrane integrity, leading to impaired metabolic activity. Limonoids such as azadirachtin are known to interfere with multiple cellular signaling pathways and may contribute to the observed effects [6].

From a safety perspective, concentrations that induce pronounced cytotoxicity exceed those typically encountered in environmental exposure; however, localized exposure, such as inhalation of aerosolized droplets during spraying or

occupational handling, may transiently elevate concentrations at the epithelial surface. A recent study further supports this concern, showing a significantly higher prevalence of respiratory symptoms and impaired lung function in workers exposed to neem oil processing compared with controls, indicating adverse effects from aerosols, dust, and associated chemicals [7].

CONCLUSION

The observed cytotoxicity in A549 cells suggests that inhaled neem oil may adversely affect the respiratory epithelium. Further studies are warranted to better characterize its safety profile under realistic exposure conditions.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract numbers 451-03-33/2026-03/200017 and 451-03-34/2026-03/200178).

REFERENCES

- [1] A.K. Dhakad, R. Kumar, R. Choudhary, S. Singh, S. Khan, P.K. Poonia, *Ind. Crops Prod.*, 2025, 231, 121155.
- [2] C. Shekhar, R. Khosya, K. Thakur, D. Mahajan, R. Kumar, S. Kumar, A.K. Sharma, *Toxicol. Rep.*, 2024, 13, 101840.
- [3] M. Mascelloni, J.M. Delgado-Saborit, N.J. Hodges, R.M. Harrison, *Toxicol. Lett.*, 2015, 237(1), 38-45.
- [4] W. Strober, *Curr. Protoc. Immunol.*, 2015, 111, A3.B.1-A3.B3.
- [5] T. Hua, X. Zhang, B. Tang, C. Chang, G. Liu, L.L. Feng, Y. Yu, D. Zhang, J. Hou, *BMC Vet. Res.*, 2018, 14, 138.
- [6] R.V. Priyadarsini, R.S. Murugan, P. Sripriya, D. Karunagaran, S. Nagini, *Free Radic. Res.*, 2010, 44(6), 624-634.
- [7] S. Subramaniam, A. Ganesan, N. Raju, N. Rajavel, M. Chenniappan, R. M. S. Mohanty, S. Angamuthu, K. Sivakumar, C. Prakash, A. Pramanik, A. K. Basak, *Indian J Occup Environ Med*, 2024, 28(2), 163-169.

COMPARATIVE ANALYSIS OF ANTIOXIDANT STATUS AND GROWTH OF MICROGREENS CULTIVATED UNDER HYDROPONIC CONDITIONS

D. Dimitrijević¹, A. Bučić¹, D. Stanković¹, V. Beškoski¹ and N. Lugonja²

¹*University of Belgrade, Faculty of Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia*

²*University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia (nikoleta.lugonja@ihtm.bg.ac.rs)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.26

ABSTRACT

Microgreens are young, small plants with high nutrient content. They were cultivated hydroponically in three media. After measuring growth parameters, the antioxidant capacity of the plant extracts was evaluated using DPPH and cyclic voltammetry. Hoagland solution influenced the greatest plant growth, and both analytical methods confirmed that the cultivation media significantly influenced the antioxidant status and redox properties of the microgreens.

INTRODUCTION

Microgreens are young edible plants that have been increasingly used in culinary applications in recent decades as food ingredients because of their distinctive aroma, texture, decorative appeal, and nutritional value. They are characterized by fully developed cotyledons and one to two pairs of true leaves and contain a high concentration of vitamins, minerals, and bioactive compounds, including antioxidants, compared with their mature counterparts. They are harvested without roots, and their early harvest stage makes them attractive for incorporation into human diet [1]. Microgreens have great potential for modern agrotechnological production systems and may serve as nutrient-rich functional food ingredients because they contain elevated levels of nutritional and bioactive compounds associated with human health [1,2]. Antioxidant capacity is an indicator of the biological properties of plant extract, reflecting their ability to scavenge free radicals and participate in electron-transfer processes. potential to neutralize free radicals and support physiological functions. The DPPH assay is commonly used to evaluate the free radical scavenging capacity of antioxidants through hydrogen atom or electron transfer mechanisms, whereas cyclic voltammetry (CV) provides information on the overall reducing capacity of electroactive compounds present in plant extracts. Electrochemical methods are “green,” eco-friendly approaches for assessing the electron-donating ability of plant antioxidants, without the need for hazardous organic solvents. In addition,

CV can be advantageous for the analysis of colored plant extracts, as the electrochemical response is not directly affected by sample coloration, which may interfere with spectrophotometric measurements. CV also provides information on the redox properties and electron-transfer behavior of the electroactive compounds, offering complementary insight into the antioxidant characteristics of complex plant matrices. The aim of this study was to compare three hydroponic cultivation media in terms of their effects on the growth and antioxidant capacity of microgreens using conventional spectrophotometric and electrochemical methods.

EXPERIMENTAL

Two different microgreens, Mung bean and Green peas (*Vigna radiata* and *Pisum sativum*), were cultivated hydroponically in three different media: demineralized water (W), Hoagland (HG) [3], and commercial compound fertilizer for growth in hydroponic systems (HI). Seeds were grown under controlled environmental conditions at 24 °C, 45% relative humidity, and 445 ppm CO₂. After 8 days of growth, plant, root, and shoot lengths were measured and expressed as mean $\pm$ SD (n=50). Microgreens were homogenized in liquid nitrogen, extracted with methanol (1:10), and radical-scavenging activity was determined spectrophotometrically using 1 mM DPPH [4]. The redox potential of the water extracts after plant homogenization was measured by cyclic voltammetry (CV). All results are presented as mean $\pm$ SD.

RESULTS AND DISCUSSION

After eight days of cultivation, both *Vigna radiata* and *Pisum sativum* microgreens developed in all three media, and microgreens cultivated in HG exhibited the greatest development compared with those grown in water and commercial hydroponic medium. Plant growth was assessed by measuring shoot and root lengths (Fig. 1). Higher biomass production in HG medium was observed for both species, indicating preferential allocation of growth to the *Pisum sativum* root system in HG compared to other media.

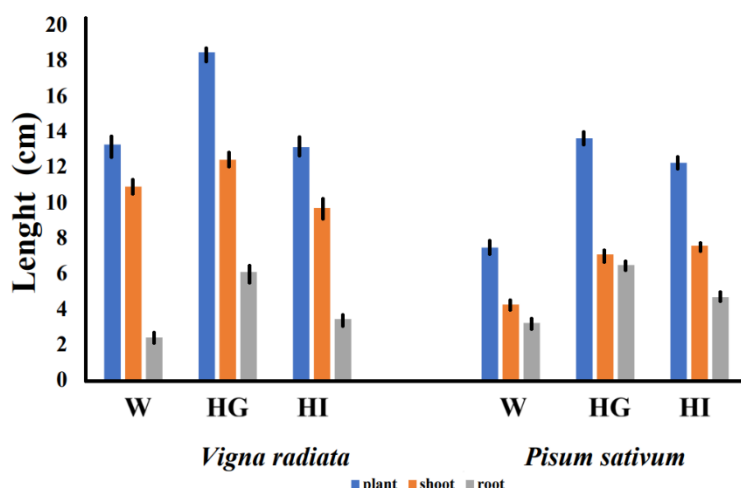


Figure 1. The effect of different media on the growth of microgreens. (W- Water, HG- Hoagland, HI- hydroponic system)

The antioxidant capacity of the microgreens was evaluated by DPPH radical scavenging assay and cyclic voltammetry (Fig. 2). Both analytical approaches revealed differences among cultivation media, although differences depended on the analytical method and plant species. The observed trends indicate that nutrient composition influences not only plant growth but also the redox properties of the microgreens. The use of DPPH and CV methods provided complementary information regarding antioxidant properties. A qualitative trend was observed, with microgreens exhibiting greater plant growth, generally showing higher redox activity in the water extracts. The higher antioxidant activity observed in nutrient-enriched media agrees with earlier findings suggesting that mineral nutrition influences the synthesis of bioactive compounds and the redox status of plants [5].

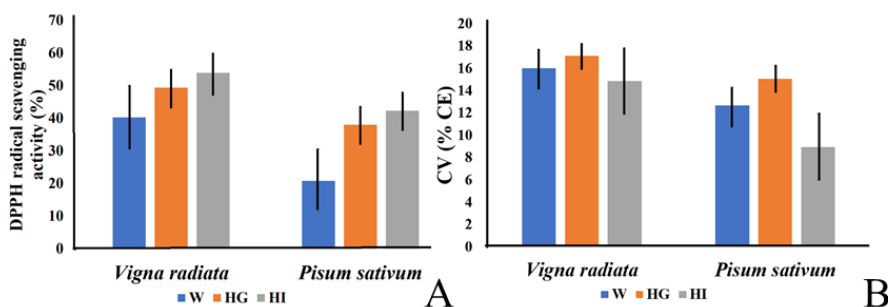


Figure 2. Comparison of antioxidant potential in microgreens determined by DPPH assay (A) and CV method (B). (W- Water, HG- Hoagland, HI- hydroponic system)

CONCLUSION

The results indicated the influence of hydroponic medium composition on the growth and antioxidant properties of microgreens. Among the tested media, the Hoagland nutrient solution showed the most favourable conditions for promoting plant growth, while both DPPH and CV confirmed that cultivation conditions affect antioxidant capacity.

Acknowledgement

This research has been financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-33/2026-03/200026 and 451-03-33/2026-03/ 200168).

REFERENCES

- [1] S. Verlinden, *Hortic. Rev.*, 2020, 47, 85-124.
- [2] S. A. Mir, M. A. Shah, M. M. Mir, *Crit. Rev. Food Sci. Nutr.*, 2017, 57(12), 2730 – 6.
- [3] P. Rajan, R. R. Lada, M. T. MacDonald, *American Journal of Plant Sciences*, 2019, 10, 1397 – 1408.
- [4] O. P. Sharma, T. K. Bhat. *Food chemistry*. 2009, 113(4), 1202 – 5.
- [5] A. Villani, M. Loi, F. Serio, F. F. Montesano, M. D’Imperio, S. De Leonardis, G. Mule, C. Paciolla, *J. Soil Sci. Plant Nutr.*, 2023, 23, 4115 – 4126.

EFFECTS OF SELENIUM SUPPLEMENTATION ON GROWTH, NDVI, AND ANTIOXIDANT CAPACITY OF *Vigna radiata* (L.) WILCZEK MICROGREENS

A. Bučić¹, D. Dimitrijević¹, S. Lekić², V. Beškoski¹ and N. Lugonja²

¹University of Belgrade, Faculty of Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia

²University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia (nikoleta.lugonja@ihtm.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.27

ABSTRACT

Microgreens are a functional food rich in vitamins, minerals, and bioactive compounds with antioxidant properties. The aim of this study was to examine the effect of selenium supplementation (0.1, 0.5, 1, and 10 mg/L) on the growth, Normalized Difference Vegetation Index (NDVI), and antioxidant capacity of *Vigna radiata* microgreens grown under hydroponic conditions. The plants were treated with different selenium concentrations during cultivation, after which plant length and NDVI value were measured. Antioxidant capacity was determined using the DPPH method. The results showed that selenium supplementation at 0.5 mg/L increased antioxidant capacity and NDVI values, whereas higher concentrations negatively affected growth and antioxidant capacity, confirming the potential of selenium for supplementation of functional foods.

INTRODUCTION

In recent years, microgreens have become increasingly prominent in the food industry, driven by heightened consumer awareness of nutrition and by their appealing appearance, texture, and diverse colors, aromas, and flavors, which have contributed to their popularity in contemporary gastronomy and high-end restaurants. Microgreens are young vegetable plants harvested shortly after the development of cotyledon leaves and can be consumed, providing substantial amounts of vitamins, minerals, dietary fiber, and bioactive compounds. Their short cultivation and harvest cycles, minimal spatial requirements, and high market value position microgreens as a significant crop for sustainable food production. A wide range of plant species is used for microgreen cultivation, with notable interspecies variation. Even small quantities of microgreens provide substantial nutritional benefits, comparable to much larger amounts of mature vegetables. Particular emphasis is placed on their content of bioactive substances, including carotenoids, polyphenols, anthocyanins, and phylloquinones, as well

as micronutrients [1, 2]. Selenium is an essential trace element that plays a critical role in antioxidant defense, immunomodulation, thyroid regulation, and various metabolic processes in humans and animals, but uneven global soil distribution leaves approximately 15% of the global population deficient. To combat hidden hunger in mineral-malnourished countries, agronomic biofortification can be the most suitable dietary intervention. In plants, low concentrations of selenium can promote growth, enhance the synthesis of bioactive compounds, and increase antioxidant capacity [3, 4]. Hydroponic systems are among the most widely used methods for cultivating microgreens, allowing plants to grow under controlled conditions by supplying nutrient solutions and regulating parameters such as light intensity, temperature, and humidity [5]. These systems facilitate precise management of mineral nutrient availability and provide an effective model for the biofortification of plants with micronutrients. This study aims to investigate the effects of selenium supplementation on the growth, NDVI, and antioxidant capacity of hydroponically cultivated *Vigna radiata* (L.) Wilczek microgreens (Mung bean).

EXPERIMENTAL

V. radiata microgreens were cultivated hydroponically in Hoagland solution supplemented with selenium at concentrations of 0-10 mg/L (Na_2SeO_3). Seeds were grown under controlled environmental conditions at 24 °C, 45% relative humidity, and 442 ppm CO_2 . After 8 days of growth, plant, root, and shoot lengths were measured and expressed as mean $\pm$ SD (n = 50). Additionally, a CI-710s SpectraVue Leaf Spectrometer - CID Bio-Science was used to obtain leaf spectra from 330 to 1100 nm and to calculate the Normalized Difference Vegetation Index (NDVI). Fresh microgreens (n = 50) were homogenized in liquid nitrogen, and Se content was detected by ICP-MS, Single Quadrupole (Thermo Fisher Scientific). Radical-scavenging activity was determined spectrophotometrically (Shimadzu UV-1280, Shimadzu Europa GmbH, Germany) in methanol-extracted (1:10) plant homogenates using 1 mM DPPH. All results are presented as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

An increase in selenium accumulation was observed in the plants, with selenium content increasing from 0.45 mg/kg DW in the control to 9.80 mg/kg DW in plants grown at the highest selenium concentration (10 mg/L). A slight increase in selenium content in plants was observed in media with 0.5 and 1 mg/L Na_2SeO_3 . The measured lengths of *V. radiata* microgreens after 8 days of growth under different selenium concentrations are shown in Figure 1A. Selenium supplementation affected growth, with the most stimulatory effect observed at 0.5 mg/L Se. At this concentration, both root and shoot elongation increased

compared to the control grown without selenium. In contrast, higher selenium concentrations showed an inhibitory effect on plant growth. A reduction in root length was observed as selenium levels increased, indicating that the root system was more sensitive to selenium-induced stress than the aerial parts of the microgreens. Additionally, the NDVI of microgreen leaves showed a slight increase at a selenium concentration of 0.5 mg/L compared to control plant leaves grown in medium without Se (Fig. 1B). This increase suggests improved photosynthetic performance and chlorophyll status of the microgreens at low selenium concentrations.

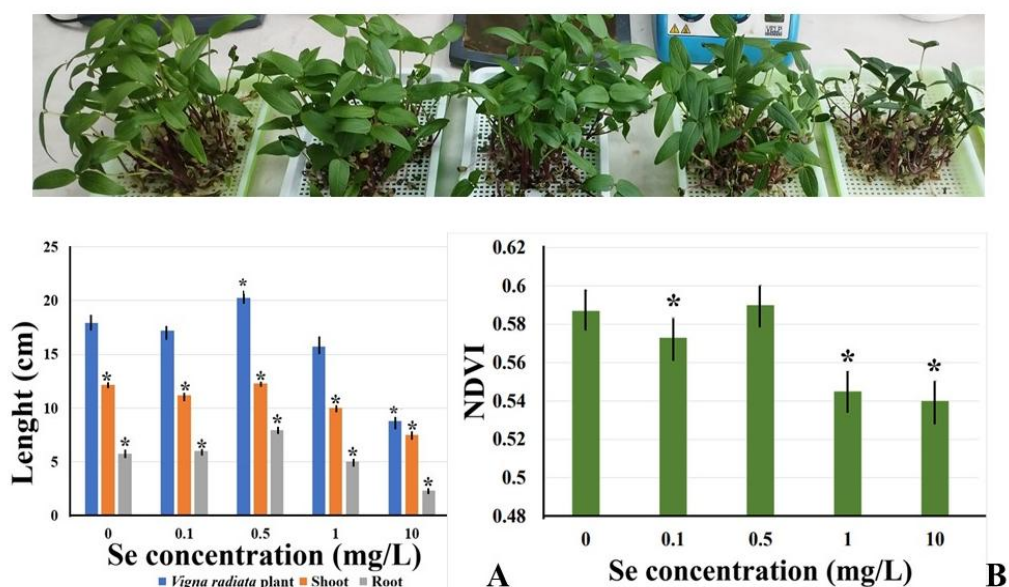


Figure 1. Effect of different selenium supplementation on hydroponic growth (A), and NDVI values (B) of *V. radiata* microgreens. Statistically significant difference compared to the control (* $p < 0.05$).

DPPH radical scavenging capacity increased in plants grown hydroponically in a medium with 0.5 mg/L selenium (Fig. 2), whereas higher selenium concentrations reduced antioxidant activity in microgreens. Low selenium concentrations may stimulate antioxidant activity, whereas excessive selenium may induce oxidative stress and reduce antioxidant capacity.

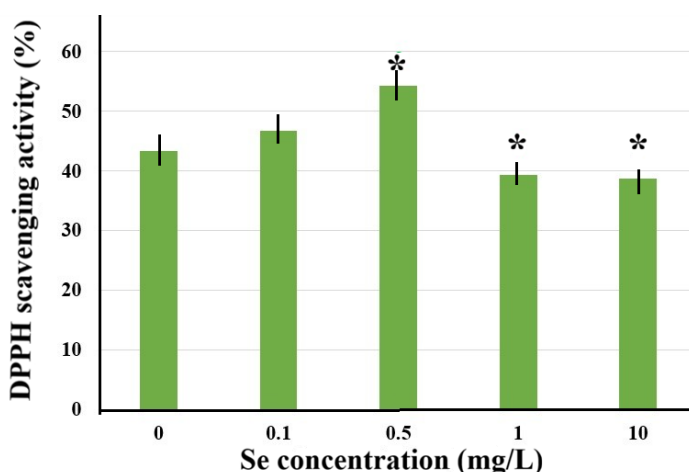


Figure 2. DPPH radical scavenging activity of *V. radiata* microgreens. Statistically significant difference compared to the control (* $p < 0.05$).

CONCLUSION

Applying selenium in hydroponic systems represents a promising strategy for improving selected functional properties of microgreens. Studying selenium's effects on the growth and antioxidant capacity of *V. radiata* microgreens helps clarify their potential as functional foods. Results show that low selenium concentrations can promote plant development by supporting metabolic activity. Therefore, 0.5 mg/L selenium appears to be the most suitable concentration for improving the growth and antioxidant capacity of *V. radiata*, whereas higher concentrations inhibited growth and antioxidant capacity.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-33/2026-03/200026 and 451-03-33/2026-03/ 200168).

REFERENCES

- [1] M. Bhaswant, D. K. Shanmugam, T. Miyazawa, C. Abe, T. Miyazawa, *Molecules*, 2023, 28, 867.
- [2] T. Seth, G. P. Mishra, A. Chattopadhyay, P. Deb Roy, M. Devi, A. Sahu, S. K. Sarangi, C. S. Mhatre, Y. A. Lyngdoh, V. Chandra, *Plants*, 2025, 14, 526.
- [3] M. Viltres-Portales, M. Sánchez-Martín, M. Llugany, R. Boada, M. Valiente, *Plant Physiology and Biochemistry*, 2024, 206, 108283.
- [4] P. J. White, M. R. Broadley, *New Phytologist*, 2009, 182, 49 – 84.
- [5] M. S. Mir, M. Shah, M. Mir, S. A. Wani, S. Rashid, A. M. Bhat. *The Pharma Innovation Journal*, 2022, 11, 1175 – 1195.

ASSESSMENT OF OXIDATIVE STRESS BIOMARKERS IN RAT LIVER AND SERUM FOLLOWING ZALEPLON ADMINISTRATION

J. Martinović¹, D. Drakulić¹, A. Todorović¹, Z. Stojanović², T. Zečević¹, V. Petković¹ and I. Guševac Stojanović¹

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (jelenazlatkovic@vinca.rs)*

²*Group for Biomaterials and Biomedical Applications, Institute of Technical Sciences of SASA, Knez Mihailova Street 35/4, 11000 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.28

ABSTRACT

Although zaleplon is widely prescribed for short-term insomnia, its effects on peripheral redox homeostasis remain poorly understood. This study investigates the impact of zaleplon administration on systemic and hepatic oxidative status in adult male Wistar rats. Animals were treated intraperitoneally with zaleplon (0.625 mg/kg) or vehicle (physiological saline) for five consecutive days. Twenty-four hours after the final treatment, serum and liver samples were collected and prooxidant-antioxidant balance (PAB), advanced oxidation protein products and lipid peroxidation markers were assessed. Zaleplon increased serum protein oxidation and lipid peroxidation, while the PAB remained unchanged. In contrast, hepatic analysis revealed a shift toward a pro-oxidative state without significant alterations in protein or lipid oxidative damage. This tissue-specific response underscores the necessity of multi-biomarker approaches to fully evaluate the peripheral redox impact of hypnotic agents.

INTRODUCTION

Zaleplon is a non-benzodiazepine “Z-drug” approved for short-term insomnia treatment [1]. Its rapid onset, ultra-short half-life, and absence of active metabolites provide a favorable pharmacokinetic profile with reduced residual effects compared with classical benzodiazepines. However, despite their perceived safety, Z-drugs are increasingly linked to misuse, dependence, and withdrawal, warranting further investigation into their systemic biological effects [2].

Since the liver is the primary site of xenobiotic metabolism, drug biotransformation can trigger reactive oxygen species generation and disrupt redox homeostasis, ultimately causing oxidative modifications to proteins and lipids [3]. Although zaleplon undergoes extensive hepatic clearance, its impact

on systemic and hepatic redox balance following repeated exposure remains insufficiently explored.

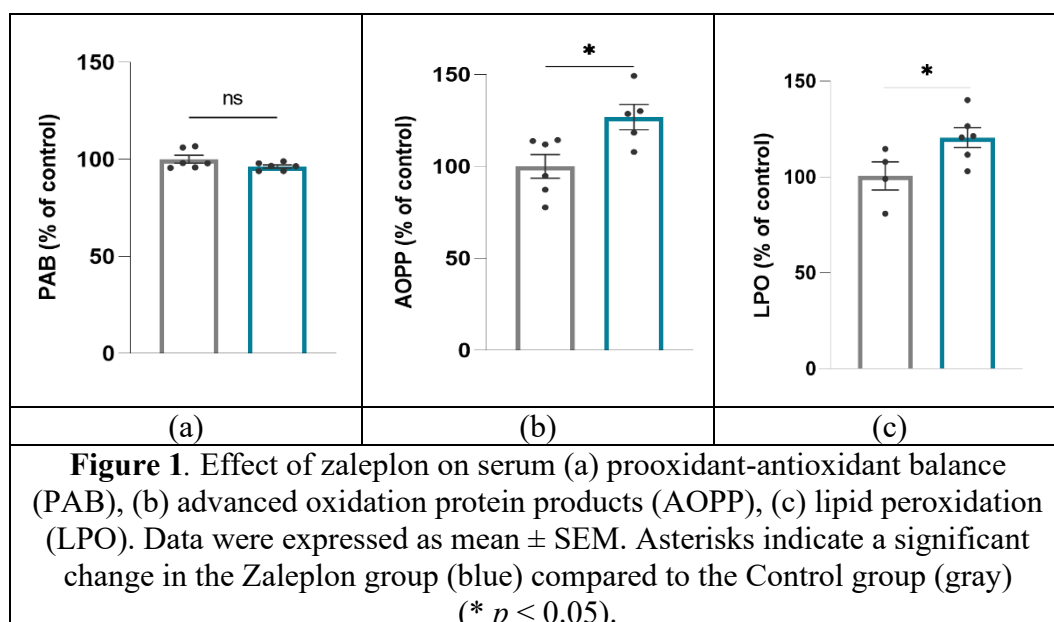
To capture these redox disturbances and potential macromolecular damage, evaluating a panel of complementary biomarkers, specifically prooxidant-antioxidant balance (PAB), advanced oxidation protein products (AOPP), and lipid peroxidation products (LPO), is essential. Therefore, this study aimed to investigate the effects of repeated zaleplon administration on systemic and hepatic oxidative status in rats.

EXPERIMENTAL

All procedures complied with EU Directive 2010/63/EU and were approved by the Ethics Committee for the Use of Laboratory Animals of the Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade (license No. 323-07-06,688/2020-05). Adult male Wistar rats, were randomly assigned to two groups, receiving daily intraperitoneal injections (08:00–09:00 h) of physiological saline (vehicle, Control group, $n=6$) or zaleplon (0.625 mg/kg, Zaleplon group, $n=6$) for five consecutive days. Twenty-four hours post-treatment, animals were decapitated (Harvard Apparatus guillotine, Holliston, MA, USA). Trunk blood was collected, centrifuged (3000 rpm, 15 min; Heraeus Labofuge GL, Hanau, Germany), and serum was stored at $-80\text{ }^{\circ}\text{C}$ alongside rapidly excised liver tissue. Analytical-grade chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Serum and hepatic PAB, AOPP and LPO (expressed as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) levels) were measured spectrophotometrically in duplicate (Tecan Sunrise microplate reader, Männedorf, Switzerland) following methods described by Guševac Stojanović et al. [4]. Results were normalized to the control group and expressed as mean $\pm$ SEM.

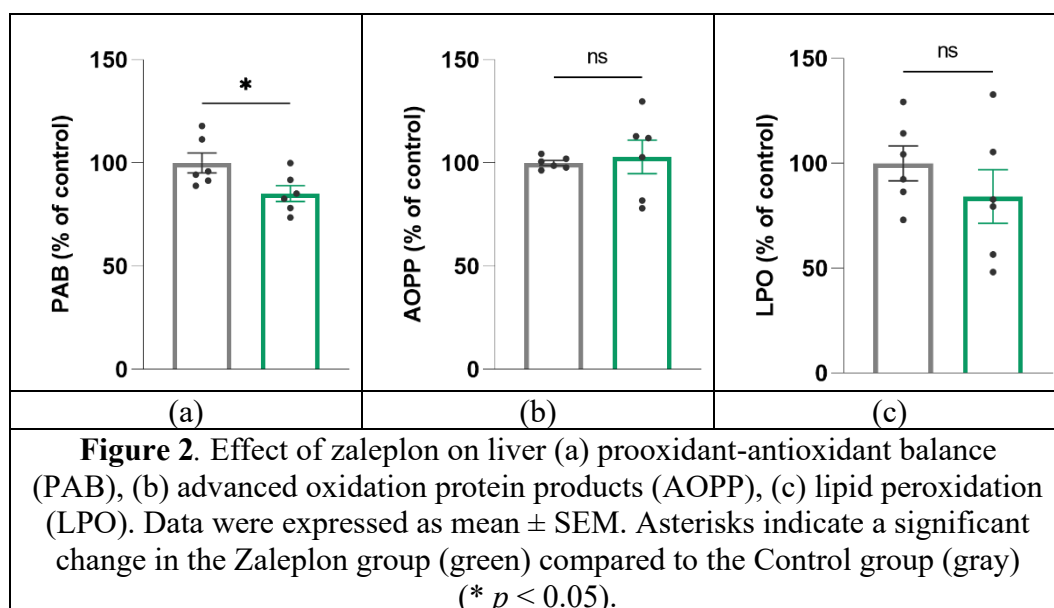
RESULTS AND DISCUSSION

Serum analysis revealed significantly increased protein oxidation [$t(9) = 2.850$, $p = 0.0191$] and lipid peroxidation [$t(9) = 2.691$, $p = 0.0247$] following zaleplon treatment, whereas the PAB remained unchanged [$t(10) = 1.678$, $p = 0.1242$] (Fig. 1). This indicates selective oxidative damage to proteins and lipids despite an unaltered global redox balance.



Since PAB reflects systemic equilibrium while AOPP and lipid peroxidation capture cumulative macromolecular damage, these markers may detect localized modifications before a global shift occurs. Alternatively, transient redox imbalances may have resolved before sampling, leaving stable oxidation products detectable.

In contrast, hepatic analysis demonstrated a significant reduction in pro-oxidative status following zaleplon treatment compared to the Control group [$t(10) = 2.39$, $p = 0.0380$], as indicated by decreased PAB levels. In contrast, markers of protein oxidation [$t(10) = 0.3577$, $p = 0.7280$] and lipid peroxidation [$t(10) = 1.039$, $p = 0.3233$] showed no significant changes (Fig. 2). The reduction in pro-oxidative status may reflect enhanced regulation of endogenous antioxidant capacity or an early adaptive response without detectable alterations in protein and lipid oxidation markers.



CONCLUSION

Repeated zaleplon administration induced tissue-specific redox alterations. While serum analysis revealed protein and lipid oxidation without changes in global redox balance, the liver exhibited reduction in pro-oxidative status and absence of macromolecular damage. This highlights tissue-specific redox responses and the importance of integrating multiple oxidative stress biomarkers for comprehensive evaluation of zaleplon effects.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-33/2026-03/200017 and 451-03-33/2026-03/200175).

REFERENCES

- [1] F. E. Dungo, C. M. Perry, *Drugs*, 2004, 64, 2071-2089.
- [2] F. Schifano, S. Chiappini, J. M. Corkery, A. Guirguis, *Front. Psychiatry*, 2019, 10, 810.
- [3] H. Jaeschke, G. J. Gores, A. I. Cederbaum, J. A. Hinson, D. Pessayre, J. J. Lemasters, *Toxicol. Sci.*, 2002, 65, 166-176.
- [4] Z. Stojanović, D. Drakulić, J. Martinović, A. Todorović, F. Veljković, N. Filipović, I. Guševac Stojanović, *Microplastics*, 2026, 5, 54.

CONSEQUENCES OF ZALEPLON DISCONTINUATION: EVIDENCE FROM THE FORCED SWIM TEST IN RATS

J. Martinović, T. Radukić and M. Zarić Kontić

*Vinča Institute of Nuclear Sciences – National Institute of the Republic of
Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade,
Serbia (jelenazlatkovic@vinca.rs)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.29

ABSTRACT

Zaleplon is a non-benzodiazepine hypnotic that selectively modulates gamma-aminobutyric acid type A receptors, primarily those containing the $\alpha 1$ subunit. Although considered a safer alternative to classical benzodiazepines, increasing evidence suggests that it may induce neuroadaptive changes following repeated administration and subsequent discontinuation. The present study investigated the effects of short-term zaleplon exposure on stress-related behavior during the early withdrawal phase in rats using the Forced Swim Test (FST). Adult male Wistar rats received zaleplon (0.625 mg/kg, i.p.) or vehicle for five consecutive days, and behavioral assessment was performed 24h after the last administration. Zaleplon-treated animals exhibited increased immobility time compared with control animals, indicating that short-term exposure to zaleplon may induce early withdrawal-related behavioral alterations, potentially associated with adaptations within inhibitory GABAergic neurotransmission.

INTRODUCTION

Zaleplon is a pyrazolopyrimidine hypnotic approved for the short-term management of insomnia. It acts as a positive allosteric modulator of gamma-aminobutyric acid type A receptors (GABA_AR), enhancing GABA-mediated inhibitory neurotransmission through increased chloride influx and neuronal hyperpolarization. Due to its selective affinity for $\alpha 1$ -containing GABA_AR, rapid onset of action, and short elimination half-life, zaleplon was initially considered to have a reduced potential for tolerance, dependence, and withdrawal compared with classical benzodiazepines [1].

However, accumulating evidence indicates that non-benzodiazepine hypnotics may also produce neuroadaptive changes following repeated exposure. Chronic stimulation of GABAergic signaling may result in compensatory alterations within inhibitory circuits, and abrupt discontinuation may lead to an imbalance between inhibitory and excitatory neurotransmission [2]. Such alterations may contribute to withdrawal-associated symptoms, including anxiety, sleep disturbances, and affective changes.

The Forced Swim Test (FST) is widely used to evaluate behavioral responses to an inescapable stressor, where increased immobility time is interpreted as enhanced passive coping behavior [3] and is frequently associated with depressive-like behavioral alterations in rodents. Since the GABAergic system is critically involved in the regulation of stress responsiveness and emotional behavior, changes induced by hypnotic exposure may influence behavioral adaptation during withdrawal. Therefore, the aim of the present study was to investigate whether short-term zaleplon administration followed by abrupt discontinuation induces behavioral alterations during the early withdrawal phase, assessed using the FST.

EXPERIMENTAL

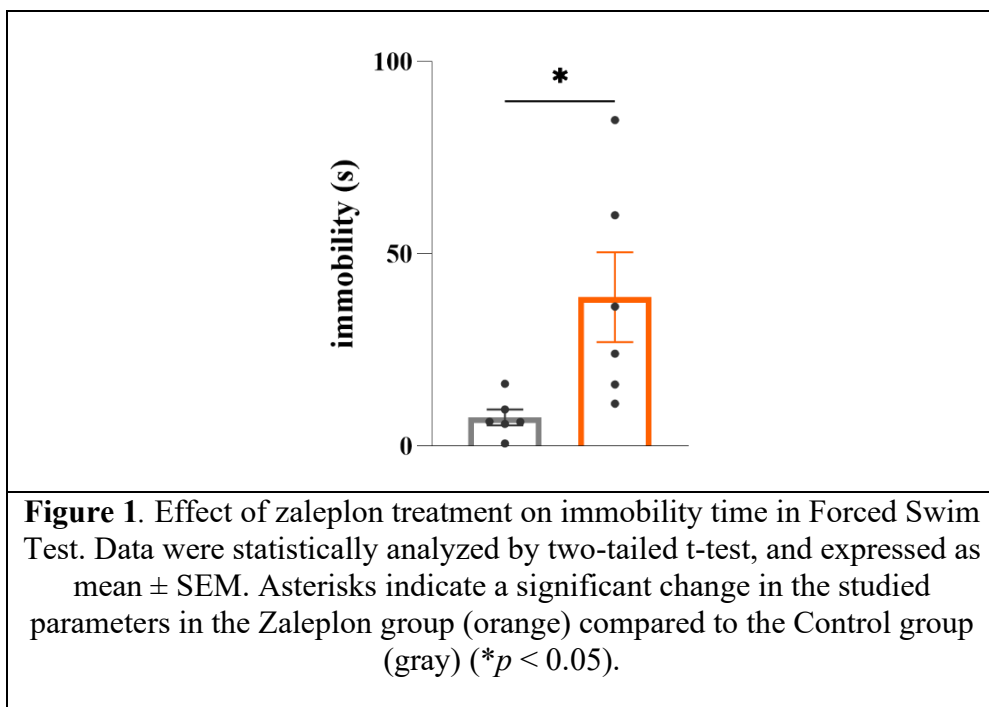
All experimental procedures were approved by the Ethics Committee for the Use of Laboratory Animals of the Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade (license number: 323-07-06,688/2020-05) and conducted in accordance with the European Communities Council Directive (2010/63/EU) for animal experiments.

Adult male Wistar rats were housed under standard laboratory conditions with a 12h light/dark cycle, controlled temperature and humidity, and ad libitum access to commercial rat pellets and tap water. Animals were randomly divided into two groups. The control group received saline (vehicle), while the experimental group received zaleplon (0.625 mg/kg, i.p.) once daily for five consecutive days. Both vehicle and zaleplon were administered between 08:00 and 09:00 AM. Behavioral assessment was performed 24 h after the final administration to evaluate behavioral changes during the early withdrawal phase.

The Forced Swim Test was conducted under standardized conditions, using a transparent plexiglass cylinder (height: 50 cm, diameter: 20 cm) filled with water ($25\pm 1^{\circ}\text{C}$) to a depth of 30 cm, ensuring the animal could not support itself by touching the bottom. Behavior was recorded during the 5-min testing session. Immobility time was quantified as the primary parameter reflecting passive coping behavior. Statistical analysis was conducted using an unpaired two-tailed Student's t-test.

RESULTS AND DISCUSSION

The unpaired two-tailed t-test revealed a statistically significant increase in immobility time in zaleplon-treated rats compared with control animals [$t(10) = 2.632$, $p = 0.0251$]. Because behavioral testing was performed 24 h after the last zaleplon administration, the observed behavioral alteration may reflect changes emerging during the early withdrawal period.



Increased immobility in the FST indicates enhanced passive coping behavior in response to an acute stress challenge. These effects may result from neuroadaptive changes induced by repeated zaleplon exposure and subsequent discontinuation. Importantly, these findings are consistent with our Open Field Test results (unpublished data), which showed no changes in locomotor activity but suggested an anxiogenic-like behavioral profile, indicating that the increased immobility is unlikely to reflect impaired motor function.

Although zaleplon exhibits selectivity for $\alpha 1$ -containing GABA_AR and was initially considered to have a lower risk of dependence-related effects, repeated modulation of GABAergic signaling may still produce compensatory alterations within inhibitory networks. Following treatment cessation, these adaptations may contribute to altered stress responsiveness and behavioral changes associated with the early withdrawal period.

The present findings indicate that short-term zaleplon exposure is sufficient to produce measurable behavioral alterations after treatment cessation, underscoring the need for further investigation into the mechanisms underlying withdrawal-related effects of selective hypnotic agents.

CONCLUSION

Short-term zaleplon administration followed by abrupt discontinuation resulted in increased immobility time in the FST during the early withdrawal phase. This behavioral alteration suggests changes in stress-coping responses following

zaleplon cessation and indicates that even brief exposure to selective hypnotic agents may induce neurobehavioral adaptations. Further studies investigating the underlying molecular mechanisms, particularly within the GABAergic system, are required to better understand the consequences of zaleplon withdrawal.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-33/2026-03/ 200017)

REFERENCES

- [1] F. Schifano, S. Chiappini, J. M. Corkery, A. Guirguis, *Int. J. Neuropsychopharmacol.* 2019; 22: 270–277.
- [2] J. Martinovic, J. Samardzic, M. Zaric Kontic, S. Ivkovic, S. Dacic, T. Major, M. Radosavljevic, D. Svob Strac. *Brain Sci.* 2023;13(12):1707.
- [3] M.L. Molendijk, E.R. de Kloet. *Behav Brain Res.* 2019; 364:1–10.

ASSESSMENT OF ANTIBACTERIAL PROPERTIES OF *Azadirachta indica* SEED OIL AGAINST SELECTED PHYTOPATHOGENIC BACTERIA

A. Bošković¹, T. Ganić¹, A. Leskovac², B. Nikolić¹, S. Petrović² and O. Medić¹

¹University of Belgrade, Faculty of Biology, Studentski trg 12-16
11000 Belgrade, Serbia. (olja.medic@bio.bg.ac.rs)

²Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia,
University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia.

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.30

ABSTRACT

The growing resistance of phytopathogenic bacteria to conventional bactericides has intensified the search for sustainable crop protection strategies. Neem (*Azadirachta indica*) seed oil is widely promoted as a botanical biopesticide with antimicrobial potential due to its complex composition and bioactive properties. However, its efficacy against phytopathogens remains poorly characterized. In this study, the antibacterial activity of neem seed oil was evaluated against four agriculturally important phytopathogenic bacteria using the broth microdilution assay. Despite its recognized bioactivity, neem seed oil did not show inhibitory or bactericidal effects against the selected strains within the tested concentration range. These findings indicate that the antimicrobial efficacy of neem-based products depends on formulation properties, chemical composition, and pathogen-specific susceptibility, highlighting the need for rigorous evaluation and optimized formulations before agricultural application.

