ChemBiotIC

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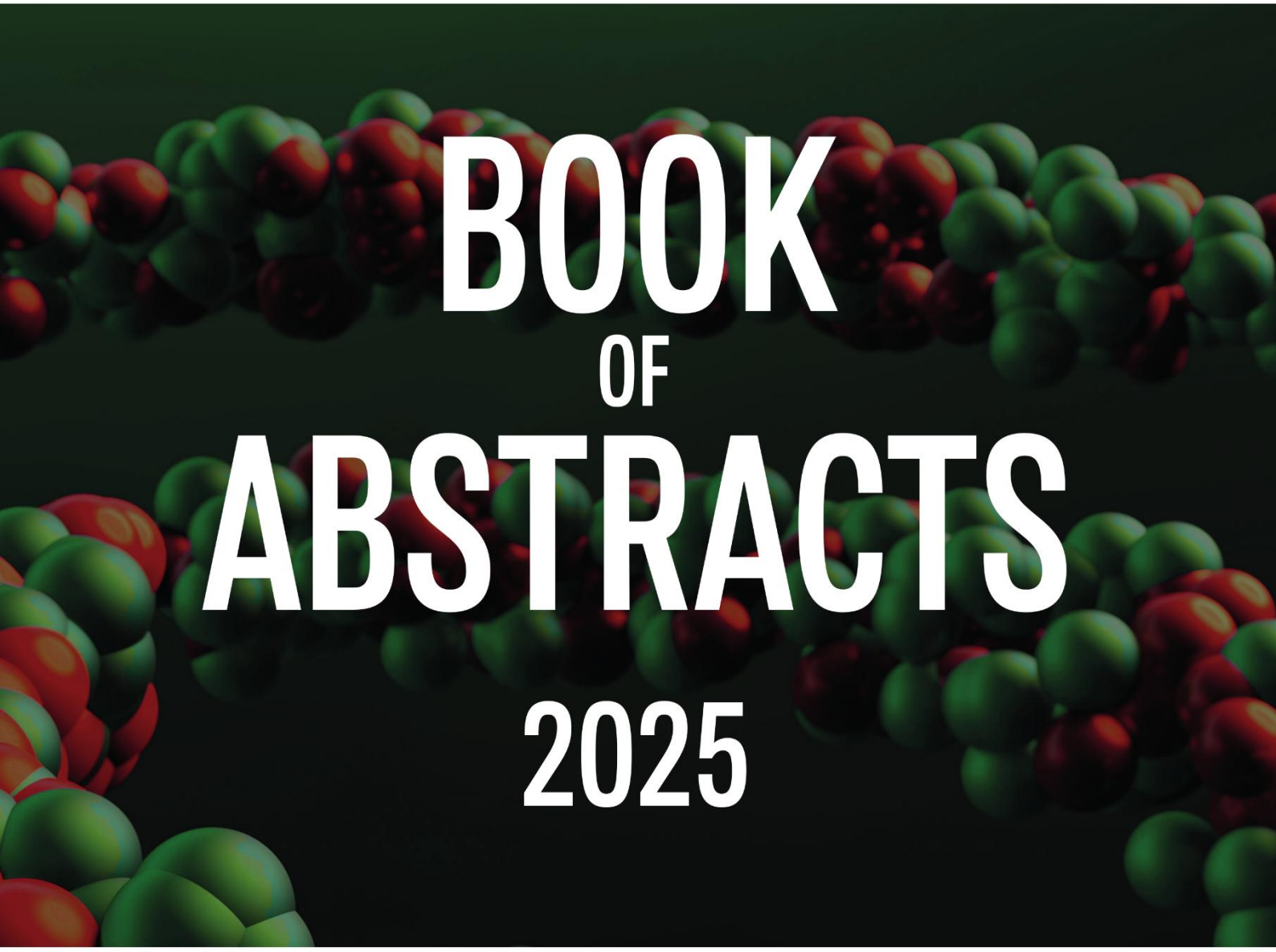

BOOK OF ABSTRACTS 2025

Wrocław University of Science and Technology

ChemBiotIC

Chemistry & Biotechnology
International Conference

BOOK OF ABSTRACTS

WUST Publishing House
Wrocław 2025

Editors
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Cover design
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Technical proofreading
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Published in the camera ready form

WUST Publishing House
Wybrzeże Wyspiańskiego 27, 50-370 Wrocław
http://www.oficyna.pwr.edu.pl
e-mail: oficwyd@pwr.edu.pl
zamawianie.ksiazek@pwr.edu.pl

ISBN 978-83-8134-005-2
https://doi.org/10.37190/ChemBiotIC_2025

Science thrives on the sharing of knowledge and experience.

In 2021, three science enthusiasts – Anna Skorupska-Stasiak PhD, Eng., Agnieszka Śliżewska PhD, Eng., and Alicja Surowiak PhD, Eng. – came up with the idea of creating an international conference that, through its interdisciplinary nature, would not only popularise scientific achievements but also connect the academic world with business.

This idea gave rise to the Chemistry & Biotechnology International Conference (ChemBiotIC) – an open, welcoming space where researchers from around the globe can exchange experiences and insights entirely online and free of charge.

The event features inspiring lectures by distinguished professors as well as practical workshops led by representatives of companies operating at the science–business interface.

ChemBiotIC is a fully secure, online event dedicated to science enthusiasts, students, and researchers.

"Even the best microscope will not contribute to science if it is kept in a wardrobe."

Ludwik Hirszfeld

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CONTENTS

PLENARY SPEAKERS

Plant biotechnology. Some case studies

Ana Cristina Figueiredo*

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Keywords: plant biotechnology, phytochemistry, biological activity

Plant biotechnology uses diverse techniques to explore and improve the metabolic properties of plants. This presentation focuses on some case studies, run at the Plant Biotechnology Laboratory, trying to respond to problems in the areas of the:

a) valorisation of resources from medicinal and aromatic plants (MAP), or underutilised biomass, with pharmacological, cosmetic and agroforestry properties,
b) quality control, chemical composition and biological potential determination of non-timber forest products, such as *água-mel*, propolis and honey,
c) screening of phytochemicals to combat agroforestry pests and diseases,
d) *in vitro* culture as an agroforestry experimental model,
e) characterization of volatile phytochemicals from agroforestry species, and
f) characterisation of host-pathogen-vector interactions in Pine Wilt Disease (PWD).

Plant biotechnology uses a set of *in vitro* plant culture techniques to, among other things, micropropagate, transform and optimize plant characteristics under controlled growth conditions. In this way, it is also possible to use these cultures as model systems for a preliminary approach to plant-organism or plant-environment interactions. Phytochemistry also uses a set of chromatography techniques to characterize the compounds produced by plants. Many of these substances, which plants use in their defense or to attract pollinators, have long been used by humans, not only in food, but also as a source of compounds with medicinal, commercial, agronomic and industrial importance. In addition to these methodologies, other, different approaches, methods and tools are used, such as analytical methods of microscopy, molecular biology and *in vitro* and *in vivo* biological activity assessment, among others, to respond to the challenges posed [1–6].

ACKNOWLEDGMENTS

Thanks to all our colleagues, both national and international, as well as to the graduating, post-graduation, Master's, and PhD students who shared their work with us in the lab over the past several years and who helped to elucidate much of the information presented here. Thanks to Fundação para a Ciencia e a Tecnologia (FCT) through previous national funds to CESAM, and now under UID/00329/2025 - Centre for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE – Global Change and Sustainability Institute.

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Advancing process control in process-driven industries with cutting-edge technology

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Keywords: lab-on-a-chip, spectroscopy, brewing, quality control, microfluidics

SG Papertronics was established to democratize access to laboratory-grade quality control within process-driven industries such as brewing. This work presents the journey from academic research to the development and deployment of the Beer-o-Meter (Figure 1), a compact analytical platform designed to enhance process control in real-world environments.

The Beer-o-Meter integrates lab-on-a-chip principles with optical spectroscopy, microfluidic systems, and IoT connectivity. These technologies were carefully engineered to ensure robust, fast, and accurate testing in brewery settings, where traditional laboratory access is often limited. Special emphasis is placed on addressing challenges related to measurement precision, device usability, and seamless integration with existing brewing workflows.

