
JSCSEN 90(12) 1425-1578(2025)

ISSN 1820-7421(Online)

Journal of the Serbian Chemical Society

Electronic version

VOLUME 90

No 12

BELGRADE 2025

Available on line at



www.shd.org.rs/JSCS/

The full search of JSCS
is available through

DOAJ DIRECTORY OF
OPEN ACCESS
JOURNALS
www.doaj.org

The **Journal of the Serbian Chemical Society** (formerly Glasnik Hemijskog društva Beograd), one volume (12 issues) per year, publishes articles from the fields of chemistry. The **Journal** is financially supported by the **Ministry of Education, Science and Technological Development of the Republic of Serbia**.

Articles published in the **Journal** are indexed in **Clarivate Analytics products: Science Citation Index-Expanded™** – accessed via **Web of Science®** and **Journal Citation Reports®**.

Impact Factor announced on 28 June, 2023: **1.000; 5-year Impact Factor: 1.100**.

Articles appearing in the **Journal** are also abstracted by: **Scopus**, **Chemical Abstracts Plus (CAplus™)**, **Directory of Open Access Journals**, **Referativni Zhurnal (VINITI)**, **RSC Analytical Abstracts**, **EuroPub**, **Pro Quest** and **Asian Digital Library**.

Publisher: **Serbian Chemical Society**, Karmegijeva 4/III, P. O. Box 36, 1120 Belgrade 35, Serbia
tel./fax: +381-11-3370-467, E-mails: **Society** – shd@shd.org.rs; **Journal** – jscs@shd.org.rs
Home Pages: **Society** – <http://www.shd.org.rs/>; **Journal** – <http://www.shd.org.rs/JSCS/>
Contents, Abstracts and full papers (from Vol 64, No. 1, 1999) are available in the

Internet Service:

Former Editors:

electronic form at the Web Site of the **Journal** (<http://www.shd.org.rs/JSCS/>).
Nikola A. Pušin (1930–1947), **Aleksandar M. Leko** (1948–1954), **Panta S. Tutundžić** (1955–1961), **Miloš K. Mladenović** (1962–1964), **Đorđe M. Dimitrijević** (1965–1969), **Aleksandar R. Despić** (1969–1975), **Slobodan V. Ribnikar** (1975–1985), **Dragutin M. Dražić** (1986–2006).

Editor-in-Chief:

Deputy Editor:

Sub editors:

Organic Chemistry

BRANISLAV Ž. NIKOLIĆ, Serbian Chemical Society (E-mail: jscs-ed@shd.org.rs)
DUŠAN SLADIĆ, Faculty of Chemistry, University of Belgrade

*Biochemistry and
Biotechnology*

Inorganic Chemistry

Theoretical Chemistry

DEJAN OPSENICA, Institute of Chemistry, Technology and Metallurgy, University of Belgrade

JÁNOS CSANÁDI, Faculty of Science, University of Novi Sad

OLGICA NEDIĆ, INEP – Institute for the Application of Nuclear Energy, University of Belgrade

BILJANA GLIŠIĆ, Faculty of Science, University of Kragujevac

MATUJA ZLATAR, Institute of Chemistry, Technology and Metallurgy, University of Belgrade

Physical Chemistry

Electrochemistry

Analytical Chemistry

Polymers

Thermodynamics

Chemical Engineering

MILOŠ MILIČIĆ, Faculty of Chemistry, University of Belgrade

LJILJANA DAMJANOVIĆ-VASILJIĆ, Faculty of Physical Chemistry, University of Belgrade

SNEŽANA GOJKOVIĆ, Faculty of Technology and Metallurgy, University of Belgrade

RADA BAOŠIĆ, Faculty of Chemistry, University of Belgrade

BRANKO DUNJIĆ, Faculty of Technology and Metallurgy, University of Belgrade

MIRJANA KIJEVCANIN, Faculty of Technology and Metallurgy, University of Belgrade

TATJANA KALUĐEROVIĆ RADOIČIĆ, Faculty of Technology and Metallurgy,

University of Belgrade

Materials

*Metallic Materials and
Metallurgy*

*Environmental and
Geochemistry*

*History of and
Education in Chemistry*

RADA PETROVIĆ, Faculty of Technology and Metallurgy, University of Belgrade

ANA KOSTOV, Mining and Metallurgy Institute Bor, University of Belgrade

VESNA ANTIĆ, Faculty of Agriculture, University of Belgrade

DRAGICA TRIVIĆ, Faculty of Chemistry, University of Belgrade

English Language

Editors:

LYNNE KATSIKAS, Serbian Chemical Society

VLATKA VAJS, Serbian Chemical Society

JASMINA NIKOLIĆ, Faculty of Technology and Metallurgy, University of Belgrade

Technical Editors:

VLADIMIR PANIĆ, Institute of Chemistry, Technology and Metallurgy, University of

Belgrade, **MARIO ZLATOVIĆ**, Faculty of Chemistry, University of Belgrade

**Journal Manager &
Web Master:**

MARIO ZLATOVIĆ, Faculty of Chemistry, University of Belgrade

Office:

VERA ČUŠIĆ, Serbian Chemical Society

Editorial Board

From abroad: **R. Adžić**, Brookhaven National Laboratory (USA); **A. Casini**, University of Groningen (The Netherlands); **G. Cobb**, Baylor University (USA); **D. Douglas**, University of British Columbia (Canada); **G. Inzelt**, Eötvös Loránd University (Hungary); **J. Kenny**, University of Perugia (Italy); **Ya. I. Korenman**, Voronezh Academy of Technology (Russian Federation); **M. D. Lechner**, University of Osnabrueck (Germany); **S. Macura**, Mayo Clinic (USA); **M. Spiteller**, INFU, Technical University Dortmund (Germany); **M. Stratakis**, University of Crete (Greece); **M. Swart**, University of Girona (Cataluna, Spain); **G. Vunjak-Novaković**, Columbia University (USA); **P. Worsfold**, University of Plymouth (UK); **J. Zagal**, Universidad de Santiago de Chile (Chile).

From Serbia: **B. Abramović**, **V. Antić**, **R. Baošić**, **V. Beškoski**, **J. Csanadi**, **Lj. Damjanović-Vasilić**, **A. Dekanski**, **V. Dondur**, **B. Dunjić**, **M. Đuran**, **B. Glišić**, **S. Gojković**, **I. Gutman**, **B. Jovančičević**, **I. Juranić**, **T. Kaluđerović**, **Radiočić**, **L. Katsikas**, **M. Kijevcanin**, **A. Kostov**, **V. Leovac**, **S. Milonjić**, **V.B. Mišković-Stanković**, **O. Nedić**, **B. Nikolić**, **J. Nikolić**, **D. Opsenica**, **V. Panić**, **M. Petkovska**, **R. Petrović**, **I. Popović**, **B. Radak**, **S. Ražić**, **D. Sladić**, **S. Sovilj**, **S. Šerbanović**, **B. Šolaja**, **Ž. Tešić**, **D. Trivić**, **V. Vajs**, **M. Zlatović**.

Subscription: The annual subscription rate is **150.00 €** including postage (surface mail) and handling. For Society members from abroad rate is **50.00 €**. For the proforma invoice with the instruction for bank payment contact the Society Office (E-mail: shd@shd.org.rs) or see JSCS Web Site: <http://www.shd.org.rs/JSCS/>, option Subscription.

Godišnja preplata: Za članove SHD: **2.500,00 RSD**, za penzionere i studente: **1000,00 RSD**, a za ostale: **3.500,00 RSD**; za organizacije i ustanove: **16.000,00 RSD**. Uplate se vrše na tekući račun Društva: **205-13815-62**, poziv na broj **320**, sa naznakom “preplata za JSCS”.

Nota: Radovi čiji su svi autori članovi SHD prioritarno se publikuju.

Odlukom Odbora za hemiju Republičkog fonda za nauku Srbije, br. 66788/1 od 22.11.1990. godine, koja je kasnije potvrđena odlukom Saveta Fonda, časopis je uvršten u kategoriju međunarodnih časopisa (**M-23**). Takođe, aktom Ministarstva za nauku i tehnologiju Republike Srbije, 413-00-247/2000-01 od 15.06.2000. godine, ovaj časopis je proglašen za publikaciju od posebnog interesa za nauku. **Impact Factor** časopisa objavljen 2024. godine je **0,700**, a petogodišnji **Impact Factor 0,900**. **Journal Citation Indicator** časopisa za period 2020–2024 je **0,16**.

CONTENTS*

Organic Chemistry

- S. S. Konstantinović, D. Z. Troter, J. B. Zvezdanović, S. M. Petrović, S. R. Savić, B. R. Danilović and J. Ćirić: Reishi (*Ganoderma lucidum*) tincture enriched with propolis or green tea from Serbia: UHPLC-DAD-ESI-MS characterization, mineral content and antibacterial activity 1425

Inorganic Chemistry

- I. S. Đorđević, S. Grubišić, D. Mitić, D. M. Popović and N. Trišović: Tuning crystal packing and reactivity through electrostatic and dispersion interactions: The case of phenytoin and its derivatives 1439

Theoretical Chemistry

- L. H. Mendoza-Huizar: A computational study to identify the metabolites in *Lippia alba* capable of inhibiting gastric cancer through BCL-2 protein inhibition 1451

Polymers

- R. Sarker, G. Chandra Sarker, A. Dhar Pranta and J. Saha: Impact of internal notation and heterobifunctional reactive dyes on color performance, mechanical and dimensional stability of cellulose-based biomaterials 1465

Materials

- M. Randelović, C. C. Sorrell, S. Koppka, A. Zarubica, M. Z. Momčilović and D. Enke: Development of a glass-ceramic composite reinforced with β -wollastonite synthesized via a hydrothermal method 1481
- D. D. Marković, V. B. Tadić, A. R. Žugić and M. M. Radetić: Sustainable synthesis of silver nanoparticles on cotton gauze for enhanced antibacterial properties 1495

Metallic Materials and Metallurgy

- G. Chunag, U. Byambasuren, S. Altantsetseg, G. Yunden, S. Sukhbaatar and D. Altansukh: Pressure oxidative leaching of copper concentrate 1513

Environmental

- I. Jovanić, A. Šajnović, S. Stojadinović, N. Burazer, B. Glavaš-Trbić and B. Jovančičević: Tri- and tetracyclic terpanes as proxies for depositional environment reconstruction for weathered siliciclastics of Internal Dinarides 1527
- N. Khakimova, M. Bogunović Koljaja, J. Simeunović, L. Landwehrkamp, S. Panglisch and I. Ivančev-Tumbas: Influence of microalgae on organic micropollutants removal from water by powdered activated carbon 1539

Letters to the Editor

- F. Fantuzzi, J. B. Maljković, M. Zlatar, C. P. Souza, H. M. Quitián-Lara, P. Nag, B. Antony, B. P. Marinković and N. J. Mason: Centennial of quantum theory in Belgrade: CEQPAS and the molecular excited state spectroscopy (MOLESs) initiative 1555

Published by the Serbian Chemical Society
Karnegijeva 4/III, P.O. Box 36, 11120 Belgrade, Serbia
Printed by the Faculty of Technology and Metallurgy
Karnegijeva 4, P.O. Box 35-03, 11120 Belgrade, Serbia

* For colored figures in this issue please see electronic version at the Journal Home Page:
<http://www.shd.org.rs/JSCS/>



J. Serb. Chem. Soc. 90 (12) 1425–1437 (2025)
JSCS–5463

Reishi (*Ganoderma lucidum*) tincture enriched with propolis or green tea from Serbia: UHPLC–DAD–ESI–MS characterization, mineral content and antibacterial activity

SANDRA S. KONSTANTINOVIĆ^{1*}, DRAGAN Z. TROTER¹, JELENA B. ZVEZDANOVIĆ¹, SANJA M. PETROVIĆ¹, SAŠA R. SAVIĆ¹, BOJANA R. DANILOVIĆ¹ and JOVAN ĆIRIĆ²

¹University of Niš, Faculty of Technology, Bulevar oslobođenja 124, 16000 Leskovac, Serbia, and ²Toplica Academy of Vocational Studies, Department of Agricultural and Food Studies, Ćirila i Metodija 1, 18400 Prokuplje, Serbia

(Received 19 June, revised 21 July, accepted 20 November 2025)

Abstract: The objective of the present study was to evaluate the differences in chemical composition and mineral content of pure Reishi (*Ganoderma lucidum*) tincture with those enriched with propolis or green tea. The chemical composition and mineral content were analyzed by UHPLC–DAD–ESI–MS and ICP–OES methods, respectively. Among all samples, Reishi/propolis tincture contained a significant amount of polyphenols, with the dominant contribution of flavonoids and phenolic acids. Regarding mineral content, P, K and Na were the most abundant in all tinctures. Reishi/propolis tincture also demonstrated the best antibacterial activity, with *Proteus vulgaris* and *Pseudomonas aeruginosa* as the most sensitive bacteria.

Keywords: antibacterial activity; green tea; ICP–OES; propolis; reishi; UHPLC–DAD–ESI–MS.

INTRODUCTION

Reishi or Lingzhi (*Ganoderma lucidum*) is one of the most popular and highly regarded medicinal fungi, demonstrating various biological and pharmacological activities, *e.g.*, anti-tumor, antimicrobial, antioxidative, anti-inflammatory, anti-hyperglycemic, anti-hyperlipidemic, anti-tyrosinase and antimutagenic, as well as cardio-, neuro-, hepato- and renal-protective, *etc.*^{1,2} Commercial *G. lucidum* products are available as powders, dietary supplements, and tea obtained from its different parts, including mycelia, spores and fruit bodies. The main component of mushrooms is water (90 mass %), while proteins, fats, carbohydrates, ash, minerals and vitamins are the remaining 10 mass % of the total content.^{2,3} The fruit body of this

*Corresponding author. E-mail: sakisandra12@yahoo.com
<https://doi.org/10.2298/JSC250619084K>

fungus is a rich source of bioactive components, such as polysaccharides, triterpenes, polyphenols, vitamins and different types of secondary metabolites (phenolic compounds, polyketides, terpenes, steroids and phenols), including more than 140 different lanostane-type triterpenoids.^{1–3} Such a versatile chemical composition explains a wide range of its pharmaceutical properties.

Combining pure Reishi tincture with other natural and biologically active extracts can improve its biological and pharmacological activities. Propolis, a resinous substance collected by bees from different plants, is another natural product considered one of the best general panaceas since ancient times.⁴ It is a complex mixture, and its chemical composition is qualitatively and quantitatively variable, depending on the bee species, geographical areas and botanical source.^{5,6} Among natural compounds found in propolis, polyphenols are the most important, mainly phenolic acids and flavonoids.^{4–6} These compounds are responsible for its numerous activities, such as antimicrobial, antioxidant, anti-inflammatory, antiproliferative, antihypertensive, spasmolytic, hepatoprotective, immune-stimulant, *etc.*^{4–6} Propolis can be found in various cosmetics, beverages, food products, and dietary supplements. Green tea, obtained from the leaves of *Camellia sinensis* L., is a widely consumed beverage worldwide. It is rich in polyphenols (particularly catechins), while its extract contains lower amounts of other compounds, such as flavonoids and phenolic acids.⁷

Thus, the aims of the current study were to determine and compare the chemical and mineral composition of pure Reishi tincture with those enriched with propolis or green tea from Serbia, as well as to evaluate their antimicrobial activity against eight bacteria. The chemical composition and mineral content were analyzed by UHPLC-DAD-ESI-MS and ICP-OES methods, respectively. These results could promote further development of the Reishi production industry in Serbia and be useful for the food, pharmaceutical, and cosmetic industries.

EXPERIMENTAL

Material

Commercial ethanolic extracts obtained by triple extraction of pure Reishi (35 %), Reishi/propolis (35 %), and Reishi/green tea (35 vol. %) were analyzed. A total of 100 ml of each tincture was evaporated by rotary evaporator at 40 °C to dryness and dried at 50 °C until reaching constant weight. The total solid matter was 4 mg·mL⁻¹ of tincture.

Chemicals and reagents

Ethanol, 96 % (Centrochem, Zemun, Serbia), and quercetin, rutin, and apigenin standards (Sigma-Aldrich, St. Louis, USA) were used. Distilled water and aqueous NaCl solution (0.9 %) were provided from Zorka Pharma d.o.o. (Šabac, Serbia). Dimethylsulfoxide (DMSO, ≥ 99 %) was purchased from Sigma-Aldrich (Munich, Germany). Solvents used in the UHPLC experiments were of HPLC or LC/MS grade. Concentrated nitric acid (65 %, Merck) and distilled water (HPLC grade, Fisher Chemical) were used for ICP-OES analysis.

UHPLC-DAD-ESI-MS analysis

The chromatographic separation was performed on a Hypersil Gold C18 column (50 mm×2.1 mm, 1.9 μ m) at 25 °C using a Dionex Ultimate 3000 UHPLC+ system equipped with a diode array (DAD) detector and LCQ fleet ion trap mass spectrometer (Thermo Fisher Scientific, USA). A mobile phase consisted of two solvents, 0.1 vol. % formic acid in water and methanol, with a gradient program at 0.25 mL·min⁻¹ flow rate.⁸ The injection volume was 1 μ L. Absorption UV/Vis spectra were recorded on a DAD-detector with a total spectral range between 200 and 800 nm. The mass spectrometric analysis was performed using a 3D-ion trap with electrospray ionization (ESI) in negative ion mode. The ESI source parameters were as follows: source voltage 4.5 kV, capillary voltage 41 V, tube lens voltage 95 V, capillary temperature 350 °C, nitrogen sheath and auxiliary gas flow 32 and 8 arbitrary units. MS spectra were acquired by full range acquisition of m/z 130–1300, with a tandem mass spectrometry analysis performed by a data-dependent scan – the collision-induced dissociation (CID) of detected molecular ion peaks ([M–H].) with normalized collision energy set at 25 eV. The ESI source parameters for positive ion mode also used for detection of pure Reishi tincture were source voltage 5.0 kV, capillary voltage 49 V, tube lens voltage 100 V, capillary temperature 350 °C, and 32 and 8 units for the auxiliary and sheath gas flow, respectively, in the full range, 130–1200, and data-dependent scan with CID set at 25 eV of detected [M+H]⁺, or cationized [M+Na]⁺ molecular ion peaks. Xcalibur software (version 2.1) was used for instrument control, data acquisition and data analysis.

ICP-OES analysis

The quantitative analysis was carried out using inductively coupled plasma-optical emission spectrometry (ICP-OES, Arcos FHE12, Spectro, Germany), following the manufacturer's guidelines. All samples from the solid (Reishi, propolis and green tea powders) and liquid (tinctures) materials were prepared through a wet digestion procedure with concentrated nitric acid, as detailed in the literature.^{9,10} Argon 5.0 (99.999 %) purity served as the carrier gas. In addition to the analytical sample, calibration standards were prepared beforehand. The calibration solution was created using Multistandard IV – a multi-elemental standard solution from Merck, which includes Ag, B, Cd, Co, Cr, Cu, Fe, Li, Mn, Na, Ni, Pb, Tl and Zn at concentrations of 1000 ppm. Parameters of the calibration lines for the determined elements are presented in the Supplementary material to this paper (Table S-I).

Antimicrobial activity

The antimicrobial activity was evaluated using the following microorganisms: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Listeria monocytogenes* (ATCC 19166), *Proteus vulgaris* (ATCC 8427), *Bacillus cereus* (ATCC11778) and *Bacillus luteus* in *haus* strain. Microorganisms were from the Microbiology Laboratory, Faculty of Technology, Leskovac. Media used for the growth of the microorganisms were Antibiotic agar No. 1 for microbiology (Merck, Darmstadt, Germany) and nutrient agar (Torlak, Belgrade, Serbia) for bacterial growth. The agar disc diffusion method was used for testing the antibacterial activity.¹¹ The mediums were sterilized in an autoclave at 121 °C under 110 kPa for 25 min. The suspension was prepared with overnight culture and adjusted to 0.5 McFarland standard. The prepared suspension contained 1×10^8 – 2×10^8 colony-forming unit (CFU)·mL⁻¹. The inoculum of 0.1 mL of suspension was added to 10 mL of medium and poured into the Petri dishes. For screening, sterilized filter paper disks (9 mm in diameter, Schleicher & Schuell) were placed on the surface of inoculated mediums and impregnated with 50 μ L of the extract sample (evaporated ethanolic extract

dissolved in DMSO, concentration 100 mg·mL⁻¹). Pure DMSO was used as a negative control. Plates were incubated for 24 h at 37 °C and results were expressed as the diameter of inhibition zones (IZ in mm). The presence of the IZ indicated the activity of the tested samples against the tested microorganisms. The following commercial standardized discs with predetermined amounts of antibiotics were used as positive controls: ciprofloxacin (5 µg), imipenem (10 µg), penicillin (6 µg, 10 IU), trimethoprim/sulfamethoxazole (1.25/23.75 µg), clindamycin (2 µg), ceftriaxone (30 µg), gentamicin (10 µg), amoxicillin + clavulanate (20/10 µg) and ceftiofur (30 µg), all from BD Sensi-Disc, GmbH, Heidelberg. All experiments were performed in triplicate.

Statistical analysis

All results were reported as mean value ± standard deviation from three measurements. Statistical comparisons were carried out by one-way ANOVA (analysis of variance) followed by Tukey's multiple comparison test by software SPSS 23.0 (IBM, USA). Differences are reflected as significant at $p < 0.05$.

RESULTS AND DISCUSSION

UHPLC-DAD-ESI-MS analysis

The typical UHPLC-DAD chromatograms recorded from DAD-signal at $\lambda = 320$ nm are shown in Fig. 1a–c.

The list of all compounds detected with their MS/UV/Vis spectral data is shown in Tables S-II–S-IV of the Supplementary material. For some detected compounds, the absorption and MS/MS spectra are shown in Supplementary material (Figs. S-1 and S-2). Mass spectra and fragmentation patterns, the order of elution from the chromatographic system, together with literature data,^{13–28} allowed the identification of some detected compounds.

Basic physiologically active components in *G. lucidum* are polysaccharides, peptidoglycans and triterpenes.^{1–3} Among 13 registered DAD-signals in pure Reishi tincture (Fig. 1a, Table S-II), signal 10 was identified as 12-acetoxycanoderic acid F, according to $[M-H]^-$, m/z 569, while ganoderic acid G type A ($[M-H]^-$, m/z 531 in negative mode and $[M+Na]^+$, m/z 555 in positive mode) was assigned to peak 12.¹⁶ Those ganoderic acids (related to triterpenoids) are the most representative components in *G. lucidum*, responsible for different biological activities. An appropriate MS/MS-fragmentation pattern in positive mode for the same compound (Fig. S-1a) confirmed the suggested compound, with cationized adducts at m/z 555 $[M+Na]^+$ and corresponding fragment ions. The corresponding UV/Vis spectrum (Fig. S-1a) of the ganoderic acid G type A exhibits an absorption maximum at 258 nm.¹⁶ The triterpenoid, often present in *G. lucidum*, lucidone A, was tentatively identified by confirming with Yan *et al.*¹⁵

The dominant class of compounds in both Reishi/propolis and Reishi/green tea tinctures are flavonoids. Rutin (Fig. 1b, Table S-III; peak No. 11), quercetin (Fig. 1c, Table S-IV; peak No. 9), and apigenin (Fig. 1c, Table S-IV; peak No. 14) were identified by their standard methanolic solutions. Hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, caffeic acid derivate and dihydroquercetin (Fig. 1c,

Table S-III; peaks No. 1, 2, 3, 4 and 5, respectively) were identified by using MassBank Record.¹⁹ For qualitative analysis of all detected signals, mass spectrometry combined with collision induced dissociation of molecular ions in data-dependent mode MS/MS was used, together with a comparison with the corresponding literature (Tables S-III and S-IV).

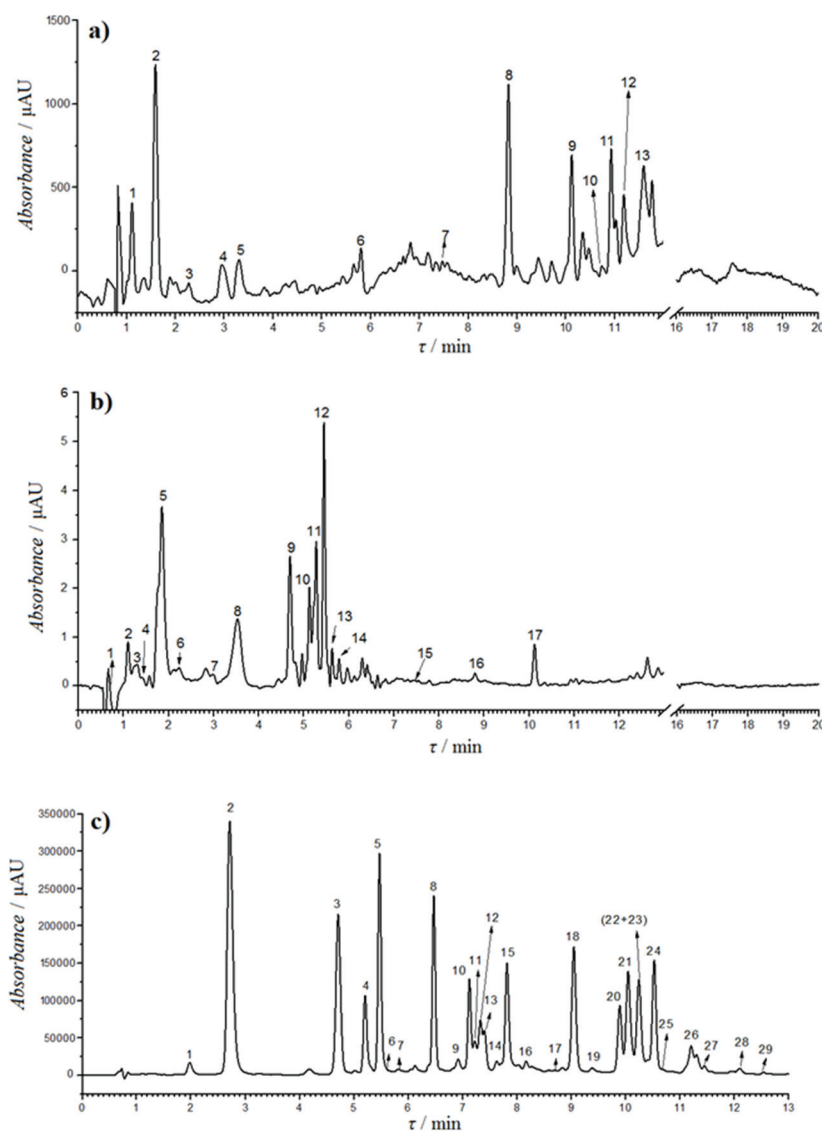


Fig. 1. UHPLC-DAD chromatograms of all tinctures (a – pure Reishi, b – Reishi/green tea and c – Reishi/propolis) at DAD signal $\lambda_{\text{det}} = 300$ nm. The corresponding peaks assignments (in the chromatograms labeled by numbers) are listed in Tables S-I–S-III, respectively.

Reishi/green tea tincture is rich in catechin, epicatechin, gallocatechin, epigallocatechin, and flavan-3-ols, characteristic of green tea. Gallocatechin is detected as signal No. 2 in the UHPLC-DAD chromatogram (Fig. 1b, Table S-III) and confirmed by the MS/MS-fragmentation pattern in negative mode and corresponding fragment ions (Fig. 2b). The UV/Vis spectrum of gallocatechin (Fig. S-1b) exhibits an absorption maximum at 287 nm.^{18,29} Reishi/green tea tincture also contains flavonols: kaempferol 3-*O*-rutinoside, kaempferol-*O*-glycoside, quercetin-glycoside and its quercetin-3-*O*-rutinoside derivative rutin (Fig. 1b, Table S-III; peaks No. 13, 14, 12 and 11, respectively) and flavones such as apigenin-glucosides: apigenin 6 (or 8)-*C*-xyloside-8(or 6)-*C*-glucoside (known as vicienin 1, IUPAC name 5,7-dihydroxy-2-(4-hydroxyphenyl)-8-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-6-[(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxyoxan-2-yl]chromen-4-one) and apigenin 6-*C*-glucosyl 8-*C*-(2"-*O*-dihydroferuloyl)-glucoside (IUPAC name 5,7-dihydroxy-2-(4-hydroxyphenyl)-6-[(2*S*,3*R*,4*R*,5*S*,6*R*)-3, 5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-8-{[2*R*,3*R*,4*S*,5*S*,6*R*)-2-[3-(4-hydroxy-3-methoxyphenyl)propanoyl]oxy-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]}chromen-4-one) (Fig. 1b, Table S-III; peaks No. 9 and 10, respectively). MS/MS fragmentation pattern in a negative mode for vicienin 1 (Fig. S-1c) presents molecular ions at *m/z* 563, 443, 383 and 353. These neutral losses are characteristics of glucosyl and pentosyl residues. The fragmentation signals at *m/z* 383 and 353 are also typical for di-*C*-glycosylflavones. UV/Vis spectra of vicienin 1 (Fig. S-1c) exhibit two absorption maxima at 272 and 334 nm, which is in accordance with the literature.²⁰ Quercetin-glycoside (Fig. 1b, Table S-III; peak No. 12) is detected as quercetin-7-*O*-glycoside, since the fragment ion at *m/z* 301 is rather abundant in 7-*O*-glycoside than in 3-*O*-glycoside (Fig. S-1d). Moreover, UV/Vis absorption maxima at 271 and 349 nm are in the range for this class of flavonols.²¹

Reishi/propolis tincture is rich in phenolic acids. Caffeic acid was detected as peak No. 2 in the UHPLC-DAD chromatogram (Fig. 1c, Table S-IV), while the corresponding UV/Vis spectrum (Fig. S-2a) contains two absorption maxima at 290 and 323 nm. An appropriate MS/MS-fragmentation pattern in negative mode (Fig. S-2a) confirmed the suggested compound with molecular ion at *m/z* 179 [M-H]⁻ and corresponding fragment ion at *m/z* 135.^{19,23} Caffeic acid derivatives were detected by peaks No. 4, 20, 21 and 22 (Fig. 1c, Table S-IV).^{19,23-25,28} Reishi/propolis tincture also contains quercetin and its derivatives dihydroquercetin or taxifolin, quercetin-*O*-rhamnoside, quercetin-3-methyl-ether and quercetin-3-dimethyl-ether (Fig. 1c, Table S-IV; peaks No. 9, 5, 7, 11 and 19) with molecular ion peaks found at *m/z* 301, 303, 447, 300 and 329, respectively. The corresponding UV/Vis spectrum of taxifolin (Fig. S-2b) contains two absorption maxima at 298 and 323 nm, while an appropriate MS/MS-fragmentation pattern in negative mode (Fig. S-2b) confirmed the suggested compound.^{19,25} Genistin, a well-known isoflavone, is identified as a peak No. 8 ([M-H]⁻, *m/z* 431) (Fig. 1c,

Table S-IV). Corresponding UV/Vis spectrum (Fig. S-2c) contains two absorption maxima at 290 and 322 nm, typical for this compound. The product ion mass spectrum of $[M-H]^-$ (Fig. S-2c) shows neutral losses of the glycan residue (162 Da) and of the glycan residue $+H^\bullet$ (163 Da) by rearrangement and cleavage, respectively, which is expected since, structurally, genistin represents genistein 7-*O*- β -D-glucoside.^{19,25} Reishi/propolis tincture is also rich in dihydroflavonol pinobanksin, its 5-methyl-ether derivative and 3-*O*-derivatives (acetate, butyrate, propionate and pentanoate), as well as methoxy-chrysin, luteolin-methyl-ether, galangin-5-methyl-ether and isorhamnetin-3-*O*-rutinoside.^{24,26,28} The tincture also contains flavanone pinocembrin (Fig. 1c, Table S-IV; peak No. 23), a natural precursor of pinobanksin. The corresponding UV/Vis spectrum (absorption maxima at 291 and 356 nm) and MS/MS-fragmentation pattern (Fig. S-2d) confirmed its presence.²⁸ Peaks No. 12, 13, 18 and 26 were unidentified derivatives of phenolic acids and flavonoids.

Mineral content

ICP-OES analysis results are presented in Table I.

TABLE I. Concentrations of macro- and microelement in samples; column values (different capital letters) and row values (different small letters) are significantly different ($p < 0.05$) by Tukey's multiple range test

Mineral	Solid material, $c / \mu\text{g}\cdot\text{g}^{-1}$			Tincture, $c / \text{mg}\cdot\text{L}^{-1}$		
	Reishi	Green tea	Propolis	Reishi	Reishi/green tea	Reishi/propolis
Ag	6.45 ^{L a}	0 ^{K b}	0 ^{N b}	0 ^{K b}	0 ^{O b}	0 ^{M b}
B	6.05 ^{M b}	3.54 ^{G c}	31.77 ^{H a}	0 ^{K e}	3.28 ^{E d}	0 ^{M e}
Ca	0 ^{O c}	112.1 ^{B b}	760.43 ^{D a}	0 ^{K c}	0 ^{O c}	0 ^{M c}
Cd	0 ^{O c}	6.8 ^{F a}	0 ^{N c}	0 ^{K c}	0 ^{O c}	0.025 ^{L b}
Co	0 ^{O b}	0.03 ^{K a}	0 ^{N b}	0 ^{K b}	0 ^{O b}	0 ^{M b}
Cr	44.76 ^{J a}	0.45 ^{J, K b}	0 ^{N c}	0 ^{K c}	0 ^{O c}	0 ^{M c}
Cu	77.02 ^{F a}	2.45 ^{H c}	8.49 ^{L b}	0.15 ^{I f}	0.83 ^{J d}	0.4 ^{I e}
Fe	1071.78 ^{C a}	2.2 ^{H c}	26.43 ^{I b}	0 ^{K e}	0.43 ^{L d}	0 ^{M e}
K	2.02 ^{N f}	11 ^{E e}	2659.07 ^{B a}	89.78 ^{B b}	49.33 ^{B c}	26.25 ^{C d}
Li	174.6 ^{E a}	2.2 ^{H b}	1.21 ^{M c}	0.1 ^{J e}	0.13 ^{M d}	0.1 ^{K e}
Mg	0 ^{O e}	320 ^{A b}	383.12 ^{E a}	4.9 ^{E c}	2.95 ^{G d}	2.05 ^{F d}
Mn	739.11 ^{D a}	0.5 ^{J, K c}	15.28 ^{J b}	0 ^{K e}	0.075 ^{N d}	0 ^{M e}
Na	0 ^{O f}	93 ^{C b}	318.14 ^{F a}	35.6 ^{C d}	48.23 ^{C c}	34.48 ^{A e}
Ni	13.31 ^{K a}	2.35 ^{H b}	0 ^{N c}	0 ^{K c}	0 ^{O c}	0 ^{M c}
Pb	45.16 ^{I a}	1.15 ^{I c}	0 ^{N e}	0 ^{K e}	1.45 ^{I b}	0.25 ^{J d}
Sr	0 ^{O e}	0 ^{K e}	11.4 ^{K a}	0.53 ^{H c}	0.73 ^{K b}	0.5 ^{H d}
Tl	47.18 ^{H a}	0.53 ^{J, K b}	0 ^{N c}	0 ^{K c}	0 ^{O c}	0 ^{M c}
Zn	48.79 ^{G a}	0.3 ^{K f}	36.13 ^{G b}	2.38 ^{G c}	2.35 ^{H d}	1.7 ^{G e}
Si	5657.26 ^{A a}	2.25 ^{H f}	35.89 ^{G b}	3.45 ^{F d}	3.08 ^{F e}	3.85 ^{E c}
P	1594.76 ^{B b}	0.88 ^{I, J f}	8692.05 ^{A a}	238.48 ^{A c}	117.58 ^{A d}	31.15 ^{B e}
S	0 ^{O f}	29.25 ^{D b}	1244.91 ^{C a}	26.2 ^{D c}	21.88 ^{D d}	6.53 ^{B e}

The difference in mineral content between crude samples and tinctures is evident, not only in a qualitative but also in a quantitative manner. A total of 21 elements was determined. Tested Reishi powder is notable for its high content of Si ($5657.26 \mu\text{g}\cdot\text{g}^{-1}$), P ($1594.76 \mu\text{g}\cdot\text{g}^{-1}$), Fe ($1071.78 \mu\text{g}\cdot\text{g}^{-1}$) and Mn ($739.11 \mu\text{g}\cdot\text{g}^{-1}$), while propolis was rich in P ($8692.05 \mu\text{g}\cdot\text{g}^{-1}$), K ($2659.07 \mu\text{g}\cdot\text{g}^{-1}$), S ($1244.91 \mu\text{g}\cdot\text{g}^{-1}$) and Ca ($760.43 \mu\text{g}\cdot\text{g}^{-1}$), followed by Mg ($383.12 \mu\text{g}\cdot\text{g}^{-1}$) and Na ($318.14 \mu\text{g}\cdot\text{g}^{-1}$). Among all crude samples, green tea powder contained the least amount of minerals, with Mg as the most abundant element ($320 \mu\text{g}\cdot\text{g}^{-1}$), followed by significantly lower quantities of Ca ($112.1 \mu\text{g}\cdot\text{g}^{-1}$), Na ($93 \mu\text{g}\cdot\text{g}^{-1}$) and S ($29.25 \mu\text{g}\cdot\text{g}^{-1}$). Although the mineral content of plants depends upon soil structure, climatic conditions, and plant species, the basic elements are more or less present in the same species. According to literature, Reishi contains minerals like Cu, Zn, Se, K, Ca, P, Mg, and Fe.³⁰ Propolis possesses Ca, K, Al, Cu, Mg, Fe and Zn in the highest amounts.³¹ The most abundant minerals found in green tea are P, K, Ca, Mg, Fe, Mn, Al, S, Si, followed by Zn, Cu and F.³² In all Reishi tinctures, the most common minerals are P, K and Na, which are very important macroelements and have a large impact on the human body. P plays a crucial and vital part in the creation of bones, teeth, nucleic acids, phosphoproteins, growth and the control of different repair processes. Its role in the conversion of food into energy, the metabolism of lipids and carbohydrates, lessening arthritis pain, and restoring proper kidney and cardiac function is also essential.³³ As two essential macroelements, K and Na are important to maintain body cellular homeostasis and metabolism, since most metabolic processes are dependent on or affected by these electrolytes. Also, these electrolytes maintain normal pH levels in extracellular fluid, regulate osmotic pressure, act as cofactors for many enzymes, and are involved in oxidation–reduction reactions.³⁴ Compared to crude samples, in all Reishi extracts, toxic elements, such as Cd and Pb, were not found or were present in insignificant amounts.

Antimicrobial activity

The results of the disc diffusion testing are presented in Table II. Both DMSO and sterile water negative controls showed no inhibition effects. The inhibition zones reveal different susceptibility of different bacteria to all tinctures.

None of the tinctures was antibacterial against *K. pneumonia*. While pure green tea tincture failed to express any antibacterial activity, both the pure Reishi and propolis tinctures demonstrated similar potential against *P. aeruginosa*, *E. coli* and *P. vulgaris*. However, propolis tincture was more active against *S. aureus* and was the only pure tincture deemed as antibacterial against *L. monocytogenes*, *B. cereus* and *B. luteus*. The antibacterial potential of different Reishi tinctures was reported in earlier studies.^{35,36}

TABLE II. Values of zones of bacterial growth inhibition (mm); column values (different capital letters) and row values (different small letters) are significantly different ($p < 0.05$) by

Tukey's multiple range test; NI – no inhibition; NT – not tested. *According to the CLSI protocol microorganisms can be considered resistant if the inhibition zone is lower than 14 mm, intermediate or susceptible-dose dependent for the zone 15–19 mm and susceptible for the zone bigger than 20 mm; bacteria: SA – *S. aureus*; PA – *P. aeruginosa*; EC – *E. coli*; KP – *K. pneumoniae*; LM – *L. monocytogenes*; BC – *B. cereus*; BL – *B. luteus*; PV – *P. vulgaris*; tincture: R – Reishi; GT – green tea; P – propolis; antibiotics: CPF – ciprofloxacin; TMF/SMT – trimethoprim/sulfamethoxazole; PCL – penicillin; CDM – clindamycin; CTX – ceftriaxone; IPN – imipenem; GTC – gentamicin; AMX/CVT – amoxicillin/clavulanate; CFX – cefoxitin

Inhibitor	SA	PA	EC	KP	LM	BC	BL	PV
R	9*±0.6 ^{Jc}	12±0.5 ^{Hb}	10±0.5 ^{Gc}	NI ^{Gd}	NI ^{Hd}	NI ^{Fd}	NI ^{Id}	15±0.5 ^{Fa}
GT	NI ^{Ka}	NI ^{Ia}	NI ^{Ha}	NI ^{Ga}	NI ^{Ha}	NI ^{Fa}	NI ^{Ia}	NI ^{Ha}
P	17±0.6 ^{Ia}	13±0.6 ^{Hb,c}	12±0.6 ^{Fc}	NI ^{Gd}	16±0.5 ^{Ga}	14±0.5 ^{Eb}	12±0.6 ^{Hc}	13±0.6 ^{Gb,c}
R/GT	11±0.6 ^{Jc}	15±0.5 ^{Ga}	12±1 ^{Fb,c}	NI ^{Gd}	NI ^{Hd}	13±0.6 ^{Eb}	NI ^{Id}	16±0.5 ^{E,Fa}
R/P	21±0.6 ^{Ga}	17±0.4 ^{Fb}	15±0.6 ^{Ec}	NI ^{Gd}	20±0.5 ^{Ea}	17±0.2 ^{Db}	15±0.3 ^{Gc}	17±0.5 ^{Eb}
CPF	26±0.6 ^{D,Ee}	40±0.5 ^{Aa}	40±0.6 ^{Aa}	31±0.8 ^{Ac}	36±0.6 ^{Bb}	30±1 ^{Ac,d}	28±1 ^{Dd,e}	26±0.6 ^{Ce}
TMF/SMT	NT ^{Kb}	NI ^{Ib}	NT ^{Hb}	NT ^{Gb}	NT ^{Hb}	NT ^{Fb}	21±0.6 ^{Fa}	NT ^{Hb}
PCL	32±1 ^{Ba}	16±0.5 ^{F,Gc}	NT ^{He}	11±1 ^{Fd}	16±0.6 ^{Gc}	13±0.5 ^{Ed}	29±1 ^{Db}	31±1 ^{Ba,b}
CDM	22±0.8 ^{F,Ga}	NI ^{Ic}	NT ^{Hc}	NT ^{Gc}	18±0.5 ^{Fb}	NT ^{Fc}	NT ^{Ic}	NT ^{Hc}
CTX	28±1 ^{C,Db}	32±0.5 ^{Ba}	32±0.6 ^{Ba}	22±0.5 ^{C,Dc}	27±0.3 ^{Cb}	21±1 ^{Cc}	14±1 ^{Gc}	31±0.3 ^{Ba}
IPN	40±1 ^{Ab}	22±1 ^{Dd}	27±0.6 ^{Cc}	26±0.6 ^{Bc}	42±1 ^{Ab}	26±0.6 ^{Bc}	46±0.6 ^{Aa}	42±0.5 ^{Ab}
GTC	24±0.8 ^{E,Fa,b}	20±0.5 ^{Ec,d}	22±1 ^{Db,c}	20±1 ^{Ec,d}	25±0.6 ^{Da}	21±0.6 ^{Cc,d}	26±0.6 ^{Ea}	19±0.5 ^{Dd}
AMX/CVT	30±0.6 ^{B,Cb}	26±0.6 ^{Cc}	12±1 ^{Fe}	21±1 ^{D,Ed}	26±0.6 ^{C,Dc}	21±1.05 ^{Cd}	44±0.6 ^{Ba}	42±1 ^{Aa}
CFX	28±0.6 ^{C,Db,c}	26±1 ^{Cc}	22±0.5 ^{Dd,e}	23±0.5 ^{Cd}	20±1 ^{Ee}	22±0.5 ^{Cd,e}	40±0.5 ^{Ca}	30±1 ^{Bb}

Compared to the pure Reishi extract, the Reishi/green tea tincture exhibited improved antibacterial effect, while Reishi/propolis tincture showed the highest activity against all tested strains, especially *S. aureus* and *L. monocytogenes*. Somewhat improved antibacterial activity of Reishi/green tea tincture compared to the pure Reishi and pure green tea tinctures can only be explained by the possible synergistic effect of the components present in Reishi and green tea. Specifically, the main catechins present in green tea (epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate) are known antimicrobials;³⁷ these catechins are detected in prepared Reishi/green tea tincture.

Interestingly, the addition of propolis in the Reishi tincture enabled it to express activity against *L. monocytogenes*, *B. luteus* and *B. cereus*. Previous studies confirmed the antibacterial potential propolis tinctures from Serbia against *L. monocytogenes*, *S. aureus* and *E. coli*.^{38–40} Although the chemical composition of propolis is quite complex, its source causes only minor variations regarding its antimicrobial activity.⁴⁰ Antimicrobial activity of propolis was associated with the presence of phenolic compounds, such as caffeic acid, quercetin, luteolin, apigenin,

p-coumaric acid, kaempferol and pinobanksin.³⁹ Since these phenolic components are also present in Reishi/propolis tincture, its improvement in antibacterial capacity is explained by both the presence of various phenolic compounds originating from Reishi and propolis, as well as their synergistic effect.

Although the Reishi/propolis tincture exhibits moderate antibacterial activity compared to some commercial options, its use may still be justified due to the multiple other potential health benefits associated with its constituents. Previously reported immunomodulatory, anti-inflammatory and antioxidant properties of both Reishi^{1–3} and propolis^{4–6} could contribute to overall wellness.

CONCLUSION

The chemical composition and mineral content of pure Reishi tincture and those enriched with propolis or green tea were compared. Among all samples, Reishi/propolis tincture contained a significant amount of polyphenols, with the dominant contribution of flavonoids and phenolic acids. Regarding mineral content, P, K and Na were the most abundant in all tinctures. Reishi/propolis tincture also exhibited the best antibacterial activity compared to the other extracts. Based on reported chemical compositions and antimicrobial activities, these Reishi-based tinctures have promising prospects as food and health supplements. The high levels of polyphenols, flavonoids, phenolic acids and essential minerals may contribute to antioxidant, immunomodulatory, and general wellness-promoting effects. Enrichment with propolis or green tea further enhances the bioactive profile, potentially supporting cardiovascular, metabolic, and immune health. Therefore, even if the antibacterial activity is moderate compared to commercial products, the combined health benefits and nutritional value make these tinctures valuable candidates for dietary supplementation. Further studies on bioavailability, dosage and clinical effects would help to fully realize their potential in human health applications.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13428>, or from the corresponding author on request.

Acknowledgements. This work was supported by the Republic of Serbia – Ministry of Science, Technological Development and Innovation (Programs for financing scientific research: 451-03-137/2025-03/200133 and 451-03-136/2025-03/200133 assigned to the University of Niš, Faculty of Technology, Leskovac).

ИЗВОД

ТИНКТУРА РЕИШИЈА (*Ganoderma lucidum*) ОБОГАЂЕНА ПРОПОЛИСОМ ИЛИ
ЗЕЛЕНИМ ЧАЈЕМ ИЗ СРБИЈЕ: UHPLC-DAD-ESI-MS КАРАКТЕРИЗАЦИЈА,
МИНЕРАЛНИ САСТАВ И АНТИБАКТЕРИЈСКА АКТИВНОСТСАНДРА С. КОНСТАНТИНОВИЋ¹, ДРАГАН З. ТРОТЕР¹, ЈЕЛЕНА Б. ЗВЕЗДАНОВИЋ¹, САЊА М. ПЕТРОВИЋ¹,
САША Р. САВИЋ¹, БОЈАНА Р. ДАНИЛОВИЋ¹ И ЈОВАН ЂИРИЋ²¹Технолошки факултет, Универзитет у Нишу, Булевар ослобођења 124, 16000 Лесковац и²Тоилчка академија сирковних ситудија, Одсек за пољопривредно-прехрамбене ситудије,
Ђирица и Методија 1, 18400 Прокупље

Циљ овог рада био је процена разлика у хемијском саставу и минералном садржају чисте реиши (*Ganoderma lucidum*) тинктура са тинктурама обогаченим прополисом или зеленим чајем. Хемијски састав и садржај минерала су анализирани применом течне хроматографије веома високих перформанси у комбинацији са детектором умрежених фотоодиода и електроспреј јонизација-масеном спектрометријом (UHPLC-DAD-ESI-MS) и оптичком емисионом спектрометријом са индуктивно спрегнутом плазмом (ICP-OES), редом. Међу овим узорцима, реиши/прополис тинктура садржи значајну количину полифенола са доминантним уделом флавоноида и фенолних киселина. У погледу минералног састава, Р, К и Na су највише присутни у свим тинктурама. Реиши/прополис тинктура је показала и најбољу антибактеријску активност, при чему су *P. vulgaris* и *P. aeruginosa* биле најосетљивије бактерије.

(Примљено 19. јуна, ревидирано 21. јула, прихваћено 20. новембра 2025)

REFERENCES

1. S. Wu, S. Zhang, B. Peng, D. Tan, M. Wu, J. Wei, Y. Wang, H. Luo, *Food Sci. Hum. Wellness* **13** (2024) 568 (<https://doi.org/10.26599/FSHW.2022.9250051>)
2. K. Sułkowska-Ziaja, M. Balik, A. Szczepkowski, M. Trepa, G. Zengin, K. Kała, B. Muszyńska, *Diversity* **15** (2023) 882 (<https://doi.org/10.3390/d15080882>)
3. S. Wachtel-Galor, J. Yuen, J.A. Buswell, I. F. F. Benzie, in *Herbal Medicine: Biomolecular and Clinical Aspects*, 2nd ed., I. F. F. Benzie, S. Wachtel-Galor, Eds., CRC Press/Taylor & Francis, Boca Raton, FL, 2011, pp. 175–200 (<https://doi.org/10.1201/b10787>)
4. A. K. Balasubramaniam, A. Elangovan, M. A. Rahman, S. Nayak, D. Swain, H. P. Babu, A. Narasimhan, V. Monga, *Fitoterapia* **183** (2025) 106526 (<https://doi.org/10.1016/j.fitote.2025.106526>)
5. N. Bhatti, Y. A. Hajam, S. Mushtaq, L. Kaur, R. Kumar, S. Rai, *Discov. Sustain.* **5** (2024) 185 (<https://doi.org/10.1007/s43621-024-00375-3>)
6. A. Sarapa, A. Peter, A. Buettner, H. M. Loos, *Eur. Food Res. Technol.* **251** (2025) 1331 (<https://doi.org/10.1007/s00217-025-04708-y>)
7. J. Sun, S. Dong, J. Li, H. Zhao, *Food Sci. Hum. Wellness* **11** (2022) 1143 (<https://doi.org/10.1016/j.fshw.2022.04.008>)
8. V. Tadić, I. Arsić, J. Zvezdanović, A. Zugić, D. Cvetković, S. Pavkov, J. *Ethnopharmacology* **199** (2017) 138 (<https://doi.org/10.1016/j.jep.2017.02.002>)
9. S. V. Dončić, J. B. Zvezdanović, S. M. Petrović, S. R. Savić, I. B. Konstantinović, D. Z. Troter, J. M. Jovanović Mirković, S. S. Konstantinović, *Adv. Technol.* **13** (2024) 5 (<https://doi.org/10.5937/savteh2401005D>)
10. S. Savić, S. Petrović, M. Petronijević, A. Cvetanović, Ž. Petronijević, *Adv. Technol.* **8** (2019) 27 (<https://doi.org/10.5937/SavTeh1901027S>)

11. *CLSI Performance Standards for Antimicrobial Susceptibility Testing*, 30th ed., CLSI supplement M100, Clinical and Laboratory Standards Institute, Wayne, PA, 2020
12. D. H. Ryu, J. Y. Cho, N. B. Sadiq, J.-C. Kim, B. Lee, M. Hamayun, T. S. Lee, H. S. Kim, S. H. Park, C. W. Nho, H.-Y. Kim, *Food Chem.* **335** (2021) 127645 (<https://doi.org/10.1016/j.foodchem.2020.127645>)
13. R. J. Robbins, *J. Agric. Food. Chem.* **51** (2003) 2866 (<https://doi.org/10.1021/jf026182t>)
14. E. de Rijke, P. Out, W. M. A. Niessen, F. Ariese, C. Gooijer, U. A. Th. Brinkman, *J. Chromatogr., A* **1112** (2006) 31 (<https://doi.org/10.1016/j.chroma.2006.01.019>)
15. Z. Yan, B. Xia, M. H. Qiu, D. L. Sheng, H. X. Xu, *Biomed. Chromatogr.* **27** (2013) 1560 (<https://doi.org/10.1002/bmc.2960>)
16. T.-Q. Chen, C. Yang, J.-G. Wu, J.-Z. Wu, *J. Chem. Pharm. Res.* **8** (2016) 730 (<https://www.jocpr.com/articles/total-triterpenoids-from-the-ultrasoniccirculating-extract-powder-of-ganoderma-lucidum-and-its-antioxidant-activity-in-v.pdf>)
17. C. C. Wyrepkowski, D. L. M. Gomes da Costa, A. P. Sinhorin, W. Vilegas, R. A. De Grandis, F. A. Resende, E. A. Varanda, L. C. dos Santos, *Molecules* **19** (2014) 16039 (<https://doi.org/10.3390/molecules191016039>)
18. D. H. Markowicz Bastos, L. A. Saldanha, R. R. Catharino, A. C. H. F. Sawaya, I. B. S. Cunha, P. O. Carvalho, M. N. Eberlin, *Molecules* **12** (2007) 423 (<https://doi.org/10.3390/12030423>)
19. *MassBank Record*, <http://www.massbank.jp> (accessed 13 May 2025)
20. Z. Benayad, C. Gómez-Cordovés, N. E. Es-Safi, *Int. J. Mol. Sci.* **15** (2014) 20668 (<https://doi.org/10.3390/ijms151120668>)
21. H.-Y. Zhao, J.-H. Sun, M.-X. Fan, L. Fan, L. Zhou, Z. Li, J. Han, B.-R. Wang, D.-A. Guo, *J. Chromatogr., A* **1190** (2008) 157 (<https://doi.org/10.1016/j.chroma.2008.02.109>)
22. X. Jiang, Y. Liu, W. Li, L. Zhao, F. Meng, Y. Wang, H. Tan, H. Yang, C. Wei, X. Wan, L. Gao, T. Xia, *PLoS One* **8** (2013) e62315 (<https://doi.org/10.1371/journal.pone.0062315>)
23. J.-P. Cornard, C. Lapouge, *J. Phys. Chem., A* **110** (2006) 7159 (<https://doi.org/10.1021/jp060147y>)
24. F. Pellati, G. Orlandini, D. Pinetti, S. Benvenuti, *J. Pharm. Biomed. Anal.* **55** (2011) 934 (<https://doi.org/10.1016/j.jpba.2011.03.024>)
25. M. Ye, W.-Z. Yang, K.-D. Liu, X. Qiao, B.-J. Li, J. Cheng, J. Feng, D.-A. Guo, Y.-Y. Zhao, *J. Pharm. Anal.* **2** (2012) 35 (<https://doi.org/10.1016/j.jpba.2011.09.009>)
26. J. M. Fuentes-Alventosa, S. Jaramillo, G. Rodríguez-Gutiérrez, P. Cermeño, J. A. Espejo, A. Jiménez-Araujo, R. Guillén-Bejarano, J. Fernández-Bolaños, R. Rodríguez-Arcos, *J. Agric. Food. Chem.* **56** (2008) 6977 (<https://doi.org/10.1021/jf8009766>)
27. F. Sánchez-Rabaneda, O. Jáuregui, I. Casals, C. Andrés-Lacueva, M. Izquierdo-Pulido, R. M. Lamuela-Raventós, *J. Mass Spectrom.* **38** (2003) 35 (<https://doi.org/10.1002/jms.395>)
28. S. I. Falcão, M. Vilas-Boas, L. M. Estevinho, C. Barros, M. R. M. Domingues, S. M. Cardoso, *Anal. Bioanal. Chem.* **396** (2010) 887 (<https://doi.org/10.1007/s00216-009-3232-8>)
29. J. Sun, F. Liang, Y. Bin, P. Li, C. Duan, *Molecules* **12** (2007) 679 (<https://doi.org/10.3390/12030679>)
30. M. F. Ahmad, A. Alsayegh, F. A. Ahmad, M. S. Akhtar, S. S. Alavudeen, F. Bantun, S. Wahab, A. Ahmed, M. Ali, E. Y. Elbendary, A. Raposo, N. Kambal, M. H. Abdelrahman, *Heliyon* **10** (2024) e25607 (<https://doi.org/10.1016/j.heliyon.2024.e25607>)
31. D. S. Ghallab, E. Shawky, M. M. Mohyeldin, A. M. Metwally, R. S. Ibrahim, *J. Adv. Pharm. Sci.* **2** (2025) 76 (<https://doi.org/10.21608/japs.2025.336494.1032>)

32. M. Radeva-Ilieva, S. Stoeva, N. Hvarchanova, K. D. Georgiev, *Foods* **14** (2025) 745 (<https://doi.org/10.3390/foods14050745>)
33. A. A. H. Ali, *J. Trace Elem. Miner.* **4** (2023) 100076 (<https://doi.org/10.1016/j.jtemin.2023.100076>)
34. M. A. Farag, B. Abib, Z. Qin, X. Ze, S. E. Ali, *Curr. Res. Nutr. Food Sci.* **6** (2023) 100450 (<https://doi.org/10.1016/j.crfs.2023.100450>)
35. M. N. Djide, Sartini, L. Rahman, N. Hasyim, *Int. J. Sci. Technol. Res.* **3** (2014) 15 (<http://www.ijstr.org/final-print/jan2014/Antibacterial-Activity-Of-Various-Extracts-From-The-Fruiting-Bodies-Of-Ganoderma-Lucidum-Growing-At-Samanea-Saman-jacq-Merr-Trunk.pdf>)
36. J. Singh, S. Gupta, S. Malviya, B. Ahrwar, *Int. J. Adv. Res.* **2** (2014) 460 (https://www.journalijar.com/uploads/31_IJAR-3328.pdf)
37. W. C. Reygaert, *Front. Microbiol.* **5** (2014) 434 (<https://doi.org/10.3389/fmicb.2014.00434>)
38. S. Ivančajić, I. Mileusnić, D. Cenić-Milošević, *Arch. Biol. Sci.* **62** (2010) 915 (<https://doi.org/10.2298/ABS1004915I>)
39. P. Ristivojević, I. Dimkić, J. Trifković, T. Berić, I. Vovk, D. Milojković-Opsenica, S. Stanković, *PLoS One* **11** (2016) e0157097 (<https://doi.org/10.1371/journal.pone.0157097>)
40. S. Stepanović, N. Antić, I. Dakić, M. Švabić-Vlahović, *Microbiol. Res.* **158** (2003) 353 (<https://doi.org/10.1078/0944-5013-00215>).



SUPPLEMENTARY MATERIAL TO
**Reishi (*Ganoderma lucidum*) tincture enriched with propolis or
green tea from Serbia: UHPLC-DAD–ESI-MS characterization,
mineral content and antibacterial activity**

SANDRA S. KONSTANTINOVIĆ^{1*}, DRAGAN Z. TROTER¹, JELENA B.
ZVEZDANOVIĆ¹, SANJA M. PETROVIĆ¹, SAŠA R. SAVIĆ¹, BOJANA R.
DANILOVIĆ¹ and JOVAN ĆIRIĆ²

¹University of Niš, Faculty of Technology, Bulevar oslobođenja 124, 16000 Leskovac, Serbia,
and ²Toplica Academy of Vocational Studies, Department of Agricultural and Food Studies,
Ćirila i Metodija 1, 18400 Prokuplje, Serbia

J. Serb. Chem. Soc. 90 (12) (2025) 1425–1437

TABLE S-I. Parameters of the calibration line for the determined elements

Element	λ / nm	R / 1	ROL / mg·L ⁻¹
Ag	224.641	0.99993	0.00532-12
	328.068	0.99995	0.00166-12
As	189.042	0.99982	0.00255-3.6
	197.262	1	0.00825-2.4
B	182.641	0.99999	0.00737-12
	249.773	0.99999	0.00643-12
Ba	233.527	0.99995	0.000115-12
Bi	190.241	0.99991	0.00353-12
	223.061	0.99996	0.00368-12
Ca	183.801	1	1.6-12
	396.847	1	0.0126-2.41
Cd	214.438	0.99989	0.000111-2.4
Ce	418.660	0.999917	0.00118-2.4
Co	228.616	0.99980	0.000327-12
Cr	283.563	0.99998	0.000435-12
Cu	224.700	0.99999	0.000907-12
	324.754	0.99999	0.000259-12
Fe	259.941	0.99995	0.00199-12
	184.950	0.99992	0.000192-12
Hg	194.227	0.99994	0.000579-12
	253.652	0.99986	0.00202-12
In	325.609	1	0.004-12
K	404.721	0.99996	0.156-60

*Corresponding author. E-mail: sakisandra12@yahoo.com

Li	323.261	1	0.798-12
	670.780	0.99997	0.0000574-1.2
Mg	285.213	0.99996	0.00206-12.1
Mn	257.611	0.99997	0.000105-12
	330.237	0.99995	0.798-12
Na	589.592	1	0.00583-1.2
Ni	231.604	0.99994	0.000474-12
P	214.914	0.99995	0.00-120
Pb	220.353	0.99998	0.00616-12
S	180.731	1	0.0029-24
Si	288.158	0.99998	0.00582-60
	251.612	0.99998	0.00124-60
Tl	190.864	0.99998	0.00172-12
Zn	213.856	0.99997	0.000082-12

TABLE S-II. The compounds detected by UHPLC-DAD-MS/MS in the pure Reishi tincture

Peak No.	t_R / min	λ_{max} / nm	$[M-H]^+$, m/z	MS/MS fragment ion peaks, m/z	Compounds assignment	Reference
1	1.11	280, 350	-	-	-	-
2	1.57	306	-	-	-	-
3	2.28	328	$[M+H]^+$ 301	^a 297.7 (100 %), 255	n.i. phenolic acid	Robbins ¹
4	2.95	280, 450	-	-	-	-
5	3.38	327	$[M+H]^+$ 301	^a 280, 264, 254, 245, 187.9 (100 %), 121	n.i. phenolic acid	Robbins ¹
6	5.81	304	$[M+H]^+$ 240	^a 217 (100 %), 199, 195	n.i. phenolic acid	Robbins ¹
7	7.46	333	175	147 (100 %), 131	n.i. phenolic acid	Robbins ¹
8	8.83	260, 366	373	329 (100 %), 305, 263, 178, 151	n.i. flavonoid	Rijke <i>et al.</i> ²
9	10.10	309	401	-	tent. Lucidone A	Yan <i>et al.</i> ³
			$[M+Na]^+$ 425	^a 407, 226, 161	-	
10	10.70	245	569	-	12-acetoxycyanoderic acid F	Chen <i>et al.</i> ⁴ ; Ryu <i>et al.</i> ⁵
11	11.00	237, 315	399	370, 353 (100 %), 337, 325, 309, 159	-	-
			$[M+Na]^+$ 423	377 (100 %), 408, 286, 159	-	-
12	11.55	258	531	-	Ganoderic acid G type A	Chen <i>et al.</i> ⁴
			$[M+Na]^+$ 555	549.8, 547, 527, 510, 497 (100 %)	Ganoderic acid G or methyl lucidenate	Chen <i>et al.</i> ⁴
13	11.65	-	$[M+Na]^+$ 555	537, 527, 508.66 (100 %), 490.62, 365, 283	Ganoderic acid G or methyl lucidenate	Chen <i>et al.</i> ⁴

^aPositive ionization mode

TABLE S-III. The compounds detected by UHPLC-DAD-MS/MS in the Reishi/green tea tincture

Peak No.	t_R / min	λ_{max} / nm	[M-H] ⁻ , m/z	MS/MS fragment ion peaks, m/z	Compounds assignment	Reference
1	0.90	-	343	191 (100 %), 169	3- <i>O</i> -galloyl quinic acid	Wyrepkowski <i>et al.</i> ⁶
2	1.13	287	305	261, 219, 179 (100 %), 175, 159, 137	Galocatechin	Markowicz Bastos <i>et al.</i> ⁷
3	1.24	324	229	183, 161 (100 %), 143	n.i.	
4	1.54	277, 337	305	261, 219, 179 (100 %), 175, 167, 137, 125	Epigallocatechin	Markowicz Bastos <i>et al.</i> ⁷
5	1.77	275	-	-	n.i.	
6	2.23	329	-	-	Phenolic acid derivative	Robbins ¹
7	3.10	274	289	245 (100 %), 231, 203, 175, 161, 137	Catechin/Epicatechin	MassBankRecord ^{8a} , Markowicz Bastos <i>et al.</i> ⁷
8	3.55	312	457	-	Epigallocatechin gallate	Markowicz Bastos <i>et al.</i> ⁷
9	4.69	272, 334	563	473, 443, 383, 353 (100 %), 297	Apigenin 6(or 8)-C-xyloside-8(or 6)-C-glucoside (vicenin 1)	Benayad <i>et al.</i> ⁹
10	5.12	259, 355	771	-	Apigenin 6-C-glucosyl 8-C-(2"-O-dihydroferuloyl)-glucoside	Benayad <i>et al.</i> ⁹
11	5.28	264, 351	609	301 (100 %), 271, 255, 228	Rutin	Standard
12	5.45	271, 349	463	343, 301 (100 %), 271, 243, 151	Quercetin-glycoside	Zhao <i>et al.</i> ¹⁰
13	5.63	266, 350	593	285 (100 %), 255, 241, 199, 185	Kaempferol 3- <i>O</i> -rutinoside	Jiang <i>et al.</i> ¹¹
14	5.80	268, 312	447	285 (100), 255, 227, 137	Kaempferol- <i>O</i> -glycoside	Jiang <i>et al.</i> ¹¹ ; Zhao <i>et al.</i> ¹⁰
15	7.45	333	583	539 (100 %), 469, 395, 243	n.i.	
16	8.85	257, 357	373	329 (100 %), 305, 263, 192, 177, 151	n.i.	
17	10.12	308	401	383, 355	Lucidone A	Yan <i>et al.</i> ³

*PR100687/PR100688

TABLE S-IV. The compounds detected by UHPLC-DAD-MS/MS in the Reishi/propolis tincture

Peak No.	t_R / min	λ_{max} / nm	$[M-H]^-$, m/z	MS/MS fragment ion peaks, m/z	Compounds assignment	Reference
1	1.97	311, 282	137	136, 109	Hydroxybenzoic acid	MassBankRecord ^{8a}
2	2.71	323, 290	179	135	Caffeic acid	MassBankRecord ^{8b} ; Cornard and Lapouge ¹²
3	4.71	310	163	119	<i>p</i> -coumaric acid	MassBankRecord ^{8c} ; Pellati <i>et al.</i> ¹³
4	5.20	323, 290	281	179, 161, 135 (100 %)	Caffeic acid derivate	-
5	5.47	323, 298	303	285 (100 %), 177	Dihydroquercetin	MassBankRecord ^{8d} ; Ye <i>et al.</i> ¹⁴
6	5.60	-	623	315, 300, 271	Isorhamnetin-3-O-rutinoside	Fuentes-Alventosa <i>et al.</i> ¹⁵
7	5.82	257, 340	447	301 (100 %), 285, 271, 255	Quercetin-O-rhamnoside	MassBankRecord ^{8e}
8	6.45	322, 290	431	268 (100 %), 239, 211, 167, 153	Genistin	Ye <i>et al.</i> ¹⁴
9	6.92	257, 370	301	-	Quercetin	Pellati <i>et al.</i> ¹³ ; standard
10	7.13	289, 320	285	267 (100 %), 252, 239, 224	Pinobanksin-5-methyl-ether	Pellati <i>et al.</i> ¹³
11	7.22	350, 252	315	300	Quercetin-3-methyl-ether	Pellati <i>et al.</i> ¹³
12	7.33	280	315	300	n.i.	
13	7.41	310, 293	349	-	n.i.	
14	7.62	338, 267	269	225, 201, 197, 183, 151, 149, 117	Apigenin	Sánchez-Rabaneda <i>et al.</i> ¹⁶ ; standard
15	7.81	293, 330	271	253	Pinobanksin	Pellati <i>et al.</i> ¹³
16	8.17	267, 350	299	284 (100), 255, 227, 179, 135	Luteolin-methyl-ether	Pellati <i>et al.</i> ¹³
17	8.70	354, 261	283	268, 239 (100 %), 211	Galangin-5-methyl-ether	Pellati <i>et al.</i> ¹³
18	9.04	312	449	421, 137	n.i.	
19	9.38	258, 358	329	314 (100), 299, 271	Quercetin-dimethyl-ether	Pellati <i>et al.</i> ¹³
20	9.89	325, 300	247	179 (100 %), 161, 135	Caffeic acid isoprenyl ester	Falcão <i>et al.</i> ¹⁷
21	10.05	327, 300	269	225, 178, 161, 134 (100 %)	Caffeic acid benzyl ester	Pellati <i>et al.</i> ¹³
22	10.16	-	247	203, 179 (100 %), 135, 121	Caffeic acid isoprenyl ester – isomer	Falcão <i>et al.</i> ¹⁷
23	10.27	291, 356	255	213 (100 %), 193, 183, 151, 145	Pinocembrin	Falcão <i>et al.</i> ¹⁷
24	10.53	296	313	271, 253 (100 %)	Pinobanksin-3-O-acetate	Falcão <i>et al.</i> ¹⁷
25	10.69	345, 266	283	268, 239, 179 (100 %), 161, 135	Methoxy-chrysin	Pellati <i>et al.</i> ¹³
26	11.21	325, 297, 250	295	211	n.i.	
27	11.45	293, 320	327	271, 253 (100 %)	Pinobanksin-3-O-propionate	Falcão <i>et al.</i> ¹⁷
28	12.10	292, 330	341	-	Pinobanksin-3-O-butyrate	Pellati <i>et al.</i> ¹³
29	12.54	293, 330	355	271, 253 (100 %)	Pinobanksin-3-O-pentanoate	Pellati <i>et al.</i> ¹³

^aPR100187; ^bKO000512; ^cPR100685; ^dBML01276; ^ePR100993

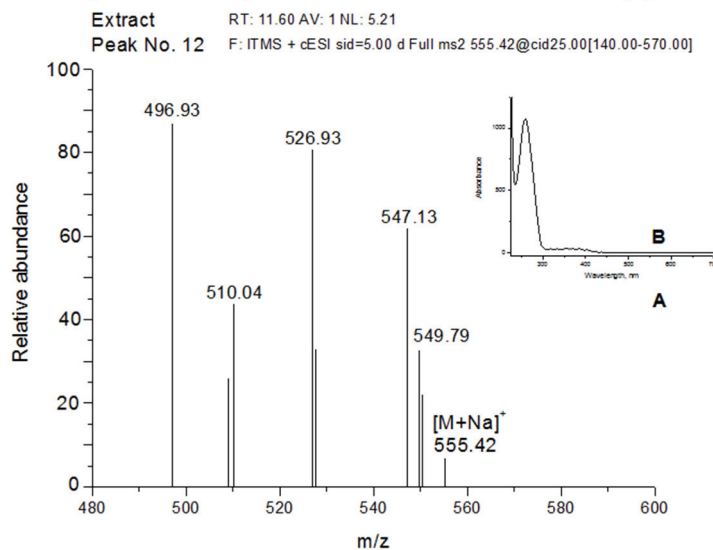
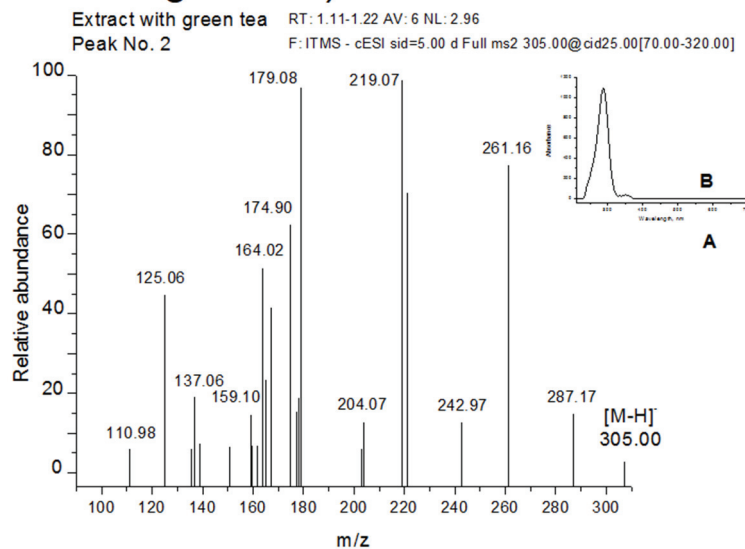
Fig. S-1. a) Ganoderic acid G type A**Fig. S-1. b) Gallocatechin**

Fig. S-1. Mass (A) and UV-Vis (B) spectra of selected compounds from chromatograms of pure Reishi tincture (a) and Reishi/green tea tincture (b-d). a) Compound No. 12 (Fig. 1a), assigned as ganoderic acid G type A (Table S-I); b) Compound No. 2 (Fig. 1b), assigned as gallocatechin (Table S-II);

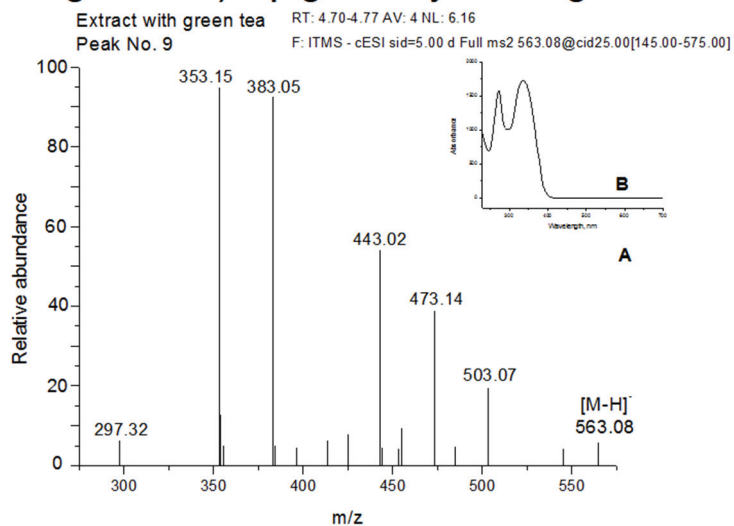
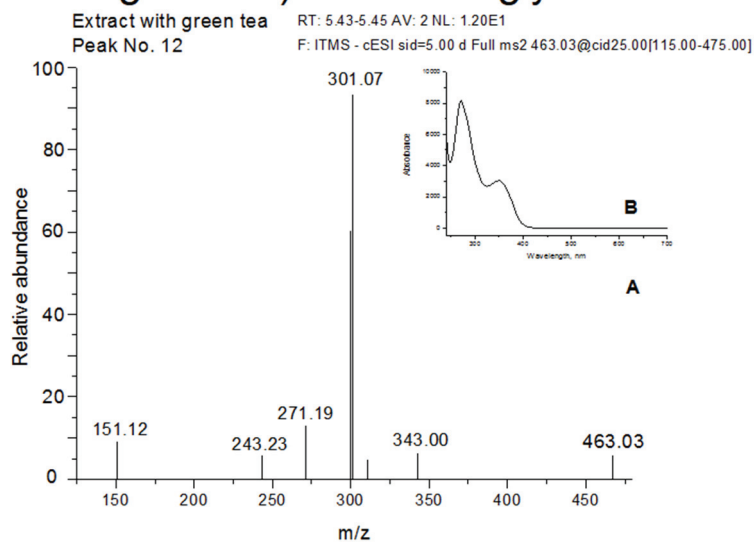
Fig. S-1. c) Apigenin-xyloside-glucoside**Fig. S-1. d) Quercetin-glycoside**

Fig. S-1. Continued c) Compound No. 9 (Fig. 1b), assigned as apigenin-xyloside-glucoside (Table S-II); d) Compound No. 12 (Fig. 1b), assigned as quercetin-glycoside (Table S-II).