INTRODUCTION

The rising prevalence of antimicrobial resistance among phytopathogenic bacteria poses a significant challenge for modern agriculture. Conventional disease control strategies rely primarily on synthetic pesticides and copper-based antimicrobial agents; however, their extensive and repeated use has contributed to the emergence of resistant bacterial strains, reduced treatment efficacy, and growing environmental and human health concerns [1]. These challenges have intensified the demand for sustainable, environmentally friendly alternatives in plant disease management. In this context, biopesticides derived from natural sources have gained increasing attention as promising tools for sustainable agriculture. Plant-based biopesticides are particularly attractive due to their biodegradability, chemical diversity, and broad spectrum of biological activities, including insecticidal, antifungal, and antibacterial effects [2]. Among them, neem (*Azadirachta indica*) seed oil has become one of the most extensively

studied botanical biopesticides. Its biological activity is primarily attributed to limonoids such as azadirachtin, along with other bioactive compounds, including nimbin and salannin, which contribute to its pesticidal and antimicrobial properties [3].

The antibacterial efficacy of neem seed oil against phytopathogenic bacteria remains insufficiently validated and inconsistent across studies. Neem oil is highly hydrophobic, so its biological activity may depend on formulation stability, emulsification efficiency, and the bioavailability of active constituents. Although antimicrobial activity is frequently attributed to neem-derived products, supporting evidence remains heterogeneous and often dependent on experimental design and extraction methodology. Phytopathogenic bacteria such as *Xanthomonas campestris* pv. *campestris*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pectobacterium carotovorum* subsp. *brasiliense*, and *Pseudomonas syringae* pv. *aptata* cause economically significant crop diseases worldwide and are often difficult to manage because of their adaptability and environmental persistence. Therefore, this study aimed to evaluate the antibacterial activity of neem seed oil against selected phytopathogenic bacteria of agricultural relevance and to assess its potential as a natural biopesticide for sustainable crop protection.

EXPERIMENTAL

Commercial cold-pressed neem (*Azadirachta indica*) seed oil (Eterra, TemaCo d.o.o., Serbia; origin: India) was evaluated for antibacterial activity against four agriculturally relevant phytopathogenic bacteria: *Xanthomonas campestris* pv. *campestris* (NCPBP 528), *Clavibacter michiganensis* subsp. *michiganensis* (CFBP 4999), *Pectobacterium carotovorum* subsp. *brasiliense* (PCB 2811), and *Pseudomonas syringae* pv. *aptata* (P21), belonging to the bacterial strain collection of the Department of Microbiology, University of Belgrade, Faculty of Biology. Neem oil emulsions were prepared in Mueller–Hinton broth using Tween 20 (Sigma-Aldrich, France) as an emulsifying agent. Antibacterial activity was assessed using the broth microdilution method in 96-well microplates by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Neem oil was tested over a concentration range of 0.078–100 mg/mL against bacterial inocula standardized to 1×10^6 CFU/mL. Streptomycin (concentration range 2–256 µg/mL) and tetracycline (concentration range 0.5–64 µg/mL) served as positive controls, while serial dilutions of Tween 20 (concentration range 0.0078–10%) were included as a solvent control. Following incubation at 30 °C for 24 h, bacterial viability was assessed using a resazurin-based assay, while bactericidal activity was confirmed by subculturing in Mueller–Hinton agar (TMMedia, India).

RESULTS AND DISCUSSION

The antibacterial activity of *Azadirachta indica* seed oil against selected phytopathogenic bacterial strains was evaluated using the broth microdilution method to determine the MIC and MBC. Tween 20, used as the emulsifying agent, did not exhibit antibacterial activity. As shown in Table 1, neem seed oil showed no inhibitory or bactericidal activity against the selected strains at the tested concentrations. In contrast, the positive controls showed expected antibacterial activity. Streptomycin exhibited MIC values ranging from ≤ 2 $\mu\text{g/mL}$ to 8 $\mu\text{g/mL}$ and MBC values from 8 $\mu\text{g/mL}$ to 32 $\mu\text{g/mL}$, while tetracycline showed pronounced activity against strain NCPPB 528 (MIC ≤ 0.5 $\mu\text{g/mL}$; MBC 2 $\mu\text{g/mL}$). Strain PCB 2811 was not sensitive to either antibiotic, indicating a high level of resistance that further emphasizes the urgent need for alternative disease management strategies.

Table 1. Minimal inhibitory and minimal bactericidal concentrations of neem seed oil.

Strain designation	Neem seed oil (mg/mL)		Streptomycin ($\mu\text{g/mL}$)		Tetracycline ($\mu\text{g/mL}$)	
	MIC	MBC	MIC	MBC	MIC	MBC
NCPPB 528	> 100	nd	8	32	≤ 0.5	2
CFBP 4999	> 100	nd	8	16	> 64	nd
PCB 2811	> 100	nd	> 256	nd	> 64	nd
P21	> 100	nd	≤ 2	8	2	32

nd- not determined in the tested concentration range

These findings contrast with several reports describing the antibacterial effects of *Azadirachta indica* extracts and formulations against certain phytopathogenic *Xanthomonas*, *Pseudomonas*, and *Agrobacterium* species [4-5], underscoring that the antimicrobial efficacy of neem-derived products is highly dependent on the extraction method, formulation, and chemical composition. Indeed, antibacterial efficacy appears to vary among neem plant parts, with leaf and bark extracts generally showing stronger activity than seed and fruit extracts [6]. Additionally, previous greenhouse and field studies suggest that neem oil formulations may contribute to disease suppression through indirect mechanisms, including modulation of plant defense responses and interference with pathogen colonization [7].

The absence of measurable antibacterial activity observed in this study may be attributed to the limited bioavailability and dispersion of hydrophobic neem oil constituents in aqueous culture systems, thereby reducing their interaction with bacterial cells. In addition, formulation-related factors and the intrinsic resistance mechanisms of phytopathogenic bacteria may further influence the antimicrobial efficacy of neem-based products. Collectively, these findings highlight the complexity of neem-based antimicrobial activity and underscore the

need for detailed chemical characterization, optimized formulations, and evaluation of isolated bioactive constituents against a broader range of phytopathogens.

CONCLUSION

Under the experimental conditions used, *Azadirachta indica* seed oil did not show direct antibacterial activity against the phytopathogenic bacteria tested. These findings underscore the importance of optimizing formulations and conducting standardized biological evaluations in developing evidence-based neem-derived biopesticides for sustainable agriculture.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract numbers 451-03-34/2026-03/200178 and 451-03-33/2026-03/200017).

REFERENCES

- [1] K. Alem, M. Dagnew, M. Gizachew, B. Gelaw, F. Moges, *Microbiologyopen*, 2025, 14(5), e70067.
- [2] E.O. Fenibo, T. Matambo, *Front. Sustain. Food Syst.*, 2025, 9, 1657000.
- [3] A.K. Dhakad, R. Kumar, R. Choudhary, S. Singh, S. Khan, P.K. Poonia, *Ind. Crops Prod.*, 2025, 231, 121155.
- [4] G. Perumal, G. Saravanan, T. Ragupathi, S. Muthusami, *Asian J. Bio Sci.*, 2008, 3(1), 130–132.
- [5] N. Goel, K. Anukrati, P.K. Paul, *J. Chem. Pharm. Sci.*, 2016, 9(4), 2547–2555.
- [6] S. Adusei, S. Azupio, *J. Chem.*, 2022, 6778554.
- [7] P.A. Abbasi, D.A. Cuppels, G. Lazarovits, *Can. J. Plant Pathol.*, 2003, 25(1), 41–48.

REMOVAL OF METHYLENE BLUE FROM AQUEOUS SOLUTION USING H₂O₂-MODIFIED HYDROCHAR

M. Milenković¹, M. Simić², S. Porobić-Katnić¹, O. Todorović¹ and M. Marinović-Cincović¹

¹ *Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (marija.kojic@vin.bg.ac.rs)*

² *Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.31

ABSTRACT

The increasing discharge of hazardous pollutants into aquatic environments demands sustainable remediation strategies. This study evaluates a biosorbent derived from food waste via hydrothermal carbonization (HTC) at 200 °C, followed by chemical modification with H₂O₂ to enhance adsorption performance. The hydrochar was tested for methylene blue (MB) removal from aqueous media at 35 °C. To elucidate equilibrium mechanisms, experimental data were fitted to Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich isotherms. The data aligned best with the Langmuir and Freundlich models, revealing a maximum adsorption capacity ($q_{\max}$) of 122 mg g⁻¹. SEM analysis revealed that HTC and H₂O₂ modification led to significant surface erosion and roughness, while preserving the underlying fibrous structure. This surface degradation and resulting roughness contributed to an increased reactive surface area. These findings confirm that H₂O₂-modified hydrochar is an efficient dye-removal solution.

INTRODUCTION

The discharge of synthetic dyes, particularly MB, into aquatic ecosystems poses severe environmental and toxicological risks due to their complex aromatic structure, persistence, and toxicity [1]. Adsorption is widely recognized as an efficient and simple remediation method, yet the high cost of commercial activated carbon highlights the need for sustainable, bio-based alternatives [2]. Food waste represents an abundant organic resource well-suited for HTC. HTC efficiently converts high-moisture biomass into hydrochars at moderate temperatures (180-260 °C) without energy-intensive pre-drying [3]. However, pristine hydrochars often suffer from limited porosity and lower functional group density [3,4]. Chemical modification with H₂O₂ serves as an eco-friendly activation strategy, enhancing surface roughness and introducing oxygen-

containing functional groups without generating secondary toxic residues. This study investigates a novel biosorbent derived from food waste via HTC at 200 °C and modified with H₂O₂ for MB removal from aqueous media. The main objectives were to evaluate its equilibrium adsorption capacity at 35 °C, characterize morphological surface changes using scanning electron microscopy (SEM), and model the adsorption mechanisms using Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich isotherms.

EXPERIMENTAL

The food waste was mixed with deionized water (1:15) and hydrothermally carbonized at 200 °C for 60 min. The washed, dried hydrochar (12 g) was stirred in 150 mL H₂O₂ solution for 180 min and dried at 60 °C. This hydrochar is labeled HC-H₂O₂. Morphology was analyzed using SEM (Thermo Fisher Scientific, USA). Batch trials evaluated hydrochar dispersions (10 mg mL⁻¹ in 50% ethanol, pH 6) with MB (Sigma-Aldrich, Germany) at 35 °C. Following centrifugation (14,500 rpm), residual dye was quantified at 664 nm via UV-Vis spectrophotometry (Perkin Elmer Lambda 35).

RESULTS AND DISCUSSION

The morphological analysis reveals significant structural transformations following hydrothermal treatment and H₂O₂ activation (Figure 1).

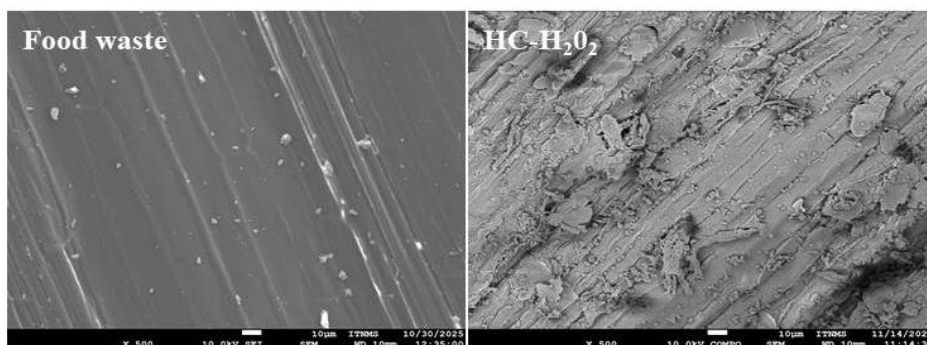


Figure 1. SEM analysis of food waste and HC-H₂O₂.

The food waste exhibits a smooth, compact surface with parallel fibrous channels. In contrast, HC-H₂O₂ shows substantial surface erosion, cracking, and increased roughness while retaining the underlying aligned matrix. This oxidative degradation and roughened texture effectively enhance the active surface area and exposure of binding sites, favoring MB adsorption.

Table 1. Adsorption parameters of HC-H₂O₂.

Freundlich isotherm	
K_f $((\text{dm}^3 \text{ mg}^{-1})^{1/n})$	0.821
n	1.23
χ^2	2.469
R^2	0.972
Langmuir isotherm	
K_L ($\text{dm}^3 \text{ mg}^{-1}$)	0.004
$q_{\max}$ (mg g^{-1})	122
χ^2	3.397
R^2	0.962
Temkin isotherm	
K_t ($\text{dm}^3 \text{ mg}^{-1}$)	1.1
b_t ($\text{J g mol}^{-1} \text{ mg}^{-1}$)	600
χ^2	33.46
R^2	0.625
Dubinin–Radushkevich isotherm	
q_{DR} (mg g^{-1})	31
K_{DR} ($\text{mol}^2 \text{ J}^{-2}$)	$1.2 \cdot 10^{-4}$
E (J mol^{-1})	64
χ^2	16.193
R^2	0.818

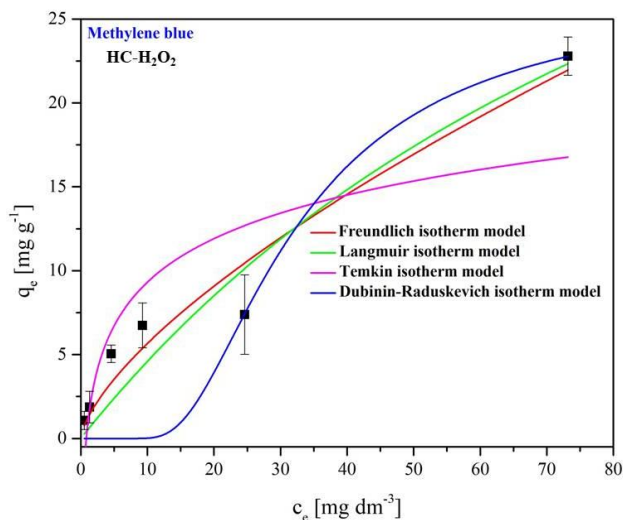


Figure 2. Adsorption isotherms of HC-H₂O₂.

This study investigates the equilibrium adsorption of MB onto a HC-H₂O₂ using Freundlich, Langmuir, Temkin, and Dubinin–Radushkevich isotherm models. Based on the R^2 and χ^2 analysis, the data were best fitted by the Freundlich model ($R^2 = 0.972$, $\chi^2 = 2.469$). The heterogeneity factor $n = 1.23$ ($1/n < 1$) confirms a highly favorable multilayer adsorption on an energetically heterogeneous surface.

The Langmuir model also showed a strong correlation ($R^2 = 0.962$), yielding a $q_{\max}$ of 122 mg g^{-1} . Additionally, the mean free energy calculated from the Dubinin–Radushkevich isotherm ($E = 0.064 \text{ kJ mol}^{-1}$) is well below 8 kJ mol^{-1} , confirming a physisorption mechanism dominated by electrostatic and weak van der Waals interactions. Overall, the sorbent exhibits high efficacy for cationic dye removal.

CONCLUSION

A highly efficient biosorbent was successfully developed from food waste via hydrothermal carbonization and H₂O₂ modification. SEM analysis confirmed enhanced surface roughness and exposed binding sites. The equilibrium data fitted well to Langmuir and Freundlich models, exhibiting $q_{\max}$ of 122 mg g^{-1} .

The mean free energy ($E = 0.064 \text{ kJ mol}^{-1}$) confirms a physical adsorption mechanism. Consequently, H_2O_2 -modified hydrochar is an effective, eco-friendly candidate for dye-contaminated wastewater treatment.

Acknowledgement

The authors are grateful to the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, for the financial support (contract no. 451-03-33/2026-03/200017 498).

REFERENCES

- [1] I. M. Jauris, S. B. Fagan, M. A. Adebayo, F. M. Machado, *Comput. Theor. Chem.*, 2016, 1076, 42-50.
- [2] A. H. Jawad, R. A. Rashid, M. A. M. Ishak, L. D. Wilson, *Desalin. Water Treat.*, 2016, 57, 25194-25206.
- [3] M. Milenković, T. Lazarević-Pašti, V. Milanković, T. Tasić, I. A. Pašti, S. Porobić-Katnić, M. Marinović-Cincović, *J. Water Process Eng.*, 2024, 60, 105204.
- [4] Y. Chen, S. A. Huang, K. Yu, J. Z. Guo, Y. X. Wang, B. Li, *Bioresour. Technol.*, 2023, 379, 129067.

INTERACTION OF QUERCETIN-CAPPED GOLD NANOPARTICLES WITH TRYPTOPHAN

T. Momić¹

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (momict@vin.bg.ac.rs)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.32

ABSTRACT

In the present work, interaction of the synthesized quercetin-capped gold nanoparticles (NPQs) and compared with that of free quercetin (Q) using UV-Vis spectroscopy. It was shown that unlike Q, the interaction of NPQs with Trp did not alter its concentration-dependent pattern of absorption changes. Moreover, this interaction was independent of the NPQ concentration and did not induce the nanoparticle aggregation.

INTRODUCTION

Flavonol quercetin (Q) (Fig. 1A), a type of natural polyphenolic possess various promising bioactive effects such as such as hypotensive, hypoglycemic, antioxidant, antiinflammatory, antibacterial, antiviral, and immunomodulatory [1]. However, its potential clinical use is hindered by its low bioavailability. As one of the possible option to overcome this limitation, the quercetin-capped gold nanoparticles (NPQs) have been synthesized. They were shown to possess antioxidant, antimicrobial, as well as anticancer activities [2].

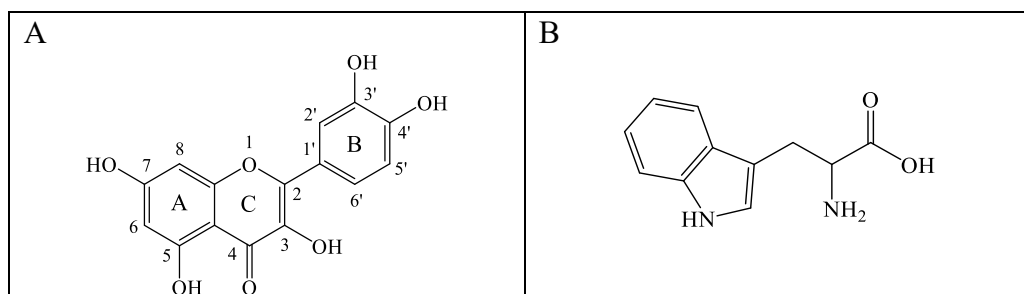


Figure 1. Molecular structures of A) quercetin, B) tryptophan

There is a general consensus that all flavonoids exert their antioxidant activity through their ability to interact with a broad range of proteins, enzymes and transcription factors rather than acting as conventional hydrogen-donating

antioxidants [3]. Previously was shown that among the amino acid (AA) residues interacting with quercetin are all hydrophobic amino acids, showing that quercetin bound to the hydrophobic region of the protein [4]. The presence of the indole ring in the chemical structure of Trp (Fig. 1B) gives high hydrophobic features to this molecule among all protein AAs [5]. Also, Trp is one of the amino acids residues which are shown to interact with Q [4].

Therefore, a better understanding of the biological activities of NPQs requires studying their interaction with proteins. In this work, the interaction of Trp with the synthesized NPQs was investigated and compared with that of free Q using UV-Vis spectroscopy.

EXPERIMENTAL

NPQs were synthesized according Milanezi et al. with slight modifications [2]. Stock solution 10^{-2} M of Q was prepared in DMSO.

In the experiments, either NPQs or free Q aqueous solution was incubated with various concentrations of Trp (10^{-6} M – 2×10^{-5} M) at 25 °C for 4 h, followed with UV-Vis spectra recording. The experiments were done with two concentrations 10^{-5} and 10^{-4} M for both, NPQs and free Q.

RESULTS AND DISCUSSION

The recorded spectra showed absorption maxima at 278, 259 and 374, and 539 nm for Trp, free Q and the NPQs, respectively, 10^{-4} M free Q and NPQs (Fig. 2 A-C). The interaction of various concentrations of Trp with the NPQs showed the same pattern of changes in the absorption maxima as for Trp alone; that is, the intensity of the absorption bands at 278 and 259 nm increased with increasing Trp concentration, whereas increasing Trp concentration did not affect the intensity of the band at 374 nm (Fig. 2 D-F). The intensity of the absorption band at 539 nm did not change, nor was a new maximum formed upon interaction of the NPQs with Trp (Fig. 2C), indicating that the nanoparticles did not aggregate. The interaction of increasing concentrations of Trp with free Q was accompanied by a decrease in the intensity of the three absorption maxima at 278, 259, and 374 nm (Fig. 2 D-F).

The results of Trp interaction with 10^{-5} M NPQs showed the same trend in changes of the absorption maxima changes as observed with 10^{-4} M NPQs (data not shown).

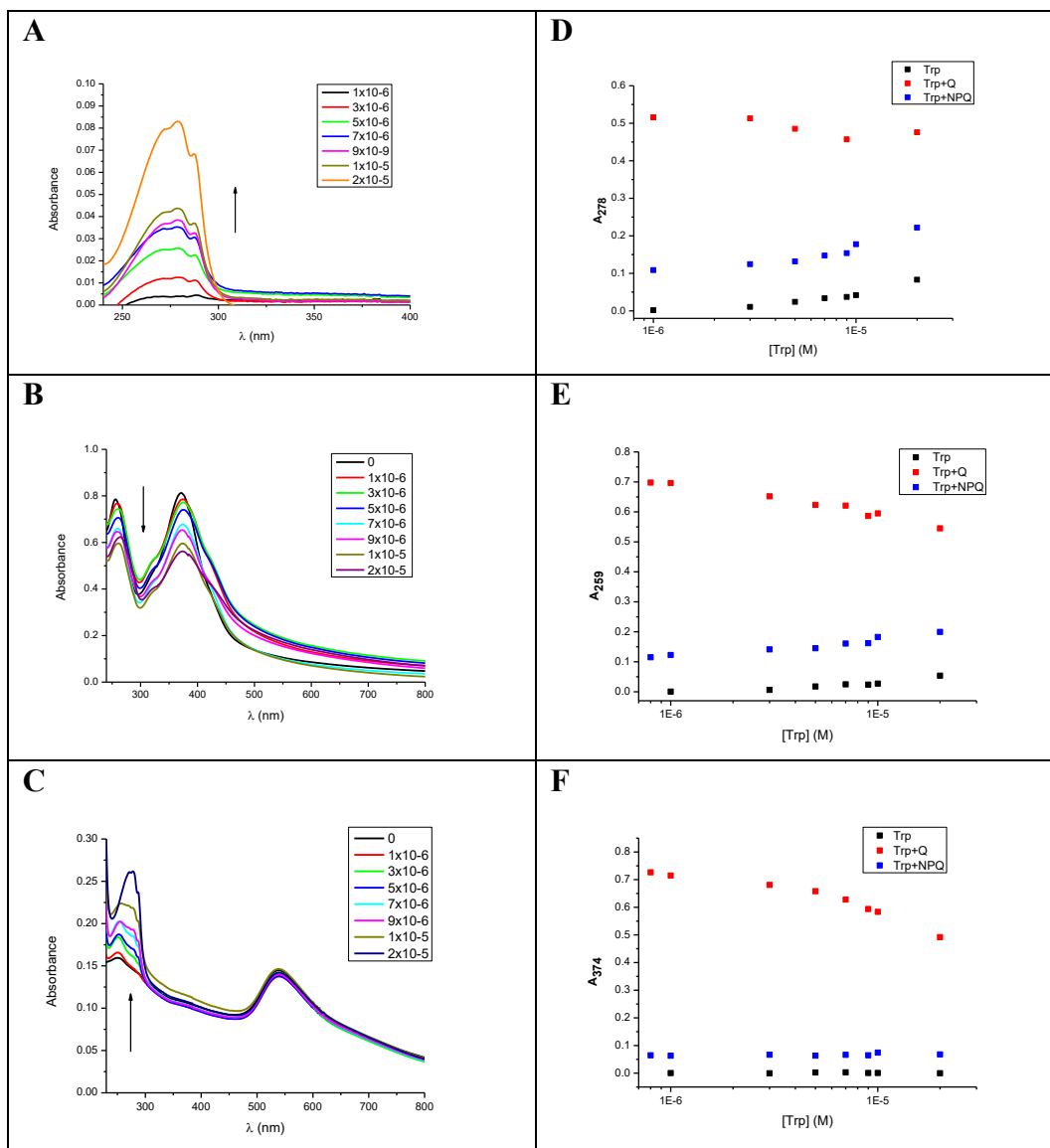


Figure 2. UV-Vis spectra of various concentrations of Trp A) alone, B) with free Q, C) with NPQs, 10^{-4} M free Q and NPQs. The changes in absorbance recorded at D) 278 nm, E) 259 nm, F) 374 nm, dependent on Trp concentrations.

CONCLUSION

The present study indicated that, unlike Q, the interaction of NPQs with Trp did not alter its concentration-dependent pattern of absorption changes. Moreover, this interaction was independent of the NPQ concentration and did not induce the nanoparticle aggregation.

Acknowledgement

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants no. 451-03-33/2026-03/200017).

REFERENCES

- [1] B. Salehi, L. Machin, L. Monzote, J. Sharifi-Rad, S.M. Ezzat, M.A. Salem, R.M. Merghany, N.M. El Mahdy, C.S. Kılıç, O. Sytar, M. Sharifi-Rad, F. Sharopov, N. Martins, M. Martorell, W.C. Cho, ACS Omega, 2020, 5(20), 11849-11872.
- [2] F.G. Milanezi, L. M. Meireles, M.M. de Christo Scherer, J.P. de Oliveira, A.R. da Silva, M.L. de Araujo, D.C. Endringer, M. Fronza, M.C.C. Guimarães, R. Scherer, Saudi Pharm. J., 2019, 27(7), 968-974.
- [3] A. Daniel Boese and E. Codorniu-Hernández, Phys. Chem. Chem. Phys., 2012, 14(45), 15682-15692.
- [4] W. Ji, M. Yang, Q. Li, J. Tuo, X. Chen, LWT, 2025, 220, 117581.
- [5] L. Palego, L. Betti, A. Rossi, G.J. Giannaccini, Amino Acids, 2016, 2016(1), 8952520.

DESIGN OF SALICYLATE-BASED IONIC LIQUID AQUEOUS BIPHASIC SYSTEMS FOR THE EXTRACTION OF A SYNTHETIC FOOD COLORANT E133

J. Mušović, D. Tekić, A. Jocić, S. Marić and A. Dimitrijević

*Vinča Institute of Nuclear Sciences – National Institute of the Republic of
Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade,
Serbia (jasmina.musovic@vin.bg.ac.rs)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.33

ABSTRACT

Food colorants play an important role in the food industry; however, their accurate determination, separation, and control are essential to ensure food quality and safety. Brilliant Blue FCF (E133) is a synthetic dye widely used in food products due to its intense color and high stability. In this study, ionic liquid-based aqueous biphasic systems (ABSs) composed of salicylate-based ionic liquids (ILs) and potassium carbonate (K_2CO_3) were investigated as alternative extraction platforms for the separation of E133. Initially, phase diagrams were constructed to evaluate the biphasic behavior of the investigated systems and to assess the influence of IL cation structure on phase formation. Subsequently, the extraction performance of selected IL-ABSs toward E133 was evaluated. The tested systems demonstrated excellent extraction efficiency, reaching 99.48% and 99.55%. These findings highlight the potential of salicylate-based IL-ABSs as efficient, tunable, and sustainable platforms for the extraction and separation of food-related compounds.

INTRODUCTION

Synthetic food colorants are widely used in the food industry to enhance the appearance and consumer acceptance of various food products. Among them, Brilliant Blue FCF (E133) is one of the most commonly applied water-soluble dyes due to its intense color, high stability, and compatibility with different food matrices [1]. However, the increasing use of synthetic colorants has highlighted the importance of reliable separation and extraction approaches for their determination, purification, and potential recovery from complex systems, contributing to improved food quality and safety.

In recent years, ILs have emerged as promising compounds for the development of sustainable separation processes due to their negligible vapor pressure, high chemical stability, and highly tunable physicochemical properties. Their ability to establish specific interactions with target molecules, including

hydrogen bonding, electrostatic interactions, and π - π interactions, enables their application as efficient extraction media.

Among different IL-based separation approaches, ABSs have gained considerable attention as environmentally favorable platforms for the extraction and separation of various compounds. Aqueous biphasic systems are liquid-liquid separation systems composed mainly of water and two phase-forming components, which, under appropriate concentrations and conditions, spontaneously separate into two distinct aqueous phases [2]. Due to differences in the affinity of solutes toward these phases, target compounds can be selectively distributed between them, enabling efficient extraction and separation. By incorporating ionic liquids as one of the phase-forming components, IL-ABSs combine the advantages of aqueous biphasic media with the tunability of ILs, allowing the selective partitioning of target molecules between coexisting aqueous phases.

In this study, aqueous biphasic systems composed of salicylate-based ILs (tetrabutylphosphonium - TBP and tetrabutylammonium - TBA) and K_2CO_3 were investigated for the extraction of E133. Initially, phase diagrams were constructed to evaluate the phase-forming ability of the investigated systems and to determine the influence of IL cation structure on biphasic behavior. Subsequently, selected ABSs were applied for the extraction of E133. The obtained results demonstrate the effectiveness of salicylate-based IL-ABSs for the extraction of E133 and highlight the importance of IL structure in designing efficient dye separation systems.

EXPERIMENTAL

Salicylate-based ILs were prepared through a neutralization reaction between the corresponding hydroxide precursors, tetrabutylphosphonium hydroxide or tetrabutylammonium hydroxide (40 wt% aqueous solutions, Sigma-Aldrich, USA), and salicylic acid ($\geq 99\%$ purity, Sigma-Aldrich, USA). The reactants were combined in an equimolar ratio, and the resulting mixtures were subjected to continuous stirring at ambient conditions to promote complete reaction. After the reaction, water was removed from the obtained mixtures under reduced pressure using a rotary evaporator (R-210 Rotavapor System, Büchi Labortechnik AG, Switzerland) to obtain the desired ILs.

Phase diagrams, representing the mass fraction of IL as a function of the mass fraction of potassium carbonate, were determined using the cloud point titration method. Aqueous mixtures of known amounts of IL and potassium carbonate were prepared and titrated with water until a clear single-phase system was obtained, followed by titration with potassium carbonate solution until turbidity appeared, indicating the onset of phase separation. The compositions at the cloud point were recorded and used to construct the phase diagrams.

The concentration of E133 was quantified using a LLG-uniSPEC 2 UV-Vis spectrophotometer (LLG Labware, Germany) by measuring the absorbance at 625 nm.

RESULTS AND DISCUSSION

Phase diagrams (Figure 1.) were determined to evaluate the phase-forming ability of the investigated ABSs with potassium carbonate. Both ILs readily formed biphasic systems; however, [TBP][Sal] showed a stronger salting-out tendency than [TBA][Sal], as indicated by its binodal curve being closer to the coordinate origin. This behavior can be attributed to the higher hydrophobicity of the [TBP]⁺ cation compared with [TBA]⁺, resulting from the larger atomic radius and lower electronegativity of phosphorus. The more diffuse charge distribution of the phosphonium cation reduces its hydration ability, facilitating IL salting-out and phase separation at lower component concentrations [3].

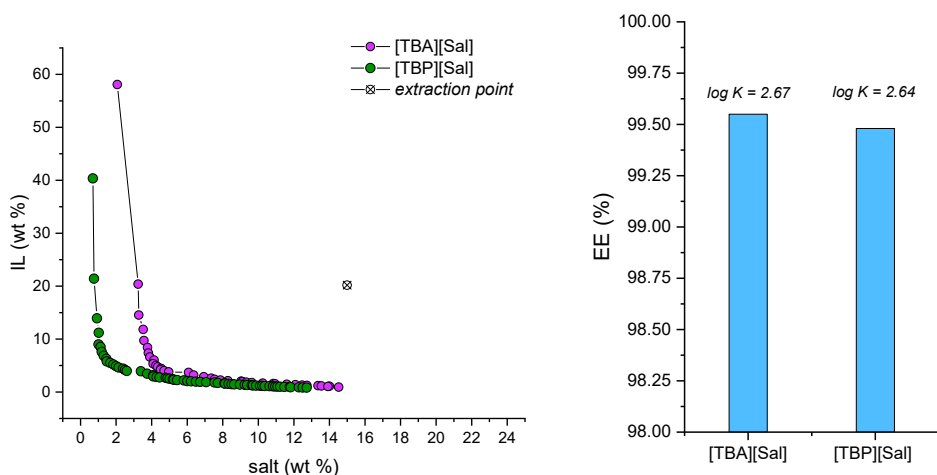


Figure 1. Phase diagrams for [TBP][Sal]/ [TBA][Sal] + K₂CO₃ at ambient conditions and extraction efficiencies of E133 obtained for [TBP][Sal]/ [TBA][Sal] + K₂CO₃ systems.

An extraction point within the biphasic region, consisting of 20 wt% IL and 15 wt% potassium carbonate, was selected for further investigation. ABSs with this composition were prepared and evaluated for the extraction of E133 dye. The obtained parameters are presented in Figure 1.

The obtained results showed that E133 dye migrated almost completely into the IL-rich phase, indicating a strong affinity of the dye toward the IL phase. This behavior can be attributed to the presence of specific interactions between the dye molecules and ILs. Consequently, high extraction efficiencies of 99.48% and 99.55% were achieved, with corresponding distribution coefficients (log K) of 2.67 and 2.64 for the ABSs containing [TBP][Sal] and [TBA][Sal], respectively.

CONCLUSION

Salicylate-based IL-ABSs combined with K_2CO_3 demonstrated excellent capability for the extraction of E133. Both investigated systems enabled efficient phase separation and almost complete dye transfer into the IL-rich phase, achieving extraction efficiencies above 99%. The higher hydrophobicity of [TBP][Sal] resulted in a slightly enhanced salting-out tendency compared with [TBA][Sal], highlighting the role of IL cation structure in tuning ABS performance. These results confirm the potential of salicylate-based IL-ABSs as efficient and sustainable platforms for the extraction of food colorants.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number: 451-03-33/2026-03/ 200017).

REFERENCES

- [1] T. Deuchande, J.F. Fundo, M.E. Pintado, A.L. Amaro, Painting the picture of food colouring agents: Near-ubiquitous molecules of everyday life – A review, *Trends in Food Science & Technology*, 2024, 143.
- [2] J.H. Santos, F.A. e Silva, S.P.M. Ventura, J.A.P. Coutinho, R.L. de Souza, C.M.F. Soares, Á.S. Lima, Ionic liquid-based aqueous biphasic systems as a versatile tool for the recovery of antioxidant compounds, *Biotechnology Progress*, 2015, 31, 70–77.
- [3] J. Mušović, D. Tekić, S. Marić, A. Jocić, D. Stanković, A. Dimitrijević, Sustainable recovery of cobalt and lithium from lithium-ion battery cathode material by combining sulfate leachates and aqueous biphasic systems based on tetrabutylphosphonium-ionic liquids, *Separation and Purification Technology*, 2024, 348, 127707.

COMPUTATIONAL BIOMIMETIC PROFILING OF MELATONIN'S EFFECTS IN WINE AGING

D. Obradović¹, B. Lončarević², T. Mitrović¹, N. Todorović Vukotić³, I. Perić³,
A. Stanisavljević Ilić³ and N. Đorđević³

¹*University of Belgrade, Institute of Physics Belgrade – National Institute of the Republic of Serbia, Pregrevica 118, 11080 Belgrade, Serbia (darija@ipb.ac.rs)*

²*University of Belgrade, Institute of Chemistry, Technology and Metallurgy – National Institute of the Republic of Serbia, Njegoševa 12, 11000 Belgrade, Serbia*

³*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.34

ABSTRACT

Melatonin is increasingly recognized as a bioactive compound that improves wine quality and stability in aging. This study combined experimental evaluation of melatonin-enriched wines with computational biomimetic profiling to investigate the underlying mechanisms. Wines supplemented with 0.05, 0.1, and 0.5 mg/kg melatonin were monitored over 6 months using the Folin–Ciocalteu index and spectrophotometric measurements at 420, 520, and 620 nm. Density functional theory (DFT), Abraham linear solvation energy relationship (LSER) descriptors, and PerMM membrane simulations characterized the electronic, intermolecular, and membrane-interaction properties of melatonin. DFT revealed higher electronic flexibility and electron-donating ability than gallic acid, while LSER and PerMM indicated favorable solvation, amphiphilic partitioning, and molecular recognition. These findings matched experimental results showing that 0.05 mg/kg melatonin best preserved phenolic compounds and wine color during aging.

INTRODUCTION

Wine is a complex hydroalcoholic system in which the stability of phenolic compounds determines antioxidant capacity, color, and sensory quality during aging. Oxidative reactions progressively modify phenolics and anthocyanin-derived pigments, leading to quality deterioration. Melatonin, an endogenous indoleamine naturally present in grapes and wines, has attracted attention because of its antioxidant and redox-regulating properties, and its supplementation has been shown to improve the preservation of phenolic compounds and wine color [1]. However, the molecular basis of these effects

remains unclear. Biomimetic computational approaches offer a useful framework for investigating these mechanisms by reproducing key physicochemical interactions in hydroalcoholic environments [2]. In this study, DFT, Abraham LSER descriptors, and PerMM membrane simulations were combined with experimental measurements of the Folin–Ciocalteu index and spectrophotometric color parameters (A420, A520, and A620) during six months of wine aging. Gallic acid was selected as a reference compound because it represents a characteristic wine phenolic with well-established antioxidant properties. Its inclusion provides a chemically relevant benchmark for comparing the electronic reactivity and intermolecular interaction properties of melatonin with those of a typical phenolic antioxidant present in the wine matrix. We hypothesized that the preservation of phenolic compounds and wine color is governed by the biomimetic molecular properties of melatonin, which can be characterized using these complementary computational approaches.

EXPERIMENTAL

Melatonin was supplemented at concentrations of 0.05, 0.1, and 0.5 mg/kg before vinification, and the wines were then produced and aged under controlled conditions for six months. Wine quality during aging was assessed using the Folin–Ciocalteu index and absorbance measurements at 420, 520, and 620 nm, corresponding to the yellow, red, and blue color components, respectively, as determined using an OenoFoss instrument. These experimental data were compared with computational biomimetic predictions. The HOMO and LUMO energies of melatonin and gallic acid were calculated using the ORCA quantum chemistry package. Geometry optimization and vibrational frequency calculations were performed at the B3LYP/def2-SVP level of theory. Frontier molecular orbital energies and the corresponding global chemical reactivity descriptors are presented in Table 1.

Table 1. HOMO–LUMO energies and derived global chemical reactivity descriptors for melatonin and gallic acid.

Analyte	EHOMO	ELUMO	ΔE	IP	EA	η	S	M	X	ω
Melatonin (eV)	−5.229	−0.488	4.742	5.229	0.488	2.371	0.211 eV ^{−1}	−2.858	2.858	1.723
Gallic acid (eV)	−6.228	−1.147	5.081	6.228	1.147	2.54	0.197 eV ^{−1}	−3.688	3.688	2.677

* $\Delta E = ELUMO - EHOMO$; $IP = -EHOMO$; $EA = -ELUMO$; $\eta = (IP - EA)/2$; $S = 1/(2\eta)$; $M = -(IP + EA)/2$; $X = (IP + EA)/2$; $\omega = \mu^2/(2\eta)$

Abraham solute descriptors were obtained from the Reaction Mechanism Generator database. The calculated descriptors included excess molar refraction (E), dipolarity/polarizability (S), hydrogen-bond acidity (A), hydrogen-bond basicity (B), gas-to-hexadecane partition descriptor (L), and McGowan characteristic volume (V). Ethanol was used to represent the alcoholic phase of

wine. Solvation free energy, enthalpy, and entropy at 298 K were also predicted, and the results are summarized in Table 2.

Table 2. Abraham LSER solute descriptors and predicted solvation thermodynamic parameters of gallic acid and melatonin in ethanol.