A critical aspect of this development was user-centered design. By collaborating closely with brewers, the system was iteratively refined to ensure intuitive operation and high adoption potential. The device enables brewers to monitor key quality parameters with minimal disruption to their processes, leading to improved consistency, efficiency, and product quality.

Looking ahead, the modular nature of the Beer-o-Meter platform offers potential for expansion into adjacent sectors. Ongoing research and industrial collaborations are expected to drive further innovation and broaden the scope of accessible, high-performance analytical tools for diverse production environments.

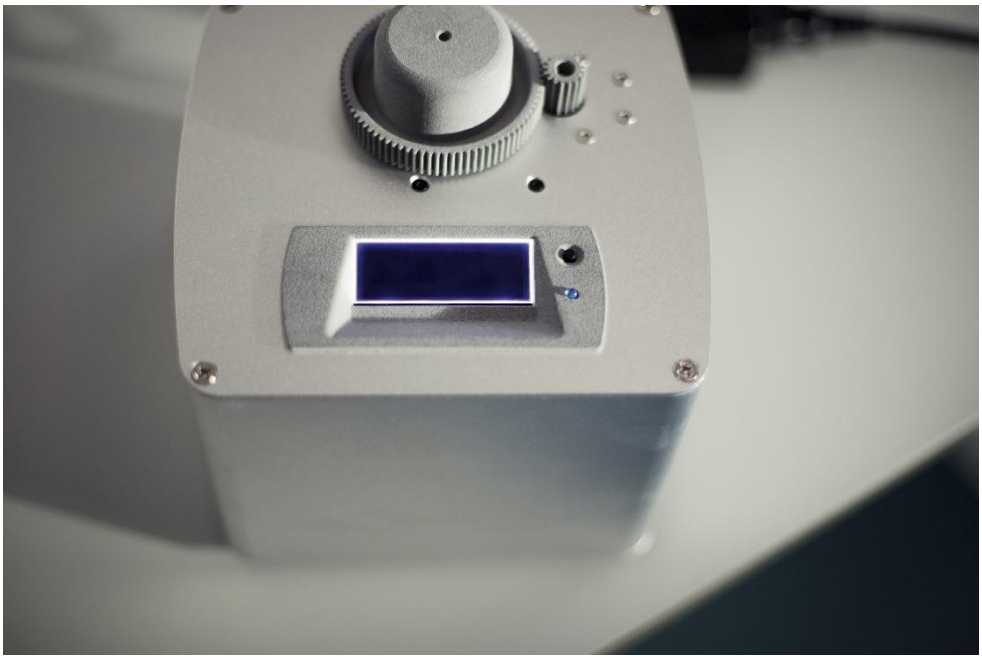

Fig. 1. Beer-o-Meter

Development of functional foods free from synthetic additives: utilizing natural extracts and circular economy principles

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Keywords: functional food, bioactive compounds, natural additives, extraction, circular economy

The increasing consumer demand for health-promoting, clean-label foods has driven the food industry to seek alternatives to synthetic additives. Simultaneously, environmental concerns and regulatory pressures have emphasized the need for sustainable, circular production models. This study has presented the development of novel functional food products free from synthetic additives, enriched with bioactive compounds extracted from mushrooms, herbs, and food processing by-products. These formulations exemplify a dual approach – enhancing nutritional value while supporting ecological and economic sustainability.

Three functional foods have been developed as representative case studies: industrial produced bio-soup enriched with mushroom extracts, an iced tea containing linden flower extract, and a plant-based yogurt fortified with a combination of mushroom and orange peel extracts. Green extraction technologies have been employed, including supercritical fluid extraction. This method has allowed for efficient recovery of bioactive compounds without the use of toxic solvents, thereby maintaining compound integrity and minimizing environmental impact.

A key innovation lies in the valorization of food industry by-products, such as citrus peels and mushroom stems, which are typically discarded despite their richness in functional compounds. The integration of these waste materials aligns with the principles of the circular economy, transforming potential waste streams into high-value ingredients while reducing environmental burden.

Chemical and biological analysis of the extracts and final new products has revealed significantly higher antioxidant and antimicrobial activities compared to conventional products containing synthetic additives. *In vitro* assays of the extracts applied in bio-soup have demonstrated notable cytotoxic potential against selected human cell lines, suggesting that these products may offer additional health-protective benefits beyond basic nutrition. These bioactivities are primarily attributed to the high content of β-glucans, polyphenols, flavonoids, and other bioactive secondary metabolites present in the natural extracts.

Despite the challenges associated with eliminating synthetic additives in industrial formulations, these products have achieved desirable sensory properties, stability, and consumer acceptability. The use of natural extracts has not only preserved quality but also contributed to the functional enhancement of the final products.

These products have been newly introduced to the market, representing a pioneering step toward the next generation of sustainable, health-oriented food solutions. Their development provides a practical model for integrating green extraction methods, natural ingredient sourcing, and circular economy principles into food innovation. This approach addresses growing consumer and regulatory expectations while delivering tangible health and environmental benefits.

Further research will focus on *in vivo* validation of the observed bioactivities, long-term formulation stability, and the potential for industrial-scale production and application.

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Direct observation of the matter using in situ transmission electron microscopy

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Keywords: imaging, in situ, diffraction, spectroscopy, microscopy

Transmission electron microscopes (TEM) have been a unique tool for observing the structures of matter for almost a century. However, the ambitions of researchers did not stop at static observations on a scale exceeding the diffraction limit of light, and subsequent decades of development brought new ideas for influencing the sample during observation. The sample was heated or cooled, and also affected by electric and magnetic fields. TEM could also be modified significantly to allow for the introduction of reactive gases or a stream of accelerated particles into its center, or for the sample itself to be placed in a small volume of liquid separated from the vacuum (Fig. 1). Such experiments provided a unique opportunity to directly observe physical or chemical processes that cannot be imaged by any other method. Interestingly, one of the most important interactions with the sample may be the electron beam itself, used for imaging. Its influence is particularly important when imaging processes in liquids, organic substances, and attempts to image living and hydrated structures [1], as well as when imaging phenomena in photosensitive materials [2].

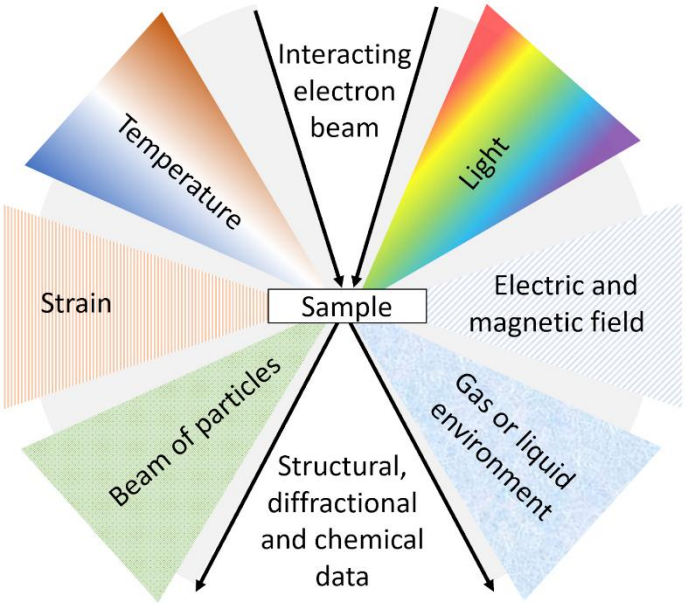

Fig. 1. Examples of in situ transmission electron microscopy interactions

ACKNOWLEDGMENTS

This work was supported by the National Science Centre, Poland (Sonata, 2023/51/D/ST11/01490), as well as the Polish-American Fulbright Commission (Fulbright Senior Award). Author would like to sincerely thank his colleagues from the research group – Dominika Benkowska-Biernacka, Olga Kaczmarczyk, Aleksandra Królicka, Amelia Maj, Patryk Obstarczyk, Adrian Matusiak, and Magdalena Wojtas.