Fig. S-2. a) Caffeic acid

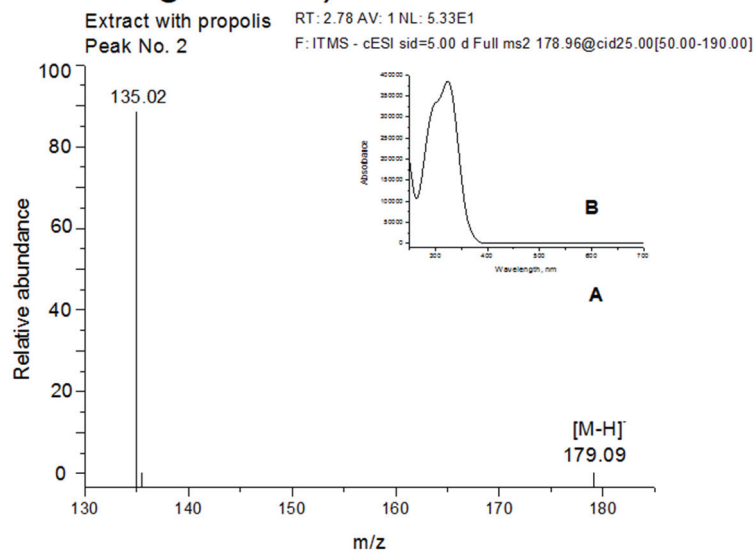


Fig. S-2. b) Dihydroquercetin

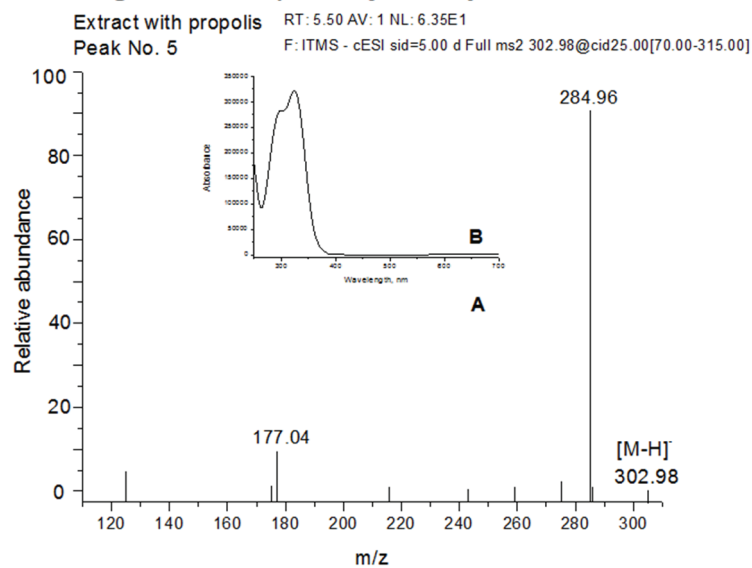


Fig. S-2. Mass (A) and UV-Vis (B) spectra of selected compounds from chromatogram of Reishi/propolis tincture. a) Compound No. 2 (Fig. 1c), assigned as caffeic acid (Table S-III); b) Compound No. 5 (Fig. 1c), assigned as dihydroquercetin (Table S-III);

Fig. S-2. c) Genistin

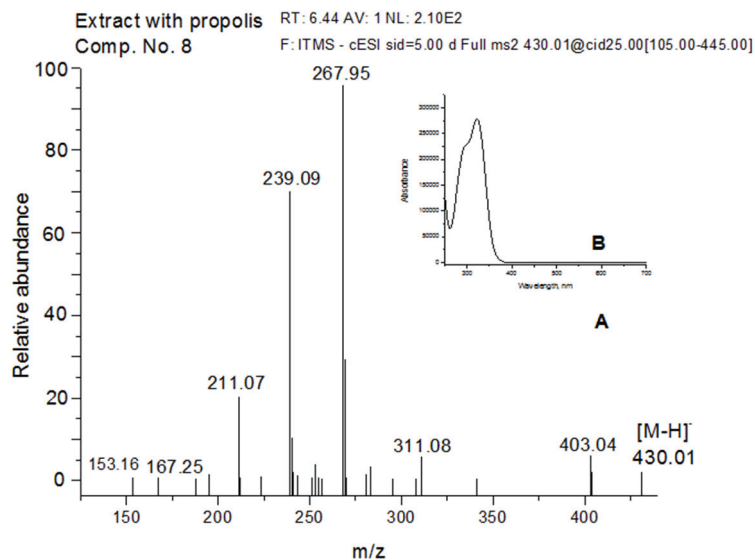


Fig. S-2. d) Pinocembrin

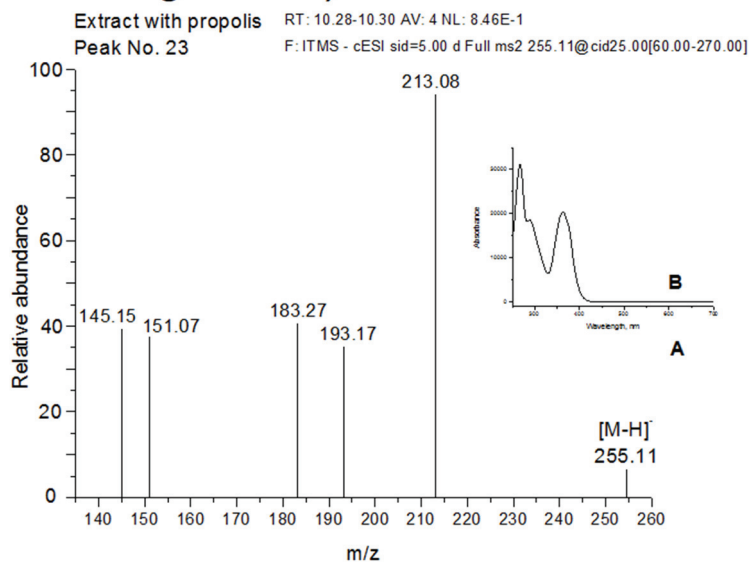


Fig. S-2. Continued c) Compound No. 8 (Fig. 1c), assigned as genistin (Table S-III); d) Compound No. 23 (Fig. 1c), assigned as pinocembrin (Table S-III).

REFERENCES

1. R. J. Robbins, *J. Agric. Food. Chem.* **51** (2003) 2866 (<https://doi.org/10.1021/jf026182t>)

2. E. d. Rijke, P. Out, W. M. A. Niessen, F. Ariese, C. Gooijer, U. A. Th. Brinkman, *J. Chromatogr. A* **1112** (2006) 31 (<https://doi.org/10.1016/j.chroma.2006.01.019>)
3. Z. Yan, B. Xia, M. H. Qiu, D. L. Sheng, H. X. Xu, *Biomed. Chromatogr.* **27** (2013) 1560 (<https://doi.org/10.1002/bmc.2960>)
4. T.-Q. Chen, C. Yang, J.-G. Wu, J.-Z. Wu, *J. Chem. Pharm. Res.* **8** (2016) 730 (<https://www.jocpr.com/articles/total-triterpenoids-from-the-ultrasoniccirculating-extract-powder-of-ganoderma-lucidum-and-its-antioxidant-activity-in-v.pdf>)
5. D. H. Ryu, J. Y. Cho, N. B. Sadiq, J.-C. Kim, B. Lee, M. Hamayun, T. S. Lee, H. S. Kim, S. H. Park, C. W. Nho, H.-Y. Kim, *Food Chem.* **335** (2021) 127645 (<https://doi.org/10.1016/j.foodchem.2020.127645>)
6. C. C. Wyrepkowski, D. L. M. Gomes da Costa, A. P. Sinhorin, W. Vilegas, R. A. De Grandis, F. A. Resende, E. A. Varanda, L. C. dos Santos, *Molecules* **19** (2014) 16039 (<https://doi.org/10.3390/molecules191016039>)
7. D. H. Markowicz Bastos, L. A. Saldanha, R. R. Catharino, A. C. H. F. Sawaya, I. B. S. Cunha, P. O. Carvalho, M. N. Eberlin, *Molecules* **12** (2007) 423 (<https://doi.org/10.3390/12030423>)
8. MassBank Record, <http://www.massbank.jp> (accessed 13 March 2020)
9. Z. Benayad, C. Gómez-Cordovés, N. E. Es-Safi, *Int. J. Mol. Sci.* **15** (2014) 20668 (<https://doi.org/10.3390/ijms151120668>)
10. H.-Y. Zhao, J.-H. Sun, M.-X. Fan, L. Fan, L. Zhou, Z. Li, J. Han, B.-R. Wang, D.-A. Guo, *J. Chromatogr. A* **1190** (2008) 157 (<https://doi.org/10.1016/j.chroma.2008.02.109>)
11. X. Jiang, Y. Liu, W. Li, L. Zhao, F. Meng, Y. Wang, H. Tan, H. Yang, C. Wei, X. Wan, L. Gao, T. Xia, *PLoS One* **8** (2013) e62315 (<https://doi.org/10.1371/journal.pone.0062315>)
12. J.-P. Cornard, C. Lapouge, *J. Phys. Chem. A* **110** (2006) 7159 (<https://doi.org/10.1021/jp060147y>)
13. F. Pellati, G. Orlandini, D. Pinetti, S. Benvenuti, *J. Pharm. Biomed. Anal.* **55** (2011) 934 (<https://doi.org/10.1016/j.jpba.2011.03.024>)
14. M. Ye, W.-Z. Yang, K.-D. Liu, X. Qiao, B.-J. Li, J. Cheng, J. Feng, D.-A. Guo, Y.-Y. Zhao, *J. Pharm. Anal.* **2** (2012) 35 (<https://doi.org/10.1016/j.jpba.2011.09.009>)
15. J. M. Fuentes-Alventosa, S. Jaramillo, G. Rodríguez-Gutiérrez, P. Cermeño, J. A. Espejo, A. Jiménez-Araujo, R. Guillén-Bejarano, J. Fernández-Bolaños, R. Rodríguez-Arcos, *J. Agric. Food. Chem.* **56** (2008) 6977 (<https://doi.org/10.1021/jf8009766>)
16. F. Sánchez-Rabaneda, O. Jáuregui, I. Casals, C. Andrés-Lacueva, M. Izquierdo-Pulido, R. M. Lamuela-Raventós, *J. Mass Spectrom.* **38** (2003) 35 (<https://doi.org/10.1002/jms.395>)
17. S. I. Falcão, M. Vilas-Boas, L. M. Estevinho, C. Barros, M. R. M. Domingues, S. M. Cardoso, *Anal. Bioanal. Chem.* **396** (2010) 887 (<https://doi.org/10.1007/s00216-009-3232-8>).



J. Serb. Chem. Soc. 90 (12) 1439–1450 (2025)
JSCS–5464

Tuning crystal packing and reactivity through electrostatic and dispersion interactions: The case of phenytoin and its derivatives

IVANA S. ĐORĐEVIĆ^{1*}, SONJA GRUBIŠIĆ¹, DRAGANA MITIĆ²,
DRAGAN M. POPOVIĆ¹ and NEMANJA TRIŠOVIĆ³

¹University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, 11001 Belgrade, Serbia, ²University of Belgrade, Innovative Centre of the Faculty of Chemistry, Belgrade, Serbia and ³University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Serbia

(Received 5 August, revised 22 August, accepted 24 October 2025)

Abstract: Hydantoin derivatives represent a versatile class of heterocycles, known for their pharmacological properties. Because drug efficacy often depends on the fine-tuning of weak intermolecular (non-covalent) interactions, analysis of the crystal structure of a drug molecule is important, as it enables deciphering its interaction profile. In this study, the crystal packing of phenytoin and its selected derivatives were examined through dimeric motifs with different recognition modes using force-field calculations and a density functional theory (DFT) approach. The relatively polar ethoxyacetyl group at the N3 position of the hydantoin ring, capable of forming hydrogen bonds, enhances the contribution of electrostatic and polar components to the total interaction energy. In contrast, the long alkyl chain promotes hydrophobic contacts, leading to dispersion forces dominating over electrostatic interactions. The reactivity of phenytoin and its derivatives were further evaluated by examining the influence of these substituents using conceptual density functional theory (CDFT) descriptors. These findings demonstrate that substituents significantly affect crystal packing and the balance of non-covalent interactions, providing valuable insights for optimizing molecular recognition and drug–target interactions in the design of new therapeutic agents.

Keywords: intermolecular interactions; hydantoin; electron density; phenytoin; global reactivity descriptors.

INTRODUCTION

Derivatives of hydantoin (imidazolidine-2,4-dione) represent a versatile class of heterocyclic compounds with important applications in pharmacology, mater-

*Corresponding author. E-mail: ivana.djordjevic@ihtm.bg.ac.rs
<https://doi.org/10.2298/JSC250805077D>

ials science and crystal engineering. This five-membered ring provides conformational rigidity and planarity, facilitating interactions with biological targets (Fig. 1).

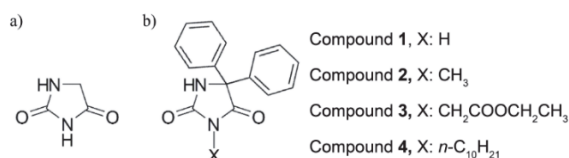


Fig. 1. Chemical structure of: a) the hydantoin and b) the phenytoin and its derivatives.

Both the carbonyl groups at positions 2 and 4 (electrophilic centres) and the NH groups at positions 1 and 3 participate in hydrogen bonding, which is crucial for interactions with biological systems. Additionally, the C5 position is a reactive methylene site suitable for functionalization. All these features contribute to the characteristic stability of hydantoin, while still providing sufficiently reactive centres for the development of new derivatives. These derivatives have a wide range of applications: in bio- and medicinal chemistry due to their pharmacological activities;¹ in agriculture as potential antimicrobial agents;² in coordination chemistry as valuable ligands with uses in electrochemical biosensors^{3,4} and anticorrosive materials.⁵ However, hydantoin derivatives are still primarily recognized for their pharmacological activities, including antimicrobial, anticonvulsant, anti-inflammatory, antidiabetic, anticancer, antiplatelet, anti-HIV and allosteric antagonist action.¹

Phenytoin (5,5-diphenylhydantoin) is a well-known anticonvulsant drug and among the first to be developed through rational drug design.^{6,7} It has been widely used for decades to manage and prevent seizures, particularly tonic-clonic and focal seizures. Disorders of the central nervous system (CNS) represent one of the major medical challenges of the twenty-first century. Despite advances in developing potential therapies for neuronal disorders, drug crossing of the blood–brain barrier (BBB) remains a significant obstacle.^{8–10} Several factors influence the activity of phenytoin. It selectively blocks voltage-dependent sodium channels (NVSC) by forming hydrogen bonds, which are critical for its anticonvulsant action.^{11–14} However, access to these channels depends on the presence of hydrophobic groups in the molecular structure. Such groups, particularly at the C5 position, enable hydantoins to interact with or cross the cell membrane. High lipophilicity is also important, enabling molecules to cross the BBB and remain in the brain for a longer time.¹⁵ On the other hand, a comparison of structurally diverse hydantoin derivatives with the same log *P* values has shown that the binding affinities can vary significantly despite similar lipophilicity.¹⁶ It appears that the orientation of the phenyl ring plays a critical role, with an optimal spatial arrangement enhancing the binding efficiency of 5-phenylhydantoins.

The crystal structure of a drug molecule plays an important role in pharmacology, as it influences solubility, stability and bioavailability (*e.g.*, different polymorphs may exhibit distinct absorption profiles). Moreover, the specific intermolecular interactions observed in the crystal structure provide valuable insights into the binding preferences of a drug molecule toward target biomolecules.¹⁷ These features are essential for the rational design of effective and selective drugs, as well as for predicting pharmacokinetically relevant properties such as lipophilicity and membrane permeability, including the ability to cross the blood–brain barrier (BBB).

Finally, improving drug efficacy often depends on the fine-tuning of weak non-covalent (non-bonding) interactions, including polar hydrogen bonds, hydrophobic $H\cdots H$ contacts and van der Waals forces. These relatively weak and long-ranged interactions have a cumulative effect that makes them crucial for molecular recognition, stability and structural organization. Because accurately modeling these interactions remains challenging, modern computational methods that incorporate dispersion effects have become indispensable tools in drug design and crystal structure prediction.^{18–21}

Based on the information and key issues outlined above, this study examines the intermolecular interactions in dimeric motifs extracted from the crystal packing of phenytoin and its N3-alkyl derivatives (Fig. 1). For this purpose, interaction energies of different molecular pairs were evaluated using two approaches: a force-field-based method and a density functional theory (DFT) approach. Furthermore, the influence of substituents was analysed through various descriptors, including electronegativity, hardness, and softness, within the framework of conceptual density functional theory (CDFT).

METHODOLOGY

The studied structures were obtained from the Cambridge Structural Database (CSD).²² All calculations were carried out using Gaussian 09,²³ CrystalExplorer 21.5²⁴ and Mercury.²⁵ The interaction energies of the dimeric motifs were evaluated with the DFT-based method, at B3LYP/6-31G(d,p) level of theory, implemented in CrystalExplorer (CE-B3LYP), while the UNI force-field approach^{26,27} was also applied for a comparative analysis. Both approaches rely on the same theoretical principle – the evaluation of intermolecular interaction energies – and therefore provide consistent criteria for the relevant dimers. Reported molecular pairs displayed the strongest stabilizing effects and played a representative role in describing the crystal packing motifs. Geometry optimizations of monomeric structures (compounds **1–4**) were carried out without constraints using the B3LYP/6-31G(d,p) level of theory in the gas phase and using the solvation model based on density (SMD) for water and dimethyl sulfoxide (DMSO), as implemented in Gaussian 09. Single-point calculations at the same level of theory were subsequently performed on the optimized geometries to obtain orbital energies in each environment. Global reactivity descriptors were then derived according to the equations provided in the Supplementary material to this paper.

RESULTS AND DISCUSSION

Structure selection

Considering the factors influencing the phenytoin activity, several of its derivatives were selected for a comparative study of preferential intermolecular interactions in their crystal packings and their molecular reactivity. Hydrogen-bond formation plays a significant role in the anticonvulsant activity of phenytoin derivatives. In contrast, hydrophobic groups at the C5 position facilitate membrane passage, enabling the molecules to reach the active site. Therefore, in addition to phenytoin, its derivatives were analysed in which the hydrogen atom at the N3 position was replaced with a methyl group, an ethoxycarbonylmethyl group, or a *n*-decyl group (Fig. 1). This selection of substituents provided structural diversity by introducing either a group capable of hydrogen bonding or short and long alkyl groups suitable for hydrophobic interactions.

Considerations of intermolecular interactions

The selected structures, extracted from the Cambridge Structural Database (CSD), crystallized in three different space groups. Compound **1**, also known as phenytoin, belongs to the orthorhombic $Pn2_1a$ space group (PHYDAN01, CCDC: 1232751).²⁸ Compound **2** and **4** crystallized in monoclinic $P2_1/n$ space group (PEPDUM01, CCDC: 2381918²⁹ and PAJMAS, CCDC: 2012925,⁸ respectively). Compound **3**, which contains a polar ester group, is reported in triclinic, $P\bar{1}$ space group (JALGEL, CCDC: 1528488).³⁰ The most relevant dimeric motifs in the crystal packing of the investigated compounds were identified and quantitatively described, providing a deeper understanding of how molecules interact and organize within the crystal structure. For this purpose, the interaction energies of molecular pairs were determined using two distinct approaches: a DFT-based method (CE-B3LYP) and the UNI force-field energy framework. First, the results obtained with the CE-B3LYP method are discussed, followed by the corresponding energy values derived from the UNI force field.

As shown in Fig. 2 and Table S-I of the Supplementary material, the D1 and D2 motifs of compound **1**, in addition to electrostatic $H\cdots O$ hydrogen bonds, also exhibit significant repulsive interactions. In contrast, motifs D3, D4, D5 and D6 are predominantly stabilized by dispersion forces.

For compound **2**, electrostatic $H\cdots O$ interactions provide the most significant energy contribution in dimer D1, whereas dispersion interactions dominate in the other dimer motifs (Fig. 3, Table S-I).

For the dimeric motif D1 of compound **3**, the electrostatic term again dominates, and the overall interaction energy shows a remarkable increase compared to the corresponding dimers of the previous two compounds (Fig. 4, Table S-I). The symmetric arrangement of the two monomers, which feature strong $N-H\cdots O$ hydrogen bonds, significantly contributes to the stability of the dimeric motif D1. Dim-

ers D2, D3 and D4 exhibit a pronounced contribution from dispersion energy, accompanied by higher total interaction energies.

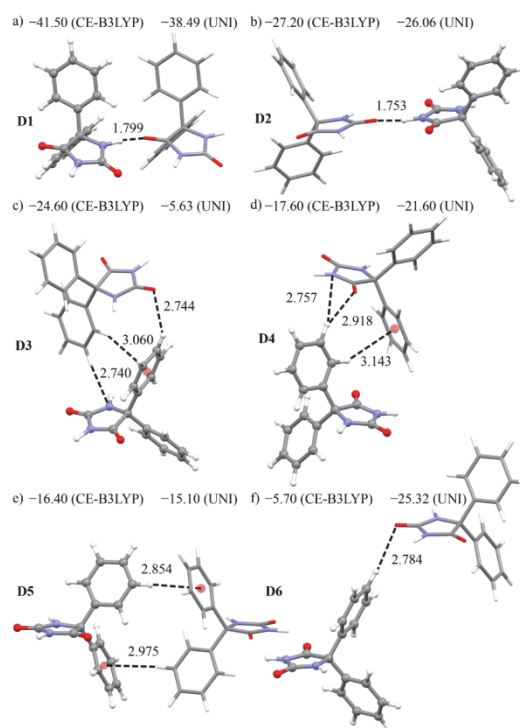


Fig. 2. Selected dimeric motifs of compound **1** with the corresponding interaction energies (in kJ mol^{-1}) calculated using the CE-B3LYP and UNI methods. The dimers were numbered according to the interaction energy values obtained from the CE-B3LYP approach.

The ball-and-stick style illustrates the asymmetric unit within the dimeric motif.

Finally, although the D1 motif of compound **4** features $\text{N-H}\cdots\text{O}$, $\text{C}(\text{sp}^3)\cdots\text{H}\cdots\text{O}$ and $\text{C}(\text{sp}^2)\cdots\text{H}\cdots\text{O}$ hydrogen bonds, dispersion interactions predominate (Fig. 5, Table S-I). A similar trend is observed for the remaining dimeric motifs, where the dispersion component dominates despite the presence of other types of interactions.

The ordering of the dimer motifs according to the interaction energies calculated with the UNI force field is mostly consistent with the results obtained using the CE-B3LYP method (Table S-I). In all studied compounds, the results for the two highest-ranked motifs are in complete agreement. Also, for compound **4**, the results from both methods correlate very well. The results presented in Table S-I reveal that the largest changes in the ranking of dimer motifs between the two methods occur for compounds **1** and **2**. For dimer D6, the interaction energy calculated using the UNI force field is significantly higher than the value obtained

with the CE-B3LYP method. For compound **3**, the UNI force field calculations indicate a perturbation in the interaction energies of the dimeric motifs D2 and D3.

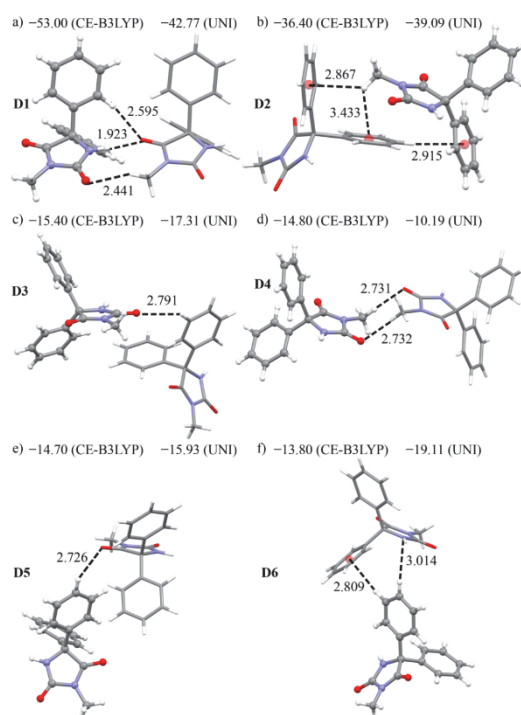


Fig. 3. Selected dimeric motifs of compound **2** with the corresponding interaction energies (in kJ mol^{-1}) calculated using the CE-B3LYP and UNI methods. The dimers were numbered according to the interaction energy values obtained from the CE-B3LYP approach. The ball-and-stick style illustrates the asymmetric unit within the dimeric motif.

The differences in dimer ranking arise because UNI (force-field) and DFT approaches estimate the relative importance of hydrogen, dispersion and electrostatic interactions differently. While DFT relies on electron density and accounts for polarization and delocalization, often highlighting polar interactions, the UNI method is purely geometric and tends to emphasize dispersive contacts.^{31–33}

Reactivity profile

Conceptual density functional theory (CDFT) has evolved into a fundamental framework within quantum chemistry for predicting molecular reactivity.³⁴ By relating the electron density distribution and the total electronic energy of a system, CDFT provides a set of global and local descriptors that quantify molecular stability, reactivity and the location of the most reactive sites. The frontier molecular orbital (FMO) theory provides an additional approach for assessing mole-

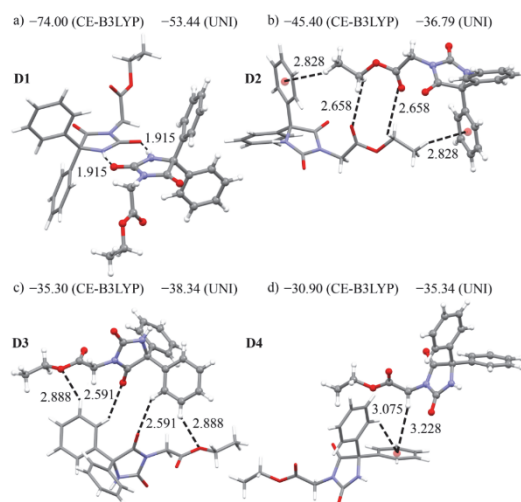


Fig. 4. Selected dimeric motifs of compound **3** with the corresponding interaction energies (in kJ mol^{-1}) calculated using the CE-B3LYP and UNI methods. The dimers were numbered according to the interaction energy values obtained from the CE-B3LYP approach. The ball-and-stick style illustrates the asymmetric unit within the dimeric motif.

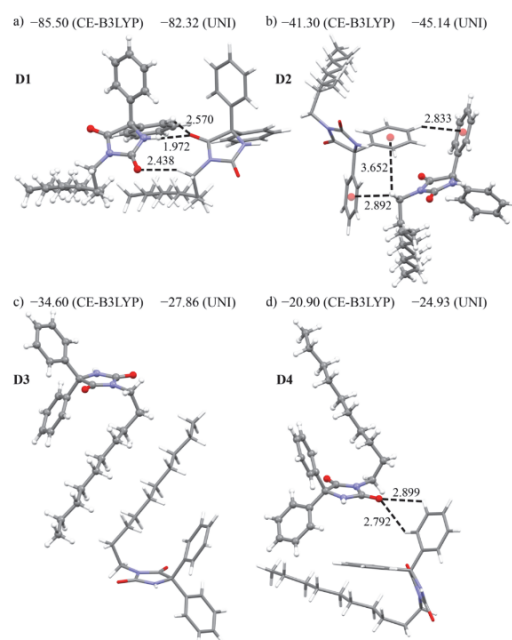


Fig. 5. Selected dimeric motifs of compound **4** with the corresponding interaction energies (in kJ mol^{-1}) calculated using the CE-B3LYP and UNI methods. The dimers were numbered according to the interaction energy values obtained from the CE-B3LYP approach. The ball-and-stick style illustrates the asymmetric unit within the dimeric motif.

cular reactivity, selectivity, and the pathways of chemical reactions.³⁵ The HOMO, as the highest occupied molecular orbital, typically represents an electron-rich region and acts as an electron donor in chemical interactions. In contrast, the LUMO, being the lowest-energy vacant orbital, corresponds to an electron-deficient region capable of accepting electrons. For this purpose, the FMO energies of the optimized monomeric structures were calculated in simulated environments of gas phase, water and DMSO, and subsequently used to determine the global reactivity descriptors (Table I), as defined by the equations provided in the Supplementary material.

TABLE I. The calculated energy of FMO (E_{HOMO} , E_{LUMO}), energy gap (ΔE_{gap}) and global reactivity parameters – ionization potential (IP), electron affinity (EA), chemical hardness (η), chemical potential (μ), electronegativity (χ) and electrophilicity index (ω) in units of eV for the studied compounds **1–4**

Compound	E_{HOMO}	E_{LUMO}	ΔE_{gap}	IP	EA	η	M	χ	ω
Gas phase									
1	−6.67	−0.72	5.96	6.67	0.72	2.98	−3.70	3.70	2.29
2	−6.62	−0.66	5.96	6.62	0.66	2.98	−3.64	3.64	2.22
3	−6.55	−0.57	5.98	6.55	0.57	2.99	−3.56	3.56	2.12
4	−6.63	−0.67	5.96	6.63	0.67	2.98	−3.65	3.65	2.24
Water									
1	−6.63	−0.79	5.85	6.63	0.79	2.92	−3.71	3.71	2.35
2	−6.62	−0.76	5.86	6.62	0.76	2.93	−3.69	3.69	2.32
3	−6.65	−0.81	5.84	6.65	0.81	2.92	−3.73	3.73	2.38
4	−6.63	−0.73	5.90	6.63	0.73	2.95	−3.68	3.68	2.30
DMSO									
1	−6.54	−0.55	5.99	6.54	0.55	2.99	−3.55	3.55	2.10
2	−6.54	−0.54	6.00	6.54	0.54	3.00	−3.54	3.54	2.09
3	−6.55	−0.56	5.99	6.55	0.56	2.99	−3.56	3.56	2.11
4	−6.54	−0.55	5.99	6.54	0.55	2.99	−3.54	3.54	2.10

Based on the HOMO–LUMO energy gap (ΔE_{gap}) values, it can be concluded that all the studied compounds exhibit similar kinetic stability in the gas phase. Positive ionization potentials (IP) and similar values of chemical hardness (η) also indicate the kinetic (energetic) stability of these compounds. In compound **3**, both frontier orbital levels lie at higher energies (less negative values), which indicates reduced overall stability. Although the values of EA , μ , χ and ω are slightly lower in absolute terms compared to the other molecules, compound **3** is expected to show a modest overall increase in chemical reactivity.

The LUMO energy values decrease more noticeably (becoming more negative) in the simulated water environment, particularly for compound **3**. In water, the LUMO level is lowered while the HOMO remains nearly unchanged, and the chemical potential (μ) decreases, making the system more stable. As a result, both electronegativity (χ) and electrophilicity (ω) increase, indicating a stronger ten-

dency of the molecule to attract electrons and a higher reactivity toward nucleophiles. In the simulated DMSO solvent, compounds **1**, **2** and **4** become energetically less stable, less electronegative and less electrophilic, due to destabilization of the LUMO level. Therefore, its reactivity towards nucleophiles is lower in DMSO than in the gas phase.

CONCLUSION

A comparison of the results using the two methods revealed that the relative ranking of the dimers depends on the method used to evaluate interaction energies. While the energy framework based on the UNI force field relies on geometric and empirical parameters, the CE-B3LYP approach derives interaction energies directly from the electron density, explicitly accounting for polarization and charge redistribution. The DFT (CE-B3LYP) method is generally regarded as more accurate, although it can sometimes place greater emphasis on polar interactions compared to the UNI approach. Nevertheless, it is worth noting that both methods identified the key dimers with the highest interaction energies (dimers D1 and D2), providing similar values and consistent relative rankings for the dominant contributors to crystal packing. The nature and strength of intermolecular interactions in dimers are strongly influenced by the substituents. Compound **3**, bearing a polar ethoxyacetyl group, shows enhanced electrostatic and hydrogen bonding contributions, while compound **4**, with a long alkyl chain, exhibits dominant dispersion (hydrophobic) interactions. In contrast, compounds **1** and **2** with smaller or non-polar substituents display weaker overall interactions, primarily governed by dispersion forces.

Overall, all studied compounds display comparable kinetic and energetic stability in the gas phase, with compound **3** being globally more reactive. Solvent effects are relevant for reactivity: in water, the stabilization of the LUMO enhances electrophilicity and nucleophile affinity, particularly for compound **3**, whereas in DMSO, the destabilization of the LUMO reduces these properties.

These findings highlight the critical role of substituent type in tuning molecular packing and interaction patterns in the crystal lattice, which can be utilized for the design and optimization of interactions between new drug candidates and their biological targets.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13485>, or from the corresponding author on request.

Acknowledgements. This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-136/2025-03/200135 and 451-03-136/2025-03/200026). We thank the COST Action CA21101 – Confined Molecular Systems: from a new generation of materials to the stars (COSY) of the European

Community for support. This research follows the United Nations 2030 Agenda for Sustainable Development Goal (SDG) 3: Good Health and Well-being.

ИЗВОД

УТИЦАЈ ЕЛЕКТРОСТАТИЧКИХ И ДИСПЕРЗИОНИХ ИНТЕРАКЦИЈА НА КРИСТАЛНУ СТРУКТУРУ И РЕАКТИВНОСТ: ПРИМЕР ФЕНИТОИНА И ЊЕГОВИХ ДЕРИВАТА

ИВАНА С. ЂОРЂЕВИЋ,¹ СОЊА ГРУБИШИЋ,¹ ДРАГАНА МИТИЋ,² ДРАГАН М. ПОПОВИЋ¹
и НЕМАЊА ТРИШОВИЋ³

¹Универзитет у Београду, Институт за хемију технологију и металургију, Институт од националног значаја за Републику Србију, Њешићева 12, 11001 Београд, ²Универзитет у Београду, Иновациони центар Хемијског факултета, Београд и ³Универзитет у Београду, Технолошко-металуршки факултет, Карнегијева 4, 11120 Београд

Деривати хидантоина представљају разноврсну класу хетероцикличних једињења, позната по својим фармаколошким својствима. Будући да ефикасност лека често зависи од финог подешавања слабих међумолекулских (нековалентних) интеракција, проучавање његове кристалне структуре је значајно јер омогућава разумевање његовог интеракцијског профила. У овој студији испитано је кристално паковање фенитоина и одабраних деривата анализом димера и различитих начина молекулског препознавања, коришћењем прорачуна заснованих на пољу сила (force-field) и теорији функционала густине (DFT). Релативно поларна етоксикарбонилметил-група у положају N3 хидантоинског прстена, са могућношћу успостављања водоничних веза, појачава допринос електростатичких и поларних компоненти укупној енергији интеракције. Супротно њој, дугачка алкил група подстиче успостављање хидрофобних контакта, услед чега дисперзионе силе постају доминантне у односу на електростатичке интеракције. Реактивност фенитоина и његових деривата додатно је испитана коришћењем дескриптора концептуалне теорије функционала густине (CDFT). Резултати показују да супституенти значајно утичу на изградњу кристалног паковања и природу нековалентних интеракција, пружајући притом значајан увид за разумевање молекулског препознавања и интеракција молекула лека са циљним местима што даље доприноси дизајнирању нових терапијских агенаса.

(Примљено 5. августа, ревидирано 22. августа, прихваћено 24. октобра 2025)

REFERENCES

1. S. Cho, S.-H. Kim, D. Shin, *Eur. J. Med. Chem.* **164** (2019) 517 (<https://doi.org/10.1016/j.ejmech.2018.12.066>)
2. W. Liu, S. Zhang, L. Xiao, Y. Wan, L. He, K. Wang, Z. Qi, X. Li, *Pest Manage. Sci.* **78** (2022) 1438 (<https://doi.org/10.1002/ps.6761>)
3. R. Rayhan, Md. S. H. Shishir, Md. A. Khaleque, Md. R. Amin, Md. R. Ali, M. A. S. Aly, S. M. Ayon, R. Saidur, T. H. Kim, Md. A. Zaed, Md. Z. Hossain, *RSC Adv.* **15** (2025) 24917 (<https://doi.org/10.1039/D5RA03128A>)
4. K. Kaewket, K. Ngamchuea, *RSC Adv.* **13** (2023) 33210 (<https://doi.org/10.1039/D3RA06175B>)
5. E. D. Akpan, O. Dagdag, E. E. Ebenso, *Coord. Chem. Rev.* **489** (2023) 215207 (<https://doi.org/10.1016/j.ccr.2023.215207>)
6. L. L. Brunton, B. C. Knollmann, *Goodman & Gilman's Pharmacological Basis of Therapeutics*, 14th ed., L. L. Brunton, B. C. Knollmann, Eds., McGraw-Hill, New York, 2022, p. 1664 (ISBN 978-1264258079)

7. M. Rask-Andersen, M. Almén, H. Schiöth, *Nat. Rev. Drug. Discov.* **10** (2011) 579 (<https://doi.org/10.1038/nrd3478>)
8. W. Guerrab, El Jemli Meryem, A. Jihane, D. Güneş, T. M. Joel, T. Jamal, I. Azeddine, A. M'Hammed, A. Katim, R. Youssef, *J. Biomol. Struct. Dyn.* **40** (2021) 8765 (<https://doi.org/10.1080/07391102.2021.1922096>)
9. A. Domínguez, A. Álvarez, B. Suárez-Merino, F. Goñi-de-Cerio, *Rev. Neurol.* **58** (2014) 213 (<https://pubmed.ncbi.nlm.nih.gov/24570360/>)
10. H. L. Wong, X. Y. Wu, R. Bendayan, *Adv. Drug Deliv. Rev.* **64** (2012) 686 (<https://doi.org/10.1016/j.addr.2011.10.007>)
11. N. Trišović, N. Valentić, G. Ušćumlić, *Chem. Cen. J.* **5** (2011) 62 (<https://doi.org/10.1186/1752-153X-5-62>)
12. J. R. Smythies, in *Progress in Drug Research*, G. H. Glaser, J. K. Penry, J. Kiffin, D. M. Woodbury, Eds., Raven, New York, 1980, p. 207
13. N. Trišović, N. Valentić, M. Erović, T. Đaković-Sekulić, G. Ušćumlić, I. Juranić, *Monatsh. Chem.* **142** (2011) 1227 (<https://doi.org/10.1007/s00706-011-0639-7>)
14. N. Trišović, T. Timić, J. Divljaković, J. Rogan, D. Poleti, M. M. Savić, G. Ušćumlić, *Monatsh. Chem.* **143** (2012) 1451 (<http://dx.doi.org/10.1007/s00706-012-0791-8>)
15. W. M. Pardridge, *Curr. Opin. Pharmacol.* **6** (2006) 494 (<https://doi.org/10.1016/j.coph.2006.06.001>)
16. M. L. Brown, G. B. Brown, W. J. Brouillette, *J. Med. Chem.* **40** (1997) 602 (<https://doi.org/10.1021/jm960692v>)
17. P. R. Spackman, L. J. Yu, C. J. Morton, M. W. Parker, C. S. Bond, M. A. Spackman, D. Jayatilaka, S. P. Thomas, *Angew. Chem. Int. Ed.* **58** (2019) 16780 (<https://doi.org/10.1002/anie.201906602>)
18. M. Stöhr, T. Van Voorhis, A. Tkatchenko, *Chem. Soc. Rev.* **48** (2019) 4118 (<https://doi.org/10.1039/C9CS00060G>)
19. D. Wu, D. G. Truhlar, *J. Chem. Theory Comput.* **17** (2021) 3967 (<https://doi.org/10.1021/acs.jctc.1c00162>)
20. C. Tantardini, A. A. L. Michalchuk, A. Samtsevich, C. Rota, A. G. Kvashnin, *Sci. Rep.* **10** (2020) 7816 (<https://doi.org/10.1038/s41598-020-64261-4>)
21. S. Tretiakov, A-K. Nigam, R. Pollice, *Chem. Rev.* **125** (2025) 5776 (<https://doi.org/10.1021/acs.chemrev.4c00893>)
22. C. R. Groom, I. J. Bruno, M. P. Lightfoot, S. C. Ward, *Acta Crystallogr., B* **72** (2016) 171 (<https://doi.org/10.1107/S2052520616003954>)
23. *Gaussian 09 (revision D.01)*, Gaussian, Inc., Wallingford, CT, 2009
24. P. R. Spackman, M. J. Turner, J. J. McKinnon, S. K. Wolff, D. J. Grimwood, D. Jayatilaka, M. A. Spackman, *J Appl. Cryst.* **54** (2021) 1006 (<https://doi.org/10.1107/s1600576721002910>)
25. C. F. Macrae, I. Sovago, S. J. Cottrell, P. T. A. Galek, P. McCabe, E. Pidcock, M. Platings, G. P. Shields, J. S. Stevens, M. Towler, P. A. Wood, *J. Appl. Cryst.* **53** (2020) 226 (<https://doi.org/10.1107/S1600576719014092>)
26. A. Gavezzotti, *Acc. Chem. Res.* **27** (1994) 309 (<https://doi.org/10.1021/ar00046a004>)
27. A. Gavezzotti, G. Filippini, *J. Phys. Chem.* **98** (1994) 4831 (<https://doi.org/10.1021/j100069a010>)
28. K. Chattopadhyay, R. A. Palmer, J. N. Lisgarten, *J. Crystallogr. Spectrosc. Res.* **23** (1993) 149 (<https://doi.org/10.1007/BF01195449>)
29. W. Guerrab, R. Akrad, M. Ansar, J. Taoufik, J. T. Mague, Y. Ramli, *IUCrData* **2** (2017) x171534 (<https://doi.org/10.1107/S2414314617015346>)

30. Y. Ramli, R. Akrad, W. Guerrab, J. Taoufik, M. Ansar, J. T. Mague, *IUCrData* **2** (2017) x170098 (<https://doi.org/10.1107/S2414314617000980>)
31. M. J. Turner, S. P. Thomas, M. W. Shi, D. Jayatilaka, M. A. Spackman, *Chem. Comm.* **51** (2015) 3735 (<https://doi.org/10.1039/C4CC09074H>)
32. M. A. Spackman, D. Jayatilaka, *CrystEngComm* **11** (2009) 19 (<https://doi.org/10.1039/B818330A>)
33. C. F. Mackenzie, P. R. Spackman, D. Jayatilaka, M. A. Spackman, *IUCrJ* **4** (2017) 575 (<https://doi.org/10.1107/S205225251700848X>)
34. P. Geerlings, F. De Proft, W. Langenaeker, *Chem. Rev.* **103** (2003) 1793 (<https://doi.org/10.1021/cr990029p>)
35. P. Politzer, F. Abu-Awwad, *Theor. Chem. Acc.* **99** (1998) 87 (<https://doi.org/10.1007/s002140050307>).

SUPPLEMENTARY MATERIAL TO

Tuning crystal packing and reactivity through electrostatic and dispersion interactions: The case of phenytoin and its derivatives

IVANA S. ĐORĐEVIĆ^{1*}, SONJA GRUBIŠIĆ¹, DRAGANA MITIĆ², DRAGAN M. POPOVIĆ¹ and NEMANJA TRIŠOVIĆ³

¹University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, 11001 Belgrade, Serbia, ²University of Belgrade, Innovative Centre of the Faculty of Chemistry, Belgrade, Serbia and ³University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Serbia

J. Serb. Chem. Soc. 90 (12) (2025) 1439–1450

Table S-I. Comparison of total interaction energies (in kJ mol⁻¹) for the dimeric motif in compound **1-4**, calculated by using the DFT (CE-B3LYP) method and the UNI force field.

Method	CrystalExplorer (CE-B3LYP)					UNI
	<i>E</i> _{ele}	<i>E</i> _{pol}	<i>E</i> _{dis}	<i>E</i> _{rep}	<i>E</i> _{tot}	<i>E</i> _{tot}
Comp. 1						
D1	-39.80	-8.50	-20.50	40.00	-41.50	-38.49
D2	-37.30	-8.30	-6.80	39.30	-27.20	-26.06
D3	-9.00	-2.00	-26.20	14.90	-24.60	-5.63
D4	-1.70	-1.40	-26.60	13.70	-17.60	-21.60
D5	-9.00	-1.00	-26.00	26.70	-16.40	-15.10
D6	-2.30	-0.80	-4.40	1.80	-5.70	-25.32
Comp. 2						
D1	-50.70	-12.10	-32.40	61.20	-53.00	-42.77
D2	-11.60	-1.80	-47.30	29.90	-36.40	-39.09
D3	-3.40	-2.80	-18.10	9.90	-15.40	-17.31
D4	-9.00	-2.20	-8.20	5.60	-14.80	-10.19
D5	-4.30	-0.70	-18.20	9.90	-14.70	-15.93
D6	-1.70	-0.80	-22.30	13.00	-13.80	-19.11
Comp. 3						
D1	-91.30	-23.70	-24.10	98.70	-74.00	-53.44
D2	-21.70	-5.20	-42.30	29.40	-45.40	-36.79
D3	-9.90	-4.70	-41.90	24.70	-35.30	-38.34
D4	-9.00	-4.00	-32.50	15.80	-30.90	-35.34
Comp. 4						
D1	-53.90	-12.60	-80.80	82.80	-85.50	-82.32
D2	-11.70	-1.80	-53.80	31.10	-41.30	-45.14
D3	-6.00	-0.40	-48.40	23.00	-34.60	-27.86
D4	-3.30	-2.90	-25.00	10.30	-20.90	-24.93

* Corresponding author. E-mail: ivana.djordjevic@ihm.bg.ac.rs

S432

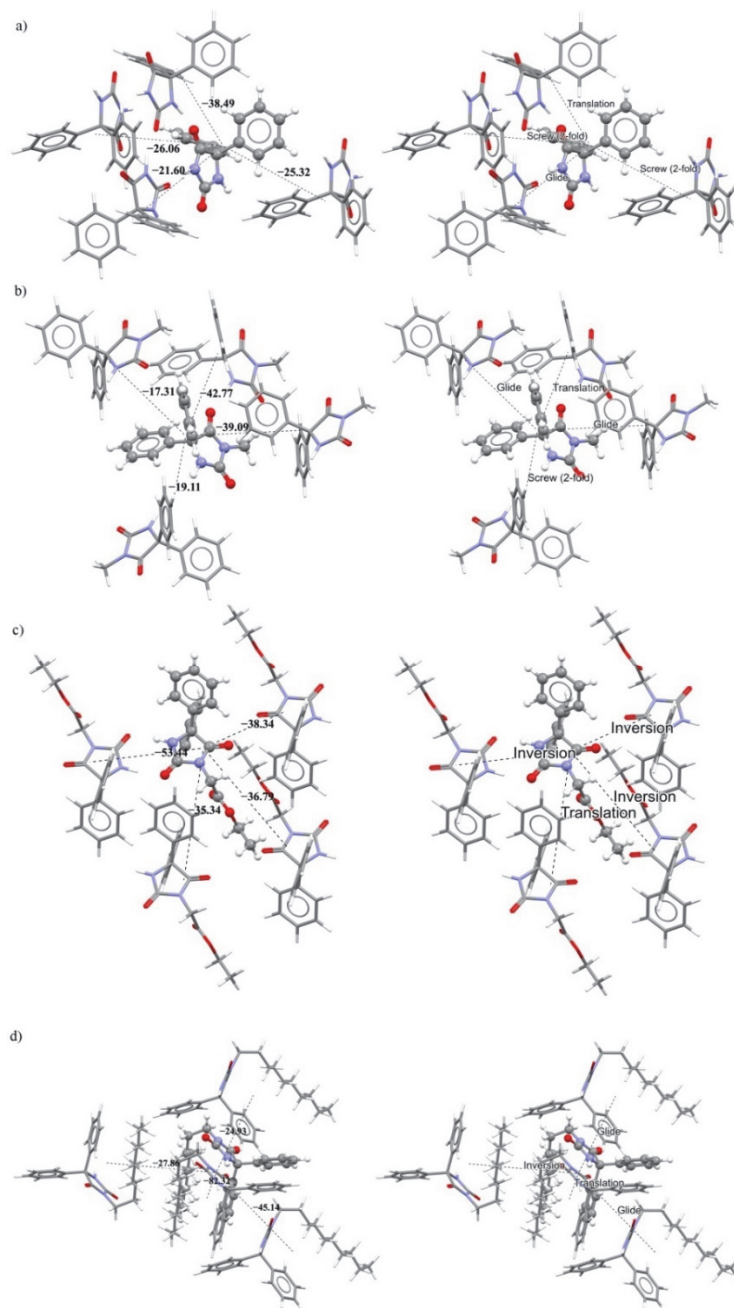


Figure S-1. The UNI force field calculated potentials (in kJ mol^{-1}) and a symmetry transformation for selected dimeric motifs in studied compounds **1-4**. The ball-and-stick style illustrates the asymmetric unit within the dimeric motif. Symmetry operations generating relevant molecular dimeric motifs: a) Identity (Translation): x, y, z; Screw axis (2-fold):

$-x, 1/2+y, -z$ (2-fold screw axis with direction $[0, 1, 0]$ at $0, y, 0$ with screw component $[0, 1/2, 0]$), Glide plane: $1/2+x, y, 1/2-z$ (Glide plane perpendicular to $[0, 0, 1]$ with glide component $[1/2, 0, 0]$); b) Identity (Translation): x, y, z ; Glide plane: $1/2+x, 1/2-y, 1/2+z$ (Glide plane perpendicular to $[0, 1, 0]$ with glide component $[1/2, 0, 1/2]$); Screw axis (2-fold): $1/2-x, 1/2+y, 1/2-z$ (2-fold screw axis with direction $[0, 1, 0]$ at $1/4, y, 1/4$ with screw component $[0, 1/2, 0]$), c) Inversion centre: $-x, -y, -z$ (Inversion at $[0, 0, 0]$), Identity (Translation): x, y, z and, d) Identity (Translation): x, y, z ; Glide plane: $1/2+x, 1/2-y, 1/2+z$ (Glide plane perpendicular to $[0, 1, 0]$ with glide component $[1/2, 0, 1/2]$); Inversion centre: $-x, -y, -z$ (Inversion at $[0, 0, 0]$); Glide plane: $1/2+x, 1/2-y, 1/2+z$ (Glide plane perpendicular to $[0, 1, 0]$ with glide component $[1/2, 0, 1/2]$).

The equations for calculation of the global reactivity parameters

Ionization potential (IP) = $-E_{HOMO}$

Electron affinity (EA) = $-E_{LUMO}$

Chemical Hardness (η) = $1/2 (IP - EA)$

Chemical potential (μ) = $-1/2(IP + EA)$

Electronegativity (χ) = $-\mu$

Electrophilicity index (ω) = $\mu^2/2\eta$



J. Serb. Chem. Soc. 90 (12) 1451–1464 (2025)
JSCS–5465

A computational study to identify the metabolites in *Lippia alba* capable of inhibiting gastric cancer through BCL-2 protein inhibition

LUIS H. MENDOZA-HUIZAR*

Universidad Autónoma del Estado de Hidalgo, Academic Area of Chemistry, Carretera Pachuca-Tulancingo Km. 4.5, CP. 42184, Mineral de la Reforma, México

(Received 7 June, revised 14 July, accepted 4 August 2025)

Abstract: In this study, a computational analysis was performed to evaluate the therapeutic potential of 73 compounds from *Lippia alba* as candidates for gastric cancer treatment. Most compounds exhibited low toxicity, except for 4-methylpent-2-enolide and β -acorenol, which demonstrated significant toxic effects. Molecular docking studies revealed that (Z)-nerolidol and 13-hydroxy-valencene both bind to the BCL2 protein, a key regulator of apoptosis in cancer cells. Furthermore, ADMET analysis predicted that both compounds are non-toxic. Molecular dynamics simulations showed that only (Z)-nerolidol maintained a stable interaction with domain 4 of BCL2, specifically with the critical Lys17 residue, which is essential for BCL2's inhibitory function. QM/MM calculations confirmed the formation of hydrogen bonds between (Z)-nerolidol and Lys17, Ile14 and Ser49, with an estimated binding energy of $-62.47 \text{ kJ mol}^{-1}$, suggesting a stable protein–ligand complex. These findings support the potential of (Z)-nerolidol as a lead compound for BCL2 inhibition and highlight its promise as a novel therapeutic agent for gastric cancer.

Keywords: *Lippia alba*; molecular docking; molecular dynamics; ONIOM; PM6.

INTRODUCTION

Gastric cancer is a malignant disease that originates from the uncontrolled growth of cells lining the stomach, along with the loss of their ability to undergo apoptosis, a crucial process for eliminating abnormal cells.¹ This disease has a significant impact on public health,^{2–4} and is the fourth most frequent type of cancer and the second leading cause of death worldwide.³ The high mortality rate and increasing incidence of gastric cancer underscore the urgent need to improve existing treatments.^{4–6} Current therapeutic options include radical surgery, chemotherapy, radiation therapy, targeted therapy and immunotherapy.^{4–6} Although these

*Correspondence E-mail: hhuizar@uaeh.edu.mx
<https://doi.org/10.2298/JSC250607059M>

approaches have demonstrated effectiveness, particularly in the early stages of the disease, they are associated with significant limitations,^{7,8} including adverse effects,^{9–11} highlighting the need to develop more selective and less toxic therapies. In this sense, an understanding of the molecular mechanisms that initiate and drive the progression of gastric cancer may help us to improve the treatments.¹²

It has been identified that the overexpression of the BCL-2 protein, associated with resistance to apoptosis and tumor development, has emerged as a key therapeutic target in gastric cancer treatment.¹³ BCL-2 plays a fundamental role in regulating apoptosis, and its inhibition could promote cell death in cancerous cells. The BH3 domain of BCL-2 facilitates the formation of heterodimers with pro-apoptotic proteins like BAX, thereby inhibiting apoptosis, while the BH4 domain (residues 1–34) neutralizes other pro-apoptotic proteins and interacts with apoptosis regulators such as Raf-1, c-Myc and the IP3R channel.¹³ This interaction is crucial because BCL-2 inhibits the opening of the IP3R channel, reducing the release of Ca^{2+} and, consequently, apoptosis mediated by this ion.¹³ Additionally, it has been suggested that the substitution of Lys17 in the BH4 domain of BCL-2 is essential for its binding and inhibition of IP3R, making Lys17 a key site for studying BCL-2 inhibition.¹³ Therefore, focusing on the BH4 domain of BCL-2 presents a novel strategy, in contrast to the traditional inhibition of the BH3 domain.¹³ Some studies even suggest that removing the BH4 domain from BCL-2 could turn it into a pro-apoptotic protein, similar to BAX.¹³ This finding has driven the investigation of natural agents that may target the BH4 domain of BCL-2 as a potential therapeutic strategy. Accordingly, several molecular-level studies have been carried out to identify natural compounds with the potential to inhibit BCL-2.^{14–18} These studies have shown that certain natural compounds can effectively act as treatments, either independently or in combination with other therapies, demonstrating promising results in the fight against gastric cancer.

In particular, metabolites from the plant *Lippia alba* have attracted attention due to their cytotoxic properties and ability to selectively induce the death of tumor cells.^{19,20} *L. alba*, a shrub from the Verbenaceae family, is traditionally used to treat gastric and intestinal diseases and has sedative properties. The composition of *L. alba* essential oils varies depending on the part of the plant used, the developmental stage and geographical conditions such as soil type and climate.²¹ It has been shown that the essential oils of *L. alba* have a cytotoxic effect, particularly by affecting the lipid metabolism of tumor cells.¹⁹ In addition to their action on the BCL-2 protein, these natural compounds could become effective therapeutic agents, either independently or in combination with other treatments.¹ However, although the therapeutic potential of *L. alba* against gastric cancer and its relationship with BCL-2 have been documented, the precise mechanism through which the plant's metabolites inhibit the protein remains unknown at the molecular level. Therefore, identifying the specific molecular interactions of these

compounds with BCL-2 would allow for the prediction and evaluation of their biological activity in the therapeutic context. In this regard, the application of ligand–receptor binding thermodynamics in drug discovery and computational chemistry constitutes a valuable tool for addressing this challenge.^{22–25} Thermodynamic analysis of binding offers critical insights into the forces governing the formation of drug–target complexes.^{22,23} There is now broad consensus that binding thermodynamics can serve as a key criterion throughout the various stages of ligand optimization in the development of novel therapeutic candidates.^{22–25} Thus, the present study analyzes the metabolites identified in *L. alba* using binding thermodynamics criteria and computational tools such as ADMET studies, Lipinski's rules, molecular docking, molecular dynamics and QM/MM methods, with the goal of identifying metabolites that may bind to the BH4 domain of the BCL-2 protein.

METHODOLOGY

In this study, we analyzed 73 metabolites from *Lippia alba*,^{26,27} as potential inhibitors of the BCL-2 protein. Studied metabolites were retrieved from PubChem,²⁸ and their IUPAC names and molecular structures are shown in Table S-I of the Supplementary material to this paper. The target protein, BCL-2, was obtained from the RCSB Protein Data Bank,²⁹ with the ID 1G5M.³⁰ Lipinski's rule of five³¹ and ADMET (absorption, distribution, metabolism, excretion and toxicity) parameters,³² for all 73 metabolites were calculated using the SwissADME,³³ (Daina *et al.*, 2017) and ADMETlab³² web servers, respectively. Molecular docking studies were conducted using the SwissDock server,³⁴ developed by the Swiss Institute of Bioinformatics (SIB). The compounds were evaluated based on their binding affinity (ΔG), and their interactions with protein residues were visualized using Chimera.³⁵ Molecular dynamics (MD) simulations were performed using GROMACS software.³⁶ A quantum mechanics/molecular mechanics (QM/MM) study was conducted to evaluate the electronic contributions to ligand–residue binding through the ONIOM methodology (our own *N*-layered integrated molecular orbital and molecular mechanics).³⁷ All quantum calculations presented in this work were carried out using the Gaussian 09 software,³⁸ and visualized with the GaussView package.³⁹ A list of the symbols used in this work is provided in Table S-II of the Supplementary material.

RESULTS AND DISCUSSION

Analysis of metabolites according to Lipinski's rule of five and ADMET parameters

The calculation of Lipinski's rule of five for the 73 metabolites evaluated showed that 53 of them met the established criteria of the rule. However, the remaining 20 metabolites violated the condition of $\log P \leq 4.15$, suggesting possible difficulties in terms of lipophilicity and, therefore, absorption, see Table S-III of the Supplementary material. Furthermore, ADMET parameter simulations were performed for all 73 metabolites using the SwissADME³³ and ADMETlab³² platforms. The results indicate that, in general, the 73 compounds meet at least four

of the five ADMET parameters, which reinforces their potential as drug candidates. However, it is important to mention that some metabolites showed unfavorable results in particular parameters. For example, 14-hydroxy-9-epi-(*E*)-caryophyllene (**6**) displayed an unfavorable distribution profile, which could hinder its bioavailability in the body. On the other hand, 4-methyl-pent-2-enolide (**9**) showed considerable toxicity, being potentially carcinogenic and corrosive to the skin and eyes. A similar profile was observed for β -acoreno (**15**), which also presented adverse results related to toxicity, which is a critical factor to consider during the selection process of metabolites for drug development.

Active site prediction on 1G5M

Drug efficacy often depends on binding to specific protein sites, traditionally identified from static structures. However, this approach overlooks protein dynamics. Cryptic pockets, transient or hidden cavities revealed through conformational changes, can be uncovered using molecular dynamics simulations, offering new targets for drug development.⁴⁰ Cryptic pockets can be identified using advanced methods such as molecular simulations, artificial intelligence or high-resolution experimental techniques.⁴⁰ In this study, we conducted a comparative analysis to identify binding sites in the static structure of the BCL-2 protein (PDB ID: 1G5M) and contrasted them with cryptic pockets predicted using deep neural networks. The active sites of the static BCL-2 structure were predicted using the PrankWeb server (<https://prankweb.cz/>),⁴¹ which identified six potential binding sites (Fig. 1a). Each of these predicted pockets exhibited varying probabilities of ligand binding, with pocket 1 showing the highest probability (0.326), followed by pockets 2 (0.134), 3 (0.087), 4 (0.039), 5 (0.033) and 6 (0.033). In terms of binding affinity, pocket 1 demonstrated the highest score (6.10), followed by pockets 2 (3.52), 3 (2.79), 4 (1.94), 5 (1.81) and 6 (1.80). These scores indicate predicted binding strength, with higher values suggesting stronger ligand interactions. This pattern supports the hypothesis that pocket 1 is the most biologically relevant binding site. Here, it is important to note that the BH4 domain (residues 1–34) has some overlap with pocket 1. However, residue Lys17, located in this domain and experimentally shown to be essential for IP3R inhibition,¹³ is not included in any of the pockets identified in the static structure. The cryptic pockets of BCL-2 were predicted using the PocketMiner server (<https://pocketminer.azurewebsites.net/>), which employs deep neural networks to predict where transient pockets may form during molecular dynamics simulations.⁴⁰ The results are shown in Fig. 1b, where residues highlighted in red and blue correspond to regions with high probabilities of cryptic pocket formation. Importantly, these regions are located within the BH4 domain, indicating a potential functional role for this domain in ligand binding. Two main subregions were identified: pocket 1a and pocket 1b. Notably, pocket

1b overlaps with static pocket 1, while pocket 1a includes Lys17 which is an experimentally validated key residue involved in IP3R inhibition.

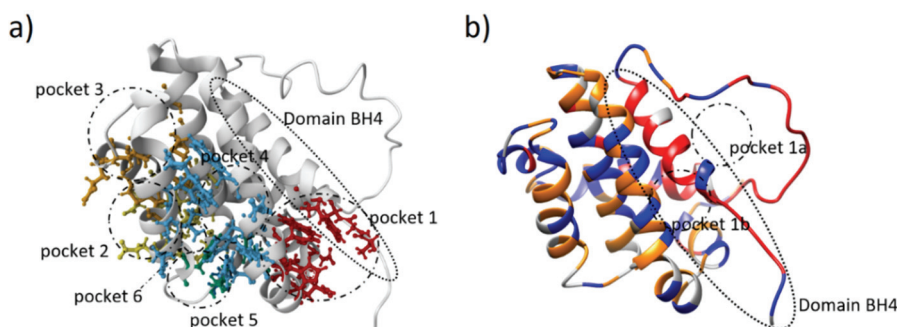


Fig. 1. Active sites on 1G5M (a); static structure (b) cryptic sites.

Binding analysis of L. alba metabolites with 1G5M through a molecular docking study

Molecular docking studies were performed using the SwissDock server³⁴ developed by SIB. This server automatically assigns docking parameters, such as box size and affinity evaluation methods, employing a rigid-flexible docking approach. Prior to docking, the protein structure was prepared by removing all solvent molecules and receptor-binding chemicals to focus the docking process on the protein's essential interaction sites. The compounds were assessed based on their binding affinity (ΔG), and their interactions with protein residues were visualized using Chimera.³⁵ These visualizations provide valuable insights into how metabolites interact with the target protein, aiding in the identification of potential inhibitors. To further explore the role of metabolite structure in inhibiting BCL-2 for the treatment of gastric cancer, the optimal ligand/protein configuration and binding affinities of each metabolite were evaluated. This analysis is essential for understanding how structural variations in metabolites influence their ability to effectively interact with BCL-2, which is critical for therapeutic development. Furthermore, evaluating the binding affinity helps to identify metabolites with higher therapeutic potential for BCL-2 inhibition, thereby contributing to the development of more effective treatments for gastric cancer. ΔG values for the 73 compounds analyzed are presented in Fig. 2. Metabolite **9**, 4-methylpent-2-enolide, exhibits the strongest binding affinity with a ΔG of $-31.13 \text{ kJ mol}^{-1}$, followed by compound **5**, 13-hydroxy-valencene ($\Delta G = -30.29 \text{ kJ mol}^{-1}$) and compound **4**, (*Z*)-nerolidol ($\Delta G = -30.25 \text{ kJ mol}^{-1}$). It is noteworthy that 4-methylpent-2-enolide specifically binds to the BH4 domain of BCL-2, interacting with residue LYS17 within pocket 1a, as shown in Fig. 1a. In contrast, compound **4** binds within pocket 1, while compound **5** is also located in the BH4 domain, near pocket 1a. From a pharmacological perspective, compound **4** has been widely reported for its anti-

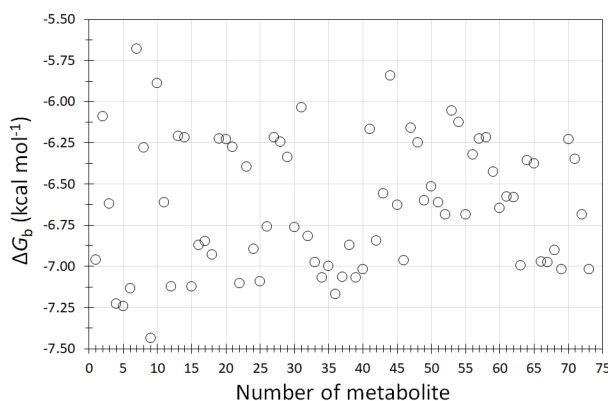


Fig. 2. $\Delta_B G$ of binding between *Lippia alba* metabolites and 1G5M.

-inflammatory, antioxidant and antimicrobial properties,^{26,27} making it a promising candidate for future therapeutic applications. Similarly, compound **5** exhibits antioxidant and anti-inflammatory activities and interacts directly with residue Lys17. In contrast, no known therapeutic properties have been reported for compound **9**. Furthermore, ADMET analysis indicates that this metabolite has significant toxicity, being potentially carcinogenic and corrosive to skin and mucous membranes, which severely limits its therapeutic potential. At the molecular level, both compound **5** and **9** display similar interactions with Lys17, including hydrogen bonds, π -alkyl, and π -sigma interactions, reinforcing the importance of this residue as an active site within the BH4 domain. However, compound **4**, despite binding outside pocket 1a and within pocket 1, does so in a region that represents the most accessible binding site in the static structure, although it initially does not establish direct interactions with Lys17. Collectively, these findings suggest that compounds **4** and **5** possess greater therapeutic potential compared to compound **9**, as they not only exhibit favorable binding affinity but also interact with key regions of BCL-2 associated with functional inhibition. Moreover, compounds **4** and **5** exhibit binding energies comparable to that of baicalin with BCL2 ($-38.5 \text{ kJ mol}^{-1}$), as reported in previous *in silico* studies, highlighting their potential as anticancer agents. This supports their potential development as effective BCL-2 inhibitors for future research.⁴² The dissociation (K_d) and binding (K_B) constants were evaluated for compounds **4** ((*Z*)-nerolidol) and **5** (13-hydroxyvalencene), respectively. The dissociation constant is related to the Gibbs energy change according to the following equation:^{43,44}

$$K_d = e^{\frac{\Delta_B G}{RT}} \quad (1)$$

In contrast, the binding constant (K_B) is the inverse of the dissociation constant and is calculated as:^{43,44}

$$K_B = e^{-\frac{\Delta_B G}{RT}} \quad (2)$$

Based on the binding free energy values, which were $-30.23 \text{ kJ}\cdot\text{mol}^{-1}$ for (Z)-nerolidol and $-30.30 \text{ kJ}\cdot\text{mol}^{-1}$ for 13-hydroxy-valencene, the K_d and K_B values were determined. For (Z)-nerolidol, K_d was $5.11 \times 10^{-6} \text{ M}$ and K_B was $1.96 \times 10^5 \text{ M}^{-1}$; whereas for 13-hydroxy-valencene, the values were $4.96 \times 10^{-6} \text{ M}$ and $2.02 \times 10^5 \text{ M}^{-1}$, respectively. The negative $\Delta_B G$ values for both compounds indicate that the binding is spontaneous and thermodynamically favorable. Moreover, the dissociation constants (K_d) in the low micromolar range suggest a moderately high affinity between (Z)-nerolidol and 13-hydroxy-valencene and BCL2. The slight difference observed between the two compounds suggests that 13-hydroxy-valencene has a slightly higher affinity (lower K_d and higher K_B), which may be attributed to minor structural variations that enhance its interaction with the active site. These values of K_d and K_B evaluated in this work fall within the range reported for BCL2 inhibition.^{30,45}

Analysis of the stability of compounds 13-hydroxy-valencene and (Z)-nerolidol in complex with 1G5M through a molecular dynamics study

To analyze the stability of compounds (Z)-nerolidol and 13-hydroxy-valencene binding to the BH4 domain of BCL-2 and its interaction with Lys17, molecular dynamics (MD) simulations were performed using the GROMACS software.³⁶ These simulations were set up by constructing a simulation box with a 1 Å margin, ensuring charge neutrality, and adding water molecules (TIP3P model) to fill the system, which was centered within a cubic box. Initially, energy minimization was performed to achieve the most stable conformation, ensuring a reasonable starting structure in terms of geometry and solvent orientation. The system was then subjected to two stages. The first one stage was performed under an NVT ensemble, allowing the system's temperature to stabilize at 300 K. The second stage involved pressure equilibration under an NPT ensemble for 1 ns. The stability of the BCL-2 complexes with (Z)-nerolidol and 13-hydroxy-valencene was evaluated throughout the 100 ns simulation. Fig. 3 shows the *RMSD* vs. simulation time graphs for these compounds. In the case of BCL2-13-hydroxy-valencene, it was displaced from pocket 1a; initially, some interactions with Lys17 at the first nanoseconds were observed; however, at the simulation proceeded, these interactions were lost, and the compound migrated to another site different from those schematized in Fig. 1. For complex BCL2-(Z)-nerolidol, the system exhibited an important fluctuation of approximately 0.8 nm at 7 ns, after which it remained stable until 100 ns. At 7 ns, compound (Z)-nerolidol migrates and binds from pocket 1 to pocket 1a and remains stable to 100 ns. The interactions of this complex are detailed in Table I, where it can be observed that there is stable

binding with the active site Lys17 during 100 ns. These results suggest that (Z)-nerolidol has more therapeutic possibilities than 13-hydroxy-valencene.

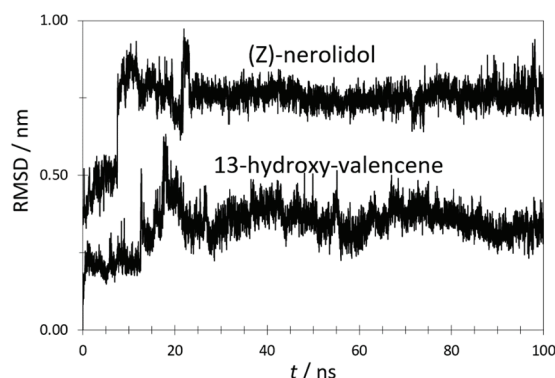


Fig. 3. *RMSD* plots from molecular dynamics simulations of the 1G5M-ligand complexes involving compounds (Z)-nerolidol and 13-hydroxy-valencene.