Compound	Solvent	E	S	A	B	L	V	ΔG_{solv} (kcal/mol)	ΔH_{solv} (kcal/mol)	ΔS_{solv} (kcal/mol·K)
Gallic acid	Ethanol	1.2777	1.5235	1.5286	0.7882	6.6293	1.1078	−18.45	−35.00	-5.55×10^{-2}
Melatonin	Ethanol	1.5838	2.3114	0.8311	1.2815	9.8516	1.8251	−20.12	−34.07	-4.68×10^{-2}

The membrane interaction profile of melatonin was evaluated using the PerMM computational platform. Simulations were performed at 298 K and pH 7.4 using the Drag method and a dioleoylphosphatidylcholine membrane model. The molecular orientation was defined using atoms C1, C2, O1, and N1. The resulting transmembrane free-energy profile was used to estimate the membrane-binding free energy (ΔG_{bind}). Permeability coefficients were additionally predicted for black lipid membrane, blood–brain barrier, Caco-2, PAMPA-DS, and plasma membrane models.

RESULTS AND DISCUSSION

Each computational approach addressed a specific aspect of biomimetic molecular behavior. DFT characterized the electronic reactivity of the molecules, Abraham LSER descriptors described intermolecular interactions within the hydroalcoholic wine matrix, and PerMM evaluated amphiphilic membrane-like interactions. Together, these methods provided a molecular basis for interpreting the experimental preservation of phenolic compounds and wine color. DFT calculations showed that melatonin had a higher HOMO energy (−5.229 eV) than gallic acid (−6.228 eV) together with a smaller HOMO–LUMO energy gap (4.742 vs. 5.081 eV), indicating greater electron-donating ability and higher electronic flexibility. In contrast, gallic acid exhibited higher ionization potential, electronegativity, and electrophilicity, consistent with a more electronically stable structure. These results suggest that melatonin readily participates in reversible electron-transfer processes, thereby contributing to the maintenance of a favorable redox environment. The Abraham LSER descriptors indicated favorable molecular recognition of melatonin within the hydroalcoholic wine matrix. Compared with gallic acid, melatonin exhibited higher excess molar refraction ($E = 1.5838$), dipolarity/polarizability ($S = 2.3114$), gas-to-hexadecane partition descriptor ($L = 9.8516$), and McGowan volume ($V = 1.8251$), whereas gallic acid showed greater hydrogen-bond acidity and melatonin higher hydrogen-bond basicity. Melatonin also displayed a more favorable solvation free energy in ethanol ($\Delta G_{\text{solv}} = -20.12$ kcal/mol vs. -18.45 kcal/mol), indicating stable incorporation into the hydroalcoholic medium while maintaining reversible non-covalent interactions. PerMM simulations further

showed that melatonin exhibits moderate membrane affinity, with a membrane-binding free energy of -2.02 kcal/mol and preferential localization at the membrane interface ($Z \approx 14$ Å). The predicted permeability coefficients for the BLM, BBB, PAMPA-DS, Caco-2, and plasma membrane models indicate efficient partitioning between aqueous and amphiphilic environments without strong retention within the lipid phase. The computational predictions agreed well with the experimental observations. Wines supplemented with 0.05 mg/kg melatonin consistently exhibited the highest Folin–Ciocalteu index together with the greatest absorbance at 420, 520, and 620 nm during six months of aging, indicating superior preservation of phenolic compounds and wine color. These findings suggest that melatonin acts as a biomimetic molecular modulator by combining favorable electronic properties, intermolecular interactions, and amphiphilic partitioning to stabilize the wine microenvironment. The lack of additional improvement at higher melatonin concentrations indicates that this protective effect depends on an optimal balance of these molecular properties rather than on melatonin concentration alone.

CONCLUSION

This study combined experimental and computational biomimetic approaches to investigate the role of melatonin in wine stabilization during aging. DFT, Abraham LSER, and PerMM analyses demonstrated that melatonin possesses favorable electronic, solvation, and membrane-interaction properties, supporting its function as a biomimetic molecular modulator. These predictions agreed well with the experimental results, with 0.05 mg/kg melatonin providing the greatest preservation of phenolic compounds and wine color. The findings highlight the potential of computational biomimetic profiling as a valuable tool for understanding wine aging and designing more stable functional wines.

Acknowledgement

This work was partially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia through the Institute of Physics Belgrade, National Institute of the Republic of Serbia, and under Grants no. 451-03-33/2026-03/200017 and 451-03-33/2026-03/200026. The authors gratefully acknowledge the winery Tri Tačke (Banatski Brestovac, Serbia) for its support during the vinification process.

REFERENCES

- [1] N. Đorđević, N. Todorović Vukotić, I. Perić, O. Keta, V. Petković, S. B. Pajović, B. Nastasijević, *Antioxidants*, 2025, 14, 504.
- [2] D. Obradović, Ł. Komsta, V. M. Petrović, I. Stojković, S. Lazović, *Microchemical Journal*, 2023, 193, 108967.

OBESITY-INDUCED HYPERLEPTINEMIA CONTRIBUTES TO METABOLIC AND INFLAMMATORY ALTERATIONS IN RAT SERUM

M. Obradović, K. Banjac, S. Zafirović and E.R. Isenović

Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (obradovic@vin.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.35

ABSTRACT

Obesity is associated with elevated levels of leptin in circulation, which is linked to serious metabolic disorders. This research aimed to investigate the link between concentrations of circulating leptin and metabolic, inflammatory, and oxidative stress markers in high-fat (HF) diet-induced obese rats. We found that consuming a HF diet significantly increased concentrations of leptin ($p<0.05$), total cholesterol ($p<0.05$), and interleukin-1 ($p<0.01$), while decreased concentration of glutathione reductase, but not significantly, in the serum of rats. These findings indicate that hyperleptinemia induced by the HF diet contributes to metabolic and inflammatory disturbance in obese rats.

INTRODUCTION

Obesity is a chronic disorder that contributes to the development of cardiovascular diseases [1]. One common characteristic of obesity is elevated leptin levels in circulation, which lead to leptin resistance [2]. Primarily, leptin resistance is defined as a condition in which leptin fails to suppress appetite and stimulate energy expenditure [3]. However, given leptin's diverse roles, impaired leptin signaling leads to serious disturbances in metabolism, redox balance, neuroendocrine function, and the immune response [2]. In addition, hyperleptinemia is associated with endothelial dysfunction, vascular inflammation, and arterial stiffness [4]. Taken together, obesity accompanied by hyperleptinemia contributes to cardiometabolic dysfunction [3]. This research aimed to investigate the link between levels of circulating leptin and metabolic, inflammatory, and oxidative stress markers in high-fat (HF) diet-induced obese rats.

EXPERIMENTAL

For all experiments, adult male Wistar rats were used. The Ethical Committee approved all experiments for Experimental Animals at the Vinca Institute (Veterinary Directorate – No. 323-07-02710/2017-05). Animals were housed

under standard laboratory conditions in cages with controlled temperature (22 ± 2 °C), a 12-hour light/dark cycle, and *ad libitum* access to water and food for 12 weeks, and split into two groups: the control group (CONT), which received a standard laboratory diet, and the HF group (HF), which received a HF diet that was enriched with 42% sunflower oil. Blood was collected from all animals, the serum was separated, and the samples were stored appropriately until further analysis. Leptin concentration in rat serum was measured using an ELISA kit, as recommended by the manufacturer. For leptin concentration, a standard curve was used to calculate concentrations, which were expressed as pg/ml. Total cholesterol concentration in rat serum was measured using an automated analyzer, and results were expressed as mmol/l. Using an ELISA kit, interleukin-1 (IL-1) concentration in rat serum were determined following the manufacturer's instructions. Concentration of IL-1 was determined from the standard curve, and results were expressed as pg/ml. An ELISA Kit was used to measure the glutathione reductase (GR) concentration in rat serum, as recommended by the manufacturer. The concentration of GR was determined from the standard curve, and results were expressed as pg/ml. Data are presented as mean $\pm$ SEM, and a two-tailed Student's t-test was used to assess statistical significance. A p-value <0.05 was considered significant. SPSS for Windows (SPSS, Chicago, IL, USA) was used for statistical analyses.

RESULTS AND DISCUSSION

Chronic overnutrition stimulates adipose tissue to produce large amounts of leptin, thereby suppressing hunger and promoting satiety. However, over time, the brain fails to recognize elevated leptin concentrations, which, in turn, leads to leptin resistance.

We found that consuming a HF diet significantly increased serum leptin concentration in rats, compared to values in rats fed a standard diet (Figure 1A). Our findings are in line with the results of other authors, who showed that the HF diet leads not only to hyperleptinemia but also to various cardiovascular disorders [5]. Hyperleptinemia is strongly associated with dyslipidemia, a condition characterized by abnormalities in lipid metabolism, including elevated blood cholesterol levels [5]. Our results showed that the concentration of total cholesterol ($p<0.05$) was higher in the serum of HF diet-fed rats than in control rats (Figure 1B). Obesity-induced hyperleptinemia contributes to low-grade inflammation by stimulating the production of pro-inflammatory cytokines [6].

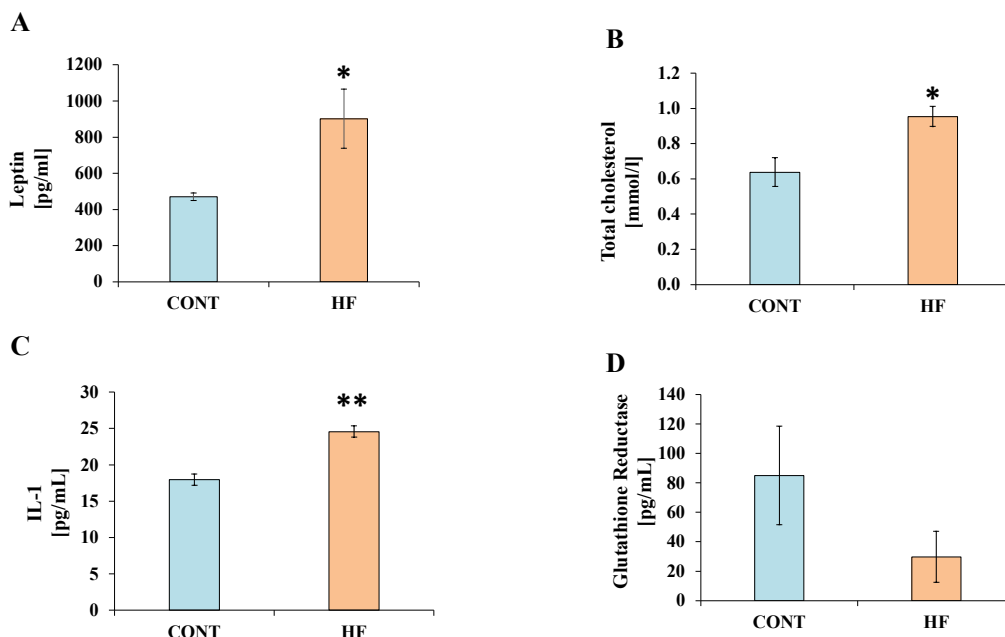


Figure 1. The concentrations of leptin, total cholesterol, IL-1, and GR in the serum of control and HF rats. GR – glutathione reductase, HF – high-fat

In addition, IL-1 may represent one of the inflammatory mediators through which hyperleptinemia contributes to metabolic and inflammatory dysfunction associated with obesity [7]. We found that the concentration of IL-1 ($p < 0.01$) was increased in the serum of HF diet-fed rats compared with the values obtained for the same parameter in control rats (Figure 1C). High circulating leptin concentration are also linked with oxidative stress and decreased antioxidant defense mechanisms [8]. Our results showed that the serum GR concentration decreased in rats fed with a HF diet compared with control rats, but the difference was not significant (Figure 1D).

CONCLUSION

The results indicate that a HF diet adversely affects the concentrations of leptin, total cholesterol, and IL-1 in the serum of obese rats. These findings suggest that hyperleptinemia induced by the HF diet contributes to metabolic and inflammatory disturbance in obese rats.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No# 451-03-33/2026-03/200017).

REFERENCES

- [1] S. Zafirovic, M. Obradovic, K. Banjac, E. Sudar-Milovanovic, A. Pajcin, J. Radovanovic, E.R. Isenovic, *Frontiers in Endocrinology (Lausanne)*, 2025, 16, 1716392.
- [2] K. Stefanakis, J. Upadhyay, A. Ramirez-Cisneros, N. Patel, A. Sahai, C.S. Mantzoros, *Metabolism - Clinical and Experimental*, 2024, 161:156056.
- [3] M. Obradovic, S. Khodahemmati, S. Zafirovic, E.R. Isenovic, *EXCLI Journal*, 2026, 25, 640-653.
- [4] M.S. Poetsch, A. Strano, K. Guan, *Frontiers in Endocrinology*, 2020, 11, 354.
- [5] L. Yan, B.M. Rust, D.G. Palmer, *Frontiers in Nutrition*, 2024, 11, 1340735.
- [6] T.V. Kirichenko, Y.V. Markina, *International Journal of Molecular Sciences*, 2022, 23, 14982.
- [7] D. Flores Gomez, S. Bekkering, R. ter Horst, B. Cossins, I.C.L. van den Munckhof, J.H.W. Rutten, L.A.B. Joosten, M.G. Netea, N.P. Riksen, *Journal of Leukocyte Biology*, 2024, 115, 374-384.
- [8] G. Pandey, M.S. Shihabudeen, H.P. David, E. Thirumurugan, K. Thirumurugan, *The Journal of Diabetes and Metabolic Disorders*, 2015, 14, 24.

PHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF SUB-CHRONIC LONG-CHAIN PFAS EXPOSURE IN MALE WISTAR RATS

I. Perić¹, A. Stanisavljević Ilić¹, N. Todorović Vukotić¹, N. Đorđević¹, S. Pejić¹, M. Pantelić¹ and V. Beškoski²

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (ivanap@vin.bg.ac.rs)*

²*Faculty of Chemistry, Department of Biochemistry, University of Belgrade, Studentski trg 12-16, 11 158 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.36

ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants associated with adverse health effects. However, differences in biological effects of individual PFAS compounds remain insufficiently characterized. This study compared physiological and behavioral effects of two structurally similar long-chain PFAS, perfluorooctanoic acid (PFOA) containing an 8-carbon backbone and perfluorononanoic acid (PFNA) containing a 9-carbon backbone, following 4 weeks of exposure to PFOA or PFNA (2.5 mg/kg/day) via drinking water in male Wistar rats. PFNA exposure resulted in reduced body mass progression compared with control and PFOA groups, accompanied by increased sucrose preference, fluid intake, and immobility. These findings demonstrate that an extra carbon-fluorine (CF₂) group between closely related PFAS compounds may result in markedly different physiological and behavioral outcomes, emphasizing the importance of compound-specific assessment of PFAS biological effects.

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) comprise a large group of more than 14 000 synthetic chemicals widely used in industrial processes and consumer products. Due to their exceptional chemical stability and extremely slow degradation, PFAS are commonly referred to as “forever chemicals”. PFAS are widely detected in surface waters, groundwater, soils, sediments, wildlife, and human biological samples [1]. PFAS contamination has also been documented in Serbian rivers and sediments [2,3]. Growing evidence on the health impacts of individual and mixed PFAS exposure highlights the need to better understand compound-specific biological effects.

Structurally, PFAS consist of fluorinated carbon chains characterized by strong carbon-fluorine bonds, contributing to their exceptional chemical stability and environmental persistence. Long-chain PFAS generally exhibit greater bioaccumulation and longer biological half-lives than short-chain compounds [4]. As persistent and bioaccumulative pollutants, PFAS have been associated with a broad spectrum of adverse health outcomes, including endocrine disruption, reproductive toxicity, organ toxicity, metabolic disturbances, immune dysfunction, and cancer risk [5]. Although PFAS share common structural characteristics, growing evidence suggests that individual compounds differ considerably in their toxicological profiles. Increasing evidence indicates that several PFAS can cross the blood-brain barrier and interfere with neurotransmission, neurodevelopment, and neuroplasticity, potentially contributing to neurobehavioral alterations and affective disturbances [6]. However, behavioral consequences of PFAS exposure remain insufficiently characterized, and direct comparisons between structurally similar compounds are scarce. Perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) are widely distributed long-chain PFAS differing by only an extra carbon-fluorine (CF₂) group that makes PFNA a slightly larger molecule. Nevertheless, this subtle structural difference may substantially influence their toxicokinetic properties, tissue accumulation, and biological activity. To our knowledge, this represents the first direct comparison of physiological and behavioral effects of PFOA and PFNA following sub-chronic exposure in male Wistar rats.

EXPERIMENTAL

One-month-old male Wistar rats were used in this study. All experimental procedures were approved by the Ethical Committee for the use of laboratory animals of the “VINČA” Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade. The study protocol was further authorized by the Ministry of Agriculture, Forestry and Water Management-Veterinary Directorate, Ethics Committee, under license no. 003912767/2025. Animals were housed in groups of 3-4 per cage under standard laboratory conditions, with food and water available *ad libitum*. Rats were divided into three groups: Control (n=13), PFOA-exposed (n=14), and PFNA-exposed (n=13). Both PFAS were administered via drinking water for four weeks at an estimated daily dose of 2.5 mg/kg. Fresh solutions were prepared daily. Body mass was monitored weekly, and dose adjustments were performed based on the average daily fluid intake of 15 mL/kg.

Behavioral testing was performed at the onset of the experiment and at the end of the fourth week of exposure. Anhedonia-like behavior was assessed using the sucrose preference (SP) test. Animals were presented with two bottles for 1 h, one containing tap water and the other containing 2% sucrose solution. The SP was calculated as the ratio of sucrose solution intake to total fluid intake and expressed as a percentage. In addition, total fluid intake during the testing period

was used to assess treatment-related changes in consummatory behavior. Since bottle exposure lasted only 1 h, these measurements reflected acute fluid consumption during testing rather than total daily fluid intake. Both SP and liquid intake measurements were performed in triplicate during each session, and average values were used for statistical analysis. Despair-like behavior was assessed using the forced swimming test (FST). During the test, animals were placed individually for 5 min in transparent plexiglass cylinders (Ø 30 cm, height 45 cm) filled with water to a depth of 30 cm. Animal behavior was recorded and analyzed by a researcher blinded to the experimental conditions. After confirmation of normality and variance homogeneity, repeated-measures ANOVA was performed, followed by Tukey's post hoc test.

RESULTS AND DISCUSSION

In toxicological studies, changes in body mass serve as an important non-specific indicator of systemic toxicity, reflecting disrupted metabolic homeostasis and potential target-organ damage resulting from toxicant exposure. Repeated-measures ANOVA revealed significant treatment-dependent alterations in body mass progression, with PFNA-exposed animals displaying reduced body mass changes from week 3 compared with controls and from week 2 compared with PFOA-exposed animals (Fig. 1). Our finding that a 4-week administration of PFNA resulted in reduced weight gain aligns with the growing consensus that PFAS may disrupt metabolic regulation and energy balance. While overt weight loss in animal models typically signifies acute systemic toxicity, human epidemiological data suggest these changes are rooted in profound metabolic suppression [7]. The disrupted weight trajectories observed in PFNA-exposed animals provide additional evidence of PFNA-associated alterations in physiological adaptation.

Neurobiological alterations can influence the ability of animals to feel pleasure, hence, evaluating the level of SP can be taken as a measure of neurobiological changes in animals. Since SP can be influenced by motivational, metabolic, and hedonic factors, these findings should not be interpreted exclusively as antidepressant-like effects. Animals exposed to PFNA exhibited a marked alteration in consummatory behavior (Fig. 2), characterized by a significant increase in both SP and total liquid intake compared to control and PFOA-exposed groups.

Baseline values were comparable among groups, whereas PFNA-exposed rats maintained fluid intake after 4 weeks, in contrast to the decline observed in controls and PFOA-treated animals. A significant time $\times$ treatment interaction was observed for total fluid intake during the SP test, while post hoc analysis revealed that PFNA-exposed animals maintained significantly higher intake values at week 4 compared with Control and PFOA groups. Maintenance of fluid intake together with elevated SP in PFNA-exposed animals may suggest altered reward-related or consummatory behavior. The elevated hedonic consumption

was strictly decoupled from mass accumulation, as PFNA-treated animals concurrently demonstrated reduced weight gain.

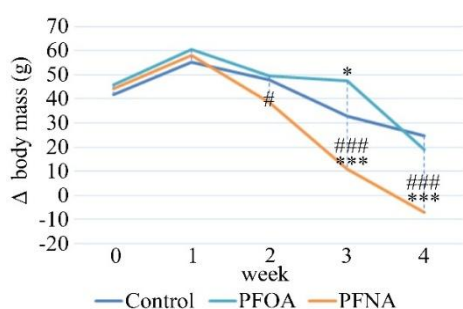


Figure 1. Weekly body mass changes (Δ) during the 4-week exposure to PFOA or PFNA. Asterisks indicate differences between PFAS-treated and control groups, whereas hashtags indicate differences between the PFOA and PFNA groups (* $p < 0.05$, *** $p < 0.001$, # $p < 0.05$, ### $p < 0.001$) at the corresponding time point.

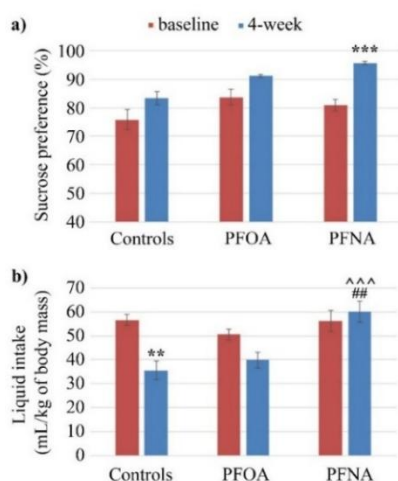


Figure 2. Effects of PFOA and PFNA exposure on (a) sucrose preference and (b) total fluid intake at baseline and after 4 weeks. Asterisks indicate within-group differences from baseline; hashtags indicate differences between PFOA and PFNA groups; carets indicate differences between PFAS-treated and control groups (** $p < 0.01$, *** $p < 0.001$, ## $p < 0.01$, ^^^ $p < 0.001$).

Increased immobility is a measure of despair-like behavior in animals and has been adopted as a measure of depressive-like behavior in translational studies. Four weeks of exposure to PFNA has resulted in increased immobility with a simultaneous decline in swimming behavior (Fig. 3). No significance in behavioral changes in FST was observed after 4-week exposure to PFOA. Thus, FST showed stronger PFNA-associated effects in neurobehavioral alterations compared to PFOA, which may be related to its capacity to induce severe neurobehavioral stress. Together with altered SP, increased immobility suggests that PFNA exposure affects multiple behavioral domains, including reward-related and stress-coping behaviors.

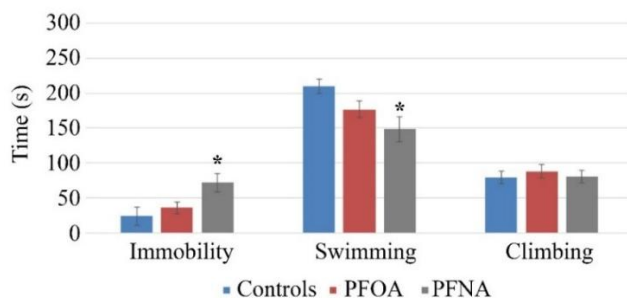


Figure 3. Immobility, swimming, and climbing behaviors following PFAS exposure. Asterisks indicate statistically significant differences between PFAS-exposed and control group (* $p < 0.05$).

CONCLUSION

Comparative analysis of 4-week exposure revealed stronger physiological and neurobehavioral effects of PFNA than PFOA. PFNA exposure resulted in reduced body mass progression, increased SP, elevated fluid intake during SP testing, and increased despair-like behavior, whereas PFOA did not produce comparable alterations in the assessed parameters. These findings indicate that a single-carbon structural difference may substantially influence PFAS biological effects, emphasizing the importance of compound-specific assessment of PFAS toxicity. Further molecular and metabolic studies are warranted to elucidate the mechanisms underlying these differences.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants no. 451-03-33/2026-03/ 200017).

REFERENCES

- [1] F. Ferretti, A. Barbarossa, A. Bardhi, *Environmental Science and Pollution Research*, 2026, 33, 1461–1489.
- [2] V.P. Beškoski, S. Takemine, T. Nakano, L. Slavković Beškoski, G. Gojgić-Cvijović, M. Ilić, S. Miletić, M.M. Vrvic, *Chemosphere*, 2013, 91, 1408–1415.
- [3] I. Antić, M. Buljovčić, R.E. Cochran, J. Živančev, M. Llorca, M. Farré, D. Rakić, R. Tautenhahn, N. Đurišić-Mladenović, *Toxics*, 2026, 14, 78.
- [4] N.K. Khalid, S. Sauvé, *npj Emerging Contaminants*, 2026, 2, 1–18.
- [5] M. Papathanasiou, H. Tzoupis, K.D. Papavasileiou, F. Dondero, D. Greco, I. Lynch, A. Afantitis, G. Melagraki, *Environmental Science: Advances*, 2026, 5, 1527–1555.
- [6] M.Y. Ko, E. Min, S.A. Hyun, M. Ka, *Behavioural Brain Research*, 2026, 514, 116348.
- [7] G. Liu, K. Dhana, J.D. Furtado, J. Rood, G. Zong, L. Liang, L. Qi, G.A. Bray, L. DeJonge, B. Coull, P. Grandjean, Q. Sun, *PLoS Medicine*, 2018, 15, e1002502.

PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF FRUCTANS ISOLATED FROM SHATAVARI AND SUMA BY MICROWAVE-ASSISTED EXTRACTION

N. Petkova¹, D. Yaneva² and D. Vassilev²

¹University of Food Technologies, 26, Maritza Blvd., 4002, Plovdiv, Bulgaria
(nadezhda_petkova@uft-plovdiv.bg)

² Technical University of Gabrovo, 4 Hadji Dimitar Str., 5300 Gabrovo, Bulgaria

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.37

ABSTRACT

The aim of the research was to characterise fructan isolated from roots of shatavari (*Asparagus racemosus* Willd.) and suma (*Pfaffia paniculata*) using microwave-assisted extraction. Its physico-chemical properties and functional properties (swelling, water and oil holding capacities, flowability and cohesiveness) were evaluated. The structure of inulin-type fructans was confirmed by FTIR spectroscopy, as the presence of 2-ketose and β (2 $\rightarrow$ 1) bonds were found. Shatavari fructan was characterized with low degree of polymerization 8 and molecular mass of 1.3 kDa, while suma polysaccharide is high-molecular fructan with degree of polymerization 24. The isolated fructans showed better oil-holding capacity than water-holding one, but low swelling properties. The research was the first detailed study for elucidation of structure and functional properties of inulin from shatavari and suma roots that reveal their potential for application in food, pharmaceutical and cosmetic purposes.

INTRODUCTION

Shatavari (*Asparagus racemosus*), is a medicinal plant from India and the Himalayas from Liliaceae family [1]. It was reported 26.7% yield of 2 $\rightarrow$ 1 linked fructo-oligosaccharides with degree of polymerization (DP) of nearly 7–8 in *Asparagus racemosus* roots with immunomodulatory activity [2].

Pfaffia glomerata (Spreng.) Pedersen, known also as suma or “Brazilian ginseng” is a medicinal herb from the Amaranthaceae family. It is distributed in Guyana, Bolivia, Argentina and Brazil [3,4]. Some authors isolated inulin with degree of polymerization (DP) of 13-15.33 [3, 4] with prebiotic activity [3]. Therefore, these two plants deserve attention for isolation of biologically active fructans. However, the information about carbohydrate composition is still insufficient and the functional properties were not evaluated well. This provokes the aim of the current research that is connected with fructan isolated from roots

of shatavari and suma using microwave-assisted extraction and evaluation of their physicochemical properties and functional properties.

EXPERIMENTAL

The organic shatavari root flour and suma root flour were purchased and obtained from FutuNatura (Slovenia). The isolation of shatavari and suma fructans was performed by boiling hot water microwave assisted extraction for 5 min [5]. The physicochemical properties were characterized [6]. The FTIR spectra were recorded on Jasco X6 (JASCO, Japan) spectrometer in the wavelength range of 4000–400 cm^{-1} , 132 scans at a resolution of 2 cm^{-1} . Color measurement was done by a portable colorimeter Model WR-10QC D 65 lighting [5]. Functional properties of fructans were evaluated [5]. True, bulk, and tapped densities, angle of repose, flowability and wettability were determined [6].

RESULTS AND DISCUSSION

The structure of shatavari and suma fructans was confirmed by FTIR spectroscopy (Fig. 1).

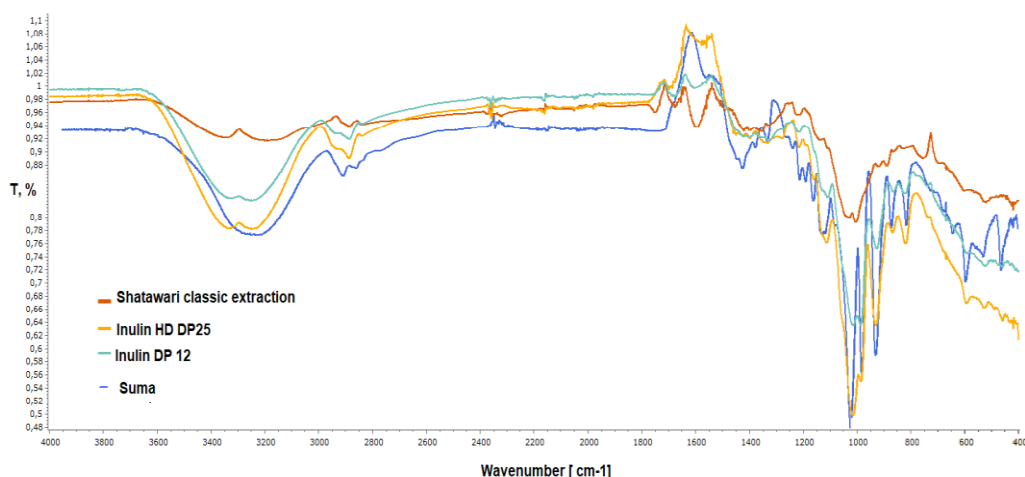


Figure 1. FTIR spectra of chicory inulin, shatavari and suma fructans.

FTIR spectra showed all typical bands for inulin-type fructans [5,6], as their bands were compared with chicory inulin standards (Frutafit HP with DP 9-12 and Raftiline HPX DP=25). The band at 3330 cm^{-1} was typical for OH groups, The bands at 1014 cm^{-1} were typical for C–O stretching vibration. The bands at 930-932 cm^{-1} showed α -D-glucopyranosyl residue, while the band at 873 cm^{-1} was due to 2-ketofuranose and β -anomer bendings in C1–H. The bands at 817-819 cm^{-1} confirmed the presence of 2-ketose in pyranosyl or furanosyl ring.

The results for the physicochemical characteristics of fructans isolated by microwave extraction were presented (Table 1). The yield of the suma

polysaccharide was low, with a low fructose content (17%) and inulin purity (10%), but it exhibited a high molecular mass and a degree of polymerization of 24 (Tab. 1). Our results were lower than the previously reported yield of 11.45 % obtained by classical hot-water extraction followed by ethanol precipitation [3]. However, the degree of polymerization of inulin from suma was higher than in some reports [3, 4]. Moreover, the colour characteristics of suma fructan were reported for the first time in this study, whereas those of shatavari were comparable to previously reported values [5]. However, shatavari fructan showed a better yield 6.8% with inulin purity of 62% and a high fructose content of 68%. The degree of polymerization is 8, and the obtained inulin from *Asparagus racemosus* can be evaluated as a low-molecular-mass fructan, part of fructooligosacchrides with a molecular mass 1375 Da. Therefore, our data on shatavari inulin were close to the reports in the literature [1, 5].

Table 1. Physicochemical characteristics of fructans isolated from shatavari and suma roots by microwave-assisted extraction

Characteristics	Shatavari inulin	Suma fructan	Inulin Frutafit HD DP 8-12
Yield, %	6.8±0.4	1.2±0.2	-
Purity, %	62.0±0.6	10.1±0.5	82.5±0.3
Fructose content, %	68.6±0.9	17.5±0.2	75.0±0.4
DP by HPLC-SEC	8	24	12
Mw, Da	1375	3951	2185
Mn, Da	1340	3757	2105
Polydispersity index	1.03	1.05	1.04
Color characteristics			
L	54.03 ± 1.50	72.81 ± 1.22	100.00±0.00
a	11.62 ± 0.47	8.27 ± 0.06	0.08±0.04
b	26.85 ± 0.91	18.11 ± 0.16	6.79±0.10
C	29.25± 0.52	19.91± 0.12	6.79±0.10
h	66.60± 0.41	65.45± 0.11	92.29±5.16

Legend: Mw- weight molecular mass, Mn – Number molecular mass, L- lightness, a – redness, b- yellowness, C- chroma, h – hue

The functional properties were summarized in Table 2. Both isolated inulin demonstrated with low swelling properties, but high oil-holding capacity, good water holding capacity and hygroscopicity. Wetting properties were close to those of the chicory inulin reference. Because of the low yield, some functional properties of suma fructan could not be determine. However, this was the first study for evaluation of the functional properties of suma fructan.

Table 2. Functional properties of isolated inulin from shatavari roots

Functional properties	Shatavari inulin	Suma fructan	Inulin Raftiline HD DP 8-12
Swelling, g/cm ³	0.17	0.09	0.74
Water-holding capacity, g water/g	5.52	3.88	0.40
Oil-holding capacity, g oil/g	8.30	8.41	0.40
Bulk density, g/mL	0.40	-	24
Tapped density, g/mL	0.47	-	18.5
True density, g/mL	0.47	-	1.11
Angle of repose, °	11.31	16.16	25.64
Higroscopicity, %	6.92	6.52	3.7
Wettability, s	11.7	11.5	9

CONCLUSION

The current study elucidated the structure, physicochemical and functional properties of inulin-type fructan from shatavari and suma roots. The obtained shatavari inulin was characterised as a low molecular mass fructan with DP 8 and an average yield, while suma fructan was a high molecular inulin. According to the obtained functional properties, shatavari inulin should be used as a taste modifier in food products with a potential prebiotic effect, because of its low molecular weight, while suma can be used as texture modifier.

REFERENCES

- [1] R.S. Singh, R. Dhaliwal, M. Puri, *J. Ind. Microbiol. Biotechnol.*, 2007, 34(10), 649 – 655.
- [2] M. Thakur, P. Connellan, M. Deseo, C. Morris, W. Praznik, R. Loeppert, V.K. Dixit, *Int. J. Biol. Macromol.*, 2012, 50(1), 77.
- [3] E. R. Caleffi, G. Krausová, I. Hyršlová, L. L. R. Paredes, M. M. dos Santos, G. L. Sassaki, R. A. C. Gonçalves, A. J. de Oliveira, *Int. J. Biol. Macromol.*, 2015, 80, 392 – 399.
- [4] S. T. C. Ribeiro da Silva, T. F. O. Castro, J. C. Cabral, M. R. P. de Abreu Filho, B. A. de Oliveira, A. J. B. and R. A. C. Gonçalves, *Bioact. Carbohydr. Diet. Fibre*, 2022, 27, 100303.
- [5] D. Yaneva, I. Hambarlyiska, N. Petkova, I. Ivanov, D. Vassilev *Bulgarian Chem Commun.* 2025, 63, 1–6.
- [6] N. Petkova, A. Petrova, I. Ivanov, I. Hambarlyiska, Y. Tumbarski, I. Dincheva, M. Ognyanov, P. Denev, *ChemEng.*, 2023, 7, 94.

GENOTOXIC EFFECTS OF NEEM SEED OIL ON HUMAN LYMPHOCYTES

A. Bošković¹, A. Leskovac², O. Medić¹, T. Ganić¹, B. Nikolić¹ and S. Petrović²

¹*University of Belgrade, Faculty of Biology, Studentski trg 12-16
11000 Belgrade, Serbia*

²*Vinča Institute of Nuclear Sciences – National Institute of the Republic of
Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade,
Serbia (sandra@vin.bg.ac.rs)*

DOI: FDSQ_WORKSHOP.2026.9.38

ABSTRACT

Neem (*Azadirachta indica*) seed oil is widely used as a biopesticide and in cosmetic formulations, yet data on its genotoxicity remain limited. This study assessed its effects in human peripheral blood lymphocytes using the cytokinesis-block micronucleus assay. A significant, concentration-dependent increase in micronuclei incidence was observed, accompanied by reduced cell proliferation across all tested concentrations. At the highest concentration, pronounced cytotoxicity was observed. These findings highlight the need for further in-depth safety evaluation of neem seed oil and clarification of the roles of its individual constituents in the observed biological effects.

INTRODUCTION

Neem (*Azadirachta indica*) seed oil is widely used in agriculture and cosmetic formulations and has also been incorporated into traditional medicinal and pharmaceutical preparations [1]. As a result of this broad, cross-sectoral application, human exposure to neem-derived products may occur through multiple routes, including dermal contact, inhalation, and oral intake as an auxiliary medical agent. Notably, certain *Azadirachta indica*-derived substances (e.g., neem seed extract) have been identified as carcinogenic, mutagenic, or toxic to reproduction (CMR) and have been prohibited in cosmetic products in the EU since 2023 [2]. Neem seed oil represents a highly complex mixture comprising more than 300 constituents, including fatty acids, triterpenes, flavonoids, saponins, catechins, and limonoids [3]. Given its complex chemical composition and increased use, concerns have arisen regarding human exposure and potential adverse effects, particularly at higher concentrations or with prolonged exposure. Genotoxicity is a critical endpoint in toxicological assessment, yet, despite its wide use, data on the genotoxic potential of neem seed oil remain limited and inconsistent, likely due to differences in composition, formulation, and experimental design. In this study, the genotoxic and cytotoxic

effects of neem seed oil in human peripheral blood lymphocytes were evaluated using the standardized cytokinesis-block micronucleus (CBMN) assay [4]. The findings provide insight into its potential genotoxic risk and contribute to its safety assessment under increasing environmental and human exposure.

EXPERIMENTAL

Cell culture and genotoxicity assessment

Commercial cold-pressed neem (*Azadirachta indica*) seed oil (Eterra, TemaCo d.o.o., Novi Sad, Serbia; origin: India) was used. Stock emulsions were prepared in DMEM (Thermo Fisher Scientific, USA) with Tween 20 (Sigma-Aldrich, France) as the surfactant. Working concentrations were obtained by serial dilution. Peripheral blood samples were collected from a healthy male donor after informed consent, in accordance with protocols approved by the Institutional Ethics Committee (approval no. 116-5-5/2023-000) and relevant ethical guidelines [5]. Heparinized whole blood was cultured in Panserin 701 (Pan-Biotech, Germany) medium. Neem seed oil was tested at concentrations ranging from 0.025 to 1 mg/mL. Untreated cultures served as controls. Tween 20 was used at final concentrations below the cytotoxicity threshold (<0.01%) [6]. Experiments were performed in triplicate. Genotoxicity was assessed using the CBMN assay of Fenech [7], following the OECD 487 Guideline [4]. At least 2000 binucleated cells per sample were analyzed using a light microscope (Optech, Germany) at 400× or 1000× magnification. Cytostatic/cytotoxic effects of neem seed oil were assessed by estimating the cytokinesis-block proliferation index (CBPI) [8], using the formula: $CBPI = [MI + 2MII + 3(MIII + MIV)]/N$, where MI-MIV represent cells with one to four nuclei, and N is the total number of cells scored. At least 500 cells per sample were analyzed [4]. Cytotoxicity (%) was calculated as: $100 - 100[(CBPI_T - 1)/(CBPI_C - 1)]$, where $CBPI_T$ is the CBPI of the test compound, and $CBPI_C$ is the CBPI of the untreated control cells [4]. Statistical analyses were performed using Student's t-test and Pearson and partial correlation analyses in Statistica 8.0 and OriginPro 8.5. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The genotoxic potential of neem seed oil was evaluated in human lymphocytes across a concentration range of 0.025–1 mg/mL. The results (Fig. 1.) show a significant, concentration-dependent increase in micronuclei (MN) incidence. The effect was evident even at the lowest tested concentration and increased progressively; at 1 mg/mL, near-complete inhibition of cell proliferation precluded reliable MN scoring, indicating cytotoxicity beyond the evaluable range (data not shown). CBPI analysis showed a parallel, concentration-dependent reduction in proliferative capacity (Fig. 1.). CBPI decreased progressively up to 0.5 mg/mL, while 1 mg/mL caused ~ 100% inhibition of proliferation (data not shown). Cytotoxicity increased accordingly, ranging from

mild effects at lower concentrations to pronounced cytotoxicity at 0.5 mg/mL ($54.41 \pm 1.47\%$) and near-complete cell cycle arrest at 1 mg/mL (Table 1).