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ORAL PRESENTATION SESSION

Green diesel and biojet fuel: emerging thermochemical routes and catalyst engineering for a low-carbon energy future

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Keywords: biofuel, biojet fuel, Green Diesel, thermochemical routes, hydroxyapatite

The growing global demand for sustainable fuels has driven the development of biojet fuel and green diesel as viable alternatives to fossil fuels, especially in the aviation and heavy transport sectors. Unlike traditional biodiesel, which is obtained through transesterification, biojet fuel and green diesel are produced via thermochemical routes, including hydrotreatment and pyrolysis of vegetable oils, animal fats, and various lipid-rich feedstocks. These processes yield fuels with physicochemical properties similar to those of fossil-derived fuels, with potentially lower environmental impact depending on feedstock and process conditions.

In recent years, research has advanced in the development of new technologies to make these processes more efficient, selective, and sustainable. Among the promising alternatives is the use of heterogeneous catalysts such as hydroxyapatite (HAP) – a calcium phosphate with a porous structure, high thermal stability, and adjustable acid-base properties. These characteristics make HAP highly attractive for catalytic applications, offering significant advantages such as catalyst reuse, reduction of contamination in the final product, and suitability for continuous operation in fixed-bed reactors. In a typical process using HAP, vegetable oils are subjected to a hydrotreatment in the presence of molecular hydrogen, promoting deoxygenation reactions that convert triglycerides into hydrocarbons compatible with aviation kerosene specifications. Moreover, hydroxyapatite can be modified by incorporating transition metals such as Ni, Co, or Pt used in the form of nanoparticles or deposited on a support, to enhance its catalytic activity, selectivity, and resistance to deactivation due to carbon deposition (coking phenomena). Current experimental investigations into green diesel production focus on optimizing reaction parameters and developing new catalytic systems, especially those based on metallic materials and metal oxides. At the same time, the range of feedstocks is expanding, aiming to increase conversion efficiency and process adaptability. Notably, one of the inherent advantages of green diesel over conventional biodiesel is its significantly lower content of oxygenated compounds, which improves both fuel stability and combustion performance. Despite recent advances, significant technical and economic challenges remain, such as catalyst stability and longevity, production costs, sustainability of feedstock sources, and compatibility with existing fuel infrastructure. As hydrogen gas is required in the hydrotreating process and its main source is often fossil-based – typically produced via steam methane reforming – the environmental benefit of these processes strongly depends on the hydrogen source used in hydrotreating. Recent research therefore focuses on replacing conventional fossil hydrogen with green hydrogen, or even on developing alternative deoxygenation reactions under inert atmospheres. In addition, the implementation of robust public policies and compliance with international certifications are essential to ensure the widespread adoption of these advanced biofuels in global markets. In this context, biojet fuel and green diesel – especially when produced via catalytic thermochemical routes employing advanced materials such as hydroxyapatite – represent a promising technological frontier that aligns environmentally with decarbonization goals and the global energy transition.

ACKNOWLEDGMENTS

This work was supported by the Research Support Foundation of the State of Rio de Janeiro (FAPERJ) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Finance Code 001).

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Optimization of phenolic compounds extraction from berberine using deep eutectic solvents

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Keywords: extraction, berberine, TPC, DPPH, deep eutectic solvents

Berberine is a bioactive isoquinoline alkaloid found in medicinal plants such as Berberis species, which is widely known for its antimicrobial, antioxidant, and anti-inflammatory properties [1]. These therapeutic effects are primarily attributed to the presence of phenolic compounds. Efficient extraction of these bioactive compounds is essential for their utilization in various scientific and functional applications. Natural deep eutectic solvents (NADESs), composed of natural primary metabolites, are now widely used as green and sustainable extraction solvents of bioactive components [2]. Choosing suitable solvents and optimized extraction conditions are essential to increasing the extraction yield and maintaining the compound integrity. In this study, a NADES composed of choline chloride and lactic acid in different molar ratio was employed as a green, biodegradable, and tunable solvent system, offering an environmentally sustainable alternative to traditional organic solvents. The extraction process was optimized using Response Surface Methodology (RSM) with a Box-Behnken design including three process parameters of extraction temperature (X_1), water content in the solvent (X_2), and liquid-to-solid ratio (X_3). Total phenolic content (TPC) and antioxidant activity will be determined using the Folin-Ciocalteu method and DPPH radical scavenging assay. These analyses will evaluate how extraction parameters affect the yield of phenolic compounds and their antioxidant activity. Therefore, this study aims to determine the optimal extraction conditions that increase phenolic yield and antioxidant potential by using NADES over conventional methods. The findings will contribute to the development of green and effective approaches for recovering bioactive compounds from berberine using environmentally friendly solvents.

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Novel citric acid-based photo-curable polyester resins for 3D printing of biocompatible tissue scaffolds for potential vascular tissue engineering applications

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Keywords: tissue regeneration, photo-crosslinkable resins, 3D-DLP printing, citric acid-based polyesters, vascular tissue engineering

The aim of the study was to develop and characterize a novel photo-crosslinkable polyester resin intended for application in 3D-DLP printing technology, with potential use in the fabrication of flexible, tubular tissue scaffolds for blood vessel regeneration.

The resin was synthesized via polycondensation of citric acid, 1,6-hexanediol, and itaconic acid. The presence of itaconic acid introduced unsaturated bonds enabling photopolymerization. A series of syntheses was conducted under various reaction conditions to determine the optimal molar ratio of components, reaction time, and temperature. The rheological properties of the obtained composition were examined in comparison with commercial resins. The crosslinking efficiency of five different photoinitiators was evaluated using FT-IR spectroscopy, and the effect of a dispersing agent on print quality was investigated. Gel permeation chromatography (GPC) was performed to determine the average molecular weights of the prepolymer, and the acid value of the formulation was assessed to estimate the material's potential biocompatibility.

The best properties were obtained at a molar ratio of 1:2:0.8 (citric acid:1,6-hexanediol:itaconic acid), a temperature of 160°C, and a reaction time of 40 minutes. The resulting resin exhibited suitable rheological behavior, good photoreactivity, and a favorable acidity in terms of biocompatibility. A significant influence of the photoinitiator type and dispersant on crosslinking rate and print quality was observed.

The developed resin demonstrated strong potential for use in 3D printing of tissue supports, particularly for vascular regeneration. Owing to its biodegradability and photo-crosslinking capability, it represents a promising material for tissue engineering applications. Further research is planned to optimize printing conditions and to conduct mechanical testing, as well as cytotoxicity and hemocompatibility assessments of the printed structures.

ACKNOWLEDGMENTS

The research was funded by the NCN PRELUDIUM project no. 2021/41/N/ST4/03362.

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Molecular nanographenes for modern technologies: synthesis and properties characterization

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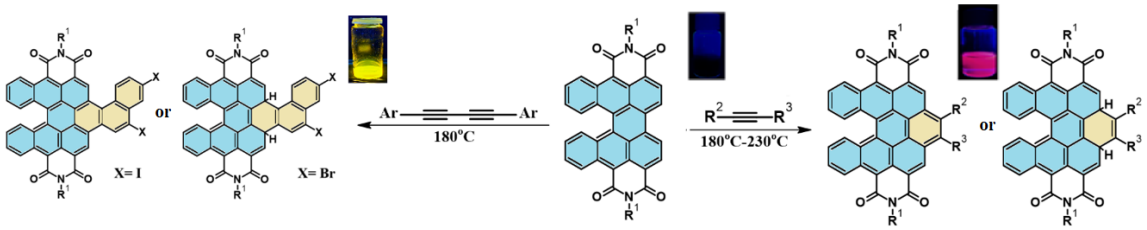
Keywords: nanographene, Diels-Alder cycloaddiction, electrochemical methods, optoelectronics

Graphene and molecular nanographenes (MNGs) play a significant role in modern technologies, ranging to dyes-industry and pharmacy to energy storage and electronics, thanks to their unique thermal, emissive, electrochemical and electron mobility properties. From organic chemistry's point of view, MNG is a polycyclic aromatic hydrocarbon (PAH) or heteroaromatic system consisting of at least 5 conjugated aromatic rings [1, 2]. Currently, almost only the "bottom-up" method enables the expansion of smaller structures into MNGs, in a controlled manner, as far as their size, shape, type, and position of heteroatoms and functional groups [3, 4]. The commonly used synthetic tool to expand the π-electron structure is the Diels-Alder (DA) cycloaddition, especially the cycloaddition in the PAH cavity (e.g., perylene or perylenebisimide) [2, 5].