TABLE I. Residues interacting with compound (Z)-nerolidol during the molecular dynamics simulation at selected time points. Only residues within interaction distance (<4.0 Å) were included

<i>t</i> / ns	Residues
0	His20, Tyr21, His94, Val93, Ile14, Tyr9, Lys17, Tyr18
10	His20, Val93, Ile14, Tyr9, Lys17
20	His20, Val93, Ile14, Tyr9, Lys17
30	His94, Val93, Tyr9, Lys17
40	His20, His94, Val93, Ile14, Tyr9, Lys17
50	His94, Val93, Ile14, Tyr9, Lys17, Tyr18
60	His94, Val93, Ile14, Tyr9, Lys17
70	Tyr21, His94, Val93, Ile14, Tyr9, Lys17
80	Tyr 21, His94, Ile14, Tyr9, Lys17, Tyr18
90	His20, His94, Val93, Ile14, Tyr9, Lys17, Tyr18
100	His20, Tyr21, His94, Val93, Ile14, Tyr9, Lys17

Also, stable and persistent interaction, between (Z)-nerolidol and residues Lys17, Ile14, Tyr9, Val93 and His94 throughout the molecular dynamics simulations, was observed. Interactions with Lys17 and Ile14 engage in hydrogen bonding, while Tyr9 contributes through π -alkyl interactions. Both types of interactions appear to play a critical role in maintaining the integrity of the ligand–protein complex.

Analysis of the molecular interactions between (Z)-nerolidol and 1G5M through ONIOM quantum calculations

The molecular dynamics study revealed that compound (Z)-nerolidol remains stable within pocket 1a of the BCL2 protein, forming predominant interactions

with residues Lys17, Ile14, Tyr9, Val93 and His94. To further characterize these interactions and understand their electronic basis, a hybrid QM/MM study was performed. Due to the size of the protein–ligand system, which makes a full quantum mechanical treatment computationally impractical, the ONIOM methodology was employed.³⁷ This approach enables accurate modeling of the active site region while maintaining a reasonable computational cost. In the applied ONIOM model, both the ligand and the residues directly involved in the active site interactions (Lys17, Ile14, Tyr9, Val93 and Ser49) were included in the high-level quantum mechanical (QM) region, while the rest of the system was treated using molecular mechanics (MM, Fig. 4). Here, it is important to mention that the selection of the theoretical levels for the QM and MM regions is fundamental for accurately calculating the properties of interest. In this context, density functional theory (DFT) calculations, while generally more accurate than semiempirical methods, can become computationally prohibitive when attempting to analyze noncovalent interactions in detail, particularly in large or flexible molecular systems such as protein–ligand complexes. In this sense, the literature reports that, for biomolecular systems or noncovalent complexes, semiempirical methods can provide reasonably accurate results at a fraction of the computational cost of DFT.⁴⁶ However, the deviations from experimental data are greater when using semiempirical methods than with DFT.⁴⁷ Considering the advantages of PM6 in reproducing experimental trends at a lower computational cost compared to DFT, in the present work the system geometry was optimized using the semiempirical PM6 method for the quantum mechanical region,⁴⁸ and the universal force field (UFF) for the molecular mechanics region.⁴⁹ This combination of these theoretical levels has proven suitable and accurate for describing the structural and electronic features of novel metal–Schiff base complexes derived from allylamine, as well as their interactions with human serum albumin, in the context of anticancer drug discovery.⁵⁰ As a result of the geometry optimization, a network of non-classical hydrogen bonds and π -alkyl interactions was identified between (*Z*)-nerolidol and several key residues within the binding site of 1G5M. Detailed analysis revealed an intramolecular hydrogen bond of 2.3 Å between the carbonyl oxygen atom of Lys17 and a hydrogen atom belonging to a methyl group of the (*Z*)-nerolidol moiety, indicative of a C–O $\cdots$ H–C interaction. Although such interactions are generally weaker than classical hydrogen bonds, they can significantly contribute to ligand conformational stability and binding specificity.⁵¹ A second C–O $\cdots$ H–C interaction was observed between the carbonyl oxygen of Lys17 and a hydrogen atom attached to carbon 4 of (*Z*)-nerolidol. Additionally, the carbonyl oxygen of Ile14 forms a weaker hydrogen bond (O $\cdots$ H, 2.4 Å) with a hydrogen atom from the methyl group at position 7 of (*Z*)-nerolidol. This interaction, also classified as a C–H $\cdots$ O non-classical hydrogen bond, is predominantly electrostatic in nature,⁵¹ but still plays a relevant role in orienting the ligand within

the binding pocket. A longer and weaker intramolecular hydrogen bond was observed between the carbonyl oxygen of Ser49 and the hydrogen atom at carbon 9 of the ligand, with a distance of 3.2 Å. This interaction is also mainly electrostatic, and while it contributes modestly to the overall binding affinity, it still supports the spatial orientation of the ligand.⁵¹ These interactions were visualized using Discovery Studio Visualizer,⁵² and are depicted in Fig. S-1 of the Supplementary material. Also, a π -alkyl interaction was identified between the π -electron cloud of the aromatic ring of Tyr9 and the terminal methyl group of (*Z*)-nerolidol, with a contact distance of 3.1 Å. Additional alkyl interactions were found between the methyl group of (*Z*)-nerolidol and Val93, with distances of 2.4 and 2.6 Å, respectively. A further alkyl interaction was observed between the methyl group of (*Z*)-nerolidol and His94, at a distance of 2.1 Å, involving hydrogen atoms from the ligand's methyl group and the imidazole ring of the residue. The binding energy (E_B) was evaluated from these ONIOM calculations through the following equation:

$$E_B = E_{\text{complex}} - (E_{\text{protein}} + E_{\text{ligand}}) \quad (3)$$

The E_{complex} is -5.531177 hartrees, $E_{\text{protein}} = -5.41322$ hartrees and $E_{\text{ligand}} = 0.094161$ hartrees. Thus, the $E_B = -0.023796$ hartrees or $-62.48 \text{ kJ mol}^{-1}$. This value indicates a favorable interaction and suggests the formation of a stable complex between BCL2 and compound **4**, supporting its potential as an inhibitor.

CONCLUSION

In this study, 73 metabolites present in the plant *Lippia alba* were evaluated, and were analyzed using Lipinski's rules and ADMET properties to assess their

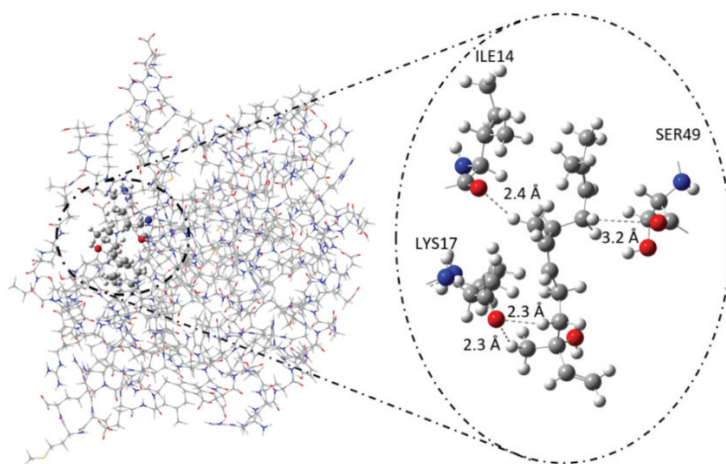


Fig. 4. ONIOM model applied to the BCL2-(*Z*)-nerolidol complex. The high-level (QM) region includes the ligand and key residues Lys17, Ile14 and Ser49. Hydrogen bond distances are shown in Å.

potential as drugs and their toxicity. Of these 73 compounds, molecular docking revealed that the compounds (Z)-nerolidol and 13-hydroxy-valencene interact with the Lys17 residue, identified as essential for binding and inhibition of IP3R in the BH4 domain of BCL-2. The molecular dynamics study indicated that only the compound (Z)-nerolidol maintains a stable binding with Lys17 for 100 ns. In particular, (Z)-nerolidol showed stable and specific interactions with key residues, which was confirmed through ONIOM calculations. These results support its potential as a lead candidate for the development of BCL2 inhibitors and justify its experimental evaluation in future studies.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13413>, or from the corresponding author on request.

Acknowledgements. LHMH thankfully acknowledges the computer resources, technical expertise, and support provided by the Laboratorio Nacional de Supercómputo del Sureste de México, CONACYT member of the network of national laboratories through the project No. 202501011N. Guanajuato National Laboratory (CONACyT 123732) is acknowledged for supercomputing resources. LHMH thanks the Universidad Autónoma del Estado de Hidalgo for the support granted for this work. LHMH acknowledges the SNII for the distinction of his membership and the stipend received.

ИЗВОД

РАЧУНАРСКА СТУДИЈА ЗА ИДЕНТИФИКАЦИЈУ МЕТАБОЛИТА У БИЉЦИ *Lippia alba* СПОСОБНИХ ДА ИНХИБИРАЈУ РАК ЖЕЛУЦА ПУТЕМ ИНХИБИЦИЈЕ ПРОТЕИНА BCL-2

LUIS H. MENDOZA-HUIZAR

Universidad Autónoma del Estado de Hidalgo, Academic Area of Chemistry, Carretera Pachuca-Tulancingo Km. 4.5, CP. 42184, Mineral de la Reforma, México

У овој студији спроведена је рачунарска анализа како би се проценио терапеутски потенцијал 73 једињења из биљке *Lippia alba* као потенцијалних кандидата за лечење рака желуца. Већина једињења има ниску токсичност, изузев 4-метилпент-2-енолида и β -акоронола, који имају значајне токсичне ефекте. Докинг анализа показала је да се (Z)-неролидол и 13-хидрокси-валенцен везују за BCL-2 протеин, кључни регулатор апоптозе у ћелијама карцинома. Додатно, ADMET анализа је предвидела да су оба једињења нетоксична. Молекулска динамика је показала да само (Z)-неролидол одржава стабилну интеракцију са доменом 4 протеина BCL-2, посебно са критичним Lys17 остатком, који је од суштинске важности за инхибиторну функцију BCL-2. QM/MM прорачуни су потврдили формирање водоничних веза између (Z)-неролидола и аминокиселинских остатака Lys17, Ile14 и Ser49, са процењеном енергијом везивања од $-62,47 \text{ kJ mol}^{-1}$, што указује на стабилан протеин–лиганд комплекс. Ови резултати указују на потенцијал (Z)-неролидола као водећег једињења за инхибицију BCL-2 и истичу његов значај као новог терапеутског агенса за лечење рака желуца.

(Примљено 7. јуна, ревидирано 30. марта, прихваћено 4. августа 2025)

REFERENCES

1. A. Sarkar, A. Paul, T. Banerjee, A. Maji, S. Saha, A. Bishayee, T. K. Maity, *Eur. J. Pharmacol.* **944** (2023) 175588 (<http://dx.doi.org/10.1016/J.EJPHAR.2023.175588>)
2. H. R. Khouzani, M. R. Maleki, A. Zackery, E. Mazloumi, M. Jalilzadeh, M. Sahebzadeh, *Heliyon* **10** (2024) e40437 (<http://dx.doi.org/10.1016/J.HELIYON.2024.E40437>)
3. M. Ilic, I. Ilic, *World J. Gastroenterol.* **28** (2022) 1187 (<http://dx.doi.org/10.3748/WJG.V28.I12.1187>)
4. R. Panahizadeh, P. Panahi, V. Asghariazar, S. Makaremi, G. Noorkhajavi, E. Safarzadeh, *Cancer Cell Int.* **25** (2025) 1 (<http://dx.doi.org/10.1186/S12935-025-03655-8>)
5. E. Morgan, M. Arnold, M. C. Camargo, A. Gini, A. T. Kunzmann, T. Matsuda, F. Meheus, R. H. A. Verhoeven, J. Vignat, M. Laversanne, J. Ferlay, I. Soerjomataram, *EClinicalMedicine* **47** (2022) 101404 (<http://dx.doi.org/10.1016/J.ECLINM.2022.101404>)
6. T. I. Mamun, S. Younus, M. H. Rahman, *Cancer Treat. Res. Commun.* **41** (2024) 100845 (<http://dx.doi.org/10.1016/J.CTARC.2024.100845>)
7. S. S. Joshi, B. D. Badgwell, *CA Cancer J. Clin.* **71** (2021) 264 (<http://dx.doi.org/10.3322/CAAC.21657>)
8. Q. Yin, Y. Zhang, X. Xie, M. Hou, X. Chen, J. Ding, *Cell Death Discov.* **11** (2025) 1 (<http://dx.doi.org/10.1038/s41420-025-02429-5>)
9. B. Zhao, W. Lv, J. Lin, *Curr. Probl. Cancer* **44** (2020) 100577 (<http://dx.doi.org/10.1016/J.CURRPROBLCANCER.2020.100577>)
10. W. Leowattana, P. Leowattana, T. Leowattana, *World J. Methodol.* **13** (2023) 79 (<http://dx.doi.org/10.5662/WJM.V13.I3.79>)
11. D. Luo, Y. Liu, Z. Lu, L. Huang, *Mol. Med.* **31** (2025) 1 (<http://dx.doi.org/10.1186/S10020-025-01075-Y>)
12. C. D. Iwu, C. J. Iwu-Jaja, *Hyg.* **3** (2023) 256 (<http://dx.doi.org/10.3390/HYGIENE3030019>)
13. Z. Liu, C. Wild, Y. Ding, N. Ye, H. Chen, E. A. Wold, J. Zhou, *Drug Discov. Today* **21** (2016) 989 (<http://dx.doi.org/10.1016/j.drudis.2015.11.008>)
14. Y. Qu, X. Yang, J. Li, S. Zhang, S. Li, M. Wang, L. Zhou, Z. Wang, Z. Lin, Y. Yin, J. Liu, N. Wang, Y. Yang, *Evid. Based Complement. Alternat. Med.* **2021** (2021) 2311486 (<http://dx.doi.org/10.1155/2021/2311486>)
15. Y. Q. Ma, M. Zhang, Z. H. Sun, H. Y. Tang, Y. Wang, J. X. Liu, Z. X. Zhang, C. Wang, *World J. Gastrointest. Oncol.* **16** (2024) 493 (<http://dx.doi.org/10.4251/WJGO.V16.I2.493>)
16. N. Bahadar, S. Bahadar, A. Sajid, M. Wahid, G. Ali, A. Alghamdi, H. Zada, T. Khan, S. Ullah, Q. Sun, *Breast Cancer Res.* **26** (2024) 114 (<http://dx.doi.org/10.1186/S13058-024-01868-9>)
17. K. Kai, J. Han-bing, C. Bing-lin, Z. Shu-jun, *Heliyon* **9** (2023) e17393 (<http://dx.doi.org/10.1016/J.HELIYON.2023.E17393>)
18. S. Dey, A. K. Singh, S. Kumar, *Biointerface Res. Appl. Chem.* **13** (2022) 1 (<http://dx.doi.org/10.33263/BRIAC135.473>)
19. S. Montero Villegas, J. F. Ciccio Alberti, X. Cortés Bratti, R. Crespo, M. Polo, M. García de Bravo, *Terc. Epoca. Rev. Cient. Facultad Ciencias Médicas* **2** (2010) 1 (<http://sedici.unlp.edu.ar/handle/10915/15469>)
20. M. A. Ramírez-García, J. E. Márquez-González, Horacio Barranco-Lampón, Gilberto López-Aguilar, *Resid.* **9** (2014) 84 (<http://dx.doi.org/10.5754/hge11946>)

21. E. E. Stashenko, B. E. Jaramillo, J. R. Martínez, *Rev. Acad. Colomb. Ciencias* **27** (2003) 579 (<http://dx.doi.org/10.18257/ssn.0370-3908>)
22. R. Claveria-Gimeno, S. Vega, O. Abian, A. Velazquez-Campoy, *Expert Opin. Drug Discov.* **12** (2017) 363 (<http://dx.doi.org/10.1080/17460441.2017.1297418>)
23. X. Du, Y. Li, Y. L. Xia, S. M. Ai, J. Liang, P. Sang, X. L. Ji, S. Q. Liu, *Int. J. Mol. Sci.* **17** (2016) 1 (<http://dx.doi.org/10.3390/ijms17020144>)
24. M. E. Popović, V. Tadić, M. Popović, *Virology* **603** (2025) (<http://dx.doi.org/10.1016/j.virol.2024.110319>)
25. W. López-Orozco, L. H. Mendoza-Huizar, G. A. Álvarez-Romero, J. de Jesús Martín Torres-Valencia, *J. Serb. Chem. Soc.* **90** (2025) 291 (<http://dx.doi.org/10.2298/JSC240428074L>)
26. L. Silva-Santos, L. Palhares Neto, N. Corte-Real, M. V. L. Sperandio, C. A. G. Camara, M. M. Moraes, C. Ulisses, *South African J. Bot.* **163** (2023) 756 (<http://dx.doi.org/10.1016/j.sajb.2023.10.024>)
27. P. T. de Souza Silva, L. M. de Souza, M. B. de Moraes, M. M. de Moraes, C. A. G. da Camara, C. Ulisses, *South African J. Bot.* **147** (2022) 415 (<http://dx.doi.org/10.1016/j.sajb.2022.01.042>)
28. S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B. A. Shoemaker, P. A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang, E. E. Bolton, *Nucleic Acids Res.* **53** (2025) D1516 (<http://dx.doi.org/10.1093/NAR/GKAE1059>)
29. H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, *Nucleic Acids Res.* **28** (2000) 235 (<http://dx.doi.org/10.1093/NAR/28.1.235>)
30. A. M. Petros, A. Medek, D. G. Nettlesheim, D. H. Kim, H. S. Yoon, K. Swift, E. D. Matayoshi, T. Oltersdorf, S. W. Fesik, *Proc. Natl. Acad. Sci. U.S.A.* **98** (2001) 3012 (<http://dx.doi.org/10.1073/pnas.041619798>)
31. C. A. Lipinski, *Drug Discov. Today Technol.* **1** (2004) 337 (<http://dx.doi.org/10.1016/J.DDTEC.2004.11.007>)
32. G. Xiong, Z. Wu, J. Yi, L. Fu, Z. Yang, C. Hsieh, M. Yin, X. Zeng, C. Wu, A. Lu, X. Chen, T. Hou, D. Cao, *Nucleic Acids Res.* **49** (2021) W5 (<http://dx.doi.org/10.1093/nar/gkab255>)
33. A. Daina, O. Michielin, V. Zoete, *Sci. Rep.* **7** (2017) 1 (<http://dx.doi.org/10.1038/srep42717>)
34. A. Grosdidier, V. Zoete, O. Michielin, *Nucleic Acids Res.* **39** (2011) W270 (<http://dx.doi.org/10.1093/NAR/GKR366>)
35. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* (2004) (<http://dx.doi.org/10.1002/jcc.20084>)
36. M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess, E. Lindah, *SoftwareX* **1–2** (2015) 19 (<http://dx.doi.org/10.1016/J.SOFTX.2015.06.001>)
37. M. Svensson, S. Humbel, R. D. J. Froese, T. Matsubara, S. Sieber, K. Morokuma, *J. Phys. Chem.* **100** (1996) 19357 (<http://dx.doi.org/10.1021/JP962071J;CTYPE:STRING:JOURNAL>)
38. *Gaussian 09, Revision A.01*, Gaussian, Inc., Wallingford, CT, 2009 (<https://gaussian.com/g09citation/>)
39. *Gaussview, Rev. 3.09*, Windows version, Gaussian Inc., Pittsburgh, PA (https://gaussian.com/508_gvw/)

40. A. Meller, M. Ward, J. Borowsky, M. Kshirsagar, J. M. Lotthammer, F. Oviedo, J. L. Ferres, G. R. Bowman, *Nat. Commun.* **14** (2023) (<http://dx.doi.org/10.1038/s41467-023-36699-3>)
41. L. Jendele, R. Krivak, P. Skoda, M. Novotny, D. Hoksza, *Nucleic Acids Res.* **47** (2019) W345 (<http://dx.doi.org/10.1093/nar/gkz424>)
42. V. Sharma, A. Gupta, M. Singh, A. Singh, A. A. Chaudhary, Z. H. Ahmed, S. U. D. Khan, S. Rustagi, S. Kumar, S. Kumar, *Front. Bioinformat.* **5** (2025) 1 (<http://dx.doi.org/10.3389/fbinf.2025.1545353>)
43. M. Popovic, *Virology* **570** (2022) 35 (<http://dx.doi.org/10.1016/j.virol.2022.03.008>)
44. M. Popovic, *Microb. Risk Anal.* **22** (2022) 100231 (<http://dx.doi.org/10.1016/j.mran.2022.100231>)
45. E. Wolf, C. Lento, J. Pu, B. C. Dickinson, D. J. Wilson, *Biochemistry* **62** (2023) 1619 (<http://dx.doi.org/10.1021/acs.biochem.2c00709>)
46. J. Fanfrlík, A. K. Bronowska, J. Řezáč, O. Přenosil, J. Konvalinka, P. Hobza, *J. Phys. Chem., B* **114** (2010) 12666 (<http://dx.doi.org/10.1021/JP1032965>)
47. J. Řezáč, J. Fanfrlík, D. Salahub, P. Hobza, *J. Chem. Theory Comput.* **5** (2009) 1749 (<https://doi.org/10.1021/ct9000922>)
48. J. J. P. Stewart, *J. Mol. Model.* **13** (2007) 1173 (<http://dx.doi.org/10.1007/s00894-007-0233-4>)
49. A. K. Rappé, C. J. Casewit, K. S. Colwell, W. A. Goddard, W. M. Skiff, *J. Am. Chem. Soc.* **114** (1992) 10024 (<https://doi.org/10.1021/ja00051a040>)
50. Z. Kazemi, H. A. Rudbari, M. Sahihi, V. Mirkhani, M. Moghadam, S. Tangestaninejad, I. Mohammadpoor-Baltork, S. Gharaghani, *J. Photochem. Photobiol., B* **162** (2016) 448 (<http://dx.doi.org/10.1016/J.JPHOTOBIO.2016.07.003>)
51. G. R. Desiraju, T. Steiner, *The weak hydrogen bond : in structural chemistry and biology*, Oxford University Press, Oxford, 2001
52. *Discovery Studio Visualiser 2019*, Dassault Systèmes, San Diego, CA, 2020 (<http://dx.doi.org/https://discover.3ds.com/discovery-studio-visualizer-download>).

SUPPLEMENTARY MATERIAL TO
**A computational study to identify the metabolites in *Lippia alba*
capable of inhibiting gastric cancer through BCL-2 protein
inhibition**

LUIS H. MENDOZA-HUIZAR*

*Universidad Autónoma del Estado de Hidalgo, Academic Area of Chemistry, Carretera
Pachuca-Tulancingo Km. 4.5, CP. 42184, Mineral de la Reforma, México*

J. Serb. Chem. Soc. 90 (12) (2025) 1451–1464

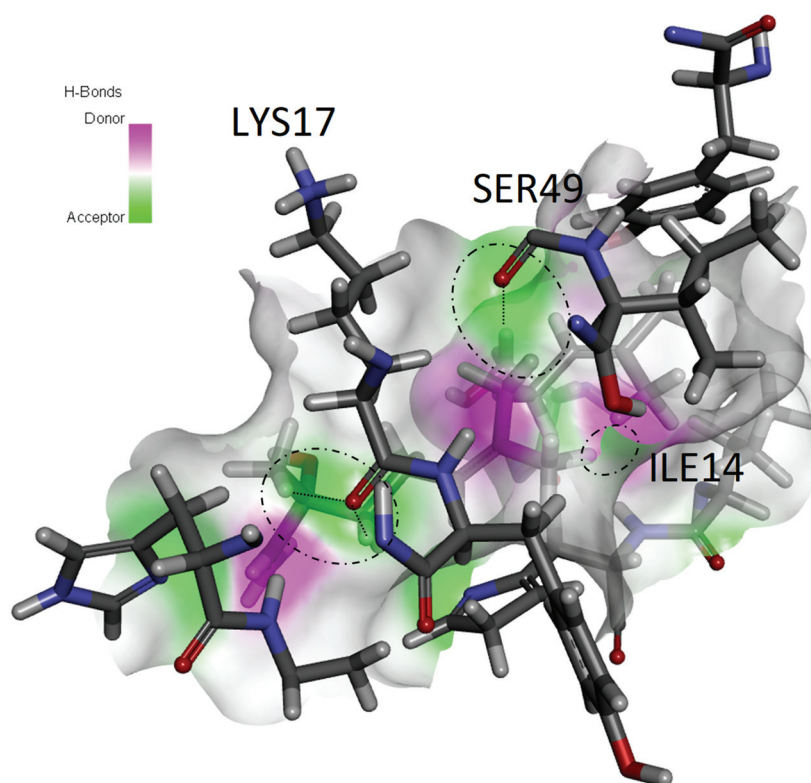
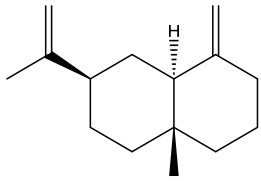
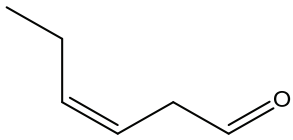
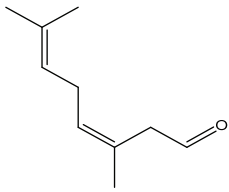


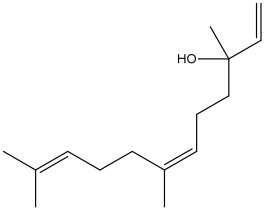
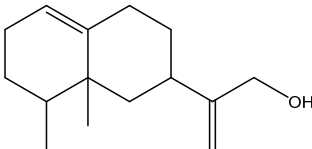
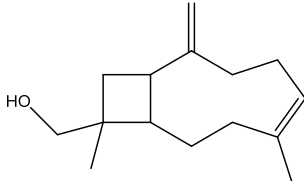
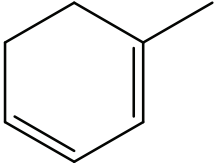
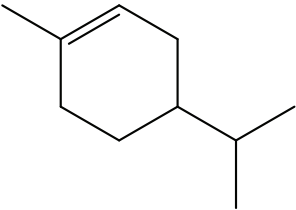
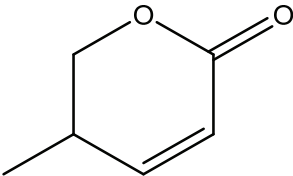
Fig. S-1. Predominant molecular interactions between Lys17, Ile14, and Ser49.

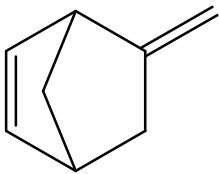
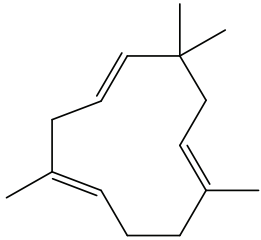
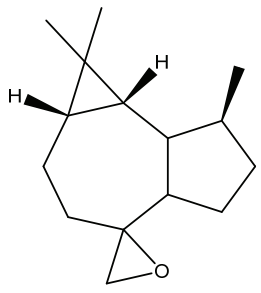
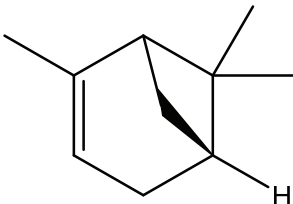
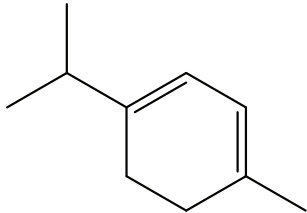
*Correspondence E-mail: hhuizar@uaeh.edu.mx

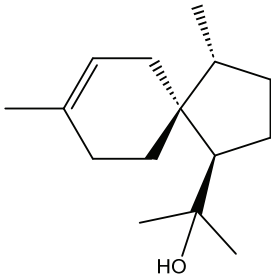
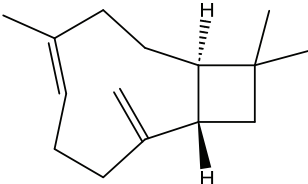
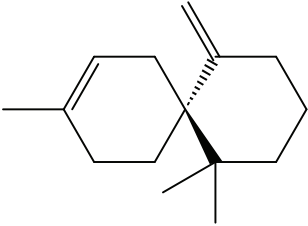
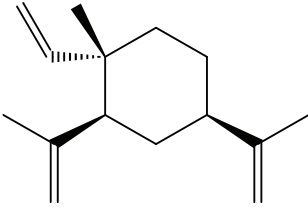
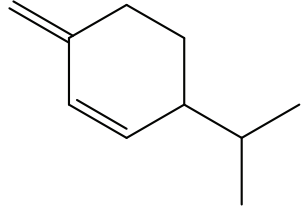
The metabolites included in this study are: 1) β -selinene, 2) (3*Z*)-hexenal, 3) (Z)-isocitral, 4) (Z)-nerolidol, 5) 13-hydroxy-valencene, 6) 14-hydroxy-9-epi-(*E*)-caryophyllene, 7) 1-methylcyclohexa-1,3-diene, 8) *p*-menth-1-ene, 9) 4-methyl-2,3-dihydro-2*H*-pyran-6-one, 10) 5-methylene-2-norbornene, 11) α -humulene, 12) allo-aromadendrene epoxide, 13) α -pinene, 14) α -terpinene, 15) β -acorenol, 16) β -caryophyllene, 17) β -chamigrene, 18) β -elemene, 19) β -phellandrene, 20) β -pinene, 21) camphene, 22) caryophyllene oxide, 23) *cis*-piperitol, 24) *cis*-pinocarvyl acetate, 25) *cis*-sabinene hydrate acetate, 26) *cis*- β -guaiene, 27) *E*-salvene, 28) ethyl pent-4-enoate, 29) eugenol, 30) eugenol acetate, 31) *exo*-2-norborneol, 32) geranial, 33) geraniol, 34) geranyl acetate, 35) geranyl butanoate, 36) geranyl isobutanoate, 37) geranyl formate, 38) germacrene D, 39) khusimol, 40) ledol, 41) limonene, 42) limonene aldehyde, 43) linalool, 44) mesitylene, 45) methyl eugenol, 46) methyl geranate, 47) 1-methylcyclohexa-1,4-diene, 48) myrcene, 49) myrcenol, 50) neoisodihydro carveol, 51) neral, 52) nerol, 53) *o*-cymene, 54) *p*-cymene, 55) santalone, 56) santolina triene, 57) terpinolene, 58) thuj-2,4(10)-diene, 59) *trans*-piperitol, 60) *trans*-ascaridol glycol, 61) *trans*-dihydrocarvone, 62) *trans*-piperitone epoxide, 63) *trans*-verbenyl acetate, 64) *trans*- β -terpineol, 65) tricyclene, 66) valencene, 67) γ -cadinene, 68) γ -elemene, 69) γ -eudesmol, 70) γ -terpinene, 71) γ -terpineol, 72) δ -selinene, 73) δ -elemene.

Table S-I. *Lippia alba* metabolites studied and biological properties^{1,2}

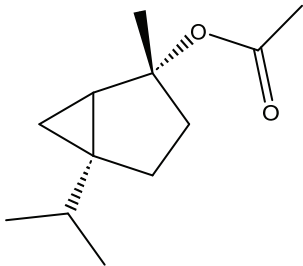
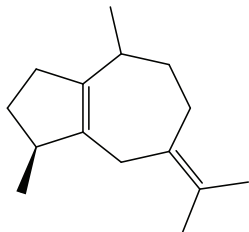
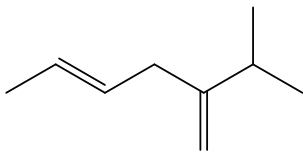
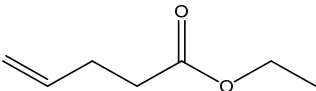
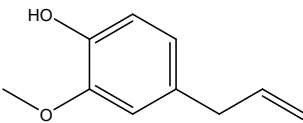
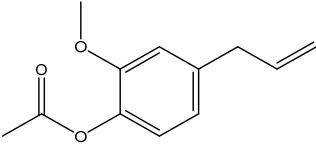
No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
1	(3 <i>R</i> ,4 <i>aS</i> ,8 <i>aR</i>)-8 <i>a</i> -methyl-5-methylidene-3-prop-1-en-2-yl-1,2,3,4,4 <i>a</i> ,6,7,8-octahydronaphthalene	β -selinene		Antifungal, antibacterial and antioxidants
2	(<i>Z</i>)-hex-3-enal	(3 <i>Z</i>)-hexenal		-
3	(<i>Z</i>)-3,7-dimethyl-3,6-octadienal	(<i>Z</i>)-Isocitral		Antispasmodic

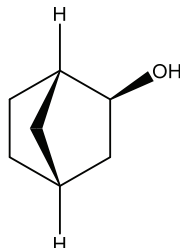
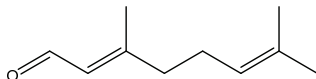
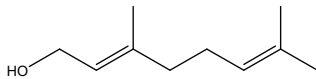
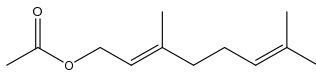
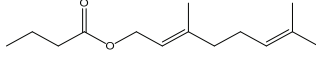
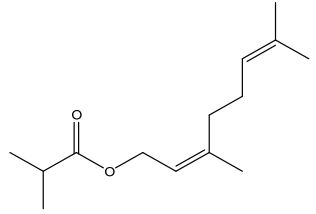
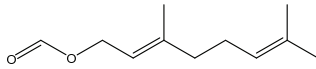
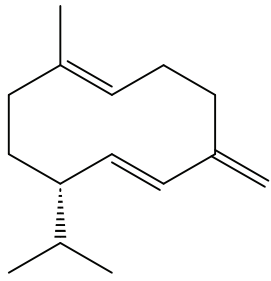
No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
4	(6Z)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol	(Z)-nerolidol		Antioxidant, antibacterial, antimycotic, antitumoral
5	(1S,4aR,7S,8aS)-1,4a-dimethyl-7-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalen-1-ol.	13-hydroxy-valencene		Antioxidant, anti-inflammatory
6	[(5Z)-6,10-dimethyl-2-methylidene-10-bicyclo[7.2.0]undec-5-enyl]methanol	14-hydroxy-9-epi-(E)-Caryophyllene		-
7	1-methylcyclohexa-1,3-diene	1-methylcyclohexa-1,3-diene		-
8	p-Menth-1-ene	p-Menth-1-ene		-
9	4-methyl-2,3-dihydro-2H-pyran-6-one	4-methyl-2,3-dihydro-2H-pyran-6-one		-

No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
10	5-methylidenebicyclo[2.2.1]-hept-2-ene	5-Methylene-2-norbornene		-
11	(1Z,4Z,8E)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene	α -humulene		Anti-inflammatory
12	(1a <i>S</i> ,7 <i>S</i> ,7 <i>bR</i>)-1,1,7-trimethylspiro[2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[<i>c</i>]azulene-4,2'-oxirane]	Allo-Aromadendrene epoxide		Antiviral
13	(1 <i>R</i> ,5 <i>R</i>)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	α -Pinene		Antioxidant, anticancer, genotoxic
14	1-methyl-4-propan-2-ylcyclohexa-1,3-diene	α -terpinene		Antioxidant

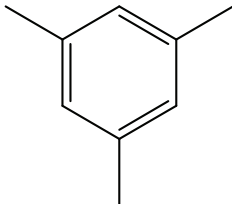
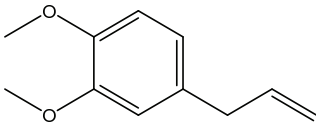
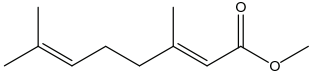
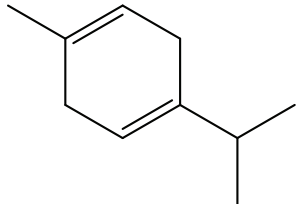
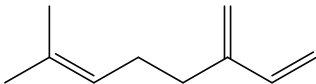
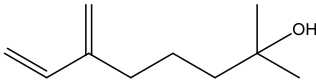
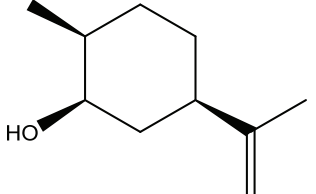
No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
15	2-[(1 <i>R</i> ,4 <i>R</i> ,5 <i>R</i>)-1,8-dimethylspiro[4.5]dec-8-en-4-yl]propan-2-ol	β -acorenol		-
16	(4 <i>E</i>)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	β -caryophyllene		Anticancer, antioxidant, antimicrobial.
17	(6 <i>R</i>)-5,5,9-trimethyl-1-methylidenespiro[5.5]undec-9-ene	β -chamigrene		-
18	(1 <i>S</i> ,2 <i>S</i> ,4 <i>R</i>)-1-ethenyl-1-methyl-2,4-bis(prop-1-en-2-yl)cyclohexane	β -elemene		Anticancer
19	3-methylidene-6-(propan-2-yl)cyclohex-1-ene	β -Phellandrene		-

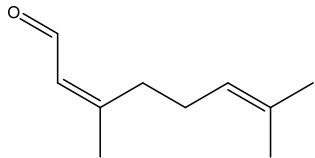
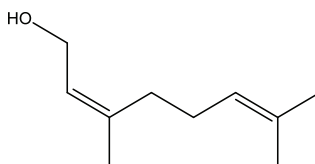
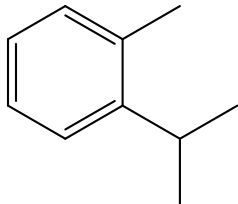
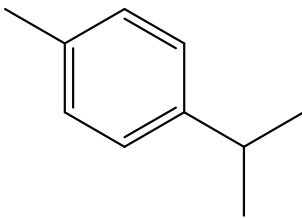
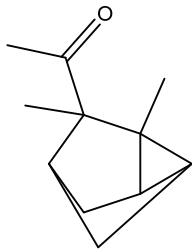
No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
20	6,6-dimethyl-2-methylidenebicyclo[3.1.1]heptane	β -pinene		Anticancer
21	2,2-dimethyl-3-methylidenebicyclo[2.2.1]heptane	Camphene		Antibacterial, antifungal, antioxidant, anticancer
22	(1 <i>R</i> ,4 <i>R</i> ,6 <i>R</i> ,10 <i>S</i>)-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.0 ^{4,6}]dodecane	Caryophyllene oxide		Anticancer, analgesic.
23	(1 <i>R</i> ,6 <i>S</i>)-3-methyl-6-propan-2-ylcyclohex-2-en-1-ol	Cis-piperitol		-
24	1 <i>S</i> ,3 <i>S</i> ,5 <i>S</i>)-6,6-dimethyl-2-methylidenebicyclo[3.1.1]hept-3-yl acetate	Cis-Pinocarvyl acetate		-

No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
25	[(2 <i>R</i> ,5 <i>S</i>)-2-methyl-5-propan-2-yl-2-bicyclo[3.1.0]hexanyl] acetate	Cis-sabinene hydrate acetate		-
26	(1 <i>R</i> ,4 <i>R</i>)-1,4-dimethyl-7-propan-2-ylidene-2,3,4,5,6,8-hexahydro-1 <i>H</i> -azulene	Cis-β-guaiene		-
27	(2 <i>E</i>)-6-methyl-5-methylenehept-2-ene	<i>E</i> -Salvene		-
28	<i>E</i> thyl pent-4-enoate	Ethyl pent-4-enoate		-
29	2-methoxy-4-prop-2-enylphenol	Eugenol		Anticancer
30	(2-methoxy-4-prop-2-enylphenyl) acetate	Eugenol acetate		Anticancer

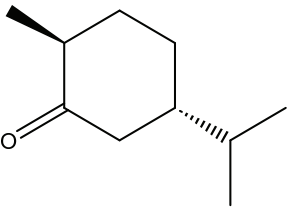
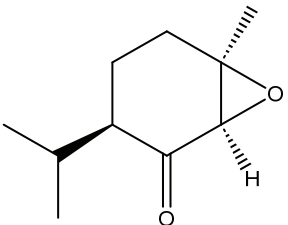
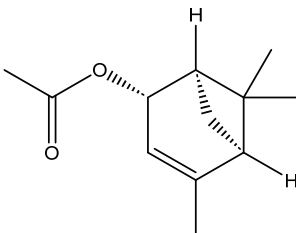
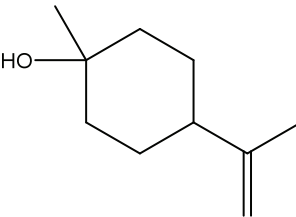
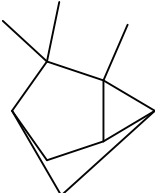
No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
31	bicyclo[2.2.1]heptan-2-ol	Exo-2-Norborneol		-
32	(2E)-3,7-dimethylocta-2,6-dienal	Geranial		Anticancer
33	(2E)-3,7-dimethylocta-2,6-dien-1-ol	Geraniol		Anticancer
34	(2E)-3,7-dimethylocta-2,6-dien-1-yl acetate	Geranyl acetate		Antibacterial and antifungal.
35	(2E)-3,7-dimethylocta-2,6-dien-1-yl butanoate	Geranyl butanoate		-
36	(2E)-3,7-dimethylocta-2,6-dien-1-yl 2-methylpropanoate	Geranyl isobutanoate		-
37	(2E)-3,7-dimethylocta-2,6-dien-1-yl formate	Geranyl formate		-
38	(1E,6E,8S)-1-methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene	Germacrene D		Antibacterial, antifungal, anti-inflammatory

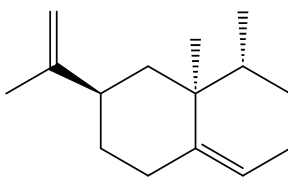
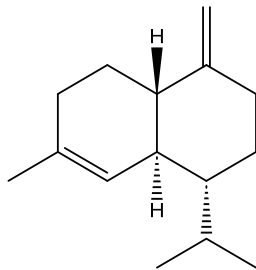
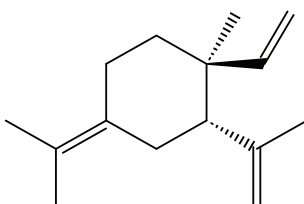
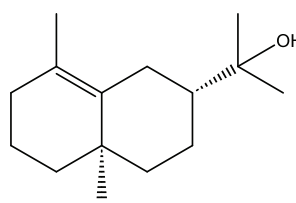
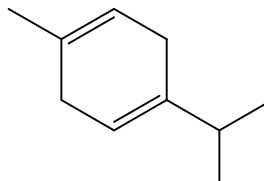
No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
39	[(1 <i>R</i> ,2 <i>S</i> ,5 <i>S</i> ,8 <i>R</i>)-7,7-dimethyl-6-methyldene-2-tricyclo[6.2.1.0 ^{1,5}]undecanyl]methanol	Khusimol		-
40	1,1,4,7-tetramethyl-2,3,4 <i>a</i> ,5,6,7,7 <i>a</i> ,7 <i>b</i> -octahydro-1 <i>aH</i> -cyclopropa[<i>e</i>]azulen-4-ol	Ledol		-
41	1-methyl-4-prop-1-en-2-ylcyclohexene	Limonene		Antioxidant, antigenotoxic, anti-inflammatory
42	3-(4-methylcyclohex-3-en-1-yl)butanal	Limonene aldehyde		-
43	(3 <i>R</i>)-3,7-dimethylocta-1,6-dien-3-ol	Linalool		Anti-inflammatory, anticancer, antimicrobial, contraceptive, analgesic, anxiolytic, antidepressant

No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
44	1,3,5-trimethylbenzene	Mesitylene		-
45	1,2-dimethoxy-4-prop-2-enylbenzene	Methyl eugenol		Anticancer
46	methyl (2E)-3,7-dimethylocta-2,6-dienoate	Methyl geranate		Antimicrobial
47	1-methylcyclohexa-1,4-diene	1-methylcyclohexa-1,4-diene		-
48	7-methyl-3-methylenoocta-1,6-diene	Myrcene		Analgesic
49	2-methyl-6-methylenooct-7-en-2-ol	Myrcenol		-
50	2-methyl-5-prop-1-en-2-ylcyclohexan-1-ol	Neoisodihydro carveol		-

No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
51	(2Z)-3,7-dimethylocta-2,6-dienal	Neral		Anticancer
52	(2Z)-3,7-dimethylocta-2,6-dien-1-ol	Nerol		Anticancer
53	1-methyl-2-(propan-2-yl)benzene	o-Cymene		Antimicrobial
54	1-methyl-4-(propan-2-yl)benzene	p-Cymene		Antimicrobial
55	1-{2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]-heptan-3-yl}ethan-1-one	Santalone		Analgesic.

No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
56	3-ethenyl-2,5-dimethylhexa-1,4-diene	Santolina triene		Anti-inflammatory, antioxidant y antibacterial
57	1-methyl-4-(propan-2-ylidene)cyclohex-1-ene	Terpinolene		-
58	1-Isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	Thuja-2,4(10)-diene		-
59	(1 <i>S</i> ,6 <i>S</i>)-3-methyl-6-(propan-2-yl)cyclohex-2-en-1-ol	Trans piperitol		Antifungal
60	2-Cyclohexene-1,4-diol, 1-methyl-4-(1-methylethyl)-	Trans-ascaridol glycol		Antioxidant.

No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
61	(2 <i>R</i> ,5 <i>R</i>)-2-methyl-5-prop-1-en-2-ylcyclohexan-1-one	Trans-dihydrocarvone		Antioxidant.
62	(1 <i>R</i> ,3 <i>R</i> ,6 <i>R</i>)-6-methyl-3-propan-2-yl-7-oxabicyclo[4.1.0]heptan-2-one	Trans-Piperitone epoxide		Antioxidant, antibacterial.
63	[(1 <i>R</i> ,2 <i>S</i> ,5 <i>R</i>)-4,6,6-trimethyl-2-bicyclo[3.1.1]hept-3-enyl]acetate	Trans-verbenyl acetate		-
64	1-Methyl-4-(1-methylvinyl)cyclohexan-1-ol	Trans-β-pterpineol		-
65	1,7,7-trimethyltricyclo[2.2.1.0 ^{2,6}]-heptane.	Tricyclene		Antioxidant.

No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
66	(3 <i>R</i> ,4 <i>aS</i> ,5 <i>R</i>)-4 <i>a</i> ,5-dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4 <i>a</i> ,5,6,7-octahydronaphthalene	Valencene		-
67	(1 <i>S</i> ,4 <i>aR</i> ,8 <i>aR</i>)-7-Methyl-4-methyldiene-1-(propan-2-yl)-1,2,3,4,4 <i>a</i> ,5,6,8 <i>a</i> -octahydronaphthalene	γ -Cadinene		Antioxidant.
68	(1 <i>S</i> ,2 <i>S</i>)-1-ethenyl-1-methyl-4-propan-2-ylidene-2-prop-1-en-2-ylcyclohexane	γ -Elemene		Larvicide.
69	(2-[(2 <i>R</i> ,4 <i>aR</i>)-4 <i>a</i> ,8-dimethyl-2,3,4,5,6,7-hexahydro-1 <i>H</i> -naphthalen-2-yl]propan-2-ol	γ -eudesmol		Antioxidant, Antimicrobial.
70	1-methyl-4-propan-2-ylcyclohexa-1,4-diene	γ -terpinene		Anticancer, antibacterial, Antifungal.

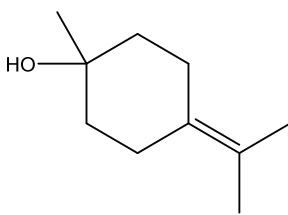
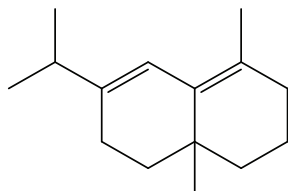
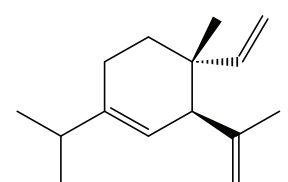
No.	IUPAC Name Metabolite ³	Common name Metabolite	Structure	Biological properties
71	1-methyl-4-propan-2-ylidenecyclohexan-1-ol	γ -terpineol		Anticancer
72	4,8a-dimethyl-6-propan-2-yl-2,3,7,8-tetrahydro-1H-naphthalene	δ -selinene		-
73	4-ethenyl-4-methyl-1-propan-2-yl-3-prop-1-en-2-ylcyclohexene	δ -elemene		Anticancer

Table S-II. Nomenclature of terms used in the manuscript

Term	Description
BCL-2	B-cell lymphoma 2
BH3	Bcl-2 Homology 3
BAX	Bcl-2-associated X protein
BH4	Bcl-2 Homology 4
Raf-1	Raf-1 proto-oncogene, serine/threonine kinase
c-Myc	cellular Myelocytomatosis oncogene
IP3R	Inositol 1,4,5-trisphosphate receptor
His20	Histidine at position 20
Tyr21	Tyrosine at position 21
His94	Histidine at position 94
Val93	Valine at position 93
Ile14	Isoleucine at position 14
Tyr9	Tyrosine at position 9
Lys17	Lysine at position 17
Tyr18	Tyrosine at position 18
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
QM/MM	Quantum Mechanics / Molecular Mechanics,
IG5M	PDB ID of BCL-2
Ile14	isoleucine amino acid residue at position 14
Tyr9	Tyrosine residue at position 9
Val93	Valine amino acid residue at position 93
His94	Histidine residue at position 94
PM6	Parametric Method 6
DFT	Density Functional Theory
UFF	Universal Force Field (UFF)

Table S-III. Results of Lipinski's Rule of Five Analysis for the 73 Evaluated Metabolites

Compound	PM ≤500	MLOGP ≤ 4.15	N or O ≤ 10	NH or OH ≤ 5
2,3,4,5,6,7,9,10,12,14,15,19,22,23,24,25,26, 27,28,29, 30,31,32,33,34,35,36,37,39,40, 41,42,43,45,46,47,48,49,50, 51,52,55,56, 57,58,59,60,61,62,63,64,69,70,71	✓	✓	✓	✓
1,8,11,13,15,16,17,18,20,21,38,44,53,54,65, 66,67,68,72,73	✓	X	✓	✓

REFERENCES

1. L. Silva-Santos, L. Palhares Neto, N. Corte-Real, M. V. L. Sperandio, C. A. G. Camara, M. M. Moraes, C. Ulisses, *South African J. Bot.* **163** (2023) 756 (<http://dx.doi.org/10.1016/j.sajb.2023.10.024>)
2. P. T. de Souza Silva, L. M. de Souza, M. B. de Morais, M. M. de Moraes, C. A. G. da Camara, C. Ulisses, *South African J. Bot.* **147** (2022) 415 (<http://dx.doi.org/10.1016/j.sajb.2022.01.042>)
3. Lexichem TK 2.7.0, 2025 (<https://docs.eyesopen.com/toolkits/python/lexichemtk/index.html#>) (Accessed July 28, 2025).



J. Serb. Chem. Soc. 90 (12) 1465–1479 (2025)
JSCS–5466

Impact of internal notation and heterobifunctional reactive dyes on color performance, mechanical and dimensional stability of cellulose-based biomaterials

RUMA SARKER¹, GAUTAM CHANDRA SARKER¹, ARNOB DHAR PRANTA^{3,4*}
and JOYKRISNA SAHA⁴

¹Golden Touch Imports Inc, Dhaka-1230, Bangladesh, ²P.N. Composite Ltd, Dhaka-1212, Bangladesh, ³ZR Research Institute for Advanced Materials, Sherpur-2100, Bangladesh and

⁴Department of Textile Engineering, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh

(Received 26 May, revised 3 July, accepted 14 July 2025)

Abstract: Internal notation factors influence the quality of cellulose-based biomaterials dyed with hetero bifunctional reactive dyes. Different internal notations of materials, such as Single Jersey, Rib and Interlock affect various coloration parameters like dye uptake, color fastness and strength. This research aims to analyze the influence of various internal notations on color performance with hetero bifunctional reactive dyes. It also aims to identify the varying shades and the manufacturing internal notation factors for achieving consistent color quality and durability across different types of cellulose-based biomaterials. Seven cellulose-based biomaterials with various internal notations (Single Jersey, 1×1 Rib, 2×1 Rib, Interlock, French Terry, Single Lacoste, Pique) were analyzed, colored with different dye uptake levels, such as light, medium and dark. The investigation measured color strength, color parameters (L^* , a^* , b^* , c^* , h), surface morphology, color fastness, mechanical strength, shrinkage and spirality. Standardized testing methods and instruments were employed for quality assessment. It has been observed that internal notation of materials significantly impacts dye absorbency with Interlock appearing brightest (K/S value 11.5). SEM analysis reveals surface morphology differences, affecting dye absorption. Colorfastness varies with fabric internal notation while bursting strength differs across fabric types.

Keywords: fabric structure; textile coloration; dyeing performance; colorfastness; quality assessment.

*Corresponding author. E-mail: arnobdhar75@gmail.com
<https://doi.org/10.2298/JSC250526052S>

INTRODUCTION

In recent years, the knitting segment of the textile industry has experienced rapid growth. The demand for weft-knitted garments has significantly increased in both domestic and export markets. Moreover, knit fabrics provide outstanding comfort qualities and have long been preferred in many kinds of clothing.^{1–4} Since they are produced on different machines with different knit stitches and conditions to create different patterns and fabric types, it is expected that they have different qualities.⁵ Efforts are being made to enhance the comfort of knitted fabric by altering fibers, yarn parameters (such as twist, bulk, count and finish), knitting parameters (including courses per inch, wales per inch, loop length and fabric weight) and post-knitting finishes (such as enzyme and chemical treatments).⁶ The Single Jersey (S. Jersey) knitted fabric properties especially the dimensional and physical properties are mainly influenced by the constituent fibers, yarn properties, knitting machine variables, processing and finishing treatments.⁷ Fabric quality refers to the various characteristics of finished fabric, which are determined by the properties of the yarn and the fabric's construction. The attributes of knitted fabrics are influenced by multiple factors, including the raw material, yarn structure, fabric internal notation, processing stages and finishing techniques.⁸ The process adopted affects the fabric's properties and its overall performance. The properties that are important for knitted fabric and maintained in the industries from the grey stage to the finished stage are GSM, dimensional stability (shrinkage and spirality), bursting strength and fastness properties.⁹ Tactile (hand) and appearance properties are also very important in all classes of fabrics.

Numerous studies have examined the effects of fibers, dye types, processing parameters and wet processing stages and sequences on knitted cotton fabric physical, dimensional and coloration properties.^{10–15} However, only a limited number of studies have focused on the influence of fabric internal notations on the physical, dimensional and coloration properties of cotton knit dyed fabrics. The research aims to investigate the impact of various internal notations of cellulose-based materials (knit fabric) on the quality parameters of fabrics dyed with hetero bifunctional reactive dyes by comparing the color strength at a particular wavelength for different knit internal notations including S. Jersey, 1×1 Rib, 2×1 Rib, Interlock, French Terry, Single Lacoste and Pique, each dyed with different shades. Additionally, the L^* , a^* , b^* , c^* and h^* values of all fabric internal notations are compared across light, medium and dark shades. Additionally, SEM analysis was conducted on dyed and undyed fabric internal notations to observe and compare morphology. Subsequently, the color fastness to washing, rubbing (both dry and wet), light exposure, and perspiration of all samples was evaluated. Moreover, the mechanical and dimensional stability features were assessed through various tests such as bursting strength, shrinkage and spirality.

EXPERIMENTAL

Materials and chemicals

Various scoured and bleached cellulose-based biomaterials with different internal notations were collected from Texeurop BD Ltd. Seven types of samples were produced in 30'' dia machine, including plain S. Jersey, French Terry, 1×1 Rib, 2×1 Rib, Interlock, S. Lacoste and Pique. These fabrics were made from 100 % cotton yarn with an areal density of 220. The fabric internal notation's knitting parameters are displayed in Table I, showing internal notations of different cellulose-based materials. The chemicals and dye stuff, sourced from Dysin Ltd., were utilized in the coloration process without any further treatment. The reactive dyes included three hetero bifunctional reactive dyes which are Levafix Amber CA (C.I. Reactive Orange 107), Levafix Fast Red CA (C.I. Reactive Red 223) and Levafix Blue CA (C.I. Reactive Blue 19) with vinyl sulphonyl and difluorochloropyrimidyl reactive groups in their molecular structure. In addition to these dyes, various chemicals played crucial roles in the coloration process. These included electrolyte glauher salt ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$), alkali soda ash (Na_2CO_3), caustic soda, hydrogen peroxide, wetting agent, leveling agent, anti-creasing agent, acetic acid (100 %) and soaping agent.

TABLE I. Knitting parameters of the fabric internal notations.

Fabric type	GSM	Yarn count	M/C dia	M/C gauge	Stitch length
S. Jersey	220	32/1(2ply)	30"	20	3.70/3.60
1×1 Rib	220	28/1	30"	18	2.60
2×1 Rib	220	28/1	36"	18	2.70
Interlock	220	24/1	30"	24	1.55
F. Terry	220	28/1	30"	24	2.75/1.35
S. Lacoste	220	22/1	30"	24	2.75
Pique	220	20/1	30"	24	3.05

Coloration process

The impact of coloration on fabric was assessed by applying light, medium, and dark shade recipes using a 30 kg Fongs dyeing machine. Three separate batches were processed for each shade. The light shade recipe included Levafix Blue CA (0.0062 %), Levafix Fast Red CA (0.079 %), and Levafix Amber CA (0.044 %), along with standard auxiliaries such as sequestering (05 g/l) and labelling agents, Glauber's salt, soda ash and acetic acid at pH 11. Dye concentrations and process parameters were proportionally adjusted for medium and dark shades. The medium shade recipe included Levafix Blue CA (1.04 %), Levafix Fast Red CA (1.96 %) and Levafix Amber CA (0.24 %), along with sequestering agent (05 g/l) and labelling agent (1.0 g/l), Glauber's salt (60 g/l), soda ash (15 g/l) and acetic acid (1 g/l) at pH 11. The dark shade recipe was prepared using Levafix Blue CA (2.40 %), Levafix Fast Red CA (1.96 %) and Levafix Amber CA (1.36 %) with the same auxiliaries at higher concentrations, including Glauber's salt (80 g/l) and soda ash (20 g/l), maintaining the pH at 11. All recipes followed a standard dyeing procedure to assess the effect of dye concentration on fabric shade depth and uniformity. Post-dyeing, the fabrics underwent slitting, stentering and compacting, followed by quality testing to evaluate coloration performance.

Characterization

The color strength (K/S) values were measured using a spectrophotometer (Datacolor 650, USA) based on the Kubelka–Munk equation: $K/S = (1-R)^2/2R$, where R is the reflectance at the

maximum absorption wavelength. Measurements were conducted under D65 illumination and a 10° standard observer. Also, the CIELab parameters were measured using the same calibrated spectrophotometer under standardized conditions. Measurements were taken with a D65 illuminant and a 10° observer angle. Color fastness to wash is tested according to ISO 105 C10:2006 and ISO 105 C06:2010, where specimens are washed with detergents and assessed for color change and staining.^{16,17} Rubbing fastness follows ISO 105 X-12:2001 E, with a test using a crock meter and grey scale evaluation.¹⁸ Shade evaluation was conducted both visually and instrumentally using a spectrophotometer for accurate color comparison. Color fastness to perspiration was assessed according to ISO 105 E04:2008, testing in both alkaline and acidic conditions. Staining was evaluated using the grey scale.¹⁹ The color fastness to light follows ISO 105-B02:2000, exposing fabrics to light and comparing color changes.²⁰ Bursting strength is measured according to ISO 13938-2:1999, assessing fabric resistance to pressure.²¹ All tests were conducted under standardized conditions using calibrated instrumentation to ensure consistency and accuracy. Dimensional stability was evaluated according to ISO 6330 and ISO 5077, assessing fabric shrinkage and deformation post-washing and drying. Spirality or skewness was measured following ISO 13935-2 by examining side seam distortion.^{22,23}

RESULTS AND DISCUSSION

Analysis of color strength performance

The K/S values of various fabric types across different shades were analyzed to evaluate the color depth and reflectivity. As shown in Fig. 1, S. Lacoste exhibited the highest K/S value in the dark shade (12.4), indicating superior color intensity. Both 1×1 Rib and S. Lacoste fabrics demonstrated strong color depth in dark shades, with K/S values of 12.1 and 12.4, respectively. S. Jersey showed a substantial increase from light (0.430) to dark (11.5) shades, highlighting its enhanced dye uptake. Pique and 2×1 Rib fabrics exhibited moderate K/S values, with Pique increasing from 0.446 to 11.4 and 2×1 Rib showing a slight decline in the dark shade (11.25) from a light value of 0.404.

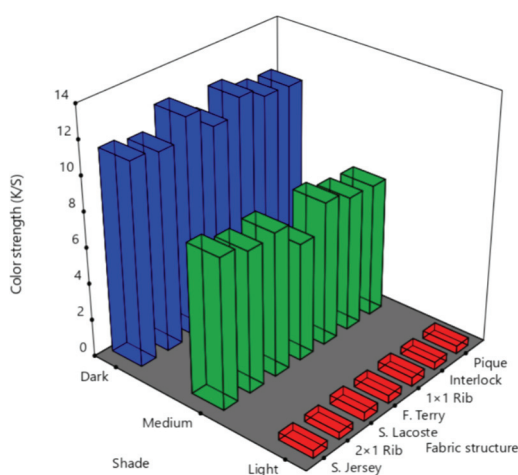


Fig. 1. Impact of internal notation and hetero bifunctional reactive dyes on color strength (K/S) of various cellulose-based biomaterials.

F. Terry and Interlock displayed similar trends, though Interlock reached a slightly higher dark shade value (11.5). Fabric types varied in color saturation, with S. Lacoste demonstrating the most consistent and intense coloration across all shades.

Analysis of lightness, red/greenness, yellow/blueness, chroma and hue

The CIE L^* , a^* , b^* , c^* and h^* values in Table II provide a detailed quantitative evaluation of the color properties across various fabric types, structures and shades. Light shades consistently show high L^* values (75–76), indicating greater brightness, while dark shades have low L^* values (24–25), reflecting higher light absorption. This pattern holds across all fabric constructions tested. The a^* and b^* values reveal tonal shifts: light shades exhibit positive a^* and b^* (red and yellow hues), medium shades show stable a^* with negative b^* (green–blue shift) and dark shades approach neutral values, indicating reduced chromaticity. Chroma (c^*) values are highest in light shades (17–18), moderate in medium shades (~18) and lowest in dark shades (~1), demonstrating desaturation with decreased lightness. Hue angle (h^*) varies from red–yellow hues in light shades (35–37°) to greenish–blue in medium shades (303–305°), with dark shades showing inconsistent values due to low lightness. These results highlight the significant effects of fabric structure and shade depth on color perception, with light and medium shades retaining distinct chromatic features, unlike the nearly neutral dark shades.

TABLE II. Impact of internal notation and hetero bifunctional reactive dyes on L^* , a^* , b^* , c^* and h^*

Fabric type	Shade	L^*	a^*	b^*	c^*	h^*
S. Jersey	Light	76.23	13.20	9.44	16.23	35.56
	Medium	30.76	10.80	–15.33	18.75	305.17
	Dark	25.02	0.20	–0.97	0.99	281.91
F. Terry	Light	75.76	13.68	10.24	17.08	36.81
	Medium	33.33	10.52	–15.37	18.62	304.39
	Dark	25.27	0.41	–1.11	1.19	290.32
1×1 Rib	Light	75.18	14.77	10.88	18.35	36.37
	Medium	31.03	10.67	–15.30	18.65	304.88
	Dark	24.18	0.35	–1.05	0.98	288.70
2×1 Rib	Light	76.24	13.86	10.38	17.31	36.83
	Medium	31.58	10.3	–15.37	18.50	303.82
	Dark	24.53	0.34	–0.55	0.65	301.91
Interlock	Light	76.07	14.28	10.74	17.87	36.94
	Medium	31.73	10.63	–15.82	19.06	303.89
	Dark	24.79	0.29	–1.23	1.27	283.23
S. Lacoste	Light	75.05	14.52	10.92	18.17	36.97
	Medium	30.80	10.17	–15.00	18.12	304.16
	Dark	23.79	0.41	–0.69	0.80	300.39
Pique	Light	75.68	13.99	10.75	17.64	37.53
	Medium	31.92	10.58	–15.41	18.69	304.49
	Dark	24.75	0.45	–0.86	0.98	297.62

Surface morphology analysis

The scanning electron microscope (SEM) provides an excellent technique for examination of surface morphology and fiber–dye adhesion, of fabric. Fig. 2 show the SEM photographs of different fabrics before and after coloration, respectively.

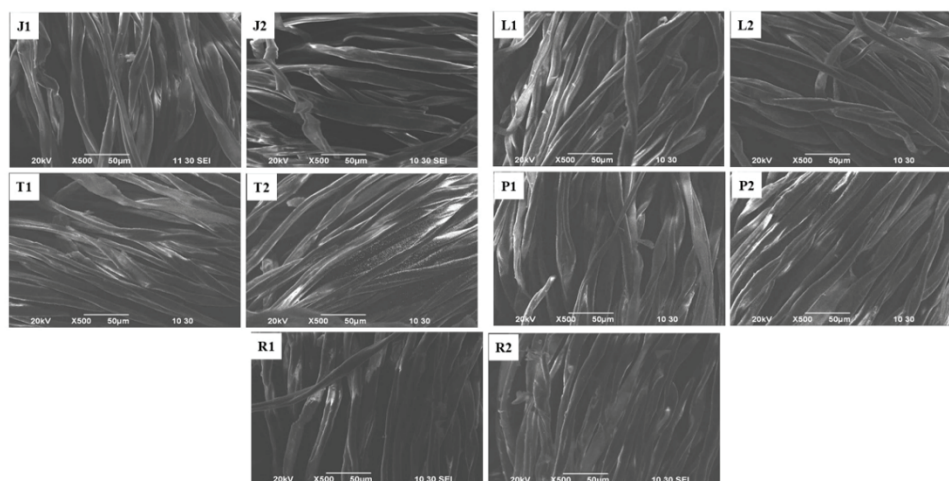


Fig. 2. SEM photographs of S. Jersey fabric: before (J1) and after c(J2)oloration; Frence Terry fabric: before (T1) and after (T2) coloration; 1×1 Rib fabric: before(R1) and after (R2) coloration; Lacoste fabric: before(L1) and after (L2) coloration; Pique fabric: before (P1) and after (P2) coloration.

Fig. 2 (J1) and (J2) illustrate the SEM images of S. Jersey fabric before and after coloration. The post-dyeing SEM analysis indicates a darker appearance, primarily due to the fabric's smooth surface and loosely arranged fibers. The surface exhibits relatively large porosity compared to other fabric structures, which likely facilitates easier and deeper penetration of dye molecules. This increased porosity may contribute to improved dye uniformity and reduced shade unevenness.²⁴ Fig. 2 (R1) and (R2) show the surface morphology of 1×1 Rib fabric before and after coloration. 1×1 Rib fabric has higher stretchability and elastic recovery than S. Jersey fabric.²⁵ Examining the image obtained from the scanning electron microscope reveals that the surface is smooth, and there is no indication of presence of fibers that are rigid and compact. There is a correlation between the size of the porosity on a surface and the ability of the dye to adhere to that surface. As a consequence, the fiber is able to absorb dye molecules, which results in the fiber taking on a darker color.

On the other hand, Fig. 2 (L1) and (L2) show the surface morphology of Lacoste fabric before and after coloration. Lacoste fabric shows low shrinkage and high extensibility.²⁶ The brighter shade observed in the SEM image of the dyed

Lacoste fabric is attributed to the cohesiveness and compactness of its fibers. This compact fiber structure, characterized by small and less porous surfaces, restricts the adherence and penetration of dye molecules, resulting in lower dye absorption. Fig. 2 (P1) and (P2) illustrate the surface morphology of Pique fabric before and after coloration. Pique fabric exhibits notable resistance to shrinkage and excellent resilience. SEM analysis reveals a characteristic grooved surface morphology with visible macrofibrils oriented along the surface. The rough texture and prominent macrofibril outlines suggest less smooth dye absorption compared to other fabrics. Similarly, Fig. 2 (T1) and (T2) show the surface morphology of Terry fabric pre- and post-coloration. The surface displays a mix of smooth and rough areas. The SEM image after dyeing indicates a more vibrant shade, which is attributed to the fabric's compactness. Terry fabric has smaller-sized porosity compared to other fabrics, which limits dye molecule absorption, contributing to its distinctive coloration.^{27,28}

Color resistance to laundry

Table III shows that, for the same dye type, color fastness to wash (color change) remains largely consistent across fabric types. Light shades for all fabrics achieved high ratings of 4–5. However, increasing shade depth led to a decrease in

TABLE III. Impact of internal notation and hetero bifunctional reactive dyes on color fastness to wash

Grey scale	Shade	S. Jersey	F. Terry	1×1 Rib	2×1 Rib	Interlock	S. Lacoste	Pique
Color change	Light	4–5	4–5	4–5	4–5	4–5	4–5	4–5
	Medium	4–5	4–5	4–5	4–5	4–5	4–5	4–5
	Dark	4	4–5	4–5	4–5	4–5	4–5	4–5
Color staining	Acetate	Light	4–5	4–5	4–5	4–5	4–5	4–5
		Medium	4–5	4–5	4–5	4–5	4–5	4–5
		Dark	4–5	4–5	4–5	4–5	4–5	4–5
	Cotton	Light	4–5	4–5	4–5	4–5	4–5	4–5
		Medium	3–4	3–4	3–4	3–4	3–4	3–4
		Dark	3	2–3	4–5	3	2–3	3
	Nylon	Light	4–5	4–5	4–5	4–5	4–5	4–5
		Medium	4–5	4–5	4–5	4–5	4–5	4–5
		Dark	4–5	4–5	4–5	4–5	4–5	4–5
	Polyester	Light	4–5	4–5	4–5	4–5	4–5	4–5
		Medium	4–5	4–5	4–5	4–5	4–5	4–5
		Dark	4–5	4–5	4–5	4–5	4–5	4–5
	Acrylic	Light	4–5	4–5	4–5	4–5	4–5	4–5
		Medium	4–5	4–5	4–5	4–5	4–5	4–5
		Dark	4–5	4–5	4–5	4–5	4–5	4–5
	Wool	Light	4–5	4–5	4–5	4–5	4–5	4–5
		Medium	4–5	4–5	4–5	4–5	4–5	4–5
		Dark	4–5	4–5	4–5	4–5	4–5	4–5

wash fastness, with medium shades rated around 4 and dark shades dropping to 3–4. No significant variation in wash fastness was observed due to fabric internal structure, indicating that dye type and shade percentage primarily influence color fastness to washing.

Color fastness to wash results for various fabric internal notations (S. Jersey, F. Terry, 1×1 Rib, 2×1 Rib, Interlock, S. Lacoste and Pique) across fibers – including acetate, cotton, nylon, polyester, acrylic and wool – reveal notable trends in color change and staining. All fabrics demonstrated excellent color change resistance, with ratings of 4–5 for light and medium shades. S. Jersey showed a slight decline to a rating of 4 for dark shades, while other fabrics maintained 4–5 ratings. Regarding color staining, synthetic fibers (nylon, polyester, acrylic) and wool consistently achieved high ratings (4–5) across all fabric structures and shade intensities, indicating superior resistance to color transfer and enhanced durability during washing. Cotton fabrics performed well under light and medium staining (ratings 4–5), but showed reduced resistance under dark staining. Specifically, S. Jersey, F. Terry, S. Lacoste and Pique exhibited lower ratings (2–3) in darker conditions, whereas 1×1 Rib and Interlock maintained higher resistance (4–5).

Color resistance to rubbing and light

Table IV shows that for the same dye type, color fastness to rubbing (color change) and light exposure do not significantly vary across light, medium and dark shades. Light shade dyeing resulted in excellent dry and wet rubbing fastness ratings (4–5) for all fabric types. For medium shades, dry rubbing ratings remained good (4–4.5), while wet rubbing exhibited variability. Pique fabric showed the lowest wet rubbing rating (2–3), followed by S. Lacoste (3), with 1×1 Rib, 2×1 Rib, and Interlock fabrics rated 3–4. S. Jersey and F. Terry fabrics had a wet rubbing rating of 3. Dark shade dyeing maintained dry rubbing ratings of 4–5 across all samples, but wet rubbing ratings varied similarly to medium shades. Fabrics with rough surfaces, such as Pique and S. Lacoste, demonstrated lower wet rubbing fastness, likely due to surface texture effects during wet rubbing.

TABLE IV. Impact of internal notation and hetero bifunctional reactive dyes on color resistance to rubbing and light expressed as light, medium and dark shading intensities

Fabric type	Dry rubbing			Wet rubbing			Light		
	Light	Medium	Dark	Light	Medium	Dark	Light	Medium	Dark
S. Jersey	4–5	3–4	4–5	4–5	3	3–4	4–5	4–5	4–5
F. Terry	4–5	4–5	4–5	4–5	3	2–3	4–5	4–5	4–5
1×1 Rib	4–5	3–4	4–5	4–5	3–4	4	4	4	4
2×1 Rib	4–5	4–5	4–5	4–5	3–4	4	4	4	4
Interlock	4–5	4–5	4–5	4–5	3–4	3–4	4–5	4–5	4–5
S. Lacoste	4–5	4–5	4–5	4–5	3	3	4	4	4
Pique	4–5	4	4–5	4–5	2–3	2–3	4	4	4

Table IV evaluates light fastness across various fabric types: S. Jersey (S/J), F. Terry, 1×1 Rib, 2×1 Rib, Interlock, Single Lacoste (S. Lacoste) and Pique. S. Jersey, F. Terry and Interlock exhibit superior light fastness (4–5), reflecting their comfort, durability and versatility for casual and activewear. The 1×1 Rib and 2×1 Rib fabrics, rated 4, provide notable elasticity suitable for stretch applications like cuffs and collars. S. Lacoste and Pique, also rated 4, offer breathability and distinctive texture, commonly used in polo and sportswear, though with slightly lower performance. This analysis highlights the specific strengths of each fabric, informing their optimal use in textile manufacturing.