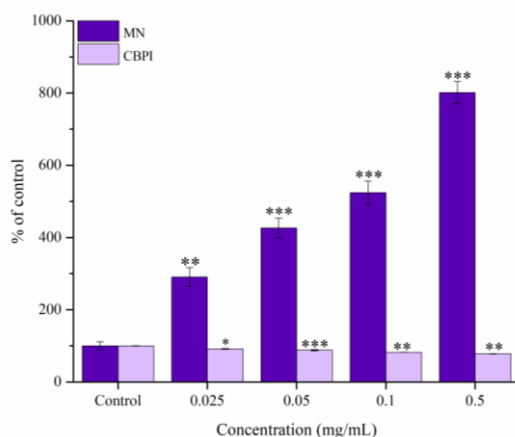


Figure 1. The MN incidence (mean $\pm$ SD) and CBPI (mean $\pm$ SD) in cultured human lymphocytes treated with neem oil, expressed as percentages relative to the control, set at 100%. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control.

A significant inverse correlation between MN incidence and CBPI ($r = -0.96$; $p = 0.0001$) suggests activation of cell cycle checkpoints, particularly at G₂/M, in response to DNA damage. Damaged cells likely undergo arrest, senescence, or apoptosis, thereby reducing proliferation and increasing genomic instability. The observed genotoxic responses align with reports on neem nanoformulations, in which DNA damage has been linked to oxidative stress and ROS generation [9]. Notably, these effects occurred at higher concentrations (1.2–2 mg/mL) than in the present study, suggesting that formulation, bioavailability, and cellular uptake influence toxicity. Evidence for constituents, such as azadirachtin, indicates that genotoxicity may arise from mitotic spindle disruption and chromosome missegregation rather than from direct DNA damage, leading to micronuclei formation [10].

Table 1. Neem seed oil–induced cytotoxicity (%) in human lymphocytes.

	Concentration of neem seed oil (mg/mL)			
	0.025	0.05	0.1	0.5
Cytotoxicity	22.06	26.47	45.59	55.88
(%)	23.53	27.94	44.11	52.94
(mean $\pm$ SD)	17.65	35.29	44.11	54.41
	21.08 $\pm$ 3.06	29.90 $\pm$ 4.72	44.60 $\pm$ 0.85	54.41 $\pm$ 1.47

Collectively, these findings suggest that neem oil–associated genotoxicity may involve both clastogenic and aneugenic mechanisms, likely driven by multiple bioactive constituents rather than a single compound.

CONCLUSION

The present findings indicate that neem seed oil compromises genomic stability and cell cycle progression in a concentration-dependent manner, raising potential toxicological concerns associated with its increasing use and possible human exposure.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract numbers 451-03-33/2026-03/200017 and 451-03-34/2026-03/200178).

REFERENCES

- [1] T. Tufail, H. Bader Ul Ain, A. Ijaz, M.A. Nasir, A. Ikram, S. Noreen, M.T. Arshad, M.A. Abdullahi, *Food Sci Nutr.*, 2025, 13(9), e70820.
- [2] Commission Regulation (EU) 2023/1490 of 19 July 2023 amending Annex II to Regulation (EC) No 1223/2009 on cosmetic products. *Official Journal of the European Union*, 2023.
- [3] V.E.d.S Gomes, P.F.J. Bócoli, J.G. Iamarino, R. Grimaldi, A.P.B. Ribeiro, L. Marangoni Júnior, *Lipidology*, 2025, 2, 9.
- [4] OECD, Test No. 487: In Vitro Mammalian Cell Micronucleus Test, 2023.
- [5] Law on Health Care Official Gazette of the Republic of Serbia, no. 25/2019.
- [6] T. Hua, X. Zhang, B. Tang, C. Chang, G. Liu, L.L. Feng, Y. Yu, D. Zhang, J. Hou, *BMC Vet. Res.*, 2018, 14, 138.
- [7] M. Fenech, *Mutat. Res.*, 1993, 285, 35-44.
- [8] J. Surrallés, N. Xamena, A. Creus, R. Marcos, *Mutat. Res.*, 1995, 342, 43-59.
- [9] J. Jerobin, P. Makwana, R. S. Suresh Kumar, R. Sundaramoorthy, A. Mukherjee, N. Chandrasekaran, *Int. J. Nanomedicine*, 2015, 10, 77–86.
- [10] P. Mosesso, L. Bohm, G. Pepe, M. Fiore, A. Carpinelli, G. Gäde, S. Nagini, A. Ottavianelli, F. Degraffi, *Toxicol. Lett.*, 2012, 213(3), 361-366.

COMPARATIVE *IN SILICO* ASSESSMENT OF DRUG-LIKENESS AND ANTIOXIDANT POTENTIAL OF SELECTED DIETARY PHENOLIC ACIDS

N. Potkonjak¹, R. Karkalić² and V. Anićijević²

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (npotkonjak@vin.bg.ac.rs)*

²*Military Academy, University of Defence in Belgrade, Veljka Lukica Kurjaka 33, 11042 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.39

ABSTRACT

In the present study, five representative dietary phenolic acids, namely p-coumaric acid, vanillic acid, caffeic acid, gallic acid, and chlorogenic acid, were comparatively evaluated using an *in silico* descriptor-based approach. Molecular weight (MW), hydrogen-bond donors (HBD), hydrogen-bond acceptors (HBA), lipophilicity (LogP), topological polar surface area (TPSA), rotatable bonds, and Lipinski Rule-of-Five compliance were analyzed to assess drug-likeness and predicted membrane permeability. Among the investigated compounds, caffeic acid provided the most balanced combination of antioxidant activity and physicochemical properties associated with oral absorption.

INTRODUCTION

Phenolic acids constitute one of the largest classes of naturally occurring phytochemicals and are widely distributed throughout fruits, vegetables, cereals, coffee, tea, medicinal plants, and numerous functional foods. These compounds contribute significantly to the antioxidant capacity of plant-derived products and have been associated with various beneficial biological effects. The *in vivo* effectiveness of phenolic acids depends not only on intrinsic chemical reactivity but also on pharmacokinetic behavior. [1]

Drug-likeness represents a conceptual framework used to assess whether a molecule possesses physicochemical characteristics commonly associated with successful orally active drugs. Among the available approaches, Lipinski's Rule of Five remains one of the most widely applied guidelines in medicinal chemistry. Phenolic acids represent an excellent model system for investigating the relationship between antioxidant activity and drug-likeness. [2]

The objective of the present study was to perform a comparative *in silico* assessment of five representative dietary phenolic acids, and to examine the relationship between antioxidant potential and drug-likeness.

EXPERIMENTAL

The molecular descriptors evaluated were molecular weight, hydrogen-bond donor count, hydrogen-bond acceptor count, octanol-water partition coefficient, topological polar surface area, and number of rotatable bonds. Drug-likeness was assessed according to Lipinski's Rule of Five. The following criteria were applied: $MW \leq 500$ Da, $HBD \leq 5$, $HBA \leq 10$, and $LogP \leq 5$. The number of violations for each compound was determined and interpreted as an indicator of predicted oral drug-likeness. We evaluated relative antioxidant potential based on established structure-activity principles for phenolic compounds. Particular attention was given to the number of phenolic hydroxyl groups, presence of catechol motifs, methoxy substitution, and expected stabilization of phenoxyl radicals following hydrogen or electron donation. [3]

RESULTS AND DISCUSSION

The investigated compounds exhibited substantial differences in molecular size, polarity, and hydrogen-bonding capacity despite sharing common aromatic acid scaffolds. Table 1 summarizes the descriptor values used for comparative analysis. Among the investigated compounds, chlorogenic acid displayed the highest molecular weight and TPSA, indicating substantially greater polarity than the remaining phenolic acids. In contrast, p-coumaric acid exhibited the lowest TPSA and the highest LogP value, suggesting superior predicted passive membrane permeability. Vanillic acid also showed a favorable descriptor profile, reflecting the permeability-supporting effect of methoxy substitution.

Table 1. Physicochemical descriptors and Lipinski parameters of selected phenolic acids.

Compound	MW (Da)	HBD	HBA	LogP	TPSA (A2)	Rotatable bonds	Violations
p-Coumaric acid	164.16	2	2	1.49	57.53	2	0
Vanillic acid	168.15	2	3	1.10	66.76	2	0
Caffeic acid	180.16	3	3	1.20	77.76	2	0
Gallic acid	170.12	4	4	0.50	97.99	1	0
Chlorogenic acid	354.31	6	8	-0.79	164.75	4	1

All compounds except chlorogenic acid fully satisfied Lipinski's criteria. Chlorogenic acid exhibited one violation due to the presence of six hydrogen-bond donor groups. These results suggest that p-coumaric acid, vanillic acid, caffeic acid, and gallic acid occupy physicochemical space generally associated with favorable oral drug-likeness. In contrast, chlorogenic acid may experience reduced passive membrane transport due to its high polarity and hydrogen-bonding capacity.

The antioxidant activity of phenolic acids is strongly influenced by hydroxylation patterns. Gallic acid contains three phenolic hydroxyl groups and therefore exhibits extensive radical stabilization through resonance mechanisms. Caffeic acid contains a catechol moiety that provides efficient electron delocalization following hydrogen donation. Chlorogenic acid retains the antioxidant characteristics of caffeic acid while introducing additional hydroxyl groups through the quinic acid moiety. Conversely, p-coumaric acid contains only a single phenolic hydroxyl group and therefore exhibits lower radical-scavenging potential. Vanillic acid occupies an intermediate position because the methoxy substituent contributes electron density while reducing hydrogen-bond donor capacity. The expected structure-based antioxidant activity follows the qualitative order: gallic acid > caffeic acid > chlorogenic acid > vanillic acid > p-coumaric acid. This ranking should be interpreted as a structural prediction until supplemented with quantitative antioxidant assay data from the literature or new experiments. A notable inverse relationship was observed between antioxidant potential and predicted drug-likeness (Fig. 1). Increasing hydroxylation generally resulted in higher antioxidant potential, increased HBD and HBA values, larger TPSA values, greater polarity, and reduced predicted membrane permeability. This relationship suggests the existence of an antioxidant activity-drug-likeness trade-off in phenolic acids.

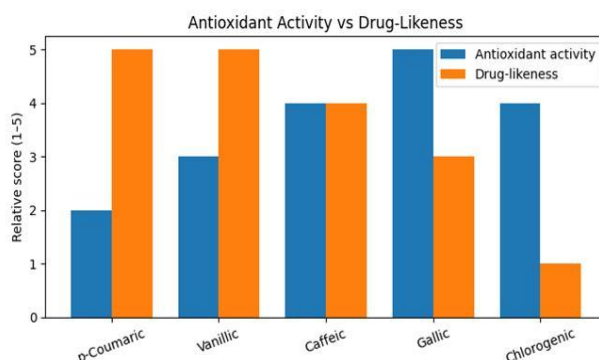


Figure 1. Structure of *p-tert*-butyldihomooxalix[4]arene tetra(diethyl)amide.

Among the investigated compounds, caffeic acid appears to occupy the most favorable position within the antioxidant activity-drug-likeness landscape. The catechol structure confers strong antioxidant activity, while molecular size, TPSA, and hydrogen-bonding characteristics remain within ranges generally associated with favorable oral absorption. This balance distinguishes caffeic acid from highly permeable but less active compounds and from highly active but more polar compounds.

CONCLUSION

The present study provides a comparative in silico assessment of drug-likeness and antioxidant potential among five representative dietary phenolic acids. The results demonstrate that structural modifications influencing antioxidant activity simultaneously affect physicochemical properties associated with oral absorption. A clear antioxidant activity-drug-likeness trade-off was observed. Increased hydroxylation enhanced predicted antioxidant activity but also increased polarity, hydrogen-bonding capacity, and topological polar surface area. These changes may reduce membrane permeability and pharmacokinetic performance. Among the investigated compounds, caffeic acid exhibited the most favorable balance between antioxidant potential and drug-like properties. This finding suggests that caffeic acid occupies an advantageous position within the structure-property landscape of phenolic acids and may represent a promising scaffold for the development of nutraceutical and pharmaceutical agents.

Acknowledgement

This work was partially supported by the Ministry for Science of the Republic of Serbia (Grants no. 451-03-33/2026-03/200017). The author NP would like to thank the Library of the ETH Zürich for providing access to historical materials and resources that supported this research.

REFERENCES

- [1] N. Kumar, N. Goel, *Biotechnology Reports*, 2019, 24, e00370.
- [2] C.-Y. Jia, J.-Y. Li, G.-F. Hao, G.-F. Yang, *Drug Discovery Today*, 2019, 25, 248-258.
- [3] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, *Advanced Drug Delivery Reviews* 1997, 23, 3-25.

CHOLINE CHLORIDE-ASCORBIC ACID DEEP EUTECTIC SOLVENT (DES) ALLEVIATES HYDROXYUREA-INDUCED OXIDATIVE DAMAGE IN TK6 CELLS

K. Radošević, M. Kostić, M. Janić, A. Valenta Šobot, S. Brković and J. Filipović Tričković

Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (ksenija.radosevic@vin.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.40

ABSTRACT

Deep eutectic solvents (DES) are a novel class of green solvents with good biocompatibility; however, their protective potential against various xenobiotics hasn't been explored. Combining choline chloride (ChCl) with the naturally occurring antioxidant, ascorbic acid (AA), could provide desirable biological effects. We investigated the potential protective effect of ChCl and AA formulated DES, ChCl:AA, against hydroxyurea (HU)-induced oxidative damage in human lymphoblastoid TK6 cells. Cell viability (50–300 µg/mL) was assessed using the Trypan blue assay, and antioxidant status was evaluated by measuring reduced glutathione (GSH). ChCl:AA treatment maintained cell viability and increased intracellular GSH. Furthermore, under HU-induced oxidative stress, ChCl:AA pretreatment significantly elevated GSH levels compared to HU treatment alone. These findings suggest that ChCl:AA enhances cellular antioxidant defenses, highlighting its potential for pharmaceutical and food applications.

INTRODUCTION

Deep eutectic solvents (DES) are a class of green solvents with growing potential in pharmaceutical and food industries owing to their ability to improve the solubility and enhance bioavailability of poorly soluble compounds. They consist of two or more components, a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), resulting in a eutectic mixture with a lower melting point compared to the individual components [1]. Choline chloride (ChCl) is one of the most commonly used HBAs, as a naturally occurring quaternary ammonium compound. Ascorbic acid (AA) is a potent water-soluble antioxidant involved in maintaining cellular redox homeostasis. Hence, combining ChCl with AA could form a DES with both good biocompatibility and beneficial antioxidant properties [2]. Hydroxyurea (HU) is a chemotherapeutic agent that can induce oxidative stress through reactive oxygen species (ROS) generation and depletion

of intracellular antioxidant defenses, leading to long-term adverse effects on healthy cells [3]. Therefore, this study aimed to evaluate the potential of choline chloride:ascorbic acid (ChCl:AA) DES (2:1 molar ratio) to mitigate the adverse effects of HU on the TK6 human lymphoblastoid cell line by monitoring cell viability and intracellular reduced glutathione (GSH) levels under HU-induced oxidative stress.

EXPERIMENTAL

TK6 cells were seeded in 24-well plates at a density of 0.05×10^6 cells per well in RPMI 1640 (Capricorn Scientific, Germany) medium supplemented with 10% Fetal Bovine Serum (FBS, Capricorn Scientific, Germany) under standard cell culture conditions, 37°C and 5% CO₂. Cells were treated with ChCl:AA (50, 100, and 300 µg/mL) for 24 h. For co-treatment experiments, cells were pretreated with ChCl:AA for 5 h before exposure to HU (100 µM). Following 24 h of incubation, cell viability was assessed using a Trypan blue exclusion assay according to the standard procedures [4]. Cell viability was expressed as the percentage of viable cells relative to the untreated control. GSH levels were determined using Ellman's method [5]. Absorbance was measured at 412 nm (Sunrise, Tecan microplate reader), and the results are expressed as percentages relative to the untreated control.

All the experiments were performed in triplicate and repeated twice. The results are presented as means $\pm$ standard errors (mean $\pm$ SE). One-way ANOVA from the SPSS software for Windows 10, version 20 (IBM, Armonk, NY, USA) was used to assess the differences between the groups, with significance set at $p < 0.05$.

RESULTS AND DISCUSSION

The cell viability assay demonstrated that after treatment with ChCl:AA at all tested concentrations (50, 100, and 300 µg/mL), cell viability remained within the range of the untreated control, indicating good biocompatibility of the tested DES in the TK6 cellular model (Figure 1A). Determination of GSH showed that ChCl:AA increased GSH levels in a concentration-dependent manner, significantly at 300 µg/mL, indicating enhancement of the cellular antioxidant defense system (Figure 1B).

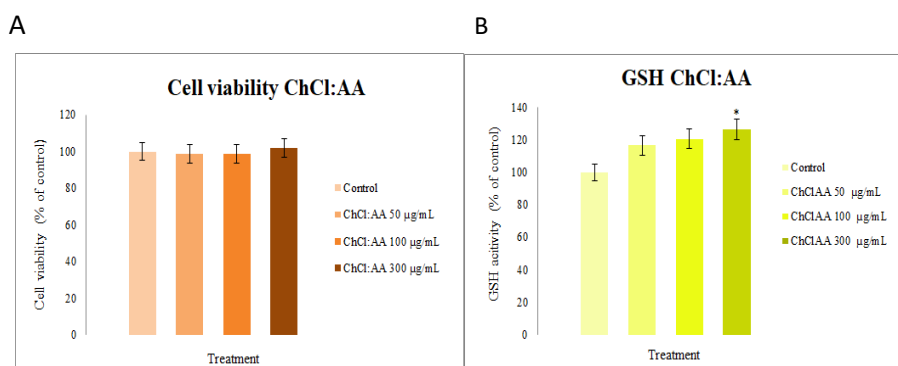


Figure 1. Effects of ChCl:AA on A) cell viability and B) GSH levels. * $p < 0.05$, treatments vs. untreated control.

Consistent with previous findings, treatment with 100 μ M HU exerted a cytotoxic effect (Figure 2A) and induced oxidative stress, seen as decreased intracellular GSH levels (Figure 2B). However, pretreatment with ChCl:AA resulted in a slight increase in the percentage of viable cells compared with HU (Figure 2A). Furthermore, DES pretreatment significantly increased intracellular GSH levels compared with HU-treated cells at all tested concentrations, indicating its promising antioxidant potential (Figure 2B). The observed increase in GSH levels may be attributed to the ascorbic acid in the DES composition, which can directly scavenge ROS and contribute to the maintenance of intracellular redox balance, thereby preserving cellular antioxidant reserves [6].

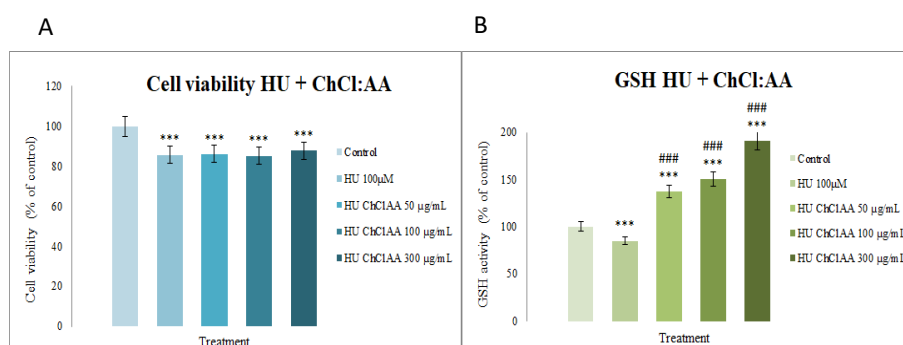


Figure 2. Effects of HU+ ChCl:AA on A) cell viability and B) GSH levels. *** $p < 0.001$, treatments vs. untreated control; ### $p < 0.001$, treatments vs. HU

These results suggest that the enhanced antioxidant defense observed following ChCl:AA treatment becomes more pronounced under oxidative stress.

However, the modest improvement in cell viability may be attributed to the relatively short pretreatment period (5 h), during which the increase in intracellular GSH may not have been sufficient to translate into a significant improvement in cell survival. Nevertheless, the significant increase in GSH levels indicates that ChCl:AA effectively enhances the cellular antioxidant response against HU-induced oxidative stress.

CONCLUSION

Taken together, these findings indicate that ChCl:AA exhibits favorable biological properties, characterized by high cellular compatibility and the ability to contribute to the maintenance of cellular redox status.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant no. 451-03-33/2026-03/ 200017 and 451-03-3627/2025-03/3456).

REFERENCES

- [1] B. M. Popović et al. Cytotoxicity profiling of choline chloride-based natural deep eutectic solvents. *Royal Society of Chemistry Advances*, 2023, 13 (6): 3520–3527.
- [2] M. Janić et al. Sustainable green extraction of selected polyphenols from *Salvia officinalis* L. with choline chloride-based NADES: Phytochemical screening and bioactivity, *Journal of Molecular Liquids*, Vol. 434, 2025.
- [3] S. Kapor, V. Čokić, J. F. Santibanez, Mechanisms of Hydroxyurea-Induced Cellular Senescence: An Oxidative Stress Connection?, *Oxidative Medicine and Cellular Longevity*, 2021, 7753857.
- [4] W. Strober, Trypan Blue Exclusion Test of Cell Viability. *Current protocols in immunology 111*, 2015, A3.b.1-a3.b.
- [5] G.L. Ellman. Tissue sulfhydryl groups, *Archives of Biochemistry and Biophysics*, 1959, 82:70–7.
- [6] A. Gęgotek, E. Skrzydlewska. Ascorbic acid as antioxidant, *Vitamins and Hormones*, 2023,121:247-70.

FLUORIDE SUBACUTE SPLEEN TOXICITY IN WISTAR RATS: BENCHMARK DOSE ANALYSIS FOR DNA DAMAGE AND ESSENTIAL ELEMENTS

J. Radovanović¹, S. Kolarević², B. Antonijević³, K. Baralić³, Z. Bulat³, E. Sudar-Milovanović¹ and Z. Mandinić⁴

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (jelenarad89@vin.bg.ac.rs)*

²*University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, Bulevar despota Stefana 142, 11000, Belgrade, Serbia*

³*Department of Toxicology "Akademik Danilo Soldatović", Toxicological Risk Assessment Center, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia*

⁴*Clinic for Pediatric and Preventive Dentistry, School of Dental Medicine, University of Belgrade, 11000 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.41

ABSTRACT

Elevated fluoride (F^-) concentrations in the environment are a real-case scenario that could negatively affect various tissues. Therefore, the current *in vivo* study evaluated subacute (28 days) F^- toxicity through the benchmark dose-response (BMD) approach in spleen tissue. Male *Wistar* rats were allocated into six groups ($n = 5$): a control group (tap water) together with five groups that were exposed to increasing F^- concentrations (10, 25, 50, 100, and 150 mg/L sodium-fluoride (NaF)) *ad libitum*. Spleen tissue was prepared for copper (Cu), iron (Fe) and DNA damage (comet assay) determination. BMD methodology for the external and internal F^- dose-response relationships was obtained via PROASTweb 70.1 software. The results showed that F^- caused a significant increase in DNA damage in all experimental groups. No significant changes were observed for Cu and Fe levels. The dose-dependent alterations were confirmed for decreasing Cu levels. The presented data provide important findings on an unexplored dose-dependent relationship between F^- exposure and possible toxicity mechanisms in the spleen tissue.

INTRODUCTION

Despite the fact that fluorides (F^-) have been known for their beneficial effects in the prevention of tooth decay for more than eight decades [1], they are constantly a subject of interest in contemporary toxicology research. Excessive

F⁻ consumption due to cumulative intake from beverages, water, air, industrial contamination and natural sources could lead to local and/or systemic toxicity [2]. According to some authors, elevated F⁻ concentrations could cause apoptosis of splenic cells, impair innate immunity and deregulate signaling pathways in spleen lymphocytes [3, 4]. To the best of our knowledge, this is the first study that has evaluated F⁻ subacute toxicity in the spleen tissue of experimental rats via dose-response methodology, with a goal to expand possible F⁻ toxicity mechanisms.

EXPERIMENTAL

The experiment, approved by the Ethical Committees of the School of Dental Medicine (Serbia, no 36/2) and the University of Belgrade, Faculty of Pharmacy (No. 323-07-11822/2018–05), was performed on 30 male albino *Wistar* rats (8 weeks old, 140-190 g). Animals (n = 5 per group, due to the 3R principle) were randomly allocated into six groups: control-tap water, while the other five groups were drinking solutions made with increasing sodium fluoride (NaF) concentrations: 10, 25, 50, 100, and 150 mg/L, for 28 days. Water (kept in the refrigerator in plastic bottles and prepared daily) and food (The Veterinary Institute, Subotica, Serbia) were provided *ad libitum*, to imitate the most common route of F⁻ exposure. The animals were euthanized by the intraperitoneal application of anesthetic ketamine (300 mg/kg) and xylazine (30 mg/kg).

The determination of zinc (Zn) and iron (Fe) concentrations was performed by flame atomic absorption spectrophotometry (FAAS) (240FS AA; Agilent Technologies, Santa Clara, CA, USA), and the protocol was in detail explained in our previously published paper [5]. Comet assay was performed in a mingle format [6] with modifications. Details on cell viability (fluorescence microscope Leica, DMLS, Austria, under magnification 200×, excitation filter 510–560 nm, barrier filter 590 nm), slide preparations and conditions for comet assay are provided in detail in our previous paper [7]. A total of 100 nucleoids were examined per each sample (Comet IV Computer Software (Perceptive Instruments, UK)). Quantification of the damage was assessed via Tail Intensity %. Nuclei with excessive levels of DNA damage were recorded for each sample by the hedgehog tool in the software. For benchmark dose–response modeling, PROASTweb 70.1 software (<https://proastweb.rivm.nl>) was used. External dosages (F⁻ in drinking solutions) represented the exposure, while internal dosages (F⁻ in tissues) indicated the actual biologically effective dose. A benchmark response (BMR) of 5% at a 90% confidence level was utilized for continuous individual data (exponential and Hill family models). BMD5 dose interval is defined by the BMDL5 (BMD lower) and BMDU5 (BMD upper) intervals.

RESULTS AND DISCUSSION

In experimental groups treated with F⁻ concentrations of 50 mg/L and higher, we observed a trend of decreasing Cu levels, without statistical significance. Fe levels remained unchanged in all the groups (Table 1).

Table 1. Bioelement concentrations in the spleen of *Wistar* rats subacutely exposed to different F⁻ concentrations.

		Groups (n=5)				
BE	C	10 mg/L F ⁻	25 mg/L F ⁻	50 mg/L F ⁻	100 mg/L F ⁻	150 mg/L F ⁻
Cu (µg/g)	1.65±0.41	2.04±1.26	2.48±0.09	1.12±0.21	0.95±0.1	1.14±0.51
	239.5	258.5	520.2	319.8	284.6	250.6
Fe (µg/g)	175.7-303.3	238.3-278.8	513.6-526.9	280.9-358.8	278.1-291	215.8-285.3

BE bioelement; C control; Cu copper; Fe iron; F⁻ fluoride. One-way ANOVA followed by Fisher's least significant difference (LSD) and Kruskal–Wallis tests, followed by Dunn's post hoc test. Results are given as means ± SDs or as the medians and interquartile ranges (25–75%).

Figure 1 summarizes comet assay data for all the experimental groups. A significant increase of DNA damage has been observed in all experimental groups exposed to F⁻ compared to the control (**p < 0.01, ***p < 0.001, ****p < 0.0001). The most prominent damage occurred within groups that were exposed to 25 and 150 mg/L F⁻ where the highest median values were observed. BMD5 analysis was performed for essential elements parameters and DNA damage measured in our study. Table 2 present the parameters that passed the criteria for dose–response modeling. External dose-dependent effects were observed for Cu, with the BMDL5 15.3 mg F⁻/L. These results showed that there is need for a constant update for F⁻ risk assessments and cautious monitoring, especially regarding water fluoridation processes, since F⁻ are usually combined with another elements, and synergistic and/or antagonistic properties should be observed [8].

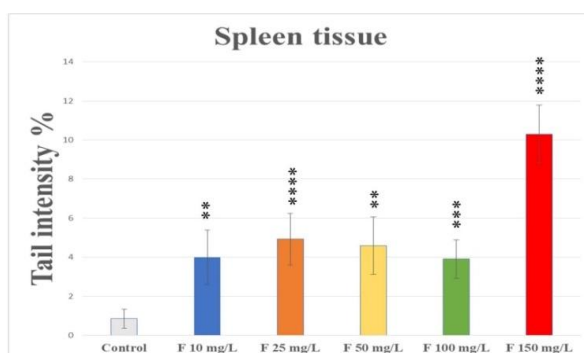


Figure 1. The level of DNA damage in the spleen assessed by the comet assay after subacute exposure to different F⁻ concentrations (10, 25, 50, 100 and 150 mg/L). One-way ANOVA followed by Fisher's least significant difference (LSD) as was used for data analysis. **p < 0.01, ***p < 0.001, ****p < 0.0001 (compared with the control group). Values represent mean ± SDs.

Table 2. External dose-dependent fluoride response for Cu in spleen tissue.

Par.	FM	Loglik.	No. Par.	AIC	BMDL5 (mg F ⁻ /L)	BMDU5 (mg F ⁻ /L)	TR
Cu	Exp. m5-	-2.14	5	14.28	15.3	48.2	+ ↓

AIC Akaike information criterion; BMDL5 lower confidence limit of the benchmark dose; BMDU5 upper confidence limit of the benchmark dose; Cu copper; FM Fitted Models; Loglik loglikelihood; No. Par. Number of parameters; Par. parameter; TR trend; ↓ negative trend.

CONCLUSIONS

Our findings reveal a trend of decreased Cu values in the groups that received moderately elevated F⁻ concentrations, suggesting Cu potential disbalance in the spleen tissue. Subacute F⁻ exposure also caused increased DNA damage in all groups, while BMD5 analysis showed a dose-dependent correlation for decreased Cu values. The presented low BMDL5 value indicates that even minimal F⁻ doses might cause measurable disturbances in essential element homeostasis and potentially impair spleen function.

Acknowledgement

This research was funded by the Ministry of Science Technological Development and Innovation of the Republic of Serbia (Contracts No# 451-03-33/2026-03/200161, No# 451-03-34/2026-03/200161 and No#451-03-33/2026-03/200017).

REFERENCES:

- [1] K. Binder, W.S. Driscoll, and G. Schützmannsky, *Caries Res*, 1978, 12 Suppl 1, 22-30.
- [2] G. Asgari, M. Salari, A. Seidmohammadi, B. Akbari-adegani, A.H. Mahvi, N. Kamari, K.E. Rastegar, and H. Faraji, *Nature*, 2025, 15, 18458.
- [3] Z. Shi, Y. Zhan, J. Zhao, J. Wang, and H. Ma, *Biol Trace Elem Res*, 2016, 173, 333-8.
- [4] H. Deng, P. Kuang, H. Cui, L. Chen, J. Fang, Z. Zuo, J. Deng, X. Wang, and L. Zhao, *Oncotarget*, 2016, 7, 67880-67900.
- [5] J. Radovanović, S. Milutinović-Smiljanić, B. Antonijević, K. Baralić, M. Ćurčić, Đ. Marić, Z. Mandinić, *J Xenobiot*, 2026, 16(2), 63.
- [6] A. Azqueta, K.B. Gutzkow, C.C. Priestley, S. Meier, J.S. Walker, G. Brunborg, and A.R. Collins, *Toxicol In Vitro*, 2013, 27, 768-73.
- [7] J. Radovanović, B. Antonijević, S. Kolarević, S. Milutinović-Smiljanić, J. Mandić, B. Vuković-Gačić, Z. Bulat, M. Ćurčić, M. Kračun-Kolarević, K. Sunjog, J. Kostić-Vuković, J.J. Marić, E. Antonijević-Miljaković, D. Đukić-Ćosić, A.B. Djordjevic, D. Javorac, K. Baralić, and Z. Mandinić, *Chemosphere*, 2021, 266, 128978.
- [8] O. Barbier, L. Arreola-Mendoza, and L.M. Del Razo, *Chem Biol Interact*, 2010, 188, 319-33.

PREFRONTAL CORTEX NEUROMODULATOR DYSREGULATION DURING BENZODIAZEPINE WITHDRAWAL IN ADULT FEMALE RATS

T. Radukić¹, J. Martinović¹, M. Dragić^{1,2}, N. Jasnić³, A. Stekić² and M. Zarić
Kontić¹

¹*VINČA Institute of Nuclear Sciences - National Institute of the Republic of
Serbia - University of Belgrade, Department of Molecular Biology and
Endocrinology, Mike Petrovica 12-14, 11351 Belgrade, Serbia
(tamara.radukic@vin.bg.ac.rs)*

²*Center for Translational Neuroscience, Faculty of Biology, University of
Belgrade, Studentski trg 16, 11158, Belgrade, Serbia*

³*Department for Animal and Human Physiology, Faculty of Biology,
University of Belgrade, Studentski trg 16, 11158, Belgrade*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.42

ABSTRACT

Chronic benzodiazepine exposure promotes dependence and withdrawal-associated neuroadaptations, yet the underlying neurochemical mechanisms remain insufficiently defined. Here, we examined alterations in dopamine and serotonin levels in the prefrontal cortex (PFC) following alprazolam withdrawal in adult female Wistar rats. Animals received escalating doses of alprazolam over 8 days, followed by abrupt cessation and assessment at 36 h withdrawal time point. Neuromodulator concentrations were quantified using high-performance liquid chromatography (HPLC). Withdrawal induced a significant increase in dopamine levels and a significant decrease in serotonin levels in the PFC. These findings indicate a disruption of neurochemical balance, suggesting that alterations in PFC monoamines may contribute to withdrawal-associated behavioral changes.

INTRODUCTION

The prefrontal cortex (PFC) is a critical brain region involved in the regulation of executive functions, emotional processing, and goal-directed behavior [1, 2]. It plays a central role in cognitive flexibility, decision-making, and inhibitory control, largely through its extensive connections with limbic and subcortical structures [1, 2]. Dysregulation of PFC activity has been strongly implicated in neuropsychiatric disorders, including anxiety, depression, and substance use disorders [3].

Benzodiazepines, such as alprazolam, are widely prescribed for the management of anxiety and related conditions due to their potentiation of γ -

aminobutyric acid (GABA)-mediated inhibitory neurotransmission. However, chronic use of benzodiazepines can lead to the development of tolerance and dependence, followed by withdrawal symptoms upon cessation. These withdrawal states are often characterized by heightened anxiety, altered emotional processing, and dysregulation of neurotransmitter/neuromodulator systems [4].

The PFC is highly sensitive to alterations in neurotransmitter balance, and both dopaminergic and serotonergic signaling within this region are essential for maintaining optimal cognitive and affective function [2, 5]. Disruption of these systems during benzodiazepine withdrawal may contribute to the behavioral and neurochemical abnormalities [6, 7]. While previous studies have investigated the effects of benzodiazepines on limbic structures such as the amygdala, less attention has been given to withdrawal-associated neurochemical changes within the PFC.

Therefore, the present study investigated dopamine and serotonin levels in the PFC at 36 h following alprazolam withdrawal in adult female Wistar rats using high-performance liquid chromatography (HPLC), providing insight into neurochemical adaptations associated with benzodiazepine withdrawal.

EXPERIMENTAL

Adult female Wistar rats were randomly divided into two experimental groups: control (vehicle-treated, 0.5 % Tween 80 in saline) and 36 h alprazolam withdrawal group. Alprazolam was administered intraperitoneally in escalating doses (3, 5, 7, and 9 mg/kg; twice daily for two days each) over 8 days, followed by abrupt cessation. Animals were housed under standard laboratory conditions with food and water available *ad libitum*. All procedures were performed in accordance with institutional ethical guidelines. Following 36 h withdrawal period animals were sacrificed, the PFC was identified using Paxinos and Watson stereotaxic atlas [8], and then carefully dissected. Tissue samples ($n = 6-7$ per group) were homogenized in deproteinization solution (1 mg tissue/10 μ L), followed by sonication (3×10 s) and centrifugation (30 min, 15,000 rpm, 4 °C). The resulting supernatants were collected for analysis using HPLC with electrochemical detection. An aliquot of each sample (40 μ L) was injected into an UltiMate 3000 HPLC system (Thermo Fisher Scientific, USA) equipped with a C18 column. The mobile phase consisted of 100 mM ammonium formate buffer (pH 3.6) and methanol, with an initial ratio of 98:2 (v/v), delivered at a flow rate of 500 μ L/min. Under these conditions, dopamine and serotonin were separated and detected using an electrochemical detector set at 850 mV and 25 °C. Data were acquired and analyzed using Chromeleon 7 Chromatography Data System (Thermo Fisher Scientific, USA). Neuromodulator concentrations were expressed as μ g/mg tissue. Data were analyzed using an unpaired two-tailed Student's t-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Dopamine and serotonin levels in the PFC were quantified following alprazolam withdrawal. Statistical analysis revealed a significant effect of withdrawal on monoamine levels.

Specifically, dopamine concentrations were significantly increased in the 36 h withdrawal group compared to controls ($p = 0.0041$; Figure 1A), indicating possible enhanced dopaminergic activity in the PFC following cessation of alprazolam treatment.

In contrast, serotonin levels were significantly decreased in the withdrawal group ($p = 0.0445$; Figure 1B), suggesting a potential reduction in serotonergic levels.

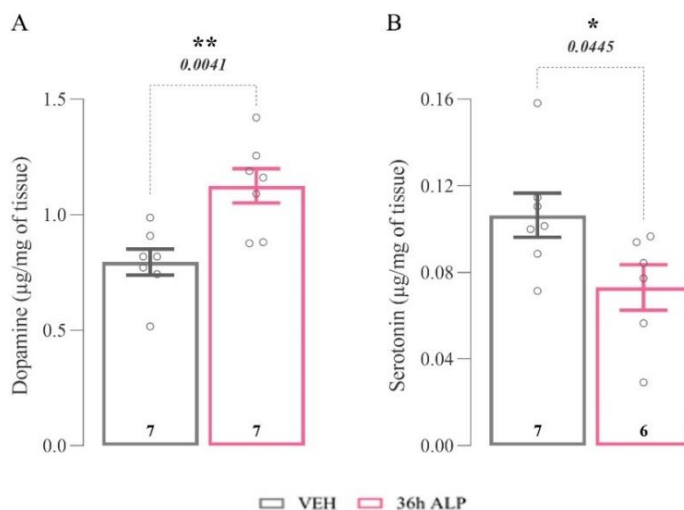


Figure 1. HPLC analysis of monoamine levels in the prefrontal cortex. (A) Dopamine concentration ($\mu\text{g}/\text{mg}$ of tissue); (B) Serotonin concentration ($\mu\text{g}/\text{mg}$ of tissue). Each dot represents an individual animal in the vehicle and alprazolam 36 h withdrawal groups. The number of animals per group is indicated within the bars (at the base of each column).

The present findings show that withdrawal from chronic alprazolam administration produces significant alterations in monoaminergic neurotransmission in the PFC, reflected by increased dopamine and decreased serotonin levels.