During this study, the focus was on PAHs whose skeleton is based on a perylene core. The research began with synthesizing a nanographene precursor, i.e., *cis*-dibenzoperylenebisimide (*cis*-DBPDI), using classical chemical synthesis (applied in an innovative manner [6]) and a completely novel electrochemical method starting from anthracene. *cis*-DBPDI was subjected to further functionalization, i.e., π-expansion according to the APEX strategy, via cycloaddition of acetylenes and butadiynes to its cavity (bay region). In this manner, several entirely new π-expanded derivatives were obtained, characterized by NMR and HRMS, and further analyzed using DFT calculations. Interestingly, some cycloaddition reactions proceeded without aromatization of the cycloadduct, which is unique when it comes to DA-cycloaddition of perylene and its derivatives. The obtained derivatives were subjected to physicochemical measurements, including UV-Vis spectroscopy, photoluminescence, and electrochemical analysis. The results so far indicate that these MNGs possess unique physicochemical properties arising from their electronic structure and distorted or/and chiral structure. MNGs, herein developed through a fully innovative approach, exhibit highly promising properties, making them attractive for applications in colorant technologies, organic electronics, and photovoltaics. The synthetic route, structures of *cis*-DBPDI and the products, and the colors of the obtained MNGs in solution are presented in Fig. 1.



R^1 – 2-ethylhexyl; Ar = *p*-*X*-Ph; R^2, R^3 = COOMe, Ph, *p*-Br-Ph, 9,9-dibutylofluoren-3-yl and others

Fig. 1. Synthetic route to MNGs

ACKNOWLEDGMENTS

Research carried out thanks to the OPUS 18 grant 2019/35/B/ST4/00115

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Exploiting synthetic biology for the production of prenylflavonoids in *Escherichia coli*

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Keywords: CRISPR, *Escherichia coli*, microbial cell factories, prenylflavonoids, synthetic biology

Prenylflavonoids are flavonoids bearing a prenylated sidechain. Among them, xanthohumol and prenylnaringenin (PN) isomers, including 3'-prenylnaringenin (3'-PN), 6-prenylnaringenin (6-PN), and 8-prenylnaringenin (8-PN) have attracted interest due to their bioactive properties. However, their low accumulation in plants limits their utilization. The construction of microbial cell factories able to produce them appeared as an alternative solution. Herein, we intended to engineer *Escherichia coli* strains able to produce PN compounds and xanthohumol using glucose as main substrate. Firstly, a step-by-step optimization was performed to construct an efficient pathway for de novo production of the precursors naringenin chalcone and naringenin. *E. coli* M-PAR-121 expressing naringenin chalcone pathway composed by tyrosine ammonia lyase (TAL) from *Flavobacterium johnsoniae* (*Fj*TAL), 4-coumarate:CoA ligase 1 (4CL-1) from *Arabidopsis thaliana* (*At*4CL), and chalcone synthase (CHS) from *Curcubita maxima* (*Cm*CHS) was able to produce 560.2 mg/L of naringenin chalcone. *E. coli* M-PAR-121 expressing naringenin biosynthetic pathway composed by *FjTAL*, *At*4CL, *Cm*CHS, and chalcone isomerase (CHI) from *Medicago sativa* (*Ms*CHI) was able to produce 769.5 mg/L of naringenin [1]. These production levels were the highest reported in *E. coli* until this moment. Since the final steps of the prenylflavonoids pathway depend on the availability of dimethylallyl pyrophosphate (DMAPP) and/or S-adenosylmethionine (SAM), clustered regularly interspaced short palindromic repeats (CRISPR) methodologies were exploited to improve the intracellular availability of both compounds. *E. coli* M-PAR-121 strain with the integration of 1-deoxy-D-xylulose-5-phosphate synthase (DXS) from *E. coli* (*Ec*DXS) (*E. coli* M-PAR-121:*Ec*DXS) expressing the soluble aromatic prenyltransferase (PT) from *Streptomyces roseochromogenes* (CloQ) (pCDFDuet_CloQ) and pRSFDuet_*Fj*TAL_*Cm*CHS_*At*4CL_*Ms*CHI was selected as the best PN producer strain, being able to produce 135.33 mg/L of 3'-PN and 8.72 mg/L of 6-PN [2]. Moreover, *E. coli* M-PAR-121:BlIDI:metK, constructed by integrating SAM synthase (metK) and isopentenyl diphosphate isomerase (IDI) from *Bacillus licheniformis* (*Bl*IDI) was selected as the best xanthohumol producing strain. Xanthohumol production reached 5.26 mg/L when pRSFDuet_*Fj*TAL_*Cm*CHS_*At*4CL was expressed in combination with CdpC3PT from *Neosartorya fischeri* and *O*-methyltransferase from *Humulus lupulus* (*Hl*OMT1) (pCDFDuet_CdpC3PT_*Hl*OMT1). These productions represent the highest reported to date in any microorganism and the first reports of *de novo* production of 3'-PN, 6-PN, and xanthohumol in *E. coli*.

ACKNOWLEDGMENTS

This work was supported by the Portuguese Foundation for Science and Technology (FCT) [UIDB/04469/2020; doi: 10.54499/UIDB/04469/2020]. D.G. acknowledges FCT for her grant [SFRH/BD/04433/2020; doi: 10.54499/2020.04433.BD]. Work in the UK (Manchester) was also supported by the Future Biomanufacturing Research Hub (EP/S01778X/1).

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Assessing antibiotic resistance in HVAC systems using culture-based and functional prediction methods

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Keywords: HVAC systems, antibiotic resistance genes, indoor air microbiome, microbial contamination, qPCR and NGS

The objective of the study was to investigate whether HVAC systems in public buildings act as secondary sources of antibiotic-resistant bacteria and resistance genes, as suggested in recent literature [1]. The presence of such microorganisms was analyzed in indoor air, HVAC components, and condensate samples. Additionally, the impact of environmental and operational factors on microbial growth and gene transfer was assessed.

Sampling was performed in selected rooms and central HVAC units. Air and surface samples were collected using culture-based methods, quantitative PCR (qPCR) targeting 16S rRNA genes for bacterial abundance, and next-generation sequencing (NGS). Quantitative and qualitative assessments included total bacterial load, phenotypic resistance profiling using selective antibiotic media, and detection of resistance traits. Functional prediction of antibiotic resistance and pathogenicity genes was conducted using PICRUSt2, filtered by NSTI < 0.15.

The results confirmed the presence of multiple antibiotic resistance genes (e.g., mecA, blaCTX-M, tet, blaOXA, ermC, ermA) and the intl1 integrase gene, which is associated with horizontal gene transfer of resistance cassettes. Dominant species included *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Escherichia coli*. Room-specific variation and seasonal differences were observed, with a higher abundance and diversity of resistance genes in winter. Ordination analyses (PCA and t-SNE) indicated distinct microbial profiles between locations and sample types, with overlap between outdoor air and central HVAC units.

In conclusion, HVAC systems were identified as environmental reservoirs and potential dissemination routes for antibiotic resistance genes in indoor environments [1, 3]. The application of PICRUSt2 enabled functional prediction of resistance potential, underlining the need for continuous monitoring, advanced bioinformatic tools, and development of standardized air quality assessment protocols [2].

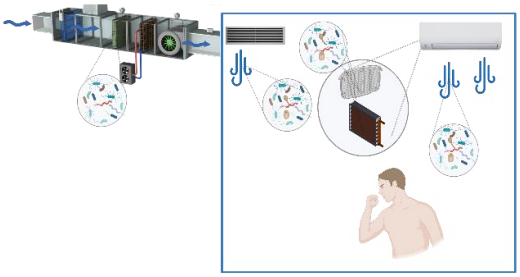

Fig. 1. HVAC systems as potential sources of airborne bacteria and antibiotic resistance genes (ARGs) in indoor environments

ACKNOWLEDGMENTS

The work was supported by the National Science Centre, Poland, under research project entitled: “Impact of condensate in air-conditioning systems on secondary microbiological pollution of indoor air”, no 2021/41/B/ST8/03402.