Color resistance to sweating

The present study evaluated the chemical resistance of multiple fabric types – specifically S. Jersey (S. Jersey), French Terry (F. Terry), 1×1 Rib, 2×1 Rib, Interlock, Single Lacoste (S. Lacoste) and Pique – against alkaline and acidic solutions, with results summarized in Table V. All fabric samples demonstrated consistently high resistance, receiving uniform grades of 4 to 5 across both acidic and alkaline exposure conditions. This uniformity underscores the intrinsic chemical stability and resilience of the fibers, irrespective of the differing fabric structures and knit notations. The consistently superior resistance ratings suggest that the material properties inherent to these fabrics play a critical role in preserving structural integrity and functional performance when subjected to variable pH environments. Such resilience is particularly advantageous for textile applications where exposure to chemical agents is frequent, indicating these fabrics' robustness and versatility in practical use scenarios. The data further reveals no significant variance in resistance between fabric types, thereby validating their comprehensive applicability across diverse industrial and commercial settings that demand high chemical durability. These findings warrant further investigation into the specific fiber compositions, manufacturing processes, and potential finishing treatments that contribute to this notable chemical stability. Optimizing these factors could lead to enhanced fabric performance and broaden their applicability in environments with stringent chemical exposure requirements.

TABLE V. Impact of internal notation and hetero bifunctional reactive dyes on color fastness to sweating; L – light; M – medium; D – dark

Grey scale		Shade	S. Jersey	F. Terry	1×1 Rib	2×1 Rib	Interlock	S. Lacoste	Pique
Fastness to perspiration (alkaline)									
Color change		L	4–5	4–5	4–5	4–5	4–5	4–5	4–5
		M	4–5	4–5	4–5	4–5	4–5	4–5	4–5
		D	4	4–5	4–5	4–5	4–5	4–5	4–5
Color staining	Acetate	L	4–5	4–5	4–5	4–5	4–5	4–5	4–5
		M	4–5	4–5	4–5	4–5	4–5	4–5	4–5
		D	4–5	4–5	4–5	4–5	4–5	4–5	4–5

TABLE V. Continued

Grey scale		Shade	S. Jersey	F. Terry	1×1 Rib	2×1 Rib	Interlock	S. Lacoste	Pique
Fastness to perspiration (alkaline)									
Color staining	Cotton	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	3-4	3-4	3-4	3-4	3-4	3-4	3-4
		D	3	2-3	4-5	3	2-3	2-3	3
	Nylon	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4-5	4-5	4-5	4-5	4-5	4-5	4-5
	Polyester	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4-5	4-5	4-5	4-5	4-5	4-5	4-5
	Acrylic	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4-5	4-5	4-5	4-5	4-5	4-5	4-5
	Wool	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Fastness to perspiration (acidic)									
Color change		L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4	4-5	4-5	4-5	4-5	4-5	4-5
Color staining	Acetate	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4-5	4-5	4-5	4-5	4-5	4-5	4-5
	Cotton	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	3-4	3-4	3-4	3-4	3-4	3-4	3-4
		D	3	2-3	4-5	3	2-3	2-3	3
	Nylon	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4-5	4-5	4-5	4-5	4-5	4-5	4-5
	Polyester	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4-5	4-5	4-5	4-5	4-5	4-5	4-5
	Acrylic	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		M	4-5	4-5	4-5	4-5	4-5	4-5	4-5
		D	4-5	4-5	4-5	4-5	4-5	4-5	4-5
Wool	L	4-5	4-5	4-5	4-5	4-5	4-5	4-5	
	M	4-5	4-5	4-5	4-5	4-5	4-5	4-5	
	D	4-5	4-5	4-5	4-5	4-5	4-5	4-5	

Analysis of mechanical behavior

Fig. 3 gives an overview of bursting strength measurement in pressure (kPa) for S. Jersey, F. Terry, 1×1 Rib, 2×1 Rib, Interlock, S. Lacoste and Pique fabric. Fabric structural difference is very significant in bursting strength measurement. For the same GSM S. Jersey fabric has the highest bursting strength rating fol-

lowed by Interlock and Pique. 2×1 Rib and F. Terry are found to have the lowest rating. For the same *GSM*, S. Jersey fabric has the highest bursting strength measurement of 227.7 kPa. The pressures needed to burst the Interlock and Pique fabrics are 214.4 and 213.3 kPa, respectively. The bursting strength of 1×1 Rib and S. Lacoste are found to be 205.1 and 195.5 kPa, respectively. The F. Terry fabric and 2×1 Rib fabrics show the lowest pressures which are 191.6 and 196.3 kPa, respectively.

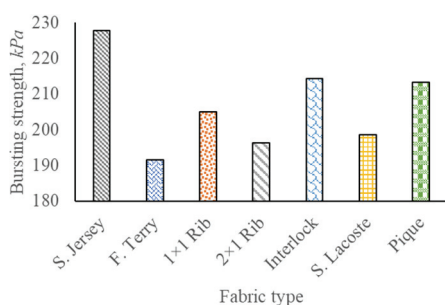


Fig. 3. Impact of internal notation on bursting strength.

Analysis of dimensional stability

Shrinkage value also differs from one fabric internal notation to another. From Fig. 4 for the S. Jersey fabric the lengthwise shrinkage is found to be -5% and the widthwise shrinkage is found to be -4% , which is acceptable. In shrinkage testing, values below 5% are generally acceptable. For the F. Terry fabric, the shrinkage in the length and width directions is found to be -6% and -5% , respectively. The shrinkage report of S. Lacoste and Pique fabrics is also found around the same. S. Lacoste and Pique show lengthwise shrinkage of -4% and -5% , respectively, and widthwise shrinkage of -5% and -6% , respectively. For a balanced internal notation like 1×1 Rib the shrinkage percentage of -4% and -6% (in lengthwise and widthwise direction). But the 2×1 Rib fabric shows the worst result among all the fabric internal notations. It is not possible to control the shrinkage percentage of 2×1 Rib fabric. The shrinkage report for the tested 2×1 Rib fabric is found to be -7% and -8% (lengthwise and widthwise). The report for Interlock fabric is also not so good. It has length shrinkage of -7% and width shrinkage of -6% .

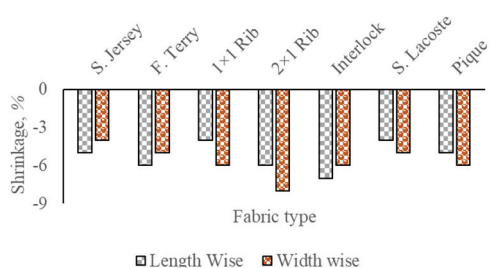


Fig. 4. Impact of internal notation on shrinkage in length and width direction.

From Fig. 5, the spirality of the S. Jersey fabric of 220 *GSM* is found to be very good which is 1%. For S. Jersey derivatives like F. Terry, S. Lacoste and Pique spirality angles are found to be different. The loop length or stitch length of F. Terry fabric is larger than that of S. Lacoste and Pique fabric. Due to the larger stitch length, the spirality of F. Terry fabric is found to be higher than that of S. Lacoste and Pique fabric. Spirality of F. Terry fabric is found to be 1.54 %. The spirality of S. Lacoste and Pique fabrics is found at 1.5 and 1.78 % respectively. Rib and Interlock fabrics are balanced fabric internal notations. So, there are fewer spiral angles. 1×1 Rib and 2×1 Rib fabrics have the same spirality angle which is 1 %. Interlock fabric has a spirality of 1.3 %.

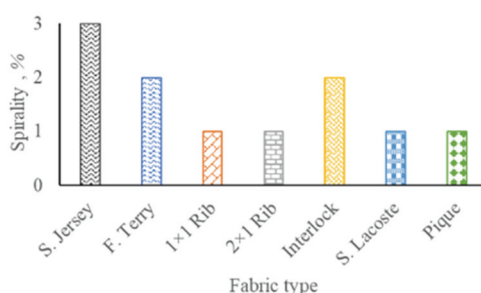


Fig. 5. Impact of internal notation on spirality.

CONCLUSION

This study presents a quantitative analysis of quality parameters across various fabric structures, including Plain Jersey, Single Jersey derivatives, Rib and Interlock. The findings indicate that the quality performance of colored cellulose-based biomaterials is significantly influenced by internal fabric structure, dye type and shade depth. Despite having the same *GSM*, variations in dye absorbency were observed due to differences in fabric construction. Visual and spectrophotometric evaluations revealed that Interlock fabric exhibited the highest brightness, followed by 1×1 Rib, while S. Lacoste appeared the duller. SEM analysis confirmed that S. Lacoste fabric has a grooved surface with prominent microfibrils and a rough texture, contributing to reduced dye absorption. Color fastness to washing (color change) was superior in light shades across all fabric types. However, staining on cotton varied with fabric structure. Dry rubbing fastness was consistent across structures but varied with shade depth, whereas wet rubbing fastness was notably affected by fabric construction. No significant differences were observed in color fastness to light and perspiration. Bursting strength differed significantly across fabric types, attributed to variations in stitch length and loop formation. S. Jersey and Interlock fabrics demonstrated higher bursting strength. Shrinkage and spirality also varied with fabric structure; S. Jersey exhibited the least shrinkage, while S. Lacoste and Pique showed moderate results. Spirality was most favorable in 1×1 and 2×1 Rib fabrics, whereas S. Jersey showed the poorest performance in this regard.

While this study offers valuable insights, certain limitations must be acknowledged. The research did not quantify the actual dye uptake through direct measurement of dye exhaustion or fixation rates. Additionally, statistical analysis, such as standard deviations or confidence intervals, was not incorporated to assess variability across samples. Future studies are encouraged to include detailed dye exhaustion profiles, fixation efficiency and statistical modeling to deepen understanding of dye–fabric interactions. Expanding the study to include alternative fiber types and environmentally friendly dyeing methods would also support broader applicability and sustainability assessment.

ИЗВОД

УТИЦАЈ ИНТЕРНЕ НОТАЦИЈЕ И ХЕТЕРО-БИФУНКЦИОНАЛНИХ РЕАКТИВНИХ БОЈА
НА ПЕРФОРМАНСЕ БОЈА, МЕХАНИЧКУ И ДИМЕНЗИОНАЛНУ СТАБИЛНОСТ
БИОМАТЕРИЈАЛА НА БАЗИ ЦЕЛУЛОЗЕ

RUMA SARKER¹, GAUTAM CHANDRA SARKER¹, ARNOB DHAR PRANTA^{3,4} и JOYKRISNA SAHA⁴

¹Golden Touch Imports Inc, Dhaka-1230, Bangladesh, ²P.N. Composite Ltd, Dhaka-1212, Bangladesh, ³ZR Research Institute for Advanced Materials, Sherpur-2100, Bangladesh и ⁴Department of Textile Engineering, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh

Фактори унутрашње нотације утичу на квалитет биоматеријала на бази целулозе обојених хетеро бифункционалним реактивним бојама. Различите унутрашње ознаке материјала, као што су Single Jersey, Rib и Interlock, утичу на различите параметре обојености као што су прихватање боје, постојаност боје и чврстоћа. Овај истраживачки рад има за циљ да анализира утицај различитих унутрашњих нотација на перформансе боја са хетеро-бифункционалним реактивним бојама. Такође намерава да идентификује различите нијансе и факторе унутрашње нотације за постизање конзистентног квалитета боја и трајности у различитим врстама биоматеријала на бази целулозе. Анализирано је седам биоматеријала на бази целулозе са различитим унутрашњим нотацијама (Single Jersey, 1×1 Rib, 2×1 Rib, Interlock, French Terry, Single Lacoste, Pique), обојених различитим нивоима уноса боје, као што су светло, средње и тамно. Истрага мери јачину боје, параметре боје (L^* , a^* , b^* , c^* , h^*), површинску морфологију, постојаност боје, механичку чврстоћу, скупљање и спиралност. Стандардизоване методе тестирања и инструменти су коришћени за процену квалитета. Примећено је да унутрашња нотација материјала значајно утиче на упијање боје где је Interlock најсветлији (K/S вредност 11,5). SEM анализа је показала површинске разлике у морфологији, што утиче на апсорпцију боје. Отпорност боја варира у зависности од унутрашње нотације тканине, док се снага пуцања разликује у зависности од врсте тканина.

(Примљено 26. маја, ревидирано 3. јула, прихваћено 14. јула 2025)

REFERENCES

1. J. Wen, B. Xu, Y. Gao, M. Li, H. Fu, *Energy Stor. Mater.* **37** (2021) 94 (<https://doi.org/10.1016/J.ENSME.2021.02.002>)
2. N. Sarwar, U. Bin Humayoun, A. A. Khan, M. Kumar, A. Nawaz, J. H. Yoo, D. H. Yoon, *J. Clean Prod.* **261** (2020) 121189 (<https://doi.org/10.1016/J.JCLEPRO.2020.121189>)
3. C. Jiang, K. Wang, Y. Liu, C. Zhang, B. Wang, *Acta Biomater.* **128** (2021) 60 (<https://doi.org/10.1016/J.ACTBIO.2021.04.047>)

4. R. Mishra, *Assessment of comfort and quality of knitted apparels* in *Advance Knitting Technology*, S. Maity, S. Rana, P. Pandit, K. Singha, Eds., Woodhead Publishing, Sawston, 2022, pp. 567–621 (<https://doi.org/10.1016/B978-0-323-85534-1.00013-1>)
5. M. S. Hoque, M. J. Hossain, M. M. Rahman, M. M. Rashid, *Heliyon* **8** (2022) e09605 (<https://doi.org/10.1016/j.heliyon.2022.e09605>)
6. S. Si, C. Sun, J. Qiu, J. Liu, J. Yang, *Appl. Mater. Today* **27** (2022) 101508 (<https://doi.org/10.1016/J.APMT.2022.101508>)
7. I. S. Čubrić, V. M. P. Matković, Ž. Pavlović, A. P. Čuden, *Materials* **15** (2022) 3306 (<https://doi.org/10.3390/ma15093306>)
8. M. A. Chowdhury, P. Pandit, *Adv. Knitting Technol.* (2022) 503 (<https://doi.org/10.1016/B978-0-323-85534-1.00002-7>)
9. S. Ahmed, M. Mohsin Ul Hoque, in *Advance Knitting Technology*, S. Maity, S. Rana, P. Pandit, K. Singha, Eds., Woodhead Publishing, Sawston, 2022, pp. 219–253 (<https://doi.org/10.1016/B978-0-323-85534-1.00011-8>)
10. L. Ammayappan, G. K. Prasad, T. Senthilkumar, G. T. V. Prabhu, S. Basak, N. K. Jha, in *The Wool Handbook*, S. Jose, S. Thomas, G. Basu, Eds., Woodhead Publishing, Sawston, 2024, pp. 1–23 (<https://doi.org/10.1016/B978-0-323-99598-6.00010-4>)
11. A. Gürses, K. Güneş, E. Şahin, in *Green Chemistry for Sustainable Textiles: Modern Design and Approaches*, N. Ibrahim, C. M. Hussain, Eds., Woodhead Publishing, Sawston, 2021, pp. 77–91 (<https://doi.org/10.1016/B978-0-323-85204-3.00008-7>)
12. H. Jamshaid, A. Khan, N. Ahmad, *J. Nat. Fibers* **20** (2023) (<https://doi.org/10.1080/15440478.2023.2253373>)
13. Y. Yang, M. Zhang, Z. Ju, P. Y. Tam, T. Hua, M. W. Younas, H. Kamrul, H. Hu, *Tex. Res. J.* **91** (2021) 1641 (<https://doi.org/10.1177/0040517520984101>)
14. A. D. Pranta, M. T. Rahaman, *Nano-Structures Nano-Objects* **39** (2024) 101243 (<https://doi.org/10.1016/j.nanoso.2024.101243>)
15. M. T. Rahaman, A. D. Pranta, M. R. Repon, M. S. Ahmed, T. Islam, *J. Open Innov. Technol. Market Complex.* **10** (2024) 100280 (<https://doi.org/10.1016/j.joitmc.2024.100280>)
16. Zalsabila, N. Yusuf, A. Arung, Syahidah, *IOP Conf. Ser. Earth Environ. Sci.* **575** (2020) 012126 (<https://doi.org/10.1088/1755-1315/575/1/012126>)
17. M. Khatri, F. Ahmed, A. W. Jatoi, R. B. Mahar, Z. Khatri, I. S. Kim, *Ultrason. Sonochem.* **31** (2016) 350 (<https://doi.org/10.1016/j.ultsonch.2016.01.020>)
18. X. Fang, Z. Zhang, Q. Wang, H. Xu, L. Tian, H. Niu, H. Zhou, *Prog. Org. Coat.* **196** (2024) 108703 (<https://doi.org/10.1016/j.porgcoat.2024.108703>)
19. R. Mongkholrattanasit, C. Saiwan, N. Rungruangkitkrai, N. Punrattanasin, K. Sriharuksa, C. Klaichoi, M. Nakpathom, *J. Text. Inst.* **106** (2015) 1106 (<https://doi.org/10.1080/00405000.2014.976957>)
20. S. Q. Jiang, E. Newton, C. W. M. Yuen, C. W. Kan, *Text. Res. J.* **76** (2006) 57 (<https://doi.org/10.1177/0040517506053827>)
21. A. Wheldrake, E. Guillemois, H. Arouni, V. Chetty, S. J. Russell, *Malar. J.* **20** (2021) 47 (<https://doi.org/10.1186/s12936-020-03571-4>)
22. S. Dhouib, F. Khedher, F. Sakli, *J. Text. Inst.* **107** (2016) 364 (<https://doi.org/10.1080/00405000.2015.1034928>)
23. M. Faulde, G. Albiez, O. Nehring, *Parasitol. Res.* **110** (2012) 2341 (<https://doi.org/10.1007/s00436-011-2769-6>)
24. A. A. Ansari, K. M. Aldajani, A. N. AlHaza, H. A. Albrithen, *Coord. Chem. Rev.* **462** (2022) 214523 (<https://doi.org/10.1016/J.CCR.2022.214523>)

25. M. S. Hoque, M. I. Shahid, A. Al Parvez, M. J. Hossain, S. M. Sheen, *AATCC J. Res.* **9** (2022) 286 (<https://doi.org/10.1177/24723444221119845>)
26. Q. Zhou, W. Wang, Y. Zhang, C. J. Hurren, Q. Li, *Text. Res. J.* **90** (2020) 2175 (<https://doi.org/10.1177/0040517520910501>)
27. C. V. T. Rigueto, J. S. Piccin, A. Dettmer, M. Rosseto, G. L. Dotto, A. P. de Oliveira Schmitz, D. Perondi, T. S. M. de Freitas, R. A. Loss, C. A. Q. Geraldi, *Ecol. Eng.* **150** (2020) 105817 (<https://doi.org/10.1016/J.ECOLENG.2020.105817>)
28. L. Jia, X. Chen, J. Xu, L. Zhang, S. Guo, N. Bi, T. Zhu, *J. Hazard. Mater.* **402** (2021) 123776 (<https://doi.org/10.1016/J.JHAZMAT.2020.123776>).



J. Serb. Chem. Soc. 90 (12) 1481–1494 (2025)
JSCS–5467

Development of a glass–ceramic composite reinforced with β -wollastonite synthesized *via* a hydrothermal method

MARJAN RANĐELOVIĆ^{1*}, CHARLES C. SORRELL², SHARON KOPPKA³,
ALEKSANDRA ZARUBICA¹, MILAN Z. MOMČILOVIĆ¹ and DIRK ENKE³

¹University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry,
Višegradska 33, 18000 Niš, Serbia, ²School of Materials Science and Engineering,
University of New South Wales, Sydney NSW 2052, Australia and ³Institute of Chemical
Technology, University of Leipzig, Linne str. 3, 04103 Leipzig, Germany

(Received 26 April, revised 20 May, accepted 25 August 2025)

Abstract: β -wollastonite (β -CaSiO₃) microfibres were successfully synthesized using a one-step, template-free hydrothermal reaction involving calcium nitrate and sodium metasilicate in an alkaline medium. The synthesis of microfibres was completed at 220 °C within only 240 min under an autogenous pressure of 19 bar (1.9 MPa). This method avoids the formation of xonotlite as an intermediate phase, eliminating the need for subsequent calcination to achieve wollastonite. X-ray diffraction (XRD) confirmed the β -wollastonite phase, while post-calcination analyses indicated enhanced crystallinity and structural characteristics. Scanning electron microscopy (SEM) revealed a needle-like morphology and N₂ adsorption-desorption analysis demonstrated a developed surface area of 26 m² g⁻¹ with notable mesoporosity. These advantageous features facilitated the integration of β -wollastonite into the synthesis of a glass–ceramic composite, which was characterized for its morphological, structural, textural, and *in vitro* bioactivity properties. The composite was prepared by mixing β -wollastonite and bioactive glass powders in a 1:4 mass ratio, followed by compaction through uniaxial pressing and sintering at 1000 °C for various time intervals. For comparison, compacted pure bioactive glass samples were also sintered under identical conditions. Structural, morphological, textural and *in vitro* bioactivity characterizations demonstrated that the incorporation of β -wollastonite led to a more uniform and narrower pore size distribution and promoted neck formation between particles, indicating its potential for bone regeneration applications.

Keywords: sintering; bioceramics; mineral; mesoporosity.

*Corresponding author. E-mail: hemija@gmail.com
<https://doi.org/10.2298/JSC250426072R>

INTRODUCTION

Wollastonite is a naturally occurring mineral with the chemical formula CaSiO_3 and two polymorphic forms; the low-temperature phase β -wollastonite and the high-temperature phase α -wollastonite (pseudowollastonite).¹ It is a member of the inosilicates with a single-chain structure and physical properties that are stable to about 1125 °C (transition temperature of β -wollastonite to α -wollastonite),² making wollastonite important in refractory ceramics production. Due to its needle-like morphology, thermal stability, low thermal expansion and low thermal conductivity, there is increasing demand for wollastonite as a substitute for short-fibre asbestos. Another boost to the industry came from its low dielectric constant and low dielectric loss at high frequency, making this material suitable for high frequency insulators.³ In the production of plastics such as nylons, phenolic moulding compounds, polyesters, polyurethanes and polyureas, wollastonite is used as filler providing reinforcement, hardening, crack resistance, high brightness and other benefits.⁴ In ceramics, wollastonite reduces warping and cracking and acts as a sintering aid which accelerates densification of ceramics and increases strength. In recent years, studies have reported improvements in strengths, shrinkage resistance and reduction in permeability for concrete mixes containing wollastonite as a cement replacement.⁵ Moreover, several studies have examined wollastonite as an adsorbent for the removal of heavy metal ions, such as As^{+5} and Cr^{+6} , from water.⁶

Another high merit for wollastonite is its good bioactivity, biocompatibility, degradability and favourable mechanical characteristics. Consequently, it is increasingly being used as a biomaterial suitable for orthopaedic applications including medical material for bone substitution and dental roots. It should be emphasized that β -wollastonite phase has better bioactivity performance than α -wollastonite. Hence, β -wollastonite nanowires may be used as strength-enhancing additives for the preparation of the ceramics or bioactive nano-composites with improved mechanical properties.³

Biomaterials are generally defined as natural or man-made materials designed for use in the manufacturing of medical devices to replace a part or function of the biological system in a safe, reliable, economic and physiologically acceptable way. Although biomaterials are primarily intended for medical applications, there are also studies in which the term “biomaterials” refers to those materials produced by living organisms.⁷

According to the type of material used, biomaterials can be classified into one of four broad categories: metals, polymers, ceramics and composites. Among them, ceramic materials are very biocompatible and show exceptional wear resistance, but they are usually stiff and brittle. In order to improve the mechanical and textural characteristics of ceramics, new structures have to be created. Those structures may include, among others, ceramic composites with various fibres used as reinforcement. Moreover, calcium silicate based glass ceramics possess excellent

biocompatibility and bioactivity, and have been considered as potential candidates for bone replacement and regeneration.^{8,9} Controlled crystallization of calcium silicate glass ceramics produces certain sodium calcium silicate phases, such as $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ which develop improved mechanical properties and enhanced stability of biomaterial under physiological conditions.¹⁰ Also, there is a wide group of biomaterials developed for bone tissue engineering based on hydroxyapatite scaffolds.^{11–13}

Needle-like bioceramics can be prepared by various techniques such as coprecipitation, sol–gel, sonochemical and hydrothermal methods. There are several studies reporting that wollastonite fibres can be obtained by calcination of tobermorite or xonotlite fibres as precursors above 800 °C. Hydrothermal process and hydrothermal microemulsion technique are also utilized to synthesize precursors for wollastonite fibres using small droplets of microemulsion as nanoreactors for the synthesis of nanoparticles with a low polydispersity.^{3,14} However, microemulsion and soft template methods are limited by the large amount of template material required and the use of organic solvents and surfactants, which are dangerous pollutants. Autoclaving has been explored by Ismail *et al.* as a safe and environment-friendly technique for wollastonite synthesis.¹⁵ However, all these techniques produce precursors that demand an additional sintering step to obtain β -wollastonite.¹⁵

Herein, we report the fabrication of needle-like wollastonite through a facile one-step hydrothermal method without any additional templates. The obtained product was employed for the bioactive composite production through a powder sintering route using bioglass as the matrix and needle-like wollastonite as the reinforcement. To study in detail the structure, morphology and sintering behaviour of the wollastonite and glass-ceramic composites, X-ray diffraction (XRD), adsorption–desorption isotherms of N_2 at -196 °C, scanning electron microscopy (SEM) equipped with EDX, FTIR and Raman spectroscopy methods were applied. *In vitro* bioactivity assessment of the composite and its potential as an implantable material was examined by immersion in simulated body fluid (SBF) at 37 °C for 21 days.

EXPERIMENTAL

Wollastonite synthesis

The procedure applied for the synthesis of wollastonite is as follows. The amount of 3.54 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ was dissolved in 35 ml of deionised water to obtain a clear solution. In a separate beaker, 3.18 g of $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ was dissolved in another 35 ml of deionised water and then dropped into $\text{Ca}(\text{NO}_3)_2$ stirred solution to obtain a milky-white suspension. The suspension was stirred for 30 min to ensure that the particles were well mixed and finely dispersed into the liquid. Subsequently, 10 ml of 5 M NaOH was added and stirring was continued for 5 min. The suspension was then transferred into a stainless steel autoclave and heated at 220 °C for 240 min at an autogenous pressure of 19 bar*, followed by cooling to room temperature naturally. After the hydrothermal reaction, the obtained suspension was filtered and washed

* 1 bar = 100 kPa

with deionised water three times. The resultant powder was dried at 105 °C for 3 h. This synthetic route is based on the following chemical reactions:



Bioglass synthesis

During the synthesis, a specific melting regime of SiO_2 , Na_2CO_3 , P_2O_5 and CaCO_3 was applied in order to obtain a composition given by the mass fraction of oxides: 45 wt. % SiO_2 , 24.5 wt. % CaO , 24.5 wt. % Na_2O and 6.0 wt. % P_2O_5 . The melting regime in a platinum crucible included heating at 1000 °C for 15 min, then heating at 1100 °C for 15 min with maintaining that temperature for the next hour. After that time, the procedure continued by heating up to 1200 °C for 15 min and keeping that temperature for another hour. The melted mass was poured onto a metal plate heated to 380 °C and allowed to cool. After cooling, the glass was crushed in an agate mortar and pulverized by grinding in a ball mill. The powder was passed through a sieve of 180 mesh.

Composite fabrication

The glass–ceramics composites were fabricated by sintering pellets (Ø 15 mm), which were previously prepared from the wollastonite and glass powder in a mass ratio of 1:4 (0.1 g + 0.4 g) by cold pressing in a laboratory hydraulic press. Components were well mixed in an ethanol suspension through sonication. Then polyvinyl alcohol (PVA) solution was added to achieve agglomeration of particles. The obtained precipitate was dried, powdered in an agate mortar and pressed in a laboratory hydraulic press at 10 t. The temperature of sintering was selected to be 1000 °C, according to our preliminary findings. The samples were heated at the chosen temperature for 15, 30, 55 and 85 min, initially cooled down to 300 °C, and then to room temperature. After cooling, the sintered samples were subjected to subsequent analysis. Pure bioglass powder was sintered under the same conditions as the composite in order to compare their physicochemical properties and to more clearly demonstrate the influence of the wollastonite phase on the characteristics of the composite.

Characterization

The microstructures of the powder samples and composites substrates were analysed by scanning electron microscopy (SEM) using a JEOL JXA-8500F and a Leo Gemini 1530 by Zeiss with an Everhart-Thornley detector for collecting secondary electrons. The accelerating voltage was 10 kV.

Structural characteristics of crystal phases were examined by X-ray diffraction, using a STADI P instrument from STOE & Cie GmbH with a Mythen1K detector. $\text{CuK}\alpha$ radiation was used at 40 kV and 40 mA. The XRD technique was used to identify the phase composition of the bioglass, wollastonite, as well as sintered glass-ceramic composites. The diffraction data were collected in the 2θ Bragg angle range from 20 to 100°. The crystallite size of the wollastonite was calculated by the Scherrer equation with data obtained from the XRD analyses. This equation is given as:

$$L = \frac{K\lambda}{\beta \cos \theta} \quad (4)$$

where L is the crystallite size, K is the Scherrer constant, λ is the X-ray wavelength, β (expressed in radians) is the width of the peak (full width at half maximum – FWHM) after correction for

instrumental peak broadening and θ is the Bragg angle. The K value used in this calculation is 0.94. The quantification of individual crystalline phases in the samples was carried out based on the reference intensity ratio (RIR) method.

For textural analyses of wollastonite, Horiba SA-9601 MP BET surface area analyser and Thermo Scientific from Surfer were used. Experimental parameters for the N_2 adsorption/desorption analysis are presented in Supplementary material to this paper.

Additional structural characterization of the wollastonite samples was performed by Raman spectroscopy using a Raman Spectrometer Renishaw inVia 2 Raman microscope with applying a laser source with a wavelength of 532 nm. All FTIR absorption spectra were recorded in the wavenumber range from 400 to 4000 cm^{-1} (transmission mode) using a Bomem spectrometer. The resolution used was 4 cm^{-1} and the number of scans was 32.

In vitro bioactivity assessment of the prepared glass-ceramics

Simulated body fluid (SBF) has nearly the same ionic composition and pH value as blood plasma. SBF is used for *in vitro* testing and evaluation of the bioactivity of artificial materials by forming an apatite layer in the fluid. Additionally, it is used for the preparation of bioactive composites that will form bone-like apatite on various substrates. For SBF preparation, 750 ml of deionized water was poured into 1 L beaker and placed onto the heated magnetic stirrer with the temperature being maintained at 37 °C. Defined quantities of the following chemicals of the highest purity have been added slowly in small increments: 7.996 g NaCl, 0.35 g $NaHCO_3$, 0.224 g KCl, 0.228 g $K_2HPO_4 \cdot 3H_2O$, 0.305 g $MgCl_2 \cdot 6H_2O$, 40 ml 1 M HCl, 0.278 g $CaCl_2$, 0.071 g Na_2SO_4 , 6.057 g Tris(hydroxymethyl)aminomethane (Tris) and a suitable volume of 1 M HCl for pH adjustments. Prepared solution was kept in a polyethylene bottle at 5–10 °C.

The glass-ceramic samples in the form of pellets were placed in polyethylene flasks containing 50 ml of simulated body fluid (SBF) in order to estimate their bioactivity. The covered flasks were kept at 37 °C for 21 days. Subsequently, the samples were removed from the SBF solution, washed with distilled water and then air-dried at room temperature. The sample was examined by an XRD analysis of the powdered glass-ceramic samples

RESULTS AND DISCUSSION

SEM

Wollastonite morphology analysed by SEM along with element mapping and distribution of the main elements are given in Fig. 1. Needle-like wollastonite particles with length up to 25 μm and very sharp ends can be noticed in Fig. 1a and b. In addition, it can be observed that larger particles are composed of aggregation and stacking of smaller ones which form broom-shaped structures at the ends. Fig. 1c–e show element mapping and distribution of the main elements in the wollastonite. EDX analysis of needles, shown in Fig. 1k, confirmed the ternary system of the wollastonite, containing the elements silicon, calcium and oxygen. However, sodium is also present in the structure probably due to its adsorption on the surface from the reaction mixture during autoclaving. Peaks for C and Pt originate from sputtered platinum and carbon which served as coating material used to coat non-conductive wollastonite samples for standard SEM observations.

From the SEM image of the calcined powder (Fig. 1f and g), it can be seen that the original shapes and sizes of wollastonite were predominantly preserved,

but a sintering process occurred giving morphologies which could be described as needles of different sizes between 2 and 20 μm in length. The edges and ends of needles are rounded and this is probably the result of surface melting of particles due to the presence of sodium on the surface which acts as a flux. Such behaviour of wollastonite at the calcination temperature could be exploited well to provide strong adhesion between wollastonite reinforcement and the glass matrix during preparation of the composite. Fig. 1h–j show element mapping of calcined wollastonite. The additional micrographs of the wollastonite at different magnifications are provided in Supplementary material (Figs. S-1 and S-2).

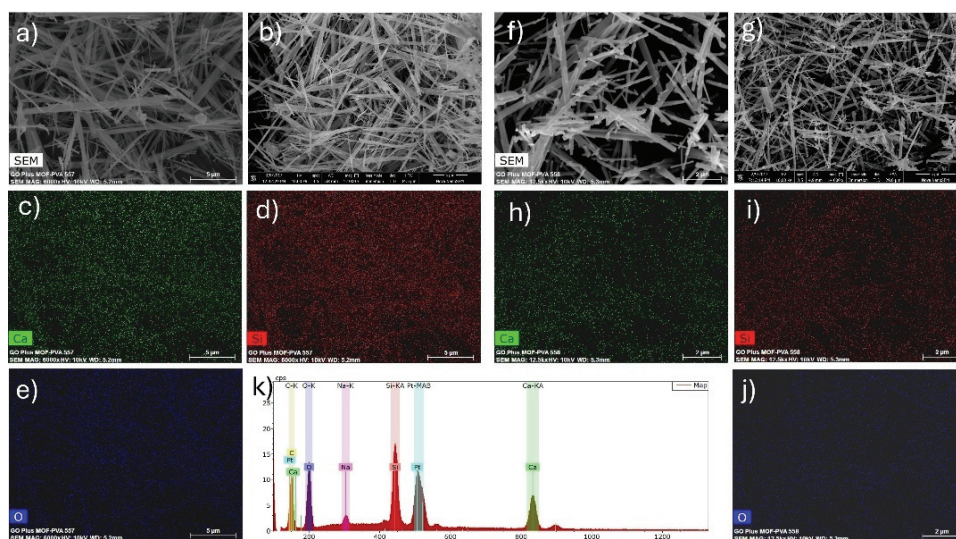


Fig. 1. SEM images of needle-shaped wollastonite along with element mapping and distribution of the main elements; a) and b) as-synthesized wollastonite and distribution of: c) Ca, d) Si, e) O, f) and g) calcined wollastonite and distribution of: h) Ca, i) Si, j) Si, k) EDX analysis of as-synthesized wollastonite.

XRD

X-ray diffraction patterns of the as-synthesized wollastonite and wollastonite after its calcination at 900 $^{\circ}\text{C}$ for 2 h are shown in Fig. 2. The observed diffraction peaks of the XRD patterns could be indexed as monoclinic wollastonite ($\beta\text{-CaSiO}_3$) with space group of $P2_1/a$ (PDF 00-066-0271). The geometry of the unit cell is defined by the following parameters: approximately $a = 15.43 \text{ \AA}$, $b = 7.33 \text{ \AA}$, $c = 7.07 \text{ \AA}$ and angles $\alpha = 90.00^{\circ}$, $\beta = 95.31^{\circ}$, $\gamma = 90.00^{\circ}$.

The position, intensities and shape of the strong diffraction peaks for the calcined sample of wollastonite coincide well to the standard values and imply that it was better crystallized than that of as-synthesized sample. Table S-I of the Supplementary material shows the positions (2θ) of the peaks, interplanar d -spacings, full

width at half maximum (*FWHM*), background intensity and areas of the peaks collected from the XRD pattern (Fig. 2). The effect of autoclaving and subsequent calcination on the crystallite size and micro strain of the β -wollastonite was calculated with data from the XRD analysis and also shown in Supplementary material (Table S-I). The results indicate an increase in average crystallite size after calcination from 46.3 to 48.6 nm, while micro strain was reduced from 0.340 to 0.234 indicating improved crystallinity and reduced internal defects. The peaks observed at 23.2, 25.3, 26.8, 28.9, 30.0, 36.2, 39.04, 41.3, 50.9 and 53.05° correspond to the (400), (002), ($\bar{2}$ 02), (202), ($\bar{3}$ 20), (412), ($\bar{4}$ 22), (040), (004) and (523) planes.

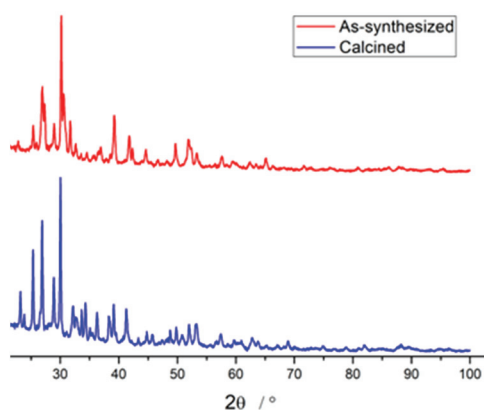


Fig. 2. XRD patterns of as-synthesized wollastonite and calcined sample at 900 °C.

BET analysis

The N₂ adsorption–desorption isotherm and the Barrett–Joyner–Halenda (BJH) pore size distribution for the as-synthesized sample of wollastonite are plotted in Fig. 3a and b, respectively. The specific surface areas as obtained by the BET method, BJH adsorption cumulative surface area of pores, BJH total pore volume and BJH average pore diameter are listed in Table S-II of the Supplementary material.

The surface area of the wollastonite ceramics was measured to be 26 m² g^{−1}. The single-point adsorption total pore volume for pores with a diameter less than 1.038 nm at $p/p^\circ = 0.01$ was found to be 0.006 cm³ g^{−1}. The average pore diameters from BJH adsorption and desorption measurements were 6.1 and 6.3 nm, respectively.

As can be seen in Fig. 3a, as-synthesized wollastonite sample showed an unusual type III isotherm pattern which is convex to the p/p° axis over its entire range. The isotherm does not exhibit a point B, but a very narrow hysteresis loop can be observed. This indicates that the adsorbent–adsorbate interaction is weak as compared with the adsorbate–adsorbate interactions.

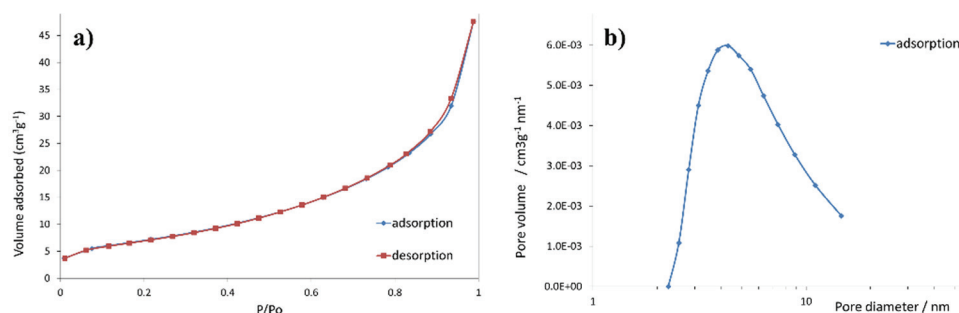


Fig. 3. a) The N₂ adsorption-desorption isotherm and b) BJH adsorption pore distribution of as-synthesized wollastonite sample.

Raman spectroscopy

The Raman spectra of as-synthesized and calcined wollastonite samples over the 100–1200 cm⁻¹ spectral range are shown in Fig. 4. This figure shows the position and relative intensity of the Raman bands that originate from an unoriented sample of wollastonite. The most intense Raman bands, listed in order of increasing wave-number at 330, 404, 580, 636, 860, 971 and 1042 cm⁻¹, align well with the β -wollastonite (2M) phase. The low-frequency bands occurring below 500 cm⁻¹ are attributed to Ca–O stretching and Si–O bending modes. The prominent bands around 636, 860, 971 and 1042 cm⁻¹ are associated with the Si–O–Si and O–Si–O vibrational stretching modes in the SiO₄ tetrahedron.^{16,17} The comparative overview of the positions of Raman bands for native and calcined wollastonite is provided in Table S-III of the Supplementary material. The observed redshift in Raman peaks after calcination at 900 °C is attributed to lattice relaxation, reduced internal strain, and increased crystallite size, as confirmed by XRD analysis.

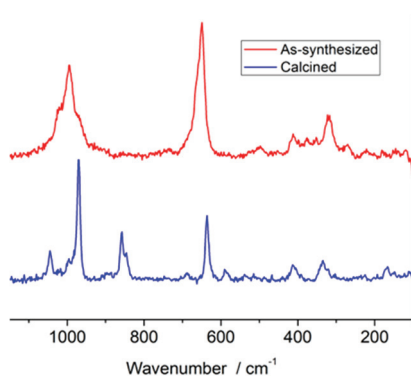


Fig. 4. Raman spectra of as-synthesized wollastonite and wollastonite after calcination at 900 °C.

FTIR spectroscopy

The FTIR spectra were used to analyse the structures of the wollastonite, as illustrated in Fig. 5. The most prominent bands are observed in the range from 850

to 1200 cm^{-1} and correspond to the vibrational modes of the asymmetric SiO_4 tetrahedron. Based on literature, the bands detected at 1087 and 1028 cm^{-1} correspond to stretching vibrations of bridging Si-O-Si , while those at 971 and 930 cm^{-1} are associated with non-bridging oxygen (Si-O) bonds.¹⁸

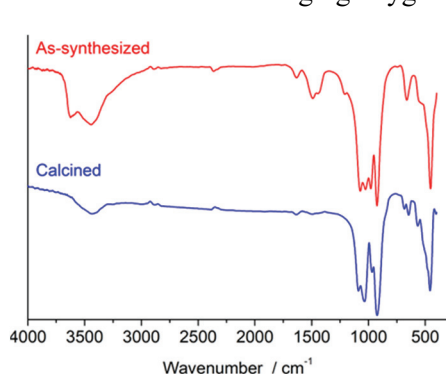


Fig. 5. FTIR spectra of as-synthesized wollastonite and wollastonite calcined at $900\text{ }^{\circ}\text{C}$.

The presence of Si-O-Ca non-bridging oxygen bonds was confirmed by the FTIR spectra of the fired powder, which exhibited vibrational modes at 903 cm^{-1} . These stretching vibrations of Si-O-Ca bonds indicate the formation of $\beta\text{-CaSiO}_3$ which is consistent with the obtained XRD patterns. Furthermore, the band peaking at 846 cm^{-1} is associated with Si-O-Ca vibrations, while the bands at 682 and 648 cm^{-1} reflect the symmetric stretching vibrations of Si-O-Si bonds. The bending vibrations of Si-O-Si bonds are detected around 567 and 452 cm^{-1} . It is important to note that many peaks in the spectrum overlap, making it challenging to identify specific bonds unambiguously.

The significant difference in the spectra was observed only in the 3625 , 1490 and 1449 cm^{-1} peaks. The last two bands were ascribed to the carbonate group. The bands at 3625 and 3438 cm^{-1} were evidence of O-H stretching vibration of the structural -OH groups and absorbed water, respectively. The band at 1637 cm^{-1} is associated with O-H bending vibration. Calcination of the sample caused decomposition of carbonates and removal of structural -OH groups.^{19,20}

Composite characterization

To highlight the influence of wollastonite on the sintering behaviour and physicochemical properties of the composites, a comparative analysis of sintered materials based on pure bioglass and wollastonite-bioglass composites is presented. Sintered samples of pure bioglass (BG) (Figs. S-3–S-6 of the Supplementary material), obtained at the same temperature but with different sintering times, exhibit a very broad pore size distribution, ranging from several nm up to $100\text{ }\mu\text{m}$, indicating a diverse range of pore sizes within their structure. This characteristic can significantly influence its adsorption properties and overall performance in biomedical applications. The specific surface area of the material is less than 1 m^2

g^{-1} , suggesting a relatively low surface area compared to other materials. Additionally, the pore volume (V_p) is within the range of $0.036\text{--}0.055\text{ cm}^3\text{ g}^{-1}$ and the highest measured porosity of the sintered bioglass is 10.7 %. (depending on sintering time), indicating a limited capacity for storing fluids. The predominant mineral in the composition of the samples is combeite, which comprises between 96 and 100 % of the material. This percentage increases with the duration of sintering. The SEM micrographs presented in Figs. S-3–S-6 indicate surface melting of the material, particle coalescence through the formation of so-called necks, and the development of globular and rounded particle morphologies.

Fig. 6a and Fig. S-7 (Supplementary material) show SEM micrograph of the composite treated at $1000\text{ }^\circ\text{C}$ for 15 min (COM 15). There are clear signs indicating the onset of the sintering process, characterized by the formation of interparticle contacts, commonly referred to as necks, which are established between the particles through weak cohesive forces. The chemical composition of the composite obtained using the EDX method indicates that the amount of sodium is about 12 %, which acts herein as a flux and facilitates the consolidation of the composite during the heat treatment. Crystallographic analysis indicates that the dominant mineral is combeite $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ (98 %), the presence of which increases the mechanical strength of the material while maintaining its biocompatibility. Sodium calcium silicate which is also known in the literature as a bioactive material was also detected in the composite, with a mass fraction of several percent. The specific surface area is less than $1\text{ m}^2\text{ g}^{-1}$, the porosity is 21 %, while the average pore diameter is 4256 nm. These results confirm that the sintering process is in the initial phase, so that there has not yet been significant densification of the material and elimination of porosity.

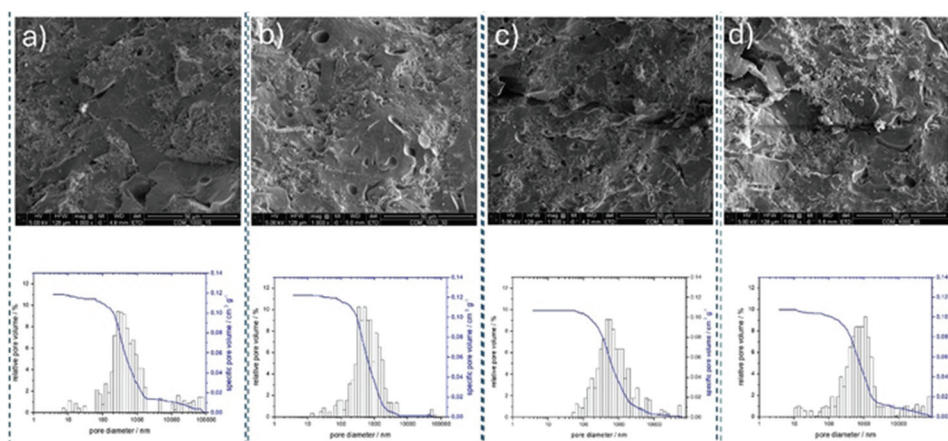


Fig. 6. SEM micrographs and pore size distributions of the composite treated at $1000\text{ }^\circ\text{C}$ for: a) 15 (COM 15), b) 30 (COM 30), c) 55 (COM 55) and d) 85 min (COM 85).

Fig. 6b and Fig. S-8 (Supplementary material) show micrographs of the composite surface after 30 min of sintering (COM 30). At this stage, the presence of large, consolidated particles that were formed during the process is observed, as well as the presence of smaller particles with an elongated morphology that indicate that sintering has not been completed yet. Despite this, the data obtained by nitrogen adsorption/desorption indicated that the porosity remained the same (21%), but a decrease in the mean pore diameter was noted, which changed to 1368 nm. The mass fraction of combeite has been shown to be at the level of the previous sample (98 %).

After calcination for 55 min (COM 55), no significant changes in the surface morphology of the composite were recorded, as can be seen in Fig. 6c and Fig. S-9 (Supplementary material). The porosity has been shown to be 19 % with the mean pore diameter of 1956 nm. The chemical changes that occur during thermal treatment led to the complete dominance of combeite minerals, whose mass fraction turned to 99.5 %. The last sample was sintered for 85 min (COM 85), and its surface morphology can be seen in Fig. 6d and Fig. S-10 (Supplementary material). In the figures, elongated particles of worm-like morphology, like spaghetti, can no longer be observed, as was the case with the previous samples. Based on morphology, it can be concluded that this is the final stage of sintering. The pore volume did not change significantly, but there was an increase in the mean pore diameter to a value of about 3928 nm. Therefore, during sintering and the formation of the liquid phase at a given temperature, the smaller pores were filled, or they joined each other into pores with a larger diameter. It should be kept in mind that sintering did not take place under high pressure. Generally, the composites typically exhibit a higher pore volume (V_p) of up to $0.122 \text{ cm}^3 \text{ g}^{-1}$ and a narrower pore size distribution compared to sintered bioglass. These properties enhance their performance and suitability in the field of biomedical engineering.

Interaction of bioactive composite COM 30 with SBF

To test the interaction of the bioactive composite with SBF, the COM 30 sample was used, as the sintering time of 30 min had been established as optimal for obtaining a mechanically stable material. After interaction with the solution, some changes were observed in the XRD diffractogram. This suggests that the crystal structure of the material remained largely unchanged, except for the appearance of small peaks associated with hydroxyapatite formation, which were just above the detection limits of the XRD method (Fig. S-11 of the Supplementary material). This result aligns with findings from other studies, as the phosphate content in SBF relative to the mass of the composite sample is minimal resulting in sparsely distributed apatite precipitates.

CONCLUSION

This study demonstrated a robust approach to the synthesis of β -wollastonite microfibres through a simple, template-free hydrothermal technique, followed by calcination at 900 °C to improve crystallinity and eliminate constitutional water. The resultant β -wollastonite exhibited a needle-like morphology with high mesoporosity and surface area, as confirmed by SEM, XRD and BET analyses. These characteristics were favourable for enhancing the structural and bioactive properties of the composite material.

The wollastonite microfibres were successfully employed as a constituent in a glass-ceramic composite, which was prepared by a powder sintering method using bioglass as the matrix. The addition of wollastonite contributed to the formation of sodium-calcium-silicate phases (mostly combeite), which improved the mechanical properties of the composite while maintaining its biocompatibility.

The calcination and sintering processes facilitated the consolidation and densification of the composite, supported neck formation between particles and led to the development of appropriate porosity suitable for the intended application. XRD analysis confirmed the dominant β -wollastonite phase with improved crystallite size and reduced microstrain post-calcination, contributing to the composite's structural stability and bioactivity. Overall, the *in vitro* bioactivity assessment in simulated body fluid highlighted the composite's potential for its use as a bioactive implant material, with surface changes suggesting the formation of bioactive phases, though further studies are needed to optimize hydroxyapatite formation.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13354>, or from the corresponding author on request.

Acknowledgement. This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreement number 451-03-137/2025-03/200124).

ИЗВОД

РАЗВОЈ СТАКЛО-КЕРАМИЧКОГ КОМПОЗИТА ОЈАЧАНОГ β -ВОЛАСТОНИТОМ
СИНТЕТИСАНИМ ХИДРОТЕРМАЛНОМ МЕТОДОМ

МАРЈАН РАНЂЕЛОВИЋ¹, CHARLES C. SORRELL², SHARON КОРРКА³, АЛЕКСАНДРА ЗАРУБИЦА¹,
МИЛАН З. МОМЧИЛОВИЋ¹ и DIRK ENKE³

¹Универзитет у Нишу, Природно-математички факултет, Департаман за хемију, Вишеградска 33,
18000 Ниш, ²School of Materials Science and Engineering, University of New South Wales, Sydney NSW
2052, Australia и ³Institute of Chemical Technology, University of Leipzig, Linne str. 3, 04103 Leipzig,
Germany

Микровлакна β -воластонита (β -CaSiO₃) су успешно синтетисана применом једно-степене хидротермалне реакције без употребе темплејта, коришћењем калцијум-нитрата и натријум-метасиликата у алкалној средини. Синтеза је изведена на температури од 220

°C за само 240 min под аутогеним притиском од 19 bar. Овим поступком избегава се формирање ксонотлита као интермедијарне фазе, чиме се елиминише потреба за наконнадном калцинацијом у циљу добијања властонита. Рендгенска дифракција (XRD) је потврдила присуство фазе β -властонита, док су анализе након калцинације указале на побољшану кристаличност и структурне карактеристике. Скенирајућа електронска микроскопија (SEM) је открила морфологију у облику иглица, а анализа адсорпције–десорпције азота је показала развијену специфичну површину од $26 \text{ m}^2 \text{ g}^{-1}$ и изражену мезопорозност. Ове повољне карактеристике омогућиле су интеграцију β -властонита у синтези стакло–керамичког композита, који је потом карактерисан у погледу морфолошких, структурних, текстурних и *in vitro* биоактивних својстава. Композит је припремљен мешањем прахова β -властонита и биоактивног стакла у масеном односу 1:4, након чега је уследило компактирање једноосним пресовањем и синтеровање на 1000°C у различитим временским интервалима. Ради поређења, компактне пастиле чистог биостакла такође су синтероване под истим условима. Структурна, морфолошка, текстуална и *in vitro* биоактивна карактеризација показала је да је додавање β -властонита довело је до уједначеније и уже расподеле величина пора и подстакло формирање веза (вратова) између честица, указујући на потенцијал композита за примену у регенерацији костију.

(Примљено 26. априла, ревидирано 20. маја, прихваћено 25. августа 2025)

REFERENCES

1. C. C. Lin, P. Shen, *Mater. Chem. Phys.* **182** (2016) 508 (<https://doi.org/10.1016/j.matchemphys.2016.07.065>)
2. E. Mazzucato, A. F. Gualtieri, *Phys. Chem. Miner.* **27** (2000) 565 (<https://doi.org/10.1007/s002690000095>)
3. K. Lin, J. Chang, G. Chen, M. Ruan, C. Ning, *J. Cryst. Growth* **300** (2007) 267 (<https://doi.org/10.1016/j.jcrysgro.2006.11.215>)
4. K. Lin, C. Lin, Y. Zeng, *RSC Adv.* **6** (2016) 13867 (<https://doi.org/10.1039/c5ra26916d>)
5. M. A. Abdelwahab, *A Reflexive Reading of Urban Space*, 1st ed., eBook ISBN 9781315565125, Taylor and Francis, London, 2018
6. N. Obradović, S. Filipović, S. Marković, M. Mitrić, J. Rusmirović, A. Marinković, V. Antić, V. Pavlović, *Ceram. Int.* **43** (2017) 7461 (<https://doi.org/10.1016/j.ceramint.2017.03.021>)
7. W. K. Lam, J. C. Y. Leong, Y. H. Li, Y. Hu, W. W. Lu, *Gait Posture* **22** (2005) 189 (<https://doi.org/10.1016/j.gaitpost.2004.09.011>)
8. V. S. Topalović, S. R. Grujić, V. D. Živanović, S. D. Matijašević, J. D. Nikolić, J. N. Stojanović, S. V. Smiljanić, *Ceram. Int.* **43** (2017) 12061 (<https://doi.org/10.1016/j.ceramint.2017.06.061>)
9. M. Riaz, R. Zia, A. Mirza, T. Hussain, F. Bashir, S. Anjum, *Mater. Sci. Eng., C* **75** (2017) 872 (<https://doi.org/10.1016/j.msec.2017.02.141>)
10. L. Adams, E. R. Essien, R. O. Shaibu, A. Oki, *New J. Glass Ceram.* **3** (2013) 11 (<http://dx.doi.org/10.4236/njgc.2013.31003>)
11. A. Logeshwaran, R. Elsen, S. Nayak, *J. Mech. Behav. Biomed. Mater.* **138** (2023) 105633 (<https://doi.org/10.1016/j.jmbbm.2022.105633>)
12. A. Iatsenko, O. Sych, A. Nikolenko, S. Stelmakh, *Results Surf. Interfaces.* **16** (2024) 100265 (<https://doi.org/10.1016/j.rsufi.2024.100265>)
13. I. W. Suh, S. R. Jang, E. M. Hia, C. H. Park, C. S. Kim, *Mater. Chem. Phys.* **326** (2024) 129865 (<https://doi.org/10.1016/j.matchemphys.2024.129865>)

14. E. Zeimaran, S. Pourshahrestani, S. F. S. Shirazi, B. Pingguan-Murphy, N. A. Kadri, M.R. Towler, *J. Non-Cryst. Solids* **443** (2016) 118 (<https://doi.org/10.1016/j.jnoncrysol.2016.04.005>)
15. H. Ismail, R. Shamsudin, M. A. A. Hamid, *Mater. Sci. Eng., C* **58** (2016) 1077 (<https://doi.org/10.1016/j.msec.2015.09.030>)
16. B. Chaudhary, Y.K. Kshetri, D.R. Dhakal, S. W. Lee, T. H. Kim, *Opt. Mater.* **135** (2023) 113326 (<https://doi.org/10.1016/j.optmat.2022.113326>)
17. J. Zhu, T. Qu, S. Niu, J. Liu, S. Liu, J. Geng, Z. Yang, A. Abulizi, *Mater. Today Sustain.* **26** (2024) 100716 (<https://doi.org/10.1016/j.mtsust.2024.100716>)
18. A. Sobhani, E. Salimi, *Ceram. Int.* **50** (2024) 26869 (<https://doi.org/10.1016/j.ceramint.2024.04.417>)
19. S. Palakurthy, S. Patel, K. V. Reddy, R. Samudrala, C. Padala, B. Manavathi, P. A. Azeem, *Ceram. Int.* **49** (2023) 36344 (<https://doi.org/10.1016/j.ceramint.2023.08.318>)
20. C. Paluszkievicz, M. Błażewicz, J. Podporska, T. Gumuła, *Vibr. Spectrosc.* **48** (2008) 263 (<https://doi.org/10.1016/j.vibspec.2008.02.020>).

SUPPLEMENTARY MATERIAL TO

Development of a glass-ceramic composite reinforced with β -wollastonite synthesized via a hydrothermal method

MARJAN RANĐELOVIĆ^{1*}, CHARLES C. SORRELL², SHARON KOPPKA³,
ALEKSANDRA ZARUBICA¹, MILAN Z. MOMČILOVIĆ¹ and DIRK ENKE³

¹University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry,
Višegradska 33, 18000 Niš, Serbia, ²School of Materials Science and Engineering, University
of New South Wales, Sydney NSW 2052, Australia, and ³Institute of Chemical Technology,
University of Leipzig, Linne str. 3, 04103 Leipzig, Germany

J. Serb. Chem. Soc. 90 (12) (2025) 1481–1494

Table S-I. The most intense peaks in XRD spectra of wollastonite.

	Position / °2 θ	<i>d</i> - spacing / Å	FWHM / °2 θ	Backgr. / cts	Area / cts*°2 θ	Crystallite size / Å	Micro strain / %
As- synthesized sample of wollastonite	22.80	3.89	0.2047	580.15	31.56	470	0.415
	25.36	3.51	0.1791	541.62	96.99	549	0.320
	26.91	3.31	0.2303	515.87	302.02	416	0.398
	28.92	3.08	0.1791	479.26	106.85	553	0.279
	30.17	2.96	0.1535	454.61	445.23	664	0.223
	36.48	2.46	0.2047	325.62	41.29	485	0.254
	39.18	2.29	0.156	295.71	201.59	706	0.163
	41.83	2.15	0.3744	278.76	280.44	216	0.500
	49.63	1.83	0.156	229.65	93.21	737	0.124
	53.29	1.71	0.3744	207.17	141.19	226	0.380
	Position / °2 θ	<i>d</i> - spacing / Å	FWHM / °2 θ	Backgr. / cts	Area / cts*°2 θ	Crystallite size / Å	Micro strain / %
Calcined sample of wollastonite	23.19	3.83	0.1535	610.89	114.8	655	0.292
	25.34	3.51	0.1535	560.4	256.45	657	0.267
	26.90	3.31	0.1791	527.31	414.11	550	0.301
	28.88	3.09	0.2047	485.04	238.59	475	0.325
	30.03	2.97	0.1535	460.53	501.07	663	0.224
	36.25	2.47	0.2047	351.64	117.99	484	0.256
	39.10	2.30	0.1791	313.28	138.35	567	0.203
	41.28	2.18	0.1279	283.91	78.91	861	0.127
	49.79	1.83	0.1535	231.04	58.97	707	0.129
	53.08	1.72	0.4605	208.03	195.23	219	0.393

* Corresponding author. E-mail: hemija@gmail.com

Table S-II. Textural parameters of as-synthesized wollastonite.

Sample Mass:	0.1844 g	Warm Free Space:	20.24 cm ³ Measured
Cold Free Space:	60.1 cm ³	Equilibration Interval:	10 s
Low Pressure Dose:	None	Sample Density:	1.0 gcm ⁻³
Automatic Degas:	No	/	/
Surface Area			m ² g ⁻¹
Single point surface area			25
BET Surface Area			26
BJH Adsorption cumulative surface area			30
BJH Desorption cumulative surface area			30
Pore Volume			cm ³ g ⁻¹
Single point adsorption total pore volume			0.006
BJH Adsorption cumulative volume of pores			0.046
BJH Desorption cumulative volume			0.047
Pore Size			nm
Adsorption average pore diameter (4V/A by BET):			0.9
BJH Adsorption average pore diameter (4V/A):			6.1
BJH Desorption average pore diameter (4V/A):			6.3

Table S-III. Mode assignments of the Raman-active vibrational modes of the wollastonite.

Assignment	As-prepared (cm ⁻¹)	Calcined (900 °C) (cm ⁻¹)
Asymmetric stretching of Si-O	992	971
Symmetric stretching of Si-O	/	894
Symmetric stretching of Si-O	/	860
Symmetric bending vibration of Si-O-Si	648	636
Bending vibrations of O-Si-O	408	404

SEM ANALYSES OF THE VARIOUS SAMPLES

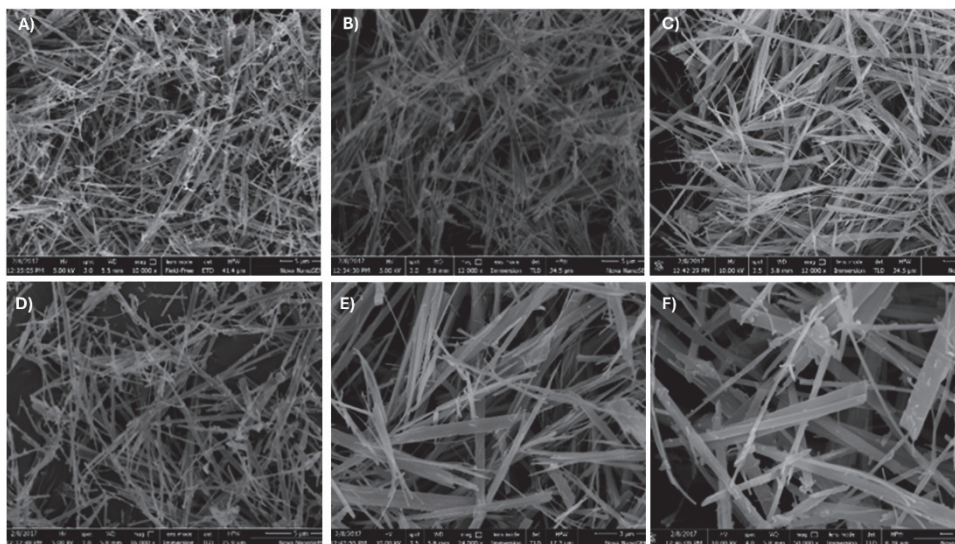


Fig. S-1. SEM micrographs of as-prepared wollastonite at different magnifications: A) 10000 x; B) 12000 x; C) 12000 x; D) 16000 x; E) 24000 x; F) 50000 x.

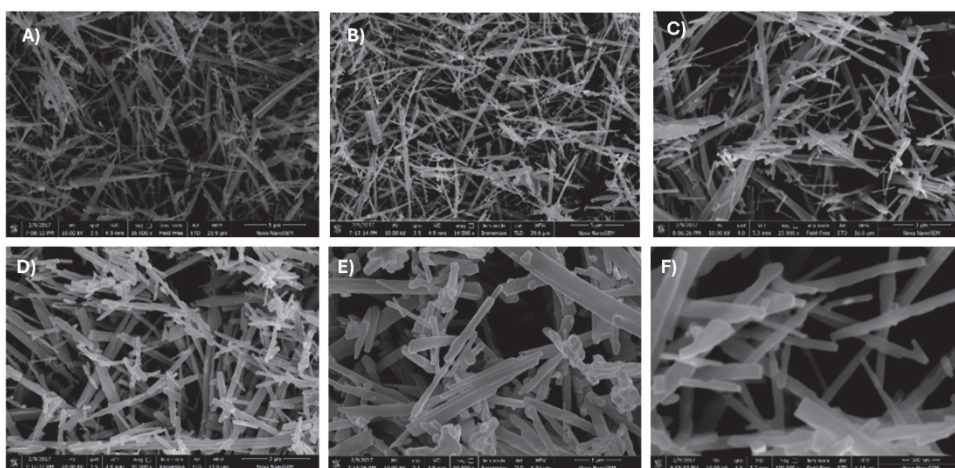


Fig. S-2. SEM micrographs of calcined wollastonite at different magnifications: A) 10000 x; B) 14000 x; C) 25000 x; D) 30000 x; E) 60000 x; F) 100000 x.

Structural and textural parameters obtained by EDX, XRD, and BET analysis are given below:

EDX analysis:

XRD analysis:

Texture data:

Figure 1 is a plot showing the pore size distribution of the poly(amide-imide) membrane. The x-axis represents the pore diameter in nanometers (nm) on a logarithmic scale, ranging from 1 to 100,000. The left y-axis represents the relative pore volume in percent (%), ranging from 0 to 12. The right y-axis represents the specific pore volume in $\text{cm}^3 \text{g}^{-1}$, ranging from 0.00 to 0.14. The distribution is characterized by a primary peak at approximately 5 nm (relative pore volume ~7.5%) and a secondary peak at approximately 1500 nm (relative pore volume ~7%). The specific pore volume curve (blue line) shows a sharp drop after 10 nm, indicating a transition from non-Fickian to Fickian behavior.

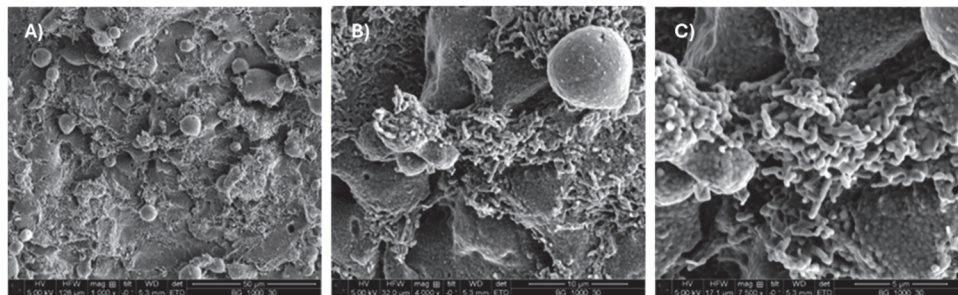
BG 1000°C 30

Figure S-4. SEM micrographs of the sintered bioglass treated at 1000 °C for 30 minutes (BG 1000°C 30) at different magnifications: A) 1000 x; B) 4000 x; C) 7500 x.

Structural and textural parameters obtained by EDX, XRD, and BET analysis are given below:

EDX analysis:

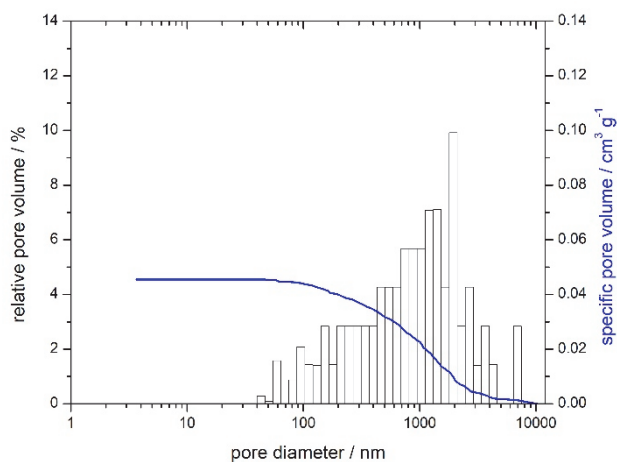
O: 40.4 wt.-%	Si: 21.0 wt.-%
Na: 15.2 wt.-%	P: 2.0 wt.-%
Ca: 21.5 wt.-%	

XRD analysis:

Combeite: 99.5 wt.-%
 Disodium calcium silicate: <1 wt.-%
 Rwp: 11

Texture data:

BET: $<1 \text{ m}^2\text{g}^{-1}$
 V_p : $0.036 \text{ cm}^3\text{g}^{-1}$
 dp : 1348 nm (average diameter)
 Porosity: 7.4 %



BG 1000°C 55

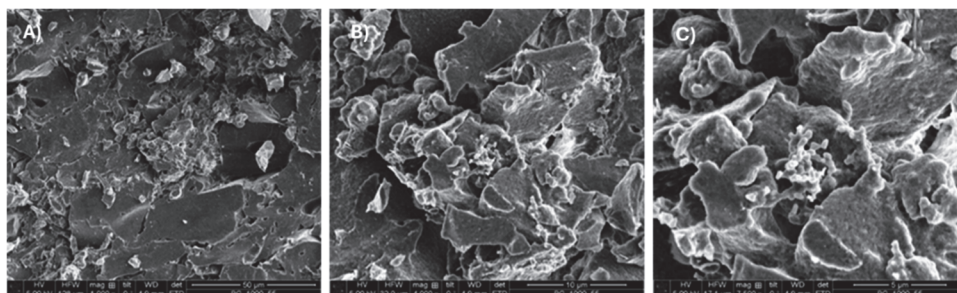


Figure S-5. SEM micrographs of the sintered bioglass treated at 1000 °C for 55 minutes (BG 1000°C 55) at different magnifications: A) 1000 x; B) 4000 x; C) 7500 x.

Structural and textural parameters obtained by EDX, XRD, and BET analysis are given below:

EDX analysis:

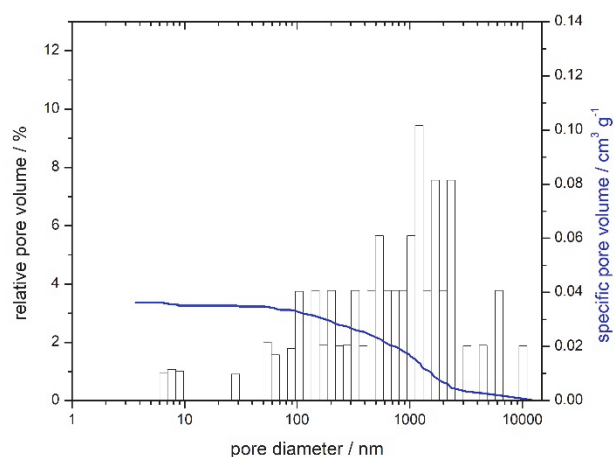
O: 40.4 wt.-%	Si: 21.0 wt.-%
Na: 15.2 wt.-%	P: 2.0 wt.-%
Ca: 21.5 wt.-%	

XRD analysis:

Combeite:	99.5 wt.-%
Disodium calcium silicate:	<1 wt.-%
Rwp:	11

Texture data:

BET:	<1 m ² g ⁻¹
V _p :	0.036 cm ³ g ⁻¹
dp:	1348 nm (average diameter)
Porosity:	7.4 %



BG 1000°C 85

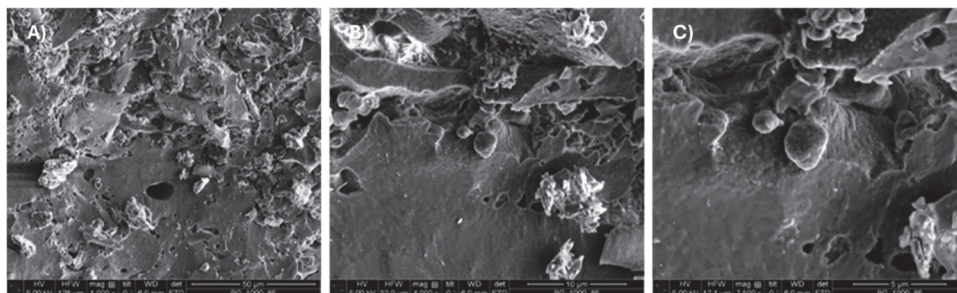


Figure S-6. SEM micrographs of the sintered bioglass treated at 1000 °C for 85 minutes (BG 1000°C 85) at different magnifications: A) 1000 x; B) 4000 x; C) 7500 x.