The observed elevation in dopamine may reflect a compensatory response to prolonged enhancement of GABAergic inhibition induced by benzodiazepines. Chronic alprazolam exposure enhances GABA_A receptor activity, leading to suppression of neuronal excitability [7]. Upon abrupt withdrawal, this inhibitory tone is removed, potentially resulting in a rebound hyperactivity of dopaminergic neurons. Such dysregulation of dopamine signaling in the PFC has been

associated with heightened arousal, anxiety, and impaired executive function, which are commonly reported during benzodiazepine withdrawal [2].

Conversely, the reduction in serotonin levels suggests impaired serotonergic transmission following withdrawal. Serotonin plays a critical role in mood regulation and emotional stability, and its decrease may contribute to withdrawal-related symptoms such as anxiety, irritability, and depressive-like states [6]. The imbalance between increased dopaminergic and decreased serotonergic activity may further exacerbate behavioral and affective disturbances.

Overall, these results indicate that alprazolam withdrawal leads to a significant neurochemical imbalance in the PFC, which may contribute to the behavioral and emotional symptoms observed during withdrawal. Further studies are needed to determine the temporal dynamics of these changes and their relationship to functional outcomes.

CONCLUSION

In conclusion, alprazolam withdrawal was associated with altered monoamine levels in the PFC, characterized by increased dopamine and decreased serotonin concentrations. These findings indicate a disruption of PFC monoaminergic balance that may contribute to withdrawal-related behavioral and affective changes. Overall, the study provides evidence for PFC monoaminergic alterations during benzodiazepine withdrawal and contributes to understanding its neurochemical basis.

Acknowledgement

The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grants: 451-03-33/2026-03/200017, 451-03-34/2026-03/200178, and 451-03-33/2026-03/200178.

REFERENCES

- [1] Friedman, N.P., Robbins, T.W., *Neuropsychopharmacology*, 47 (2022) 72–89.
- [2] Ott, T., Nieder, A., *Trends Cogn. Sci.*, 23 (2019) 213–234.
- [3] Stuss, D.T., *J. Int. Neuropsychol. Soc.*, 17 (2011) 759–765.
- [4] Ait-Daoud, N., Hamby, A.S., Sharma, S., Blevins, D., *J. Addict. Med.*, 12 (2020) 4–10.
- [5] Albert, P.R., Vahid-Ansari, F., Luckhart, C., *Front. Behav. Neurosci.*, 8 (2014) 199.
- [6] Celada, P., Puig, M.V., Artigas, F., *Front. Integr. Neurosci.*, 7 (2013) 25.
- [7] Rizvi, S.J., Kennedy, S.H., Sunderji, N., Zahra, Z., Sproule, B.A., Lim, B., *Int. Clin. Psychopharmacol.*, 35 (2020) 243–253.
- [8] Paxinos, G., Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press, 2013.

PROCESS OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF POLYPHENOLS FROM COCOA BEAN SHELLS AND THEIR ANTIOXIDANT POTENTIAL

S. Rakin¹, M. Petrović², M. Rakin², D. Zarić³, D. Bezbradica² and A. A. Jovanović¹

¹University of Belgrade, Institute for the Application of Nuclear Energy INEP, Banatska 31b, 11080 Belgrade, Serbia (sandra.rakin@inep.co.rs)

²University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia

³Innovation Centre Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.43

ABSTRACT

Food waste is a valuable source of bioactive compounds, and cocoa bean shells (*Theobroma cacao* L.) are rich in phenolic compounds with antioxidant potential. This study optimized ultrasound-assisted extraction of polyphenols by varying solvent type, solid-to-solvent ratio, and time, and evaluated the extracts' antioxidant activity. The results showed that extraction conditions significantly affected both total polyphenol content (TPC) and IC₅₀ values. The highest TPC was obtained with 50% ethanol, while optimal conditions were a 1:30 ratio and 30 min extraction. Antioxidant activity improved with higher TPC, but only a weak correlation was observed ($r = -0.37$), indicating that activity depends not only on total phenolics but also on the extract's chemical profile. Ultrasound-assisted extraction was confirmed as an efficient method for obtaining antioxidant-rich cocoa bean shell extracts.

INTRODUCTION

Food waste represents a significant environmental problem, but it can also serve as a source of bioactive compounds, dietary fibers, and natural antioxidants for the development of functional food products. Valorization of food waste materials contributes to the principles of the circular economy, the reduction of environmental pollution, and the more efficient use of agro-industrial by-products [1,2]. Cocoa bean shells from *Theobroma cacao* L. are a valuable by-product rich in flavonoids, phenolic acids, methylxanthines (theobromine and caffeine), dietary fibers, phytosterols, amino acids, and microelements, thus showing significant antioxidant and functional potential [1,3]. Ultrasound-assisted extraction allows for shorter extraction times, lower solvent consumption, and higher efficiency of bioactive components separation due to

the cavitation effect and improved mass transfer [4]. Therefore, this study aimed to optimize the ultrasound extraction process from cocoa shell *via* evaluation of polyphenol yield and antioxidant potential of developed extracts.

EXPERIMENTAL

The optimization of the extraction from cocoa bean shells was performed by varying the solid-to-solvent ratio (1:10, 1:20, 1:30, and 1:40), the extraction medium (30%, 50%, and 70% ethanol, and water), and time (15, 30, 45, and 50 min), using an ultrasound bath. Total polyphenol content (TPC) was measured using the previously described Folin-Ciocalteu method [4]. Antioxidant potential of the obtained extracts was evaluated using the DPPH• assay by measuring absorbance changes at 517 nm [5].

RESULTS AND DISCUSSION

The effects of ultrasound extraction conditions on the TPC and antioxidant activity of cocoa bean shell extracts are presented in Table 1.

Table 1. Total polyphenol content (TPC) and antioxidant potential of cocoa bean shell extracts obtained by ultrasound extraction.

Extraction conditions	TPC (mg GAE*/g plant material)	IC ₅₀ DPPH• (mg/mL)
1:10, 30% EtOH, 30 min	12.09±1.18 ^c	60.71±2.00 ^d
1:20, 30% EtOH, 30 min	14.32±1.04 ^{bc}	29.65±1.61 ^b
1:30, 30% EtOH, 30 min	15.77±1.23 ^b	21.99±0.55 ^a
1:40, 30% EtOH, 30 min	14.22±1.11 ^{bc}	23.44±1.02 ^a
1:20, H ₂ O, 30 min	9.46±0.74 ^d	39.46±1.42 ^c
1:20, 50% EtOH, 30 min	18.18±1.07 ^a	29.27±0.75 ^b
1:20, 70% EtOH, 30 min	9.00±0.44 ^d	29.26±1.00 ^b
1:20, 30% EtOH, 15 min	13.22±0.91 ^c	29.00±1.24 ^b
1:20, 30% EtOH, 45 min	16.24±1.25 ^b	29.97±0.87 ^b
1:20, 30% EtOH, 60 min	12.42±0.75 ^c	30.50±1.34 ^b

*GAE, gallic acid equivalent; IC₅₀, concentration for 50% inhibition of DPPH radicals; EtOH, ethanol; different letters in superscript indicate statistically significant differences between samples (n = 3, p < 0.05, one-way ANOVA, Duncan *post hoc* test).

The results presented in Table 1 show that the ultrasound extraction conditions significantly affect the TPC in cocoa bean shell extracts. The highest TPC was recorded with 50% ethanol (~18.18 mg gallic acid equivalent/g of plant material), indicating that the mixture of water and ethanol provides more efficient extraction of phenolic compounds compared to pure water or higher ethanol concentrations. This result is in accordance with literature data stating that mixtures of water and ethanol often show higher efficiency in polyphenol extraction compared to pure solvents, due to better solubility of different groups of phenolic compounds [4,6]. Increasing the solid-to-solvent ratio to 1:30 led to an increase in the TPC value, while further increase to 1:40 had no positive effect. Also, extending the extraction time to 45 min increased the TPC, while after 60 min, there was a decrease, probably due to the degradation of sensitive phenolic compounds and possible oxidation of the extracted components [4,7]. The results obtained indicate that all tested variables are key factors for the efficient extraction of polyphenols from cocoa bean shells. The results of the IC₅₀ DPPH analysis indicate that the extraction conditions also significantly affect the antioxidant activity of cocoa bean shell extracts (Table 1). Namely, lower IC₅₀ values indicate a higher ability to neutralize free radicals. The most pronounced antioxidant activity was achieved at the solid-to-solvent ratio of 1:30 and 1:40 with 30% ethanol for 30 min, where the lowest IC₅₀ values were recorded (~21.99 and ~23.44 mg/mL, respectively). Water extract showed significantly weaker antioxidant activity (~39.46 mg/mL), while the ratio of 1:10 yielded the highest IC₅₀ value (~60.71 mg/mL), indicating the lowest extraction efficiency of antioxidants. Changes in ethanol concentration and extraction time did not significantly affect the IC₅₀ values. The results confirm that selecting an appropriate solvent type and solid-to-solvent ratio increases the antioxidant potential of cocoa bean shell extracts. The Pearson correlation analysis showed a weak-to-moderate negative correlation between TPC and IC₅₀ values ($r = -0.37$). The negative sign of the correlation indicates that an increase in polyphenol content is accompanied by a decrease in IC₅₀ values, *i.e.*, an increase in the antioxidant activity. However, the obtained correlation is not statistically significant ($p > 0.05$), which suggests that the antioxidant capacity of the extracts does not depend solely on the TPC, but also on the composition and structure of individual phenolic compounds present in the extracts [8].

CONCLUSION

Based on the results obtained, it can be concluded that the ultrasound extraction conditions significantly affect the TPC and the antioxidant activity of cocoa bean shell extracts. The most effective solvent was 50% ethanol, while the optimal ratio and extraction time were 1:30 and 30 min, respectively. Although the increase in TPC was followed by an improvement in antioxidant activity, correlation analysis showed only a weak relationship between these parameters.

The data confirm that ultrasonic extraction is an effective procedure for obtaining antioxidant-rich extracts from cocoa bean shells and indicate their potential for further valorization as a functional ingredient.

Acknowledgement

This research was supported by the Ministry of Science, Technological Development, and Innovation of Serbia (451-03-136/2026-03/200019 and 451-03-34/2026-03/200135); United Nations Sustainable Development Goal 3 (Good Health and Well-Being).

REFERENCES

- [1] T. Belwal, C. Cravotto, S. Ramola, M. Thakur, F. Chemat, G. Cravotto, *Foods*, 2022, 11(6), 798.
- [2] A. Baiano, *Molecules*, 2014, 19(9), 14821-14842.
- [3] A. Gil-Ramírez, S. Cañas, I. Monedero Cobeta, M. Rebollo-Hernanz, P. Rodríguez-Rodríguez, V. Benítez, S. M. Arribas, M. A. Martín-Cabrejas, Y. Aguilera, *Future Foods*, 2024, 10, 100461.
- [4] A. Jovanović, V. Đorđević, G. Zdunić, K. Šavikin, D. Pljevljakušić, B. Bugarski, *Hem. Ind.*, 2016, 70(4), 391-398.
- [5] A. Ahmoda, A. Pirković, V. Milutinović, M. Milošević, A. Marinković, A. A. Jovanović, *Plants*, 2025, 14, 352.
- [6] J. F. Osorio-Tobón, *J. Food Sci. Technol.* 2020, 57(12), 4299-4315.
- [7] F. Chemat, Zill-e-Huma, M. K. Khan, *Ultrason. Sonochem.*, 2011, 18(4), 813-835.
- [8] N. Balasundram, K. Sundram, S. Samman, *Food Chem.*, 2006, 99(1), 191-203.

TARTRAZINE CALIBRATION BY BRIGGS RAUSCHER REACTION AND UV/VIS METHOD: COMPARATIVE STUDY

J. Senćanski¹, J. Maksimović², N. Potkonjak³ and M. Pagnacco⁴

¹*University of Belgrade - Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, Kneza Višeslava 1, Belgrade, 11030, Serbia
(jelena.sencanski@imsi.bg.ac.rs)*

²*Faculty of Physical Chemistry, University of Belgrade, Studentski Trg 12-16, 11000 Belgrade, Serbia*

³*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia*

⁴*University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.44

ABSTRACT

This paper compares two methods for obtaining analytical curves of tartrazine: by the standard UV/VIS method and by the oscillatory Briggs Rauscher (BR) reaction. The aim of this paper is to calculate and compare the lowest limit of detection (LOD) and the lowest limit of quantification (LOQ) for both methods. Although UV/VIS spectrophotometry exhibited lower LOD and LOQ values, the Briggs-Rauscher reaction represents a simple and cost-effective alternative for tartrazine determination, providing satisfactory analytical performance.

INTRODUCTION

Tartrazine is an organic dye, often used in the food, medical, pharmaceutical, and textile industries [1-5]. Because of its potentially harmful effects, including the possibility of being poisonous and triggering cancer in humans, detecting low tartrazine concentrations in aqueous solution is important [6]. This paper describes the Briggs Rauscher (BR) reaction as a method for tartrazine determination that, according to the literature, has not previously been applied for this purpose [7].

EXPERIMENTAL

To establish the UV/VIS calibration curve, a stock solution was prepared by dissolving 0.0157 g of standard tartrazine (Sigma-Aldrich) in 100 mL of distilled water. This stock was subsequently diluted 5-, 10-, 25-, 50-, and 100-fold to generate a series of working standards for the analytical curve. All

spectrophotometric measurements were conducted at an ambient temperature of approximately 25 °C using an Agilent 8453 spectrophotometer, scanning across a wavelength range of 200 nm to 1100 nm.

To establish the BR calibration curve, all experiments were performed in a closed, thermostated ($T = 37.1$ °C), well-stirred reactor ($\sigma = 900$ rpm). The time evolution of the BR system was monitored potentiometrically with an ion-sensitive I⁻ electrode as the working electrode and a Hg/HgSO₄ electrode as the reference. The initial concentrations of the reactants were: [Malonic acid]⁰ = 0.0789 mol dm⁻³, [MnSO₄]⁰ = 0.00752 mol dm⁻³, [HClO₄]⁰ = 0.03 mol dm⁻³, [KIO₃]⁰ = 0.0752 mol dm⁻³, [H₂O₂]⁰ = 1.176 mol dm⁻³. 50 μ L of Tartrazine solutions of different concentrations (5.86×10^{-4} mol dm⁻³; 2.84×10^{-2} mol dm⁻³, 5.71×10^{-2} mol dm⁻³, 8.52×10^{-2} mol dm⁻³, 1.13×10^{-1} mol dm⁻³, 1.69×10^{-1} mol dm⁻³, 2.25×10^{-1} mol dm⁻³, 2.80×10^{-4} mol dm⁻³) were added to the reaction mixture 30 s after the initiation of the reaction with hydrogen peroxide.

RESULTS AND DISCUSSION

Figure 1 a) presents the UV/VIS and b) BR results for tartrazine analytical curves obtained as written in the figure's description.

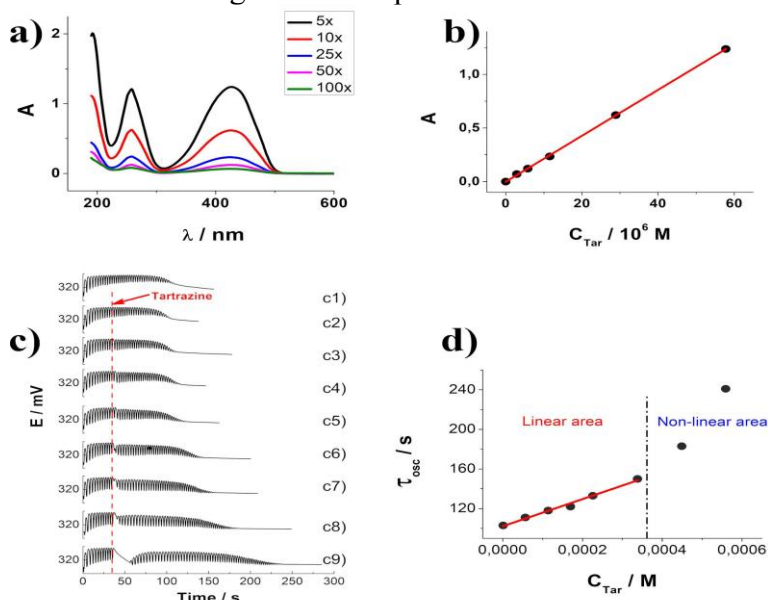


Figure 1. a) The standard tartrazine water solutions UV/VIS spectra 5.78×10^{-5} mol dm⁻³ (black line), 2.89×10^{-5} mol dm⁻³ (red line), 1.16×10^{-5} mol dm⁻³ (blue line), 5.78×10^{-6} mol dm⁻³ (violet line) and 2.89×10^{-6} mol dm⁻³ (green line) and b) analytical UV/VIS curve at 426 nm, c) Basic BR oscillogram and BR oscillograms obtained with the addition of various concentrations of tartrazine: 1.17×10^{-6} mol dm⁻³ (c2), 5.67×10^{-5} mol dm⁻³ (c3), 1.14×10^{-4} mol dm⁻³ (c4), 1.70×10^{-4} mol dm⁻³ (c5), 2.26×10^{-4} mol dm⁻³ (c6), 3.38×10^{-4} mol dm⁻³ (c7), 4.49×10^{-4} mol dm⁻³ (c8), and 5.59×10^{-4} mol dm⁻³ (c9), d) The BR oscillatory period, τ_{osc} , dependence on the tartrazine concentration.

UV/VIS analytical curve shows a linear area supported by the Lambert-Beer law, matching the coefficient a with the reference [8]. In contrast, the BR reaction shows two areas: the linear and non-linear ones. In order to calculate LOD and LOQ values, only the linear area of the BR reaction was taken into account. LOD and LOQ values for each method were calculated according to the formulas listed in Table 1.

Table 1. LOD and LOQ values for each method-formulas and the results.

Formulas	UV/VIS method $A=(21400\pm200)\times C + (0.001\pm0.005)$	BR reaction $\tau_{osc}=(137000\pm8000)\times C + (102\pm2)$
$LOD = 3.3 \times \frac{\delta}{slope}$	1.1×10^{-6}	4.3×10^{-5}
$LOQ = 10 \times \frac{\delta}{slope}$	3.0×10^{-6}	1.3×10^{-4}

Where $\delta = \sqrt{\frac{\sum(y_i - y_{exp})^2}{(n-1)}}$

n is the number of measurements, and y is the oscillatory period for the BR method, while it is absorbance for the UV-VIS method.

The UV/VIS spectrophotometric method yielded superior analytical performance compared to the Briggs–Rauscher (BR) matrix method, achieving significantly lower LOD and LOQ values. Notably, the LOQ for the UV/VIS method was approximately two orders of magnitude lower than that of the BR method. This enhanced sensitivity is primarily attributed to the lower concentration range utilized to construct the UV/VIS calibration curve, as well as the lower δ obtained, due to better repeatability and trueness.

CONCLUSION

This work provides a comparison of two methods to obtain analytical curves for tartrazine determination in order to calculate LOD and LOQ. Those two methods included the Briggs Rauscher oscillatory reaction as a nonstandard method for tartrazine calibration, and UV/VIS as a standard one. UV/VIS spectrophotometry exhibited lower LOD and LOQ values, indicating superior analytical sensitivity. The higher detection limits obtained with the Briggs-Rauscher method are mainly associated with its lower sensitivity under the investigated experimental conditions. Nevertheless, the Briggs-Rauscher reaction represents a simple and cost-effective alternative for tartrazine determination and may be suitable for applications where ultra-low detection limits are not required.

Acknowledgement

This work was partially supported by the Ministry for Science of the Republic of Serbia (451-03-33/2026-03/200053, 451-03-33/2026-03/200146 and 451-03-33/2026-03/200026, 451-03-33/2026-03/200017).

REFERENCES

- [1] J. König, *Colour Additives for Foods and Beverages*, 2015, 35–60.
- [2] M. Ramesh, A. Muthuraman, *Natural and Artificial Flavoring Agents and Food Dyes*, 2018, 1–28.
- [3] M.A. Abud, R. Simon, *Encyclopedia of Food Allergy*, 2024, 828–839.
- [4] N.H. Choulis, *Side Eff. Drugs Annu.*, 2010, 32, 891–902.
- [5] K. Walton, R. Walker, J.J.M. van de Sandt, J.V. Castell, A.G.A.A. Knapp, G. Kozianowski, M. Roberfroid, B. Schilter, *Food Chem. Toxicol.*, 1999, 37, 1175–1197.
- [6] M. Visternicu, A. Săvucă, V. Rarinca, V. Burlui, G. Plavan, C. Ionescu, A. Ciobica, I.-M. Balmus, C. Albert, M. Hogas, *Toxics*, 2025, 13, 771.
- [7] M. C. Pagnacco, J. P. Maksimović, T. M. Mudrinić, Z. D. Mojović, Z.P. Nedić, *J. Electroanal. Chem.*, 849, 2019, 113369.
- [8] J. Masłowska, J. Janiak, *Chem. Anal.*, 1996, 41, 855–864.

THE FIRST REPORT ON THE MICROSCOPIC EXAMINATION OF THE BRIGGS RAUSCHER REACTION AND ITS MODIFICATION WITH TARTRAZINE

J. Senćanski¹, J. Maksimović², A. Radulović³, M. Kalaba³, U. Čakar⁴ and M. Pagnacco⁵

¹*Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, University of Belgrade,*

Kneza Višeslava 1, 11030 Belgrade, Serbia (jelena.sencanski@imsi.bg.ac.rs)

²*Faculty of Physical Chemistry, University of Belgrade, Studentski Trg 12–16, 11000 Belgrade, Serbia,*

³*Institute of General and Physical Chemistry, University of Belgrade, Studentski Trg 12–15,*

⁴*University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, 11 000 Belgrade, Serbia and*

⁵*University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia.*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.45

ABSTRACT

This work aims to microscopically examine the solid residues obtained after evaporation of the Briggs-Rauscher (BR) reaction mixture, as well as the effect of tartrazine addition. Individual BR components, the complete BR mixture, pure tartrazine, and the BR system containing tartrazine were evaporated and analyzed by optical microscopy and SEM. Distinct morphological differences were observed, particularly after tartrazine addition.

INTRODUCTION

Tartrazine is an organic dye widely used as a colorant in the food, pharmaceutical, and related industries [1,2]. The BR reaction is a well-known oscillating chemical system [3]. However, microscopic characterization of solid residues obtained after evaporation of the BR reaction mixture has received little attention. Therefore, optical microscopy and SEM were applied to compare the morphology of BR residues in the absence and presence of tartrazine.

EXPERIMENTAL

For morphological characterization, the individual components of the BR reaction, the complete BR reaction mixture, pure tartrazine, and the BR reaction containing tartrazine were prepared in separate Petri dishes. The BR mixture consisted of 0.7 mL malonic acid (0.282 mol dm⁻³), 0.5 mL MnSO₄

(0.0376 mol dm⁻³), 0.5 mL HClO₄ (0.15 mol dm⁻³), 0.5 mL KIO₃ (0.376 mol dm⁻³), and 0.3 mL H₂O₂ (9.8 mol dm⁻³). The tartrazine-containing system was prepared by adding 0.001 g of tartrazine to the complete BR mixture, while pure tartrazine was prepared by dissolving 0.001 g in 1 mL of distilled water. All samples were allowed to evaporate under ambient conditions (approximately 25 °C, atmospheric pressure) for 48 h, yielding solid residues suitable for microscopic analysis.

Optical microscopy was performed using a Geefantech transmission microscope at magnifications of 160×, 400×, and 1600×. Representative micrographs were selected after examining multiple regions of each sample to ensure that the observed features reflected the characteristic morphology.

Scanning electron microscopy (SEM) was carried out using a JEOL JSM-6460LV microscope (JEOL Ltd., Tokyo, Japan). Prior to imaging, the samples were mounted on aluminum stubs using conductive adhesive tape and sputter-coated with gold (BAL-TEC SCD 005, Balzers, Liechtenstein) to minimize charging effects. Particle sizes were determined using SemAfore 5.21 software.

RESULTS AND DISCUSSION

The evaporated samples were subjected to microscopic analysis, under magnifications ranging from 160 to 400 and up to 1600×. To the best of our knowledge, optical microscopic characterization of the evaporated BR system is reported here for the first time.

Figure 1 a) shows optical microscopy characterization of evaporated samples for each BR system component, of the complete BR system, of tartrazine itself, and of BR with tartrazine. Potassium iodate shows hexagonal particles, malonic acid exhibits a chain-like morphology, while the H₂O₂ sample shows a spider-web-like appearance. BR reaction, when evaporated, shows agglomerated particles containing partly defined hexagonal granules and a chain morphology with snowflake features (Figure 1 a). Under the optical microscope, tartrazine itself possesses granules full of small needles, while the mixture of tartrazine and BR reaction shows hexagonal granules that become more agglomerated compared to the BR ones.

To have better insight into the morphology of the same samples, they underwent SEM analysis (Figure 1b).

The fractured plate morphology with well-defined plate edges ranging from 4 to 12 μm was observed for KIO₃, while a gravelly morphology was observed for HClO₄, ranging from 0.7 to 4 μm. Large, broken particles were observed for malonic acid, while irregular particles of 2–7 μm were present in the MnSO₄ sample. BR has a gravelly morphology with particles ranging from 0.9 to 6 μm, showing a similar morphology to the mixture morphologies of HClO₄ and MnSO₄.

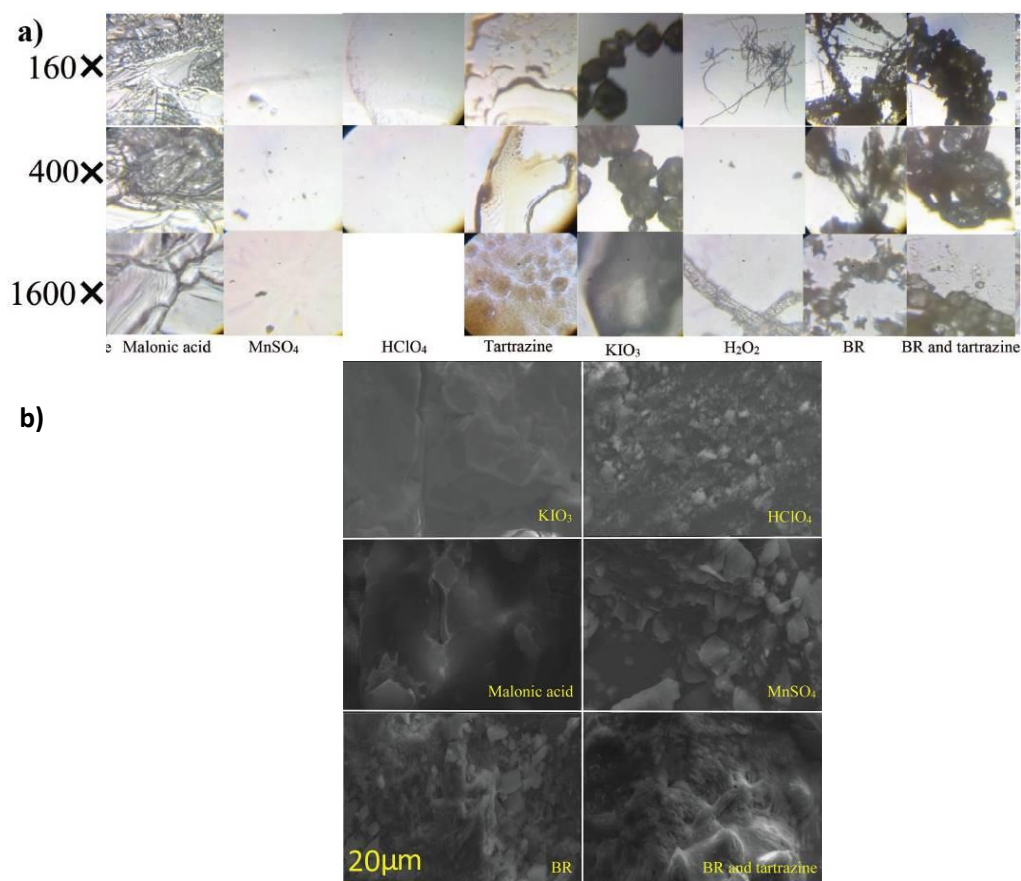


Figure 1. A) optical microscopy characterization of evaporated samples for each BR system component (as marked), of the complete BR system, of tartrazine itself and of BR with tartrazine under the magnifications of 160 to 400 and to 1600 $\times$; **B)** SEM characterization of the same samples, as marked in each figure.

After the addition of tartrazine to the BR system, the BR morphology has changed into a microporous form, possessing particles 0.3 to 1.3 micrometer-sized and 1.4–1.9 μm pores. This change in morphology indicates that the presence of tartrazine affects the formation and organization of the solid residue obtained from the BR system (Figure 1b).

CONCLUSION

This work presents microscopic characterization of solid residues obtained from the BR reaction system. Optical microscopy and SEM revealed clear morphological differences after tartrazine addition, including changes in particle size and structure. These results indicate that tartrazine affects the morphology and organization of the solid residue formed after evaporation of the BR reaction mixture.

Acknowledgement

This work was partially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants no. 451-03-33/2026-03/ 200161 and 451-03-34/2026-03/ 20016, 451-03-33/2026-03/200051, 451-03-33/2026-03/200053, 451-03-33/2026-03/200146 and 451-03-33/2026-03/200026).

REFERENCES

- [1] K Walton, R Walker, J.J.M van de Sandt, J.V Castell, A.G.A.A Knapp, G Kozianowski, M Roberfroid, B Schilter, *Food and Chemical Toxicology*, 1999, 37, 12, 1175-1197.
- [2] R. Gentry, T. Greene, G. Chappell, I. Lea, S. Borghoff, C. Yang, J. Rathman, J. V. Ribeiro, B. Hobocienski, A. Mostrag, J. Rodricks, H. Clewell, *Food and Chemical Toxicology*, 2021, 151, 112097.
- [3] M. C. Pagnacco, J. P. Maksimović, T. M. Mudrinić, Z. D. Mojović, Z.P. Nedić, *Journal of Electroanalytical Chemistry*, 2019, 849, 113369.

FORMULATION OF FUNCTIONAL INGREDIENT BASED ON ENRICHED SKIMMED GOAT'S MILK WITH GRAPE POMACE EXTRACT USING CENTRAL COMPOSITE DESIGN

I. D. Sredović Ignjatović, D. D. Milinčić, S. P. Stanojević, A. Ž. Kostić and M. B. Pešić

University of Belgrade – Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia (isredovic@agrif.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.46

ABSTRACT

The aim of this research was to establish the optimal composition of functional ingredients based on skimmed goat's milk and grape pomace seed extract. A central composite design was used to test the influence of grape pomace content and pH on total phenolic content (TPC), antioxidant (ABTS, DPPH, and FRP) and chelating (CHE) properties as response variables of analyzed mixtures. Significant linear regression was observed for TPC, ABTS^{•+} radical scavenging activity, and FRP, while significant linear and squared regression was determined for DPPH[•] scavenging activity and chelating ability. No significant correlation between tested responses and the interaction term was detected. By applying the desirability function, optimal conditions were set, and predicted values of responses were compared to experimental results. Good agreement was obtained only for TPC and FRP, contrary to other properties whose experimental values were much higher than predicted.

INTRODUCTION

The modern way of life has shifted the consumers' interest toward nutritious, safe, and healthy food products. Designing new food products with improved nutritive and/or techno-functional properties raises the question of what proportions ingredients should be mixed? Answering this question encompasses experimental work, and one way for planning and conducting experiments is using a statistical approach called design of experiments (DoE) [1,2]. The principles of DoE gained more attention in food processing during the last few decades as a tool for quality control, cutting down production costs, developing new products, or optimizing food processes [3,4].

In this paper, optimization of nutritional properties of functional formulation based on skimmed goat milk enriched with grape pomace seed extract was performed using central composite design. The aim was to summarize results of the statistical approach, analyzing advantages and

shortcomings of applying a statistical approach in optimization of new functional ingredients. The nutritive properties of tested formulations were assessed using total phenolic content (TPC), antioxidant activity (tested by ABTS, DPPH, and FRP assays), and chelating ability (CHE) as dependent (response) variables. Employing the desirability function as a multiple response optimization tool, the optimal mixture was determined.

EXPERIMENTAL

Grape seeds of the Prokupac grape variety ("Wine house Milinčić") were manually separated from the fermented pomace and ground in a coffee grinder (Bosch MKM 6003 UC, BSH Hausgerate GmbH, Munich, Germany). Fresh goat's milk was obtained from a local dairy and immediately skimmed prior to use.

Grape pomace seed extracts (GPE) were prepared as previously described by Pešić et al. [5], with acidified 80% aqueous methanol (0.1% HCl) (1:20, m/v ratio) for 1 h under continuous shaking at room temperature. The obtained filtrates were combined, and the solvent was removed under reduced pressure at 40 °C. The dried extract was reconstituted in MilliQ water, then lyophilized and stored in a freezer at -20 °C until further use. Samples of skimmed goat milk were heat-treated (90 °C, 10 min) (TM) and prepared according to the methodology previously described by Pesic et al. [6]. Milk samples were spray-dried and stored in a freezer until further use.

The effects and interactions of two independent variables on total phenolic content (TPC), antioxidant activity (tested by ABTS, DPPH and FRP assays) and chelating ability (CHE) of prepared functional additives were investigated by central composite experimental design (CCD). The testing of selected factors was performed at three levels (low, medium and high). Grape pomace seed extract content ($w(\text{GPE})$, % (m/m)) was tested at 0.1, 0.55, and 1.0%, while pH was tested at 5.5, 6.25, and 7.0. The experimental matrix was obtained according to the selected CCD using MINITAB software (Release 16, Minitab Inc., State College, PA). A full factorial design with a total of 13 experimental runs was performed, with 4 cube points, 5 central points in the cube, and 4 axial points. The experimental measurements of all dependent variables were done in triplicate, and the average was taken as the response.

The spray-dried thermally treated goat milk powder was reconstituted with milliQ water (1:10 w/v, 10%). The prepared milk was divided into three glasses to adjust the desired pH (7.0; 6.25; and 5.50). Then, the suggested amounts of lyophilized grape pomace extract were added to the prepared milk samples to obtain 13 mixtures according to the experimental matrix. The mixtures were mixed on a mechanical shaker for 1h and then sonicated for 30 minutes to ensure the best solubilization.

To determine the total phenolic content, the procedure described by Kostić et al. [7] was used. The TPC was expressed as mg gallic acid equivalents per 100 mL

(mg GAE/100 mL). Antioxidant assays, such as ferric reducing power (FRP), ABTS^{•+} scavenging activity (ABTS), DPPH[•] scavenging activity (DPPH), and ferrous ion-chelating capacity (CHE) of the prepared samples were evaluated as previously described [7,8]. The results for both radical scavenging assays and FRP were expressed as mg Trolox equivalents per 100mL of sample (mg Trolox/100mL). Results for FCC were expressed as mg EDTA equivalents per 100mL of sample (mg EDTA/100mL). A microplate Reader was used for all measurements (ELISA Plate Reader).

RESULTS AND DISCUSSION

RSM comprises a group of mathematical relationships that rely on the fit of empirical models to the experimental data acquired in a factorial experimental design. The experimental data were represented using a second-order polynomial equation that considers linear, square, and interaction terms [9]. Experimental results were statistically analyzed. The confidence interval of each individual factor and their combinations at a 95% confidence level were evaluated.

The relative magnitudes of the process variable effects were determined on the basis of the factor effect and the corresponding *p*-value. The estimated effects and regression coefficients, validated with an ANOVA test (data not shown), were used to determine the way in which investigated factors influence analyzed nutritional properties of prepared functional products. Regression equations including only statistically significant terms (*p*<0.05), as well as correlation coefficients, are summarized in Table 1.

Table 1. Second-order polynomial equations and regression coefficients of the response variables.

Response	Regression equation	<i>R</i> ² (%)
TPC (mg GAE/100 mL)	$y = 49.13 + 4.58 \cdot w(\text{GPE}) + 4.50 \cdot \text{pH} + \varepsilon$	73.24
ABTS ^{•+} (mg Trolox/100 mL)	$y = 44.47 + 4.37 \cdot \text{pH} + \varepsilon$	74.02
DPPH [•] (mg Trolox/100 mL)	$y = 28.89 + 3.37 \cdot w(\text{GPE}) - 5.23 \cdot (w(\text{GPE}))^2 + \varepsilon$	87.83
FRP (mg Trolox/100 mL)	$y = 24.82 + 11.92 \cdot w(\text{GPE}) + \varepsilon$	92.91
CHE (mg EDTA/100 mL)	$y = 92.29 + 17.44 \cdot \text{pH} - 6.78 \cdot (\text{pH})^2 + \varepsilon$	95.87

By comparing the estimated *p*-values and the established criterion (*p*=0.05), it was found that linear terms significantly influence the TPC in analyzed mixtures. The satisfactory coefficient of variation (*R*²=73.24%) and no significant lack of fit (*p*>0.05) indicated that there was a linear correlation between the response variable and independent factors. The response surface plot presented in Fig. 1a

showed that the highest total phenolic content (TPC) was observed in the mixture prepared at the maximum investigated levels of independent variables (pH and $w(\text{GPE})$).

Analysis confirmed that pH significantly influenced ABTS^{•+} radical scavenging activity ($p < 0.05$). Linear increase of antioxidant activity with increase of pH value at which functional mixtures were prepared is evident from the response surface plot (Fig. 1b). Influence of pH value on ABTS radical scavenging activity is more pronounced at higher grape pomace seed extract content. The coefficient of variation was $R^2 = 74.02\%$, indicating a satisfactory degree of correlation between the response variable and independent factors.

Contrary to TPC and ABTS, for DPPH scavenging activity, it was found that linear and square terms of grape pomace seed extract were significant for system response ($p < 0.05$). A good correlation coefficient of $R^2 = 87.73\%$ indicated a high degree of correlation between the response variable and independent factors and a high degree of fitting. The DPPH radical scavenging activity (Fig. 1c) linearly increases with an increase in grape pomace seed extract content ($w(\text{GPE})$), reaching a maximum and curvature at $w(\text{GPE}) = 0.55\%$ (m/m). From the response plot, although pH is not a statistically significant parameter, maximum antioxidant activity measured by the DPPH assay was recorded for mixtures prepared at pH 6.25.

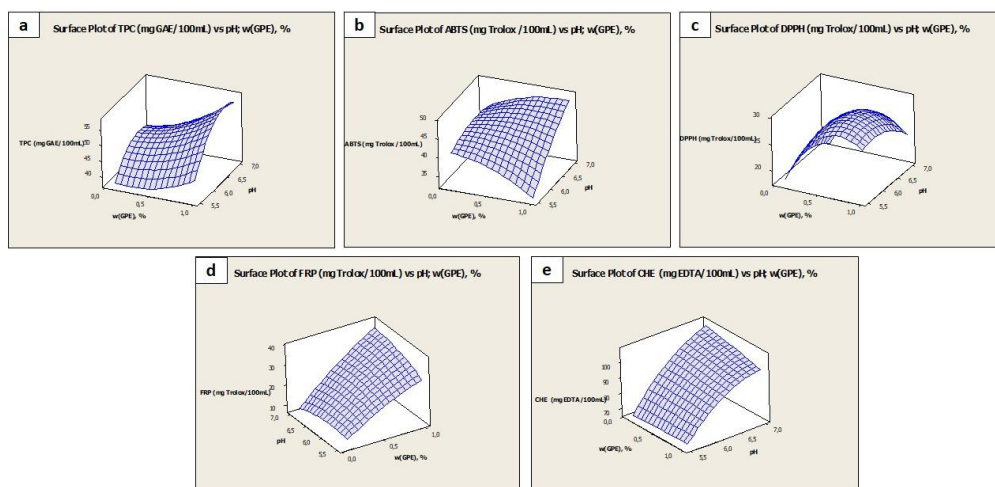


Figure 1. Response surface plot for: (a) Total phenolic content; (b) ABTS^{•+} radical scavenging activity; (c) DPPH[•] radical scavenging activity; (d) Ferric reducing power; (e) Ferric chelating ability

Ferric reducing power was highly correlated with the investigated parameters ($R^2 = 92.91\%$), with a high degree of fitting between the response variable and linear grape pomace seed extract content term. The response surface plot (Fig. 1d) showed the effect of pH and grape pomace seed extract (GPE) content on the

tendency to reduce ferric ions (FRP). From this plot, it can be seen that antioxidant activity increased with an increase in GPE content, and the highest antioxidant activity was observed at the maximum investigated levels of pH and GPE.