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Optimization of culture conditions for fungal-mediated synthesis of silica nanoparticles

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Keywords: nanosilica, *Fusarium culmorum*, respirometric activity, OxiTop®, biotransformation

Fungi are well known for their ability to synthesize nanoparticles. While chemical methods for nanosilica synthesis are well established and relatively cost-effective, biosynthetic approaches have the potential to produce materials with unique properties or hybrid structures [1]. Nevertheless, the mechanism behind the fungal-mediated synthesis of silica nanoparticles remains largely unknown. This study aimed to optimize culture conditions for the conversion of silica into nanoparticles by *Fusarium culmorum*. This species has previously shown promise as a biocatalyst for nanosilica synthesis from plant waste, particularly corn cob husks [2]. A crucial first step in elucidating the underlying mechanism was to examine how the enzymatic activity of the fungal biocatalyst can be influenced by optimized culture conditions. This study, therefore, evaluated the effects of various cultivation media - Czapek-Dox Medium (CDM), Potato Dextrose Broth (PDB), Maltose Glucose Medium (MG), and Maltose Glucose Yeast Extract Peptone Medium (MGYP) - on the growth and respiratory activity of *F. culmorum*. Respiratory activity was measured using the OxiTop® system and served as an indirect indicator of the fungus's biocatalytic potential. The aim was to identify the culture conditions that would promote the development of an active enzymatic system suitable for further investigation. Subsequently, the biotransformation of Na_2SiO_3 was carried out over a period of three days, with continuous monitoring of fungal respiratory activity.

The study demonstrated that fungal growth rates were significantly affected by the composition of the medium: the more nutrient-rich the medium, the faster the growth. In MGYP, the most nutrient-dense medium, *F. culmorum* entered the logarithmic phase after just one day and reached the stationary phase 24 hours later. By contrast, in the minimal CDM medium, the logarithmic phase began on the second day and lasted for 48 hours. Regarding biotransformation, the study showed that when the biocatalyst was cultivated in minimal media, the presence of the silicon compound increased the aerobic activity of fungal cells more than when the biocatalyst was cultivated in rich media. These growth patterns are valuable for selecting optimal culture conditions for future biotransformation experiments. Furthermore, they provide a starting point for studying the effect of substrate availability on fungal metabolism. To improve our understanding of the bioconversion mechanism, future work will compare the respiratory activity of *F. culmorum* in different media with the structural characteristics of mycosynthesized products, as visualized by transmission electron microscopy (TEM).

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From waste to value: culture medium based on potato wastewater for cultivation of biocontrol yeast *Metschnikowia pulcherrima*

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Keywords: media optimization, biocontrol, *Metschnikowia pulcherrima*, potato wastewater, fractional factorial design

In the face of a growing global population, one of the key challenges for modern science is the sustainable production of food. An important aspect in this context is the use of biological plant protection agents (BCA), which, given the increasing resistance of phytopathogens to chemical pesticides, might provide a safe and effective alternative for controlling plant diseases. The inspiration for this study was the combination of this idea with the need to manage agro-industrial waste materials.

The study investigated the possibility of using potato wastewater, generated during starch production [1], as a culture medium component for the cultivation of biocontrol yeast *Metschnikowia pulcherrima*. The composition of the medium and the process parameters were optimized using the fractional factorial design 3^{3-1}, including glycerol concentration, pH, and temperature as independent variables and optical density (OD_{600}), determined spectrophotometrically, as the dependent variable. Based on the optimization results, a bioreactor culture (working volume: 2 L) was carried out under the following conditions: initial pH ~5 (pH of wastewater), temperature of 23°C, and glycerol concentration of 80 g/l. After 41 hours of cultivation, a high biomass concentration of *M. pulcherrima* d.4.1 strain was achieved – 42.3 g/l (determined gravimetrically), along with 97.78 % glycerol utilization (determined by HPLC).

Additionally, *in vitro* antagonistic activity tests were conducted using both the yeast suspension and the post-culture supernatant against three strains of the phytopathogen *Alternaria alternata* (CBS 116329, CBS 117143, CBS 120829). The yeast suspension exhibited strong inhibitory effects on both the growth and sporulation of the tested filamentous fungi, whereas the supernatant showed no antifungal activity.

Given these results, future research directions may include scaling up the cultivation process to pilot scale, replacing pharmaceutical glycerol with crude glycerol, and extending pathogen inhibition studies to include *in vivo* and field tests.

ACKNOWLEDGMENTS

The research was financially supported by the project VEFAF - Designing a versatile and environmentally friendly alginate-based fibre platform and assessing the impact on the soil environment (Project OPUS 25 UMO-2023/49/B/NZ9/00959) funded by the National Science Center (NCN).

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Combined phage–antibiotic therapy for the eradication of skin pathogens

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Keywords: bacteriophages, antibiotics, synergy, skin pathogens

With the alarming rise in antibiotic resistance, modern medicine faces a critical challenge: developing effective alternative treatments while optimizing the use of existing antibiotics [1]. Combination therapies that enhance antibacterial efficacy and reduce antibiotic dosage have gained particular attention. Among the most promising strategies is the integration of bacteriophages – viruses that selectively target bacteria – with conventional antibiotics [2].

The aim of our study was to evaluate the effectiveness of combined phage–antibiotic therapy against key skin pathogens: *Staphylococcus aureus*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* [3]. We used highly specific bacteriophages targeting each pathogen: CS1 (*S. aureus*), TWORT (*S. hyicus*), ALEX (*S. epidermidis*), and JG004 (*P. aeruginosa*), in combination with a selection of commercially available antibiotics with diverse mechanisms of action: daptomycin, vancomycin, oxacillin, tobramycin, polymyxin B, and ciprofloxacin. The efficacy of each combination was tested in two *in vitro* models: liquid cultures (OD_{600} measurement, 24 h) and solid media (spot-test assay). All experiments were performed in triplicate.

Our results revealed a pronounced synergistic effect in many phage–antibiotic combinations. In the liquid culture model, the strongest growth inhibition was observed for *P. aeruginosa* (JG004 + tobramycin, JG004 + polymyxin B) and *S. hyicus* (TWORT + vancomycin). In the solid medium model, the largest inhibition zones were recorded for *S. aureus* and *S. epidermidis* when treated with phages in combination with oxacillin, daptomycin, or vancomycin.

These findings demonstrate that phage–antibiotic combination therapy can be an effective strategy for combating skin infections, particularly those caused by multidrug-resistant strains. The observed synergy suggests the potential for reducing antibiotic doses without compromising therapeutic efficacy. These results form a basis for further preclinical research aimed at optimizing combined therapies and evaluating their clinical applicability.

ACKNOWLEDGMENTS

This work was supported by the Wrocław University of Environmental and Life Sciences (Poland) as the Ph.D. research program "Bon doktoranta SD UPWr" from the subsidy increased by the minister responsible for higher education and science for the period 2020–2026 in the amount of 2% of the subsidy referred to Art. 387 (3) of the Act of 20 July 2018 – Law on Higher Education and Science, obtained in 2019.