Structural and textural parameters obtained by EDX, XRD, and BET analysis are given below:

EDX analysis:

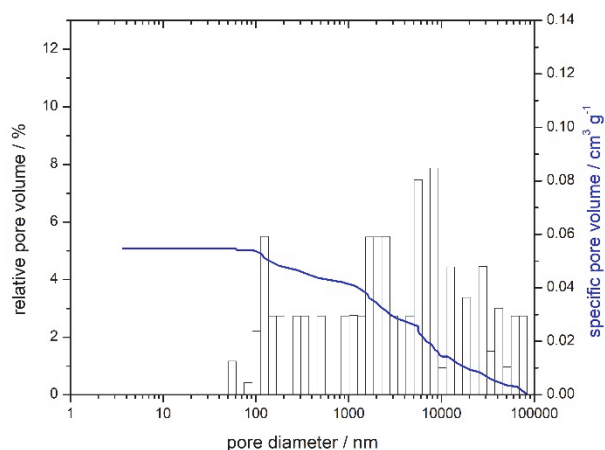
O: 44.5 wt.-%	Si: 20.5 wt.-%
Na: 17.4 wt.-%	P: 1.7 wt.-%
Ca: 15.9 wt.-%	

XRD analysis:

Combeite: 100 wt.-%
 Disodium calcium silicate: 0 wt.-%
 Rwp: 11

Texture data:

BET: $<1 \text{ m}^2\text{g}^{-1}$
 V_p : $0.055 \text{ cm}^3\text{g}^{-1}$
 dp : 11104 nm (average diameter)
 Porosity: 10.7 %



COM 1000°C 15

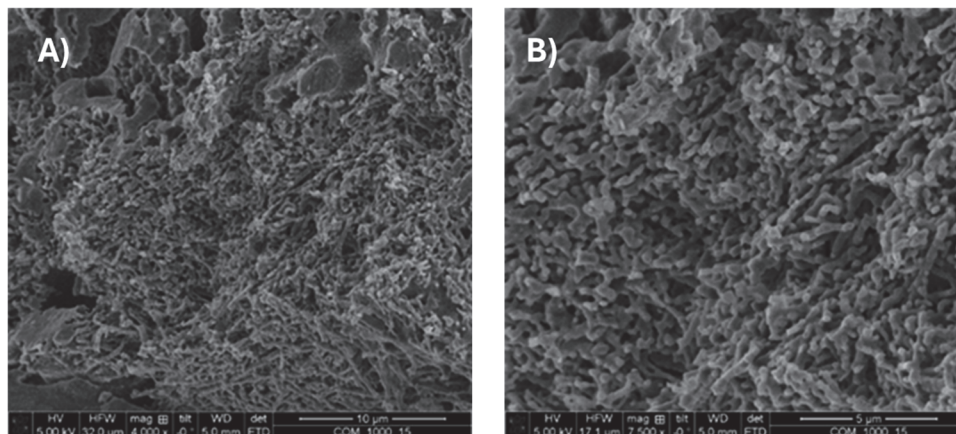


Figure S-7. SEM micrographs of the composite treated at 1000 °C for 15 minutes (COM 15) at different magnifications: A) 4000 x; B) 7500 x.

Structural and textural parameters obtained by EDX, XRD, and BET analysis are given below:

EDX analysis:

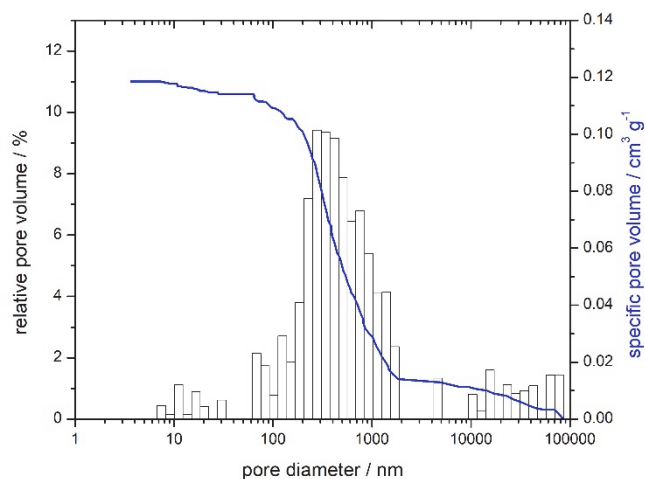
O: 40.0 wt.-% Si: 21.8 wt.-%
 Na: 12.5 wt.-% P: 1.4 wt.-%
 Ca: 24.3 wt.-%

XRD analysis:

Combeite: 98.5 wt.-%
 Disodium calcium silicate: 1.5 wt.-%
 Rwp: 10

Texture data:

BET: $<1 \text{ m}^2\text{g}^{-1}$
 Vp: $0.119 \text{ cm}^3\text{g}^{-1}$
 dp: 4256 nm (average diameter)
 Porosity: 20.7 %



COM 1000°C 30

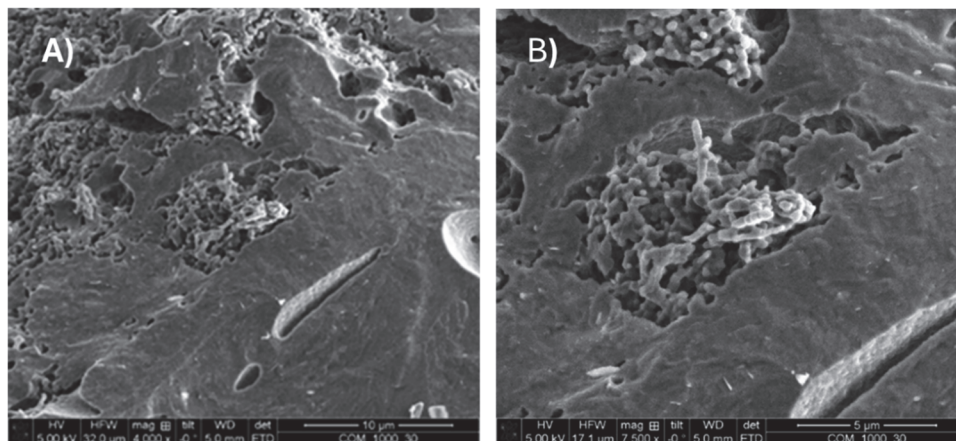


Figure S-8. SEM micrographs of the composite treated at 1000 °C for 30 minutes (COM 30) at different magnifications: A) 4000 x; B) 7500 x.

Structural and textural parameters obtained by EDX, XRD, and BET analysis are given below:

EDX analysis:

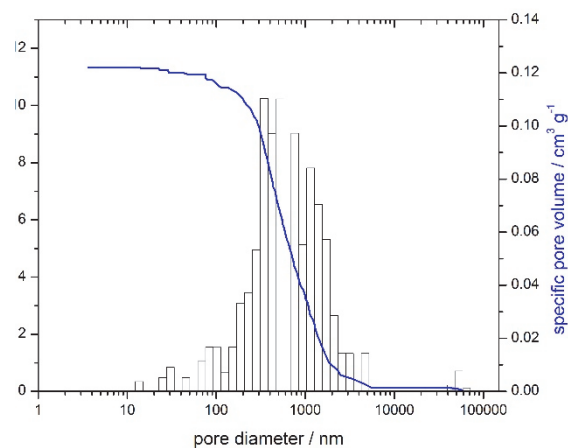
O: 45.2 wt.-%	Si: 21.1 wt.-%
Na: 14.5 wt.-%	P: 1.3 wt.-%
Ca: 17.8 wt.-%	

XRD analysis:

Combeite:	98.5 wt.-%
Disodium calcium silicate:	1.5 wt.-%
Rwp:	

Texture data:

BET:	<1 m ² g ⁻¹
V _p :	0.122 cm ³ g ⁻¹
dp:	1368 nm (average diameter)
Porosity:	21.2 %



COM 1000°C 55

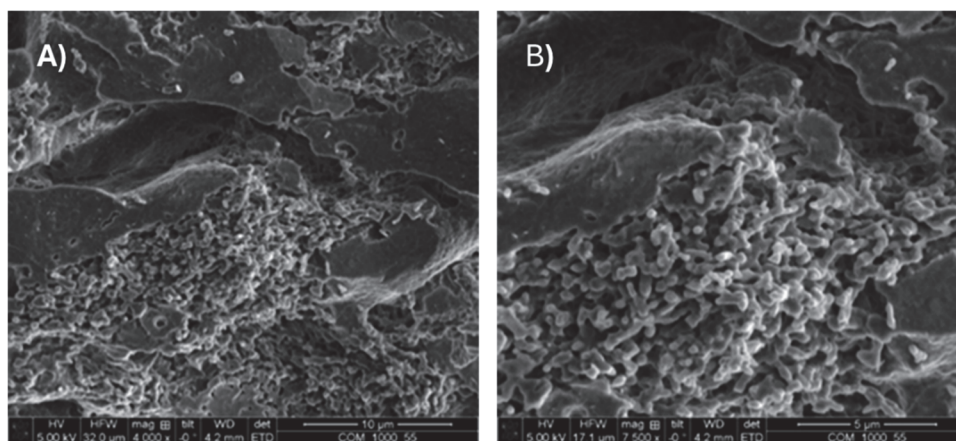


Figure S-9. SEM micrographs of the composite treated at 1000 °C for 55 minutes (COM 55) at different magnifications: A) 4000 x; B) 7500 x.

Structural and textural parameters obtained by EDX, XRD, and BET analysis are given below:

EDX analysis:

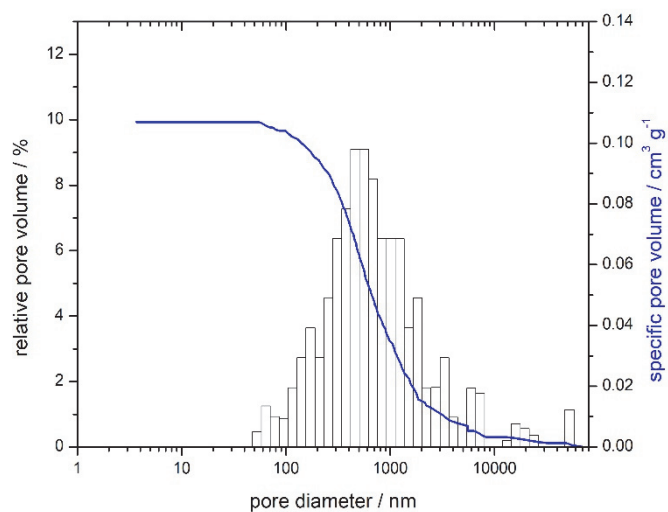
O: 40.0 wt.-%	Si: 21.3 wt.-%
Na: 12.5 wt.-%	P: 1.2 wt.-%
Ca: 25.1 wt.-%	

XRD analysis:

Combeite:	99.5 wt.-%
Disodium calcium silicate:	<1 wt.-%
Rwp:	11

Texture data:

BET:	<1 m ² g ⁻¹
V _p :	0.107 cm ³ g ⁻¹
dp:	1956 nm (average diameter)
Porosity:	19 %



COM 1000°C 85

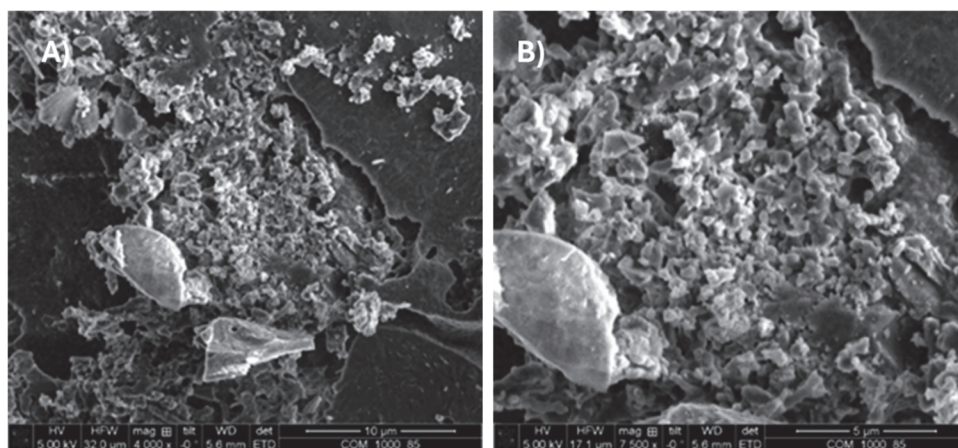


Figure S-10. SEM micrographs of the composite treated at 1000 °C for 85 minutes (COM 85) at different magnifications: A) 4000 x; B) 7500 x.

Structural and textural parameters obtained by EDX, XRD, and BET analysis are given below:

EDX analysis:

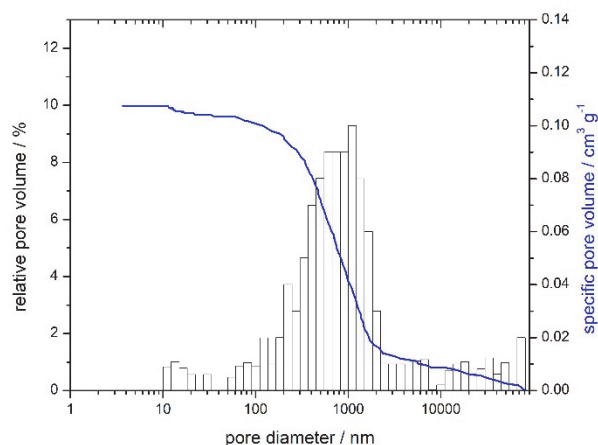
O: 45.1 wt.-%	Si: 21.1 wt.-%
Na: 14.9 wt.-%	P: 1.1 wt.-%
Ca: 17.8 wt.-%	

XRD analysis:

Combeite: 99.5 wt.-%
 Disodium calcium silicate: <1 wt.-%
 Rwp: 11

Texture data:

BET: <1 m²g⁻¹
 V_p: 0.108 cm³g⁻¹
 dp: 3928 nm (average diameter)
 Porosity: 19.1 %



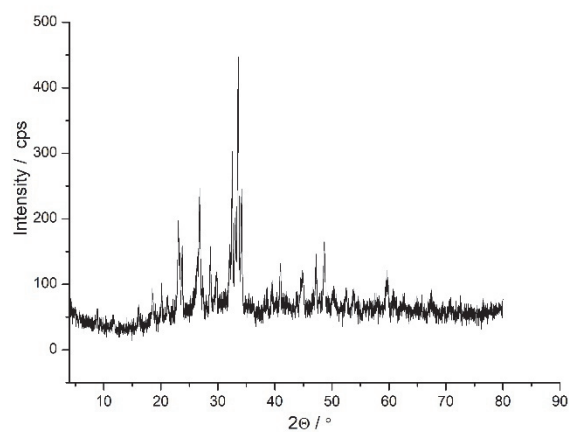


Figure S-11. XRD pattern of COM 30 after interaction with the SBF solution. Peaks at 26.0°, 31.8°, and 33.1° correspond to the (002), (211), and (300) crystal planes of hydroxyapatite.



J. Serb. Chem. Soc. 90 (12) 1495–1511 (2025)
JSCS–5468

Sustainable synthesis of silver nanoparticles on cotton gauze for enhanced antibacterial properties

DARKA D. MARKOVIĆ^{1*}, VANJA B. TADIĆ², ANA R. ŽUGIĆ² and MAJA M. RADETIĆ³

¹University of Belgrade, Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, Mihajla Petrovića Alasa 12–14, Belgrade, 11351, Serbia, ²University of Belgrade, Institute for Medical Plant Research “Dr Josif Pančić”, Tadeuša Košćuška 1, 11000 Belgrade, Serbia and ³University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia

(Received 3 June, revised 15 July, accepted 13 September 2025)

Abstract: Wound protection is a critical step in preventing or reducing infections, as well as in preventing the transmission of infections between patients. Wound dressings are essential medical products that cover wounds and facilitate healing. The present study examines the possibility of using Ag nanoparticles (NPs) to impart antibacterial activity to cotton gauze, a commonly used disposable wound dressing material. The *in situ* synthesis of Ag-based NPs on cotton gauze was achieved using extracts from *Populus x euramericana* (PE) and *Ailanthus altissima* (Mill.) Swingle (AA) leaves. Major phytochemical compounds in the extracts were quantified using HPLC. FESEM and EDS mapping analyses confirmed the presence of Ag-based NPs across the fibre surfaces of both samples. The average size of NPs synthesized in the presence of PE and AA extracts was 88±26 and 82±23 nm, respectively. A comparable amount of silver was found in the sample obtained with PE (14.22±0.23 µmol/g) and in the sample synthesized using AA extract (13.63±1.40 µmol/g). The synthesized samples achieved maximum bacterial reduction against Gram-negative bacteria *Escherichia coli* and Gram-positive *Staphylococcus aureus*.

Keywords: cotton gauze; Ag nanoparticles; plant extracts; green synthesis; antibacterial activity.

INTRODUCTION

Skin plays an important role in protecting the body from harmful external factors.^{1–2} Diabetes, diseases, accidents or burns can all damage the skin and cause wounds, as can minor cuts and surgical procedures.³ Chronic wounds are usually caused by infection, while acute wounds are sometimes prone to infection. Surgical wounds are especially vulnerable and can be colonised by a variety of bacteria,

*Corresponding author. E-mail: darka.markovic@vin.bg.ac.rs
<https://doi.org/10.2298/JSC250603074M>

such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella spp.* and *Enterobacter spp.*^{4–6} These microorganisms frequently cause wound infections that can progress to sepsis, pneumonia, or meningitis in patients.⁷ Hence, wound management has been a major challenge in medicine worldwide. Wound protection is an important step in preventing or reducing infections, as well as in preventing the transmission of infections between patients.^{8–9} Wound dressings are crucial medical products that cover wounds and support healing.¹ Cotton-based medical gauze is a type of sterile, disposable dressing that is commonly used to treat wounds due to its softness, breathability, affordability, and lack of skin irritation.^{2,10} The primary issue with cotton gauze is that it cannot prevent or treat infections because bacteria in humid environments can readily stick to the surface of hydrophilic cotton fibres, enter the fibres and can create a biofilm, which acts as a reservoir for the growth of pathogens and poses a health risk to humans.¹⁰ Consequently, antimicrobial treatments are crucial for mitigating and preventing microbial proliferation. Antimicrobial agents for antimicrobial finishing of textile materials can be classified as organic, inorganic, or natural.¹¹ The more studied types of antimicrobial agents include biguanides, triclosan, quaternary ammonium salts, metallic silver, zinc oxide, triclosan, herbal extracts and certain natural and metal complex colourants.^{12–16} Owing to their excellent antimicrobial activity against a wide range of microorganisms and low cytotoxicity, silver nanoparticles (Ag NPs) have received much interest. Among various methods that have been used for the synthesis of Ag NPs, such as microwave-assisted reduction, salt reduction, reverse micelles, and ultrasonic irradiation, the widely used and investigated method is the reduction of silver nitrate in aqueous solution, using a reducing agent such as sodium borohydride (NaBH₄) or hydrazine.^{17–18} Exploitation of conventional strong reducing agents enables the synthesis of smaller NPs, but the toxicity of such reducing agents has prompted research into more environmentally friendly routes.

Green synthesis of Ag NPs includes utilising bacteria, fungi, enzymes, polysaccharides and plant extracts as reducing agents for metal ions.^{17–18} Owing to its natural abundance and low cost, various plants and their parts (roots, leaves, seeds, fruits) have been extensively investigated in the synthesis of colloidal Ag NPs.¹⁹ Despite the substantial studies on the green synthesis of Ag NPs, research on the green synthesis of these NPs on textile fibres remains limited. One possible explanation might be the lack of adequate reproducibility in terms of NPs shape, size and stability due to the variability in chemical composition (*e.g.*, phenolic compounds and flavonoids), which is closely tied to geographical location, weather and environmental conditions. Moreover, the use of plants can be complicated because they are seasonal. In terms of *ex situ* strategies for synthesizing Ag NPs, it is commonly difficult to achieve monodisperse NPs, which are beneficial for multiple applications, including textiles.²⁰

Sodium alginate and aloe vera inkjet print onto cotton fabric were used as a sustainable reducing agent for *in situ* synthesis of Ag NPs.²¹ Antimicrobial cotton fabric has been fabricated using whey protein isolate as both a reducing agent and stabilizer for Ag NPs.¹⁷ *Spirulina*, a microalga rich in proteins, polysaccharides, vitamins, and phenolic could be successfully employed for antimicrobial finishing of cotton with Ag NPs.¹⁸ Maghimaa *et al.* synthesized the metallic Ag NPs from the aqueous extract of *Curcuma longa* L. leaf and evaluated their antimicrobial and wound healing potential of Ag NPs-coated cotton fabric.²² It has been shown that Black rice (*Oryza sativa* L.) could be used for *in situ* green synthesis of Ag NPs on carboxymethyl chitosan modified cotton substrate.²³ *In situ* phytosynthesis of Ag NPs on cotton fabric was also performed using extracts of plant materials from food waste (green tea leaves (*Camellia sinensis* (L.) Kuntze), avocado seed (*Persea americana* Mill.) and pomegranate peel (*Punica granatum* L.) and invasive alien species (the Japanese knotweed rhizome (*Fallopia japonica* Houtt.), the staghorn sumac fruit (*Rhus typhina* L.), and goldenrod flowers (*Solidago canadensis* L.)).²⁴ Štular *et al.* synthesized Ag NPs in the presence of sumac leaf extract onto polysiloxane modified cellulose fibres.²⁵

Our previous studies have revealed that extracts of *Paliurus spina-christi* Mill., *Juglans regia* L., *Humulus lupulus* L. and *Sambucus nigra* L. could be successfully used for *in situ* synthesis of Ag-based NPs on cotton fabric modified with citric acid.²⁶ Pomegranate peel extract (*P. granatum*) was also utilised for the simple *in situ* synthesis of Ag-based NPs on chitosan-modified viscose fibres.²⁷ Considering that a variety of phytochemicals found in different plant extracts may serve as reducing, stabilizing and capping agents, our intention was to extend previous research by utilizing the extract of cultivated hybrid *Populus x euramericana* (Dode) Guinier I-214, Salicaceae (buds) and weed species *Ailanthus altissima* (Mill.) Swingle, Simaroubaceae (leaves) for *in situ* synthesis of Ag-based NPs on cotton gauze to impart antibacterial activity. *P. euramericana* is a fast-growing plantation tree that is extensively exploited in the wood industry, but thin branches with buds are regarded as waste. On the other hand, *A. altissima* (Tree-of-heaven) is an invasive species that causes serious damage to the ecosystem, economy, and public health in the United States and Europe.²⁸ To the best of our knowledge, there is only one paper that has addressed the exploitation of bark extract *A. altissima* for the synthesis of Ag NPs.²⁹ Furthermore, to our knowledge, no research has been conducted on the *in situ* synthesis of Ag-based nanoparticles on textile substrates using extracts derived from *P. euramericana* and *A. altissima*. Major phytochemical compounds in the extract were quantified by HPLC. Morphological and chemical changes on the fibre surface were assessed by FESEM and FTIR, respectively. The total content of silver and the released amounts of Ag⁺ were quantified by AAS. Antibacterial activity of cotton gauze with Ag-based NPs was tested against Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus*.

EXPERIMENTAL

Materials

Populus x euramericana (Dode) Guinier I-214, Salicaceae was provided by “Srbija sume”, Belgrade in March 2024, while *Ailanthus altissima* (Mill.) Swingle, Simaroubaceae was collected in the forest of Košutnjak (N: 44°46'34", E: 20°25'56") in Belgrade at the beginning of June 2024. The voucher specimens were deposited and identification was performed at the Faculty of Forestry, University of Belgrade.

Nitric acid and sodium chloride were purchased from Zorka Pharma, Serbia. Centrohem, Serbia, provided silver nitrate. The microbial inoculum was prepared in tryptone soya broth, agar, and yeast extract provided by Torlak, Serbia. Cotton gauze was supplied by Diva (Serbia) and used without further purification.

Preparation of plant extracts

The plants *P. euramericana* (PE) and *A. altissima* (AA) were selected for the synthesis of Ag-based NPs. The plant extracts were prepared as decocts by adding 6.00 g of buds or leaves (previously milled) to 100 mL of distilled water. The vessel containing the extract was sealed and heated to 80°C for 1 h. Prepared extracts/decocts were filtered and immediately used for the synthesis of Ag-based NPs.

HPLC analysis of PE and AA leaves decoction

Comprehensive analyses of the tested decoctions, from the aspect of their chemical profile, were achieved using HPLC analysis. “Fingerprinting” of the investigated samples was carried out using a 1200 HPLC system (Agilent Technologies) equipped with a Phenomenex, HydroRP 80, 150 mm×4.6mm column using two mobile phases (phase A being 0.1 M solution of phosphoric acid, and phase B pure acetonitrile). The flow rate was 0.800 mL/min, with photodiode-array (PDA) detection (UV at 260). Best peak separation was achieved using the following combination: 2 % B (0 min); 2–10 % B (0–5 min); 10 % B (5–15 min), 10–15 % B (15–20 min) and 15–60 % (20–45 min). For poplar buds, the slightly modified method was used, namely the flow rate was 1.00 mL/min, with photodiode-array (PDA) detection (UV at 360 nm; for catechin at 260 nm). Elution was performed 11 % B (0 min); 11–25 % B (0–5 min); 25 % B (5–10 min), 25–40 % B (10–20 min), 40 % B (20–35 min), 45–55 % B (35–50 min) and 50–60 % B (50–60 min). Before injection, the samples of PE and AA (*c* = 24.6 and 8.6 mg/mL, respectively) were filtered through a PTFE membrane filter. For standards gallic acid, neochlorogenic acid, chlorogenic acid, procyanidin B1, catechin, caffeic acid, epigallocatechin gallate, *p*-coumaric acid, ferulic acid, taxifolin, rutin, 3,5-di-*O*-caffeoylquinic acid, naringin, rosmarinic acid, *t*-cinnamic acid, quercetin, pinobanksin, naringenin, pinocembrin, chrysin, syringic acid, myricitrin, ellagic acid, isoquercetin, quercitrin, kaempferol-3-*O*-glucoside, apigenin-7-*O*-glucoside and diosmin (Chem Faces, China) used in the investigation, the concentrations were 0.07, 0.3, 0.42, 0.14, 0.40, 0.52, 0.32, 0.50, 0.43, 0.30, 0.44, 0.39, 0.28, 0.43, 0.58, 0.21, 0.20, 0.38, 0.46, 0.47, 0.19, 0.50, 0.34, 0.52, 0.28, 0.49 and 0.38 mg/mL, respectively. The injection volume of the standard solutions and the tested extract was 4 µL. Identification was based on overlay curves and the retention times. After spectra matching succeeded, results were confirmed by spiking with the respective standard to achieve a complete identification using the peak purity test. The peaks that did not fulfil these requirements were not quantified. Quantification was performed through external calibration with the standard.

The total phenolic content was determined by the Folin–Ciocalteu method.³⁰ 100 mL of methanol solution of the investigated sample (starting concentration being 5.66 and 6.97 mg/mL, for heaven of tree and poplar, respectively) was mixed with 0.75 mL of 10-fold diluted Folin–

–Ciocalteu reagent and allowed to stand at 22 °C for 5 min; 0.75 mL of sodium bicarbonate (60 g/L) solution was added to mixture. After 90 min at 22 °C, absorbance was measured at 725 nm. Gallic acid (0–100 mg/L) was used for calibration of a standard curve. The calibration curve showed the linear regression at $R^2 > 0.99$, and the results are expressed as mg of gallic acid equivalents per g dry weight (DW). Triplicate measurements were taken and data were presented as mean $\pm$ standard deviation (SD).

The percentage content of tannins was calculated using the method described in the European Pharmacopoeia 11.0.³¹ Briefly, the investigated extracts were treated with the phosphomolybdotungstic reagent in an alkaline medium after and without treatment with the hide powder reagent. The absorbance was measured by UV–Vis spectrophotometer HP 8453 (Agilent Technologies, USA), at a max of 760 nm. The percentage content of tannins expressed as pyrogallol (mass %), was calculated from the difference in absorbance of total polyphenols ($A1$) and polyphenols not adsorbed by the hide reagent ($A2$), using the following expression:

$$(62.5(A1 - A2) m2)/(A3 \times m1) \quad (1)$$

where $m1$ represents the mass of the sample to be examined, in grams; and $m2$ and $A2$ represent the mass, in grams and the absorbance of pyrogallol, respectively. The results represent the mean of three determinations.

In situ synthesis of Ag-based NPs on the modified samples

One gram of cotton gauze (COG) was soaked in 30 mL of a 20 mM solution of AgNO_3 for one hour. Afterwards, the sample was rinsed twice with deionised water and placed in the freshly prepared solution of plant extract. The synthesis step was conducted at 60 °C for 1 h. Subsequently, the samples were thoroughly rinsed with distilled water and left to dry at room temperature. Cotton gauze containing Ag-based NPs synthesized in the presence of PE and AA were marked as COG–Ag–PE and COG–Ag–AA, respectively. Simultaneously, COG samples were put in PE and CC extracts at 60 °C for 1 h. After rinsing, samples were marked as COG–PE and COG–AA, respectively. The synthesis route is schematically presented in Fig. 1.

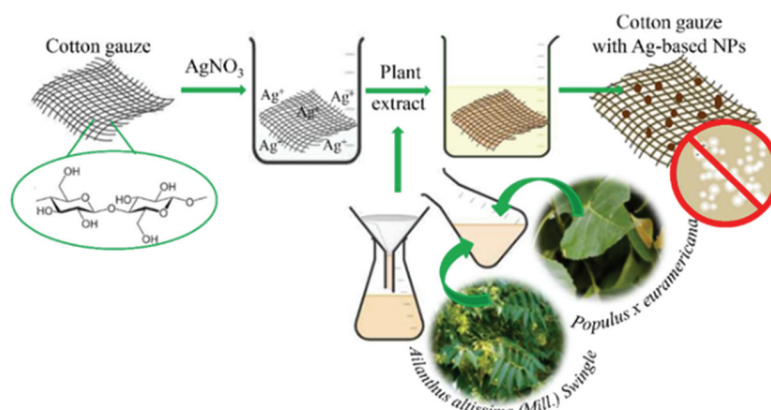


Fig. 1. Schematic presentation of green *in situ* synthesis of Ag-based NPs onto cotton gauze.

FESEM analysis

The morphology of fibres with embedded Ag-based NPs, and the qualitative analysis of the chemical composition and distribution of silver over the surface were investigated by field

emission scanning electron microscopy equipped with an energy dispersive X-ray spectrometer (FESEM-EDS, FEI SCIOS 2 dual beam). The samples were recorded under high vacuum with an acceleration voltage of 7 kV. The EDS mapping acquisition time was 30 min. To define the size and size distribution of NPs, the obtained FESEM images were analysed using the open-access imaging software tool ImageJ.

FTIR analysis

Fourier transform infrared (FTIR) spectra of the COG, COG-PE, COG-AA, COG-Ag-PE and COG-Ag-AA samples were recorded in the ATR mode using a Nicolet iS5 FTIR spectrometer (Thermo Scientific) at 4 cm^{-1} resolution, in the wavenumber range $500\text{--}4000\text{ cm}^{-1}$.

The total amount of Ag

The total amount of Ag in the samples was measured using atomic absorption spectroscopy (AAS). The measurements were conducted in the absorption mode at a wavelength 328.1 nm. The COG-Ag-PE and COG-Ag-AA samples were immersed in a nitric acid solution ($\text{H}_2\text{O}:\text{HNO}_3 = 1:2$) and subsequently analysed using AAS after 24 h.

Colour measurements

The colour coordinates of the samples, represented as CIE L^* , a^* and b^* , were measured utilising a Datacolour SF300 spectrophotometer under the D65 illuminant, employing the 10° standard observer.

Antibacterial test

Antibacterial activity of the samples with Ag-based NPs was tested against Gram-negative bacteria *E. coli* ATCC 25922 and Gram-positive bacteria *S. aureus* ATCC 25923 using the standard test method for determination of the antimicrobial activity of immobilised antimicrobial agents under dynamic contact conditions ASTM E 2149-01 (2001). Bacterial inoculums were cultivated in the tryptone soya broth (3 mL) supplemented with 0.6 % yeast extracts at 37°C and left for 18 h (late exponential stage of growth). Freshly grown bacterial cultures were diluted in sterile physiological saline solution to obtain an inoculum with an initial number of cells of *ca.* 10^7 CFU/mL . 50 mL of sterile physiological saline solution (pH 7.2) was inoculated with 0.5 mL of the bacterial inoculum. One gram of the control COG, COG-Ag-PE, and COG-Ag-AA samples (previously sterilised in an autoclave for 15 min) were placed in the flask with physiological saline solution and microorganisms at 37°C and shaken for 2 and 24 h. After these time intervals, 1 mL aliquots from the flask were taken and diluted with physiological saline solution, and 1 mL of the solution was placed onto a tryptone soya agar supplemented with 0.6 % yeast extracts. After 24 h of incubation at 37°C , the zero time (initial number of bacteria colonies), 2 and 24 h counts of viable bacteria were made.

The percentage of bacteria reduction (R , %) was calculated using the following equation:

$$R = 100 \frac{C_0 - C}{C_0} \quad (2)$$

where C_0 (CFU/mL – colony forming units) is the number of bacteria colonies on the control sample (COG) and C (CFU/mL) is the number of bacteria colonies on the fabric with Ag-based NPs.

Release study

To assess the release capability of Ag^+ from the samples, 0.25 g of COG-Ag-PE and COG-Ag-AA were immersed in 25 mL of physiological saline solution (9 g/L NaCl) and main-

AAS analysis was also conducted to assess the concentration of Ag^+ that were released from COG-Ag-PE and COG-Ag-AA samples after 2 and 24 h in the physiologically saline solution, under the conditions utilized in the antimicrobial test

rs, seeds, stems and roots) possess a high bioreduction
varied reactive constituents, including polyphenols, ter-
g sugars, polysaccharides and amino acids. Among these,
s and alkaloids are recognised as the most effective red-
ding agents,³² as they inhibit the growth and aggregation
a. Fig. 2 presents the HPLC spectra of the PE and AA
The chemical composition of the PE and AA decoction
II, respectively.

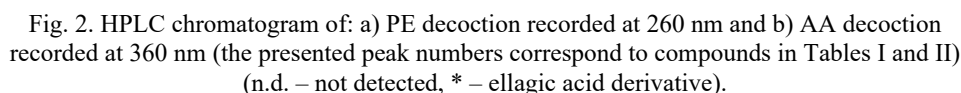


TABLE I. Chemical composition of PE decoct (the type of the extract preparation, whose preparation was explained in the Methodology); DW extr – dry weight of the analysed extract

No.	Compound, mg g ⁻¹ DW extr	<i>Populus x euramericana</i> (Dode) Guinier I-214, Salicaceae, bud decoction
1	Gallic acid	55.30±1.05
2	Neochlorogenic acid	7.46±0.38
3	Procyanidin B1	22.55±1.26
4	Catechin	6.42±0.22
5	Chlorogenic acid	4.83±0.12
6	Caffeic acid	20.15±1.01
7	Epigallocatechin gallate	1.15±0.05
8	<i>p</i> -Coumaric acid	6.04±0.19
9	Ferulic acid	5.39±0.15
10	Taxifolin	9.18±0.33
11	Rutin	0.75±0.02
12	Quercitrin	0.64±0.03
13	3,5-di- <i>O</i> -Caffeoylquinic acid	1.98±0.09
14	Naringin	11.91±0.35
15	Rosmarinic acid	27.17±0.71
16	<i>t</i> -Cinnamic acid	15.48±0.44
17	Quercetin	0.80±0.02
18	Pinobanksin	17.32±0.63
19	Naringenin	0.76±0.02
20	Pinocembrin	5.06±0.09
21	Chrysin	0.11±0.01
Total phenolic compounds, mg GAE/g		278.1±2.15
Tannin content, %		0.05±0.01

TABLE II. Chemical composition of AA decoct

No.	Compound, mg g ⁻¹ DW extr	<i>Ailanthus altissima</i> (Mill.) Swingle, Simaroubaceae leaf decoction
1	Gallic acid	6.07±0.02
2	Neochlorogenic acid	6.07±0.02
3	Chlorogenic acid	14.58±0.11
4	Syringic acid	21.17±0.21
5	Myricitrin	1.33±0.01
6	Ellagic acid	5.84±0.03
7	Isoquercetin	6.89±0.10
8	Quercitrin	1.71±0.03
9	Kaempferol-3- <i>O</i> -glucoside	0.96±0.02
10	Apigenin-7- <i>O</i> -glucoside	0.55±0.01
11	Diosmin	0.22±0.01
Total phenolic compounds, mg GAE/g		247.15±1.05
Tannin content, %		0.17±0.01

The aqueous leaf extracts of PE and AA are rich in various biomolecules/phytochemicals (Fig. 2). Tables I and II show that the total phenolic content found

in PE and AA extracts were 278.1 ± 2.15 and 247.15 ± 1.05 mg GAE/g, respectively. The total tannin content was 0.05 and 0.17 % in PE and AA extracts, respectively. The HPLC analysis revealed the predominant presence of simple phenolic compounds, flavan-3-ols, flavanoneols and polyphenolic acids (gallic acid, procyanidin B1, caffeic and rosmarinic acids, Fig. 2a, Table I) in PE, while AA was rich in chlorogenic, syringic and ellagic acids (Fig. 2b, Table II). Namely, comparing PE and AA, the PE extract had a higher content of total phenolic compounds, represented by phenolic acids (rosmarinic, caffeic and *t*-cinnamic acids) and gallic acid and flavan-3-ols derivatives (catechin, procyanidins), while the AA extract, although rich in phenolic constituents, was less abundant in gallic and phenolic acids (the highest content was detected for chlorogenic and syringic acids). The flavonoids were detected in both investigated samples. These compounds can interact with the metal ion *via* the hydroxyl group and form metal NPs through the bioreduction process.³² The presence of flavonoids and phenolic acids with hydroxyl and ketonic groups in the extract could be responsible for the formation of metal nanoparticles. These compounds could be adsorbed on the surface of metal nanoparticles, possibly through π -electron interaction in the absence of other strong ligating agents.³³ Namely, tautomeric transformation of flavonoids and phenolic acids from enol to keto form is followed by the release of reactive hydrogen atoms that can reduce Ag^+ to Ag^0 .³²

The initial analysis of the synthesis process for Ag-based nanoparticles was validated through visual confirmation of the COG sample's colour shift from white to dark brown (Fig. 3a). It is well documented that the biosynthesis of Ag-based NPs produces brown-coloured solutions or solid samples.^{25–26,32,34} Our recent study revealed that the *in situ* synthesis of Ag-based NPs using extracts from *P. spina-christi*, *J. regia*, *H. lupulus* and *S. nigra* resulted in the colouration of cotton samples in various shades of brown.²⁶ Stular *et al.* reported that cotton samples coated with various concentrations of Ag NPs synthesized in the presence of sumac leaf extracts were coloured in shades ranging from yellow to brown.²⁵ The colour change was quantified by determining the CIE $L^*a^*b^*$ coordinates (Fig. 3b). Apparently, the colour of the samples changed considerably after the synthesis of Ag-based NPs, resulting in darker reddish and more yellowish shades of the samples (Fig. 3a and b).

Fig. 3c illustrates the change in the shape of the reflectance curves after coating with plant extracts, and the biosynthesis of Ag-based NPs. The reflectance intensity and shape of the reflectance curve of the COG altered after being treated with plant extracts, especially in the wavelength range of 400–500 nm. An additional decrease in reflectance intensity was observed after the *in situ* synthesis of Ag-based NPs. The shape of the reflectance curves for COG–Ag–PE and COG–Ag–AA is significantly different across the entire measuring range, particularly in the high-energy region, where a slight drop of reflectance intensity appeared. This sug-

gests the emergence of the plasmon resonance band of Ag NPs at approximately 475 nm. Similar results were reported for cotton fabric loaded with colloidal Ag NPs.³⁵

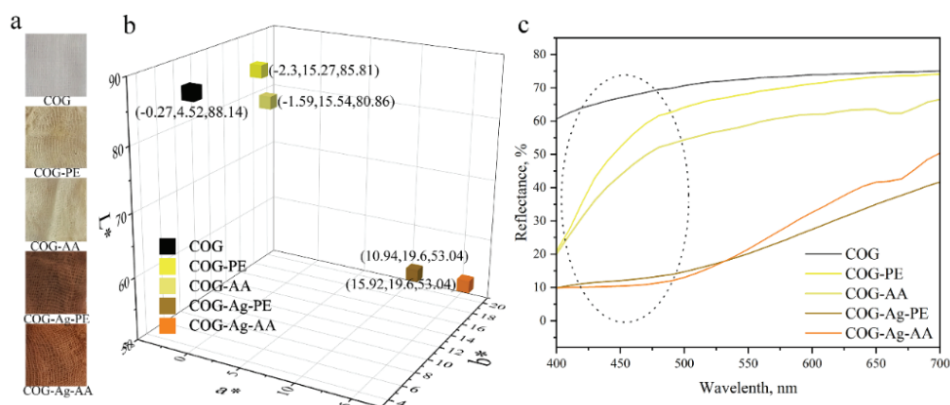


Fig. 3. The photographs (a), $L^*a^*b^*$ coordinates (b) and reflectance spectra (c) of the samples.

In comparison to the COG–Ag–PE surface (Fig. 4), a smaller number of spherical NPs was visible on the COG–Ag–AA surface (Fig. 5). Additionally, the size distribution demonstrated the presence of smaller NPs ranging from 29 to 140 nm, with a mean size of 82 ± 23 nm. AAS analysis showed that the total content of silver in the COG–Ag–AA sample was 13.63 ± 1.40 $\mu\text{mol/g}$ (1.47 ± 0.15 mg/g). Given that the total silver content in COG–Ag–PE and COG–Ag–AA is comparable, the reduced quantity of synthesized NPs in the COG–Ag–AA sample (Fig. 5) may indicate that a portion of Ag^+ was merely adsorbed onto the cotton fibres. This may be attributed to the concentration of gallic acid, recognized as an effective reducing and stabilizing agent,³⁶ which was found to be nearly nine times lower in the AA extract compared to that in the PE extract (Tables I and II). Nevertheless, an in-depth investigation into the effect of gallic acid on the formation of Cu-based NPs demonstrated that a single component in the extract cannot solely account for the overall results; rather, it is the synergistic interaction of the various phytochemicals present that plays a crucial role.³⁶

In general, the variation in the overall quantity of Ag NPs is the consequence of the differing total phenolic content observed in the samples (Tables I and II). Larger amounts of silver ($17\text{--}27$ $\mu\text{mol/g}$) on the cotton samples were accomplished in our previous study, where *P. spina-christi*, *J. regia*, *H. lupulus* and *S. nigra* leaves extracts were employed for *in situ* synthesis of Ag-based NPs.²⁶ The observed outcomes may be attributed to prior modifications of cotton using citric acid, which introduces carboxyl groups that improve the material's capacity to adsorb Ag^+ . The size distribution and the overall quantity of silver obtained in this research fell within the findings reported by Štular *et al.*²⁵

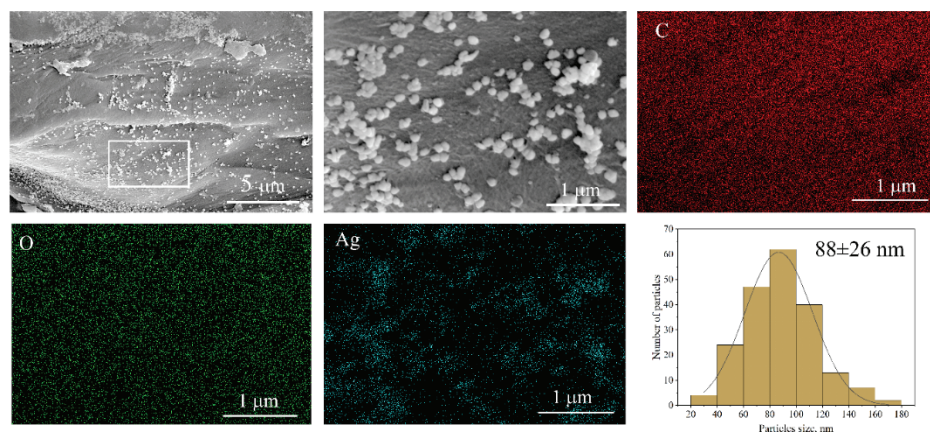


Fig. 4. FESEM micrographs, mapping and particle size distribution of COG-Ag-PE sample.

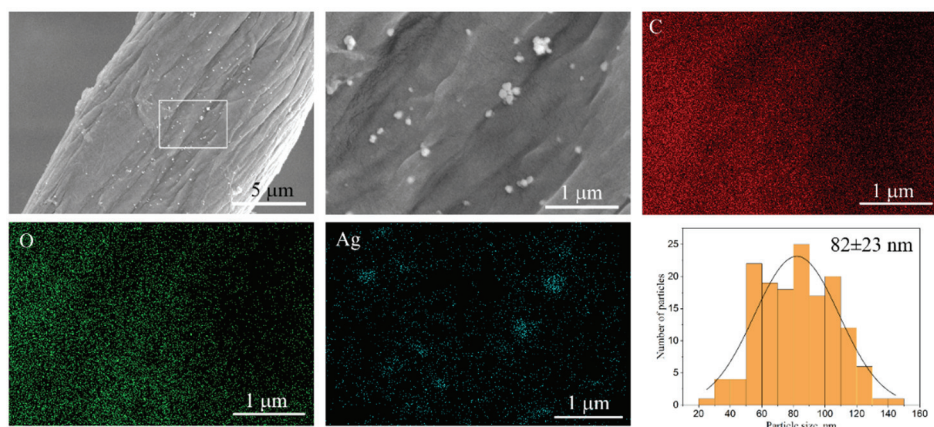


Fig. 5. FESEM micrographs, mapping and particle size distribution of COG-Ag-AA.

The FTIR spectra of the COG, COG-PE, COG-AA, COG-Ag-PE and COG-Ag-AA are shown in Fig. 6. The bands characteristic of cellulose are visible in all spectra. The band located between $3500\text{--}3200\text{ cm}^{-1}$ is ascribed to the hydrogen-bonded O-H stretching in cellulose. The bands centred at 2898 cm^{-1} and $1427, 1364, 1315$ and 1280 cm^{-1} are related to C-H asymmetric stretching, C-H in-plane bending vibrations, C-H bending vibrations, C-H wagging vibrations and C-H deformation stretch vibrations, respectively. The OH in-plane bending vibration is located at 1203 cm^{-1} . The band centred at 1640 cm^{-1} could be related to adsorbed water molecules. The bands at $1156, 1105, 1050, 1031\text{ cm}^{-1}$ correspond to asymmetric bridge C-O-C, asymmetric in-plane ring stretching and C-O stretching, respectively. The band at 900 cm^{-1} originates from asymmetric out-of-phase ring stretching at the C1-O-C4 glucosidic bond.³⁷ FTIR spectra of gauze loaded with

herbal extracts are similar to those of the control gauze. Keeping in mind that herbal extracts are cellulose in origin, the overlapping of bands has occurred.

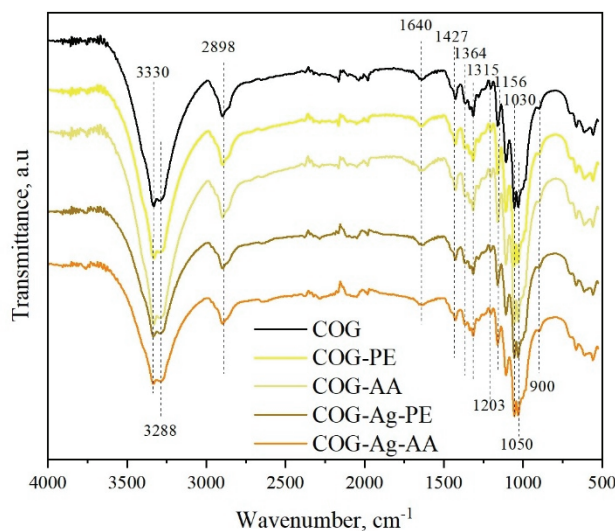


Fig. 6. FTIR spectra of COG, COG-PE, COG-AA, COG-Ag-PE and COG-Ag-AA samples.

Table III summarises the results of antibacterial reduction of *E. coli* and *S. aureus* after 2 and 24 h of contact with the synthesized samples. The results indicated that COG and COG samples loaded only with plant extracts did not exhibit any antibacterial activity towards the investigated pathogens. The *in situ* synthesis of Ag-based NPs ensured a 99.9% reduction of *S. aureus* just after 2 h of contact with the COG-Ag-PE sample. This sample did not provide sufficient reduction of *E. coli* after 2 h of contact. However, a significant reduction of 99.9 % was achieved after 24 h of contact. The COG-Ag-AA sample ensured a 98.9 % reduction of *S. aureus* after 2 h of contact, and maximum bacterial reduction after 24 h. The COG-Ag-AA sample provided a 99.9 % reduction of *E. coli* after 24 h of contact. Both samples exhibited greater antibacterial activity against Gram-positive bacteria during shorter contact times than against Gram-negative bacteria, where bacteriostatic activity was attained. Despite differences in cell wall structure, it has been well known that Ag NPs have similar antibacterial activity against Gram-positive and Gram-negative bacteria.³⁸ Apparently, a higher silver content or a longer contact time is necessary for the complete inhibition of Gram-negative strains.

Several mechanisms for silver antibacterial activity have been postulated so far: *i*) absorption of free silver ions, which disrupts DNA replication and ATP synthesis; *ii*) production of reactive oxygen species (ROS) by Ag NPs and silver ions; and *iii*) Ag NPs may also cause direct damage to the cell membrane, which could result in the leakage of cell contents.³⁸ In order to provide a more comprehensive

understanding of the antibacterial effects of Ag-based NPs synthesized on cotton gauze, the quantity of silver leached from the sample during the antibacterial evaluation was measured. AAS analysis revealed that 2.33 ± 0.05 and 2.55 ± 0.04 $\mu\text{g/ml}$ of Ag^+ were released from the COG–Ag–AA sample after 2 and 24 h, respectively. The COG–Ag–PE samples showed a similar trend of Ag^+ release, with 2.51 ± 0.28 and 2.54 ± 0.01 $\mu\text{g/ml}$ of Ag^+ released after 2 and 24 h, respectively. The synthesis method, colloidal concentration, NPs size and various other factors significantly affect the minimal inhibitory concentration (*MIC*) of Ag NPs. The estimated *MIC* values for Ag NPs against *E. coli* and *S. aureus* were observed to be in the range of 6.75–320 and 13.5–80 $\mu\text{g/ml}$, respectively, varying according to the size of the NPs.³⁹ Moreover, the *MIC* for both microorganisms spans from 0.25 to 400 $\mu\text{g/ml}$, depending on the synthesis method utilized.³⁹ Some studies indicated that the *MIC* of Ag NPs against *E. coli* varied from 5.087–8.8 $\mu\text{g/ml}$.⁴⁰ Another research revealed that the *MIC* of Ag NPs against *E. coli* was 3.38 mg/l and for *S. aureus* 13.5 mg/l.⁴¹ Given that the reported *MIC* values are significantly higher than the concentration of Ag^+ released from the tested samples, it can be concluded that in our study direct contact with NPs plays a significant role in antibacterial action. Furthermore, the negligible difference observed in the concentrations of Ag^+ released after 2 and 24 h supports this conclusion, demonstrating that the length of contact time plays a crucial role in the antibacterial effect.

TABLE III. Antibacterial activity (CFU/mL) of samples with Ag NPs

Sample	Bacteria							
	<i>E. coli</i>				<i>S. aureus</i>			
	2 h contact	<i>R</i> / %	24 h contact	<i>R</i> / %	2 h contact	<i>R</i> / %	24 h contact	<i>R</i> / %
Inoculum	4.1×10^6	–	4.3×10^6	–	1.8×10^6	–	1.8×10^6	–
COG	8.0×10^5	–	3.0×10^5	–	2.7×10^4	–	2.3×10^4	–
COG–PE	7.7×10^5	3.75	2.8×10^5	–	3.2×10^4	–	1.0×10^4	–
COG–AA	6.9×10^5	13.8	3.5×10^5	–	2.9×10^4	–	3.0×10^4	–
COG–Ag–PE	6.4×10^5	20.0	400	99.9	0	100	0	100
COG–Ag–AA	1.6×10^5	80.5	500	99.9	2.9×10^2	98.9	0	100

Cotton gauze containing Ag NPs as a potential medical textile should be in direct contact with human skin and bodily fluids (blood or lymph). Therefore, understanding the properties of the NPs and their effects on the body and bodily fluids is crucial. To simulate body fluid, a physiological saline solution was selected as a release medium, as its salinity is close to that of blood or lymph. Fig. 7 illustrates the amounts of Ag^+ released from the samples in the physiological saline solution within 24 h. A larger amount of Ag^+ was released from the COG–Ag–AA sample compared to the COG–Ag–PE sample. This supports the assumption that some portion of Ag^+ is loosely bound to the cotton fibres in the COG–Ag–AA sample. The maximum amount of Ag^+ released from the COG–Ag–PE and COG–Ag–AA

samples in the physiological solution was 0.714 ± 0.003 and 1.025 ± 0.020 $\mu\text{mol/g}$, respectively. An analysis of the total amount of silver in the COG–Ag–PE and COG–Ag–AA samples, in conjunction with the maximum release of Ag^+ after 24 h, revealed that approximately 95 and 92 % of the silver was retained in the samples, respectively. These findings are comparable to cotton samples with Ag-based NPs synthesized in the presence of *Juglans regia* and *Humulus lupulus* extracts.²⁶

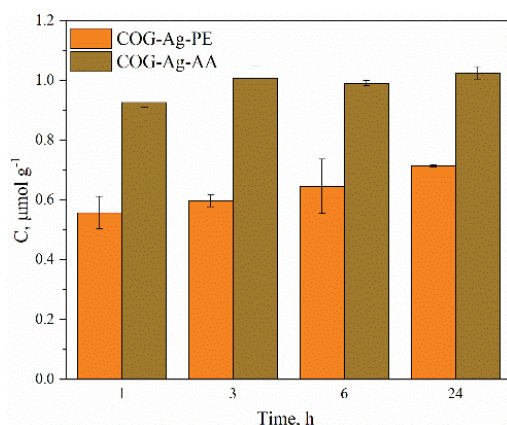


Fig. 7. Release of Ag^+ from the samples into physiological saline solution.

CONCLUSION

This study revealed that extracts from *P. euramericana* and *A. altissima* leaves could be utilized for the synthesis of Ag-based NPs on cotton gauze. FESEM analysis and EDS mapping showed that spherical, mostly single Ag-based NPs were evenly distributed across the surface of the cotton fibres. The total amount of silver in the samples, as well as the size and shape of the NPs, depend only negligibly on the applied plant extracts. Even though the total silver content is similar, a higher number of nanoparticles with larger sizes was detected in the sample derived from the *P. euramericana* extract, suggesting that a certain amount of silver ions was only adsorbed onto the samples synthesized using *A. altissima* (Mill.) Swingle leaves extract. Antibacterial tests revealed time-dependent antibacterial activity. However, the concentration of silver in all samples was sufficient to provide excellent antibacterial activity against *E. coli* and *S. aureus* after 24 h. Release studies demonstrated that 92–95 % of the silver was retained in the samples after a 24 h exposure to a physiological saline solution, suggesting a high level of stability in the samples.

Acknowledgments. This work was financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200017, 451-03-136/2025-03/200135 and 451-03-136/2025-03/200003). The authors appreciate Prof. dr. Slobodan D. Milanović from the Faculty of Forestry, University of Belgrade, for collecting *A. altissima* leaves.

ИЗВОД

ОДРЖИВА СИНТЕЗА НАНОЧЕСТИЦА СРЕБРА НА ПАМУЧНОЈ ГАЗИ ЗА ПОБОЉШАНА АНТИБАКТЕРИЈСКА СВОЈСТВА

ДАРКА Д. МАРКОВИЋ¹, ВАЊА Б. ТАДИЋ², АНА Р. ЖУГИЋ² и МАЈА М. РАДЕТИЋ³¹Универзитет у Београду, Институт за нуклеарне науке „Винча“ – Институт од националног значаја, Михајла Пейровића Аласа 12–14, Београд, ²Универзитет у Београду, Институт за проучавање лековитих биља „Јосиф Панчић“, Тадеуша Кошћушка 1, 11000 Београд и ³Универзитет у Београду, Технолошко–металуршки факултет, Карнегијева 4, 11000 Београд

Заштита рана је кључни корак у превенцији или смањењу инфекција, као и преношења инфекција између пацијената. Завоји за ране су неопходни медицински производи који покривају ране и олакшавају зарастање. У овом раду се испитује могућност коришћења наночестица сребра за обезбеђивање антибактеријске активности памучној гази, често коришћеном материјалу за једнократну употребу за завоје за ране. *In situ* синтеза наночестица на бази сребра на памучној гази постигнута је коришћењем екстракта из листова *Populus x euramericana* (PE) и *Ailanthus altissima* (Mill.) Swingle (AA). Главна фитохемијска једињења у екстрактима су квантификована помоћу HPLC. FESEM и EDS анализа мапирања површине потврдила су присуство наночестица на бази сребра на површини влакана оба узорка. Просечна величина наночестица синтетизованих у присуству екстракта PE и AA износила је 88 ± 26 nm односно 82 ± 23 nm. Сличан садржај сребра пронађен је у узорку добијеном са екстрактом PE ($14,22 \pm 0,23$ $\mu\text{mol/g}$) у поређењу са узорком синтетизованим коришћењем екстракта AA ($13,63 \pm 1,40$ $\mu\text{mol/g}$). Синтетизовани узорци остварују максималну редукцију Грам-негативних бактерија *Escherichia coli* и Грам-позитивних бактерија *Staphylococcus aureus*.

(Примљено 3. јуна, ревидирано 15. јула, прихваћено 23. септембра 2025)

REFERENCES

1. F. Feng, Z. Zhao, J. Li, Y. Huang, W. Chen, *Prog. Mat. Sci.* **146** (2024) 101328 (<https://doi.org/10.1016/j.pmatsci.2024.101328>)
2. S. Lv, Y. Liu, C. Liu, J. Yin, D. An, S. Deng, G. Liu, *Ind. Crop. Prod.* **224** (2025) 120384 (<https://doi.org/10.1016/j.indcrop.2024.120384>)
3. U. L. Bristi, A. Rahman, S. B. Malitha, O. Rahman, S. Shoukat, *Sci. Rep.* **14** (2024) 31650 (<https://doi.org/10.1038/s41598-024-80318-0>)
4. O. S Boateng, K. H. Matthews, H. N. E. Stevens, G. M. Eccleston, *J. Pharm. Sci.* **97** (2008) 2892 (<https://doi.org/10.1002/jps.21210>)
5. K. Rahim, S. Saleha, X. Zhu, L. Huo, A. Basit, O. L. Franco, *Microb. Ecol.* **73** (2017) 710 (<https://doi.org/10.1007/s00248-016-0867-9>)
6. M. M. Mihai, M. Preda, I. Lungu, C. M. Gestaki, M. I. Popa, A. Holban, *Int. J. Mol. Sci.* **19** (2018) 1179 (<https://doi.org/10.3390/ijms19041179>)
7. A. Hanczvikkel, A. Vig, A. Tóth, *J. Ind. Text.* **48** (2019) 1113 (<https://doi.org/10.1177/1528083718754901>)
8. C. Z. Bueno, A. M. Moraes, *Mat. Sci. Eng., C* **93** (2018) 671 (<https://doi.org/10.1016/j.msec.2018.07.076>)
9. A. Basu, K. Heitz, M. Strømme, K. Welch, N. Ferraz, *Carbohydr. Polym.* **181** (2018) 345 (<https://doi.org/10.1016/j.carbpol.2017.10.085>)
10. G. Liu, J. Xiang, Q. Xia, K. Li, T. Lan, L. Yu, *Cellulose* **26** (2019) 1383 (<https://link.springer.com/article/10.1007/s10570-018-2110-y>)

11. Y. Dai, H. Li, J. Wan, L. Liang, J. Yan, *Ind. Crop. Prod.* **208** (2024) 117871 (<https://doi.org/10.1016/j.indcrop.2023.117871>)
12. B. Simončič, B. Tomšič, *Text. Res. J.* **80** (2010) 1721 (<https://doi.org/10.1177/0040517510363193>)
13. M. Radetić, *J. Mater. Sci.* **48** (2013) 95 (<https://doi.org/10.1007/s10853-012-6677-7>)
14. V. Bhandari, S. Jose, P. Badanayak, A. Sankaran, V. Anandan, *Ind. Eng. Chem. Res.* **61** (2022) 86 (<http://dx.doi.org/10.1021/acs.iecr.1c04203>)
15. S. Milovanovic, M. Stamenic, D. Markovic, M. Radetic, I. Zizovic, *J. Supercrit. Fluids* **84** (2013) 173 (<http://dx.doi.org/10.1016/j.supflu.2013.10.003>)
16. R. Nawaz, S. T. R. Naqvi, B. Fatima, N. Zulfiqar, M. U. Farooq, M. N. ul Haq, D. Hussain, A. Javeed, A. Rasul, L. Jafri, S. t Majeed, W. Q. Khan, *Sci. Rep.* **12** (2022) 2493 (<https://doi.org/10.1038/s41598-022-06391-5>)
17. S. Srisod, K. Motina, T. Inprasit, P. Pisitsak, *Prog. Org. Coat.* **120** (2018) 123 (<https://doi.org/10.1016/j.porgcoat.2018.03.007>)
18. A. M. Abdelgawad, E. Shaban, D. A. Elsherbiny, R. A. El-Sherbiny, H. Farouk, I. El-Tantawy El-Sayed, *Fib. Polym.* **26** (2025) 1223 (<https://doi.org/10.1007/s12221-025-00880-w>)
19. M. Khan, M. R. Shaik, S. F. Adil, S. T. Khan, A. Al-Warthan, M. H. Siddiqui, M. N. Tahir, W. Tremel, *Dalton Trans.* **47** (2018) 11988 (<https://doi.org/10.1039/c8dt01152d>)
20. M.A. Kakakhel, W. Sajjad, F. Wu, N. Bibi, K. Shah, Z. Yali, W. Wang, *J. Hazard. Mater. Adv.* **1** (2021) 100005 (<https://doi.org/10.1016/j.hazadv.2021.100005>)
21. A. Soleimani-Gorgani, O. Avinc, R. Alborz, *J. Clean. Prod.* **386** (2023) 135796 (<https://doi.org/10.1016/j.jclepro.2022.135796>)
22. M. Maghimaa, S. A. Alharbi, *J. Photochem. Photobiol., B* **204** (2020) 111806 (<https://doi.org/10.1016/j.jphotobiol.2020.111806>)
23. Z. Yu, J. Liu, H. He, Y. Wang, Y. Zhao, Q. Lu, Y. Qin, Y. Ke, Y. Peng, *Cellulose* **28** (2021) 1827 (<https://doi.org/10.1007/s10570-020-03639-z>)
24. N. Čuk, M. Šala, M. Gorjanc, *Cellulose* **28** (2021) 3215 (<https://doi.org/10.1007/s10570-021-03715-y>)
25. D. Štular, E. Savio, B. Simončič, M. Šobak, I. Jerman, I. Poljanšek, A. Ferri, B. Tomšič, *Appl. Surf. Sci.* **563** (2021) 150361 (<https://doi.org/10.1016/j.apsusc.2021.150361>)
26. A. Krkobabić, M. Radetić, A. Zille, A. I. Ribeiro, V. Tadić, T. Ilic-Tomic, D. Marković, *Molecules* **29** (2024) 1447 (<https://doi.org/10.3390/molecules29071447>)
27. A. Krkobabić, M. Radetić, H.H. Tseng, T.S. Nunney, V. Tadić, T. Ilic-Tomic, D. Marković, *Appl. Surf. Sci.* **611** (2023) 155612 (<https://doi.org/10.1016/j.apsusc.2022.155612>)
28. W. Ding, H. Li, J. Wen, *Ecol. Ind.* **143** (2022) 109408 (<https://doi.org/10.1016/j.ecolind.2022.109408>)
29. M. Arshad, A. Khan, Z. H. Farooqi, M. U. M. A. Waseem, S. A. Shah, M. Khan, *Mat. Sci.-Pol.* **36** (2018) 21 (<https://dx.doi.org/10.1515/msp-2017-0100>)
30. Y.S. Velioglu, G. Mazza, L. Gao, B.D. Oomah, *J. Agricul. Food Chem.* **46** (1998) 4113 (<https://doi.org/10.1021/jf9801973>)
31. *European Pharmacopeia*, 11th ed., Council of Europe, Strasbourg, 2023
32. S. S. R. Albeladi, M. A. Malik, S. A. Al-thabaiti, *J. Mat. Res. Tech.* **9** (2020) 10031 (<https://doi.org/10.1016/j.jmrt.2020.06.074>)
33. C. Tamuly, M. Hazarika, S. Ch. Borah, M. R. Das, M. P. Boruah, *Colloids Surfaces, B* **102** (2013) 627 (<https://doi.org/10.1016/j.colsurfb.2012.09.007>)
34. N. Ahmad, S. Bhatnagar, S. S. Ali, R. Dutta, *Int. J. Nanomed.* **10** (2015) 7019 (<https://doi.org/10.2147/ijn.s94479>)

35. V. Ilić, Z. Šaponjić, V. Vodnik, B. Potkonjak, P. Jovančić, J. Nedeljković, M. Radetić, *Carbohydr. Polym.* **78** (2009) 564 (<https://dx.doi.org/10.1016/j.carbpol.2009.05.015>)
36. A. Krkobabić, D. Marković, A. Kovačević, V. Tadić, M. Radoičić, T. Barudžija, T. Ilic-Tomic, M. Radetić, *Fib. Polym.* **23** (2022) 954 (<https://doi.org/10.1007/s12221-022-4639-5>)
37. C. Chung, M. Lee, E. Choe, *Carbohydr. Polym.* **58** (2004) 417 (<https://doi.org/10.1016/j.carbpol.2004.08.005>)
38. N. Durán, M. Durán, M. B. de Jesus, A. B. Seabra, W. J. Fávaro, G. Nakazato, *Nanomed. Nanotechnol. Biol. Med.* **12** (2016) 789 (<http://dx.doi.org/10.1016/j.nano.2015.11.016>)
39. C. M. Crisan, T. Mocan, M. Manolea, L. I. Lasca, F. A. Tăăran, L. Mocan, *Appl. Sci.* **11** (2021) 1120 (<https://doi.org/10.3390/app11031120>)
40. I. S. Sandí, S. C. Fuentes, R. P. Reyes, J. R. V. Baudrit, D. B. Menezes, G. M. O. Vásquez, *Biotechnol. Rep.* **40** (2023) e00816 (<https://doi.org/10.1016/j.btre.2023.e00816>)
41. L. Hochvaldová, D. Panáček, L. Váľková, R. Večeřová, M. Kolář, R. Pucek, L. Kvítek, A. Panáček, *Commun. Biol.* **7** (2024) 1552 (<https://doi.org/10.1038/s42003-024-07266-3>)



Pressure oxidative leaching of copper concentrate

GUNCHIN CHUNAG¹, ULZIIDELGER BYAMBASUREN¹, SARNAI ALTANTSETSEG¹,
GANCHIMEG YUNDEN¹, SUKHBAT SUKHBAAATAR²
and DORJGOTOV ALTANSUKH^{1*}

¹Department of Chemical Engineering, School of Applied Sciences, Mongolian University of Science and Technology, Ulaanbaatar 14191, Mongolia and ²Industrial Policy Department, Ministry of Industry and Mineral Resources, Ulaanbaatar, Mongolia

(Received 22 April, revised 17 May, accepted 17 June 2025)

Abstract: This study investigated the oxidative leaching of copper concentrate using a mixture of ferric ions and sulfuric acid solutions. We examined the effects of various parameters, including temperature, initial sulfuric acid concentration and slurry filtration conditions. At lower temperatures (150 °C), the leaching process resulted in the elemental sulfur and jarosite minerals formed in the solid residue. In contrast, at higher temperatures (190 °C), the solid residue contained jarosite and hematite, the most elemental sulfur-oxidizing to sulfuric acid. Under optimal conditions, a leaching temperature of 190 °C, a concentrate-to-leaching solvent (Fe^{3+} 5 g L⁻¹ and H_2SO_4 50 g L⁻¹) ratio of 1:8, an oxygen pressure of 1.0 MPa, and a solid phase particle size of less than 20 μm the dissolution rate of copper reached 98 % after 3 h. When the sulfuric acid concentration increased from 30 to 100 g L⁻¹, the amount of copper increased from 40 to 48 g L⁻¹. Furthermore, rapid filtering of the leaching solution without cooling helped retain most of the iron in the solid phase, resulting in a relatively pure solution. The solid residue was analyzed using X-ray diffraction and scanning electron microscopy.

Keywords: hydrometallurgy; ferric ion; chalcopyrite acidic leaching.

INTRODUCTION

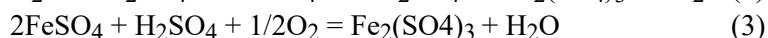
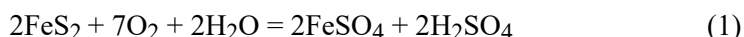
The largest copper production plants are the Erdenet and Oyu Tolgoi in Mongolia. Copper products exported between 2018 and 2023, including copper concentrate, is 8.49 million tons, and cathode copper is 66.92 thousand tons.¹ Currently, cathode copper production is less than 1 % of total exports, which means the basic research and production of deep processing and purification technologies for copper concentrate have taken the attention of researchers in Mongolia.

*Corresponding author. E-mail: E-mail: gotov@must.edu.mn
<https://doi.org/10.2298/JSC250422044C>

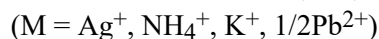
Chalcopyrite is one of the most abundant minerals, accounting for about 70 % of the world's copper reserves.^{2–4} Galena, sphalerite, pyrite, arsenic, antimony bismuth, silver and gold also occurred as associated with the minerals.^{4–6} Hydrometallurgical processing is a more efficient and environmentally friendly technology for sulfide sulfur-containing concentrates than the pyrometallurgical method.⁷ Research has been increasingly conducted to separate the base metals copper and zinc from the residual solids during the autoclave dissolution of metal-containing sulfide concentrates, thereby transferring them to solution and separating silver and gold as products.⁴

Sulfuric acid is a reagent that does not decompose under the influence of temperature, has a high boiling point, is non-volatile, is reasonably cheap, does not interfere with subsequent production processes and is recycled in the leaching process. The mineral chalcopyrite forms the majority of copper concentrates, with a high melting point and low solubility. The dissolution rate could be decreased due to the layer of elemental sulfur coat the surface of the mineral during the leaching. Hydrometallurgical processing of sulfide-containing copper concentrates is developing in atmospheric and pressure leaching (autoclave leaching).^{8,9} Depending on the temperature, autoclave leaching is classified as low-temperature (<120 °C), medium-temperature (120–180 °C) and high-temperature (>180 °C).¹⁰ Sabina Matuska *et al.* reported that the copper dissolution rate increased from 85 to 98 % when the oxygen pressure increased from 0.5 to 2 MPa at 140 °C within 3 h in 1 M sulfuric acidic solution.¹¹

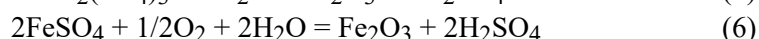
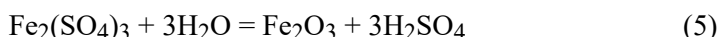
The iron-containing minerals in copper concentrate are chalcopyrite and pyrite. When these minerals are treated with a sulfuric acid solution at 180–200 °C, Fe³⁺ is formed by the dissolution of chalcopyrite and Fe²⁺ by the dissolution of pyrite. At the same time, the sulfide sulfur is converted to the sulfate sulfur to form free sulfuric acid, and ferric ion hydrolysis reactions occur, as shown in the following equations (forming jarosite and hematite):⁶



The reaction for the formation of the jarosite mineral:

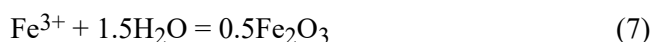


The reaction for the formation of hematite mineral:



There are results reported as mentioned above according to technology on pressure leaching of sulfide concentrate for recovery metals.^{9,12} However, a lack

of research results regarding hot filtration, that is, rapid separation of the solid–liquid phase, is an effective factor in controlling the ferric ion content in the leaching solution, which is transferred directly to the electrowinning process. Ferric ions act to increase the consumption of electrical energy during electrowinning. Rapidly separating a solution from a solid phase, *i.e.*, during the hot filtration process, ferric ions at high temperatures can undergo hydrolysis according to the following equation and precipitate out of the solution in the form of hematite:



However, when the solution is cooled, the reaction reverses, causing the iron to dissolve, and the solution becomes contaminated with ferric ions. Therefore, when the solution is filtered rapidly without cooling, most of the iron remains in the solid phase, and the solution has a relatively high purity.^{13,14}

In this work, copper concentrate from the “Erdenet” mining plant in Mongolia was used in pressure oxidative leaching at a temperature range of 150 and 190 °C. We focused on comparing the leaching efficiency of iron and copper at various parameters, including temperature, initial sulfuric acid concentration and slurry filtration conditions.

EXPERIMENTAL

Sample preparation

Copper concentrate from the “Erdenet” mining plant in Mongolia was milled in a ring mill for 5 min. Due to the small size of the concentrate, a wet sieving method was employed after milling, as it is challenging to dry sieve material of this fineness. The milled concentrate was sieved through a 25 µm sieve. After sieving, the concentrate was dried at 60 °C before being analyzed and used for further experiments.

Reagents

The leaching solutions were prepared by dissolving analytical grade $\text{Fe}_2(\text{SO}_4)_3$, CuSO_4 , and H_2SO_4 (Xilong Scientific Co. Ltd. in China) in deionized water. For the study on the effect of temperature, the initial concentrations of H_2SO_4 and Fe^{3+} were chosen as 50 and 5 g L⁻¹, respectively, within a temperature range of 150–190 °C. To investigate the effect of sulfuric acid concentration, the initial concentrations of H_2SO_4 , Fe^{3+} and Cu^{2+} were chosen as 50, 5 and 30 g L⁻¹, respectively, with sulfuric acid concentrations ranging from 30 to 100 g L⁻¹.