According to the results of estimated regression coefficients and ANOVA test, chelating properties of analyzed mixtures were statistically influenced by the linear term and square term of pH. The high correlation coefficient ($R^2=95.87\%$) showed a high degree of fitting between chelating properties and varied parameters. It is important to emphasize that the pH square regression term was not only statistically significant, but also negatively influenced the analyzed response parameter. Maximum chelating properties, as shown on the response surface plot in Fig. 1e, were recorded for products prepared with the lowest grape pomace extract content and at the highest investigated pH value.

To obtain overall desirability, all five responses were taken into account, and the importance of every variable relative to others was chosen. In this process, importance equal to 1 for all five responses was selected. After that, the weights were assigned to individual responses. The weights determine how important it is for the individual desirability function to be close to maximum or minimum. Weights were chosen to be 1 [4]. According to the established data set, overall desirability was $D=0.9467$ for a mixture prepared with 0,703 % (m/m) grape pomace seed extract at pH 6.36. An excellent agreement between predicted and obtained results was achieved for FRP antioxidant activity and total phenolic content. Significantly higher experimental than predicted results were recorded for antioxidant activity measured with ABTS, DPPH assays and for ferrous ion chelating properties.

CONCLUSION

To prepare an optimized milk/GPE functional ingredient, a surface response design methodology was used. The effects and interactions of two independent variables, pH and GPE content, on total phenolic content (TPC), antioxidant activity (tested by ABTS, DPPH and FRP assays) and chelating ability (CHE) of milk/GPE mixtures were investigated by central composite experimental design (CCD). The results revealed that TPC was linearly correlated with the investigated parameters; ABTS was satisfactorily correlated with the linear term of pH; DPPH and FRP values were highly correlated with both the linear and square term of the content of grape pomace seed extract, while results for chelating ability were correlated with the linear and square term of pH. The desirability function determined that an optimal mixture can be prepared with the addition of 0,703 % (m/m) grape pomace seed extract in 10% milk at pH 6.36. An excellent agreement between the predicted and obtained results was achieved for the FRP antioxidant activity and TPC. Significantly higher experimental than predicted results were recorded for DPPH, ABTS activity, and CHE properties.

Acknowledgement

This research was funded by the Science Fund of the Republic of Serbia, FUNRPO Project, #Grant No. 7744714 and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No, 451-03-34/2026-03/200116).

REFERENCES

- [1] S. L. C. Ferreira, M. M. Silva Junior, C. S. A. Felix, D. L. F. da Silva, A. S. Santos, J. H. Santos Neto, C. T. de Souza, R. A. Cruz Junior, A. S. Souza, *Food Chem.*, 2019, 273, 3-8
- [2] M. Yolmeh, S. M. Jafari, *Food Bioprocess. Technol.*, 2017, 10, 413-433.
- [3] M. Madoumier, G. Trystram, P. Sébastien, A. Collignan, *Trends Food Sci. Technol.*, 2019, 86, 1-15.
- [4] M. Lučić, N. Potkonjak, I. Sredović Ignjatović, S. Lević, Z. Dajić-Stevanović, S. Kolašinac, M. Belović, A. Torbica, I. Zlatanović, V. Pavlović, A. Onjia, *Foods*, 2023, 12, 2468.
- [5] M. B. Pešić, D. D. Milinčić, A.Ž. Kostić, N.S. Stanisavljević, G. N. Vukotić, M. O. Kojić, U. M. Gašić, M. B. Barać, S. P. Stanojević, D. A. Popović, N. R. Banjac, Ž. Lj. Tešić, *Food Chem.*, 2019, 284, 28–44.
- [6] M.B. Pesic, M. B. Barac, S. P. Stanojevic, N. M. Ristic, O. D. Macej, M. M. Vrvic, *Small Rumin. Res.* 2012, 108, 77–86.
- [7] A. Ž. Kostić, D. D. Milinčić, N. S. Stanisavljević, U. M. Gašić, S. Lević, M. O. Kojić, Ž. Lj. Tešić, V. Nedović, M. B. Barać, M. B. Pešić, *Food Chem.*, 2021, 351, 129310.
- [8] D. D. Milinčić, A. Ž. Kostić, U. M. Gašić, S. Lević, S. P. Stanojević, M. B. Barać, Ž. Lj. Tešić, V. Nedović, M. B. Pešić, *Biomolecules*, 2021, 11, 965.
- [9] M. A. Bezerra, R. E. Santelli, E. P. Oliveira, L. S. Villar, L.A. Escaleira, *Talanta*, 2008, 76, 965–977.

THE IMPACT OF HIGH-FAT DIET ON LIVER PARAMETERS AND ANTIOXIDANT SIGNALLING

J. Stanimirović, K. Banjac, M. Obradović and E. R. Isenović

Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (julijana@vin.bg.ac.rs)

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.47

ABSTRACT

Due to present-day lifestyle habits, obesity is a disease of modern society. Obesity is characterized by excessive fat accumulation and lipid disturbance, which further leads to alterations in liver enzyme activity and redox signaling. We aimed to explore the effect of a high-fat (HF) diet on the concentrations of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) in serum, and levels of nuclear factor erythroid 2-related factor 2 (NRF2) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) in rat liver. Our results showed a significant elevation of ALP concentration and no change in AST and ALT concentrations in serum. Additionally, protein levels of NRF2 and PGC1- α were reduced in liver of HF diet-fed rats compared to control rats. These findings indicate an increased inflammatory response and impaired antioxidant defense signaling in the liver of HF diet-fed rats, predisposing them to the development of insulin resistance.

INTRODUCTION

Obesity has emerged as one of the major public health challenges of modern society, largely driven by contemporary lifestyle habits. Excessive fat accumulation and lipid disturbance are key characteristics of obesity, which consequently lead to oxidative stress and insulin resistance [1]. Ample evidence demonstrates that high-fat (HF) diet induces intracellular and systemic lipid overload which affects liver parameters, such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) [2, 3]. Furthermore, HF diet affects the hepatic antioxidant defense where nuclear factor erythroid 2-related factor 2 (NRF2) takes a central role, promoting oxidative stress and insulin resistance development [4, 5]. Additionally, obesity impairs peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1- α) in the liver, which contributes significantly to insulin resistance and non-alcoholic fatty liver disease [6, 7]. The objective of this study was to elucidate the effect of HF diet on liver parameters in serum and hepatic NRF2/PGC1- α signaling in rats.

EXPERIMENTAL

The study was conducted on adult male Wistar rats. Animals were kept in cages under standardized laboratory conditions: a controlled temperature (22 ± 2 °C), a 12-hour light/dark cycle, and *ad libitum* water and food. The animals were randomly assigned to one of two experimental groups: a control group (CONT, $n = 7$), fed a standard laboratory food, and a HF diet group (HF, $n = 7$), fed a standard laboratory food enriched with 42% sunflower oil. After 12 weeks, animals were anesthetized and euthanized, and blood samples were obtained by cardiac puncture for subsequent analyses. From each blood sample the serum was separated and preserved to further analysis. In addition, the liver was excised and stored at -80 °C until analysis. The experimental protocols were approved by the official Vinca Institute's Ethical Committee for Experimental Animals and Veterinary Directorate – No. 323-07-02710/2017-05. Serum enzyme concentrations of ALP, ALT, and AST were determined using an automated analyzer according to the manufacturer's instructions and expressed as units per liter (U/L). Furthermore, the protein expression of NRF2 and PGC1- α in rat liver was determined using Western blot analysis as we previously showed [1].

RESULTS AND DISCUSSION

To elucidate the impact of HF diet on hepatic function and redox regulation, concentrations of ALP, ALT and AST in serum and the levels of protein expression of NRF2 and PGC1- α in rat liver were analyzed.

Obesity contributes to hepatocyte damage, which can be recognized via changes in the concentrations of various biomarkers of hepatic dysfunction in circulation [2]. Previous studies have reported elevated serum concentrations of ALP, ALT, and AST enzymes in rats fed a HF diet [8, 9]. We observed that HF diet significantly increased serum ALP concentration in rats, relative to rats fed a standard diet ($p < 0.001$) (Figure 1A). However, the serum ALT and AST concentrations were not significantly altered following the HF diet (Figures 1B and 1C). To investigate the impact of feeding with an HF diet on antioxidant defense signaling, we evaluated the expression of the transcriptional factor NRF2, which is also involved in lipid metabolism. Our results suggest that HF diet suppresses hepatic NRF2 protein expression ($p < 0.001$), which is associated with oxidative stress and, consequently, insulin resistance (Figure 1E).

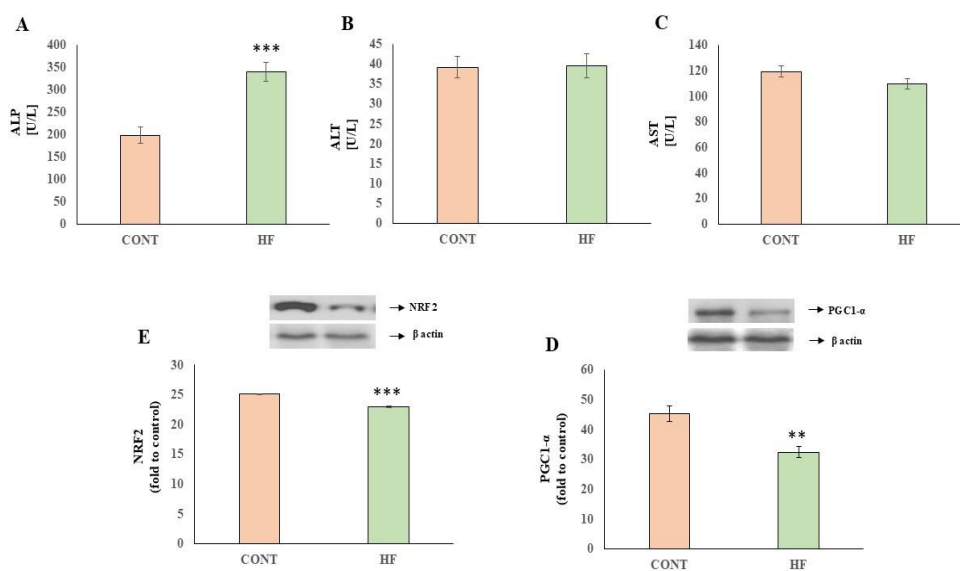


Figure 1. The ALP (A), ALT (B), and AST (C) concentrations in serum and protein expression, and representative blots of PGC1- α (D) and NRF2 (E) in the liver of control and HF rats. ALP - alkaline phosphatase, ALT - alanine aminotransferase, AST - aspartate aminotransferase, HF – high-fat, PGC1- α - peroxisome proliferator-activated receptor gamma coactivator 1-alpha, NRF2 - nuclear factor erythroid 2-related factor 2.

Consistent with our results, the literature indicates that HF diet decreases NRF2 protein expression in the liver [4, 5]. The transcriptional coactivator PGC1- α is involved in hepatic fatty acid oxidation by activating peroxisome proliferator-activated receptor target genes, thereby regulating metabolic processes under different dietary conditions. In our study obesity leads to reduction of PGC1- α protein expression ($p < 0.01$) in the liver (Figure 1D). Furthermore, results from other studies demonstrated that HF diet decreased the level of PGC1- α mRNA expression, which contributes significantly to insulin resistance and development of non-alcoholic fatty liver disease [6, 7].

CONCLUSION

These findings suggest that HF diet adversely affects hepatic function, as evidenced by significant elevation in serum ALP concentration and reduced hepatic protein expression of NRF2 and PGC1- α in the liver of obese rats relative to control rats. These findings indicate an increased inflammatory response and impaired antioxidant defense signaling in the liver of HF diet-fed rats, predisposing them to the development of insulin resistance.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No# 451-03-33/2026-03/ 200017).

REFERENCES

- [1] J. Stanimirovic, M. Obradovic, A. Jovanovic, E. Sudar-Milovanovic, S. Zafirovic, S.J. Pitt, A.J. Stewart, and E.R. Isenovic, *Nitric Oxide*, 2016, 54, 51-9.
- [2] S. Lasker, M.M. Rahman, F. Parvez, M. Zamila, P. Miah, K. Nahar, F. Kabir, S.B. Sharmin, N. Subhan, G.U. Ahsan, and M.A. Alam, *Scientific Reports*, 2019, 9, 20026.
- [3] A. Curcio, A. Romano, S. Cuzzo, A.D. Nicola, O. Grassi, D. Schiaroli, G.F. Nocera, and M. Pironti, *Medicina (Kaunas)*, 2020, 56.
- [4] P. Liu, A. Anandhan, J. Chen, A. Shakya, M. Dodson, A. Ooi, E. Chapman, E. White, J.G.N. Garcia, and D.D. Zhang, *Redox Biology*, 2023, 59, 102570.
- [5] S.M. Alamri, L.N. Al-Harbi, G.M. Alshammari, N.A. Albadr, A. Saleh, and M.A. Yahya, *Scientific Reports*, 2025, 15, 34888.
- [6] M. Azizollahi, Z. Nasehi, M. Derakhshan, and F. Zadhoush, 2025, 2025, 1565994.
- [7] Y. Abrahams, T. Willmer, O. Patel, E. Samodien, C.J.F. Muller, S. Windvogel, R. Johnson, and C. Pfeiffer, *Biochemical and Biophysical Research Communications*, 2023, 680, 25-33.
- [8] Y.M. Ezz-Eldin, M.G. Ewees, A.A. Azouz, and M.M. Khalaf, *Naunyn-Schmiedeberg's Archives of Pharmacology*, 2024, 397, 9067-9079.
- [9] A.Z. Mohd Rafie, A. Syahir, W.A.N. Wan Ahmad, M.Z. Mustafa, and A.R. Mariatulqabtiah, *Evidence-Based Complementary and Alternative Medicine*, 2018, 2018, 6371582.

CHOLINIUM SALICYLATE AS A HYDROTROPIC MEDIUM FOR IMPROVED RESVERATROL SOLUBILIZATION IN WATER AND PRESERVATION OF ANTIOXIDANT ACTIVITY

D. Tekić, J. Mušović, A. Jocić, S. Marić and A. Dimitrijević

*Vinča Institute of Nuclear Sciences – National Institute of the Republic of
Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade,
Serbia (danijela.tekic@vin.bg.ac.rs)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.48

ABSTRACT

Resveratrol (RSV) is a naturally occurring polyphenolic compound that has attracted considerable attention in recent decades due to its pronounced antioxidant activity and therapeutic potential, including anti-inflammatory, cardioprotective, and anticancer effects. However, its broader application is significantly limited by poor aqueous solubility. In this study, aqueous solutions of cholinium salicylate ([Ch][Sal]) were investigated as a potential medium for improving RSV solubilization. The influence of ionic liquid concentration on RSV solubility was evaluated to gain insight into the interactions governing the dissolution process. Additionally, the antioxidant activity of RSV in [Ch][Sal] aqueous media was assessed to determine whether the ionic liquid environment affects its functional properties.

INTRODUCTION

Resveratrol is widely recognized for its diverse biological activities, which have contributed to its growing interest in pharmaceutical, nutraceutical, and cosmetic applications [1]. This compound is biologically active in its trans configuration; however, under exposure to light, elevated temperature (above ~37 °C), oxygen, and basic media, it readily undergoes isomerization to the cis form, which exhibits significantly reduced biological activity [2]. Additionally, the practical application of RSV remains limited by its poor aqueous solubility, which leads to low bioavailability and challenges in formulation within water-based systems.

Various strategies have been explored over the years to overcome these limitations for RSV and other poorly water-soluble molecules. More recently, ionic liquids (ILs) have emerged as promising alternative media for enhancing the solubility of such compounds [3]. Owing to their highly tunable physicochemical properties, amphiphilic character, and ability to establish specific intermolecular interactions, ILs can act as efficient hydrotropic systems

capable of significantly improving the dissolution behavior of hydrophobic molecules.

Among different IL families, cholinium-based ionic liquids have attracted particular interest due to their favorable environmental profile and relatively low toxicity [4]. Their ability to participate in hydrogen bonding and stabilize dissolved molecules in aqueous media makes them attractive systems for solubilization of bioactive compounds.

Therefore, in this study, the influence of aqueous solutions of cholinium salicylate ([Ch][Sal]) on RSV solubilization behavior was investigated. Initially, the concentration of IL was varied from ~2 to ~25 wt% to evaluate its influence on RSV solubilization enhancement. In order to ensure that the functional properties of RSV were preserved under these conditions, its antioxidant activity was additionally evaluated in IL media at both lower (5 wt%) and higher (20 wt%) concentrations. Overall, the proposed method represents a potential solution to the problem of poor solubility and the practical utilization of bioactive compounds in aqueous formulations.

EXPERIMENTAL

Cholinium salicylate was synthesized via a neutralization reaction using cholinium hydroxide (46 wt% in water) solution (Sigma-Aldrich - USA) and salicylic acid (purity $\geq 99\%$, Sigma-Aldrich - USA), in an equimolar ratio. The obtained solution was stirred for an additional period to ensure reaction completion, followed by water removal under reduced pressure using a rotary evaporator (R-210 Rotavapor System, Büchi Labortechnik AG, Switzerland).

Resveratrol concentration was determined using a high-performance liquid chromatography (HPLC) system (Azura HPLC, Germany) equipped with a C18 column and diode-array detector (DAD). Separation was performed using a methanol/water gradient elution at a flow rate of 1 mL/min, injection volume of 20 μ L, and column temperature of 30 °C. Quantification was performed at 306 nm, and all solutions were prepared in 0.1% formic acid in water (pH = 2.70).

The antioxidant activity of RSV in IL media was evaluated using the DPPH radical scavenging assay. A DPPH stock solution (0.2 mM) was prepared in methanol. An aliquot of 10 μ L of RSV solution at its maximum solubility in aqueous ionic liquid media (containing 5 and 20 wt% IL) was mixed with 990 μ L DPPH solution and incubated in the dark at room temperature for 30 min. Antioxidant activity was simultaneously evaluated in ethanol at the same RSV concentrations to assess the influence of the IL medium. The decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer (LLG-uniSPEC2, Germany).

RESULTS AND DISCUSSION

The presence of IL in aqueous media significantly enhanced RSV solubility, achieving up to a 50-fold increase compared to water (Figure 1). This confirms their strong solubilizing (hydrotropic) effect through RSV-IL and IL-water interactions that disrupt water structure and favor solute stabilization. Solubility enhancement can be potentially attributed to π - π interactions between the aromatic salicylate anion and RSV and hydrogen bonding, further stabilizing the solute in solution.

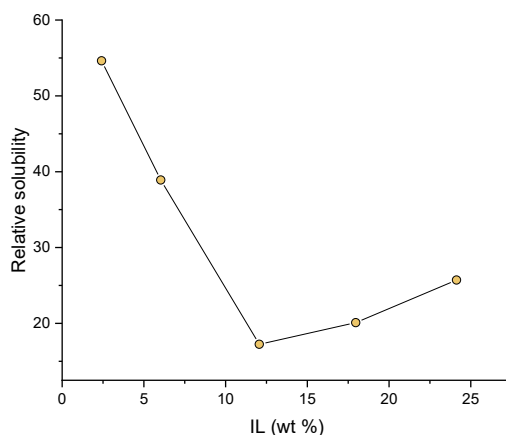


Figure 1. Dependence of the relative solubility of RSV on the IL mass percentage in aqueous media.

Additionally, IL concentration had a clear impact: the solubility increase was more pronounced at lower concentrations, where RSV-IL interactions dominate. At higher concentrations, enhanced IL-IL self-association likely reduces the availability of free IL species for direct interaction with RSV, leading to a less efficient incremental increase.

The system operates in a hydrotropic regime within the studied range (≤ 1 M), as no distinct minimum hydrotrope concentration was observed and only a weak sigmoidal trend was detected. This suggests gradual solubilization without formation of well-defined IL-rich aggregates.

After establishing that the ionic liquid significantly enhances the solubility of RSV, its antioxidant activity was subsequently evaluated.

The inhibition percentage of RSV in 5 wt% IL media at its maximum solubility resulted in 40.12% inhibition, while in 20 wt% IL media the inhibition was 33.06%. For comparison, at the same RSV concentrations in ethanol, inhibition values of 39.45% and 33.56% were obtained, respectively. These results indicate that the IL medium does not adversely affect the antioxidant activity of RSV, confirming that its radical scavenging capability is preserved in IL-based systems.

CONCLUSION

Ionic liquids demonstrated a significant enhancement of RSV aqueous solubility, achieving up to a 50-fold increase compared to water, confirming their strong hydrotropic capability and tunable solvation behavior. The influence of IL concentration was found to be crucial, with more pronounced solubilization effects observed at lower concentrations. Importantly, despite the strong solubilization effect, the antioxidant activity of RSV remained preserved in IL media. This indicates that the IL environment does not negatively affect the intrinsic radical scavenging properties of RSV. Overall, the results highlight the functionality of ILs as efficient solubilizing media that maintain the bioactivity of RSV, supporting their potential application in the formulation and processing of poorly water-soluble bioactive compounds.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number: 451-03-33/2026-03/ 200017).

REFERENCES

- [1] M. Koushki, N. Amiri-dashatan, N. Ahmadi, H.A.M. Rezaei-tavirani, Resveratrol: A miraculous natural compound for diseases treatment, *Food Science & Nutrition*, 2018, 6, 2473–2490.
- [2] Š. Zupančič, Z. Lavrič, J. Kristl, Stability and solubility of trans-resveratrol are strongly influenced by pH and temperature, *European Journal of Pharmaceutics and Biopharmaceutics*, 2015, 93, 196–204.
- [3] A.F.M. Cláudio, M.C. Neves, K. Shimizu, J.N. Canongia Lopes, M.G. Freire, J.A.P. Coutinho, The magic of aqueous solutions of ionic liquids: Ionic liquids as a powerful class of catanionic hydrotropes, *Green Chemistry*, 2015, 17, 3948–3963.
- [4] S. Shahriari, L.C. Tomé, J.M.M. Araújo, L.P.N. Rebelo, J.A.P. Coutinho, I.M. Marrucho, M.G. Freire, Aqueous biphasic systems: A benign route using cholinium-based ionic liquids, *RSC Advances*, 2013, 3, 1835–1843.

PRELIMINARY *IN SILICO* ADMET EVALUATION OF A NOVEL 1,4-DIHYDROPYRIDINE DERIVATIVE: A COMPARATIVE STUDY WITH BENIDIPINE

S. J. Tufegdžić¹, M. B. Živković¹, I. T. Novaković¹ and D. M. Sladić²

¹University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Studentski trg 12–16, 11000 Belgrade, Serbia (srdjan.tufegdzc@ihtm.bg.ac.rs)

²University in Belgrade, Faculty of Chemistry, Studentski trg 12–16, 11000 Belgrade, Serbia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.49

ABSTRACT

C4 stereochemistry is an important feature of 1,4-dihydropyridine (DHP) calcium channel blockers, but its impact on ADMET properties remains insufficiently explored. A novel unsymmetrical DHP derivative bearing an optically active (–)-menthyl ester was synthesized as a mixture of C4 diastereoisomers and evaluated *in silico* using pkCSM and ADMETlab 2.0, with benidipine as a reference drug. The new derivative showed favorable predicted absorption and distribution properties and more favorable predicted hERG and AMES profiles than benidipine, while a high predicted DILI probability was observed for both compounds. These findings support further experimental evaluation of the novel DHP scaffold, including the role of C4 stereochemistry in its ADMET properties.

INTRODUCTION

L-type calcium channels are well-established targets of 1,4-dihydropyridine (DHP) calcium channel blockers. Unsymmetrical ester substitution, common among newer DHPs, renders the C4 position stereogenic, and pronounced stereoselectivity in pharmacological activity has been reported [1,2]. Increasing evidence suggests that C4 stereochemistry may also affect pharmacokinetic and safety profiles [3]. In this study, a simplified unsymmetrical DHP bearing an optically active (–)-menthyl ester was designed to afford a diastereomeric mixture of C4 stereoisomers. The new DHP derivative was synthesized using a modified Hantzsch approach, and its *in silico* ADMET profile was comparatively evaluated against benidipine (Ben) as a reference DHP drug.

EXPERIMENTAL

Synthesis of 3-ethyl 5-((1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl) (*R/S*)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (C) was accomplished in three steps (Figure 1). Ethyl benzylideneacetoacetate (A) was

prepared according to the reported procedure [4], while menthyl acetoacetate (B) was synthesized by catalyst-free, solvent-free microwave-assisted transesterification of ethyl acetoacetate with (–)-menthol [5]. The target DHP derivative C was obtained by Hantzsch cyclocondensation of A and B in refluxing ethanol using ammonium carbonate as the ammonia source [6]. The product was obtained as a mixture of two C4 diastereoisomers in an approximately 6:4 ratio, as determined by GC-MS analysis. Its structure was further supported by IR spectroscopy.

In silico predictions were performed using pkCSM [7] and the ADMETlab 2.0 web server [8].

RESULTS AND DISCUSSION

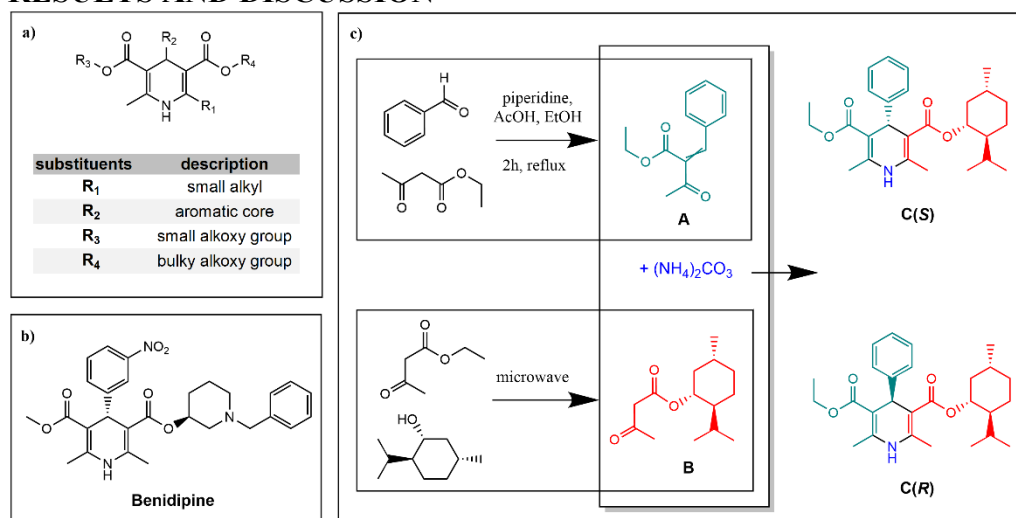


Figure 1. (a) General structure of 1,4-dihydropyridines (DHPs); (b) structure of benidipine (Ben); (c) synthetic route to the novel DHP derivative.

The general DHP scaffold, the structure of benidipine, and the synthetic route to C, including the preparation of the β -ketoester precursors, are presented in Figure 1. The target derivative was obtained as a mixture of two C4 diastereoisomers in an approximately 6:4 ratio, as determined by GC-MS analysis.

Predicted molecular properties and drug-likeness parameters of C and benidipine are summarized in Table 1. The novel DHP derivative C exhibited higher predicted lipophilicity and lower molecular weight, while both compounds satisfied the drug-likeness criteria with only one Lipinski Rule of Five violation. Compared with the reference drug, C showed lower TPSA, fewer rotatable bonds, and fewer hydrogen-bond acceptors.

Table 1. Molecular properties and drug-likeness of the novel DHP derivative (C) and benidipine (Ben).

(MW – molecular weight; log *P* – lipophilicity; rotB – number of rotatable bonds; HBA/HBD – number of hydrogen bond acceptors/donors; TPSA – topological polar surface area (Å²); *n*_{viol} – number of violations of Lipinski Rule of Five criteria; Ref. – recommended reference ranges for drug-likeness and oral bioavailability)

Compound	MW ^a	log <i>P</i> ^a	rotB ^a	HBA ^a	HBD ^a	TPSA ^b	<i>n</i> _{viol}
C	440	5.49	6	5	1	65	1
Ben	506	4.21	7	8	1	111	1
Ref.	≤500	≤5	≤10	≤10	≤5	≤140	≤1

^apkCSM ^bADMETlab2.0

Predicted ADMET profiles of C and benidipine as a reference DHP drug are compared in Table 2.

Table 2. Comparative predicted ADMET profiles of the novel DHP derivative (C) and benidipine (Ben).

(Compd. – compound; IA – intestinal absorption (%); VDss – steady-state volume of distribution (log L/kg); CYP – number of interacting cytochrome P450 isoforms; CL – clearance (mL/min/kg); hERG – hERG inhibition (cardiotoxicity); DILI – drug-induced liver injury; AMES – AMES mutagenicity)

Compd.	IA ^a	VDss ^a	BBB ^a	CYP ^b	CL ^b	hERG ^b	DILI ^b	AMES ^b
C	92	0.39	-0.13	7	15.1	0.642	0.969	0.012
Ben	86	0.61	-0.77	7	17.1	0.698	0.966	0.564

^apkCSM ^bADMETlab2.0

Both compounds showed favorable predicted absorption and distribution, with C exhibiting higher predicted IA and BBB penetration probability, consistent with its higher lipophilicity and lower TPSA, whereas benidipine showed a higher predicted VDss. C and benidipine were predicted to interact with seven CYP isoforms, and both showed high predicted total clearance. A high predicted DILI probability was observed for both compounds, whereas C exhibited more favorable predicted hERG and, particularly, AMES profile than benidipine.

CONCLUSION

The novel unsymmetrical DHP derivative was successfully synthesized as a mixture of C4 diastereoisomers and exhibited a favorable predicted drug-likeness and ADMET profile. Compared with benidipine, the new derivative showed more favorable predicted hERG and AMES profiles, while both compounds exhibited a high predicted probability of DILI. These findings support the

potential of the new DHP scaffold for further experimental pharmacological and ADMET evaluation.

Acknowledgement

This work was partially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants no. 451-03-33/2026-03/200026, and 451-03-136/2026-03/200168).

REFERENCES

- [1] A. L. Wang, C. Iadecola, G. Wang, *J. Geriatr. Cardiol.*, 2017, 14, 67 – 72.
- [2] G. Ananchenko, J. Novakovic, J. Lewis, Amlodipine Besylate in: Profiles of Drug Substances, Excipients, and Related Methodology, Vol. 37, H.G. Brittain (Ed.), Academic Press, Oxford, 2012, 31 – 77.
- [3] D. B. Tikhonov, B. S. Zhorov, *J. Gen. Physiol.*, 2023, 155, e202313418.
- [4] M. A. Buil, M. Calbet, M. Castillo, J. Castro, C. Esteve, M. Ferrer, P. Forns, J. Gonzalez, S. Lopez, R. S. Roberts, S. Sevilla, B. Vidal, L. Vidal, P. Vilaseca, *Eur. J. Med. Chem.*, 2016, 113, 102 – 133.
- [5] M. Gianotti, G. Martelli, G. Spunta, E. Campana, M. Panunzio, M. Mendoza, *Synth. Commun.*, 2000, 30, 1725 – 1730.
- [6] P. A. Hopes, A. J. Parker, I. Patel, *Org. Process Res. Dev.*, 2006, 10, 808 – 813.
- [7] <https://biosig.lab.uq.edu.au/pkcsim/prediction>
- [8] <https://admetmesh.scbdd.com/service/evaluation/index>

VALORIZATION OF SPENT COFFEE GROUNDS INTO CARBON-BASED ADSORBENTS FOR CRYSTAL VIOLET REMOVAL FROM WATER

T. Terzić¹, V. Milanković¹, N. Radivojević¹, I. Pašti², V. Anićijević³, T. Lazarević-Pašti¹ and C. Unterweger⁴

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia*

²*University of Belgrade – Faculty of Physical Chemistry, Studentski Trg 12–16, 11158 Belgrade, Serbia*

³*Military Academy, University of Defence in Belgrade, Veljka Lukica Kurjaka 33, 11042 Belgrade, Serbia*

⁴*Wood K Plus—Kompetenzzentrum Holz GmbH, Altenberger Strasse 69, 4040 Linz, Austria (c.unterweger@wood-kplus.at)*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.50

ABSTRACT

Spent coffee grounds represent an abundant food-related waste material that can be converted into value-added carbon-based materials for environmental applications. In this study, two carbon adsorbents prepared from spent coffee grounds were evaluated for the removal of crystal violet (CV) from water. The prepared materials were characterized by SEM and BET analysis, while CV adsorption was investigated in batch mode. The maximum adsorption capacities were 360 and 374 mg g⁻¹, confirming the high efficiency of both materials. These results indicate that spent coffee grounds can be efficiently valorized as low-cost precursors for sustainable adsorbents for the treatment of dye-contaminated water.

INTRODUCTION

Crystal violet is a synthetic cationic dye whose release into wastewater may cause environmental concern due to its persistence, strong coloration, and potential toxicity [1]. Adsorption represents an efficient and environmentally suitable strategy for dye removal, particularly when low-cost carbon-based materials are used as adsorbents [2]. In this study, spent coffee grounds were valorized as a biomass precursor for the preparation of two carbon-based adsorbents and evaluated for the removal of CV from water, highlighting the potential of food waste-derived materials for sustainable water remediation.

EXPERIMENTAL

Spent coffee grounds (100% Arabica) were collected from the coffee machine at the Wood K plus office in Linz. Half of the sample was additionally extracted in boiling water for 1 h, filtered and washed three times with boiling water. Both samples were dried for 24 h at 105 °C. Afterwards the samples were carbonized in a GERO HTK8 chamber furnace at 900 °C in N₂ atmosphere (200 L h⁻¹) using the following program: 10 °C min⁻¹ up to 200 °C – 1 °C min⁻¹ up to 600 °C – 10 °C min⁻¹ up to 900 °C – 30 min isothermal at 900 °C. For each carbonized sample, 10 g were subsequently activated in a Nabertherm RSR rotary tube furnace at 870 °C for 60 min using a CO₂ flow of 80 L h⁻¹. The activated carbons were denoted as “SCG_a60” and “wSCG_a60” for the sample from the original SCG and the washed one, respectively.

The prepared materials were characterized by scanning electron microscopy (SEM). Nitrogen adsorption–desorption measurements were performed, and the specific surface area was calculated by the Brunauer-Emmett-Teller (BET) method.

CV adsorption was investigated in batch mode using deionized water for solution preparation. Experiments were performed at pH 7, 25 °C, with an adsorbent concentration of 1 mg mL⁻¹ and a contact time varied from 1 to 1440 min. After adsorption, the suspensions were centrifuged, and the residual CV concentration was determined by UV-Vis spectrophotometry. Control experiments were performed without adsorbent, while removal efficiency and adsorption capacity were calculated according to $\text{Uptake} = 100\% \times (C_0 - C_{\text{eq}})/C_0$, where C_0 represents the initial CV concentration, and C_{eq} corresponds to the equilibrium concentration.

RESULTS AND DISCUSSION

SEM micrographs showed irregular, porous, and fragmented carbonaceous structures for both samples, with visible surface roughness and cavities that may contribute to the accessibility of adsorption sites for CV removal.

The specific surface area of the prepared materials, determined by the BET method, was 436 m² g⁻¹ for sample SCG_a60 and 525 m² g⁻¹ for sample wSCG_a60, confirming that both materials possessed a well-developed porous structure, with sample wSCG_a60 showing a slightly higher surface area.

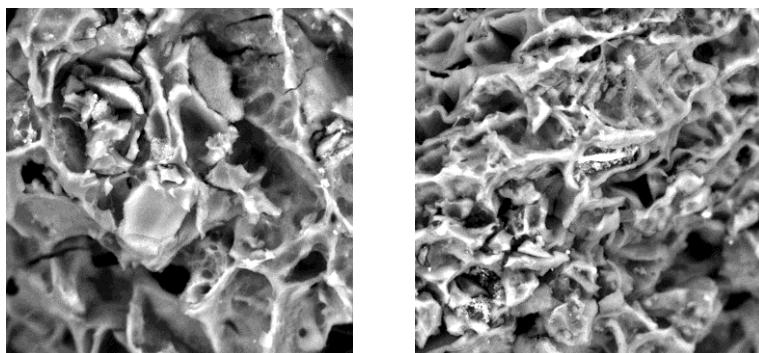


Figure 1. SEM micrographs of SCG_a60 (left) and wSCG_a60 (right)

The adsorption behavior of CV on the prepared carbon materials was further evaluated using kinetic and isotherm models to assess adsorption rate, model fit, and maximum adsorption capacity. Non-linear pseudo-first-order (PFO) and pseudo-second-order (PSO) models were used. For both materials, the PSO model provided a slightly better fit (Table 1), suggesting that both adsorbents exhibited similar kinetic behavior, with sample wSCG_a60 exhibiting a slightly higher calculated q_e value. The time of equilibrium was determined as 1440 min. The adsorption isotherms were fitted using the Freundlich and Langmuir models (Table 2), with the Langmuir model showing better agreement with the experimental data. The maximum adsorption capacities were 360 mg g^{-1} for sample SCG_a60 and 374 mg g^{-1} for sample wSCG_a60, confirming the high efficiency of both materials toward CV. The somewhat better performance of sample wSCG_a60 is consistent with its higher specific surface area.

Table 1. Kinetic parameters of CV ($5 \times 10^{-6} \text{ mol dm}^{-3}$) adsorption on investigated materials (1 mg mL^{-1}) at 25°C

Material	PFO			
	$q_e \text{ (mg g}^{-1}\text{)}$	$k_1 \text{ (min}^{-1}\text{)}$	χ^2	R^2
SCG_a60	1.793	0.032	0.026	0.940
wSCG_a60	1.883	0.045	0.023	0.948
	PSO			
	$q_e \text{ (mg g}^{-1}\text{)}$	$k_2 \text{ (mg min}^{-1} \text{ g}^{-1}\text{)}$	χ^2	R^2
SCG_a60	1.879	0.024	0.015	0.965
wSCG_a60	1.958	0.036	0.018	0.960

Table 2. Parameters of the Freundlich and Langmuir isotherm models for CV adsorption at an adsorbent dose of 1 mg mL⁻¹

Material	Freundlich isotherm			
	$K_F ((\text{dm}^3 \text{ mg}^{-1})^{1/n})$	n	χ^2	R^2
SCG_a60	53.483	2.734	2681.795	0.856
wSCG_a60	34.808	2.272	683.154	0.960
	Langmuir isotherm			
	$K_L (\text{dm}^3 \text{ mg}^{-1})$	$q_{\text{max}} (\text{mg g}^{-1})$	χ^2	R^2
SCG_a60	0.078	359.935	671.074	0.964
wSCG_a60	0.034	373.634	20.699	0.999

CONCLUSION

Spent coffee grounds were successfully converted into carbon-based adsorbents for the removal of CV from water. Both materials showed high adsorption capacities, while sample wSCG_a60 exhibited slightly better performance, consistent with its higher specific surface area. These results highlight spent coffee grounds as a promising, low-cost precursor for sustainable adsorbents for the remediation of dye-contaminated water.

Acknowledgement

This work was funded by the Serbian Ministry of Science, Technological Development, and Innovations (grant numbers 451-03-34/2026-03/200146 and 451-03-33/2026-03/200017). The authors also acknowledge support from the Serbian Academy of Sciences and Arts (project no. F-49), the Serbian Ministry of Defence, Republic of Serbia (project code: VA-TT/1/22-24), and the COMET Programme operated by the FFG (WOOD, 892416).