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Fully biobased multilayer composites reinforced with epoxy resin: a green route to recycle cellulose based trays

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Keywords: recycling, thermoset, reinforced composites, biobased epoxy resin, eutectic harderner

In order to modify french production and consumption, EGalim [1a] and AGEC [1b] law have been adopted by french parliament to suppress single use plastic in public catering to 2040 [2]. Application of the solutions brought by AGOREES workgroup is restricted by financial and technical aspect [3]. Several issues were noticed, but the creation of fully new cleaning process have convinced the city of Nice to use single use biobased cellulose trays. However, plastics cover used to coat the cellulose part is complex to recycle [4, 5]. In order to satisfy the 3R decree, proof recyclability of those waste were studied by the conversion of the cellulosic trays into fully biobased thermosets composites with an innovative epoxy resin based on a full biobased eutectic hardener [6]. The composition of cellulosics trays used in the Nice caterings were studied. In accordance of tray's thermal resistance, an innovative epoxy resin developed in the team, were formulated, and the physical properties were measured. This Epoxy resin was produced by mixing biobased vegetal epoxy oil (EVO) with also an biobased eutectic hardener. Tensile tests, Dynamic and thermal mechanical analysis (DMA) and Scanning electronic microscopy (SEM) were conducted to study interactions between fibers and the thermosets matrix. The compositions of each type of trays were successfully made. Thus, negative interaction between plastic covering and epoxy resin had proscribed the production of multilayer composites. However, these composites were successfully produced by using the polymeric film collected from one cellulosic tray. To the extent of embed coating film onto trays, Polycaprolactone were chosen by manufacturer as adhesive thanks to his low melting temperature [7]. Good reinforcement were observed with cured impregnated cellulose trays. An increase of Young's modulus and yield strength were measured. Hybrid composites made by the combination of thermosets sheet layered with adhesives films have attained honorable mechanical performance (2100+ MPa and 40+ MPa of Tensile modulus and strength). This work had given enthusiastic outlook to produce inexpensive fully biobased composites for non-structural application. Scale up can be accessible after resolving water permeability issues. Then, this is the first description of reinforced cellulose composites made with a non-toxic biobased eutectic hardener.

Fig. 1 Project logo

ACKNOWLEDGMENTS

The authors express theirs thanks to the University of Nice, the City of Nice, the French ecological transition agency (ADEME) for funding this research.

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Sustainable development of chewy candy formulations with porcine hydrolysates exhibiting antioxidant and antimicrobial activities

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Keywords: functional gummy candies, protein hydrolysates, porcine liver, antioxidant activity

Porcine liver protein hydrolysates were added to functional chewy candies in this study to improve their antibacterial and antioxidant qualities. Pepsin and papain were used to create hydrolysates, which were then added to matrices made of gelatin and agar. The best formulations were determined by consumer acceptance testing, and they were then examined for physicochemical characteristics, antibacterial effects, microbiological stability, and antioxidant activity (ABTS•+, DPPH•). The maximum antioxidant activity was demonstrated by the agar-based sample GC5Pa24Ag (papain, 24 h), which had scavenging capabilities of DPPH• and ABTS•+ of 49.14 ± 1.00 and 67.6 ± 0.98, respectively ($p < 0.05$). While GC5Pa24Ag exhibited the widest antimicrobial spectrum, pepsin-hydrolyzed samples exhibited higher inhibition against *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* than the control. According to colour analysis, 2 g of hydrolysate produced stronger yellow-pink tones ($p < 0.05$), but 5 g induced a shift to blue-green colours. Sample GC5Pa24Ag exhibited the highest hardness ($p \leq 0.05$), whereas the use of a lower hydrolysate content (2 g) combined with as the gelling agent resulted in a noticeably softer texture in samples GC2Pe3Gl, GC2Pe6Gl, and GC2Pe24Gl. Agar-based gummy candies demonstrated superior microbiological stability, showing no detectable bacterial, yeast, or mold growth throughout the 21-day storage period.

A small change, a big impact? N-Donors at the C9 position of fluorene

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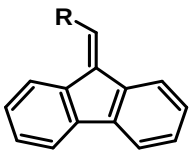
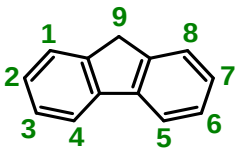
Keywords: fluorene derivatives, dibenzofulvene derivatives, Knoevenagel condensation, N-donor substituents, optoelectronics materials

The ongoing pursuit of chemical compounds with precisely defined physicochemical properties suitable for organic electronics and photovoltaic applications continues to be a key focus of scientific research. Recently, derivatives of dibenzofulvene (DBF) (Fig. 1a), created by altering the C9 position of fluorene (Fig. 1b), have attracted growing attention. These molecules are particularly valued for their adjustable physicochemical properties and versatile application potential [1-3]. Additionally, modifications at the 2 and 7 positions offer further opportunities to finely tailor their physicochemical traits for specific technological purposes [1–3].

This study aimed to synthesize and investigate the impact of N-donor substituents at the C9 position on the physicochemical properties of six dibenzofulvene derivatives, in comparison to unsubstituted fluorene[2]. The structures of the synthesized compounds were confirmed using NMR spectroscopy. Optical, electrochemical, and spectroelectrochemical properties were examined to evaluate the influence of N-donor substituents on the molecular behavior. Additionally, DFT calculations were performed to support experimental findings. The results demonstrate that even minor structural modifications at the C9 position significantly affect the absorption and emission characteristics of the studied compounds. Notably, the presence of N-donor groups enhances the electrochemical properties of the derivatives. The experimentally determined values closely align with theoretical predictions obtained through DFT calculations. The study confirms that new dibenzofulvene-based compounds with N-donor substituents show promising physicochemical properties, making them potential candidates for use in optoelectronic devices. Further research could focus on optimizing their structure for specific applications such as dye-sensitized solar cells (DSSCs), perovskite solar cells (PSCs), polymer light-emitting diodes (PLEDs), and electrochromic materials [1–3].



(a)



(b)

Fig. 1. (a) The structure of dibenzofulvene derivative, R – substituent; (b) the numbering of atomic positions in fluorene

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Construction and characterization of artificial short L-asparaginases

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Keywords: L-asparaginase, enzyme, leukemia, protein engineering

Antitumor activity of L-asparaginases was discovered in 1953 when Kidd first reported that guinea pig (*Cavia porcellus*) serum inhibited the growth of leukemic cells *in vivo* [1]. Since then, L-asparaginases have become widely known for their antileukemic properties and classified into five types, based on their biochemical and structural features [2]. A few years ago, a new type of L-asparaginases was discovered in *Rhodospirillum rubrum* [3] (Fig. 1), closely related to the enzymes of types I and II (the latter being currently used in therapy). The new L-asparaginases were shortened forms of the aforementioned proteins and not only did they retain their enzymatic activity, but also turned out to have new interesting properties, such as the ability to enter cancerous cells and to inhibit their telomerase in a yet unknown manner [4].

In the presented research, artificial "short" forms were constructed based on the most important type I L-asparaginases. The enzymes were expressed heterologously in *Escherichia coli*, purified and subject to kinetic measurements and thermal stability evaluation with NanoDSF. Three-dimensional structures of the novel enzymes were predicted with AlphaFold3.

Short forms of type I L-asparaginases expressed well in *E. coli*, however, they also exhibited a tendency to aggregate and remain in the insoluble fraction. Nevertheless, produced proteins were able to retain their enzymatic activity. Thermal stability of the short forms was usually lowered in comparison to their counterparts incorporating a full L-asparaginase domain. The results indicate that it is vital that short L-asparaginases be investigated as they shed a light on the mechanism of catalysis of their full counterparts and potentially constitute a novel group of anticancer agents.

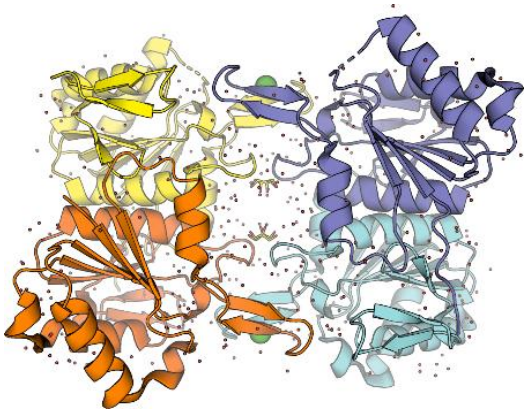

Fig. 1. Structure of short L-asparaginase from *Rhodospirillum rubrum* (PDB ID: 1UOO)

ACKNOWLEDGMENTS

Work funded through the programme "Excellence Initiative – Research University" at Jagiellonian University.

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Mass spectrometric analysis of thermal degradation products of betacyanin pigments obtained from *H. polyrhizus* fruit extract

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Keywords: betalains, betacyanins, thermal degradation, spectrometry, *H. polyrhizus*

Betalains belong to a group of nitrogen-containing organic compounds. They are natural hydrophilic pigments, which are divided into yellow-orange betaxanthins and red-purple betacyanins. Due to their labile nature, they are sensitive to environmental conditions, including temperature, pH value, and light. Under unfavorable conditions, betacyanins undergo degradation through decarboxylation and dehydrogenation reactions [1].