Apparatus and procedure

The pressure oxidation dissolution experiments were conducted using a Hastelloy autoclave (model BFK-1/8, manufactured by Welhal Borui Chemical Machinery Co. Ltd., China) equipped with a Teflon vessel. This autoclave features a PID controller, which ensures accurate temperature control within ±1 °C. Throughout the experiments, both the oxygen pressure and temperature were carefully monitored. In each experiment, 240 ml of leaching solvent was added to 30 g of milled copper concentrate sample with a particle size of 25 µm. The leaching process was performed at 150, 170 and 190 °C under an oxygen pressure of 1 MPa. The slurry was agitated with an anchor impeller at 150 rpm for three hours to enhance oxygen saturation in the leaching solution. After completing the dissolution process, the heating was turned off,

and the autoclave was allowed to cool to 80 °C. Subsequently, the resulting slurry was filtered to separate the solid residue from the pregnant leach solution. The solid and liquid phases were then analyzed using scanning electron microscopy (SEM, TM-1000, Hitachi Inc., Japan), X-ray diffraction (XRD, Diffractis 583 X-ray generator, Enraf-Nonius, the Netherlands) and inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer NexION 300Q ICP-MS, USA) for mineralogical and chemical analysis. The following equation was used to determine the metal leaching rate:

$$\varepsilon_{\text{Me}} = 100 \frac{m_{\text{R}} C_{\text{R}}}{m_{\text{F}} C_{\text{F}}} \quad (8)$$

where m_{R} is a mass of solid residue after leaching, g; C_{R} is a content of metal in solid residue, %; m_{F} is a mass of metal concentrate, g; C_{F} is a content of metal in concentrate, %.

RESULTS AND DISCUSSION

Raw material analysis

The compositional characteristics of the copper concentrate were analyzed using SEM/EDS, XRD and ICP-OES equipment. The results from the SEM/EDS analysis (Fig. 1) indicate that the concentrate has a relatively large particle size of approximately 200 μm . It suggests that milling is necessary for effective process autoclave leaching. The content of the main elements and minerals in the concentrate was determined through ICP-OES and XRD analyses; the results are presented in Tables I and II.

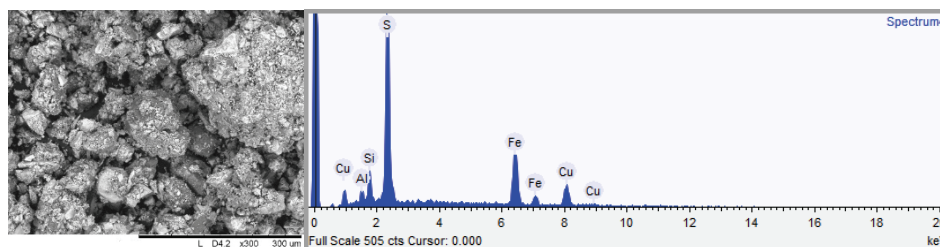


Fig. 1. SEM image and EDS spectra of the copper concentrate.

TABLE I. The content of the main elements, mass %

Sample	Element				
	Al	Si	S	Fe	Cu
Copper concentrate	1.46	3.6	30.05	25.8	22.65

TABLE II. The content of the minerals, mass %

Sample	Mineral			
	CuFeS_2	FeS_2	SiO_2	$\text{CaAlSi}_2\text{O}_8$
Copper concentrate	47.83	23.79	17.13	11.24

The analysis of the X-ray spectrum reveals that the concentrate is primarily composed of three elements: Cu at 22.65 %, Fe at 25.8 % and S at 30.5 %. Furthermore, the X-ray diffraction analysis identifies the main minerals shown as as chalcopyrite (CuFeS_2) at 47.83 % and pyrite (FeS_2) at 23.79 %. The remaining minerals consist of barren rock.¹⁵

Effect of temperature

The pressure oxidation dissolution experiments were conducted at 150, 170 and 190 °C, respectively, with an oxygen pressure of 1 MPa for 3 h. The results, shown in Fig. 2, indicate that, as the temperature increased from 150 to 190 °C, the leaching rate of copper and concentration of free sulfuric acid increased when the leaching rate of iron decreased.

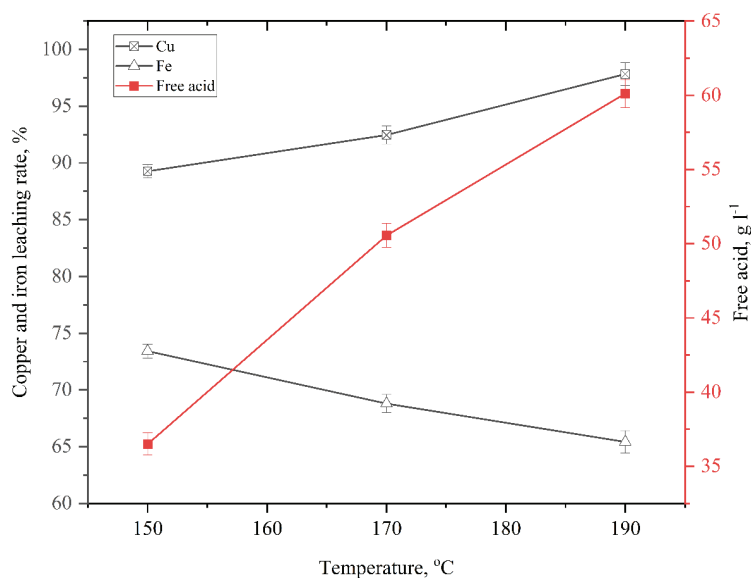


Fig. 2. The effect of temperature on copper and iron leaching (initial concentration sulfuric acid 50 g L⁻¹).

The pregnant leach solution and solid residue from the slurry were analyzed using ICP-OES and XRD. The results are presented in Tables III and IV.

TABLE III. The composition of both the pregnant leach solution and solid residue

Temperature °C	The composition of the pregnant leach solution, g L ⁻¹			pH of the pregnant leach solution	The composition of the solid residue, mass %		
	Cu ²⁺	Fe ³⁺	SO ₄ ²⁻		Cu	Fe	S (total)
150	19.0	31.5	123.9	0.87	4.5	16.8	39.09
170	21.2	30.5	144	0.56	3.73	18.3	30.32
190	22.5	26.7	147	0.56	1.34	24.5	24.3

TABLE IV. Mineral composition (mass %) of solid residue after the leaching

Temperature °C	Mineral					
	NaAlSi ₃ O ₈	S	FeS ₂	SiO ₂	CuFeS ₂	[H ₃ O ⁺]Fe ₃ (SO ₄)(OH) ₆
150	28.45	23.26	16.36	13.64	10.75	7.69
170	22.70	18.51	12.52	19.71	12.88	5.27
190	23.45	3.17	45.79	15.11	3.84	3.74

Table IV presents the results of the XRD analysis of the solid residue, indicating that the mass of the undissolved solid portion contains minerals such as quartz, albite, anorthite, chalcopryrite, pyrite and various other sulfide minerals. A comparison of the mineral composition between the concentrate and the solid residue after dissolution shows that elemental sulfur may be formed during the dissolution process. The elemental sulfur of the total sulfur in the solid residue after dissolution decreased when the dissolution temperature increased.

The SEM image illustrates that small pellets or granular particles of elemental sulfur are predominant at low temperatures (Fig. 3).

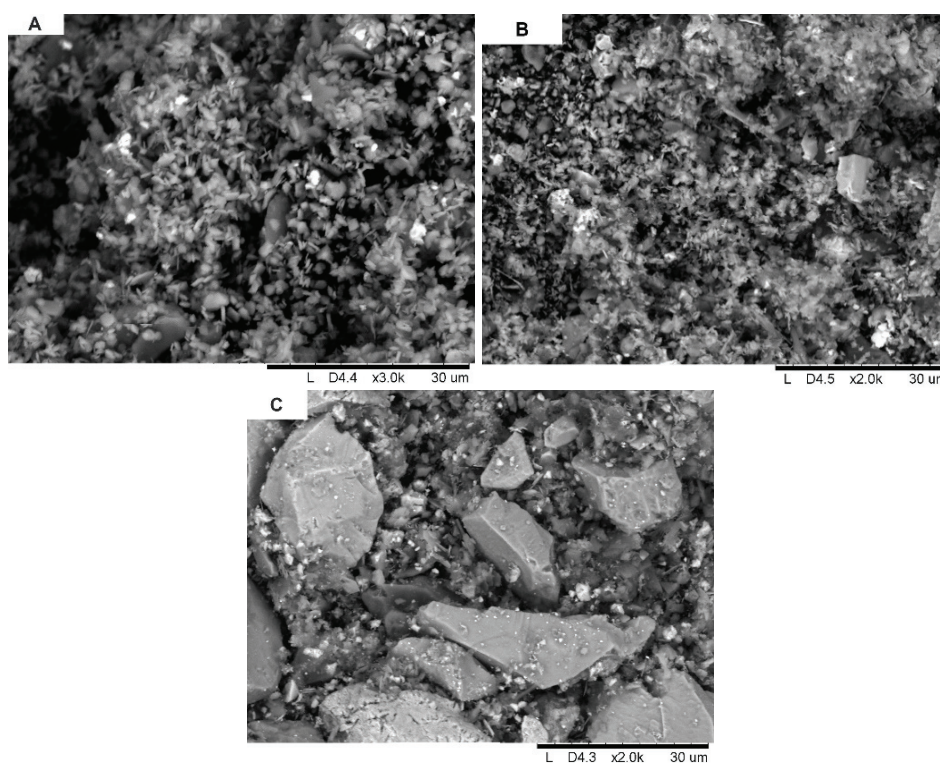


Fig. 3. SEM image of solid residue after leaching at 150 (A), 170 (B) and 190 °C (C).

According to the SEM/EDX analysis of the solid residue at 190 °C dissolution, elemental sulfur accounted for 3.17 % of the total sulfur. When the dissolution temperature was increased from 150 to 190 °C, the amount of undissolved chalcopyrite in the solid residue decreased from 10.75 to 3.84 %, and the amount of elemental sulfur decreased and was transferred to the solution in the form of sulfate, increasing the amount of free sulfuric acid. However, the content of the pyrite was increased from 16.36 to 45.79 %. The dissolution of pyrite minerals decreased with increasing temperature. The results showed that pyrite was more insoluble than chalcopyrite due to the relative hardness of chalcopyrite and pyrite 3.5–4 and 6–6.56, respectively. Moreover, during milling, the particle size of pyrite is bigger than that of chalcopyrite, which can be affected by reducing the specific surface area and reducing dissolution.¹⁵

Effect of sulfuric acid concentration and filtration condition

When the sulfuric acid concentration was increased from 30 to 100 g L⁻¹ at the leaching temperature of 150 and 190 °C, the leaching rate of copper and iron was compared in Figs. 4 and 5. As shown in Fig. 4, the copper ion content varied from 40 to 43.8 g L⁻¹ when the solution was filtered with and without cooling, which was relatively close. However, the concentration of iron ions increased from 22 to 27 g L⁻¹ and from 31.3 to 34 g L⁻¹ when the solution was filtered with and without cooling, respectively (at 150 °C).

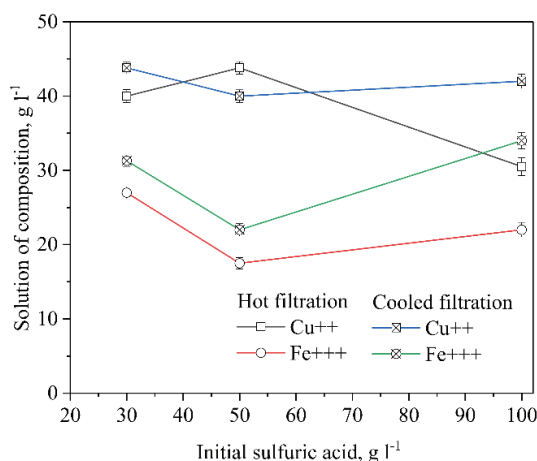


Fig. 4. The effect of sulfuric acid concentration and filter condition on copper and iron dissolution at 150 °C.

Fig 5 illustrates the effects of increasing sulfuric acid concentration on metal dissolution at 190 °C. As the concentration of sulfuric acid rises from 30 to 100 g L⁻¹, the leaching rate of copper slightly decreases while the leaching rate of iron increases. When the solution was rapidly filtered from the autoclave while still hot,

the concentration of copper ions ranged from 41.3 to 48 g L⁻¹, and the concentration of iron ions increased from 7.3 to 14 g L⁻¹. Conversely, when the solution was filtered after cooling, the concentration of iron ions in the solution increased from 31.3 to 37.14 g L⁻¹. Notably, at a sulfuric acid concentration of 50 g L⁻¹, copper dissolution peaked, resulting in an iron ion concentration of 7.5 g L⁻¹. These results indicate that rapidly separating the solution from the solid phase without allowing it to cool after pressure leaching is crucial for reducing ferric ions in the solution, as iron is precipitated as hematite during the oxidation pressure leaching process.

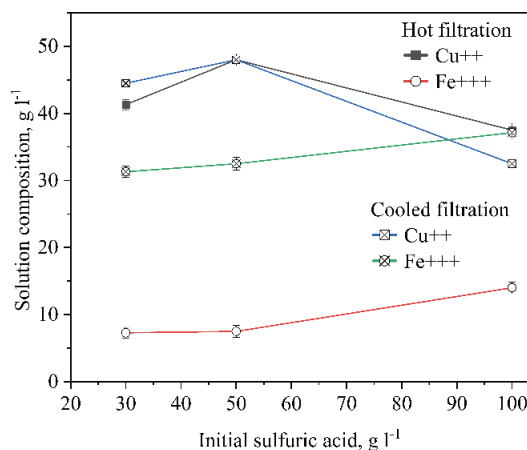


Fig. 5. The effect of sulfuric acid concentration and filter condition on copper and iron dissolution at 190 °C.

The X-ray diffraction patterns of the solid residue obtained from rapid filtration (without cooling) at temperatures of 150 and 190 °C are presented in Fig. 6. The peaks corresponding to elemental sulfur and jarosite are generated at low temperatures (150 °C). At 190 °C, new peaks associated with the minerals jarosite and hematite were detected in the XRD pattern of the solid residue. The peaks of elemental sulfur disappeared at 190 °C, which indicates that the sulfur oxidized to sulfuric acid at high temperatures.

Fig. 7 illustrates the X-ray diffraction patterns of the solid residue formed filtration after cooling. A general symptom was that a new peak for elemental sulfur was identified at 2θ 27.78°, and the intensity of the peaks for the mineral pyrite generally decreased. This analysis suggests that elemental sulfur formed by the decomposition of chalcopyrite minerals transitioned from a solid to a liquid state between 150 and 190 °C, resulting in a reduced quantity of sulfur in the hot-filtered sample. Furthermore, during the cooling of the slurry, new elemental sulfur formed on the surface of the solid phase, as indicated by the X-ray diffraction results. The analysis results suggest that the amount of elemental sulfur detected

in solid residue obtained from rapid filtration (without cooling) is expected to be low due to the elemental sulfur formed by the decomposition of chalcopyrite minerals must have transitioned from a solid to a liquid state at 150 and 190 °C. However, the X-ray diffraction pattern shows that new elemental sulfur is formed on the surface of the solid phase during the cooling of the slurry.

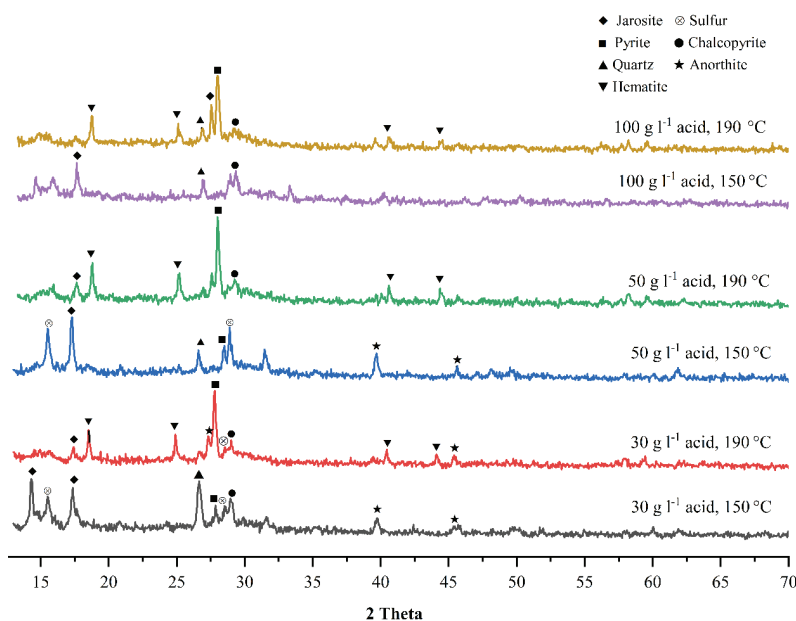


Fig. 6. X-ray diffraction pattern of the solid residue formed rapid filtration (without cooling) of an autoclave solution.

Table V presents the analysis of free sulfuric acid content in leaching solution. It was observed that the amount of free sulfuric acid in the solution increased, while the peak for elemental sulfur was nearly absent in the solid residue at 190 °C (Fig. 6 and Table V). It suggests that the sulfide sulfur in the concentrate was converted to sulfate. Moreover, hematite in the solid residue (190 °C) indicates that iron sulfate, formed by the reaction, decomposed at this temperature to yield free sulfuric acid and hematite. As shown in Fig. 7, the concentration of iron ions in the solution after rapid filtration decreased. This reduction can be expected to lead to the hydrolysis of iron ions in the solution, resulting in the formation of hematite in the solid residue.^{7,16,17}

Based on the above mentioned results, the iron content in the solution obtained from filtration with cooling increased from 7.5 to 37.5 g L⁻¹ (Fig. 4), the intensity of the peak corresponding to the pyrite mineral decreased, and a new peak for hematite mineral was detected (Fig. 7). It suggests that the following reaction may occur.^{18–20}

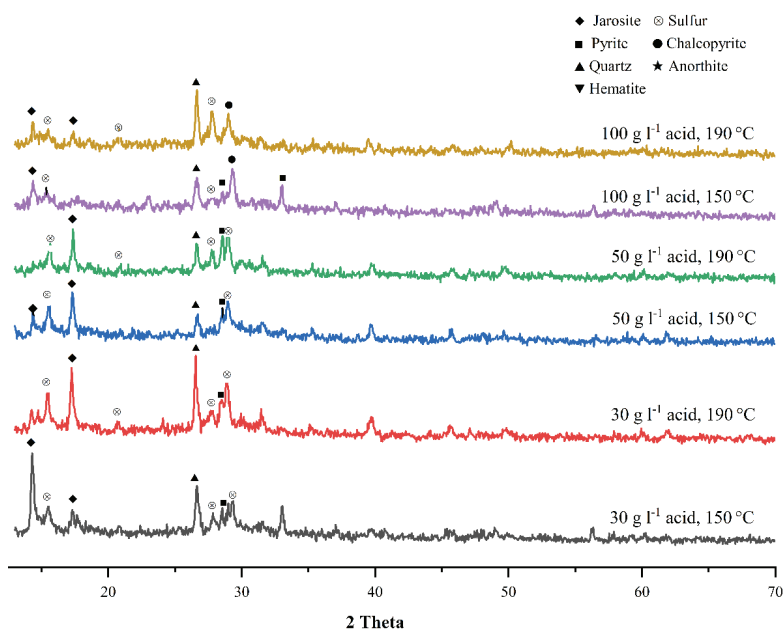


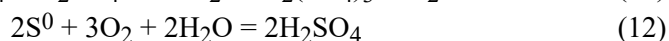
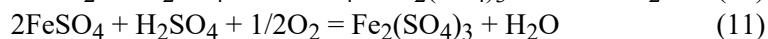
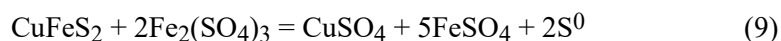
Fig. 7. X-ray diffraction pattern of the solid residue formed filtration (with cooling) of an autoclave solution.

TABLE V. Amount of free sulfuric acid in the leaching solution

H ₂ SO ₄ initial concentration, g L ⁻¹	Temperature, °C	Filtration condition	Free H ₂ SO ₄ , g L ⁻¹
30	150	Without cooling	28.59
		With cooling	22.12
30	190	Without cooling	64.63
		With cooling	40.49
50	150	Without cooling	46.78
		With cooling	42.72
50	190	Without cooling	88.11
		With cooling	46.99
100	150	Without cooling	62.15
		With cooling	62.04
100	190	Without cooling	117.6
		With cooling	92.925

Reactions in the system CuSO₄ – H₂SO₄ – O₂ – H₂O:

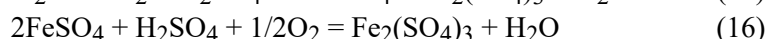
The main reaction that occurs at 150 °C:



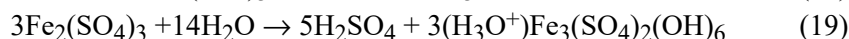
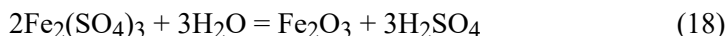
Jarosite formation reaction at 150 °C:



The main reaction that occurs at 190 °C:



Hematite formation reaction at 190 °C:



CONCLUSION

1. When the dissolution temperature was increased from 150 to 190 °C, the amount of copper transferred to the solution increased from 89.27 to 97.84 %, while the dissolution of iron decreased from 73.42 to 65.42 %. However, the amount of elemental sulfur in the solid residue decreased from 39.09 to 24.3 %, and the amount of iron increased.

2. When the sulfuric acid content was increased from 0.3 to 1 M, the amount of copper transferred from the slurry to the solution increased from 40 g to 48 g L⁻¹. However, for iron, the content in the solution increased from 22 to 27 g L⁻¹ and from 31.3 to 34 g L⁻¹ when the slurry was filtered without (rapidly) and with cooling.

3. Most of the sulfur in the initial concentrate remains as elemental sulfur in the solid residue, and the acid content in the solution decreases at low temperatures. However, at high temperatures, elemental sulfur is oxidized to sulfate, leading to an increase in the acid content of the solution.

4. Based on XRD analysis and experimental results, it can be concluded that as the dissolution temperature increases, the solubility of pyrite minerals decreases. As a result, iron ions in the solution undergo hydrolysis, forming new minerals such as jarosite and hematite. Therefore, the amount of iron transferred to the solution reduces while increasing the concentration of free sulfuric acid.

5. It was confirmed that the rapid separation of the solution from the solid phase without cooling after pressure leaching is crucial for decreasing ferric ions in the solution. If rapid filtration of the leach solution is not employed, the leaching solution will contain a significant amount of ferric ions. This high concentration of ferric ions creates issues in the subsequent neutralization step, as many copper ions will adsorb onto the iron hydroxide, making the slurry excessively thick. This thickness is a challenge in separating the solid and liquid phases during neutralization.

ИЗВОД

ОКСИДАТИВНО ЛУЖЕЊЕ БАКАРНОГ КОНЦЕНТРАТА ПОД ПРИТИСКОМ

GUNCHIN CHUNAG¹, ULZIIDEGER BYAMBASUREN¹, SARNAI ALTANTSETSEG¹, GANCHIMEG YUNDEN¹,
SUKHBAT SUKHBAATAR² и DORJGOTOV ALTANSUKH¹

¹Department of Chemical Engineering, School of Applied Sciences, Mongolian University of Science and Technology, Ulaanbaatar 14191, Mongolia и ²Industrial Policy Department, Ministry of Industry and Mineral Resources, Ulaanbaatar, Mongolia

Ова студија је истраживала оксидативно лужење бакарног концентрата коришћењем смеше јона гвожђа(III) и раствора сумпорне киселине. Испитали смо утицаје различитих параметара, укључујући температуру, почетну концентрацију сумпорне киселине и услове филтрације суспензије. На нижим температурама (150 °C), процес лужења је резултирао формирањем елементарног сумпора и минерала јарозита у чврстом остатку. Насупрот томе, на вишим температурама (190 °C), чврсти остатак је садржао јарозит и хематит, при чему се већина елементарног сумпора оксидовала у сумпорну киселину. Под оптималним условима, температура лужења од 190 °C, однос концентрата и растварача за лужење (Fe^{3+} 5 g L⁻¹ и H_2SO_4 50 g L⁻¹) од 1:8, притисак кисеоника од 1.0 МПа и величина честица чврсте фазе мања од 20 μm , стопа растварања бакра достигла је 98 % након три сата. Када је концентрација сумпорне киселине повећана са 30 на 100 g L⁻¹, количина бакра је повећана са 40 на 48 g L⁻¹. Даље, брзо филтрирање раствора лужења без хлађења помогло је да се већина гвожђа задржи у чврстој фази, што је резултирало релативно чистим раствором. Чврсти остатак је анализиран помоћу рендгенске дифракције (XRD) и скенирајуће електронске микроскопије (SEM).

(Примљено 22. априла, ревидирано 17. маја, прихваћено 17. јуна 2024)

REFERENCES

1. 2021/08, <https://mrpam.gov.mn/public/pages/169/2021.08.stat.report.mon.pdf> (accessed: april 2025)
2. N. Tumen-Ulzii, B. Gunchin, *J. Serb. Chem. Soc.* **88** (2023) 1149 (<https://doi.org/10.2298/JSC230114057T>)
3. S. Wang, *Jom* **57** (2005) 48 (<https://doi.org/10.1007/s11837-005-0252-5>)
4. A. A. Baba, K. I. Ayinla, F. A. Adekola, M. K. Ghosh, O. S. Ayanda, R. B. Bale, A. R. Sheik, S. R. Pradhan, *Int. J. Min. Eng. Miner. Process.* **1** (2012) 1 (<https://doi.org/10.5923/j.mining.20120101.01>)
5. J. Cháidez, J. Parga, J. Valenzuela, R. Carrillo, I. Almaguer, *Metals (Basel)* **9** (2019) 189 (<https://doi.org/10.3390/met9020189>)
6. B. Han, B. Altansukh, K. Haga, Y. Takasaki, A. Shibayama, *J. Sustain. Metall.* **3** (2017) 528 (<https://doi.org/10.1007/s40831-017-0135-3>)
7. K. Takatori, H. Kato, A. Yoshimura, Y. Matsuno, *Mining, Metall. Explor.* **38** (2021) 1477 (<https://doi.org/10.1007/s42461-021-00400-3>)
8. M. Sokić, B. Marković, S. Stanković, Ž. Kamberović, N. Štrbac, V. Manojlović, N. Petronijević, *Metals (Basel)* **9** (2019) 1173 (<https://doi.org/10.3390/met9111173>)
9. F. Saloojee, F. K. Crundwell, *J. South Afr. Inst. Min. Metall.* **116** (2016) 517 (<https://doi.org/10.17159/2411-9717/2016/v116n6a5>)
10. S. Heguri, S. Asano, A. Idegami, *J. MMIJ* **131** (2015) 470 (<https://doi.org/10.2473/journalofmmij.131.470>)

11. S. Matuska, K. Ochromowicz, T. Chmielewski, *Physicochem. Probl. Miner. Process.* **54** (2018) 781–792 (<https://bibliotekanauki.pl/articles/110420.pdf>)
12. E. Uzun, M. Zengin, Ý. Atýlgan, *Mater. Tehnol.* **50** (2016) 395 (<https://doi.org/10.17222/mit.2015.091>)
13. E. M. Córdoba, J. A. Muñoz, M. L. Blázquez, F. González, A. Ballester, *Hydrometallurgy* **93** (2008) 81 (<https://doi.org/10.1016/j.hydromet.2008.04.015>)
14. J. Gega, W. Walkowiak, *Physicochem. Probl. Miner. Process.* **46** (2011) 155 (https://www.dbc.wroc.pl/Content/10110/46_2011.pdf)
15. K. J. Nyembwe, E. Fosso-Kankeu, F. Waanders, M. Mkandawire, *Trans. Nonferrous Met. Soc. China (Engl. Ed.)* **31** (2021) 2139 ([https://doi.org/10.1016/S1003-6326\(21\)65644-3](https://doi.org/10.1016/S1003-6326(21)65644-3))
16. S. J. Petrović, G. D. Bogdanović, M. M. Antonijević, M. Vukčević, R. Kovačević, *Metals (Basel)* **13** (2023) 1818 (<https://doi.org/10.3390/met13111818>)
17. S. J. Petrović, G. D. Bogdanović, M. M. Antonijević, *Trans. Nonferrous Met. Soc. China (Engl. Ed.)* **28** (2018) 1444 ([https://doi.org/10.1016/S1003-6326\(18\)64788-0](https://doi.org/10.1016/S1003-6326(18)64788-0))
18. H. B. Zhao, M. H. Hu, Y. N. Li, S. Zhu, W. Q. Qin, G. Z. Qiu, J. Wang, *Trans. Nonferrous Met. Soc. China (English Ed.)* **25** (2015) 303 ([https://doi.org/10.1016/S1003-6326\(15\)63605-6](https://doi.org/10.1016/S1003-6326(15)63605-6))
19. Y. Xing, C. Wei, Z. Deng, X. Li, M. Li, *Sci. Rep.* **14** (2024) 24490 (<https://doi.org/10.1038/s41598-024-75857-5>)
20. C. Li, Z. Deng, C. Wei, G. Fan, X. Li, M. Li, Y. Wang, *Hydrometallurgy* **178** (2018) 294 (<https://doi.org/10.1016/j.hydromet.2018.05.012>).



J. Serb. Chem. Soc. 90 (12) 1527–1536 (2025)
JSCS–5470

Tri- and tetracyclic terpanes as proxies for depositional environment reconstruction for weathered siliciclastics of Internal Dinarides

IVANA JOVANIĆ¹, ALEKSANDRA ŠAJNOVIĆ², SANJA STOJADINOVIC^{2*},
NIKOLA BURAZER², BOJAN GLAVAŠ-TRBIĆ¹ and BRANIMIR JOVANČIČEVIĆ³

¹Geological Survey of Serbia, Rovinjska 12, Belgrade, Serbia, ²University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, Belgrade, Serbia and ³University of Belgrade, Faculty of Chemistry, Studentski trg 12–16, Belgrade, Serbia

(Received 27 December 2024, revised 4 February, accepted 11 April 2025)

Abstract: This study examines the potential of using tri- and tetracyclic terpanes to determine the depositional environment of sediments lacking preserved fossils and whose biomarker distribution has been disrupted by biodegradation, weathering, or other alteration processes. In the case of sediments from Jazovnik, Snagovo, Drina and Gučevo (Internal Dinarides), tricyclic terpanes have demonstrated easy applicability and strong predictive power for distinguishing depositional settings. This study analysed siliciclastic sediments exposed to prolonged chemical weathering, influencing the distribution of hydrocarbons, and resulting in highly abundant branched hydrocarbons and the presence of unresolved complex mixtures (UCMs). The abundances and interrelationships of tri- and tetracyclic terpanes (C₂₀–C₂₄TT, C₂₆TT, C₂₄TeT) showed valuable results. The triangular diagram incorporating C₂₀ + C₂₂%, C₂₁% and C₂₃% has proven particularly effective in distinguishing between swamp, fluvial-deltaic, freshwater lacustrine, and marine-saltwater lacustrine depositional environments. Once again, this method clearly depicts different sedimentary environments, providing valuable insights into the geological history of the studied area.

Keywords: chemical weathering; biodegradation, organic matter; branched alkanes; unresolved complex mixture; paleoenvironmental settings.

INTRODUCTION

Reconstructing the conditions of paleoenvironmental deposition represents one of the most difficult challenges in organic geochemistry. Traces of changes in paleoenvironmental settings are preserved in molecular structures incorporated into the organic matter (OM) of sedimentary rocks, representing the "guardians"

* Corresponding author. E-mail: sanja.stojadinovic@ihtm.bg.ac.rs
<https://doi.org/10.2298/JSC241227026J>

of important information about biogeochemical transformations of OM. Therefore, the application of specific organic-geochemical parameters based on the distribution and abundance of individual classes of saturated and aromatic hydrocarbons is one of the most widely used approaches in interpreting paleoredox conditions, paleoclimate and paleosalinity of the environment, which is most often in connection with other scientific disciplines such as stratigraphy, sedimentology, mineralogy, petrography, *etc.* However, the reconstructions of depositional environments based on organic proxies are often ambiguous or even impossible due to the intense and prolonged chemical weathering that affects the distribution of hydrocarbons, resulting in high relative abundances of branched hydrocarbons and the presence of unresolved complex mixtures (UCMs).^{1,2} Only a few proxies are available for assessing depositional environments, and they rarely allow unambiguous interpretations. Among them, the tricyclic terpanes seem to be the most useful.²

The tricyclic terpanes are the organic compounds ubiquitous in sediments and oils spanning the geological record.^{1,3} Their ubiquities and ability to participate in solving a wide range of different geochemical and geological problems. Abundance, clear origin and resistance to biodegradation (robustness) allow them to have been widely applied in geochemical analyses to determine the oil-to-oil correlations,⁴ the oil-to-source correlations,^{5,6} the thermal maturity,⁷ as well as the depositional environment characteristics⁸ and the OM inputs.^{9–11} In the identifying depositional settings, the specific tricyclic terpanes (*e.g.*, C₁₉ + C₂₀, C₂₁, C₂₃TT) are key for determining of the origin of OM: C₂₃ TTs are often associated with marine environments and can indicate the presence of planktonic organic matter,^{1,3} C₂₁TT is typically derived from the degradation of higher plant material, such as terrigenous plants,⁹ while C₂₂TTs are similar to C₂₁TT also derived from higher plant material, but can show variations based on the maturity and depositional environment.⁵

With all said, the idea behind this work is to reconstruct the depositional settings using tri- and tetracyclic terpanes in sediments of Jazovnik, Snagovo, Drina and Gučevo (Internal Dinarides) that have participated in three sedimentary cycles and characterise the absence of preserved fossils. For this reason, the sediments that were exposed to prolonged chemical weathering, and almost all of them contain over 50 % SiO₂, were considered in this study.

EXPERIMENTAL

Geological settings

Upper Cretaceous–Eocene carbonate and siliciclastic sediments (Fig. 1) were formed as sedimentary cover in two Internal Dinaridic domains: Jadar-Kopaonik and Drina-Ivanjica,^{12,13} close to the Sava zone. The Neoproterozoic–Carboniferous basement metamorphics, predominantly belonging to green schist, facies comprise the Drina-Ivanjica domain while Jadar unit consist of Paleozoic clastics and carbonates. These rocks are overlaid and are overlaid by the Lower Triassic continental to shallow marine meta (clastics) and the Middle to Upper

Triassic shallow marine to basinal formations.^{14,15} The Jadar-Kopaonik and the Drina-Ivanjica thrust sheets are bonded by ophiolites and ophiolitic mélange of western Vardar tectonic unit or thrust sheet.^{12,16}

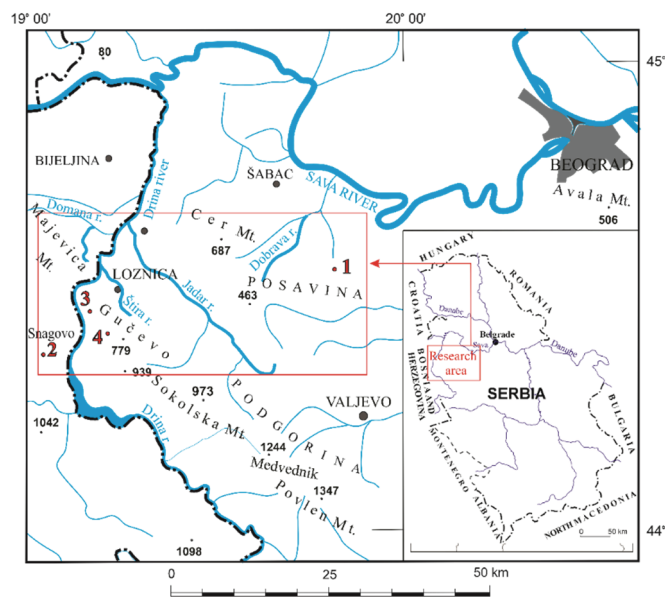


Fig. 1. Map of the investigated area, with localities that have been sampled.
Legend: 1. Jazovnik; 2. Snagovo; 3. Drina River; 4. Gučevo Mt.

Methods

All analysed samples were crushed using a jaw crusher, pulverised in a mill, and later sieved through a 63 μm sieve.

The Soxhlet extraction method was used to isolate the soluble organic matter (bitumen). An azeotropic mixture of dichloromethane and methanol (88:12 volume ratio) served as the solvent. Elemental sulphur was removed by adding copper to the mixture. Afterwards, the saturated hydrocarbon fraction was isolated from bitumen using column chromatography, using the silica gel and aluminium oxide (in a 2:1 mass ratio) as the adsorbent, while *n*-hexane was employed as the eluent. Subsequently, the total saturated fraction was analysed using gas chromatography–mass spectrometry (GC–MS) in total ion chromatogram (TIC) mode. This analysis was conducted with an Agilent 7890A gas chromatograph equipped with an HP-5MS column (30 $\text{m} \times 0.25 \text{ mm}$, 0.25 μm film thickness, with helium as the carrier gas at a flow rate of 1.5 cm^3/min), coupled to an Agilent 5975C mass selective detector. For a detailed analysis of alkanes, branched alkanes and tricyclic terpanes, the typical mass fragments were analysed: m/z 71, 127 and 191, respectively.

RESULTS AND DISCUSSION

The total ion current (TIC) chromatograms of an aliphatic fraction for most investigated samples are characterised by the presence of an unresolved complex mixture (UCM). The unresolved complex mixture often indicates matured organic

matter or a significant contribution of degraded or reworked organic matter.^{1,3} The TIC chromatograms of the two representative samples are shown in Fig. 2. This distribution reflects prolonged chemical weathering to which the sediments from Drina, Gučevo and Snagovo were exposed. The mentioned sediments passed through three cycles of sedimentation (see Geological settings), which influence hydrocarbon distributions, resulting in highly abundant branched hydrocarbons and the presence of UCMs (Fig. 2).

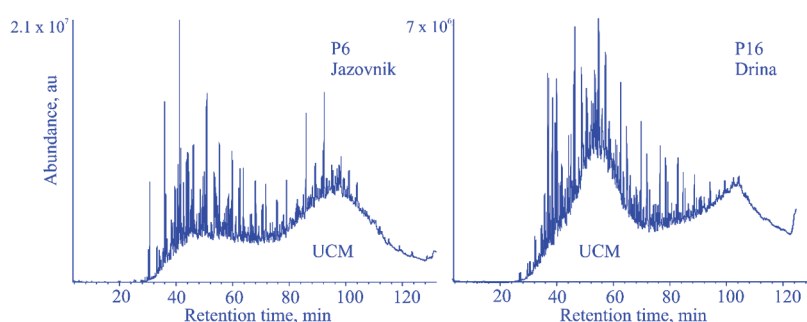


Fig. 2. Total ion currents (TICs) of investigated samples.

Further, *n*-alkanes are absent or present in low abundance in most samples, except for P1–P8 (Jazovnik). In these samples, even homologs predominate with a maximum at *n*-C₁₈alkane (Fig. 3). At the same time, branched alkanes, monitored using ion *m/z* 71, are present in all samples. These hydrocarbons have been identified in cyanobacterial cultures,¹⁷ as well as modern and ancient sediments associated with cyanobacterial mat assemblages.^{18–21} The highly abundant branched alkanes in a large group of studied sediments suggest the presence of microbiologically reworked OM.

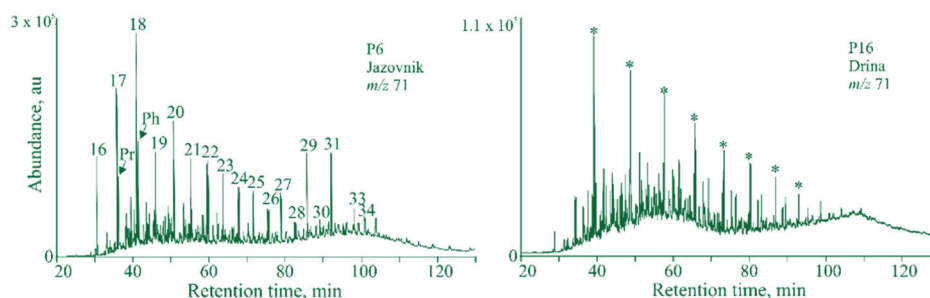


Fig. 3. Fragmentograms of *m/z* 71 for *n*-alkanes and isoprenoids. *n*-Alkanes are labelled according to their carbon number; Legend: Pr – pristane; Ph – phytane; * – 5,5-diethylalkanes.

However, the literature data indicate that tricyclic terpanes (TT) are significantly more resistant to chemical changes and can be a reliable tool for determining

the depositional environment.^{1,3} For example, some studies show that the difference in the distributions of tricyclic and tetracyclic terpane (TeT) ratios can help distinguish sediments and crude oils derived from different sedimentary environments.^{4,6,9} In the aliphatic fraction, a series of tricyclic terpanes were identified, including C₂₀TT, C₂₁TT, C₂₂TT, C₂₃TT, C₂₄TT and C₂₆TT, as well as C₂₄TeT

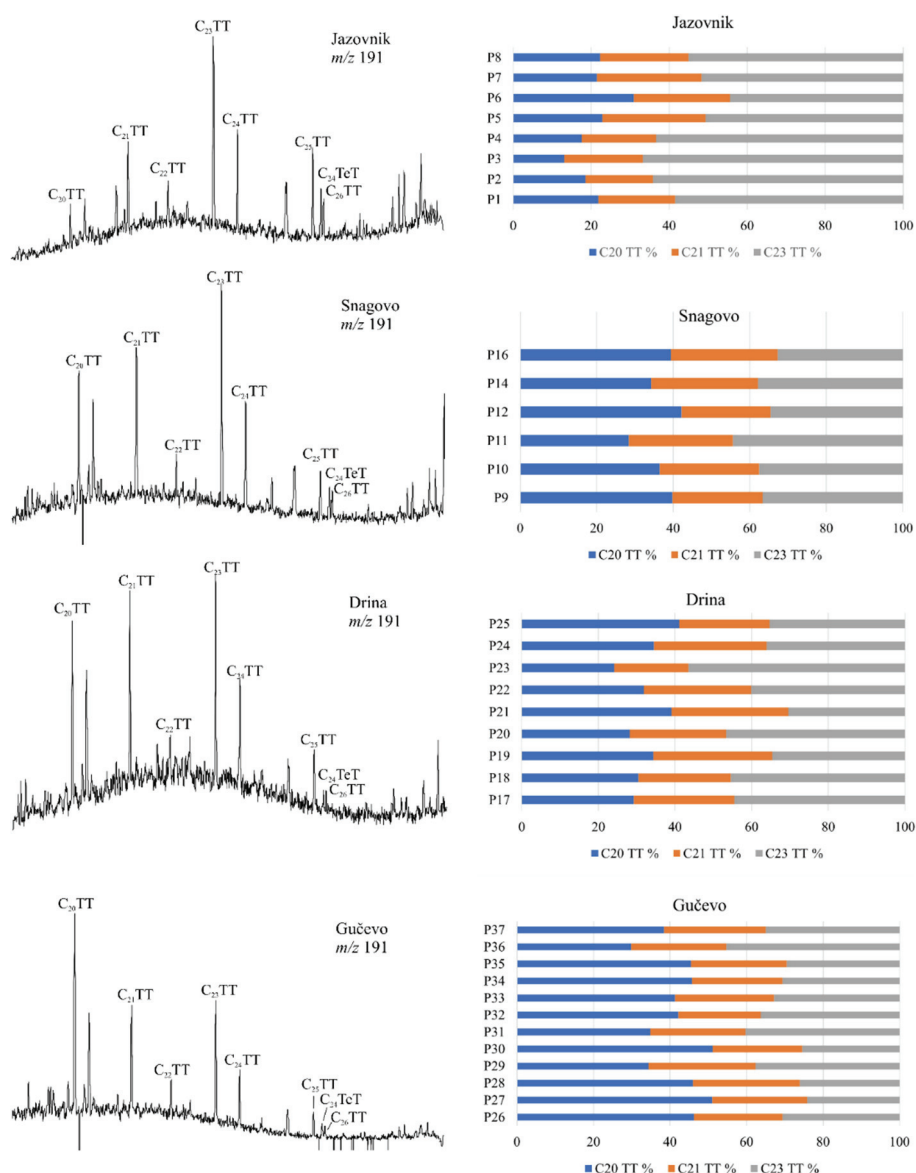


Fig. 4. C₁₉-C₂₆ TT distribution in *m/z* 191 mass chromatograms for representative samples.

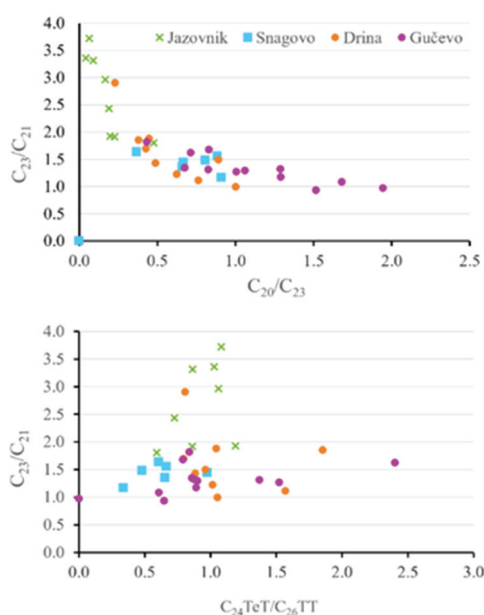
and C₂₆TeT. The representative chromatograms of the *m/z* 191 ion are shown in Fig. 4, while the geochemical parameters based on the abundance of certain tricyclic and tetracyclic terpanes are summarised in Table I. The C₂₃ tricyclic terpene is typically the dominant homolog among C₂₀–C₂₃ tricyclic terpanes in Jazovnik samples (P1–P8), where C₂₃TT > 50 % (Table I, Figs. 4–6). The C₂₃TT domination is characteristic of the marine, reducing marine carbonate setting or saline lacustrine depositional environment.^{22–24} Since Jazovnik sediments are from the Cretaceous–Paleogene boundary, it can be assumed that they were deposited in an environment that is the remnant of a former sea or in a lake environment that often resembles a marine sedimentation environment in its characteristics. Opposite to the Jazovnik, most samples from Gučevo are characterised by a predominance of C₂₀ and C₂₁TT (Table I, Figs. 4–6). Domination of these tricyclic terpanes is characteristic of terrigenous input.^{1,25} The other examined samples from Drina and Snagovo are formed in a mixed depositional environment between continental and marine depositional settings (Table I, Figs. 4–6).

TABLE I. Organic geochemical parameters based on the abundance of tricyclic and tetracyclic terpanes; Legend: TTs – tricyclic terpanes, TeTs – tetracyclic terpanes, C₂₀ + C₂₂ (%) = 100(C₂₀ + C₂₂TTs)/(C₂₀ + C₂₁ + C₂₂ + C₂₃TTs), C₂₁ (%) = 100C₂₁TT/(C₂₀ + C₂₁ + C₂₂ + C₂₃TTs), C₂₃ (%) = 100C₂₃TT/(C₂₀ + C₂₁ + C₂₂ + C₂₃TTs)

Sam.	Formation	C ₂₃ /C ₂₁ TTs	C ₂₁ /C ₂₀ TTs	C ₂₀ /C ₂₃ TTs	C ₂₄ /C ₂₆ TeTs	C ₂₀ +C ₂₂ (%)	C ₂₁ (%)	C ₂₃ (%)
P1	Jazovnik	2.96	2.03	0.166	1.061	21.85	19.71	58.44
P2		3.72	4.22	0.064	1.082	18.55	17.25	64.20
P3		3.36	7.14	0.042	1.028	13.12	20.16	66.73
P4		3.31	3.39	0.089	0.863	17.58	19.11	63.32
P5		1.92	2.30	0.226	0.862	22.86	26.45	50.69
P6		1.80	1.16	0.477	0.591	30.96	24.62	44.43
P7		1.93	2.61	0.198	1.190	21.48	26.82	51.70
P8		2.43	2.17	0.189	0.727	22.31	22.62	55.07
P9	Snagovo	1.55	0.73	0.885	0.667	39.78	23.58	36.64
P10		1.44	1.04	0.667	0.976	36.48	26.00	37.52
P11		1.64	1.66	0.368	0.606	28.39	27.17	44.44
P12		1.48	0.84	0.807	0.480	42.14	23.33	34.54
P13		0.00	0.00	0.000	0.000	0.00	0.00	0.00
P14		1.36	1.12	0.659	0.654	34.24	27.91	37.85
P15		0.00	0.00	0.000	0.000	0.00	0.00	0.00
P16		1.17	0.55	0.910	0.338	39.37	27.96	32.67
P17	Drina	1.70	1.38	0.427	0.795	29.23	26.22	44.55
P18		1.88	1.19	0.445	1.044	30.45	24.13	45.42
P19		1.12	1.18	0.762	1.568	34.38	31.01	34.61
P20		1.86	1.42	0.379	1.852	28.20	25.13	46.67
P21		1.00	1.00	1.001	1.052	39.07	30.49	30.43
P22		1.43	1.44	0.487	0.884	31.83	28.05	40.11
P23		2.91	1.51	0.228	0.805	24.11	19.42	56.47
P24		1.23	1.31	0.623	1.014	34.46	29.44	36.10

TABLE I. Continued

Sam.	Formation	C_{23}/C_{21} TTs	C_{21}/C_{20} TTs	C_{20}/C_{23} TTs	C_{24}/C_{26} TeTs	$C_{20}+C_{22}$ (%)	C_{21} (%)	C_{23} (%)
P25	Gučevo	1.50	0.75	0.890	0.962	41.06	23.61	35.32
P26		1.32	0.59	1.287	0.874	46.24	23.13	30.63
P27		0.97	0.53	1.942	0.000	51.05	24.80	24.15
P28		0.93	0.71	1.515	0.647	46.00	27.91	26.09
P29		1.35	1.10	0.674	0.857	34.37	27.98	37.65
P30		1.09	0.55	1.680	0.607	51.14	23.39	25.47
P31		1.63	0.86	0.712	2.400	34.91	24.79	40.30
P32		1.68	0.72	0.830	0.789	42.19	21.56	36.25
P33		1.27	0.78	1.006	1.522	41.31	25.83	32.86
P34		1.30	0.73	1.059	0.900	45.79	23.60	30.62
P35		1.18	0.66	1.289	0.890	45.48	25.04	29.47
P36		1.82	1.27	0.431	0.838	29.89	24.85	45.26
P37		1.32	0.92	0.826	1.371	38.45	26.58	34.97

Fig. 5. The cross plots of C_{23}/C_{21} vs. C_{20}/C_{23} TTs and C_{23}/C_{21} vs. $C_{24}Te/C_{26}Te$ TTs for investigated samples.

A recent study introduced a ternary diagram that enables the differentiation of depositional environments of sediments based on the analysis of C_{19} – C_{23} TTs.^{4,11,26} In general, the rocks with abundant terrigenous organic matter input exhibit higher concentrations of $C_{19}TT$ and $C_{20}TT$, while a predominance of $C_{21}TT$ characterises sediments deposited in freshwater lacustrine environments.⁶ A slightly modified ternary plot ($C_{22}TT + C_{20}TT$, $C_{21}TT$ and $C_{23}TT$, Fig. 6) suggests that Jazovnik sediments fall within zone IV, indicating a brackish/marine depositional environment. Some Drina samples belong to the same zone, while others are within zone III, indicating a mixed floodplain/freshwater lacustrine

depositional environment. In contrast, the Gučevo samples fall within zone II, indicating the fluvial-deltaic depositional settings. Besides, the most of Snagovo samples overlap between the Zone III (floodplain/freshwater lacustrine environment) and the Zone IV (brackish/marine environment).

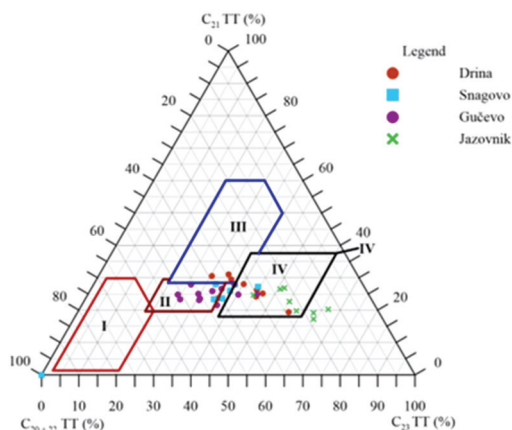


Fig. 6. A triangular diagram of C20+C22TT, C21TT and C23TT depicting distinct depositional settings. Legend: I – Swamp facies, II – Fluvial deltaic, III – Floodplain/freshwater lacustrine, IV – Brackish/marine (modified and adapted from Xiao *et al.*, 2019c, Bao *et al.*, 2023, and Xiao *et al.*, 2024)^{10,11,26}

The distribution of tri- and tetracyclic terpanes indicates that the examined sediments were deposited in distinct depositional environments. The sediments from Jazovnik were formed in brackish/marine depositional environments, while the sediments from Gučevo were deposited under continental fluvial-deltaic settings. On the other hand, the transitions in sedimentary conditions were observed during the formation of the Drina and Snagovo sediments. For some sediments, a salt enrichment within the alluvial system was noticed.

Moreover, the cross-plots of C_{23}/C_{21} vs. C_{20}/C_{23} TTs and C_{23}/C_{21} vs. C_{20}/C_{23} TTs (Fig. 5) and the ternary diagram C_{19} – C_{23} TTs (Fig. 6) more reliably depicted paleoenvironmental settings under which investigated sediments were formed.

CONCLUSION

The study focused on tracking changes in hydrocarbon distributions of siliciclastic sediments from Jazovnik, Snagovo, Drina and Gučevo (Internal Dinarides) exposed to prolonged chemical weathering. A large group of studied sediments participated in three sedimentary cycles, containing over 50 % SiO_2 and are characterized by the absence of preserved diagnostic fossils. Biodegradation, chemical weathering and/or other alteration processes influenced the distribution of hydrocarbons, resulting in highly abundant branched hydrocarbons and UCMs. In such cases, tri- and tetracyclic terpanes proved susceptible to transitions in paleoenvironmental settings since they are well-known for their resistance to the above-mentioned processes.

The results showed that tri- and tetracyclic terpanes effectively determined the changes in the depositional environments of examined sediments. The sediments

from Jazovnik were formed in the brackish/marine lacustrine depositional environments, while the sediments from Gučevo were deposited under continental fluvial-deltaic settings. On the other hand, the transitions in sedimentary conditions were observed during the formation of the Drina and Snagovo sediments, which resulted from the salt enrichment within the alluvial system. The conditions were changing between the floodplain/freshwater lacustrine and brackish/marine. Once again, this approach clearly demonstrates the differences in depositional settings of the Jazovnik, Snagovo, Drina and Gučevo sediments, offering some valuable insights into the geological history of the studied area.

Acknowledgments. The study was partly financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract numbers 451-03-136/2025-03/200026 and 451-03-136/2025-03/200168).

ИЗВОД

РЕКОНСТРУКЦИЈА СРЕДИНЕ ТАЛОЖЕЊА ЕРОДОВАНИХ СИЛИЦИКЛАСТИЧНИХ СЕДИМЕНАТА ИНТЕРНИХ ДИНАРИДА НА ОСНОВУ РАСПОДЕЛЕ И ОБИЛНОСТИ ТРИ- И ТЕТРАЦИКЛИЧНИХ ТЕРПАНА

ИВАНА ЈОВАНИЋ¹, АЛЕКСАНДРА ШАЈНОВИЋ², САЊА СТОЈАДИНОВИЋ², НИКОЛА БУРАЗЕР²,
БОЈАН ГЛАВАШ-ТРБИЋ¹ и БРАНИМИР ЈОВАНЧИЧЕВИЋ³

¹Геолошки завод Србије, Ровињска 12, 11000 Београд, ²Универзитет у Београду, Институт за хемију, технологију и металургију, Њешићева 12, 11001 Београд и ³Универзитет у Београду, Хемијски факултет, Студентски трг 12–16, 11001 Београд

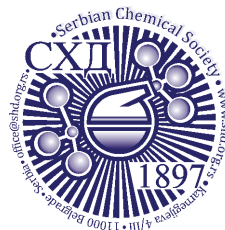
Ова студија испитује могућност коришћења три- и тетрацикличних терпана за карактеризацију средине таложења седимената који немају очуване фосиле и чија је расподела биомаркера нарушена биоразградњом или другим процесима. Анализирани су силицикластични седименти који су били изложени продуженом хемијском и атмосферском деловању на стене, које је утицало на расподелу угљоводоника. То је резултирало високом обилношћу разгранатих угљоводоника и присуством нераздвојене комплексне смеше једињења (UCM). Из тог разлога је релативна обилност и расподела три- и тетрацикличних терпана (C_{20} – C_{24} ТТ, C_{26} ТТ, C_{24} ТеТ), као резистентних биомаркера, коришћена за процену услова таложења. Триангуларни дијаграм који укључује $C_{20} + C_{22}\%$, $C_{21}\%$ и $C_{23}\%$ показао се посебно ефикасним за разликовање мочварне, флувијално-делтне, слатководне и морско-слане језерске средине седиментације. Овај приступ је јасно разграничио депозиционе средине седимената из Јазовника, Снагова, Дрине и Гучева (унутрашњи Динариди), пружајући вредан увид у геолошку историју проучаваног подручја.

(Примљено 27. децембра 2024, ревидирано 4. Фебруара, прихваћено 11. априла 2025)

REFERENCES

1. K. E. Peters, C. C. Walters, J. M. Moldowan, *The biomarker Guide, Vol. 2: Biomarkers and Isotopes in Petroleum Exploration and Earth History*, Cambridge University Press, Cambridge, 2005 (ISBN: 0-521-83763-4)
2. A. Wang, C. Li, L. Li, R. Pu, Z. Yang, N. Zhu, K. Guo, *Front. Earth Sci.* **11** (2023) 1128692 (<https://doi.org/10.3389/feart.2023.1128692>)
3. S. Tao, C. Wang, J. Du, L. Liu, Z. Chen, *Mar. Pet. Geol.* **67** (2015) 460 (<https://doi.org/10.1016/j.marpetgeo.2015.05.030>)

4. H. Xiao, M. Li, J. Liu, F. Mao, D. Cheng, Z. Yang, *Mar. Pet. Geol.* **103** (2019) 351 (<https://doi.org/10.1016/j.marpetgeo.2019.03.004>)
5. E. Grosjean, G. D. Love, A. E. Kelly, P. N. Taylor, R. E. Summons, *Org. Geochem.* **45** (2012) 77 (<https://doi.org/10.1016/j.orggeochem.2011.12.006>)
6. H. Xiao, T.G. Wang, M. Li, H. Lai, J. Liu, F. Mao, Y. Tang, *J. Petrol. Sci. Eng., B* **172** (2019) 360 (<https://doi.org/10.1016/j.petrol.2018.09.082>)
7. P. Farrimond, J.C. Bevan, A.N. Bishop, *Org. Geochem.* **30** (1999) 1011 ([https://doi.org/10.1016/S0146-6380\(99\)00091-1](https://doi.org/10.1016/S0146-6380(99)00091-1))
8. S. M. B. De Grande, F. R. Aquino Neto, M. R. Mello, *Org Geochem.* **20** (1993) 1039 ([https://doi.org/10.1016/0146-6380\(93\)90112-O](https://doi.org/10.1016/0146-6380(93)90112-O))
9. C. Ekweozor, O. Strausz, in *Advances in Organic Geochemistry*, M. Bjorøy, P. Albrecht, C. Cornford, K. de Groot, G. Eglinton, E. Galimov, D. Leythaeuser, R. Pelet, J. Rullkötter, G. Speers, Eds., Wiley, Chichester, 1983, pp. 746–766
10. H. Xiao, M. J. Li, Z. Yang, Z. Zhu, *Geochemistry* **48** (2019c) 161 (<https://doi.org/10.19700/j.0379-1726.2019.02.006>)
11. H. Xiao, L. Meijun, B.J. Nettersheim, *Chem. Geol.* **652** (2024) 122023 (<https://doi.org/10.1016/j.chemgeo.2024.122023>)
12. S. M. Schmid, D. Bernoulli, B. Fügenschuh, L. Matenco, S. Schefer, R. Schuster, M. Tischler, K. Ustaszewski, *Swiss J. Geosci.* **101** (2008) 139 (<https://doi.org/10.1007/s00015-008-1247-3>)
13. S. M. Schmid, B. Fügenschuh, A. Kounov, L. Maţenco, P. Nievergelt, R. Oberhänsli, J. Pleuger, S. Schefer, R. Schuster, B. Tomljenović, K. Ustaszewski, D. J. J. van Hinsbergen, *Gondwana Res.* **78** (2020) 308 (<https://doi.org/10.1016/j.gr.2019.07.005>)
14. I. Đoković, *Geoloski anali Balkanskoga poluostrva* **49** (1985) 143 (in Serbian)
15. D. Spahić, B. Glavaš-Trbić, S. Đajić, T. Gaudenyi, *Geoloski anali Balkanskoga poluostrva* **79** (2018) 57 (<https://doi.org/10.2298/GABP1802057S>) (in Serbian)
16. S. Karamata, in *Tectonic Development of the Eastern Mediterranean region*, A.H.F. Robertson, D. Mountrakis, Eds., Special Publications, Geological Society, London, 2006, pp. 155–178
17. V. M. Dembitsky, I. Dor, I. Shkrob, M. Aki, *Russ. J. Bioorg. Chem.* **27** (2001) 110 (<https://doi.org/10.1023/A:1011385220331>)
18. J. Shiea, S. C. Brassell, D. M. Ward, *Org. Geochem.* **15** (1990) 223 ([https://doi.org/10.1016/0146-6380\(90\)90001-G](https://doi.org/10.1016/0146-6380(90)90001-G))
19. N. Robinson, G. Eglinton, *Org. Geochem.* **15** (1990) 291 ([https://doi.org/10.1016/0146-6380\(90\)90007-M](https://doi.org/10.1016/0146-6380(90)90007-M))
20. R. E. Summons, M. R. Walter, *Am. J. Sci., A* **290** (1990) 212
21. F. Kenig, J. S. Sinninghe Damsté, N. L. Frewin, J. M. Hayes, J. W. De Leeuw, *Org. Geochem.* **23** (1995) 485 ([https://doi.org/10.1016/0146-6380\(95\)00049-K](https://doi.org/10.1016/0146-6380(95)00049-K))
22. R. Wang, J. Fu, *Int. J. Salt Lake Res.* **6** (1997) 25 (<https://doi.org/10.1007/BF02441867>)
23. Z. Yangming, W. Huanxin, S. Aiguo, L. Digang, P. Dehua, *Appl. Geochem.* **20** (2005) 1875 (<https://doi.org/10.1016/j.apgeochem.2005.06.003>)
24. S. Huang, G. Xu, F. Xu, W. Wang, H. Yuan, Z. Yan, X. Lin, M. Zhang, *Energy Explor. Exploit.* **38** (2020) 2296 (<https://doi.org/10.1177/0144598720915533>)
25. A. D. Hanson, S. C. Zhang, J. M. Moldowan, D. G. Liang, B. M. Zhang, *AAPG Bull.* **84** (2000) 1109 (<https://doi.org/10.1306/A9673C52-1738-11D7-8645000102C1865D>)
26. Y. Bao, Y. Hu, H. Huang, J. Meng, R. Zheng, *ACS Omega* **8** (2023) 41885–41896 (<https://doi.org/10.1021/acsomega.3c06835>).



J. Serb. Chem. Soc. 90 (12) 1537–1551 (2025)
JSCS–5471

Influence of microalgae on organic micropollutants removal from water by powdered activated carbon

NADIJA KHAKIMOVA¹, MINJA BOGUNOVIĆ KOLJAJA^{2*}, JELICA SIMEUNOVIĆ¹,
LUCAS LANDWEHRKAMP³, STEFAN PANGLISCH³⁻⁶
and IVANA IVANČEV-TUMBAS²

¹University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia, ²University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 1, 21000 Novi Sad, Serbia, ³Chair for Mechanical Process Engineering and Water Technology, University of Duisburg-Essen, Lotharstr. 1, 47057 Duisburg, Germany, ⁴IWW Water Centre, Moritzstraße 26, 45476 Mülheim an der Ruhr, Germany, ⁵DGMT German Society for Membrane Technology e.V., Universitäts str. 2, 45141 Essen, Germany and ⁶Centre for Water and Environmental Research (ZWU), Universitäts str. 2, 45141 Essen, Germany

(Received 29 December 2024, revised 9 April, accepted 24 July 2025)

Abstract: This study investigates how two morphologically distinct microalgae, *Chlorella vulgaris* and *Arthrospira platensis*, at different growth phases, affect the adsorption of ibuprofen, caffeine and diclofenac onto two powdered activated carbons (AC1 and AC2). Dechlorinated tap water (DCTW) matrix was used with microalgae added and experiments were performed with/without pre-chlorination and with/without filtration through 0.45 µm filter to assess the influence of total and dissolved algal organic matter. Organic micropollutants (OMPs) were analyzed using gas chromatography coupled with mass spectrometry. Results indicate that the effect of microalgae morphology on OMPs' removal efficiency is different for different carbons. Species and growth phase-dependent variations were observed in some cases. *A. platensis* in the stationary phase in the water reduced diclofenac removal by AC2 (from 99 % to range of 44–62 %), while *C. vulgaris* in the exponential phase reduced it to the range of 16–74 % in comparison to effectiveness of AC2 in DCTW without microalgae (99 %). Removal of uncharged caffeine remained stable, suggesting minimal influence from algal matrices or AC variability. For negatively charged ibuprofen and diclofenac the observed effects were more variable and not always consistent, likely due to limitations in experimental methodologies.

*Corresponding author. E-mail: minja.bogunovic@dh.uns.ac.rs
<https://doi.org/10.2298/JSC241229071K>

Keywords: adsorption; algal organic matter; drinking water treatment; ibuprofen; caffeine; diclofenac.

INTRODUCTION

The growing presence of organic micropollutants (OMPs) in freshwater systems poses a significant challenge to environmental sustainability and public health.¹ In a recent study, over 600 chemicals (pesticides, biocides, pharmaceuticals) were screened across various European streams.² This extensive study found that in three-quarters of the sites investigated across 22 European river basin the concentrations of these chemicals often surpassed the safety limits set to protect freshwater ecosystems from harmful pollution.² Some of the most frequently detected compounds (> 25 % of sites) included 1,3-diphenylguanidine, sucralose, gabapentin-lactam, 1*H*-benzotriazole, candesartan, DEET, lamotrigine, oxypurinol, melamine, caffeine and guanylurea.² Caffeine (CF) was particularly notable, with its presence confirmed in more than 25 % of the sampling sites, with a maximum concentration of more than 1 µg L⁻¹ at several sites.² According to the review study by Luo *et al.*³ average concentrations or range of ibuprofen (IB) concentrations in surface water were found to be 0.98, nd–8.0, nd–1417, 1.0–67, <15–414, 5.0–280, 0.3–100 and nd–77 ng L⁻¹ for Canada, France, China, Greece, Korea, Taiwan, UK and US, respectively, while environmental concentrations of diclofenac (DCF) exceeding 100 ng L⁻¹ have been detected in many water bodies around the world, including Antarctica (1,000 ng L⁻¹).⁴ These OMPs are extensively used and recognized as anthropogenic markers within the group of pharmaceuticals and personal care products. A typical water treatment plant uses flocculation, coagulation, filtration and disinfection to remove suspended solids and microorganisms. However, they are not specifically engineered to address the removal of micropollutants.^{5,6} Among the various strategies employed to mitigate the impact of OMPs, adsorption techniques using powdered activated carbon, granulated activated carbon and also including biological activated carbon have emerged as effective treatment methods.^{5,7,8} The large surface area and high porosity of powdered activated carbon (PAC) provides numerous adsorption sites, enabling it to remove contaminants at very low concentrations.⁹ Also, PAC's implementation requires minimal modifications to the existing infrastructure of drinking water treatment plants, and its dosage can be easily adjusted to accommodate variations in raw water quality.⁵ However, the presence of natural organic matter (NOM) in surface waters particularly dissolved organic matter (DOM) introduces significant challenges to the adsorption capacity of PAC.¹⁰ NOM is known to negatively impact the adsorption of organic micropollutants by, first, directly competing for adsorption sites and secondly, because NOM molecules between 200 and 700 Da can cause blockage of the AC pores.^{11,12} Nam *et al.*¹³ highlighted in their study on adsorption characteristics of hydrophilic and hydrophobic OMPs (initial concentration of 100 ng L⁻¹),

including DCF and CF, that the presence of DOM in surface river water significantly decreases the adsorption capacity of PAC (doses of 1, 5 and 20 mg L⁻¹), especially for hydrophobic molecules. Based on their findings, DOM alters the surface charge of PAC and competes with OMPs for adsorption sites on the PAC surface. The impact of NOM is especially pronounced in surface waters, where algal blooms form algal organic matter (AOM).¹⁴ Total AOM includes both intracellular and extracellular dissolved organic matter originating from algae. The presence of high-molecular-weight fractions (*e.g.*, *MW* range 1,100–10,000 Da) in algal-rich waters, need higher PAC doses to achieve effective removal than the dose which is needed for lower *MW* range (20–1,100 Da). When present, charge balance and interaction potential of diverse amphoteric groups in low molecular weight NOM and proteinaceous low-aromaticity substances can cause their effective adsorbability, while high MW moieties have the tendency to block the pores of AC.¹¹ While PAC can effectively adsorb hydrophobic components of AOM, it has limited adsorption capacity for polysaccharides and proteins, which are the main components in AOM.¹⁵ In the 1990s, microalgae research in drinking water focused on optimizing physical removal processes to prevent algal toxin release, later shifting to disinfection by-product (DBP) formation after chlorination.^{16,17} Chlorination, used as disinfection method during drinking water treatment, disrupts algal cells, releasing intracellular organic matter which reacts with chlorine to form DBPs, which can also block active sites on PAC or alter its surface properties.^{18,19} AOM is a complex DBP precursor in water treatment, with its composition, influenced by algal species and environmental conditions, affecting DBP formation potential and nature.¹⁷ However, there is a lack of information regarding the influence of pre-chlorination on AOM structure and its consequences for subsequent activated carbon treatment steps. Similarly, how the presence of total and dissolved AOM originating from different microalgae growth phases and species influences OMP removal through adsorption on PAC is not fully understood. According to Tian *et al.*²⁰ AOM levels from green algae *Chlorella vulgaris* and *Scenedesmus obliquus* during the stationary phase increased under simulated conditions mimicking karst environments, with more cell metabolites being released. The stationary phase was characterized by a decrease in amino groups and an increase in aromatic protein-like compounds, while the exponential phase exhibited higher levels of amino structural material. When it comes to influence of morphology onto efficiency of water treatment for microalgae removal, Yu *et al.*²¹ study emphasized the significance of divalent ions in removal of algae during a two-step process including first, preoxidation with KMnO₄, followed by coagulation with polyaluminium chloride. It was shown that complexation of divalent ions improved the removal of algae, and filamentous *Pseudanabaena* sp. was more efficiently removed compared to unicellular *Chlorella* sp. Ma *et al.*²² investigated how microalgal morphology affects their removal efficiency in conventional coagulation–sedimentation.

mentation–filtration water treatment processes, finding that smaller algae with irregular shapes, such as thorns or flagellae were more difficult to remove. Laksono *et al.*²³ studied ultrafiltration membrane fouling by AOM substances and found that biopolymers, building blocks, and hydrophobic organic carbon significantly affected membrane performance. Algae lysing notably increased fouling severity. They also observed that microalgae cell shape and size influenced fouling: helical cells primarily blocked membrane pores, causing strong fouling, while rectangular and cylindrical cells caused milder, combined fouling.²³ Furthermore, related to investigating on how growth phase affect treatment efficiency, Park *et al.*²⁴ found that toxic colonial cyanobacterial species *Microcystis aeruginosa* at the stationary growth phase had higher cell density and extracellular organic matter, and both the exponential and stationary phases could be fully inactivated by chlorination during drinking water treatment, with faster toxin degradation occurring at the stationary phase. But based on our knowledge no research has examined how pre-chlorination in distinct growth phases influence the efficacy of PAC for OMP adsorption. For this lab-case study, we chose unicellular *Chlorella vulgaris* and filamentous *Arthrospira (Spirulina) platensis* in two growth phases (exponential and stationary) due to their distinct morphological differences and well-documented growth characteristics. Three widely prevalent in surface waters contaminants with varying hydrophilicities represented by different distribution coefficient ($\log D$) values were chosen – ibuprofen ($\log D = 0.45$), caffeine ($\log D = 0.28$) and diclofenac ($\log D = 1.37$). $\log D$ refers to corrected $\log P$ (octanol water partitioning coefficient) at pH 7.4 (Table S-I of the Supplementary material to this paper).

The aim of this study is to assess how the distinct morphological differences and growth phases of microalgae *C. vulgaris* and *A. platensis* impact the adsorption of three different OMPs under various conditions, including the presence of total (tAOM) and dissolved algal organic matter (dAOM) and the effects of pre-chlorination. Although microalgae are present as mixed cultures in nature, we studied them separately to isolate their individual effects. By filling this gap, we seek to get more fundamental knowledge which might result in improved treatment strategies for water containing microalgae and emerging micropollutants in the future.