REFERENCES

- [1] M. Kumar, A. Mishra, S.K. Patel, J. Kushwaha, S. Singh, V. Mishra, D. Singh, V. Singh, B.S. Giri, R.R. Singhanian, D. Singh, *Bioengineering*, 2025, 12, 1043.
- [2] R. Kurtić, T. Tasić, V. Milanković, V.J. Anićijević, L. Rakočević, N. Potkonjak, C. Unterweger, I.A. Pašti, T. Lazarević-Pašti, *Processes*, 2025, 13, 3357.

ELEMENTAL PROFILE AND NUTRITIONAL ASSESSMENT OF DIFFERENT AVOCADO CULTIVARS

M. Vukomanović¹, B. Simonović², I. Grbić¹, A. Krstić³ and N. Pantelić¹

¹Department of Chemistry and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

(marko.vukomanovic@agrif.bg.ac.rs)

²Institute for Medical Research, National Institute of the Republic of Serbia, Dr Subotića 4, 11029 Belgrade, Serbia

³Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.51

ABSTRACT

The mineral composition of three avocado (*Persea americana* Mill.) cultivars (Arad, Pinkerton, and Hass) was determined by inductively coupled plasma optical emission spectrometry (ICP-OES), followed by a theoretical assessment of their nutritional contribution based on the recommended daily intake of essential minerals. Potassium was the predominant macroelement in all cultivars, while zinc was the most abundant microelement. Arad showed the highest concentrations of most essential elements, whereas Pinkerton was characterized by the highest calcium content. The results demonstrated cultivar-dependent differences in mineral composition and indicated that the investigated avocado cultivars are good dietary sources of potassium and manganese, with additional contributions of zinc and iron.

INTRODUCTION

Avocado (*Persea americana* Mill.) is a nutritionally important fruit whose mineral composition depends on both cultivar and growing conditions. Essential macro- and microelements present in avocado play important physiological roles in human nutrition, while their concentrations may vary considerably among cultivars, making their characterization important for evaluating fruit nutritional quality [1].

The present study investigated the elemental composition of three commercially important avocado cultivars (Arad, Pinkerton, and Hass). In addition, a theoretical assessment of their nutritional value was performed based on the content of essential macro- and microelements in order to evaluate their contribution to the recommended dietary intake.

EXPERIMENTAL

ICP-OES Analysis

A total of ten elements were analysed in avocado samples: calcium (Ca), cadmium (Cd), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), lead (Pb), phosphorus (P), sulphur (S) and zinc (Zn), using optical emission spectrometry with inductively coupled plasma (ICP-OES) technique, according to previously described procedures [2].

Dietary Intake of Nutritional Elements

Dietary intake (nutritional contribution) of essential elements (K, Ca, Mg, Fe, Mn, and Zn) from 100 g of avocado was calculated based on the concentrations of elements, compared with nutritional reference values (NRV) and expressed in percentage. NRV values are established by the National Institutes of Health and the Institute of Medicine [3].

RESULTS AND DISCUSSION

Content of the Major and Trace Elements

The presence and concentrations of ten elements were examined by ICP-OES technique. Comparison was done based on the content of five macroelements (K, Ca, Mg, P and S) and five microelements (Cd, Fe, Mn, Pb and Zn).

The concentrations of the major macroelements determined in the three avocado varieties are presented in Table 1. Potassium was the predominant macroelement in all three avocado varieties. This is in accordance with the literature, showing potassium as the most abundant element in avocados [4]. Magnesium, phosphorus, sulfur, and calcium were also found in relatively large quantities. Arad consistently exhibited the highest concentrations of potassium (15,320.07 mg/kg), magnesium (248.30 mg/kg), phosphorus (130.39 mg/kg), and sulfur (94.71 mg/kg), indicating a greater capacity for the accumulation of these essential minerals. Potassium content in Arad was approximately 1.5-fold higher than in Pinkerton and more than 2.5-fold higher than in Hass. In contrast, Pinkerton was distinguished by its calcium concentration (130.37 mg/kg), which was more than twice that of Arad (60.53 mg/kg) and almost twofold higher than Hass (71.25 mg/kg).

Among the microelements (Table 1), zinc was the most abundant, followed by iron and manganese. Arad contained the highest concentrations of zinc (17.61 mg/kg) and manganese (5.52 mg/kg), while iron levels were comparable in Arad (16.88 mg/kg) and Hass (16.98 mg/kg) but substantially lower in Pinkerton (6.67 mg/kg). Although cadmium was detected only at trace levels (up to 0.03 mg/kg), differences were observed in lead accumulation, ranging from 0.32 mg/kg in Pinkerton to 1.81 mg/kg in Arad.

Table 1. Content of macro- and microelements (mg/kg $\pm$ SD) in the tested avocado cultivars.

Element	Arad	Pinkerton	Hass
K	15320 $\pm$ 190	10160 $\pm$ 167	5868 $\pm$ 72
Mg	248.3 $\pm$ 1.8	182.8 $\pm$ 0.6	163.0 $\pm$ 2.8
Ca	60.53 $\pm$ 0.03	130.4 $\pm$ 2.2	71.25 $\pm$ 0.05
P	130.4 $\pm$ 0.2	111.8 $\pm$ 0.7	78.46 $\pm$ 0.04
S	94.71 $\pm$ 0.14	77.47 $\pm$ 0.22	56.80 $\pm$ 1.77
Cd	0.02 $\pm$ 0.01	<0.01	0.03 $\pm$ 0.01
Fe	16.88 $\pm$ 1.78	6.67 $\pm$ 0.20	16.98 $\pm$ 0.03
Mn	5.52 $\pm$ 0.10	4.00 $\pm$ 0.12	4.07 $\pm$ 0.03
Pb	1.81 $\pm$ 0.01	0.32 $\pm$ 0.01	1.24 $\pm$ 0.02
Zn	17.61 $\pm$ 0.05	14.51 $\pm$ 0.07	13.32 $\pm$ 0.03

Dietary Intake of Nutritional Elements

The estimated contribution of avocado consumption to the recommended daily intake varied considerably among the analyzed minerals (Table 2).

Potassium provided the greatest nutritional contribution, supplying 17.3–45.1% of the daily requirement for men and 22.6–58.9% for women per 100 g, highlighting avocado as an excellent dietary source of this essential macroelements. Manganese also contributed substantially, covering 17.4–24.0% and 22.2–30.7% of the recommended intake for men and women, respectively. Zinc provided a moderate contribution (12.1–22.0%), while iron supplied up to 21.2% of the recommended intake for men but only 3.7–9.4% for women because of their higher physiological requirements. In contrast, magnesium contributed modestly (3.9–7.8%), whereas phosphorus and calcium accounted for less than 2% of the recommended daily intake.

CONCLUSION

The investigated avocado cultivars showed distinct differences in their mineral composition. Arad contained the highest overall levels of essential macro- and microelements, while Pinkerton was characterized by the highest calcium content. The nutritional assessment indicated that all cultivars represent good dietary sources of potassium and manganese, with additional contributions of zinc and iron. These findings confirm the nutritional importance of avocado and the influence of cultivar on its mineral composition.

Table 2. Daily intake estimations of selected macro- and microelements through the consumption of 100 g of tested avocado cultivars.

Element	Daily Intake (mg/100 g)	RDA/AI* (F) (mg/day)	RDA/AI* (M) (mg/day)	% Intake (F)	% Intake (M)
Mg	16.30-24.83	320	420	5.1-7.8	3.9-5.9
P	7.85-13.04	700	700	1.1-1.9	1.1-1.9
K	586.84-1532.01	2600	3400	22.6-58.9	17.3-45.1
Ca	6.05-13.04	1000	1000	0.6-1.3	0.6-1.3
Mn	0.40-0.55	1.8	2.3	22.2-30.7	17.4-24.0
Fe	0.67-1.70	18	8	3.7-9.4	8.3-21.2
Zn	1.33-1.76	8	11	16.7-22.0	12.1-16.0

* AI: Adequate Intake; RDA: Recommended Daily Allowance; M: male 31–50 years old; F: female 31–50 years old.

Acknowledgement

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant numbers: 451-03-34/2026-03/ 200116 and 451-03-33/2026-03/ 200017).

REFERENCES

- [1] A. Rojas-García, E. Fuentes, M. L. Cádiz-Gurrea, L. Rodríguez, M. C. Villegas-Aguilar, I. Palomo, D. Arráez-Román, A. Segura-Carretero, *Antioxidants*, 2022, 11, 1049.
- [2] United States Environmental Protection Agency, Method 6010D (SW-846): Inductively Coupled Plasma-Atomic Emission Spectrometry, Revision 4, Washington, DC, 2014.
- [3] National Institutes of Health Office of Dietary Supplements, Nutrient Recommendations and Databases.
- [4] N. A. Ford, P. Spagnuolo, J. Kraft, E. Bauer, *Foods*, 2023, 12, 2516.

SAFETY EVALUATION AND ANTIVIRULENCE ACTIVITY OF *Symphytum officinale* ETHANOL EXTRACT

S. Vuletić¹, J. Jocković¹, N. Borjan², A. Ignjatijević¹, T. Anđić¹ and S. Spasović¹

¹University of Belgrade, Faculty of Biology,

Studentski trg 16, 11000 Belgrade, Serbia (stefana.d@bio.bg.ac.rs)

²Institute of Meat Hygiene and Technology, Kacanskog 13, 11040 Belgrade, Serbia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.52

ABSTRACT

The increased frequency of bacterial resistance nowadays leads to the necessity for novel therapeutic strategies targeting bacterial virulence rather than its viability. Among numerous pathogens, *Staphylococcus aureus* represents a serious problem due to its resistance and numerous virulence factors. Thus, the aim of the study was to chemically characterize *Symphytum officinale* extract and investigate its antibacterial, antivirulence, and toxicity potential. LC-MS/MS analysis identified rosmarinic acid as the predominant compound. Although the extract showed no antibacterial activity against *S. aureus*, it inhibited staphyloxanthin production up to 26.5 %. Moreover, the MTT assay on the MRC-5 cell line demonstrated no cytotoxic effect at tested concentrations (0.03125 – 1 mg/mL). These findings suggest that *S. officinale* may represent a promising source of natural antivirulence compounds, but further investigations are needed.

INTRODUCTION

Antibacterial resistance has become one of the most significant global public health challenges that reduces the effectiveness of conventional antibiotics. This problem emphasizes the urgent need for novel agents, targets, and alternative strategies. Among these, antivirulence approaches have attracted considerable attention since they attenuate bacterial pathogenicity rather than inhibit their growth [1]. *Staphylococcus aureus* represents one of the most important pathogens responsible for a wide range of community- and hospital-acquired infections due to its resistance, ability to form biofilms, and multiple virulence factors. One of its key virulence factors is staphyloxanthin, a golden carotenoid pigment that protects bacterial cells from oxidative stress and host immune responses [2]. Natural products, particularly medicinal plants, attract attention since they are a valuable source of bioactive compounds and may potentially provide plausible alternatives to antibiotics. Besides efficacy, the safety profile of natural products is crucial for their potential pharmaceutical application,

highlighting the importance of simultaneous evaluation of biological activity and cytotoxicity [3]. *Symphytum officinale* L. (Boraginaceae), known as comfrey, is traditionally used for the treatment of inflammatory conditions and wound healing. It contains a wide variety of bioactive constituents including phenolics, flavonoids, saponins, alkaloids, tannins, etc [4]. Hence, the aim of the study was to chemically characterize the chemical profile of *S. officinale* extract and to evaluate its antibacterial activity, effect on staphyloxanthin production, and cytotoxicity.

EXPERIMENTAL

The extract of *S. officinale* root (Institute „Dr Josif Pančić”, Belgrade) was prepared using a 24-hour extraction method described by Ignjatijević et al. [5], with 70% ethanol as the solvent. The chemical profile of the ethanol extract was analyzed by LC-MS/MS [6, 7]. Antibacterial activity of the extract (0.0156 – 2 mg/mL) was assessed by applying the microdilution assay, with resazurin used to determine minimal inhibitory concentrations (MIC) [8]. The MIC assay was conducted on *S. aureus* ATCC 29213 and *S. aureus* MRSA ATCC 43300 strains. Inhibition of staphyloxanthin production was determined as described by Vuletić et al. [9] using 250 µg/mL extract treatment. In order to investigate the cytotoxicity of the extract, an MTT assay was performed on the MRC-5 cell line (concentration range 0.0325 – 2 mg/mL) according to Ignjatijević et al. [5].

RESULTS AND DISCUSSION

The obtained results of LC-MS/MS analysis are presented in Table 1. They revealed that rosmarinic acid was the most abundant compound in *S. officinale* extract, followed by caffeic acid, while other compounds were identified in markedly low concentrations (coumaric and neochlorogenic acid, rutin, quercitrin).

Table 1. Chemical analysis of *S. officinale* extract.

Compound	µg/g dw
Caffeic acid	200
Chlorogenic acid	15.5
Rosmarinic acid	9955.7

Although no antibacterial activity was observed in the tested concentration range, previous studies demonstrated antibacterial activity of *S. officinale*. Differences in extraction procedures, solvent type, plant origin, and growth conditions may influence its chemical composition and consequently its biological activity [8]. Furthermore, it was demonstrated that *S. officinale* extract inhibited staphyloxanthin production of both tested strains at a concentration of 250 µg/mL

(Figure 1). The production was inhibited by 26.5% for *S. aureus* ATCC 29213 (Figure 1a) and 25.6% for *S. aureus* MRSA ATCC 43300 (Figure 1b).

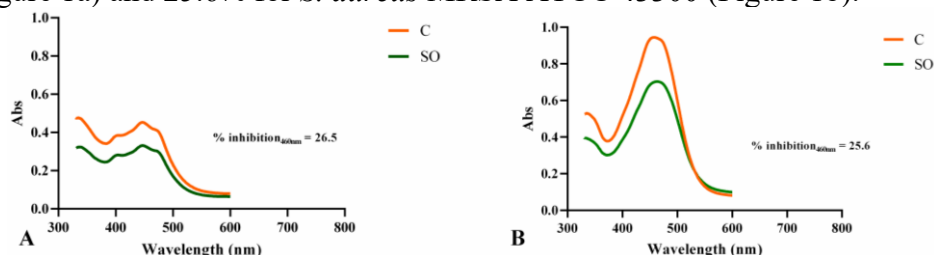


Figure 1. Absorption spectra of staphyloxanthin under the influence of *S. officinale* extract in the *S. aureus* ATCC 29213 (A) and *S. aureus* ATCC 43300 MRSA (B) strains. C-control; SO – treatment with extract.

Considering the fact that inhibiting pigment synthesis could make cells more sensitive to the effects of therapeutic agents, these results are important. To the best of our knowledge, this is the first report demonstrating the effect of *S. officinale* extract on staphyloxanthin production, supporting its previously described antivirulence potential [4].

MTT assay revealed that the extract did not exhibit cytotoxicity toward the MRC-5 cell line at the tested concentrations (Figure 2), supporting its favorable safety profile.

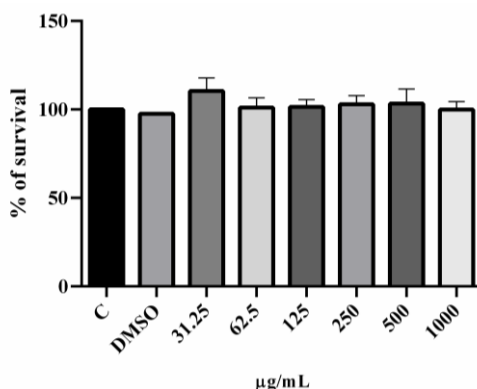


Figure 2. Cytotoxic potential of *S. officinale* extract on MRC-5 cell line.

Rosmarinic acid, identified as the dominant component of the extract, has previously been reported to possess antimicrobial and antivirulence properties, suggesting it may contribute to the observed antivirulence activity [10]. Nevertheless, the biological activity of *S. officinale* extract is likely the result of the synergistic action of multiple phytochemicals. Taken together, these findings indicate that although comfrey extract did not exhibit antibacterial activity, it

reduced *S. aureus* virulence without affecting human cell viability, supporting its potential as a safe source of natural antivirulence agents.

CONCLUSION

The obtained results revealed that the *S. officinale* extract is rich in phenolic compounds, with rosmarinic acid being the most abundant constituent. Even though no direct antibacterial activity was observed, the extract inhibited staphyloxanthin production of both tested *S. aureus* strains and showed no cytotoxicity toward the MRC-5 cell line. These findings highlight the potential of *S. officinale* as a promising source of safe natural compounds and warrant further investigation of its underlying mechanisms.

Acknowledgement

This work was partially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants no. 451-03-34/2026-03/200178 and 451-03-33/2026-03/200178). UN Sustainable Development Goals SDG3: Good Health and Well-being.

REFERENCES

- [1] R. Dehbanipour, Z. Ghalavand, *Germs*, 12(2), 262, 2022.
- [2] R. A. Elmesseri, S. E. Saleh, H. M. Elsherif, I. S. Yahia, K. M. Aboshanab. *Antibiotics*, 11(3), 298, 2022.
- [3] S. E. Rossiter, M. H. Fletcher, W. M. Wuest. *Chemical reviews*, 117(19), 12415-12474, 2017.
- [4] X. Liu, X. Huang, X. Song. *Frontiers in Pharmacology*, 17, 1793084, 2026.
- [5] A. Ignjatijević, T. Anđić, M. Lješević, B. Nikolić, T. Ganić, S. Spasović, S. Vuletić. *Pharmaceutics*, 17(9), 1134, 2025.
- [6] G. A. Pereira, H. S. Arruda, D. R. de Morais, N. M. P. Araujo, G. M. Pastore, *Food chemistry*, 310, 125857, 2020.
- [7] U. R. Rinik, J. E. Kim, E. Lee, O. Kwon, B.H. Jung. *Journal of Chromatography B*, 1232, 123957, 2024.
- [8] S. Đukanović, S. Cvetković, B. Lončarević, M. Lješević, B. Nikolić, N. Simin, K. Bekvalac, D. Kekić, D. Mitić-Ćulafić, *Industrial Crops and Products*, 158, 2020.
- [9] S. Vuletić, T. Ganić, B. Lončarević, S. Cvetković, B. Nikolić, M. Lješević, D. Mitić-Ćulafić. *Comprehensive Plant Biology*, 49(1), 1-14, 2025.
- [10] M. Kostić, M. Stojković, D. Soković, M. South African Journal of Botany, 146, 521-527, 2022.

PORTABLE COLORIMETRIC DETECTION OF Hg^{2+} IN WATER USING ECO-FRIENDLY PAPER-BASED SENSOR

N. Radivojević¹, T. Terzić¹, S. Knežević Graovac¹, S. Graovac², V. Rajić¹, M. Milanković¹, N. Potkonjak¹, S. Weheabby³, I. Pašti⁴, T. Lazarević-Pašti¹ and V. Milanković¹

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia*

²*Faculty of Physics, University of Belgrade, POB 44, 11001 Belgrade, Serbia*

³*Measurement and Sensor Technology, Chemnitz University of Technology, Chemnitz 09126, Germany (saddam.weheabby@etit.tu-chemnitz.de)*

⁴*University of Belgrade - Faculty of Physical Chemistry, Studentski Trg 12-16, 11158 Belgrade, Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.53

ABSTRACT

Mercury is among the most hazardous heavy metals in aquatic systems due to its toxicity, persistence, and bioaccumulation, posing significant risks to ecosystems and human health. This study presents a sustainable, and low-cost approach for Hg^{2+} detection based on green-synthesized silver nanoparticles (AgNPs). AgNPs were prepared using an ethanolic extract of *Juglans regia* as a natural reducing and stabilizing agent and immobilized onto filter paper to fabricate a portable colorimetric sensor for Hg^{2+} detection. The sensing mechanism is based on the specific interaction between Hg^{2+} and AgNPs, producing a distinct color change visible to the naked eye. The sensor exhibited a linear detection range from 1×10^{-3} to 1×10^{-8} mol dm^{-3} , with a limit of detection of 1.065×10^{-8} mol dm^{-3} . The proposed platform offers eco-friendly synthesis, low production cost, portability, and instrument-free operation, making it a promising candidate for rapid on-site monitoring of Hg^{2+} contamination in water.

INTRODUCTION

Mercury is one of the most toxic and persistent heavy metals in aquatic environments, posing serious risks to ecosystems and human health. Although conventional techniques such as ICP-MS and AAS provide excellent sensitivity, they require expensive instrumentation and laboratory facilities, limiting their use for rapid on-site monitoring [1]. Silver nanoparticles (AgNPs) have emerged as promising colorimetric probes due to their localized surface plasmon resonance response toward Hg^{2+} . Immobilization of AgNPs onto cellulose filter paper further enhances portability and ease of use, although many reported sensors rely on chemically synthesized nanoparticles [2]. In this work, AgNPs were green-synthesized using *Juglans regia* extract and immobilized onto filter

paper to fabricate a portable colorimetric sensor for Hg^{2+} detection. The proposed platform combines sustainable nanoparticle synthesis with a simple paper-based format, offering a sensitive, low-cost, and environmentally friendly approach for rapid mercury monitoring in water.

EXPERIMENTAL

AgNPs were synthesized via a green route using an ethanolic extract of *Juglans regia* as both reducing and stabilizing agent. The nanoparticles were characterized by UV-Vis, FTIR, SEM-EDX, XRD, DLS, zeta potential, and ICP-OES analyses. A paper-based sensing platform was fabricated by immersing filter paper in the AgNP suspension, followed by air drying. Colorimetric detection of Hg^{2+} was evaluated by exposing the AgNP-coated paper to Hg^{2+} solutions of different concentrations. Sensor selectivity was investigated against various potentially interfering metal ions and common inorganic anions. Color changes were quantified from digital images using a custom Python/OpenCV image-processing algorithm based on a yellow index derived from RGB values. Analytical performance was assessed in terms of linear range, limit of detection, limit of quantification, and recovery using Hg^{2+} -spiked tap water samples.

RESULTS AND DISCUSSION

Green synthesis using *Juglans regia* extract successfully produced silver nanoparticles, as confirmed by UV-Vis, FTIR, DLS, XRD, FESEM-EDX, ICP-OES, and zeta potential analyses. UV-Vis spectroscopy showed the characteristic LSPR band at 405 nm, while XRD confirmed the formation of crystalline face-centered cubic silver. FESEM revealed predominantly spherical nanoparticles with an average diameter of approximately 30 nm, and EDX verified Ag as the major elemental component. FTIR spectra indicated that plant-derived biomolecules acted as reducing and stabilizing agents, whereas DLS and zeta potential measurements confirmed the formation of a moderately stable colloidal suspension suitable for sensor fabrication.

The colloidal AgNPs responded to several metal ions, with Hg^{2+} producing the most pronounced color change. However, the colloidal system showed limited visual selectivity, particularly at low concentrations. To improve performance, AgNPs were immobilized onto filter paper (Figure 1), producing a stable sensing platform.

The paper-based sensor exhibited a distinct color change only in the presence of Hg^{2+} , while no visible response was observed for other tested metal ions or common inorganic anions, even at much higher concentrations, demonstrating excellent selectivity.

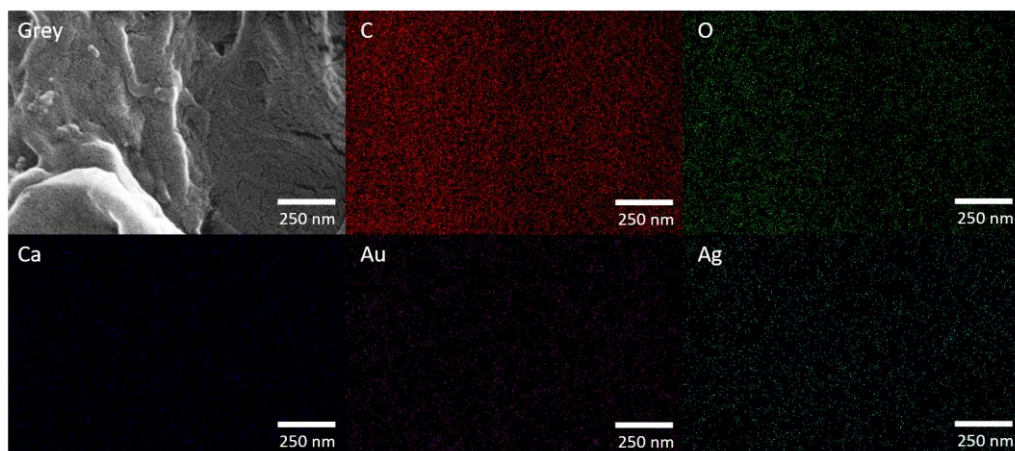


Figure 1. FESEM micrograph ($150000\times$ magnification) and corresponding EDX elemental mapping of AgNPs@FP

The AgNPs@FP sensor showed a clear concentration-dependent color change over the Hg^{2+} concentration range of $1\times 10^{-8} - 1\times 10^{-3} \text{ mol dm}^{-3}$ (Figure 2). Image analysis based on the yellow index produced a linear calibration curve with a LOD of $1.065\times 10^{-8} \text{ mol dm}^{-3}$ and an LOQ of $3.263\times 10^{-8} \text{ mol dm}^{-3}$, enabling reliable nanomolar detection without sophisticated instrumentation.

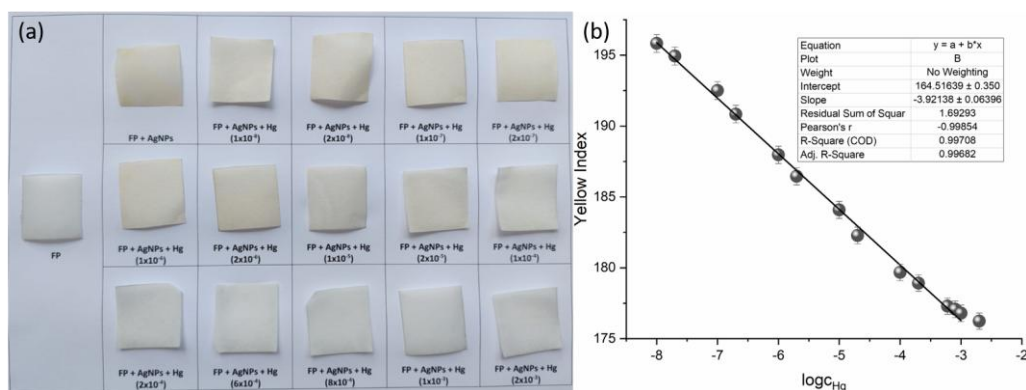


Figure 2. (a) Visual response of AgNPs@FP synthesized using *Juglans regia* extract, after exposure to increasing concentrations of Hg^{2+} (from 1×10^{-8} to $2\times 10^{-3} \text{ mol dm}^{-3}$); (b) Calibration curve showing the linear dependence of yellow index on the logarithm of Hg^{2+} concentration.

The applicability of the sensor was evaluated using Hg^{2+} -spiked tap water. Recoveries ranged from 96% to 102%, confirming excellent accuracy and negligible matrix effects without any sample pretreatment. These results demonstrate the suitability of the proposed paper-based sensor for rapid environmental monitoring (Table 1).

Table 1. Detection of Hg^{2+} in spiked tap water samples using AgNPs@FP sensor.

Spiked concentration (mol dm^{-3})	Found concentration (mol dm^{-3})	Recovery (%)
1.0×10^{-3}	$(9.8 \pm 0.1) \times 10^{-4}$	98 ± 1
1.0×10^{-4}	$(1.02 \pm 0.04) \times 10^{-4}$	102 ± 4
1.0×10^{-5}	$(9.9 \pm 0.4) \times 10^{-6}$	99 ± 4
1.0×10^{-6}	$(1.01 \pm 0.03) \times 10^{-6}$	101 ± 3
1.0×10^{-7}	$(9.7 \pm 0.2) \times 10^{-8}$	97 ± 2
1.0×10^{-8}	$(9.6 \pm 0.5) \times 10^{-9}$	96 ± 5

CONCLUSION

This study demonstrates that green-synthesized silver nanoparticles obtained using *Juglans regia* extract can be successfully immobilized onto filter paper to create a portable and highly sensitive colorimetric sensor for Hg^{2+} detection. The developed platform enables nanomolar detection ($\text{LOD} = 1.065 \times 10^{-8} \text{ mol dm}^{-3}$) over a broad linear range without requiring sophisticated instrumentation. Combining plant-mediated synthesis with a simple paper-based format provides a sustainable, low-cost, and user-friendly approach for rapid on-site monitoring of mercury in water.

Acknowledgement

This work was funded by the Serbian Ministry of Science, Technological Development, and Innovations (grant numbers 451-03-34/2026-03/200146, 451-03-33/2026-03/200017, and 451-03-33/2026-03/200162). The authors also acknowledge support from the Serbian Academy of Sciences and Arts (project no. F-49).

REFERENCES

- [1] S. Ali, M. Mansha, N. Baig, S. A. Khan, The Chemical Record, 2022, 22, 7.
- [2] S. Wang, W.-J. Chen, A. Du, Y. Kou, X. Xu, D. Hu, Z. Lu, Advanced Composites and Hybrid Materials, 2025, 8, 3.

ALPRAZOLAM WITHDRAWAL ALTERS DOPAMINE AND SEROTONIN LEVELS IN THE CAUDATE-PUTAMEN OF FEMALE WISTAR RATS

T. Radukić¹, M. Dragić^{1,2}, N. Jasnić³, A. Stekić² and M. Zarić Kontić¹

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (marinazaric@vin.bg.ac.rs)*

²*Center for Translational Neuroscience, Faculty of Biology, University of Belgrade, Studentski trg 16, 11158, Belgrade, Serbia*

³*Department for Animal and Human Physiology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11158, Belgrade*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.54

ABSTRACT

Benzodiazepine (BZD) withdrawal is associated with neurochemical adaptations that contribute to withdrawal symptoms; however, monoaminergic alterations within the caudate-putamen (CPu) remain poorly understood. This study investigated dopamine and serotonin concentrations in the CPu following early alprazolam withdrawal in adult female Wistar rats. Animals received an escalating alprazolam treatment regimen (3–9 mg/kg, i.p.) followed by a 36 h withdrawal period. Monoamine concentrations were determined using high-performance liquid chromatography (HPLC). Compared with vehicle-treated animals, the withdrawal group exhibited significantly reduced dopamine and increased serotonin concentrations, suggesting that alprazolam withdrawal disrupts dopaminergic-serotonergic balance within CPu.

INTRODUCTION

Benzodiazepines (BZDs) remain among the most frequently prescribed medications for treating anxiety and fear related disorders and insomnia. However, prolonged use may result in tolerance and physical dependence, while abrupt discontinuation often precipitates a withdrawal syndrome characterized by anxiety, irritability, autonomic disturbances, and cognitive impairment [1, 2]. Accumulating evidence suggests that, in addition to adaptive changes in GABAergic neurotransmission, alterations in monoaminergic systems contribute to withdrawal symptoms. Enhanced serotonergic activity has been associated with withdrawal-induced anxiety, and both serotonergic and dopaminergic pathways are important modulators of behavioral responses during withdrawal [3, 4].

Although previous studies have primarily focused on limbic regions, the involvement of the caudate-putamen (CPu) in BZD withdrawal remains poorly understood. Therefore, the present study aimed to determine dopamine and serotonin concentrations in the CPu during early withdrawal from alprazolam, one of the most prescribed BZDs worldwide.

EXPERIMENTAL

All experimental procedures were approved by the Ethics Committee for the Use of Laboratory Animals of the VINČA Institute of Nuclear Sciences and the Veterinary Directorate of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (Approval No. 003447280/2025, August 25, 2025).

Adult female Wistar rats were housed under controlled environmental conditions (12 h light/dark cycle, constant temperature and humidity) with *ad libitum* access to standard laboratory food and water. Animals were randomly divided into two experimental groups: control (vehicle-treated, 0.5 % Tween 80 in saline, VEH) and 36 h alprazolam withdrawal group (36h ALP, $n = 6$ animals per both groups). Alprazolam was administered intraperitoneally in escalating doses (3, 5, 7, and 9 mg/kg; twice daily for two days each) over 8 days, followed by abrupt cessation. Following the final administration, animals underwent a 36 h withdrawal period before tissue collection.

The animals were euthanized by rapid decapitation using a guillotine for small laboratory animals (Harvard Apparatus, USA). Immediately after decapitation, brains were rapidly removed and placed on an ice-cooled surface. The CPu was carefully dissected bilaterally according to the rat brain atlas of Paxinos and Watson [5], approximately corresponding to anteroposterior coordinates from +2.28 to -0.12 mm relative to bregma.

Dopamine and serotonin concentrations were quantified using high-performance liquid chromatography (HPLC). Tissue samples were homogenized in deproteinization solution (1 mg tissue/10 μ L), followed by sonication (3×10 s) and centrifugation (10 min, 13,500 rpm). Subsequently, 40 μ L of the obtained supernatant was injected into an UltiMate 3000 HPLC system (Thermo Fisher Scientific, USA) equipped with a reversed-phase C18 analytical column. Separation was achieved using a mobile phase consisting of 100 mM ammonium formate buffer (pH 3.6) and methanol (98:2, v/v) delivered at a flow rate of 500 μ L/min. Detection was performed electrochemically at an oxidation potential of 850 mV and a column temperature of 25 °C. Chromatographic data were acquired and processed using Chromeleon 7 Chromatography Data System (Thermo Fisher Scientific, USA). Dopamine and serotonin concentrations were expressed as μ g/mg of tissue.

Data are expressed as mean $\pm$ SEM. Differences between the VEH and 36 h ALP groups were analyzed using an unpaired Student's t-test, with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Compared with the VEH group, animals subjected to 36 h ALP withdrawal exhibited a significant reduction in dopamine concentration in the CPu ($t = 2.788$, $p = 0.0192$; Figure 1A). In contrast, serotonin concentration was significantly increased in the 36 h ALP group relative to VEH group ($t = 2.97$, $p = 0.0141$; Figure 1B), indicating a disruption of local monoaminergic homeostasis during the discontinuation phase.

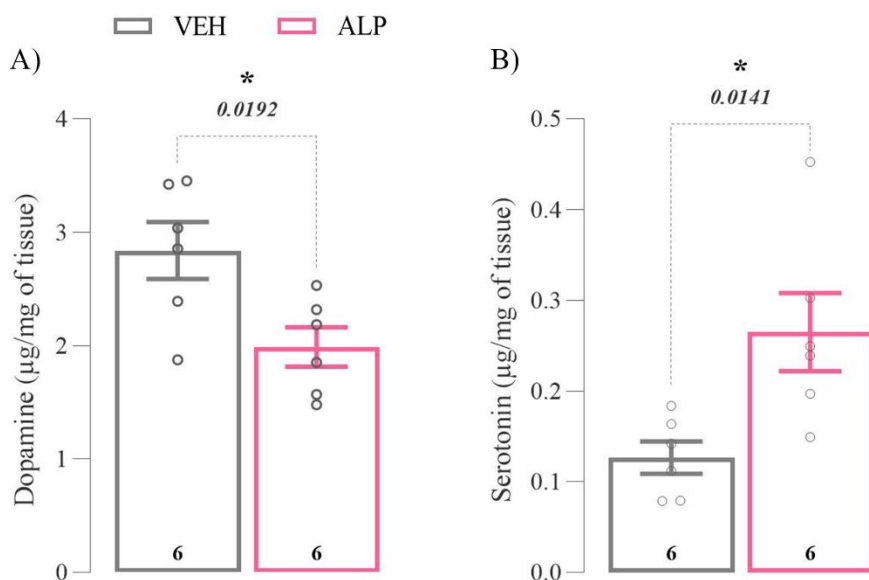


Figure 1. HPLC analysis of dopamine and serotonin levels in the CPu. (A) Dopamine concentration (µg/mg of tissue); (B) Serotonin concentration (µg/mg of tissue). Each dot represents an individual animal. The number of animals per group is indicated within the bottom of each bar.

These findings complement our previous unpublished observations demonstrating neurochemical alterations in the hippocampus, amygdala and prefrontal cortex, as well as increased anxiety-like behavior and impaired stress-coping strategy following 36 h alprazolam withdrawal. Previous studies have primarily associated BZD withdrawal with serotonergic alterations in limbic brain regions, particularly the amygdala and hippocampus [3, 4]. The present findings extend these observations by identifying the CPu as an additional brain region affected following alprazolam abrupt discontinuation, further supporting the involvement of widespread monoaminergic neuroadaptations in the BZD withdrawal syndrome.

The observed changes in dopamine and serotonin concentrations may reflect an early neurochemical adaptation to abrupt alprazolam discontinuation. Future studies combining behavioral assessment with neurochemical and molecular

analyses will be important for clarifying the contribution of CPu monoaminergic signaling to the development of BZD withdrawal syndrome.

CONCLUSION

Thirty-six hours of alprazolam withdrawal disrupts monoaminergic neurotransmission within the CPu, as demonstrated by reduced dopamine and elevated serotonin concentrations. These findings identify the CPu as a previously underexplored target of BZD withdrawal-induced neuroadaptations and provide a foundation for future investigations into the mechanisms underlying withdrawal-related behavioral disturbances.

Acknowledgement

The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grants: 451-03-33/2026-03/200017, 451-03-34/2026-03/200178, and 451-03-33/2026-03/200178.

REFERENCES

- [1] A. N. Edinoff, C. A. Nix, J. Hollier, C. E. Sagrera, B. M. Delacroix, T. Abubakar, E. M. Cornett, A. M. Kaye, A. D. Kaye, *Neurol. Int.*, 2021, 13, 594-607.
- [2] A. M. Cardona-Acosta, N. Meisser, N. I. Vardeleon, H. Steiner, C. A. Bolaños-Guzmán, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2024, 136, 111137.
- [3] L. Dos Santos, T. G. de Andrade, F. G. Graeff, *J. Psychopharmacol.*, 2010, 24, 725-731.
- [4] C. Fernandes, N. Andrews, S. E. File, *Pharmacol. Biochem. Behav.*, 1994, 49, 359-362.
- [5] G. Paxinos, C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press, London, 2014.

EVALUATION OF URINARY AND SERUM RENAL BIOMARKERS FOLLOWING ACUTE MICROPLASTIC EXPOSURE IN MALE RATS

T. Zečević¹, Z. Stojanović², D. Drakulić¹, A. Todorović¹, F. Veljković¹, V. Petković¹ and I. Guševac Stojanović¹

¹*Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade, Serbia (tijana.zecevic02@gmail.com)*

²*Institute of Technical Sciences of SASA, University of Belgrade, Knez Mihailova Street 35/4, 11000 Belgrade, Republic of Serbia*

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.55

ABSTRACT

Microplastic particles (MPs) smaller than 5 mm are ubiquitous environmental pollutants, with concerning potential impacts on human health. This study focused on early changes in renal function following a single oral dose (35 mg/kg) of mechanically generated irregular polyethylene terephthalate (PET) MPs of two different sizes (~41 µm and ~106 µm) in male *Wistar* rats. Renal function was evaluated 24 h post-exposure using serum biochemical parameters and urinalysis. Serum urea and creatinine were measured as indicators of glomerular filtration, while urinalysis assessed the presence of blood and leukocytes. Exposure to MPs resulted in a significant elevation of serum urea and creatinine levels compared with controls, but no blood or leukocytes were present in urine. These findings indicate that acute exposure to MPs may induce early functional changes in the kidney before overt signs of urinary tract injury and inflammation become apparent, suggesting early physiological responses.

INTRODUCTION

Plastic materials have widespread industrial benefits, but have recently come to light as a serious pollutant with potential risk to the ecosystem and human health. The term microplastics is used to describe polymeric matrices or synthetic solid particles of regular or irregular shape that can be categorized as either primary or secondary microplastics [1]. Microplastic particles (MPs) range in size from 1 µm to 5 mm, and are insoluble in water. They have been detected in a wide array of shapes and polymeric structures, and their distribution is vast - from drinking and marine water, agroecosystems and foods, to the atmosphere [1].

Consequently, human exposure to MPs is mainly through ingestion, inhalation or dermal contact. Multiple studies have reported accumulation of MPs in

different organs and tissues, as well as their cellular uptake, raising concerns about possible adverse biological effects of these polymers [2-3].