The process of thermal degradation was conducted on one-step or two-step purified extracts from the fruits of *Hylocereus polyrhizus*. The first purification step involved the use of column chromatography with a weak anion exchange (WAX). Part of the resulting eluate was further purified using a silica-based ODS column. The eluates obtained in this way were subjected to thermal degradation at 70°C in the presence of three different buffers: acetate (pH range 3–5), citrate (pH 3–6), and phosphate (pH 6–8). The heating process was carried out using 96-well plates placed in a laboratory oven. The experiment lasted 3.5 hours, with samples taken every 30 minutes for analysis.

Based on analyses performed by liquid chromatography combined with diode array detection and tandem mass spectrometry with electrospray ionization (LC-DAD-ESI-MS), decarboxylated and dehydrogenated forms of the dominant betacyanin – betanin and its derivatives: phyllocactin and hylocerenin, were identified as products of thermal degradation. These included, among others: 17-decarboxy-betanin, 2,17-bidecarboxy-phyllocactin, and 2-decarboxy-xanphyllocactin.

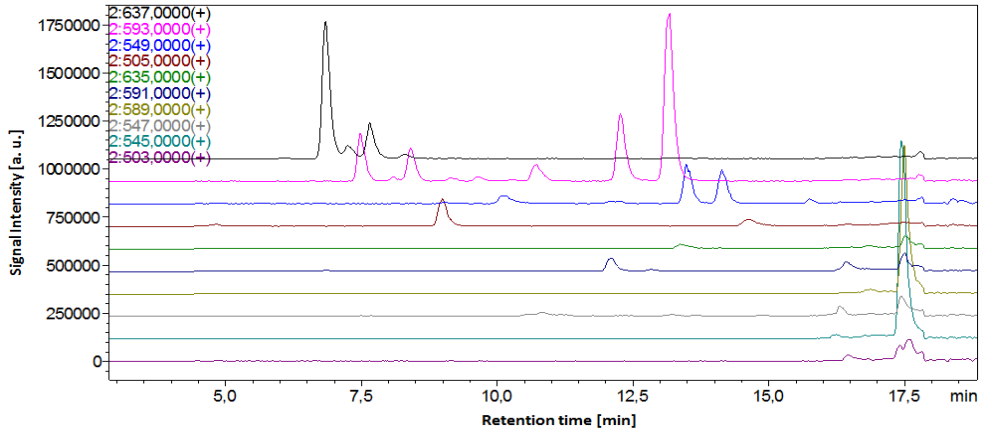

Fig. 1. Chromatogram of selected ions monitored with LC-MS technique in the SIM (Selected-Ion Monitoring) mode for the phyllocactin derivatives from the heated purified fruit extract of *H. polyrhizus*

ACKNOWLEDGMENTS

The research was funded by the National Science Centre (NCN) under the project contract number: 2021/41/N/NZ9/03046 (for the years 2022-2026).

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Induced Circular Dichroism (ICD) of amyloid fibrils functionalized with dyes

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Keywords: amyloid fibrils, chirality, chirality induction, circular dichroism, supramolecular systems

Amyloid fibrils are mostly associated with neurodegenerative diseases, however due to their interesting properties (e.g., stability, stiffness, strength) the research interest has also focused on the possible application of fibrils as nanomaterials. Although various amyloid-forming proteins differ in sequence, the resulting fibrils share common features, such as hierarchical organization, *cross-β* pattern and binding of amyloid-specific dyes [1]. However, amyloid fibrils exhibit remarkable structural diversity referred to as the structural polymorphism, which is manifested with various morphologies, depending on the number and arrangement of protofilaments [2]. Interesting aspect of fibrils, that also adds to their complexity, is their chirality, which is exhibited on different levels of fibrillar architecture starting from amino acids up to the mesoscopic organization [3].

Here we investigated the supramolecular systems consisting of amyloid fibrils and simple organic dyes using Electronic Circular Dichroism (ECD). For fibrils and dyes alone CD signal in the visible range is not observed. However, when non-chiral dyes interact with chiral structure of fibrils the Induced CD (ICD) signal in visible range is observed due to formation of supramolecular assemblies and chirality induction in organic molecules. To better understand the obtained systems ECD in the far-UV region and Vibrational Circular Dichroism (VCD) measurements were also carried out. ECD in the far-UV region is used to characterize the secondary structure of the protein aggregates, while VCD allows to study chirality of protofilaments [3].

The obtained results show that fibrils prepared from different proteins (hen egg white lysozyme, human lysozyme, insulin) and obtained under different conditions (agitation, NaCl) bind the dyes differently and exhibit variabilities in ICD signal. These studies show that ICD of amyloid-bound dyes may be a valuable method for distinguishing different types of amyloid fibrils as well as elucidating the complexity of fibrils' structure. Moreover, observation of the ICD indicates the possibility of generating Circularly Polarized Luminescence (CPL) from such systems, which can in the future be the basis for the design of CPL-active materials for applications in optoelectronics.

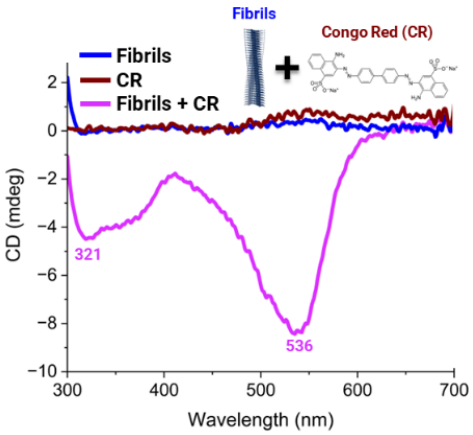

Fig. 1. ICD spectra of fibrils functionalized with Congo Red (CR), CR and fibrils alone

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This research has been funded by the program "Excellence Initiative – Research University" at the Jagiellonian University (project no. WSPR.WCh.1.10.2024.44).

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Media composition as a crucial factor influencing the growth and EPS profile of *Limnospira* sp.

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Keywords: cyanobacteria, exopolysaccharides, growth curves

Biotransformation performed by cyanobacteria provides an optimistic and sustainable route for obtaining target products, as these photoautotrophic organisms utilize carbon dioxide as a carbon source and enable, in situ, cofactor regeneration. Since, they combine photo- and biocatalysis, which is especially valuable in a field of green chemistry and sustainability. One potential application of their enzymatic systems can be the biotransformation of cinnamic acid, what can result in obtaining products of antioxidant properties, e.g., caffeic acid. Additionally, according to zero waste philosophy, the post-biotransformational biomass could be used in processes such as bioremediation and/or biostimulation of plants, where exopolysaccharides (EPS) play a major role in metal ion sequestration and in protecting plants from biotic and abiotic stresses.

The study aims to determine optimal conditions for the biotransformation of cinnamic acid by preliminarily chosen cyanobacterial catalysts *Limnospira maxima* and *Limnospira indica*, as well as establishing the EPS profile and concentration depending on the cultivation media and process duration. *L. indica* and *L. maxima* were cultured on Spirulina medium (SM) - medium designated for those species, yet where cinnamic acid is unstable, as well as on BG-11 medium commonly used for cyanobacteria growing under stationary conditions and on rotary shaker under controlled conditions (temperature: 30°C, light: 47.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with access to carbon dioxide). Over a 21-day period, 1 mL samples were aseptically collected daily from triplicate cultures, and cell growth was monitored spectrophotometrically via absorbance measurements. EPS concentration was determined via the phenol-sulphuric assay. Preliminary results indicate the most suitable time for cinnamic acid addition. The observed EPS trends also offer a basis for exploring biomass valorization strategies. It is imperative to optimise cyanobacterial cultivation conditions in the early stages of biotransformation studies to maximize both catalytic performance and later industrial applications.