EXPERIMENTAL

The experiments aimed to assess how different microalgae types (*C. vulgaris* and *A. platensis*) and growth phases (exponential and stationary) influence the adsorption efficiency of powdered activated carbon for removing OMPs, focusing on the effects of tAOM and dAOM. The number of algae used in the experiments ranged from 2×10^8 to 8×10^8 cells L^{-1} . The dAOM is the fraction remained after glass fiber filtration (GF filter 0.6 μm , Macherey-Nagel, Germany), and subsequent membrane filtration (MF filter 0.45 μm , Sartorius, USA). Furthermore, effects of usually applied pre-chlorination in waterworks were investigated. 1 mg L^{-1} Cl_2 was added into the matrix containing algae for 30 min and afterwards dechlorination was performed. In this way, it was possible to investigate how the potential change of both, chlorinated tAOM and chlorinated dAOM structure and content affect the OMPs' adsorption. Adsorption tests

were performed on a shaker (KS 501-IKA, Germany) with shaking intensity of 180 rpm. Nominal concentration of selected OMPs was $1.5\text{--}2\text{ }\mu\text{g L}^{-1}$. Applied dose (5 mg L^{-1}) of two selected powdered activated carbons was 2 h in contact with water sample that is close to real application rather than reaching equilibrium. The scheme of experimental methodology is shown in Fig. S-1 of the Supplementary material. Adsorption tests were performed in 1 L bottles in a way that 0.7 L of each prepared matrix spiked with OMP (nominal Co, $1.5\text{--}2\text{ }\mu\text{g L}^{-1}$) was shaken with 5 mg L^{-1} of AC1 or AC2 for 2 h. Afterwards, samples are filtered through glass-fiber filter ($0.6\text{ }\mu\text{m}$, Macherey-Nagel) and afterwards through $0.45\text{ }\mu\text{m}$ membrane filter (Sartorius) to remove PAC. First 300 mL of aliquot was discarded. From 400 mL collected, 250 mL was taken for further gas chromatography/mass spectrometry (GC/MS) analysis. Analysis of the OMPs was performed by the in-house developed GC/MS method.²⁵ Solid-phase extraction (60 mg, Oasis[®] HLB, WatersTM, USA) was used to prepare water samples for the analysis of selected OMPs. Details of the tested OMPs sample preparation and analysis are given in the Supplementary material, section Analytical method. Full method validation performed in DCTW is given in Supplementary material, Table S-III. For this study five water matrices were used for investigation (Fig. S-1): *i*) dechlorinated tap water (DCTW) without algae addition; *ii*) DCTW with total algal organic matter (tAOM); *iii*) DCTW with dissolved algal organic matter (dAOM); *iv*) pre-chlorinated tAOM; *v*) pre-chlorinated dAOM. All the details related to materials (water matrices, microalgae selection and cultivation, OMP stock solution preparation and PAC) used are given in Supplementary material, section Material. All adsorption tests were performed once within set I, and after nine months, some of them were repeated (set II). Repeatability of experiments without microalgae addition within both batches was tested for experiment testing adsorption on AC2 in DCTW (matrix (*i*), Fig. S-1), in 5 and 3 replicates, respectively. Repeatability of experiments with microalgae addition (matrix (*ii*), Fig. S-1) was tested once in triplicate for stationary phase of each microalgae type. Quality control procedures of experimental methodology and GC/MS measurement are shown in Supplementary material, section Quality control procedures. Dissolved organic carbon (DOC) method characterization and obtained values for each of the tested matrices are presented in Tables S-IV and S-V of the Supplementary material.

RESULTS AND DISCUSSION

How microalgal morphology and growth phase affect the OMPs removal from different water matrices by two activated carbons is presented in Figs. 1–3.

Ibuprofen (IB)

The removal of IB with and without the presence of different species of microalgae, in their different growth phases (exponential vs. stationary), in the presence of different type of AOM (tAOM vs. dAOM), and with/without chlorination is presented in Fig. 1 (*A. platensis*, Fig. 1a and c vs. *C. vulgaris*, Fig. 1b and d). For the clarity of the following discussion we adopted the term “significant difference” between two removals if the error bars do not overlap and if the closest results of two cases which are compared, differ by more than 30 % (acceptance limit for quality control samples of the Environmental Protection Agency (EPA), method 525.3)).²⁶

In DCTW without algae addition the removal efficiency of IB was $47\pm 14\%$ by AC1 (Fig. 1a and b, *i*). In matrix containing *A. platensis* in both phases of growth

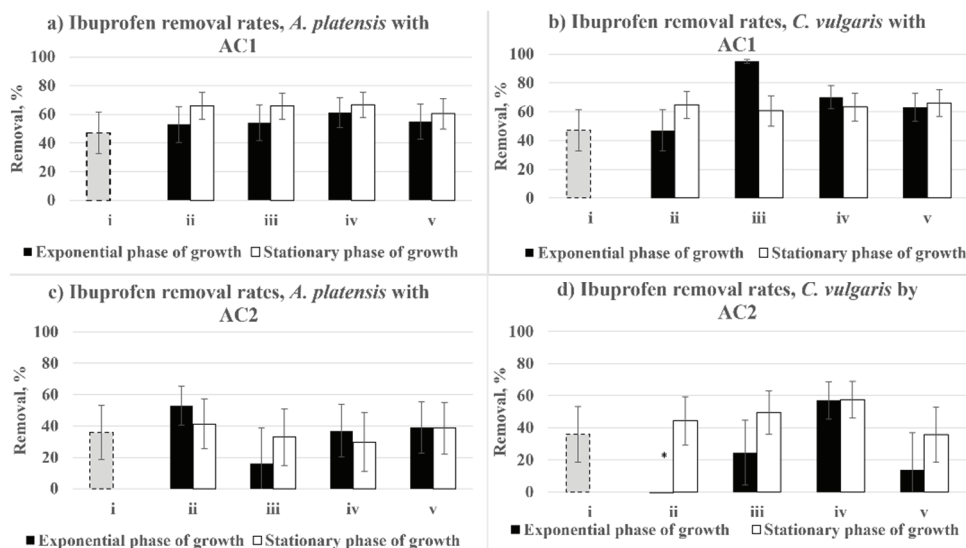


Fig. 1. Efficiency of IB removal using PACs (dose 5 mg L⁻¹). i) DCTW without microalgae addition (average value of five replicates); ii) non-chlorinated matrix with tAOM; iii) non-chlorinated matrix with dAOM; iv) pre-chlorinated with tAOM; v) pre-chlorinated with dAOM. * – No removal efficiency; Letters a–d indicate ibuprofen removal rates for *A. platensis* with AC1 (a), *C. vulgaris* with AC1 (b), *A. platensis* with AC2 (c) and *C. vulgaris* with AC2 (d). Error bars show the error of the relative removal. The number of cells in matrices containing *A. platensis* in exponential and stationary phase of growth were 8×10^8 and 4×10^8 cells L⁻¹, respectively. For *C. vulgaris* these numbers in exponential and stationary phase of growth were 2×10^8 and 5×10^8 cells L⁻¹, respectively.

the removal efficiency ranged between 53 ± 13 and 66 ± 9 % (Fig. 1a, ii) showing no significant change in comparison to DCTW without algae addition (47 ± 14 %, Fig. 1a, i). Similarly, in the second set of experiments (Table S-VI of the Supplementary material), matrix (ii), which contained *A. platensis* in the exponential phase of growth, no significant difference compared to DCTW (51 ± 3 % vs. 77 ± 6 %, respectively) was observed, indicating good repeatability after nine months. A significant increase of removal efficiency was noticed in the presence of *C. vulgaris* in exponential phase in dAOM matrix (Fig. 1b, iii) in comparison to DCTW matrix (95 ± 1 vs. 47 ± 14 %). Similarly, significant difference (48 %) in removal of IB between non-chlorinated tAOM (removal 47 ± 14 %) and dAOM (removal 95 ± 1 %) in matrix containing *C. vulgaris* in exponential phase was also noticed (Fig. 1b, ii and iii), indicating difference between competition mechanisms between tAOM and dAOM matrices under given conditions. The set of repeated experiments after nine months did not include any of these tests, so there was no additional confirmation of the effect of AOM, originating from *C. vulgaris* in exponential phase. Same matrix showed a slight but still not significant difference between non-chlorinated algae containing matrix dAOM (removal 95 ± 1 %, Fig. 1, b, iii) and pre-

-chlorinated algae containing matrix with dAOM (removal 63 ± 10 %, Fig. 1b, v) which, if present could have indicated potential effect of pre-chlorination on competitiveness of the AOM moieties. However, this finding was also not possible to confirm for particular pair of tests since only one of the tests was repeated. In the matrix containing *C. vulgaris* in the stationary phase of growth (Fig. 1b, ii, iii, iv, v) removal of IB by AC1 remained constant (from 61 ± 11 to 66 ± 9 %). When it comes to the other activated carbon, AC2, similar removal efficiency of IB in DCTW matrix without algae addition (36 ± 17 %, Fig. 1c and d, i) in comparison with AC1 (47 ± 14 %) was observed. In a matrix containing *A. platensis* in both growth phases, no significant change was observed compared to DCTW without algae addition (36 ± 17 %), Fig. 1c, ii–v). For the same matrices containing *A. platensis* in the stationary phase no significant differences were observed (removal rates ranged between 30 ± 19 and 41 ± 16 %, Fig. 1c, ii–v). Likewise, in most of the tests repeated after nine months for the same algae and growth phase, no significant differences were found between the removals observed in set I and II (Arth-s, AC2, Table S-VI). Only one of four tests had significant differences (Arth-s, matrix (iv), Table S-VI). Also, tests in stationary phase did not show significant decreases of removal in comparison to DCTW matrix without algae except the matrix (iv) (Arth-s, AC2, Table S-VI). In the non-chlorinated tAOM matrix containing *C. vulgaris* in exponential growth phase no removal was observed (Fig. 1d, ii), indicating high potential for competition of the tAOM when compared to DCTW without algae addition (36 ± 17 %). However, this was not confirmed in repeated tests after nine months when removal rate was 54 ± 12 % (Chlor-e, matrix (ii), AC2, Table S-VI). Similarly, significant removal rate differences were noticed between non-chlorinated tAOM (Fig. 1d, matrix (ii), no removal) and pre-chlorinated tAOM (Fig. 1d, iv, 57 ± 12 %), indicating that pre-chlorination can positively affect adsorption. However, during the second set of experiments opposite effect was observed between non-chlorinated tAOM (54 ± 12 %) and pre-chlorinated tAOM (no removal), indicating contradicting effect of higher potential for competition in pre-chlorinated samples. For matrices containing *C. vulgaris* in the exponential growth phase, a slight difference was observed between pre-chlorinated tAOM (57 ± 12 %) compared to pre-chlorinated dAOM (14 ± 23 %, Fig. 1d, iv and v). Repeated experiments (Table S-VI, Chlor-e, AC2, matrices (iv) and (v)) showed somewhat higher removal efficiency in pre-chlorinated dAOM matrix (28 ± 19 %) in comparison to pre-chlorinated tAOM matrix (no removal). For matrices containing *C. vulgaris* in the stationary growth phase, the removal rates by AC2 stabilize between 36 ± 17 and 58 ± 11 % (Fig. 1d, ii–v) with no significant difference. Although differences between growth phases were observed, only for non-chlorinated tAOM matrix containing *C. vulgaris* (Fig. 1d, ii) the impact of algal growth phase cannot be conclusively confirmed. In the exponential growth phase no removal was observed while in the stationary phase an increase of 44 ± 15 % was noticed. The

difference in cell number between exponential and stationary phases exceeded 50 % (Fig. 1b and d), which is well above the known 20–30 % uncertainty of the hemocytometer counting method. While these differences reflect realistic physiological states of algae, they may have also contributed to observed variability in IB removal. Therefore, due to differences in both physiological composition and biomass quantity, the effect of growth phase cannot be reliably isolated in this case. Repeated experiments again did not confirm such an effect showing no difference between measurements in two phases of growth (54 ± 12 and 56 ± 12 %, Table S-VI). In the second set of experiments, the difference in cell density between growth phases was within the 20–30 % measurement uncertainty, suggesting that the growth phase did not have a meaningful effect on IB removal (3×10^8 and 4×10^8 cell L^{-1} , Table S-VI).

Caffeine (CF)

Results for CF removal across all matrices and both microalgae species are shown in Fig. 2.

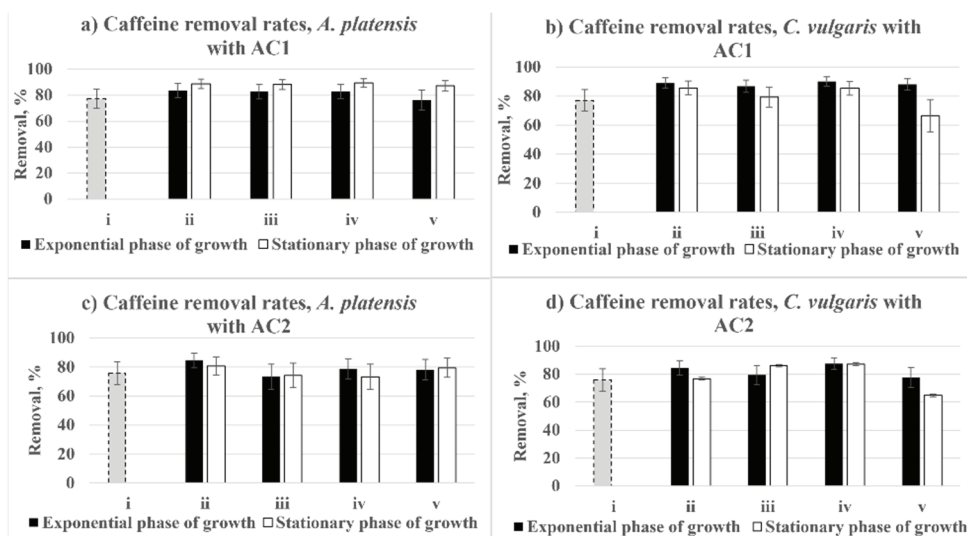


Fig. 2. Efficiency of CF removal using PACs (dose 5 mg L^{-1}); i) DCTW without microalgae addition (average value of five replicates); ii) non-chlorinated matrix with tAOM; iii) non-chlorinated matrix with dAOM; iv) pre-chlorinated with tAOM; v) pre-chlorinated with dAOM. Letters a–d indicate caffeine removal rates for *A. platensis* with AC1 (a), *C. vulgaris* with AC1 (b), *A. platensis* with AC2 (c) and *C. vulgaris* with AC2 (d). Error bars show the error of the relative removal. The number of cells in matrices containing *A. platensis* in exponential and stationary phase of growth were 8×10^8 and 4×10^8 cells L^{-1} , respectively. For *C. vulgaris* these numbers in exponential and stationary phase of growth were 2×10^8 and 5×10^8 cells L^{-1} , respectively.

Caffeine removal rates in DCTW without microalgae were 77 ± 7 % for AC1 and 76 ± 8 % for AC2. Removal rates remained essentially stable (from 66 ± 11 to 90 ± 3 % for AC1 and from 65 ± 11 to 87 ± 4 % for AC2) with no significant differences in removal across the matrices and conditions (Fig. 2). In the second set of experiments removals were 93 ± 2 and 92 ± 3 % for AC1 and AC2, respectively (Table S-VI). Algae addition caused the change of rates in the ranges of 76 ± 4 to 88 ± 8 % for AC1 and 64 ± 12 to 92 ± 3 % for AC2. Only one sample with *A. platensis* in stationary phase showed high discrepancy with a removal rate of 19 ± 26 %.

Diclofenac (DCF)

Results for DCF removal across all matrices and both microalgae species are shown in Fig. 3.

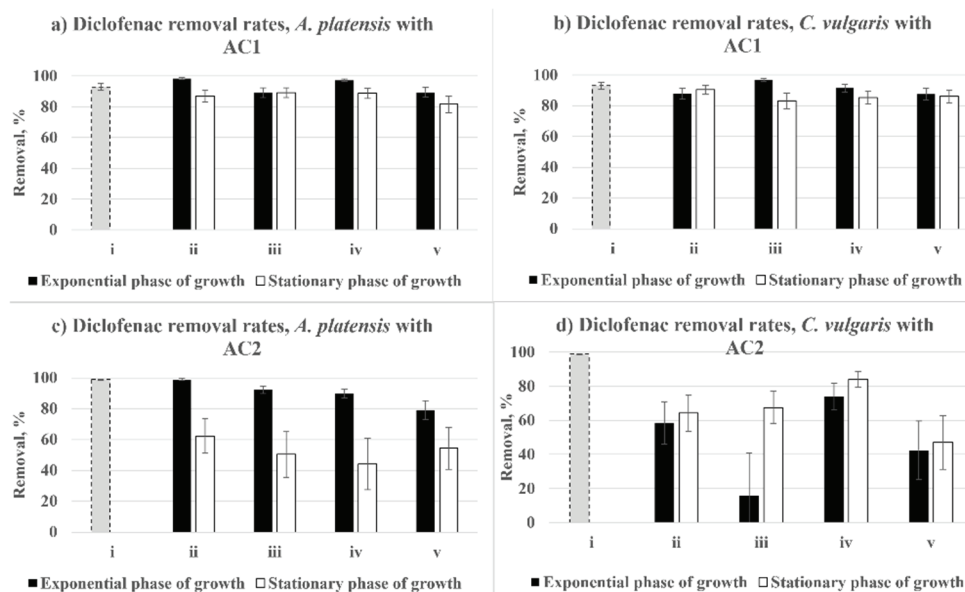


Fig. 3. Efficiency of DCF removal using PACs (dose 5 mg L⁻¹). i) DCTW without microalgae addition (average value of five replicates); ii) non-chlorinated matrix with tAOM; iii) non-chlorinated matrix with dAOM; iv) pre-chlorinated with tAOM; v) pre-chlorinated with dAOM. Letters a–d indicate caffeine removal rates for *A. platensis* with AC1 (a), *C. vulgaris* with AC1 (b), *A. platensis* with AC2 (c) and *C. vulgaris* with AC2 (d). Error bars show the error of the relative removal. The number of cells in matrices containing *A. platensis* in exponential and stationary phase of growth were 8×10^8 and 4×10^8 cells L⁻¹, respectively.

For *C. vulgaris* these numbers in exponential and stationary phase of growth were 2×10^8 and 5×10^8 cells L⁻¹, respectively.

In experiments using DCTW without microalgae, removal rates for DCF were 93 ± 2 % for AC1 and 99 ± 0.3 % for AC2 (Fig. 3, i). A second set of experiments after nine months (Table S-VI) showed repeatable removals of 90 ± 3 and 85 ± 4 %, respectively.

respectively. None of the factors (morphology, growth phase – even difference in cell number between exponential and stationary phases often exceeded 50 %, chlorination or AOM type) had a significant effect on DCF removal with AC1 for both *A. platensis* and *C. vulgaris*, as removal rates ranged from 82 ± 5 to 99 ± 0.5 % (Fig. 3a) and 83 ± 5 to 97 ± 1 % (Fig. 3b), respectively, across all matrices. Good repeatability of experiments was achieved after nine months, when removal values ranged between 69 ± 9 and 90 ± 3 % (which is in line with the acceptance limit for quality control samples).²⁷ All repeated matrices with AC1 showed insignificant difference (≤ 30 %) compared to first adsorption set (Table S-VI). In the case of AC2 use it was shown that the stationary phase of *A. platensis* significantly decreased DCF removal rates in all tested matrices in comparison to removal in DCTW without algae (they are from 44 ± 17 to 62 ± 11 %, Fig. 3c, *ii–v*), but the difference between tested matrices remains insignificant. It should be noted that the result may have been influenced by the approximately twofold lower cell number in the stationary phase of growth, and therefore the effect of the growth phase cannot be clear. In the repeated experiments with *A. platensis* in the stationary phase after nine months, similar results were obtained. Pre-chlorinated tAOM (matrix (*iv*)) showed the highest removal drop compared to DCTW without microalgae (30 ± 21 vs. 85 ± 4 %, respectively, Table S-VI). Contrarily, for *C. vulgaris*, significant differences in DCF removal range with AC2 (Fig. 3d) were observed when microalgae were in the exponential growth phase (the removal rate dropped sharply from 99 ± 0.3 % in DCTW to 58 ± 12 % in non-chlorinated tAOM and 16 ± 25 % in the non-chlorinated dAOM matrix (Fig 3d, *i–iii*). However, this was not clearly confirmed between the tAOM and dAOM matrix in the second set of experiments after nine months (Table S-VI, matrices *ii* and *iii* showed 74 ± 8 and 39 ± 18 % respectively). In this case, the difference in cell number between exponential growth phases during the first and second set was within the 20–30 % measurement uncertainty, without expectation of algal cell influence. Similarly, a significant difference was not possible to show in the matrix containing *C. vulgaris* in the exponential growth phase for AC2 removal efficiency in the pre-chlorinated dAOM matrix (43 ± 17 %) in comparison to pre-chlorinated tAOM (removal efficiency 74 ± 8 %) (Fig. 3d, *iv* and *v*). Similar effect was obtained in the repeated experiment (Table S-VI) as well as in the case when water matrix contained *C. vulgaris* in the stationary phase between pre-chlorinated tAOM and dAOM (84 ± 5 vs. 47 ± 16 %, respectively, Fig. 3d, *iv* and *v*). Repetition for this pair of tests was not performed.

Performance of activated carbons in matrices without algae differed to some extent in comparison to literature findings. Thus, Westerhoff *et al.*²⁷ reported lower removals of 16 and 39 % for IB and DCF respectively (PAC dose 5 mg L^{-1} , contact time 4 h) in surface waters spiked with 100 ng L^{-1} OMPs, while, similarly to our results. Nam *et al.*¹³ observed high removal (> 90 %) of DCF from distilled

water, while in surface water removal under the same experimental conditions (at $C_o = 100 \text{ ng L}^{-1}$, PAC dose 5 mg L^{-1} , contact time 4 h), was around 80 %. Snyder *et al.*²⁸ found that IB, CF and DCF average removal from four surface water was 15, ~70 and ~40 %, respectively, at a PAC dose of 5 mg L^{-1} ($C_o = 100 \text{ ng L}^{-1}$, contact time 4 h). The reasons for those differences in comparison to our study are most likely due to the use of different matrices (DCTW *vs.* surface water) and a higher OMPs initial concentration in this study ($1.5\text{--}2 \text{ }\mu\text{g L}^{-1}$ *vs.* 100 ng L^{-1}). Furthermore, PAC type, dose and different contact time can also influence results. High removal efficiency (~80 %) of hydrophilic CF ($\log D = 0.28$) by PAC is similar to results of Nam *et al.*¹³ and Piel *et al.*²⁹ It could be due to a strong affinity of heterocyclic-N group in his molecular structure for the carbonaceous surfaces, and specific sorbate-sorbent interactions.¹³ Notably, the removal of IB was less efficient compared to that of CF and DCF, indicating potential differences in adsorption behavior based on the compounds' molecular properties (Table S-I). In all cases, effects depended on the type of carbon and on the type of substance which was tested. Microalgae morphology and growth had an effect in some cases, but their influence was more context-dependent and secondary influenced by the type of PAC used. In general, it was observed that addition of microalgae increased the value of DOC by more than 13 %, which corresponds to the measurement accuracy of the method for measuring DOC (Tables S-IV and S-V). Rare significant differences for IB removal were observed in the presence of *C. vulgaris* during the exponential growth phase in the first set of single experiments for AC1, particularly between matrices containing tAOM and those with only dAOM. In the case of AC2, AOM had significant effects in certain instances but not clearly confirmed in repeated experiments. The removal of CF remained stable and was not affected by the influences of investigated matrix. The removal of DCF significantly decreased in presence of algae only when AC2 is used in matrices containing *A. platensis* in the stationary growth phase and in some cases when *C. vulgaris* was in exponential growth phase. One can hypothesize that the opposite effects of tAOM and dAOM obtained by filtration (increase of IB removal by AC1 and decrease of DCF removal by AC2 in matrix (iii) containing *C. vulgaris* in the exponential growth phase), might be caused by the chemical composition of the dAOM and characteristics of OMPs. In this phase of growth *C. vulgaris*, has high lipid (20–38 %) and protein (42–58 %) content.³⁰ *A. platensis*, has lower lipid (5–20 %) and higher protein (50–70 %) content.^{31,32} This may cause different interactions in between dAOM and PAC adsorption sites as well as formation of complexes of dAOM and OMPs of different characteristics. The lipid fraction of DOC may promote IB and DCF focus through complex formation, while proteinaceous compounds on AC can release amino acids, increasing competition for adsorption sites, especially for hydrophobic OMPs like DCF (*A. platensis*, stationary phase, AC2). Aromatic rings in IB and DCF also enable electron donor-acceptor complexes and

π - π interactions with AC functional groups, enhancing removal.³³ Negatively charged IB and DCF seem to be more affected by those matrix effects changes than neutral caffeine. Those interactions can further change when AOM structure is changed by pre-chlorination. According to Pivokonsky *et al.*¹¹ chlorination can lead to the reduction in adsorption due to the increased hydrophilicity and molecular size changes. In this study, a slight adsorption decrease of DCF was observed, but also a consistent increase in adsorption in the case of IB, which was not sufficiently supported by the confirmatory experiments, which often yielded contradictory results. Inconsistent effects in repeated experiments likely resulted from uncertainties in matrix preparation and DOC composition, including variations in DOC quality between sets. The second set of measurements showed good reproducibility, with removal efficiency differences over 30 % in few cases, but significant measurement uncertainties prevent confirming all effects. Impacts on charged molecules are evident but inconsistent.

CONCLUSION

This study evaluated the impact of the morphological characteristics and growth phases of *C. vulgaris* and *A. platensis* on the adsorption of IB, CF and DCF from water under various conditions, including the presence of total and dissolved algal organic matter and pre-chlorination. It was confirmed that different carbons have different ability to compensate for matrix variability. The results confirm that the removal efficiency of the uncharged CF remained stable across all tested conditions, suggesting minimal influence from algal matrices or PAC variability. For negatively charged IB and DCF, the observed effects were more variable. Significant differences were observed between algal matrices and PAC types, particularly for *C. vulgaris* in the exponential growth phase and *A. platensis* in the stationary phase. These effects were not consistently reproduced, likely due to variability in living systems, including changes in DOC structure and composition causing inconsistent interactions. The findings underscore the complexity of adsorption in living systems, where DOC variability and PAC interactions hinder reproducibility. Inconsistent effects highlight the need for further investigation into DOC behavior and its interactions. Future studies should assess the process in real surface waters with natural organic matter and algae, under varying environmental and seasonal conditions, to evaluate its robustness and scalability for practical application.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13180>, or from the corresponding author on request.

Acknowledgement. This research is funded from German federal budget by the DLR Project Management Agency acting on behalf of Federal Ministry for Education and Research

(Bundesministerium für Bildung und Forschung BMBF), grant number 01DS21012A, funding period 01.06.2021–31.12.2024.

ИЗВОД

УТИЦАЈ МИКРОАЛГИ НА УКЛАЊАЊЕ ОРГАНСКИХ МИКРОПОЛУТАНАТА ИЗ ВОДЕ АКТИВНИМ УГЉЕМ У ПРАХУ

NADIA KHAKIMOVA¹, MIHA BOGUHOVIĆ KOĴAJA², JEĴICA SIMEUNOVIĆ¹, LUCAS LANDWEHRKAMP³, STEFAN PANGNISCH³⁻⁶ и ИВАНА ИВАНЧЕВ-ТУМБАС²

¹Универзитет у Новом Сагу, Природно-математички факултет, Дејарман за биологију и екологију, Трј Досијеја Обрадовића 3, 21000 Нови Сад, ²Универзитет у Новом Сагу, Природно-математички факултет, Дејарман за хемију, биохемију и заштитну живојне средине, Трј Досијеја Обрадовића 3, 21000 Нови Сад, ³Chair for Mechanical Process Engineering and Water Technology, University of Duisburg-Essen, Lotharstr. 1, 47057 Duisburg, Germany, ⁴IWW Water Centre, Moritzstraße 26, 45476 Mülheim an der Ruhr, Germany, ⁵DGMT German Society for Membrane Technology e.V., Universitäts str. 2, 45141 Essen, Germany и ⁶Centre for Water and Environmental Research (ZWU), Universitäts str. 2, 45141 Essen, Germany

У овом раду испитан је утицај две врсте микроалги различите морфологије *Chlorella vulgaris* и *Arthrospira platensis*, у различитим фазама раста, на адсорпцију ибупрофена, кофеина и диклофенака на 2 врсте активног угља у праху. Дехлорисана чесменска вода са додатком микроалги је коришћена као матрица. Таква матрица у експериментима је коришћена са предхлорисањем и филтрацијом кроз филтер од 0,45 μm или без њих, како би се проценио утицај укупне и растворене алгалне органске материје. Одабрани органски микрозагађивачи (ОМР) анализирани су гасном хроматографијом са масеном спектрометријом. Резултати показују да је ефекат морфологије микроалги на ефикасност уклањања ОМР различит за различите угљеве. У неким случајевима примећене су варијације зависне од врсте микроалги и фазе раста. Присуство *A. platensis* у стационарној фази у води смањило је уклањање диклофенака активним угљем 2 (са 99 % на опсег од 44–62 %), док је присуство *C. vulgaris* у експоненцијалној фази изазвало пад на опсег од 16–74 % у поређењу са ефикасношћу активног угља 2 у дехлорисаној чесменској води без микроалги (99 %). Уклањање неутралног кофеина је стабилно, што указује на минималан утицај алгалне матрице или типа активног угља. За негативно наелектрисан ибупрофен и диклофенак, уочени ефекти су били променљиви и недоследни услед ограничења у експерименталним методологијама.

(Примљено 29. децембра 2024, ревидирано 9. априла, прихваћено 24. јула 2025)

REFERENCES

1. P. A. Inostroza, R. Massei, R. Wild, M. Krauss, W. Brack, *Environ. Pollut.* **231** (2017) 339 (<https://doi.org/10.1016/j.envpol.2017.08.015>)
2. S. Finckh, E. Carmona, D. Borchardt, O. Büttner, M. Krauss, T. Schulze, S. Yang, W. Brack, *Environ. Int.* **183** (2024) 108371 (<https://doi.org/10.1016/j.envint.2023.108371>)
3. Y. Luo, W. Guo, H. H. Ngo, L. D. Nghiem, F. I. Hai, J. Zhang, J. Liang, X. Wang, *Sci. Total Environ.* **473–474** (2014) 619 (<https://doi.org/10.1016/j.scitotenv.2013.12.065>)
4. T. Di Lorenzo, M. Cifoni, M. Baratti, G. Pieraccini, W. D. Di Marzio, D. M. P. Galassi, *Environ. Pollut.* **287** (2021) 117315 (<https://doi.org/10.1016/j.envpol.2021.117315>)
5. D. Sang, N. Cimetiere, S. Giraudet, R. Tan, D. Wolbert, P. Le Cloirec *J. Water Process Eng.* **49** (2022) 103190 (<https://doi.org/10.1016/j.jwpe.2022.103190>)

6. M. Huerta-Fontela, M. T. Galceran, F. Ventura, *Water Res.* **45** (2011) 1432 (<https://doi.org/10.1016/j.watres.2010.10.036>)
7. P. Schumann, M. Muschket, D. Dittmann, L. Rabe, T. Reemtsma, M. Jekel, A. S. Ruhl, *Water Res.* **235** (2023) 119861 (<https://doi.org/10.1016/j.watres.2023.119861>)
8. J. P. Gutkoski, E. E. Schneider, C. Michels, *J. Environ. Manage.* **349** (2024) 119434 (<https://doi.org/10.1016/j.jenvman.2023.119434>)
9. M. Campinas, C. Silva, R. M. C Viegas, R. Coelho, H. Lucas, M. J. Rosa, *J. Water Process Eng.* **40** (2021) 101833 (<https://doi.org/10.1016/j.jwpe.2020.101833>)
10. R. Guillosoou, J. Le Roux, R. Mailler, C. S. Pereira-Derome, G. Varrault, A. Bressy, E. Vulliet, C. Morlay, F. Nauleau, V. Rocher, J. Gasperi, *Water Res.* **172** (2020) 115487 (<https://doi.org/10.1016/j.watres.2020.115487>)
11. M. Pivokonsky, I. Kopecka, L. Cermakova, K. Fialova, K. Novotna, T. Cajthaml, R. K. Henderson, L. Pivokonska, *Sci. Total Environ.* **799** (2021) 149455 (<https://doi.org/10.1016/j.scitotenv.2021.149455>)
12. Q. Li, V. L. Snoeyink, B. J. Mariñas, C. Campos, *Water Res.* **37** (2003) 4863 (<https://doi.org/10.1016/j.watres.2003.08.018>)
13. S. W. Nam, D. J. Choi, S. K. Kim, N. Her, K. D. Zoh, *J. Hazard. Mater.* **270** (2014) 144 (<https://doi.org/10.1016/j.jhazmat.2014.01.037>)
14. M. Pivokonsky, J. Naceradska, I. Kopecka, M. Baresova, B. Jefferson, X. Li, R. K. Henderson, *Crit. Rev. Environ. Sci. Technol.* **46** (2016) 291 (<https://doi.org/10.1080/10643389.2015.1087369>)
15. Y. Zhang, X. Wang, H. Jia, B. Fu, R. Xu, and Q. Fu, *Sci. Total Environ.* **671** (2019) 351 (<https://doi.org/10.1016/j.scitotenv.2019.03.371>)
16. W. W. Carmichael, *J. Appl. Bacteriol.* **72** (1992) 445 (<https://doi.org/10.1111/j.1365-2672.1992.tb01858.x>)
17. L. de S. Leite, L. A. Daniel, T. Bond, *Environ. Sci. Water Res. Technol.* **9** (2023) 2787 (<https://doi.org/10.1039/D3EW00674C>)
18. J. Fan, L. Rao, Y. T. Chiu, T. F. Lin, *Water Res.* **102** (2016) 394 (<https://doi.org/10.1016/j.watres.2016.06.053>)
19. T. Garoma, R. E. Yazdi, *BMC Plant Biol.* **19** (2019) 18 (<https://doi.org/10.1186/s12870-018-1614-9>)
20. L. Tian, Z. Zhang, Z. Wang, P. Zhang, C. Xiong, Y. Kuang, X. Peng, M. Yu, Y. Qian, *Front. Environ. Sci.* **11** (2023) 1112522 (<https://doi.org/10.3389/fenvs.2023.1112522>)
21. M. Yu, Y. Qian, M. Ni, Z. Wang, P. Zhang, *Chemosphere* **354** (2024) 141733 (<https://doi.org/10.1016/j.chemosphere.2024.141733>)
22. M. Ma, R. Liu, H. Liu, J. Qu, *J. Water Supply: Res. Technol. – AQUA* **56** (2007) 41 (<https://doi.org/10.2166/aqua.2007.062>)
23. S. Laksono, I. M. ElSherbiny, S. A. Huber, S. Panglisch, *Chem. Eng. J.* **420** (2021) 127723 (<https://doi.org/10.1016/j.cej.2020.127723>)
24. K.-Y. Park, S.-Y. Choi, S.-K. Ahn, J.-H. Kweon, *J. Hazard. Mater.* **408** (2021) 124864 (<https://doi.org/10.1016/j.jhazmat.2020.124864>)
25. P. Cuderman, E. Heath, *Anal. Bioanal. Chem.* **387** (2006) 1343 (<https://doi.org/10.1007/s00216-006-0927-y>)
26. US EPA Method 525.3, *Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction And Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS)*, Washington, DC, 2012
27. P. Westerhoff, Y. Yoon, S. Snyder, E. Wert, *Environ. Sci. Tech.* **39** (2005) 6649 (<https://doi.org/10.1021/es0484799>)

28. S. A. Snyder, S. Adham, A. M. Redding, F. S. Cannon, J. DeCarolis, J. Oppenheimer, E. C. Wert, Y. Yoon, *Desalination* **202** (2007) 156 (<https://doi.org/10.1016/j.desal.2005.12.052>)
29. S. Piel, S. Blondeau, J. Pérot, E. Baurès, O. Thomas, *Water Qual. Res. J.* **48** (2013) 121 (<https://doi.org/10.2166/wqrjc.2013.138>)
30. C. Safi, B. Zebib, O. Merah, P. Y. Pontalier, C. Vaca-Garcia, *Renew. Sustain. Energy Rev.* **35** (2014) 265 (<https://doi.org/10.1016/j.rser.2014.04.007>)
31. B. Maddiboyina, H. K. Vanamamalai, H. Roy, Ramaiah, S. Gandhi, M. Kavisri, M. Moovendhan, *J. Basic Microbiol.* **63** (2023) 573 (<https://doi.org/10.1002/jobm.202200704>)
32. S. C. Silva, T. Almeida, G. Colucci, A. Santamaria-Echart, Y. A. Manrique, M. M. Dias, L. Barros, Á. Fernandes, E. Colla, M. F. Barreiro, *Colloids Surfaces, A* **648** (2022) 129264 (<https://doi.org/10.1016/j.colsurfa.2022.129264>)
33. S. Ebrahimzadeh, B. Wols, A. Azzellino, F. Kramer, J. P. van der Hoek, *J. Water Process Eng.* **47** (2022) 102792 (<https://doi.org/10.1016/j.jwpe.2022.102792>).

SUPPLEMENTARY MATERIAL TO
**Influence of microalgae on organic micropollutants removal
from water by powdered activated carbon**

NADIJA KHAKIMOVA¹, MINJA BOGUNOVIĆ KOLJAJA^{2*}, JELICA SIMEUNOVIĆ¹,
LUCAS LANDWEHRKAMP³, STEFAN PANGELISCH³⁻⁶
and IVANA IVANČEV-TUMBAS²

¹University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg
Dositeja Obradovića 3, 21000 Novi Sad, Serbia, ²University of Novi Sad, Faculty of Sciences,
Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja
Obradovića 1, 21000 Novi Sad, Serbia, ³Chair for Mechanical Process Engineering and
Water Technology, University of Duisburg-Essen, Lotharstr. 1, 47057 Duisburg, Germany,
⁴IWW Water Centre, Moritzstraße 26, 45476 Mülheim an der Ruhr, Germany, ⁵DGMT
German Society for Membrane Technology e.V., Universitäts str. 2, 45141 Essen, Germany,
and ⁶Centre for Water and Environmental Research (ZWU), Universitäts str. 2, 45141 Essen,
Germany. TABLE S1. Results of ICP-OES analysis of metal content in bio-oil samples

J. Serb. Chem. Soc. 90 (12) (2025) 1537–1551

The scheme of experimental methodology is shown in Fig S-1.

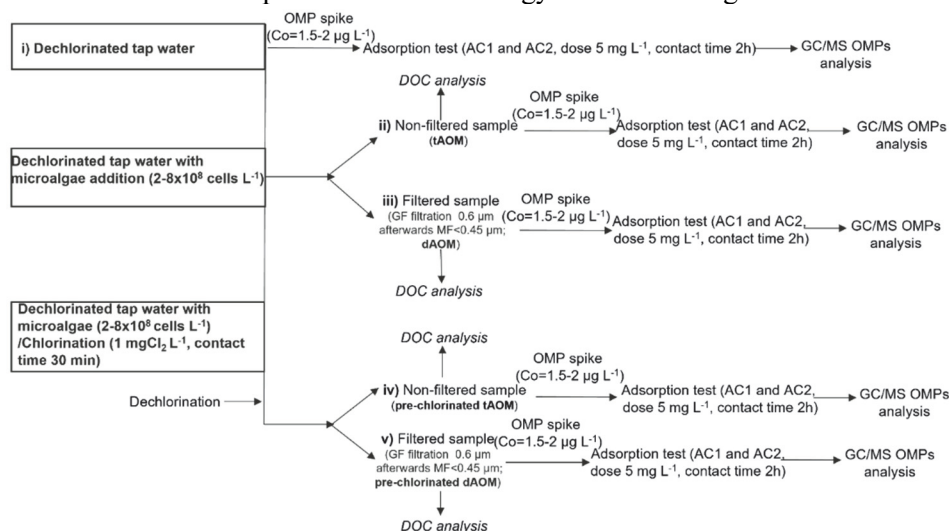


Figure S-1. Work methodology.

* Corresponding author. E-mail: minja.bogunovic@dh.uns.ac.rs

MATERIALS

Powdered activated carbons

Two types of commercial PAC were used for conducting all experiments. According to manufacturer's specifications, AC1 has an iodine number $900 \pm 100 \text{ mg g}^{-1}$, while AC2, which is obtained from carbonized coconut shell, has an iodine number $1000\text{--}1100 \text{ mg g}^{-1}$.

Microalgae selection and cultivation

For this study, two microalgae were selected based on their different morphology and talus: a) *Chlorella vulgaris*, a unicellular green alga, has a spherical shape and size that varies from 2 to 10 μm , and is known for its high lipid content (20–38 %) ^{1,2} and b) *Arthrospira (Spirulina) platensis* with filamentous structure and filaments length from 400–700 μm , ³ known for its rapid growth and high protein content (50–70 %) ^{3, 4, 5}. These differences in morphology and biochemical composition are expected to influence their interaction with micropollutants and PAC and the efficiency of their removal in drinking water treatment processes. The microalgae strains used in this study were obtained from the Novi Sad culture collection of cyanobacteria and microalgae (NSCCC) at the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad. The strains were cultivated under photoautotrophic growth conditions for 15–21 days to reach the exponential phase, and further up to 45 days to reach the stationary phase.

Chlorella vulgaris: Cultivated using commercial sterilized Algae Broth (Sigma Aldrich) under 12 h light and dark cycles and $T = 22\text{--}24^\circ\text{C}$. Medium composition is: 0.05 g L^{-1} of NH_4Cl , 0.058 g L^{-1} of CaCl_2 , 0.25 g L^{-1} of K_2HPO_4 , 0.003 g L^{-1} of FeCl_3 , 0.513 g L^{-1} of MgSO_4 , and 1 g L^{-1} of NaNO_3 .

Arthrospira (Spirulina) platensis: Cultivated for 45 days in sterilized Spirulina-Ogawa-Terui (SOT) (Ogawa T, 1970) medium under 12 h light and dark cycles and $T = 22\text{--}24^\circ\text{C}$. Medium composition is: NaHCO_3 16.8 g, K_2HPO_4 0.5 g, NaNO_3 2.5 g, NaCl 1.0 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.04 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g, $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ 0.08 g, trace mineral mix A5 1.0 mL.

The number of algal cells was counted using the standard direct counting method under a microscope (Kern Optic, OBN 135, Germany) with a hemocytometer plate and expressed as cells per L.

Organic micropollutants

For this study, investigated organic micropollutants are ibuprofen (IB), caffeine (CF) and diclofenac sodium salt (DCF), all purity $\geq 99\%$, purchased from Sigma Aldrich. Aqueous stock solutions ($\sim 5 \text{ mg L}^{-1}$) of substances were prepared in distilled water by sonication for 3 h and filtration through a $0.45 \mu\text{m}$ membrane filter (Sartorius, US). They were further diluted and used for spiking the water matrices (i, ii, iii, iv and v, Fig.S1) to achieve nominal concentrations of OMPs

around 1.5-2 $\mu\text{g L}^{-1}$ Table SI summarizes the physico-chemical properties of the selected compounds.

Table S-I. Chemical and physical properties of the analyzed organic micropollutants

Substance	MW ^a (g/mol)	ACD/logD ^b pH=7.4	pKa at 25 °C ^a	Charge at pH 7.4
Ibuprofen (IB)	206.28	0.45	5.3	-1
Diclofenac (DCF)	296.1	1.37	4.15	-1
Caffeine (CF)	194.19	0.28	14	0

^aPubChem 2024⁶; ^bChemSpider, 2024⁷; Abbreviations: MW - Molecular Weight; ACD/logD - Distribution Coefficient at pH=7.4.

Water matrices

Tap water from Novi Sad, Serbia was used for preparation of five water matrices:

i) Dechlorinated tap water without algae addition (DCTW); Dechlorination is performed with solution of Na-sulphite ($C=0.1 \text{ g mL}^{-1}$) in a way that 0.1 mL is added per 5 mg $\text{Cl}_2 \text{ L}^{-1}$ of residual chlorine.⁸ A part of DCTW was taken and spiked with selected OMPs to obtain nominal concentrations of 1.5-2 $\mu\text{g L}^{-1}$. For each matrix enrichment, aqueous solutions of OMPs were used. For each type of microalgae and phase of growth following type of matrices were prepared:

ii) DCTW with total algal organic matter (tAOM); In 12 L of DCTW 80 mL of culture (*A. platensis* or *C. vulgaris*, exponential or stationary phase of growth) was added. After algae addition, solution was slowly mixed for 10 min at 60 rpm and during the mixing three aliquots of 10 mL were taken and mixed as a composite sample in a beaker, afterwards, a composite sample (30 mL) was taken for algae abundance analysis using the standard direct counting method. This way the constant number of algae after repeating the experiment 3 times, was proven to be $2-8 \times 10^9 \text{ cells L}^{-1}$. Then, the whole mixture is transferred into tank filled with 90 L of DCTW and slowly mixed for 10 min at 60 rpm, after which, the abundance analysis is performed again to reach the number of algae of $2-8 \times 10^8 \text{ cells L}^{-1}$, in order to simulate algal abundance characteristic for eutrophic surface water. The solution was left undisturbed at room temperature under 12 h light and dark cycles for 24 hours to allow algae adaptation. After this period, the sample was mixed at 60 rpm for 10 minutes before measuring the algae abundance again. A part of this sample from the tank was used and spiked with selected OMP, in the same way as explained for the previous matrix (i);

Before the experiments, method for measuring the algae abundance was tested five consecutive times to demonstrate measurement consistency. The obtained values are presented in Table SII. These tests confirmed that algal densities consistently ranged from $1-3 \times 10^8 \text{ cells L}^{-1}$. In the main experiments, algal densities varied from $2-8 \times 10^8 \text{ cells L}^{-1}$, likely due to the use of fresh algal cultures in each run. Nonetheless, for future studies, standardizing the initial algal density

across all experiments could enhance methodological consistency and enable more accurate comparisons between different experimental conditions.

Table S-II. Microalgae abundance consistency in five separate test replications.

Number of cells in separate experiments taken from the 100 L water tank after culture addition and adaptation (cells L ⁻¹)	
<i>A. platensis</i>	<i>C. vulgaris</i>
1.0×10 ⁸	2.4×10 ⁸
3.0×10 ⁸	2.1×10 ⁸
2.95×10 ⁸	1.9×10 ⁸
1.2×10 ⁸	3.5×10 ⁸
1.15×10 ⁸	2.0×10 ⁸

iii) DCTW with dissolved algal organic matter (dAOM); Preparation: a part of matrix ii) was filtered through glass-fiber filter (0.6 µm, MACHEREY-NAGEL, Germany) and afterwards through 0.45 µm membrane filter (Sartorius, US), to obtain the solutions with dAOM. After filtration matrix was spiked with selected OMP, in the same way as explained for the matrix (i);

iv) Pre-chlorinated tAOM - a part of the matrix ii) is chlorinated with the dose of 1 mg Cl₂ L⁻¹, and left for 30 min to react and afterwards dechlorinated again based on measurement of actual concentration of chlorine in the samples, and after that spiked with selected OMPs as previously explained, to avoid the contact of chlorine and OMPs;

v) Pre-chlorinated dAOM - a part of the matrix ii) is chlorinated with the dose of 1 mg Cl₂ L⁻¹ and left for 30 min to react and afterwards dechlorinated based on measurement of actual concentration of chlorine in the samples. Sample is further filtrated through glass-fiber filter (0.6 µm, MACHEREY-NAGEL, Germany) and afterwards through 0.45 µm membrane filter (Sartorius, US). Upon filtration it was spiked with selected OMPs.

ANALYTICAL METHODS

Sample preparation and GC/MS OMPs analysis

In an aliquot of the sample (250 mL), the pH value (pH=2) is adjusted using cc of HCl (p.a >37 %, Centrochem). Sample was spiked with the 25 µL of mecoprop internal standard working solution (PESTANAL® analytical 99.6 % (HPLC) Sigma-Aldrich, c=1 µg L⁻¹), and pre-concentrated with the Oasis® HLB (3cc, 60 mg) SPE cartridge (Waters, Massachusetts, USA) according to the following procedure: a) Conditioning – 3x1 mL of mixture ethyl-acetate/methylene-chloride 1:1 (EtAc – Sigma Aldrich, 99 %; DCM for pesticide residue analysis, Chromasolv™, Honeywell), than 3x1 mL of methanol (for pesticide residue analysis, Chromasolv™, Honeywell), and 3x1 mL of acidic laboratory water (pH ≈ 2); b) Sample loading (flow 5 mL min⁻¹); c) Sorbent drying under vacuum for 1h and d) Elution with 3 aliquots of 1 mL EtAc/DCM (1:1) with

20 min retention of the last solvent portion. Further, solvent was removed under N₂ at room temperature. Extracts were quantitatively transferred to vials by washing the walls of the cuvette with 0.5 mL of toluene (for pesticide residue analysis, Chromasolv™, Honeywell). Before that, 1 µL of working solution of the second internal standard for chromatography check, phenanthrene d-10 ($c=0.4 \mu\text{g L}^{-1}$) is added to vial and dried under N₂ and afterwards derivatization was performed using 100 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide MSTFA (Synthesis grade, Sigma-Aldrich) at 60 °C for 1 h.⁹

IB, CF and DCF were measured by GC/MS (Agilent 7890B GC with 5977A MSD). The column Agilent J&W Scientific, DB-5 MS (30 m × 0.25 mm × 0.25 µm; Agilent, USA) was applied with helium as the carrier gas in constant flow (1 mL min⁻¹). 2 µL of the extract was injected in the splitless mode at 250 °C (purge flow 30 mL min⁻¹ at 0.75 min). Temperature program applied is 100 °C–2 min; 15 °C min⁻¹ up to 180 °C; 30 °C min⁻¹ up to 230 °C which was held 5 min and afterwards 20 °C min⁻¹ up to 270 °C which was held 3 min. Method characterization is shown in Table SIII.

Table S-III. GC/MS method characterization

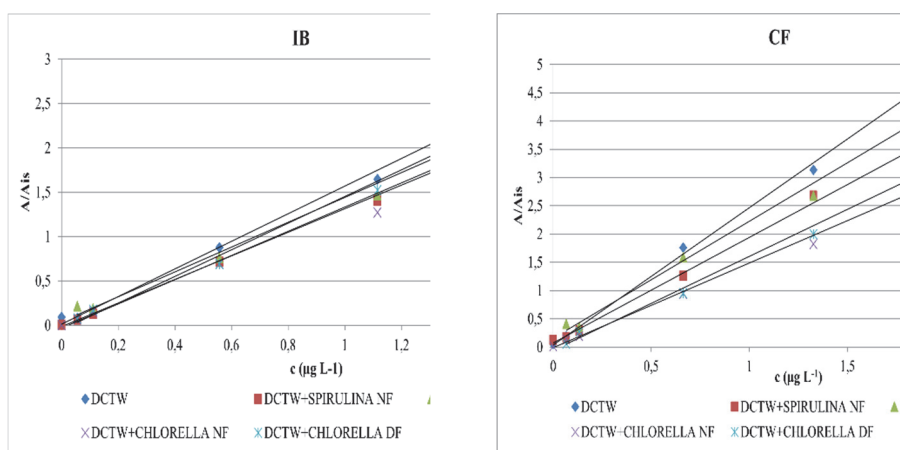
OMP	Target ion, qualifier ion	Calibration		Method repeatability, RSD, %		Instrument repeatability, RSD, %		Bias %		MDL*	PQL**
		Range (µg L ⁻¹)	R ²	at 0.5	at 1.5	at 0.5	at 1.5	at 0.5	at 1.5		
IB	160, 263, 117	0.05-2.12	0.999	3	4	5	2	8	2	0.02	0.08
CF	194, 109, 82	0.05-2.01	0.994	2	6	2	4	0.3	6	0.03	0.091
DCF	214, 242, 367	0.05-1.89	0.995	10	2	2	2	3	4	0.024	0.071

*MDL- method detection level. **PQL- practical quantitation level

Calibration was done in DCTW in triplicate and average values are presented in Table SIII. MDL is calculated from the standard deviation (SD) of seven replicates in DCTW spiked with 0.05 µg L⁻¹ of selected OMPs.⁸ PQL is assessed as SD×10 which is in accordance with the rule that PQL is usually 3-5 times higher than MDL.⁸ Method repeatability is determined in a DCTW as the relative standard deviation of three measurements of different extracts. Instrument repeatability is determined as the relative standard deviation of three measurements in the same extract. Method and instrument repeatability are satisfactory (≤10 %) for all compounds and for both concentration levels (at 0.5 and 1.5 µg L⁻¹). Biases at both concentration levels were very satisfactory (≤8 %). They are determined based on the difference between measured calibration concentration and the expected calibration concentration (at 0.5 and 1.5 µg L⁻¹).

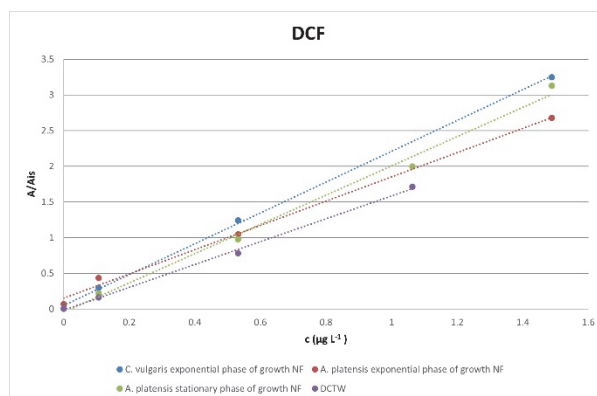
For calibrations in algal matrices, linearity was confirmed in range 0.1-1.5 µg L⁻¹ (Fig. S2). To cover variations in detector sensitivity, the peak area of each

analyte was divided by the peak area of the internal standard mecoprop (A/A_{is}). In applied linear range, no significant differences of A/A_{is} ratios were observed for the great majority of calibration points in DCTW and DCTW matrix containing algae (e.g. U.S. EPA Method 525.3 allows $\pm 30\%$ change in signal for quality control checks at mid- and high-calibration level points, while at the minimum level reporting $\pm 50\%$ of change from the expected value is allowed (U.S. EPA, 2012)¹⁰).



a) IB

b) CF



c) DCF

Fig. S2. Calibrations in DCTW and algal matrices; a) for IB; b) for CF and c) for DCF; DCTW- dechlorinated tap water; *Spirulina* NF – DCTW with *A. platensis* (*Spirulina*) addition (6.7×10^8 cells L^{-1}) and OMPs without filtration; *Spirulina* DF – DCTW with *A. platensis* (*Spirulina*) addition (6.7×10^8 cells L^{-1}) and OMPs double filtered through glass-fiber and afterwards $0.45 \mu m$ membrane filter; CHLORELLA NF – DCTW with *C. vulgaris* addition (3.5×10^8 cells L^{-1}) and OMPs without filtration; CHLORELLA DF – DCTW with *C. vulgaris* addition (3.5×10^8 cells L^{-1}) and OMPs filtered through a $0.45 \mu m$ membrane filter.

Thus, having the linear calibrations and assuming their validity for the samples before and after the treatment in each adsorption experiment, simple removal calculations were applied only from ratio of OMPs chromatographic signals and internal standard mecoprop (IS) (Eq. 1):

$$R = \frac{S_0 - S_i}{S_0} \cdot 100\% \quad (1)$$

whereas:

R denotes the relative removal of OMPs in percent

S_0 is the ratio of the peak area of the target ion of the OMP and the peak area of the internal standard target ion in the sample before the adsorption experiment (A_0/A_{IS0} , proportional to the initial concentration in the samples)

S_i is the ratio of the peak area of the target ion of the OMP and the peak area of the internal standard target ion in the sample after the adsorption experiment (A_i/A_{ISi} , proportional to the final concentration in the samples)

The samples of each matrix before treatment were filtered by the same procedure (GF and afterwards by MF) as the samples after PAC treatment.

Since the values before and after the adsorption experiment are measured independently from each other, the overall error of the relative removal can be calculated by the variance formula¹¹:

$$S_R = \sqrt{\left(\frac{\partial R}{\partial S_{S0}}\right)^2 \cdot S_{S0}^2 + \left(\frac{\partial R}{\partial S_{Si}}\right)^2 \cdot S_{Si}^2} \quad (2)$$

whereas:

S_R – overall error of the relative removal R

S_{S0} and S_{Si} are the measurement errors of S_0 and S_i . Combined uncertainty of OMPs analysis in DCTW matrix was used for their calculation. It was assessed based on adopted acceptance limit of 30% and the biases of recovery data up to 24% (see the sub-section *QC of GC/MS measurements*).

Solution of Eq. 2 results in Eq. 3:

$$s_R = \sqrt{\left(\frac{s_i}{s_0}\right)^2 \cdot s_{s0}^2 + \left(\frac{-1}{s_0}\right)^2 \cdot s_{si}^2} \quad (3)$$

DOC ANALYSIS

DOC in matrices was tracked using a TOC analyzer (liquiTOCII, Elementar, Germany). Method characterization and the results are shown in Table SIV and SV.

Table S-IV. DOC method characterization.

Linearity (mg L ⁻¹)	0.56–25
MDL (mg L ⁻¹)	0.25
PQL (mg L ⁻¹)	0.56
Trueness (%)	94–101
Precision at 1 mg L ⁻¹ (%)	13

Table S-V. Initial DOC values in matrices in the first and second experiment set.

Matrix	DOC, mgC/L		DOC difference between two sets, %	Impact of algae addition on DOC content in comparison to DCTW, %	
	I set	II set		I set	II set
DCTW blank	1.35	1.96	31	-	-
iiArth-e	1.29	1.81	29	4	-8
iiiArth-e	1.49	-	-	9	-
ivArth-e	2.23	-	-	39	-
vArth-e	1.65	-	-	18	-
DCTW blank	-	1.50	-	-	-
iiChlor-e	1.64	1.77	7	-	15
iiiChlor-e	1.97	1.78	-11	-	16
ivChlor-e	1.46	1.67	13	-	10
vChlor-e	1.54	1.93	20	-	22
DCTW blank	-	1.43	-	-	-
iiArth-s	1.70	1.69	-1	-	15
iiiArth-s	1.99	1.81	-10	-	21
ivArth-s	1.87	1.84	-2	-	22
vArth-s	2.29	1.96	-17	-	27
DCTW blank	-	1.84	-	-	-
iiChlor-s	1.58	1.49	-6	-	-23
iiiChlor-s	1.78	1.76	-1	-	-5
ivChlor-s	-	-	-	-	-
vChlor-s	1.77	1.95	9	-	6

Arth – *A. platensis*, Chlor – *C. vulgaris*, e – exponential growth phase, s – stationary growth phase. ii – non-chlorinated matrix with tAOM; iii – non-chlorinated matrix with dAOM; iv – pre-chlorinated matrix with tAOM; v – pre-chlorinated matrix with dAOM.; - not measured

RESULTS

Table S-VI. Comparison of the removal efficiency obtained in two set of experiments

Type of water matrix	Microalgae	Number of algae · 10 ⁸ (cells L ⁻¹)		Removal rates for AC1 ± error of the relative removal, %						Removal rates for AC2 ± error of the relative removal, %					
				IB		CF		DCF		IB		CF		DCF	
		I set	II set	I set	II set	I set	II set	I set	II set	I set	II set	I set	II set	I set	II set
i	without	-	-	47±14	77±6	77±7	93±2	93±2	90±3	36±17	69±8	76±8	92±3	99±0.3	85±4
	Arth-e	8	6	53±13	51±3	83±5	81±6	99±0.5	74±8	53±13	-	84±5	-	98±1	-
ii	Arth-s	4	5	66±9	-	89±4	-	87±4	-	41±16	47±14	81±6	89±4	62±11	63±11
	Chlor-e	2	3	47±14	-	89±4	-	88±4	-	NR	54±12	86±5	88±4	58±12	74±8
	Chlor-s	5	4	65±9	-	86±5	-	91±3	-	44±15	56±12	77±8	92±3	64±11	84±5
	Arth-e	8	-	54±12	-	83±6	-	89±3	-	16±23	-	73±9	-	92±2	-
iii	Arth-s	4	5	66±9	-	88±4	-	89±3	-	33±18	34±18	74±8	75±8	50±15	57±13
	Chlor-e	2	3	95±1	44±15	87±4	76±4	97±1	69±9	25±20	17±22	79±7	66±11	16±25	39±18
	Chlor-s	5	4	61±11	-	79±7	-	83±5	-	50±14	53±13	86±5	88±4	68±10	73±8
	Arth-e	8	-	61±10	-	83±6	-	97±1	-	37±17	-	79±7	-	90±3	-
iv	Arth-s	4	5	66±9	-	89±3	-	89±3	-	30±19	NR	73±9	19±26	44±17	30±21
	Chlor-e	2	3	70±8	63±10	90±3	88±8	91±3	78±7	57±12	NR	87±4	64±12	74±8	44±16
	Chlor-s	5	4	63±10	-	85±5	-	85±4	-	58±11	-	87±4	-	84±5	-
	Arth-e	8	-	55±12	-	76±8	-	89±3	-	39±16	-	78±7	-	79±6	-
v	Arth-s	4	5	60±11	-	87±4	-	82±5	-	39±17	39±17	79±7	80±7	54±14	60±12
	Chlor-e	2	3	63±10	-	88±4	-	88±4	-	14±23	28±19	78±7	79±7	43±17	54±14
	Chlor-s	5	4	66±9	-	66±11	-	86±4	-	36±17	33±18	65±11	81±6	47±16	74±8

Arth – *A. platensis*, Chlor – *C. vulgaris*, e – exponential growth phase, s – stationary growth phase. i – DCTW without algae; ii – non-chlorinated matrix with tAOM; iii – non-chlorinated matrix with dAOM; iv – pre-chlorinated matrix with tAOM; v – pre-chlorinated matrix with dAOM; - not repeated in second set experiments; NR – no removal

Based on the algal cell counts presented in Table SVI, it can be concluded that the deviation in algal abundance between first and second of experimental runs is not significant, as it falls within the known measurement uncertainty for algal quantification (20–30%)¹². A significant difference was observed between growth phases, as the difference in cell density between the exponential and stationary phases often exceeded 50%, which is well above the known 20–30% uncertainty of the hemocytometer counting method. While these differences reflect realistic physiological states of algae, they may have also contributed to the observed variability in OMP removal.

Quality control procedures (QC)

QC of experimental methodology

Adsorption test without algae - Experiment repeatability was checked by the analysis of five independent replicates of adsorption tests for AC2 in dechlorinated tap water (matrix (i), dose 5 mg L⁻¹, 2 h contact time, Figure S1). The relative standard deviation (RSD) of signal normalized for IS peak (A/A_{IS}) was for IB, CF

and DCF 17, 2 and 0.8 %, respectively. Nine months later the triplicate of the same test gave RSD of 2 %, 1 % and 1 %, for IB, CF and DCF, respectively.

Adsorption test with algae – Repeatability was checked by three independent adsorption tests in matrix (ii), with AC2 dose 5 mg L⁻¹ and 2 h contact time (Figure S1). Both microalgae in stationary phase (4 and 5x10⁸ cells L⁻¹) were tested. RSD of signal normalized for IS peak (A/A_{IS}) in case of *C. vulgaris* was for IB 12 %, for CF 1 % and for DCF 0.1 %. In the case of *A. platensis* RSD of signal normalized for IS peak (A/A_{IS}) was ≤6 % for all OMPs.

Repeatability of adsorption tests in time - 14 experiments out of 32 were repeated after nine months (three for AC1 and 11 for AC2). Obtained results are shown in Table SVI. The values from the first set of adsorption test and the same tests repeated after 9 months were compared. All tests for AC1 in matrix containing algae showed differences less than 30 % for CF and for DCF, while for IB one showed 50 % difference, which is higher than expanded uncertainty of the analysis (38 %). For AC2, no tests with algae for DCF showed significant differences in removal rates, while for CF and IB, one and three tests had very low repeatability, respectively. A possible reason for this could be the fact that the matrices with algae are living systems and/or accuracy of preparing the solution varies as shown before in addition to the fact that accuracy of counting method of algal cells is between 20-30 %.¹² The applied criteria for declaring significant differences (> 30%) related to OMPs analysis is same as mentioned above.

QC of GC/MS measurements

Recovery test

An independent control standard check (OMPs c=0.5-0.7 µg L⁻¹) as the mid-calibration point was processed and analyzed exactly like a sample in laboratory routine work during one year. Single, same extract was repeatedly analyzed within one batch of measurements after each 10 samples (5-20 measurements of control standard within a batch). In total, data for 6 such batches were available during one year. Recoveries for all OMPs were between 78-124 %. During the analysis of the samples from the first set of experiments, this independent control standard check showed biases from expected values in the range 9-24 % with RSD of recovery values in the range of 1-2 % for all OMPs. Similarly, in the case of the second set of experiments, after nine months, biases from expected recovery values were in the range 7-20 % with RSD for recoveries of 3-4 % for all OMPs. Similar to the methodology provided in ISO/DIS 11352:2024¹³ standard, Annex C4, and based on the mentioned recovery values, combined uncertainty was calculated and obtained as 19 % for IB, 21 % for CF and 23 % for DCF.

Extraction and derivatization were monitored by check of internal standard mecoprop which was added into samples before extraction. For a total of 116 samples (32 experiments and 14 repeated, in total 12 batches of GC/MS analysis) RSD of target ion peak surface was 15 %, regardless of the water matrix used. In

the same time IS phenanthrene d-10 was used to assess the work of instrument without sample preparation and, only reflects gas-chromatography procedure. RSD of target ion peak surface was 19 %.

REFERENCES

1. Y. Liang, N. Sarkany, N., and Y. Cui, *Biotechnol. Lett.*, **31** (2009) 1043 (<https://doi.org/10.1007/s10529-009-9975-7>)
2. C. Safi, B. Zebib, O. Merah, P. Y. Pontalier, and C. Vaca-Garcia, *Renew. Sustain. Energy Rev.* **35** (2014) 265 (<https://doi.org/10.1016/j.rser.2014.04.007>)
3. C. Van Eykelenburg, On the morphology and ultrastructure of the cell wall of *Spirulina platensis*. *Antonie Van Leeuwenhoek* **43** (1977) 89 (<https://doi.org/10.1007/BF00395664>)
4. B. Maddiboyina, H. K. Vanamamalai, H. Roy, H., Ramaiah, Gandhi, S., Kavisri, M., and M. Moovendhan, *J. Basic Microbiol.*, **63** (2023) 573 (<https://doi.org/https://doi.org/10.1002/jobm.202200704>)
5. S. C. Silva, T. Almeida, G. Colucci, A. Santamaria-Echart, Y. A. Manrique, M. M. Dias, L. Barros, Â. Fernandes, E. Colla, and M. F. Barreiro, (2022). *Colloids Surf. A: Physicochem. Eng. Asp.*, **648** (2022) 129264 (<https://doi.org/10.1016/j.colsurfa.2022.129264>)
6. PubChem. Online: <https://pubchem.ncbi.nlm.nih.gov/> (Last Accessed December 11, 2024).
7. ChemSpider properties predicted by ACD/Labs. Online. <https://www.chemspider.com/> (Last Accessed December 11, 2024)
8. APHA-AWWA-WEF, Standard methods for the examination of water and wastewater, Edited by R.B. Baird, A. D. Eaton, and E. W. Rice (2017)., 23rd edition, Washington, D.C., American Public Health Association.
9. P. Cuderman, E. Heath, *Anal. Bioanal. Chem.* **387** (2006) 1343 (<https://doi.org/10.1007/s00216-006-0927-y>)
10. US EPA Method 525.3: Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction And Capillary Column Gas Chromatography/ Mass Spectrometry (GC/MS) (2012)
11. H.H Ku, *J. Res. Nat. Bur. Stand. Sec. C: Eng. Inst.*, **70C** (1966) 263
12. J. W. G. Lund, C. Kipling and E. D. Le Cren, *Hydrobiologia*, **11** (1958) 143 (<http://dx.doi.org/10.1007/BF00007865>)
13. ISO/DIS 11352: Water quality - Estimation of measurement uncertainty based on validation and quality control data (2024).



J. Serb. Chem. Soc. 90 (12) 1553–1561 (2025)
JSCS–5472

LETTER TO THE EDITOR

Centennial of quantum theory in Belgrade: CEQPAS and the molecular excited state spectroscopy (MOLESs) initiative

FELIPE FANTUZZI¹, JELENA B. MALJKOVIĆ², MATIJA ZLATAR³, CAUÊ P. SOUZA¹,
HEIDY M. QUITIÁN-LARA⁴, PAMIR NAG⁵, BOBBY ANTONY⁶,
BRATISLAV P. MARINKOVIĆ^{2*} and NIGEL J. MASON^{7**}

¹School of Natural Sciences, University of Kent, Park Wood Rd, Canterbury CT2 7NH, UK,
²Institute of Physics, University of Belgrade, Pregrevica 118, Belgrade, Serbia, ³Institute of
Chemistry, Technology and Metallurgy, Njegoševa 12, 11000, Belgrade, Serbia, ⁴Max Planck
Institute for Extraterrestrial Physics, Gießenbachstraße 1, Garching 85748, Germany, ⁵J.
Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Dolejškova 2155/3,
182 23 Prague, Czech Republic, ⁶Department of Physics, Indian Institute of Technology (ISM),
Dhanbad, 826004, Jharkhand, India and ⁷School of Engineering, Mathematics and Physics,
University of Kent, Park Wood Rd, Canterbury CT2 7NH, UK

(Received 18 November, revised 20 November, accepted 25 November 2025)

Abstract: The first week of November 2025 saw two events in Belgrade celebrating one hundred years of quantum mechanics and its impact on chemistry and molecular science. The Centennial of Quantum Theory: Progress in Atomic and Molecular Structure (CEQPAS) brought together researchers from across Europe and beyond to reflect on the scientific legacy of quantum theory and to showcase current advances in atomic and molecular spectroscopy, collision processes relevant to plasma research, chemical bonding, quantum chemistry, and the design of functional molecules, astrochemistry, the use of AI/ML for the analysis and generation of atomic and molecular data and wider applications of quantum science. Immediately after CEQPAS, a new international initiative was launched: Molecular Excited State spectroscopy (MOLESs), whose primary goal is to develop an atlas of molecular electronic structure and excited-state spectroscopy that rigorously integrates experiment and theory. Together, CEQPAS and MOLESs align historical perspective with future capability, positioning Belgrade as a regional hub for quantum-enabled molecular science.

Keywords: atomic and molecular spectroscopy; collision processes; plasma physics; machine learning for spectroscopy; electronic structure; quantum chemistry.

*,** Corresponding authors. E-mail: (*)bratislav.marinkovic@ipb.ac.rs,
(**)n.j.mason@kent.ac.uk
<https://doi.org/10.2298/JSC251118083F>

In 1925, quantum mechanics emerged from an intellectual rupture. Werner Heisenberg, then a young research assistant in Göttingen, retreated to the North Sea island of Helgoland to recover from hay fever and confront the mounting contradictions of atomic theory.¹ Working late into the night with tables of spectral lines, he set aside unobservable electron orbits and rebuilt the theory using only measurable quantities such as transition frequencies and intensities. The result was his *quantum-theoretical reinterpretation* paper.² Heisenberg later described the moment he realised the mathematics worked: he felt he was looking “through the surface of atomic phenomena into a strangely beautiful interior”, became “far too excited to sleep”, and went to the southern tip of the island where he climbed a rock and waited for the sunrise.

Within months, Born and Jordan recast Heisenberg’s scheme into matrix mechanics, a formalism that recovered selection rules and line strengths drawn from spectroscopy.³ Pauli introduced the exclusion principle,⁴ and Uhlenbeck and Goudsmit proposed electron spin to explain fine structure and the anomalous Zeeman effect.⁵ Early in 1926, Schrödinger formulated wave mechanics and derived the hydrogen spectrum, later showing its equivalence to matrix mechanics,⁶ while Born provided the statistical interpretation that linked calculated amplitudes to observed line intensities.⁷ Throughout these developments, spectroscopy supplied both the puzzles and the tests; it was spectroscopy that compelled quantum theory into being.

A century after Heisenberg’s Helgoland breakthrough, the historical arc turned toward Belgrade. In the first week of November 2025, two landmark international events were organised by two institutes of national importance: the Institute of Physics Belgrade (IPB) and the Institute of Chemistry, Technology, and Metallurgy (ICTM). IPB hosted the international conference Centennial of Quantum Theory: Progress in Atomic and Molecular Structure (CEQPAS, Fig. 1).⁸ Serbian-based researchers were joined by colleagues from Europe, India, New Zealand and Latin America, gathering not only to celebrate the intellectual leap of 1925 but also to examine how that leap continues to shape modern science.

The opening lecture was delivered by Nigel Mason (University of Kent, UK), who reconnected the historical origin of quantum mechanics to present-day scientific challenges. Mason argued that spectroscopy was central to the birth of quantum mechanics, having first revealed the breakdown of classical physics, and remains the method most closely linked to quantum principles. He noted that major reformulations of quantum theory have often followed advances in spectroscopy, from early optical emission studies to modern free-electron lasers. Looking ahead, THz spectroscopy was highlighted as the next frontier for probing intermolecular forces and structural dynamics in soft matter.^{9,10} Mason also warned that progress relies not only on instrumentation but on retaining expertise, as the field risks losing specialised knowledge faster than it can be renewed. He underscored that spectro-

scopic data underpin developments in quantum computing, astrophysics, environmental chemistry and nanotechnology, and called for long-term stewardship through open repositories, shared protocols and sustained training. The opening session, which included representation from the British Embassy in Belgrade and coverage by Radio Television of Serbia, marked the growing recognition of quantum science as a driver of collaboration and technological progress.



Fig. 1. CEQPAS conference group photo. Credits: Dušan Pantelić & Bojan Živojinović.