Although the kidney represents a crucial organ for the elimination of xenobiotics, information on its susceptibility to acute MPs exposure remains limited [4]. Due to the valuable role of the kidneys in the maintenance of overall physiological homeostasis, the need for assessment of renal function in the context of MPs exposure is a crucial part of any toxicological and biomedical research [5]. This is most commonly done using serum biomarkers such as urea and creatinine, while urinalysis parameters including the presence of blood and leukocytes can be additional indicators of structural damage and inflammation.

Accordingly, this study aimed to assess the effects of a single dose of MPs on renal function in male *Wistar* rats using serum biomarkers and urinalysis 24 h post - exposure.

EXPERIMENTAL

MPs were prepared from polyethylene terephthalate (PET) bottles that were mechanically filed, as previously described [6].

The approved experiments (protocol authorization number 323-07-03460/2021-05) were performed on young adult male *Wistar* rats ($n = 12$; body weight 300 - 350 g) maintained under standard laboratory conditions. The animals were randomly assigned into three experimental groups: the control group (Q), which received 2.5 mL of Milli-Q water; and two groups treated with different sizes of MPs dispersed in 2.5 mL of Milli-Q, in sizes of 41 μm (S) or 106 μm (L), both at doses of 35 mg/kg. All treatments were administered as a single dose by oral gavage. Urine samples were collected prior to sacrifice, 24 h post - exposure. Analysis was performed using visual colorimetric comparison with commercial reagent test strips (Insight Urinalysis Reagent Strips, Acon Laboratories, USA). The parameters assessed included hematuria, the presence of blood, and the presence of leukocytes, both monitored semi-quantitatively according to the manufacturer's colorimetric scale. Following trunk blood collection and clotting at room temperature, the samples were centrifuged (3000 rpm for 15 min utilizing Heraeus Labofuge GL (Heraeus, Germany)) to obtain serum, which was used for biochemical analysis. Renal function was evaluated by measuring urea and creatinine levels using standard enzymatic colorimetric assays, as indicators of glomerular filtration capacity.

Data, presented as mean $\pm$ SEM relative to the control, were analyzed using one-way ANOVA followed by appropriate post hoc tests for multiple comparisons vs. the control group in GraphPad Prism software (version 8.02, USA). Differences were considered statistically significant at $p < 0.05$. Urinary blood and leukocyte presence was assessed semi - quantitatively and analyzed descriptively.

RESULTS AND DISCUSSION

To assess the potential effect on renal physiological functions, we measured the serum levels of urea and creatinine. As shown in Figure 1., serum levels of urea were significantly elevated in both MPs groups compared to control ($p < 0.05$ for S and L vs. Q), suggesting that PET particles of both sizes similarly affect their clearance and metabolic processes associated with kidney function. A significant increase was also detected in creatinine levels compared to the control group ($p < 0.05$ for S and L vs. Q), further supporting an alteration in renal filtration - related processes. Together, these changes indicate an early functional response of the kidney following acute PET MPs exposure.

Table 1. Effects of acute oral exposure to polyethylene terephthalate microplastics on the presence of blood and leukocytes in urine of male *Wistar* rats 24 h after treatment. Data are presented descriptively (– indicates no blood or leukocytes detected).

Groups Parameters	Q	S	L
Hematuria	–	–	–
Leukocytes	–	–	–

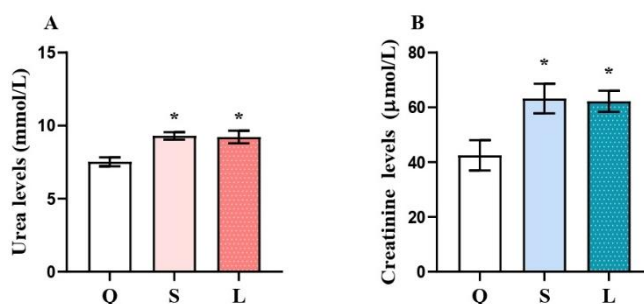


Figure 1. Effects of acute oral exposure to polyethylene terephthalate microplastics on serum A) urea and B) creatinine levels in male *Wistar* rats 24 h after treatment. Data are presented as the mean ± SEM relative to the control (* $p < 0.05$).

As shown in Table 1., neither hematuria nor leukocytes were present in the urine of experimental groups. This absence indicates that acute PET MPs exposure did not result in detectable urinary tract injury or inflammatory changes within the investigated time frame.

Taken together, the observed changes in serum renal biomarkers accompanied by the absence of urinary abnormalities suggest that acute PET MPs exposure primarily affects renal physiological processes during the early phase after exposure. The combined assessment of serum and urinary parameters provides a broader view of renal responses and indicates that functional alterations may occur before detectable manifestations of renal injury.

CONCLUSION

Acute oral exposure to PET MPs results in early alterations of renal - related parameters in male Wistar rats, highlighting the rapid physiological response of the kidney following microplastic intake. These findings provide further insight into the short - term effects of microplastic exposure and support the need for continued investigation of their biological consequences.

Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-33/2026-03/200017 and 451-03-33/2026-03/200175).

REFERENCES

- [1] Campanale, C., Massarelli, C., et al., *Int. J. Environ. Res. Public Health*, 2020, 17, 1212.
- [2] Jenner, L.C., Rotchell, J.M., et al., *Sci. Total Environ.*, 2022, 831, 154807.
- [3] Ragusa, A., Svelato, A., et al., *Environ Int.*, 2021, 146, 106274.
- [4] Alamilla-Sanchez, M., Alcalá Salgado, M.A., et al., *Pathophysiology*, 2025, 32, 33.
- [5] La Porta, E., Exacoustos, O., et. al., *Int. J. Mol. Sci.*, 2023, 24, 14391.
- [6] Guševac Stojanović, I., Drakulić, D., et al., *Toxics*, 2024, 12, 167.

STEROIDAL SEMICARBAZONES VERSUS ETHYL-CARBAZATES: DRUG-LIKENESS AND ADMET PROFILE

M. B. Živković¹, S. J. Tufegdžić¹, N. O. Đorđević² and I. T. Novaković¹

¹University of Belgrade, Institute of Chemistry, Technology and Metallurgy,
Studentski trg 12–16, 11000 Belgrade, Serbia

(marijana.zivkovic@ihtm.bg.ac.rs)

²Vinča Institute of Nuclear Sciences – National Institute of the Republic of
Serbia, University of Belgrade, Mike Petrovića Alasa 12-14, 11351 Belgrade,
Serbia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.56

ABSTRACT

Steroidal semicarbazones and ethyl carbazates represent structurally diverse derivatives with biological relevance, while their integrated ADMET profiles remain insufficiently explored. This study evaluated the drug-likeness, ADMET properties and DPPH radical scavenging activity of steroidal bis-semicarbazones (SCs) and corresponding bis-ethyl carbazates (ECs). Drug-likeness and ADMET parameters were predicted using Molinspiration, SwissADME, pkCSM and ADMETlab 2.0, while antioxidant activity was assessed by the DPPH assay. SC derivatives exhibited higher TPSA values, more hydrogen-bond donors, and stronger predicted P-gp substrate propensity, whereas EC analogs generally showed higher predicted BBB permeability. Most compounds displayed favorable ADMET profiles. However, negligible DPPH radical scavenging activity was observed. These findings provide insight into structure–property relationships governing the physicochemical and ADMET characteristics of steroidal SCs and ECs.

INTRODUCTION

Steroidal scaffolds represent privileged structures in medicinal chemistry due to their structural diversity and wide range of biological activities. Structural modification of steroidal scaffolds by introducing semicarbazone (SC) or ethyl carbazate (EC) functionalities may alter their physicochemical properties and consequently affect their pharmacokinetic behavior and biological activity [1,2]. Although the investigated steroidal SCs and ECs were previously synthesized and evaluated for cytotoxic and antimicrobial activities [3], their radical scavenging activity and integrated *in silico* ADMET characteristics remained unexplored. Therefore, this study aimed to perform a comparative *in silico* and *in vitro* evaluation of two series of androstane derivatives, focusing on drug-

likeness, ADMET properties, and antioxidant activity assessed by the DPPH assay.

EXPERIMENTAL

Drug-likeness properties were evaluated using the Molinspiration [4], whereas ADMET parameters were predicted using SwissADME, pkCSM and ADMETlab 2.0 web tools [5–7].

The antioxidative activity of steroidal bis-SCs and bis-ECs was determined using the standard DPPH (2,2-diphenyl-1-picrylhydrazyl) assay [8].

RESULTS AND DISCUSSION

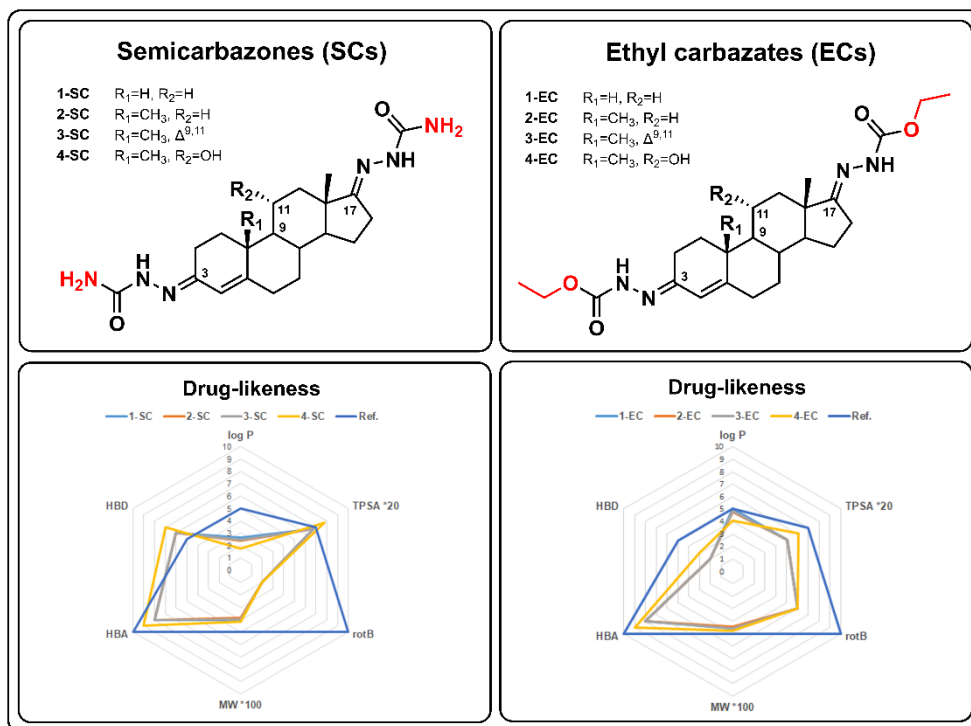


Figure 1. Chemical structures and predicted drug-likeness radar charts of steroidal bis-semicarbazones and bis-ethyl carbazates.

(log P – lipophilicity; TPSA – topological polar surface area ($\text{\AA}^2$); rotB – number of rotatable bonds; MW – molecular weight; HBA/HBD – number of hydrogen bond acceptors/donors; Ref. – recommended ranges)

The chemical structures and predicted drug-likeness profiles of the investigated steroidal derivatives are shown in Figure 1. SC derivatives displayed higher TPSA values and a higher number of hydrogen-bond donors than EC analogs, while EC derivatives were characterized by higher lipophilicity.

Table 1 summarizes the predicted ADMET profiles of steroidal SCs and ECs. Most derivatives demonstrated favorable predicted Caco-2 permeability, while

4-SC stood out with markedly reduced permeability, consistent with its low GI absorption. This distinct behavior may be related to the physicochemical characteristics of 4-SC, which exhibited the lowest lipophilicity, the highest TPSA value, and the greatest hydrogen-bonding potential among the investigated derivatives.

Table 1. Predicted ADMET parameters of steroidal SCs and ECs. (Compd. – Compound; GI – gastrointestinal absorption; Caco-2 – Caco-2 permeability (logPapp, 10⁻⁶ cm/s); BBB – blood–brain barrier permeability (logBB); P-gp – P-glycoprotein substrate propensity; hERG – predicted hERG inhibition probability; AMES – predicted Ames mutagenicity probability)

Compd.	GI ^a	Caco-2 ^b	BBB ^b	P-gp ^c	hERG ^c	AMES ^c
1-SC	High	0.81	-0.90	0.987	0.10	0.58
1-EC	High	0.70	-0.56	0.005	0.33	0.67
2-SC	High	0.80	-0.95	0.988	0.15	0.86
2-EC	High	0.70	-0.55	0.003	0.44	0.87
3-SC	High	0.71	-0.98	0.978	0.00	0.95
3-EC	High	0.71	-0.57	0.004	0.04	0.95
4-SC	Low	-0.03	-1.05	0.991	0.07	0.86
4-EC	High	0.63	-1.03	0.117	0.28	0.88

^aSwissADME; ^bpkCSM; ^cADMETlab2.0

Higher BBB permeability was generally observed for EC derivatives compared to the corresponding SC analogs, with the exception of the C11-hydroxylated series. The stronger P-gp substrate propensity predicted for SC derivatives may be associated with their higher polarity and greater hydrogen-bonding capacity relative to the EC series. Both series showed moderate CYP inhibition potential and low predicted clearance values (<5 mL/min/kg), with no pronounced inter-series differences. Favorable cardiotoxicity profiles were predicted for most derivatives, with only two EC analogs exhibiting moderate hERG-related risk. Predicted mutagenicity profiles varied among the compounds, indicating the need for further toxicological evaluation.

All investigated derivatives exhibited negligible DPPH radical scavenging activity under the applied experimental conditions. These findings indicate that neither the semicarbazone nor the ethyl carbazate functionality confers appreciable direct radical scavenging activity within the investigated steroidal series.

CONCLUSION

The present study demonstrated that structural variation between steroidal semicarbazones and corresponding ethyl carbazates influences their predicted ADMET profiles, particularly parameters related to membrane permeability and

P-gp substrate propensity. Despite generally favorable predicted drug-likeness and safety profiles, neither series exhibited appreciable DPPH radical scavenging activity. These findings contribute to a better understanding of the structure–property relationships governing the physicochemical and ADMET characteristics of steroidal semicarbazone- and ethyl carbazate-based derivatives.

Acknowledgement

This work was partially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants no. 451-03-33/2026-03/200026, 451-03-33/2026-03/200017).

REFERENCES

- [1] H. Beraldo, D. Gambino, *Mini Rev. Med. Chem.*, 2004, 4, 31–39.
- [2] M. Krátký, N.-H. Hounghbedji, J. Vinšová, *Eur. J. Med. Chem.*, 2024, 279, 116835.
- [3] M. B. Živković, I. T. Novaković, I. Z. Matić, D. M. Sladić, N. M. Krstić, *Steroids*, 2019, 148, 36–46.
- [4] <https://www.molinspiration.com/>
- [5] <https://www.swissadme.ch/index.php>
- [6] <https://biosig.lab.uq.edu.au/pkcsim/prediction>
- [7] <https://admetmesh.scbdd.com/service/evaluation/index>
- [8] M. S. Blois, *Nature*, 1958, 181, 1199–1200.

STRUCTURE-PROPERTY RELATIONSHIP IN STEROIDAL HYDANTOINS: *IN SILICO* EVALUATION OF DRUG-LIKENESS AND ADMET PROFILES

M. B. Živković¹, R. Z. Pavlović¹, P. B. Stanić² and B. M. Šmit²

¹University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Studentski trg 12–16, 11000 Belgrade, Serbia
(marijana.zivkovic@ihtm.bg.ac.rs)

²University in Kragujevac, Institute for Informational Technologies, Liceja kneževine Srbije 1A, 34000 Kragujevac, Serbia

DOI: 10.68725/FDSQ_WORKSHOP.2026.9.57

ABSTRACT

Steroidal hydantoin (HDTs) represent promising scaffolds for the development of biologically active compounds, including potential antitumor agents. In this study, the drug-likeness and ADMET properties of six structurally diverse steroidal HDTs, including mono- and bis-hydantoin derivatives, were evaluated *in silico* using Molinspiration, pkCSM, and ADMETlab 2.0. Most derivatives satisfied the recommended drug-likeness criteria. Mono-HDTs exhibited higher predicted permeability and intestinal absorption, whereas bis-derivatives showed lower clearance, suggesting slower elimination. All compounds demonstrated low predicted risks of mutagenicity and cardiotoxicity. Overall, the obtained results highlight steroidal HDTs, particularly mono-functionalized derivatives, as promising candidates for further synthesis and biological evaluation.

INTRODUCTION

Cancer is the second leading cause of noncommunicable disease-related death worldwide [1], following cardiovascular diseases, highlighting the continuous need for the development of novel antitumor agents. Steroidal scaffolds represent privileged structures in medicinal chemistry due to their favorable membrane permeability and broad potential for structural derivatization, which may yield compounds with enhanced biological activity, including antitumor effects [2]. Numerous biologically active hydantoin derivatives have been reported to date [3]; however, steroidal hydantoin (HDTs) remain insufficiently explored. Therefore, the aim of this study was to perform a preliminary *in silico* evaluation of the drug-likeness and ADMET properties of structurally diverse steroidal hydantoin derivatives in order to identify promising candidates for future synthesis and biological evaluation.

EXPERIMENTAL

Drug-likeness parameters were assessed using the Molinspiration calculator [4] while ADMET parameters were calculated using the pkCSM and ADMETlab 2.0 software [5–6].

RESULTS AND DISCUSSION

Six steroidal HDTs differing in the C-17 substitution pattern were evaluated *in silico* (Fig. 1), including mono-hydantoin derivatives of androstenedione (A), progesterone (P), testosterone (T), and cholestane (C), as well as bis-hydantoin derivatives of the A and P scaffolds, since only these scaffolds possess two carbonyl groups capable of hydantoin ring formation.

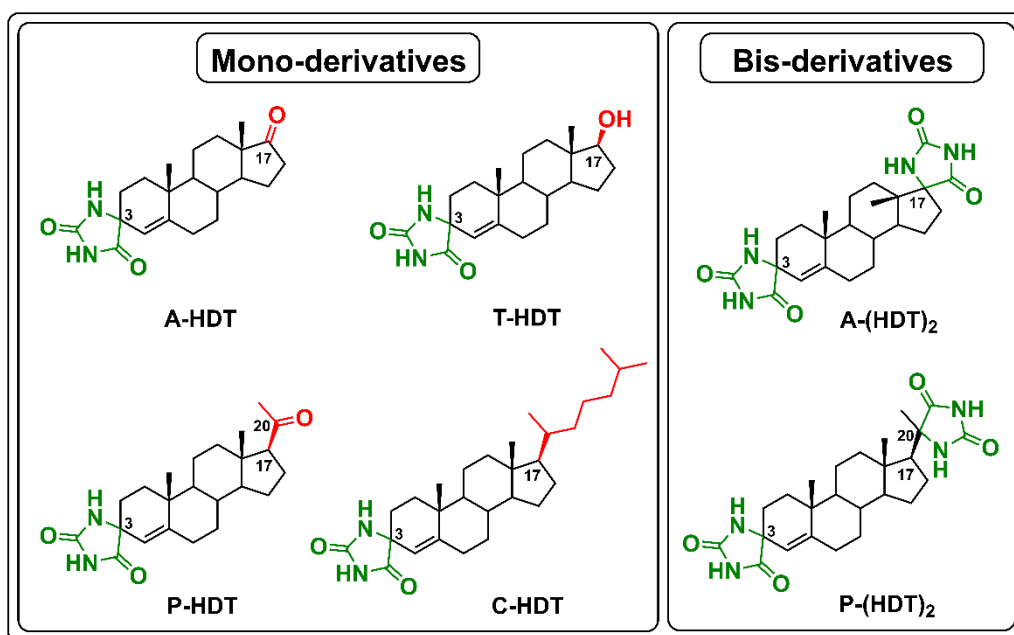


Figure 1. Chemical structures of the evaluated steroidal mono- and bis-hydantoin.

Drug-likeness parameters are presented in Table 1. Except for the highly lipophilic C-HDT ($\log P \approx 7$), which shows a single Lipinski violation, all analyzed derivatives fall within ranges generally associated with favorable oral bioavailability. Compared to mono-HDTs, bis-derivatives exhibit higher MW, HBA/HBD counts, and TPSA values, suggesting increased hydrogen-bonding capacity and reduced membrane permeability potential.

Table 1. Predicted drug-likeness descriptors of steroidal hydantoins.
(Compd. – compound; log P – lipophilicity; MW – molecular weight; HBA/HBD – number of hydrogen bond acceptors/donors; n_{viol} – number of Ro5 violations; TPSA – topological polar surface area (Å²); rotB – number of rotatable bonds; Ref. – recommended reference ranges for drug-likeness)

Compd.	log P	MW	HBA	HBD	n _{viol}	TPSA	rotB
A-HDT	2.56	356	5	2	0	75	0
A-(HDT)₂	2.07	427	8	4	0	116	0
P-HDT	3.31	385	5	2	0	75	1
P-(HDT)₂	2.82	455	8	4	0	116	1
T-HDT	2.75	358	5	3	0	78	0
C-HDT	6.94	455	4	2	1	58	5
Ref.	≤5	≤500	≤10	≤5	≤1	≤140	≤10

To further evaluate the pharmacokinetic and safety profiles of HDTs, predicted ADMET parameters were analyzed (Tab. 2).

Water solubility (Ws) ranged from 0.05 to 13.6 mg/L, with the lowest value predicted for C-HDT and the highest for T-HDT. Mono-derivatives exhibited higher predicted Caco-2 permeability (>0.9) and intestinal absorption (94–96%) than bis-derivatives (~0.4 and 82%, respectively). Moderate VD_{ss} values were predicted for A-HDT and T-HDT, while low Fu values (0.03–0.23) suggested extensive plasma protein binding. BBB permeability indicated moderate brain penetration, reduced for bis-derivatives.

Most compounds were predicted to undergo metabolism via CYP1A2, CYP2C19, CYP2C9, and CYP3A4. Mono-HDTs showed moderate clearance (7.4–14 mL/min/kg), whereas bis-derivatives exhibited lower clearance (1.2–3.1 mL/min/kg), suggesting slower elimination. All compounds demonstrated low predicted risk of hERG inhibition (0.2–2.7%) and AMES mutagenicity (1.4–10.0%), together with similar hepatotoxicity probabilities (~0.3). Overall, mono-derivatives displayed more favorable absorption-related properties than bis-HDTs, whose profiles were mainly influenced by increased molecular size and polarity.

Table 2. Predicted ADMET parameters of steroidal hydantoins.
(Compd. – compound; Caco-2 – Caco-2 permeability (logPapp, 10⁻⁶ cm/s); IA – intestinal absorption (%); VDss – steady-state volume of distribution (log L/kg); Fu – unbound plasma fraction; BBB – blood–brain barrier permeability (logBB); CL – clearance (mL/min/kg); AMES – predicted Ames mutagenicity probability; hERG – predicted hERG inhibition probability)

Compd.	Caco-2	IA	VDss	Fu	BBB	CL	AMES	hERG
A-HDT	0.90	94	0.12	0.17	-0.02	9.6	6.6	0.5
A-(HDT)₂	0.45	82	-0.43	0.23	-0.63	1.2	10.0	0.4
P-HDT	1.10	96	-0.20	0.03	-0.01	14.0	1.4	0.6
P-(HDT)₂	0.41	82	-0.49	0.14	-0.47	3.1	2.6	0.2
T-HDT	0.94	94	-0.10	0.18	-0.40	9.5	3.0	1.0
C-HDT	1.20	94	-0.19	0.00	-0.32	7.4	2.0	2.7

CONCLUSION

Overall, the analyzed steroidal HDTs exhibited favorable drug-likeness properties, with only the highly lipophilic C-HDT showing a single Lipinski violation. Mono-derivatives displayed more favorable absorption- and clearance-related ADMET properties than bis-derivatives, while all compounds demonstrated low predicted risks of mutagenicity and cardiotoxicity. These findings highlight steroidal hydantoins, particularly mono-functionalized derivatives, as promising candidates for further synthesis and biological evaluation as potential antitumor agents.

Acknowledgement

This work was partially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants no. 451-03-33/2026-03/200026, 451-03-33/2026-03/200378).

REFERENCES

- [1] https://www.who.int/health-topics/cancer#tab=tab_1
- [2] M. B. Živković, I. Z. Matić, M. V. Rodić, I. T. Novaković, A. M. Krivokuća, D. M. Sladić, N. M. Krstić, *J. Steroid Biochem. Mol. Biol.*, 2017, 174, 72 – 85.
- [3] S. H. Cho, S. H. Kim, D. Shin, *Eur. J. Med. Chem.*, 2019, 164, 517 – 545.
- [4] <https://www.molinspiration.com/>
- [5] <https://biosig.lab.uq.edu.au/pkcsmprediction>
- [6] <https://admetmesh.scbdd.com/service/evaluation/index>

AUTHOR INDEX

<i>Ahmoda, Rabiea</i>	0009-0005-1739-3343	73
<i>Anićijević, Vladan V.</i>	0000-0001-8731-1378	57, 150, 196
<i>Anđić, Tamara</i>	0009-0005-4419-6396	204
<i>Antonijević, Biljana</i>	0000-0002-3996-454X	158
<i>Aykut, Abdurrahman</i>	0000-0002-2671-9393	22
<i>Balić, Kerim</i>	/	13
<i>Banjac, Katarina</i>	0000-0001-6100-3887	133, 184
<i>Baralić, Katarina</i>	0000-0003-3290-2204	158
<i>Bego, Tamer</i>	0000-0002-1350-7084	13
<i>Bezbradica, Dejan</i>	0000-0002-4385-1433	166
<i>Beškoski, Vladimir</i>	0000-0002-6372-4706	97, 101, 137
<i>Blagojević, Stevan</i>	0000-0002-0451-5912	85
<i>Borjan, Nikola</i>	0000-0003-4067-3755	204
<i>Bošković, Anastasija</i>	/	93, 113, 146
<i>Brankov, Milan</i>	0000-0002-5462-6477	49
<i>Brković, Snežana</i>	0000-0002-8584-5648	57, 65, 154
<i>Bučić, Anđela</i>	/	97, 101
<i>Bulat, Zorica</i>	0000-0001-9830-8772	158
<i>Čakar, Uroš</i>	0000-0002-7449-1960	39, 61, 174
<i>Čebela, Maria</i>	0000-0001-6120-6924	39
<i>Čechová, Ludmila</i>	0000-0002-1554-4903	16
<i>Čolović, Mirjana B.</i>	0000-0001-5037-9985	45
<i>Čalić, Milica</i>	0009-0001-7459-9453	85
<i>Dimitrijević, Aleksandra</i>	0000-0002-8346-9909	125, 188
<i>Dimitrijević, Dunja</i>	/	97, 101
<i>Domijan, Ana-Marija</i>	0000-0001-5645-9732	32, 45
<i>Dostál, Martin</i>	0009-0009-2008-038X	16
<i>Dragić, Milorad</i>	0000-0003-4855-6131	162, 212
<i>Dragičević, Vesna</i>	0000-0003-1905-7931	49
<i>Drakulić, Dunja</i>	0000-0003-1448-9639	53, 105, 216
<i>Džafić, Suvad</i>	/	13
<i>Đorđević, Brižita</i>	0000-0002-4582-0809	39
<i>Đorđević, Neda O.</i>	0000-0003-4063-1635	129, 137, 220
<i>Đurđević, Mladen M.</i>	0009-0007-8235-4003	1
<i>Elezović, Alisa</i>	0000-0003-0559-1463	13
<i>Filipović Tričković, Jelena</i>	0000-0001-5450-0842	65, 89, 154
<i>Gajski, Goran</i>	0000-0002-1886-1453	45
<i>Ganić, Tea</i>	0000-0001-8600-4392	93, 113, 146
<i>Gelo, Ružica</i>	/	13
<i>Gerić, Marko</i>	0000-0002-5886-4106	45
<i>Gogoi, Bhoirob</i>	0000-0003-4408-8254	18, 37
<i>Gojković, Živan</i>	0000-0002-7916-5427	69

<i>Goksen, Gulden</i>	0000-0002-5432-7936	22
<i>Graovac, Stefan</i>	0000-0001-5849-4451	208
<i>Grbić, Ivana</i>	/	200
<i>Gulišija, Ivana</i>	0009-0008-7622-1420	13
<i>Gutić, Sanjin</i>	0000-0002-5780-8022	57
<i>Guševac Stojanović, Ivana</i>	0000-0001-5307-2970	53, 105, 216
<i>Hamplová, Marie</i>	0009-0003-2251-7182	32
<i>Hoxha, Fatjon</i>	0000-0001-9445-7316	28
<i>Ignjatijević, Ana</i>	0009-0004-6109-0713	204
<i>Ilić, Tijana</i>	0000-0002-2533-5152	39, 61
<i>Isenović, Esma R.</i>	0000-0002-0012-2636	133, 184
<i>Islary, Pungbilii</i>	0000-0002-8284-6266	37
<i>Ivanovska, Aleksandra</i>	0000-0001-6846-9583	30
<i>Janić, Marijana</i>	0000-0002-0080-2201	65, 89, 154
<i>Janković, Marija</i>	0000-0002-2255-7163	5
<i>Jasnić, Nebojša</i>	0000-0003-0333-333X	162, 212
<i>Ječmenica Dučić, Marija</i>	0000-0002-6437-519X	69
<i>Jocić, Ana</i>	0000-0001-6731-8396	57, 125, 188
<i>Jocković, Jelena</i>	/	204
<i>Jovanović, Aleksandra A.</i>	0000-0001-5394-0125	73, 77, 166
<i>Jovanović, Marina</i>	0000-0001-9617-1151	81
<i>Jovanović, Miloš</i>	0000-0003-0039-0929	77
<i>Kaiser, Jozef</i>	0000-0002-7397-125X	16
<i>Kalaba, Milica</i>	0000-0003-0989-7398	85, 174
<i>Karkalić, Radovan</i>	0000-0002-8074-7264	150
<i>Klopchevska, Jana</i>	/	31
<i>Knežević Graovac, Sanja</i>	0000-0002-9371-4066	208
<i>Kodranov, Igor</i>	0000-0001-5871-1487	61
<i>Kokunešoski, Maja</i>	0000-0003-1732-002X	69
<i>Kolarević, Stoimir</i>	0000-0002-6938-8803	158
<i>Kostić, Aleksandar Ž.</i>	0000-0002-1012-4029	178
<i>Kostić, Mila</i>	0009-0007-7948-5731	65, 89, 154
<i>Kozáková, Zuzana</i>	/	16
<i>Krgović, Nemanja</i>	0000-0003-3611-9911	77
<i>Krstić, Aleksandar</i>	0000-0001-7418-6723	200
<i>Krstić, Danijela Z.</i>	0000-0002-9192-8087	45
<i>Krstić, Jugoslav</i>	0000-0003-0321-0698	57
<i>Lasić, Dinka</i>	/	13
<i>Lazarević-Pašti, Tamara</i>	0000-0001-6220-2079	57, 196, 208
<i>Lazić, Ana</i>	0009-0002-4259-2625	5
<i>Lekić, Stefan</i>	0000-0002-0407-7887	101
<i>Leskovac, Andreja</i>	0000-0002-6293-5237	93, 113, 146
<i>Lončarević, Branka</i>	0000-0002-2226-511X	129
<i>Lugonja, Nikoleta</i>	0000-0002-5093-4448	97, 101
<i>Maksimović, Jelena</i>	0000-0001-7138-6666	39, 170, 174

<i>Mandinić, Zoran</i>	0000-0003-2622-6043	158
<i>Marinković, Aleksandar</i>	0000-0003-3239-5476	73
<i>Marinović-Cincović, Milena</i>	0000-0001-6197-1511	117
<i>Marić, Slađana</i>	0000-0002-3982-2664	125, 188
<i>Marković, Olivera S.</i>	0000-0001-5830-1445	1
<i>Martinović, Jelena</i>	0000-0001-8641-1051	105, 109, 162
<i>Medić, Olja</i>	0000-0002-0350-9318	93, 113, 146
<i>Milanković, Milica</i>	0000-0001-5379-6020	208
<i>Milanković, Vedran</i>	0000-0002-7706-9036	57, 196, 208
<i>Milenković, Marija</i>	0000-0002-6496-8091	117
<i>Milinčić, Danijel D.</i>	0000-0002-2476-9426	178
<i>Milošević, Milena</i>	0000-0002-4949-5062	73
<i>Mitrović, Tatjana</i>	0000-0002-5991-1445	129
<i>Momić, Tatjana</i>	0000-0003-2596-4474	65, 121
<i>Mušović, Jasmina</i>	0000-0001-6985-2701	125, 188
<i>Nikolić, Biljana</i>	0000-0003-1765-2454	93, 113, 146
<i>Nikolić, Željka</i>	0000-0002-2922-0256	85
<i>Nikolov, Jovana</i>	0000-0002-9331-8151	5
<i>Novaković, Irena T.</i>	0000-0001-9881-1204	192, 220
<i>Njuhović, Azemina</i>	/	13
<i>Obradović, Darija</i>	0000-0001-5678-034X	129
<i>Obradović, Milan</i>	0000-0002-4769-2652	133, 184
<i>Omeragić, Elma</i>	0000-0002-5996-4926	13
<i>Pagnacco, Maja</i>	0000-0002-1299-7974	39, 170, 174
<i>Pantelić, Marija</i>	0000-0001-8457-0585	137
<i>Pantelić, Nebojša</i>	0000-0003-1843-9890	200
<i>Parac-Vogt, Tatjana</i>	0000-0002-6188-3957	45
<i>Pavićević, Kristina</i>	0009-0003-3743-2894	5
<i>Pavlović, Radoslav Z.</i>	0000-0003-1216-3950	224
<i>Pašti, Igor</i>	0000-0002-1000-9784	57, 196, 208
<i>Pejić, Snežana</i>	0000-0003-4573-2371	137
<i>Perić, Ivana</i>	0000-0001-6878-7581	129, 137
<i>Petkova, Nadezhda</i>	0000-0002-5870-9157	142
<i>Petković, Vladana</i>	0000-0003-0538-3322	53, 105, 216
<i>Petrović, Maja</i>	0000-0002-1053-7739	166
<i>Petrović, Sandra</i>	0000-0003-0930-6455	93, 113, 146
<i>Pešić, Miloš P.</i>	0000-0002-1982-040X	1
<i>Pešić, Mirjana B.</i>	0000-0001-9779-1686	178
<i>Pjanović, Rada</i>	0000-0002-0817-182X	77
<i>Porobić-Katnić, Slavica</i>	0000-0003-2163-2261	117
<i>Potkonjak, Nebojša N</i>	0000-0002-7390-1470	150, 170, 208
<i>Pořízka, Pavel</i>	0000-0002-8604-7365	16
<i>Pravdić, Danijel</i>	0000-0002-0281-2024	13
<i>Radivojević, Nevena</i>	0000-0002-2582-8619	57, 196, 208
<i>Radovanović, Jelena</i>	0000-0002-3005-7943	158

<i>Radošević, Ksenija</i>	0009-0001-4881-9715	65, 89, 154
<i>Radukić, Tamara</i>	0009-0000-5236-7953	109, 162, 212
<i>Radulović, Aleksandra</i>	0000-0001-8591-2946	174
<i>Rajić, Vladimir</i>	0000-0001-7053-572X	208
<i>Rakin, Marica</i>	0000-0001-5791-942X	166
<i>Rakin, Sandra</i>	0009-0007-4016-3882	166
<i>Rikalo, Aleksandar</i>	0000-0002-3433-2009	5
<i>Rizvanbegović, Harun</i>	/	13
<i>Ružić, Jovana</i>	0000-0002-8076-0012	69
<i>Sarap, Nataša</i>	0000-0003-4686-3920	5
<i>Savić, Nada</i>	0000-0002-6593-3499	45
<i>Senčanski, Jelena</i>	0000-0002-2020-0435	39, 170, 174
<i>Simić, Milena</i>	0000-0001-7790-1681	49, 117
<i>Simonović, Bogdana</i>	0009-0008-1851-3505	200
<i>Sladić, Dušan M.</i>	0000-0002-1000-9813	192
<i>Spahić, Lemana</i>	0000-0002-0922-6335	13
<i>Spasović, Stefana</i>	0000-0001-9214-4682	204
<i>Sredović Ignjatović, Ivana D.</i>	0000-0002-2471-9242	178
<i>Stanić, Petar B.</i>	0000-0002-3655-6452	224
<i>Stanisavljević Ilić, Andrijana</i>	0000-0002-4723-9145	129, 137
<i>Stanimirović, Julijana</i>	0000-0002-0929-3482	184
<i>Stanojević, Slađana P.</i>	0000-0001-5740-8267	178
<i>Stanković, Dalibor</i>	0000-0001-7465-1373	97
<i>Stekić, Andjela</i>	0000-0002-2353-2937	162, 212
<i>Stojanović, Zoran</i>	0000-0002-5989-0031	53, 105, 216
<i>Sudar-Milovanović, Emina</i>	0000-0002-2273-5310	158
<i>Šavikin, Katarina</i>	0000-0002-2086-9593	77
<i>Šenk, Milena</i>	0000-0002-4732-8586	49
<i>Šmit, Biljana M.</i>	0000-0001-9799-4723	224
<i>Štefanović, Mario</i>	0000-0002-3965-3231	13
<i>Tasić, Aleksandra</i>	0000-0002-8361-5697	85
<i>Tekić, Danijela</i>	0000-0003-2102-3116	125, 188
<i>Tenji, Dina</i>	0000-0002-7657-943X	81
<i>Terzić, Tamara</i>	0000-0003-1988-4064	57, 196, 208
<i>Tešić, Živoslav</i>	0000-0002-5162-3123	85
<i>Todorović, Ana</i>	0000-0002-2074-1637	53, 105, 216
<i>Todorović, Nataša</i>	0000-0001-8694-8860	5
<i>Todorović Vukotić, Nevena</i>	0000-0003-2806-1991	129, 137
<i>Todorović, Ognjen</i>	0009-0000-5184-5239	117
<i>Tomić, Nina</i>	0000-0003-0086-0054	81
<i>Tosif, Mensuri M.</i>	0000-0002-9077-5429	22
<i>Tošić, Miloš</i>	0000-0002-6159-379X	89
<i>Tufegdžić, Srđan J.</i>	0000-0002-5397-1092	192, 220
<i>Unterweger, Christoph</i>	0000-0003-4399-5813	196
<i>Valenta Šobot, Ana</i>	0000-0002-8429-1399	65, 89, 154

<i>Vassilev, Dragomir</i>	0000-0003-0579-894X	142
<i>Veljković, Filip</i>	0000-0002-4471-334X	216
<i>Verbić, Tatjana Ž.</i>	0000-0002-6348-1644	1
<i>Višinková, Jana</i>	/	16
<i>Vozár, Tomáš</i>	0009-0001-6414-9918	16
<i>Vukadinović, Jelena</i>	0000-0003-0999-9677	49
<i>Vukomanović, Marko</i>	0009-0004-5535-0961	200
<i>Vuletić, Stefana</i>	0000-0001-8868-474X	204
<i>Vulin, Irina</i>	0000-0002-3184-9774	81
<i>Weheabby, Saddam</i>	0000-0002-7577-900X	208
<i>Yaneva, Dobromira</i>	0009-0004-4565-1656	142
<i>Zafirović, Sonja</i>	0000-0002-5486-0079	133
<i>Zarić Kontić, Marina</i>	0000-0003-1635-9766	109, 162, 212
<i>Zarić, Danica</i>	0000-0002-0026-3949	166
<i>Zečević, Tijana</i>	0009-0008-7630-6184	53, 105, 216
<i>Zlámalová Gargošová, Helena</i>	0000-0001-8309-8012	32
<i>Živković, Jelena</i>	0000-0002-9684-2637	77
<i>Živković, Marijana B.</i>	0000-0002-1978-1308	192, 220, 224

Organized by



Department of Physical Chemistry,
Vinča Institute of Nuclear Sciences-National Institute of the Republic of Serbia
University of Belgrade



Society of Physical Chemists of Serbia

Supported by



Ministry of Science, Technological Development and Innovation
of the Republic of Serbia