What followed during CEQPAS demonstrated this idea in concrete scientific terms, with spectroscopy and quantum theory appearing in contexts that could not have been imagined by Heisenberg in 1925. Andjelka Kovačević adapted correlation spectroscopy, originally developed for molecular systems, to analyse variability in supermassive black holes using quantum neural process models,¹¹ effectively treating accretion discs as spectroscopic systems and enabling tests of scenarios in which ultralight bosons form gravitationally quantised states. Larissa Lopes Cavalcante (University of Otago, New Zealand) examined cryogenic hydrocarbon and nitrogen-bearing ices relevant to Titan, showing through infrared spectroscopy and vacuum-ultraviolet photolysis that the degree of molecular ordering, crystalline or amorphous, controls both spectral signatures and photoreactivity.¹² Milan Milovanović extended the astrochemical perspective by benchmarking the vibrational and electronic spectra of fullerenes and their hydrogenated derivatives, identifying computational strategies suitable for astronomical assignments. Bratislav Marinović then led a guided tour of the IPB facilities, presenting the Omicron High-Resolution Hemispherical Analyser (OHRHA),¹³ a shielded crossed-beam spectrometer capable of resolving electron-atom interactions with sub-0.1 eV precision, alongside the UGRA (UGaona RAspodela, Angular Distribution) system,¹⁴ which measures angular-dependent differential cross sections and underpins the derivation of integral and momentum-transfer cross sections essential for plasma, atmospheric and environmental modelling. These contributions all pointed to the same realis-

ation: spectroscopy is not only a tool for identifying molecular structure, but also our means of accessing chemistry in environments that cannot be reached physically, from planetary surfaces to the vicinity of black holes.

CEQPAS then shifted from spectroscopy to collisions. David Field (Aarhus University, Denmark) presented low-energy electron collisions in condensed molecular films and introduced *spontelectrics*: spontaneously polarised thin films where internal electric fields alter electron scattering.¹⁵ Bobby Antony (Indian Institute of Technology Dhanbad, India) presented an overview of modern electron–molecule scattering theory,¹⁶ outlining how current methods address a wide range of energies while still facing challenges for complex polyatomic and spin-relativistic targets, and showing how recent advances are beginning to make these systems tractable. Pamir Nag (J. Heyrovský Institute of Physical Chemistry, Czech Republic) showed that dissociative electron attachment can require symmetry lowering for bond cleavage to occur, providing an example where allowed and forbidden pathways depend on transient geometry.¹⁷ Nikola Cvetanović showed how Stark spectroscopy serves as a non-intrusive probe of strong electric fields in low-temperature plasmas, using line splitting and shifts of hydrogen and helium emissions to map sheath fields that develop near plasma boundaries.¹⁸ Nikola Škoro showed how non-thermal atmospheric-pressure plasma jets act as reactors that generate and deliver reactive oxygen and nitrogen species into water, illustrating how such plasmas can be harnessed for plasma-based water treatment with relevance to environmental remediation, agriculture and medicine.¹⁹ The first day concluded with public lectures by Nenad Vukmirović, on quantum aspects of electron transport in solar-cell materials and Slobodan Bubnjević, on the early history of quantum mechanics through key figures of the Solvay conferences. Framed by these broader perspectives, the day's programme reinforced that collision processes, once central to the derivation of quantum mechanics, now underpin applications in plasma agriculture, plasma medicine and environmental technologies.

The second day opened with a session on physical chemistry. After briefly linking quantum mechanics and spectroscopy to the design of functional molecules, including therapeutics relevant to neglected tropical diseases,²⁰ Felipe Fantuzzi (University of Kent, UK) argued that many core chemical concepts, devised before quantum mechanics, must be reformulated in a quantum language, recasting the foundations of chemistry itself. He then demonstrated that chemical bonding is a manifestation of quantum interference.²¹ Cauê Souza (University of Kent, UK) presented a new computational protocol to reproduce the laboratory vacuum-ultraviolet absorption spectra of cryogenic ices, providing phase-sensitive benchmarks relevant to planetary and interstellar environments.^{22,23} Dušan Veljković introduced strategies to reduce sensitivity in energetic materials by tuning local electrostatic potential,²⁴ and the discussion highlighted opportunities for collabor-

ation with forensic science and dual-use research areas. Astrochemistry returned in the contribution by Heidy M. Quitián-Lara (Max Planck Institute for Extra-terrestrial Physics, Germany), who showed how high-resolution spectroscopy and radiation-induced fragmentation experiments, paired with quantum-chemical calculations, can map the stability, reactivity and formation pathways of interstellar molecules and interpret astronomical observations.^{25,26} The session concluded with a quantum-chemical study of non-covalent sulphur interactions by Ivana Veljković,²⁷ extending the σ -hole concept to rationalise chalcogen bonding.

The programme then turned to the conceptual foundations of quantum mechanics and to the emerging role of artificial intelligence (AI) and machine learning (ML) in physical science. Nenad Simonović discussed quantum diffraction and interference in atomic processes and showed that time-resolved superpositions of electron wave packets generate dynamic and exchange interference, enabling phase retrieval and the clear discrimination of competing scattering channels.²⁸ Igor Salom revisited the Wigner's friend paradox, challenging assumptions about the boundary between quantum and classical descriptions of observers. Saša Dujko re-examined the Franck–Hertz experiment with modern diagnostics, showing that energy exchange and electron spatial relaxation in gases are richer than the classic picture suggests.²⁹ Ivan Belča showed that an AI system can autonomously generate a complete spectroscopy workflow, raising the question of whether AI-generated datasets may eventually complement or replace new experiments. Vladimir Srećković demonstrated how small molecules shape astrophysical spectra through collisions and radiation, emphasising the need for accurate cross sections, robust databases and non-local thermodynamic equilibrium models, and noted that AI can help interpolate sparse data and guide future measurements.³⁰ Dimitrije Maletić reviewed how AI and ML support modern high-energy and nuclear-physics experiments, from event selection and pattern recognition to real-time decision systems. Lastly, Vladimir M. Petrović examined the capacities and limits of current AI, arguing that understanding it requires moving beyond black-box use toward transparent mechanisms tested across both physical and virtual environments.

The conference closed with remarks by Jelena Maljković, who emphasised that CEQPAS had showcased substantial progress, yet also revealed how much remains to be done. Across spectroscopy, collision science, physical chemistry, astrochemistry and AI, a recurring message emerged: quantum mechanics is not a finished theory, and spectroscopy remains the discipline that reveals where its limits and opportunities lie. Many speakers highlighted the need for coordinated infrastructures, openly accessible data and sustained training to ensure that experimental and computational knowledge does not dissipate as facilities evolve and generations change. CEQPAS demonstrated that quantum science has become a collective, international endeavour that links fundamental questions to applications with societal impact, from plasma medicine to environmental remediation and space missions.

Immediately after CEQPAS, the MOLEcular Excited State spectroscopy (MOLESSs)³¹ initiative was launched at ICTM's historic building, where the Serbian Chemical Society was founded in 1897. MOLESSs seeks to create a modern atlas of molecular excited states, taking inspiration from Melvin Robin's Higher Excited States of Polyatomic Molecules book series.^{32–34} The consortium will assemble experimental and theoretical excited-state spectra across molecular families, phases and spectral regions, accompanied by reproducible protocols, benchmark datasets and fully accessible digital resources. If CEQPAS revisited the century that spectroscopy shaped, MOLESSs prepares the century that spectroscopy will define; where CEQPAS celebrated the discovery of quantisation, MOLESSs commits to charting the landscape of electronic structure. CEQPAS looked back to 1925; MOLESSs looks forward.

In 1925, spectroscopy forced scientists to abandon classical pictures and create quantum mechanics. In 2025, spectroscopy again calls for collective action, not to reinvent quantum mechanics, but to organise and share the data required to use it. Belgrade, for one week in November, became a focal point where the legacy of quantum theory met its future applications in chemistry and molecular sciences, linking spectroscopy to planetary science, plasma medicine and data-driven discovery. The arc from Heisenberg in Helgoland to CEQPAS and MOLESSs is continuous: spectroscopy reveals structure, quantum mechanics explains it, and the next century of discovery will depend on the ability to preserve, organise and interpret the spectra that have always been quantum mechanics' most important teacher.

Acknowledgments. The authors acknowledge the framework of the International Year of Quantum Science and Technology (IYQ2025), proclaimed by the United Nations and coordinated with the support of UNESCO, under which CEQPAS and MOLESSs were organised. This work was supported by the Science Fund of the Republic of Serbia (grant no. 6821), Atoms and (bio)molecules – dynamics and collisional processes on short time scale (ATMOLCOL). F.F. gratefully acknowledges funding from the Royal Society ISPF International Collaboration Awards 2024 (grant no. ICAO\R1\241112) for the NUBIAN project. M.Z. thanks the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (451-03-136/2025-03/200026). The CEQPAS conference and MOLESSs initiative are aligned with the United Nations Sustainable Development Goals, notably SDG 4 (Quality Education), SDG 9 (Industry, Innovation and Infrastructure), SDG 13 (Climate Action), and SDG 17 (Partnerships for the Goals), by fostering international collaboration, advancing scientific infrastructure, promoting sustainable applications of quantum-enabled molecular science, and ensuring the long-term stewardship of knowledge.

ИЗВОД
СТОГОДИШЊИЦА КВАНТНЕ ТЕОРИЈЕ У БЕОГРАДУ: CEQPAS И MOLESs
ИНИЦИЈАТИВА

FELIPE FANTUZZI¹, ЈЕЛЕНА Б. МАЉКОВИЋ², МАТИЈА ЗЛАТАР³, CAUÊ P. SOUZA¹, HEIDY M. QUITIÁN-LARA⁴,
PAMIR NAG⁵, BOBBY ANTONY⁶, БРАТИСЛАВ П. МАРИНКОВИЋ² и NIGEL J. MASON⁷

¹*School of Natural Sciences, University of Kent, Park Wood Rd, Canterbury CT2 7NH, UK,* ²*Институт за физику, Универзитет у Београду, Предревница 118, Београд,* ³*Институт за хемију, технологију и металургију, Универзитет у Београду, Њешићева 12, 11000 Београд,* ⁴*Max Planck Institute for Extra-terrestrial Physics, Gießenbachstraße 1, Garching 85748, Germany,* ⁵*J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Dolejškova 2155/3, 182 23 Prague, Czech Republic,* ⁶*Department of Physics, Indian Institute of Technology (ISM), Dhanbad, 826004, Jharkhand, India* и ⁷*School of Engineering, Mathematics and Physics, University of Kent, Park Wood Rd, Canterbury CT2 7NH, UK*

Прва недеља новембра 2025. године у Београду била је обележена одржавањем два догађаја посвећена стогодишњици квантне механике и њеном утицају на хемију и молекулске науке. Конференција *Centennial of Quantum Theory: Progress in Atomic and Molecular Structure* (CEQPAS) окупила је истраживаче из Европе и света како би дискутовали о научном наслеђу квантне теорије и представили актуелна достигнућа у атомској и молекулској спектроскопији, сударним процесима релевантним за физику плазме, анализи хемијске везе и дизајну функционалних молекула, астрохемији, примени вештачке интелигенције и машинског учења у анализи и генерисању атомских и молекулских података, као и у ширим применама квантне науке. Одмах по завршетку CEQPAS покренута је нова међународна иницијатива: *MOLEcular Excited State spectroscopy* (MOLESs), чији је примарни циљ развој атласа електронске структуре и спектроскопије ексцитованих стања молекула који ће ригорозно интегрисати експеримент и теорију. Заједно, CEQPAS и MOLESs повезују историјску перспективу са будућим могућностима, позиционирајући Београд као регионални центар молекулских наука заснованих на квантној механици.

(Примљено 18. новембра, ревидирано 20. новембра, прихваћено 25. новембра 2025)

REFERENCES

1. M. Durrani, *Phys. World* **38** (2025) 35 (<https://dx.doi.org/10.1088/2058-7058/38/08/28>)
2. W. Heisenberg, *Z. Phys.* **33** (1925) 879 (<https://dx.doi.org/10.1007/BF01328377>)
3. M. Born, P. Jordan, *Z. Phys.* **34** (1925) 858 (<https://dx.doi.org/10.1007/BF01328531>)
4. W. Pauli, *Z. Phys.* **31** (1925) 765 (<https://dx.doi.org/10.1007/BF02980631>)
5. G. E. Uhlenbeck, S. Goudsmit, *Naturwissenschaften* **13** (1925) 953 (<https://dx.doi.org/10.1007/BF01558878>)
6. E. Schrödinger, *Phys. Rev.* **28** (1926) 1049 (<https://dx.doi.org/10.1103/PhysRev.28.1049>)
7. M. Born, *Z. Phys.* **38** (1926) 803 (<https://dx.doi.org/10.1007/BF01397184>)
8. *Centennial of Quantum Theory: Progress in Atomic and Molecular Structure – CEQPAS 2025*, J. B. Maljković, M. Zlatar, Eds., *Book of Abstracts*, Belgrade, 2025 (<https://dx.doi.org/10.5281/zenodo.17467615>)
9. D. V. Mifsud, P. A. Hailey, A. Traspas Muñia, O. Auriacombe, N. J. Mason, S. Ioppolo, *Front. Astron. Space Sci.* **8** (2021) 757619 (<https://dx.doi.org/10.3389/fspas.2021.757619>)
10. X. Fu, Y. Liu, Q. Chen, Y. Fu, T. J. Cui, *Front. Phys.* **10** (2022) 869537 (<https://dx.doi.org/10.3389/fphy.2022.869537>)
11. A. B. Kovačević, *Appl. Spectrosc.* **79** (2025) 227 (<https://dx.doi.org/10.1177/00037028241241308>)

12. L. Lopes Cavalcante, E. C. Czapinski, H. E. Maynard-Casely, M. L. Cable, N. Chaouche-Mechidal, R. Hodyss, C. Ennis, *Phys. Chem. Chem. Phys.* **26** (2024) 26842 (<https://dx.doi.org/10.1039/D4CP03437F>)
13. J. J. Jureta, A. R. Milosavljević, B. P. Marinković, *Int. J. Mass Spectrom.* **365–366** (2014) 114 (<https://dx.doi.org/10.1016/j.ijms.2014.03.002>)
14. J. Vukalović, B. P. Marinković, J. Rosado, F. Blanco, G. García, J. B. Maljković, *Phys. Chem. Chem. Phys.* **26** (2024) 985 (<https://dx.doi.org/10.1039/D3CP05052A>)
15. A. Cassidy, M. R. S. McCoustra, D. Field, *Acc. Chem. Res.* **56** (2023) 1909 (<https://dx.doi.org/10.1021/acs.accounts.3c00094>)
16. I. Chakraborty, N. Sinha, B. Antony, *Radiat. Phys. Chem.* **216** (2024) 111421 (<https://dx.doi.org/10.1016/j.radphyschem.2023.111421>)
17. P. Nag, M. Tarana, J. Fedor, *Phys. Rev., A* **103** (2021) 032830 (<https://dx.doi.org/10.1103/PhysRevA.103.032830>)
18. N. Cvetanović, M. M. Martinović, B. M. Obradović, M. M. Kuraica, *J. Phys., D* **48** (2015) 205201 (<https://dx.doi.org/10.1088/0022-3727/48/20/205201>)
19. A. Kumar, N. Škoro, W. Gernjak, N. Puač, *Eur. Phys. J., D* **75** (2021) 283 (<https://dx.doi.org/10.1140/epjd/s10053-021-00283-5>)
20. E. R. S. Paz, C. P. Souza, J. C. De Oliveira, R. G. Almeida, C. Herrera-Acevedo, S. Lakoh, G. A. M. Jardim, E. N. da Silva Júnior, F. Fantuzzi, *ChemistryOpen* (2025) e202500465 (<https://dx.doi.org/10.1002/open.202500465>)
21. T. M. Cardozo, D. W. Oliveira De Sousa, F. Fantuzzi, M. A. C. Nascimento, in *Compr. Comput. Chem.*, Elsevier, Amsterdam, 2024, p. 552 (<https://dx.doi.org/10.1016/B978-0-12-821978-2.00027-1>)
22. J. Zhang, A. T. Muiña, D. V. Mifsud, Z. Kaňuchová, K. Cielinska, P. Herczku, K. K. Rahul, S. T. S. Kovács, R. Rácz, J. C. Santos, A. T. Hopkinson, L. Craciunescu, N. C. Jones, S. V. Hoffmann, S. Biri, I. Vajda, I. Rajta, A. Dawes, B. Sivaraman, Z. Juhász, B. Sulik, H. Linnartz, L. Hornekær, F. Fantuzzi, N. J. Mason, S. Ioppolo, *Mon. Not. R. Astron. Soc.* **533** (2024) 826 (<https://dx.doi.org/10.1093/mnras/stae1860>)
23. M. D. Dickers, D. V. Mifsud, N. J. Mason, F. Fantuzzi, *Space Sci. Rev.* **221** (2025) 106 (<https://dx.doi.org/10.1007/s11214-025-01228-9>)
24. D. S. Kretić, I. S. Veljković, D. Ž. Veljković, *Chemistry* **5** (2023) 1843 (<https://dx.doi.org/10.3390/chemistry5030126>)
25. J. Londoño-Restrepo, S. Gómez, H. M. Quitián-Lara, F. Fantuzzi, A. Restrepo, *Chem. Sci.* **16** (2025) 3051 (<https://dx.doi.org/10.1039/D4SC07986H>)
26. H. M. Quitián-Lara, J. Londoño-Restrepo, S. Gómez, K. V. García-González, A. Restrepo, N. J. Mason, P. Caselli, H. M. Boechat-Roberty, F. Fantuzzi, *Mon. Not. R. Astron. Soc.* **539** (2025) 3778 (<https://dx.doi.org/10.1093/mnras/staf595>)
27. D. S. Kretić, V. B. Medaković, I. S. Veljković, *Comput. Theor. Chem.* **1230** (2023) 114381 (<https://dx.doi.org/10.1016/j.comptc.2023.114381>)
28. N. S. Simonović, D. B. Popović, A. Bunjac, *Atoms* **11** (2023) 20 (<https://dx.doi.org/10.3390/atoms11020020>)
29. S. Dujko, J. Atić, D. Bošnjaković, R. D. White, P. Stokes, K. R. Hamilton, O. Zatsarinny, K. Bartschat, M. S. Rabasović, D. Šević, B. P. Marinković, D. V. Fursa, I. Bray, R. P. McEachran, F. Blanco, G. García, D. B. Jones, L. Campbell, M. J. Brunger, *Plasma Sources Sci. Technol.* **30** (2021) 115019 (<https://dx.doi.org/10.1088/1361-6595/ac3343>)
30. V. Vujčić, B. P. Marinković, V. A. Srećković, S. Tošić, D. Jevremović, L. M. Ignjatović, M. S. Rabasović, D. Šević, N. Simonović, N. J. Mason, *Phys. Chem. Chem. Phys.* **25** (2023) 26972 (<https://dx.doi.org/10.1039/D3CP03752E>)

31. *MOLEcular Excited State spectroscopy – MOLESs*, <https://www.moless-spectroscopy.org/>
32. M. B. Robin, *Higher Excited States of Polyatomic Molecules, Volume I*, Elsevier, New York, 1974 (<https://dx.doi.org/10.1016/B978-0-12-589901-7.X5001-0>)
33. M. B. Robin, *Higher Excited States of Polyatomic Molecules, Volume II*, Elsevier, New York, 1975 (<https://dx.doi.org/10.1016/B978-0-12-589902-4.X5001-1>)
34. M. B. Robin, *Higher Excited States of Polyatomic Molecules, Volume III*, Elsevier, Orlando, FL, 1985 (<https://doi.org/10.1016/B978-0-12-589903-1.X5001-2>).



Contents of Volume 90

NUMBER 1

Organic Chemistry

- G. S. Mrđan, S. S. Vlasiavljević, P. N. Knežević, I. N. Nikolić, D. G. Tenji and B. M. Matijević: Investigating the therapeutic potential of monothiocarbohydrazones: A comprehensive *in vitro* evaluation of antioxidant, antimicrobial and cytotoxic activities..... 1

- A. Rašović: The interaction between 4-oxothiazolidine-2-ylidene thioamides and iodine: A regioselective two-component 4-oxothiazolidine-2-ylidene thioamide to thiazolo-[3,2-c]pyrimidine transformation mediated by iodine..... 13

Biochemistry and Bioengineering

- A. M. Al Hindawi, Z. M. Abd Al-Aama, J. K. Al-Sabbagh and M. Awad: Zinc oxide nanoparticles: An experimental study of synthesis, characterization and biological activity 27

Theoretical Chemistry

- K. N. Mishra, H. C. Upadhyay and P. Verma: Monte Carlo optimization-based QSAR modelling of *Staphylococcus aureus* inhibitory activity of coumarin-1,2,3-triazole hybrids 39

Physical Chemistry

- I. Zakiryanova, I. Korzun, E. Nikolaeva and A. Bovet: The lithium oxide solubility in molten fluoride system $\text{CeF}_3\text{--FLiNaK}$ 53

Analytical Chemistry

- R. Karuppiyahya, S. G. Arunachalam, S. V. Sampath, S. Ramanathan, A. T. Subramaniam, R. Ramasamy, A. Paulmurugan and J. Munusamy: Analytical method development and validation of antifungal drugs in updated ointment formulation using UV spectroscopy and RP-HPLC..... 67

- H. A. H. Barzani, H. Saadi Ali and Y. Yardim: Determination of indacaterol from inhaler capsules by square-wave voltammetry at the surface of the boron-doped diamond electrode..... 77

Materials

- M. Bošković, S. Stanković, J. V. Živković and A. M. Veselinović: Monte Carlo optimization based QSAR modeling of the cytotoxicity of acrylic acid-based dental monomers ... 95

Environmental

- S. Z. Mohammadi, F. Mousazadeh, L. Salajegheh-Tezerji, B. Lashkari and E. Bani-Asadi: Preparation, characterization and evaluation of nano manganese dioxide coated on alumina as a new adsorbent for the effective removal of phenol from aqueous samples 109

- M. Can and M. Keskin: Green synthesis, characterization and biochemical properties of waste walnut (*Juglans regia* L.) inner shell-based silver nanoparticles..... 123

NUMBER 2

Organic Chemistry

- J. B. Martinov Nestorov, G. V. Janjić and M. M. Petković Benazzouz: Theoretical evaluation of pectin therapeutic potential in relation to degree of methylation 137
- M. R. Petković, J. M. Kotur-Stevuljević, P. M. Jovanović, M. D. Jovanović, N. M. Mitrović, M. R. Simić, G. D. Tasić and V. M. Savić: Synthesis and *in vitro* study of redox properties of pyrrole and halogenated pyrrole derivatives 149

Biochemistry and Bioengineering

- B. Ramli, M. R. Mokred, A. Hamiani, S. B. Benzine, C. K. Bendeddouche, M. T. Lao, M.-L. Fauconnier and N. Kambouche Bouzidi: Chlorogenic acid with cytotoxic activity and other constituents from *Anacyclus valentinus* from Algeria..... 163

Inorganic Chemistry

- Dj. D. Minić, K. G. Marković, A. S. Kesić, M. Z. Grujović, S. M. Marković, A. M. Torbica and N. H. Djukić: The effects of silver nanoparticles synthesized with an aqueous extract of *Agrimonia eupatoria* L. on winter wheat and barley varieties 175

Theoretical Chemistry

- R. L. Swagat Shrestha, P. Neupane, S. Dhital, N. Parajuli, B. Maharjan, T. Shrestha, S. Bharati, B. P. Marasini and J. Adhikari Subin: Selected phytochemicals as potent acetylcholinesterase inhibitors: An *in silico* prediction..... 187

Analytical Chemistry

- N. T. Ngan and T. H. D. Nguyen: Chemical composition and bioactivities of *Phellinus pini* extracts and quality evaluation of healthy drinks prepared from the mushroom 201
- S. Azizi and H. Z. Mousavi: Malachite green removal by *Eryngium caeruleum* ash..... 215

Materials

- Z. Fei, Y. Zhang, Z. Wang, Y. Duan and B. Zhang: Enhancing fire resistance in wood with high-water retention silica gel: A promising flame-retardant solution..... 233

Environmental

- L. Sablii, O. Obodovych and V. Sydorenko: Efficiency of physical–chemical treatment of wastewater of the paper and cardboard factory 247
- I. I. Al-Khateeb, Y. M. Al-Obaidi and S. M. Hussain: Refinement technique for nano-cellulose extraction from corn cobs as a green material for environmental sustainability 257

NUMBER 3

Organic Chemistry

- H. G. Ramos, J. A. de Oliveira Lima, H. C. dos Santos, C. C. do Nascimento, L. H. K. Queiroz-Junior and M. da Paz Lima: Phytochemical investigation from wood residues of *Dalbergia spruceana* Benth (Short communication)..... 271

Biochemistry and Bioengineering

- C. Ozay, B. C. Alkac, A. Temel and M. Pehlivan: Investigation of the bioactivities of *Saponaria mesogitana* methanolic extract along with its phytochemical composition 277

Theoretical Chemistry

- W. López-Orozco, L. H. Mendoza-Huizar, G. A. Álvarez-Romero and J. D. J. M. Torres-Valencia: A computational study of the potential bioactivity of hibiscus and garcinia acids against SARS-CoV-2 291

Electrochemistry

- M. Chopin-Doroteo, L. Mendoza, E. Lima and E. Krötzsch*: A simple method for identification of native collagen by reversed-polarity electrophoresis: Short report (Short communication)..... 305

Analytical Chemistry

- M. Biuki, H. Z. Mousavi, M. Arvand and H. Fallah Moafi*: Application of magnetite nanoparticle-modified walnut shell as an adsorbent for the removal of the organic dye Coomassie Brilliant Blue R-250..... 311

Chemical Engineering

- Ö. Gök*: Recovery of copper from pre-concentrated printed circuit boards (PCBs) by catalyzed acidic leaching 325

Materials

- N. Jumrus, M. Thapinta, P. Chaiwong, A. Pantawan, T. Kumpika, W. Thongpan, N. Jhuntama, E. Kantarak, W. Sroila, R. Rianyai, P. Singjai and W. Thongsuwan*: Coating technology on mortar surface for extending service life of on-site building construction..... 339

Metallic Materials and Metallurgy

- S. Rengarajan, G. Kasirajan and R. A. Kumar*: Corrosion studies, parameter effects, and surface morphology of AA5052-AA6101T6 friction stir welded joints 351

Environmental

- M. Keskin*: Waste corn silk for eco-friendly silver nanoparticles: Green synthesis, characterization and determination of enzyme inhibition properties..... 369
- G. B. Aydin*: A comparative study on ecological risk assessment of some potentially toxic elements accumulation in surface sediment of stagnant and running water ecosystems in Meriç delta wetland, Turkish Thrace 383

NUMBER 4

Organic Chemistry

- G. B. Krstić, M. B. Jadranin, D. Z. Savić, V. V. Tešević, N. M. Todorović, Lj. V. Vujisić and S. M. Milosavljević*: The first evidence of lumiphorbol as a metabolite of the *Euphorbia* species 393

Biochemistry and Bioengineering

- D. B. Zejak, B. T. Popović, A. P. Leposavić, V. R. Spalević and V. V. Tešević*: Chemical characterization and differentiation of Montenegrin plum spirits obtained by two techniques of traditional batch distillation..... 401

Theoretical Chemistry

- N. J. B. Agosto*: *In silico* molecular docking and ADMET prediction of *Ginkgo biloba* biflavonoids as dual inhibitors of human HMG-CoA reductase and α -amylase..... 415

Physical Chemistry

- A. Y. Galashev, Y. P. Zaikov, K. A. Abramova, O. R. Rakhmanova and Y. S. Mochalov*: Molecular dynamics simulation of uranium nitride oxidation 431

Electrochemistry

- B.-O. Taranu*: Different electrode modification protocols for evaluating the water-splitting properties of a P(V)-metalloporphyrin..... 447

Analytical Chemistry

- İ. Tegin, B. M. F. Fattah, M. Fidan, O. Acar and E. Yabalak*: An effective and facile approach for the determination of bioactive components of *Rheum ribes* in the Kurdish state of Iraq and Siirt region in Turkey..... 477

Chemical Engineering

- C. Bilgiç, N. Karakehya and A. Voelkel*: Thermal, morphological and surface properties of composite materials with perlite reinforcement 479

Materials

- J. Lj. Petrović, Ž. Radovanović, B. Obradović, Đ. Janačković and R. Petrović*: Modification of surface properties and photocatalytic performance of pure and oxygen-doped graphitic carbon nitride via DBD plasma treatment 497

Environmental

- F. Z. Benhachem, H. Mahroug, M. Bendjelloul, A. Miraoui, E. H. Elandalousi, K. Oukebdane and R. Halfaoui*: Enhanced adsorption properties of Algerian bentonite modified by starch and glycerol for methylene blue and methyl red retention 513
- Sushma, A. Keshav and M. Ramachandran*: Biosorptive removal of cobalt (II) ion from wastewater using pomegranate peel activated carbon as biosorbent..... 529

NUMBER 5

Organic Chemistry

- K. Komatović, A. Matošević, M. Zlatović, D. Sladić, A. Bosak and D. M. Opsenica*: Hybrids of 4-aminoquinolines and adamantane as inhibitors of AChE 545

Biochemistry and Bioengineering

- M. Mokhnache, B. Hani, F. Dogan Koca, G. Unal, N. A. Rahman, A. Basma, N. M. Bozkurt, A. A. M. Bachir and H. Daoud*: Evaluation of antimicrobial, anticancer and neuroprotective activities of silver nanoparticles (AgNPs) green-synthesized using a red pigment produced by *Streptomyces* sp. A23 strain isolated from Algerian bee pollen..... 561
- N. Gligorijević, N. Svržić, B. Dojčinović, D. Stanković, S. Combet, S. Minić and M. Nikolić*: Optimizing alginate immobilization of food-derived C-phycoerythrin: Structural and functional characterization 579

Inorganic Chemistry

- H. Abdul Rahim, N. Nordin, B. H. Yahaya, Y. W. Lye and A. H. Lahuri*: Electrochemical synthesis and anticancer inhibitory effect of copper(II)-diclofenac/decanoic acid complexes on MCF 7 breast cancer cells 593

Theoretical Chemistry

- F. Motaghi and H. Mohammadi-Manesh*: Enhancing hydrogen evolution reaction using transition metal atoms on 6,6,12-graphyne: A DFT study 609

Physical Chemistry

- M. M. Keshtiban, A. Nikoo and B. Massoumi*: Synthesis and characterization of nano Fe₂CuAl₂O₇ as a reusable catalyst for Biginelli reaction 623

Analytical Chemistry

- V. Mitic, J. Nikolic, M. Dimitrijevic, V. Stankov Jovanovic and G. Stojanovic*: Application of principal component and hierarchical cluster analysis to classify different edible *Cucurbita* based on *in vitro* antioxidant activity 639

Materials

- M. Marković, M. Gorgievski, N. Štrbac, V. Grekulović, M. Zdravković, M. Marković and D. Stanković*: Analysis and statistical modeling of copper ions biosorption onto calcined chicken eggshell..... 661

Environmental

- M. T. Divrik*: Assessment of water quality in Yapialtin Dam Lake (Sivas, Turkey) during dry and rainy seasons using various parameters and water quality indices..... 681
- N. Palić, M. Vukčević, M. Maletić, M. Mirković, M. Ristić, A. Perić Grujić and K. Trivunac*: Amino-starch derivates for adsorption of specific pharmaceuticals and pesticides in contaminated water: Examination in both spiked and real water samples 693

NUMBER 6

Biochemistry and Bioengineering

- S. Srivastava, P. Srivastava, G. Raj Dwivedi, V. Ramanathan and H. C. Upadhyay*: Green synthesis of silver nanoparticles utilizing *Gardenia latifolia* extract, characterization and *in vitro* antibacterial activity 709

Theoretical Chemistry

- I. Temer, A. Mostefai and A. Rahmouni*: Conceptual DFT study based on the characterization of the local electrophilicity and nucleophilicity for intramolecular Diels–Alder reaction of the *trans* isomers of 4-[(4*E*)-4,6-heptadien-1-yl]-2-cyclohepten-1-one 727
- M. Bigović, I. S. Veljković, J. Petrović and D. Ž. Veljković*: Quantum-chemical study of C–H...O interactions between HTcO₄ and aromatic amino acids 741

Physical Chemistry

- P. Taba, ST. Fauziah, H. Natzir, N. H. Soekamto, R. S. Sarungallo, N. W. Nahru, Y. Astuti and I. I. Amin*: Synthesis and characterization of zeolite obtained from Toraja natural montmorillonite 753
- S.-Y. Tang, X.-X. Cao, T.-T. Di and X.-Y. Li*: Catalytic performance of palladium/degraded chitosan for acetylene selective hydrogenation 767

Analytical Chemistry

- Z. Bozhinoska, Z. Zhivikj, S. Ubavkova, M. Bujaroska Perkovicj, V. Poposka, A. Stankov and T. Petreska Ivanovska*: Analyzing food supplements to enhance erectile system function for the presence of undeclared sildenafil and tadalafil using HPLC-DAD (Short communication) 781

Polymers

- F. Mustafayeva, N. Kakhramanov, G. Martynova and N. Koseva*: Effect of different compatibilizers and aluminium hydroxide amount on the thermophysical and thermomechanical properties of polypropylene random copolymer/aluminium hydroxide composites 789

Environmental

- D. Deswati, E. Emriadi, S. M. Aura, W. E. Fitri, S. Suparno and A. Putra*: Microplastic accumulation and reduction in shellfish (*Polymesoda bengalensis*) using NaCl solution..... 803

- I. R. Milošević, S. Živković, M. Momčilović, Ž. Višnjić-Jeftić, M. Veselinović, I. D. Marković and D. M. Marković: Field experiment on the uptake of lead, strontium, cobalt and nickel in the wood and bark of spruce (*Picea abies* L.) and Douglas-fir (*Pseudotsuga menziesii* Mirb.) 823

NUMBER 7–8

Organic Chemistry

- M. Koravović, M. Kovačević, M. Jovanović, G. Tasić, A. Mayasundari, G. Nishiguchi, B. Marković, Z. Ranković and V. Savić: Comparative *in silico/in vitro* analysis of pharmacokinetic profiles of BET inhibitors 837
- B. Anić Marković, I. Cvijetić, M. Milošević, A. Marinković, Lj. Tolić Stojadinović, S. Drmanić and J. Nikolić: *In vitro* antioxidant activity of nicotinic acid hydrazides: Experimental and theoretical study 857

Biochemistry and Bioengineering

- İ. Özgen, A. Gül, C. Baltacı, İ. Öner and E. Aydoğmuş: Determination of antibacterial properties and chemical composition of pyrolysis liquid from feeding wastes of mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) adults and larvae 869

Theoretical Chemistry

- S. Kumar, N. Kumar, R. Kumar and K. K. Mishra: The role of non-covalent interactions in the solvation dynamics of metronidazole in water: A theoretical study 883
- M. M. Petković-Benazzouz, I. Radović, D. V. Milojkov and G. V. Janjić: Molecular docking's pioneering role in materials science. Metal ion site-preference in fluorapatite 899

Physical Chemistry

- M. Ristić, N. Krstevski, M. Marković, A. Šajić and M. Kuzmanović: Influence of pressure and mixing with air on the equilibrium composition of laser-induced plasma on ODS steel 915
- M. Febriansyah, D. K. Putri, A. T. Nabila, I. Radiansyah, M. Nurrahman and I. N. Candra: Innovation in printer ink based on carbon quantum dots (CQDs) derived from styrofoam waste for anti-counterfeiting document technology 929

Analytical Chemistry

- C. Son Yue, C. Fung Loke, S. Hong Teo, H. Hong Teo, S. Iiangovan and K. Niang Chee: Acetic acid liquid–liquid extraction and UHPLC-DAD detection of polycyclic aromatic hydrocarbons in toasted and fried foods 943

Chemical Engineering

- S. Mansouri and S. Cheknoun: Ruthenium(III)-monosubstituted Keggin-type polyoxotungstate: Synthesis, characterization and application in the catalytic methanation of carbon dioxide 957

Environmental Chemistry

- T. Marjanović, M. Bogunović Koljaja, J. Prodanović, B. Barta Holló and I. Ivančević-Tumbas: Influence of dissolved organic carbon nature on adsorption of ibuprofen, caffeine and diclofenac by powdered activated carbon from water 973
- D. Tahirović M. Balaban, T. Muhić Šarac, E. Članjak-Kudra, M. Smajlović, F. Čaklovica and V. Antić: Arsenic in Bosnia and Herzegovina market seafood: Effects of matrix modifiers on measured concentration 987

History of and Education in Chemistry

- A. Kargopoulos, C. Skordoulis, P. Giannakoudakis and A. Mavropoulos*: Teaching chemical reactions to upper secondary school students: A laboratory approach..... 1001

NUMBER 9

Organic Chemistry

- P. H. Mohamadabad, D. Setamdideh and F. Ghanbary*: Synthesis of poly(itaconic acid) and its application for synthesis of pyrrolinones as a reusable homogeneous catalyst ... 1015

Biochemistry and Bioengineering

- S. Jampour-Kolour and H. Dezhampanah*: Binding elucidation of azo dye with DNA via spectroscopic approaches and molecular docking techniques..... 1027

Inorganic Chemistry

- S. Budania, A. M Kumar, P. Chand and A. Jain*: Dibutyltin(IV) formulations: Synthetic aspect, spectroscopic interpretation and computational calculation 1041

Theoretical Chemistry

- A. Chaoui, A. Bouakkadia, N. Kertiou and H. Haddag*: Environmental behavioral controls of polychlorinated biphenyls: Prediction of the soil sorption coefficient (K_{oc}) using multiple linear regression..... 1055

Physical Chemistry

- L. Lestari, S. Raharjo, I. N. Sudiana, L. O. Rusman and N. Sulastrri*: Effect of microwave power level and processing time on the quality of palm shell charcoal (*Elaeis guineensis* Jacq.) briquettes..... 1069

Electrochemistry

- M. A. Tudoran and B.-O. Taranu*: Water-splitting electrocatalytic properties and computational characterization of a symmetrically substituted porphyrazine..... 1089

Polymers

- J. Milinković Budinčić, L. Petrović, M. Aleksić, J. Fraj, Lj. Đekić, S. Bučko, J. Katona, Lj. Spasojević, J. Ostojić, Ž. Radonić and M. Bosnić*: Chitosan/sodium dodecyl sulphate microcapsules preparation: Influence of applied method 1105

Metallic Materials and Metallurgy

- C. I. Abilov, I. B. Bakhtiyarly, S. H. Sadigova, M. Sh. Hasanova and E. K. Gasimova*: Ni–Bi–Sn ternary system liquidus surface projection..... 1119

Environmental

- E. Adak, M. Keskin and H. Arslan*: The potential of electrochemical pesticide analyses by green synthesized, waste olive leaves-based, silver nanoparticles 1131

NUMBER 10

Biochemistry and Bioengineering

- J. Z. Marinković, M. D. Jakovljević, I. Stanisavljević, M. Obradović, K. Mijačić, N. Gajović, B. Simović Marković, M. Jovanović, M. Živković, S. Rajković and M. Jurišević*: DNA/bovine serum albumin interaction studies and immunomodulatory effects of dinuclear platinum(II) complex with aromatic 1,5-naphthyridine bridging ligand 1147

Inorganic Chemistry

- K. B. Sakhare, K. N. Sarwade, H. U. Joshi and M. A. Sakhare: Green route for efficient synthesis of metal complexes of 4-bromo-2-((*E*)-((2-hydroxyphenyl)imino)methyl)-6-((*E*)-(3-nitrophenyl)diazenyl)phenol and its anti-hyperglycemia, anticancer and antimicrobial assessment 1161

Theoretical Chemistry

- A. Laoud, A. Belafriekh and M. Alaqarbeh: *N*-Phenyl-3-sulfamoyl-benzamide derivatives as anti-Hepatitis B virus agent candidates. Integrated computational studies 1175

Physical Chemistry

- N. Mustafayeva, E. Zeynalov, A. Huseynov, Y. Nagiyev, M. Nadiri and M. Maharramova: Investigation of multi-walled carbon nanotubes catalytic activity by means of the model aerobic oxidation reaction 1189

Analytical Chemistry

- N. H. Sazali, M. Miskam, F. B. M. Suah and N. Y. Rahim: Optimization of deep eutectic solvent based liquid phase microextraction of PAHs in environmental samples using response surface methodology 1203

Chemical Engineering

- G. Akar Şen: Synthesis of zeolite LTA from kaolin and its model CBC and WAC performance: Optimized by central composite design 1223

Geochemistry

- E. Hukić, M. Kašanin-Grubin, M. Subašić, T. Tosti, S. Đogo-Mračević, S. Štrbac and S. Stojadinović: Impact of drying, freezing and re-wetting events soil leachate in acidic versus calcareous soils 1241

Environmental

- L. Imas-Garay, E. de Carvalho Lourenço, F. Rubio, A. T. Toci and M. Boroski: Unified method for multiresidue pesticide analysis in corn and sediment 1253

History of and Education in Chemistry

- G. Özveren, B. Turan, M. Ulucan and C. Tosun: University students' scientific knowledge levels regarding chemical reaction arrows and electron arrows 1267

NUMBER 11

Biochemistry and Bioengineering

- A. Ahmedi and M. Abouseoud: Development of biopolymer encapsulated enzyme for efficient acetaminophen degradation 1285

Inorganic Chemistry

- R. Dai, Y. Wang, Hua Zhang and Z. Kong: A novel Zn(II) coordination compound exhibits selective and sensitive detection of Fe³⁺ and acetylacetone 1303

Theoretical Chemistry

- P. J. P. Tjitda, F. O. Nitbani, T. D. Wahyuningsih, Y. M. Abanit and F. R. Soeharto: Computational insights into the inhibitory potential of dihydroorotate dehydrogenase by natural compounds in *Artocarpus champeden* as antimalarial agents 1317

Polymers

- A. Bouharaoua, H. Merine and Y Ramli: Optimisation of the controlled release of valsartan via cellulose acetate butyrate and poly(butylene succinate) microspheres: Influence of formulation conditions 1331

Materials

- S. K. Shah, S. Karki and A. Bhattarai*: Comparative study of micellization and surface properties of cationic and anionic surfactants in acetonitrile–water mixed media 1353

Chemical Engineering

- K. Youcef, Z. Driss, M. Foukrach, S. Rosa and T. Baki*: A CFD investigation of the performance of stirred tanks 1369

Metallic Materials and Metallurgy

- J. N. Pejić, B. V. Jegdić, B. M. Radojković, A. R. Simović, D. D. Marunčić, B. Z. Jugović and A. S. Popović*: Investigation of corrosion causes and failures in the interior metal components of an automobile 1383

Environmental

- T. T. H. Nguyen, N. T. Vu, H. M. Le and H. S. Nguyen*: Effect of pyrolysis temperature and time of Robusta coffee husk on yield and product characteristics 1401
- S. Mortazavi, M. Sillanpää and D. Bose*: Kinetic and equilibrium comparison of methylene blue and basic blue 41 adsorption by silica fume 1415

NUMBER 12

Organic Chemistry

- S. S. Konstantinović, D. Z. Troter, J. B. Zvezdanović, S. M. Petrović, S. R. Savić, B. R. Danilović and J. Ćirić*: Reishi (*Ganoderma lucidum*) tincture enriched with propolis or green tea from Serbia: UHPLC-DAD–ESI-MS characterization, mineral content and antibacterial activity 1425

Inorganic Chemistry

- I. S. Đorđević, S. Grubišić, D. Mitić, D. M. Popović and N. Trišović*: Tuning crystal packing and reactivity through electrostatic and dispersion interactions: The case of phenytoin and its derivatives 1439

Theoretical Chemistry

- L. H. Mendoza-Huizar*: A computational study to identify the metabolites in *Lippia alba* capable of inhibiting gastric cancer through BCL-2 protein inhibition 1451

Polymers

- R. Sarker, G. Chandra Sarker, A. Dhar Pranta and J. Saha*: Impact of internal notation and heterobifunctional reactive dyes on color performance, mechanical and dimensional stability of cellulose-based biomaterials 1465

Materials

- M. Randelović, C. C. Sorrell, S. Koppka, A. Zarubica, M. Z. Momčilović and D. Enke*: Development of a glass–ceramic composite reinforced with β -wollastonite synthesized via a hydrothermal method 1481
- D. D. Marković, V. B. Tadić, A. R. Žugić and M. M. Radetić*: Sustainable synthesis of silver nanoparticles on cotton gauze for enhanced antibacterial properties 1495

Metallic Materials and Metallurgy

- G. Chunag, U. Byambasuren, S. Altantsetseg, G. Yunden, S. Sukhbaatar and D. Altansukh*: Pressure oxidative leaching of copper concentrate 1513

Environmental

- I. Jovanić, A. Šajnović, S. Stojadinović, N. Burazer, B. Glavaš-Trbić and B. Jovančičević*: Tri- and tetracyclic terpanes as proxies for depositional environment reconstruction for weathered siliciclastics of Internal Dinarides 1527

<i>N. Khakimova, M. Bogunović Koljaja, J. Simeunović, L. Landwehrkamp, S. Panglisch and I. Ivančev-Tumbas: Influence of microalgae on organic micropollutants removal from water by powdered activated carbon</i>	1537
---	------

Letters to the Editor

<i>F. Fantuzzi, J. B. Maljković, M. Zlatař, C. P. Souza, H. M. Quitián-Lara, P. Nag, B. Antony, B. P. Marinković and N. J. Mason: Centennial of quantum theory in Belgrade: CEQPAS and the molecular excited state spectroscopy (MOLESS) initiative</i>	1553
Contents of Volume 90	1563
Author Index	1573



Author Index

- Abanit, Y. M., 1317
Abd Al-Aama, Z. M., 27
Abdul Rahim, H., 593
Abilov, C. I., 1119
Abouseoud, M., 1285
Abramova, K. A., 431
Acar, O., 461
Adak, E., 1131
Adhikari Subin, J., 187
Agosto, N. J. B., 415
Ahmed Alien, M. B., 561
Ahmedi, A., 1285
Al Hindawi, A. M., 27
Alaqarbeh, M., 1175
Aleksić, M., 1105
Ali, H. S., 77
Alkac, B. C., 277
Al-Khateeb, I. I., 257
Al-Obaidy, Y. M., 257
Al-Sabbagh, J. K., 27
Altansukh, D., 1513
Altantsetseg, S., 1513
Amin, I. I., 753
Anić Marković, B., 857
Antić, V., 987
Antony, B., 1555
Arslan, H., 1131
Arunachalam, S. G., 67
Arvand, M., 311
Astuti, Y., 753
Awad, M., 27
Aydin, G. B., 383
Aydoğmuş, E., 869
Azizi, S., 215

Álvarez-Romero, G. A., 291

Bakhtiyarly, I. B., 1119

Baki, T., 1369
Balaban, M., 987
Baltaci, C., 869
Bani-Asadi, E., 109
Barta Holló, B., 973
Barzani, H. A., 77
Basma, A., 561
Belafriekh, A., 1175
Belhadj, H., 561
Bellahouel Benzine, S., 163
Bendeddouche, C. K., 163
Bendjelloul, M., 513
Benhachem, F. Z., 513
Bharati, S., 187
Bhattarai, A., 1353
Bigović, M., 741
Bilgiç, C., 479
Biuki, M., 311
Bogunović Koljaja, M., 973, 1539
Boroski, M., 1253
Bosak, A., 545
Bose, D., 1415
Bosnić, M., 1105
Bošković, M., 95
Bouakkadia, A., 1055
Bouharaoua, A., 1331
Bovet, A., 53
Bozhinoska, Z., 781
Bozkurt, N. M., 561
Bučko, S., 1105
Budania, S., 1041
Bujaroska Perković, M., 781
Burazer, N., 1527
Byambasuren, U., 1513

Can, M., 123
Candra, I. N., 929
Cao, X.-X., 767

- Chaiwong, P., 339
Chand, P., 1041
Chaoui, A., 1055
Chee, K. N., 943
Cheknoun, S., 957
Chopin-Doroteo, M., 305
Chunag, G., 1513
Combet, S., 579
Cvijetić, I., 857

Čaklović, F., 987
Članjak-Kudra, E., 987

Ćirić, J., 1425

Da Paz Lima, M., 271
Dai, R., 1303
Danilović, B. R., 1425
Daoud, H., 561
De Carvalho Lourenço, E., 1253
De Oliveira Lima, J. A., 271
Deswati, D., 803
Dezhampanah, H., 1027
Dhital, S., 187
Di, T.-T., 767
Dimitrijević, M., 639
Divrik, M. T., 681
Do Nascimento, C. C., 271
Dojčinović, B., 579
Dos Santos, H. C., 271
Driss, Z., 1369
Drmanić, S., 857
Duan, Y., 233
Dwivedi, G. R., 709

Djukic, N. H., 175

Đekić, L., 1105
Đogo-Mračević, S., 1241
Đorđević, I. S., 1439

Elandaloussi, E. H., 513
Emriadi, E., 803
Enke, D., 1481

Fallah Moafi, H., 311
Fantuzzi, F., 1555
Fattah, B. M. F., 461
Fauconnier, M.-L., 163

Fauziah, S., 753
Febriansyah, M., 929
Fei, Z., 233
Fidan, M., 461
Fitri, W. E., 803
Foukrach, M., 1369
Fraj, J., 1105

Gajović, N., 1147
Galashev, A. Y., 431
Gasimova, E. K., 1119
Ghanbary, F., 1015
Giannakoudakis, P., 1001
Glavaš-Trbić, B., 1527
Gligorijević, N., 579
Gorgievski, M., 661
Gök, Ö., 325
Grekulović, V., 661
Grubišić, S., 1439
Grujović, M. Z., 175
Gül, A., 869

Haddag, H., 1055
Halfaoui, R., 513
Hamiani, A., 163
Hasanova, M. S., 1119
Hukić, E., 1241
Huseynov, A., 1189
Hussain, S. M., 257

Ilangoan, S., 943
Imas-Garay, L., 1253
Ivančev-Tumbas, I., 973, 1539

Jadranin, M. B., 393
Jain, A., 1041
Jakovljević, M. D., 1147
Jampour-Kolour, S., 1027
Janačković, Đ., 497
Janjić, G. V., 137, 899
Jegdić, B. V., 1383
Jhunta, N., 339
Joshi, H. U., 1161
Jovančičević, B., 1527
Jovanić, I., 1527
Jovanović, M. D., 149
Jovanović, M., 1147
Jovanović, M., 837
Jovanović, P. M., 149

- Jugović, B. Z., 1383
Jumrus, N., 339
Jurišević, M., 1147

Kakhramanov, N., 789
Kambouche Bouzidi, N., 163
Kantarak, E., 339
Karakehya, N., 479
Kargopoulos, A., 1001
Karki, S., 1353
Karuppiyahya, R., 67
Kasirajan, G., 351
Kašanin-Grubin, M., 1241
Katona, J., 1105
Kertiou, N., 1055
Keshav, A., 529
Keshtiban, M. M., 623
Kesić, A. S., 175
Keskin, M., 123, 369, 1131
Khakimova, N., 1539
Knežević, P. N., 1
Koca, F. D., 561
Komatović, K., 545
Kong, Z., 1303
Konstantinović, S. S., 1425
Koppka, S., 1481
Koravović, M., 837
Korzun, I., 53
Koseva, N., 789
Kotur-Stevuljević, J. M., 149
Kovačević, M., 837
Krötzsch, E., 305
Krstevski, N., 915
Krstić, G. B., 393
Kumar, A. M., 1041
Kumar, N., 883
Kumar, R. A., 351
Kumar, R., 883
Kumar, S., 883
Kumpika, T., 339
Kuzmanović, M., 915

Lahuri, A. H., 593
Landwehrkamp, L., 1539
Lao, M. T., 163
Laoud, A., 1175
Lashkari, B., 109
Le, H. M., 1401
Leposavić, A. P., 401

Lestari, L., 1069
Li, X.-Y., 767
Lima, E., 305
Loke, C. F., 943
López-Orozco, W., 291
Lye, Y. W., 593

Maharjan, B., 187
Maharramova, M., 1189
Mahroug, H., 513
Maletić, M., 693
Maljković, J. B., 1555
Mansouri, S., 957
Marasini, B. P., 187
Marinković, A., 857
Marinković, B. P., 1555
Marinković, J. Z., 1147
Marjanović, T., 973
Marković, B., 837
Marković, D. D., 1495
Marković, D. M., 823
Marković, I. D., 823
Marković, K. G., 175
Marković, M., 661
Marković, M., 661
Marković, M., 915
Marković, S. M., 175
Martinov Nestorov, J. B., 137
Martynova, G., 789
Marunkić, D. D., 1383
Mason, N. J., 1555
Massoumi, B., 623
Matijević, B. M., 1
Matošević, A., 545
Mavropoulos, A., 1001
Mayasundari, A., 837
Mendoza, L., 305
Mendoza-Huizar, L. H., 291, 1451
Merine, H., 1331
Mijačić, K., 1147
Milinković Budinčić, J., 1105
Milojkov, D. V., 899
Milosavljević, S. M., 393
Milošević, I. R., 823
Milošević, M., 857
Minić, Dj. D., 175
Minić, S., 579
Miraoui, A., 513
Mirković, M., 693

- Mishra, K. K., 883
Mishra, K. N., 39
Miskam, M., 1203
Mitic, V., 639
Mitić, D., 1439
Mitrović, N. M., 149
Mochalov, Y. S., 431
Mohamadabad, P. H., 1015
Mohammadi, S. Z., 109
Mohammadi-Manesh, H., 609
Mohd Suah, F. B., 1203
Mokhnache, M., 561
Mokred, M. R., 163
Momčilović, M. Z., 1481
Momčilović, M., 823
Monica Aura, S., 803
Mortazavi, S., 1415
Mostefai, A., 727
Motaghi, F., 609
Mousavi, H. Z., 215
Mousazadeh, F., 109
Mrđan, G. S., 1
Muhić Šarac, T., 987
Munusamy, J. M., 67
Mustafayeva, F., 789
Mustafayeva, N., 1189

Nabila, A. T., 929
Nadiri, M., 1189
Nag, P., 1555
Nagiyeu, Y., 1189
Nahru, N. W., 753
Nasrat, A. R., 561
Natzir, H., 753
Neupane, P., 187
Ngan, N. T., 201
Nguyen, H. S., 1401
Nguyen, T. H. D., 201
Nguyen, T. T. H., 1401
Nikolaeva, E., 53
Nikolic, J., 639
Nikolić, I. N., 1
Nikolić, J., 857
Nikolić, M., 579
Nikoo, A., 623
Nishiguchi, G., 837
Nitbani, F. O., 1317
Nordin, N., 593
Nurrahman, M., 929

Obodovych, O., 247
Obradović, B., 497
Obradović, M., 1147
Opsenica, D. M., 545
Ostojić, J., 1105
Oukebdane, K., 513
Ozay, C., 277

Öner, İ., 869
Özgen, İ., 869
Özveren, G., 1267

Palić, N., 693
Panglisch, S., 1539
Pantawan, A., 339
Parajuli, N., 187
Paulmurugan, A., 67
Pehlivan, M., 277
Pejić, J. N., 1383
Perić Grujić, A., 693
Petković Benazzouz, M. M., 137, 899
Petković, M. R., 149
Petreska Ivanovska, T., 781
Petrović, J. Lj., 497
Petrović, J., 741
Petrović, L., 1105
Petrović, R., 497
Petrović, S.M., 1425
Poposka, V., 781
Popović, A. S., 1383
Popović, B. T., 401
Popović, D. M., 1439
Pranta, A. D., 1465
Prodanović, J., 973
Putra, A., 803
Putri, D. K., 929

Queiroz-Junior, L. H. K., 271
Quitián-Lara, H. M., 1555

Radetić, M. M., 1495
Radiansyah, I., 929
Radojković, B. M., 1383
Radonić, Ž., 1105
Radovanović, Ž., 497
Radović, I., 899
Raharjo, S., 1069
Rahim, N. Y., 1203

- Rahmouni, A., 727
Rajković, S., 1147
Rakhmanova, O. R., 431
Ramachandran, M., 529
Ramanathan, S., 67
Ramanathan, V., 709
Ramasamy, R., 67
Ramli, B., 163
Ramli, Y., 1331
Ramos, H. G., 271
Randelović, M., 1481
Ranković, Z., 837
Rašović, A., 13
Rengarajan, S., 351
Rianyoi, R., 339
Ristić, M., 693
Ristić, M., 915
Rosa, S., 1369
Rubio, F., 1253
Rusman, L. O., 1069

Sablii, L., 247
Sadigova, S. H., 1119
Saha, J., 1465
Sakhare, K. B., 1161
Sakhare, M. A., 1161
Salajegheh-Tezerji, L., 109
Sampath, S. V., 67
Sarker, G. C., 1465
Sarker, R., 1465
Sarungallo, R. S., 753
Sarwade, K. N., 1161
Savić, D. Z., 393
Savić, S. R., 1425
Savić, V. M., 149, 837
Sazali, N. H., 1203
Setamdideh, D., 1015
Shah, S. K., 1353
Shrestha, R. L. S., 187
Shrestha, T., 187
Sillanpää, M., 1415
Simeunović, J., 1539
Simić, M. R., 149
Simović Marković, B., 1147
Simović, A. R., 1383
Singjai, P., 339
Skordoulis, C., 1001
Sladić, D., 545
Smajlović, M., 987

Soeharto, F. R., 1317
Soekamto, N. H., 753
Sorrell, C. C., 1481
Souza, C. P., 1555
Spalević, V. R., 401
Spasojević, Lj., 1105
Srivastava, P., 709
Srivastava, S., 709
Sroila, W., 339
Stanisavljević, I., 1147
Stankov Jovanovic, V., 639
Stankov, A., 781
Stanković, D., 579, 661
Stanković, S., 95
Stojadinović, S., 1241, 1527
Stojanovic, G., 639
Subašić, M., 1241
Subramaniam, A. T., 67
Sudiana, I. N., 1069
Sukhbaatar, S., 1513
Sulastri, N., 1069
Suparno, S., 803
Sushma, 529
Svrzić, N., 579
Sydorenko, V., 247

Şen, G. A., 1223

Šajić, A., 915
Šajnović, A., 1527
Štrbac, N., 661
Štrbac, S., 1241

Taba, P., 753
Tadić, V. B., 1495
Tahirović, D., 987
Tang, S.-Y., 767
Taranu, B.-O., 447, 1089
Tasić, G. D., 149, 837
Tegin, I., 461
Temel, A., 277
Temer, I., 727
Tenji, D. G., 1
Teo, H. H., 943
Teo, S. H., 943
Tešević, V. V., 393, 401
Thapinta, M., 339
Theodoro Toci, A., 1253
Thongpan, W., 339

- Thongsuwan, W., 339
Tjitda, P. J. P., 1317
Todorović, N. M., 393
Tolić Stojadinović, Lj., 857
Torbica, A. M., 175
Torres-Valencia, J. de J. M., 291
Tosti, T., 1241
Tosun, C., 1267
Trišović, N., 1439
Trivunac, K., 693
Trotter, D. Z., 1425
Tudoran, M. A., 1089
Turan, B., 1267

Ubavkova, S., 781
Ulucan, M., 1267
Unal, G., 561
Upadhyay, H. C., 39, 709

Veljković, D. Ž., 741
Veljković, I. S., 741
Verma, P., 39
Veselinovic, A. M., 95
Veselinović, M., 823
Višnjić-Jeftić, Ž., 823
Vlaisavljević, S. S., 1
Voelkel, A., 479
Vu, N. T., 1401
Vujisić, Lj. V., 393
Vukčević, M., 693

Wahyuningsih, T. D., 1317
Wang, Y., 1303
Wang, Z., 233

Yabalak, E., 461
Yahaya, B. H., 593
Yardim, Y., 77
Youcef, K., 1369
Yue, C. S., 943
Yunden, G., 1513

Zaikov, Y. P., 431
Zakiryanova, I., 53
Zarubica, A., 1481
Zavvar Mousavi, H., 311
Zdravković, M., 661
Zejak, D. B., 401
Zeynalov, E., 1189
Zhang, B., 233
Zhang, H., 1303
Zhang, Y., 233
Zhivikj, Z., 781
Zlatar, M., 1555
Zlatović, M., 545
Zvezdanović, J. B., 1425

Živković, J. V., 95
Živković, M., 1147
Živković, S., 823
Žugić, A. R., 1495

Subject Index of Vol. **90** and List of Referees in 2025 are given in the electronic form at the Internet address of the Journal of the Serbian Chemical Society: <http://www.shd.org.rs/JSCS>

End of Volume 90.



Volume 90 (2025)

Subject index

- (+)-JQ1, 837
2-Alkylidene-4-oxothiazolidine, 13
3D-QSAR, 1175
4-Aminoquinolines, 545
5 Phosphodiesterase inhibitors, 781
5-Flourouracil, 1161
6LU7, 291

β-Glucosides, 163

Ab initio calculations, 741
Acarbose, 1161
Accumulation, 823
Acetonitrile, 1353
AChE, 545
Acid-hydrolysis, 257
Actinomycetes, 561
Activation, 1069
Adamantane, 545
ADME analysis, 837
ADMETlab 2.0, 837
Adsorption capacity, 529
Adsorption, 215, 311, 693, 1539
Adulteration, 781
Aggregate, 447
AgNPs, 709, 1495
Agricultural impact, 1253
Agricultural waste, 1131
AIM, 883
Air mixing, 915
Akaganeite, 1383
Alembic, 401
Algal organic matter, 1539
Alginate, 579
Alloys, 1119
Alumina, 109
Aluminium hydroxide, 789

Aluminum alloys, 351
Alumofloc, 247
Amorphous and crystalline fragments, 431
Amorphous silica, 233
Anacyclus valentinus, 163
Analcime, 753
ANOVA, 1223
Antibacterial activity, 27, 39, 869, 1425, 1495
Antibiofilm, 277
Anticancer properties, 137, 593
Anti-corrosion, 339
Anti-diabetic, 1161
Antimicrobial agents, 1, 137, 561
Antimigration, 277
Antioxidant activity, 1, 123, 149, 175, 201, 277, 369, 461, 639
Argon, 431
Ash content, 1069
Atomic and molecular spectroscopy, 1555

BET, 623
Bilobetin, 415
Binding affinity, 187, 415
Binding energy, 899
Bioceramics, 1481
Biochar properties, 1401
Biodegradation, 1527
Biofabrication, 709
Bioindicators, 803
Biological activity, 1, 857
Biological synthesis, 1131
Biosorption, 661
Biowaste pyrolysis, 869
Blend of fatty acid metal soap and amide, 789
Boron-doped diamond electrode, 77

- Box–Behnken design, 661, 1203
Branched alkanes, 1527
- Caffeine, 1539
Calcined chicken eggshell, 661
Calcium alginate, 1285
Calculation, 431
Calf thymus DNA, 1027
Cancer risk, 987
Cancer, 561
Cancrinite, 753
Car, 1383
Carbonization, 1069
Carboxylic acid, 1041
Catalyst, 1189
Catalytic gorge, 187
Cereals, 175
Cerium fluoride, 53
Cerium oxyfluoride, 53
Chalcopyrite acidic leaching, 1513
Chemical reaction arrows, 1267
Chemical reactions, 1001
Chemical weathering, 1527
Chemometric approach, 639
Chitosan, 767, 1105
Chlorination, 247
Chlorogenic acid, 163
Cholinesterase, 545
Clobetasol propionate, 67
CO₂, 957
Coagulation, 247, 973
Coefficient, 1175
Collision processes, 1555
Colorfastness, 1465
Combustion morphology, 233
Compatibilizer, 789
Composite resin, 95
Composite, 513
Computer-aided drug discovery, 415
Contact angle, 1353
Controlled release, 1331
Conversion, 767
Coordination compound, 1303
Copper(II) complex, 593
Copper, 325
CORAL software, 39
Correlation analysis, 1089
Correlation intensity index, 39
Corrosion, 1383
- Cotton gauze, 1495
C-phycocyanin, 579
Cr(VI), 497
Cucurbita genus, 639
Cumene, 1189
CVD reactor, 1189
Cycloaddition, 727
Cycloalkenone, 727
Cytokines, 1147
Cytotoxicity, 1, 95, 163, 277
- Decomposition temperature, 789
Decomposition, 1285
Degradation, 767
Density functional theory, 609
Dental material, 95
Detection and quantification, 1253
DFT, 857, 1041
Diabetes mellitus, 369, 415
Diagnostic test, 1267
Dibutyltin(IV) complexes, 1041
Diclofenac, 593, 1539
Diene, 727
Dissolved organic carbon surrogates, 973
Diterpenes, 393
Docking study, 137
Double distillation, 401
Drinking water treatment, 1539
Drug development, 837
Drug resistance, 709
Drug, 883
Dye removal, 1415
Dyeing performance, 1465
Dyes, 513
- Eco-friendly, 369
Ecological risk indices, 383
Electrocatalyst, 447, 1089
Electrochemical methods, 1383
Electrochemical synthesis, 593
Electron arrows, 1267
Electron density, 1439
Electronic structure, 1555
Electrostatic model, 899
Emulsion, 1105
Environmental chemistry, 1055
Environmental matrices, 1253
Environmental monitoring, 1253
Environmental variables, 681

- Epoxy coatings, 1383
Equilibrium plasma composition, 915
Equilibrium, 661
ETAAS, 987
Ethylene, 727
Euphorbia nicaeensis, 393
Euphorbiaceae, 393
Extracellular matrix, 305
- Fabaceae, 271
Fabric structure, 1465
Fabriesite, 753
Fatty acid, 593
Fatty acid-based deep eutectic solvent, 1203
Ferric ion, 1513
Ferrofluid, 1203
Flagyl, 883
Flavonoids, 163, 271, 461
FLiNaK, 53
Fluorescence sensing, 1303
Fluorescence spectroscopy, 1027
Fluorescence, 929
Food, 1253
Forest soil, 1241
Formulation conditions, 1331
Fragmentation, 727
Free energy changes, 187
Freundlich, 529
- Gastrointestinal infection, 883
GC-FID-MS, 401
Geometrical stability, 187
Global reactivity descriptors, 1439
Green chemistry, 1015
Green microextraction, 1203
Green synthesis, 123, 175, 561, 1495
Green tea, 1425
- H. pylori*, 123
HBV, 1175
Healthy drinks, 201
HER, 609, 1089
Heterocyclization, 13
Hibiscus, 291
High-performance liquid chromatography, 67
Homogeneous catalyst, 1015
HPLC, 277, 781, 943
HPLC-DAD-MS, 163
HR-TEM, 709
Hydantoin, 1439
Hydrogen bond, 741
Hydrogen evolution reaction, 447
Hydrogenation, 957
Hydrometallurgy, 1513
Hydrothermal method, 753, 1223
Hypercholesterolemia, 415
- Ibuprofen, 1539
ICP-OES, 1425
IMDA, 727
Imino derivatives, 857
Immobilization, 579
Immune response, 1147
Impedance study, 351
Indacaterol maleate, 77
Index of ideality of correlation, 39
Industrial waste, 1415
Inhaler capsules, 77
Inhibition, 369
Intermolecular interactions, 1439
Inverse gas chromatography, 479
Ions, 1241
Isolation, 257
Isotherm, 215, 311, 1415
- Kaolin, 1223
Keggin-type polyoxometalate, 957
Kinetic parameters, 1189
Kinetics, 215, 311, 513, 661
- Laboratory course, 1001
Laboratory experiment, 1241
Lagoon lake, 383
Langmuir, 529
LC-MS/MS analysis, 869
Leaching, 325
LIBS, 915
Limestone, 1241
Lippia alba, 1451
Liquid waste, 247
Lithium oxide, 53
Low temperatures, 175
Low-cost adsorbent, 215
Lumiphorbol, 393
- Machine learning for spectroscopy, 1555

- Macrocycle, 447
Magnetic nanocomposite, 311
Malaria, 1317
Malaysian food, 943
Maleic anhydride functionalized homopolypropylene, 789
Maleic anhydride functionalized polypropylene copolymer, 789
Mass loss, 233
Matrix effect, 943
Matrix modifier, 987
Mechanical durability, 339
Melting temperature, 789
Mercury ions, 579
Meriç River Delta, 383
Mesoporosity, 1481
Metal complexes, 857
Metal-containing carbon nanotubes, 1189
Methane, 957
Methyltrichlorosilane, 339
Miconazole nitrate, 67
Microbial pigment, 561
Microcapsules, 1105
Microencapsulation, 1331
Microplastics, 803
Microscopic process, 233
Microstructure, 351
Mineral, 1481
Mixed metal oxides, 623
Mixing, 1369
Modification, 693
Modified carbon paste electrode, 1131
Molecular descriptors, 1055
Molecular docking, 291, 1175, 1317, 1451
Molecular dynamics simulation, 187
Molecular dynamics, 291, 1317, 1451
Molecular modeling, 1027
Molten fluoride, 53
Monte Carlo optimization, 95
MRSA, 709
Multivariate analyses, 681
Mushroom, 201

N,B-codoped CQDs printer ink, 929
Nano manganese dioxide, 109
Nano, 257
Nanocatalyst, 623
Nanocomposite, 109
Nanoparticle, 593, 1131

Native protein separation methods, 305
Natural product, 1317
Natural resistance, 271
NBO, 883
Neoflavonoid, 271
Neuroprotective activity, 561
Nitrogen content, 1401
Nonvariant reactions, 1119

ODS steel, 915
One-pot synthesis, 623
ONIOM, 1451
Organic matter, 1527
Organic pollutants, 247, 693
Organic solvent, 943
Oxidative stress, 149
Oxygen evolution reaction, 447, 1089
Oxygen rate, 1189
Oxygen, 431

Paleoenvironmental settings, 1527
Partition coefficient, 1055
PCA, 401
Peroxidase, 1285
Pertechentic acid, 741
Pharmaceutical effluent, 1285
Pharmaceuticals, 973
Phase diagram, 53, 1119
Pharmacokinetic properties, 415
Phellinus pini, 201
Phenol, 109, 201, 277
Phenolic compounds, 461
Phenytoin, 1439
Phosphorus content, 1401
Photocatalytic reduction, 497
Phytochemical composition, 869
Phytostabilization, 823
Plant extracts, 1495
Plasma physics, 1555
Plasmodium falciparum, 1317
Platinum(II) complex, 1147
PM6, 1451
Poly(itaconic acid), 1015
Polyacrylamide gel electrophoresis, 305
Polyacrylamide, 233
Polychlorinated biphenyls, 1055
Polymer carriers, 1331
Polymethyl methacrylate, 479
Polypropylene random copolymer, 789

- Polystyrene, 479
Polyvinyl alcohol, 479
Polyvinylpyrrolidone, 479
Pores, 1069
Potassium content, 1401
Potato starch, 693
Power number, 1369
Pressure, 915
Primary crystallization, 1119
Printed circuit board, 325
Proline, 175
Prooxidant, 149
Propolis, 1425
Protein electric charge, 305
Pseudo-second order, 529
Pterocarpan, 271
Push–pull effect, 13
Pyridinecarboxylic acid derivatives, 857
Pyrolysis mechanism, 1401
Pyrroles, 149
Pyrrolin-2-ones, 1015
- QSAR, 95
QSAR/QSPR study, 1055
Quality assessment, 1465
Quantum chemical calculations, 1089
Quantum chemistry, 1555
- Recovery, 325
Reish, 1425
Removal, 109
Reservoir, 681
Reuse, 693
Rhubarb, 461
ROS, 857
Rushton turbine, 1369
- Salicylic acid, 67
Salt solutions, 803
Sandstone, 1241
Schiff-base, 1161
Selectivity, 767
Self-cleaning, 339
Semiconductor, 497, 529
Sesame seeds, 27
Sexual dysfunction, 781
Shaking table, 325
Shellfish, 803
Silver nanoparticles, 123
- Simulation, 1369
Single distillation, 401
Single-atom catalysts, 609
Sintering, 1481
SMD, 883
SMILES, 95
Sodium dodecyl sulfate, 1105
Soil additive, 1401
Sol–gel auto-combustion, 623
Solubility, 53
Solvothermal reaction, 1303
Sonication, 257
Spirulina, 579
Splenocytes, 1147
Square-wave voltammetry, 77
Stability, 579
Structural attributes, 39
Structure, 431
Styrofoam, 929
Substituted pyrazolones, 1041
Superhydrophobic, 339
Surface tension, 1353
Surfactant, 1353
Sustainability, 369, 1131
Synthesis of zeolite, 1223
Synthesis, 149
Synthesized azo dyes, 1027
- Target hazard quotient, 987
Tenebrio molitor, 869
Tetracycline, 1161
Textile coloration, 1465
Thermal conditions, 1241
Thermodynamics, 215, 311
Thiazolopyrimidine, 13
Thiocarbohydrazones, 1
Total flavonoid content, 639
Total phenolic content, 639
Toxic elements assessment, 383
Trace elements, 823
Transfer factors, 823
Tree seedlings, 823
Turbulent flow field, 1369
Types of polymers, 803
- UHPLC-DAD-ESI-MS, 1425
University students, 1267
Unresolved complex mixture, 1527
Upper secondary school, 1001

UV spectroscopy, 67, 1027

Validation, 943

Valsartan, 1331

Volatile compounds, 401

Volatile matter, 1069

Voltammetry, 1131

Waste management, 123, 369

Wastewater, 513, 1415

Water content, 1069

Water treatment, 497, 1415

Weight loss method, 351

X-ray crystallography, 1303

Zeolite LTA, 1223

Zinc(II), 1303

ZnO-NPs, 27