Organic emitters for light-emitting electrochemical cells (LECs): synthesis, physicochemical properties and application prospects

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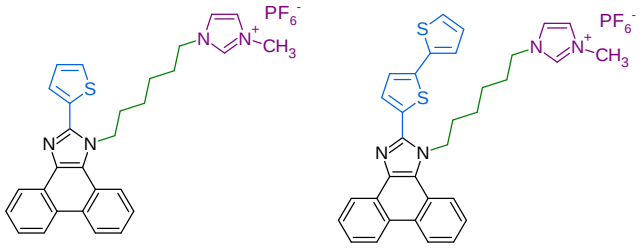
Keywords: phenanthroimidazole derivatives, electroluminescence, light emitting devices

The increasing number of electrical devices is associated with a growing demand for electricity, which poses significant challenges for modern technology in terms of its efficient generation, transmission, and utilization. In response to these needs, there is a growing interest in designing energy-saving solutions such as light-emitting electrochemical cells (LECs) [1–2]. In recent years, phenanthro[9,10-d]imidazole derivatives have attracted considerable attention as active materials in such devices. These compounds are characterized not only by favorable physicochemical properties but also by simple and cost-effective synthesis [3].

The aim of this study was the synthesis and physicochemical characterization of new phenanthro[9,10-d]imidazole derivatives that can be emitters in LEC devices (Scheme 1). The obtained compounds differ in their substituents at the C2 position – 2-thiophene and 2,2′-bithiophene groups were introduced. The synthesis involved a condensation reaction, followed by alkylation, and a final condensation step. The synthesized derivatives were subjected to thermal, optical, and electrochemical property analyses. Based on the results, the influence of the C2 substituent type on the physicochemical properties of the compounds was evaluated, allowing for a preliminary assessment of their suitability as active materials in LECs. The collected data represent a significant contribution to the development of LEC technology, enhancing the potential of these devices for use in modern lighting systems and consumer electronics.



Scheme 1. The obtained phenanthro[9,10-d]imidazole derivatives investigated for application as emitters in LEC devices

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This work was supported by Competition No. 15 for financing scientific activities in the Doctoral School – edition October 2024.

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Cellulose nanocrystals with tunable hydrophobicity for the separation of fine mineral particles

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Keywords: cellulose nanocrystals, flotation, flocculation, fine particles

Aggregation processes have significant industrial importance, enabling the separation of different phases, particularly solids from liquids. These processes are especially crucial in systems used for water purification and mineral processing, where it is necessary to separate valuable metal ore components from gangue. Destabilization mechanisms primarily rely on the adsorption of chemical substances at phase interfaces, which modify the physicochemical properties of their surfaces. Typically, aluminum and iron salts (coagulants), synthetic polymers, and surfactants are employed for this purpose. Despite their high efficiency, these substances are not environmentally benign and can significantly affect the well-being of living organisms. Consequently, extensive research has been focused for many years on developing environmentally friendly substitutes.

This study proposes a novel approach utilizing amine-functionalized cellulose nanocrystals (ANC) as flocculants for fine particles. ANC were synthesized through a two-step procedure involving cellulose oxidation using sodium periodate, followed by reductive amination with aliphatic amines (methylamine, n-butylamine, and n-hexylamine) in the presence of 2-picoline borane as a reducing agent.

To precisely control the degree of hydrophobicity, and therefore the extent of amine substitution, mathematical models were developed. These models allowed for the prediction of how critical reaction parameters, such as the quantity of sodium periodate, reaction time, and temperature, influence aldehyde content – an essential parameter for the second stage of cellulose chemical processing.

The proposed methodology, enabled the preparation of nanomaterials exhibiting a water contact angle ranging from approximately 50° to 100°, with controlled increments of hydrophobicity of about 6.6°.

Analysis of phosphorus, copper, and thallium content in milk available on the polish market

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Keywords: multi-element analysis, ICP-OES spectrometry, microwave-assisted mineralization, milk contamination, heavy metals

The aim of the research was to assess the potential contamination of cow's milk available on the Polish market with phosphorus, copper, and thallium. These elements were selected due to their diverse impacts on human health: phosphorus as a macroelement, copper as a microelement, and thallium as a toxic heavy metal with high bioaccumulation capacity [1]. Thallium, even at trace levels, is acutely toxic to humans. It causes a number of different symptoms, such as ulcers, alopecia, polyneuropathy, internal bleeding, heart muscle damage, or even death. Copper, though essential, may also become toxic at elevated levels, leading to liver damage and neurodegenerative effects. Regular consumption of milk containing these metals – even at low levels – poses a risk, particularly to children and individuals with compromised detoxification systems [2].

Five milk samples from different producers were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES) following microwave-assisted acid digestion. Phosphorus, copper, and thallium contents were quantified, and the results were statistically processed: normality and variance homogeneity tests were conducted to determine the appropriate post hoc analyses.

Phosphorus concentrations ranged from 952.5 to 1015 mg/kg and were comparable to the results reported in the literature. Copper levels were within safe limits in four brands, but for one brand, the concentration exceeded the reference values by up to tenfold, averaging 0.504 mg/kg. Thallium was detected in all samples at concentrations significantly exceeding historical and literature values, with some measurements reaching up to 0.435 mg/kg – over nineteen times higher than previously reported results.

The phosphorus content in the tested milk was within acceptable ranges, indicating no abnormal enrichment. Copper concentrations were generally safe, although a concerning anomaly was identified in one brand. The presence of elevated thallium across all samples is alarming and suggests contamination. This study highlights the urgent need for routine monitoring of both essential and toxic elements in dairy products to ensure food safety. Particular attention should be paid to thallium, given its severe toxicity. Further research should focus on identifying sources of contamination and evaluating the potential health risks associated with chronic exposure to trace thallium through dietary intake.

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Utilization of potato wastewater and sugar beet molasses for the production of killer yeast biomass *Wickerhamomyces anomalus*

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Keywords: *Wickerhamomyces anomalus*, biomass, biocontrol, potato wastewater, sugar beet molasses

Wickerhamomyces anomalus yeast are microorganisms with significant biotechnological potential, particularly in combating fungal plant pathogens. Their effectiveness stems from various mechanisms of action, including competition for nutrients, biofilm formation, production of volatile compounds, hydrolytic enzymes, and killer toxins harmful to other microorganisms. Thanks to these properties, W. anomalus effectively inhibits the growth of pathogens such as *Botrytis cinerea*, *Fusarium oxysporum*, and *Aspergillus flavus*, both under laboratory conditions and in practice [1, 2].

In this study, potato processing wastewater – rich in nitrogen and minerals – along with beet molasses as a carbon source, were used for the cultivation of *W. anomalus* biomass. The process was optimized using the fractional factorial design 3(k−p), analyzing the impact of pH (4, 5, 6), temperature (16, 22, and 28°C), and molasses concentration (10, 15, 20% w/v) on yeast growth efficiency. After determining the optimal conditions, the process was scaled for bioreactor culture, confirming the feasibility of effective biomass production on a pilot scale.

The obtained results demonstrate that the use of inexpensive, waste-derived raw materials from the agri-food industry can serve as an effective and environmentally friendly alternative to conventional media, supporting the development of sustainable technologies aligned with the principles of a circular economy.

ACKNOWLEDGMENTS

The research was financially supported by the project VEFAF - Designing a versatile and environmentally friendly alginate-based fibre platform and assessing the impact on the soil environment (Project OPUS 25 UMO-2023/49/B/NZ9/00959) funded by the National Science Center (NCN).

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Molecular docking: predicting interactions between vitamin B12 derivatives and transport proteins

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Keywords: vitamin B_{12}, Pt(II) complexes, transport proteins, Troyan horse drug delivery, molecular docking

Designing drug carriers via molecular docking represents a contemporary approach. It enabled the simulation and prediction of interactions between transport proteins and carrier structures at the molecular level, thereby facilitating the identification of suitable compounds to enhance both the efficiency and precision of therapeutic delivery. The increasing incidence of resistance to conventional chemotherapy and the systemic toxicity associated with non-selective drug distribution have underscored the need for novel targeting strategies in oncology. Vitamin B_{12} (cyanocobalamin) was selected as a carrier scaffold owing to its natural transport pathway, which involves sequential binding to intrinsic factor in the gastrointestinal tract and to transcobalamin I and II in the bloodstream, culminating in receptor-mediated cellular uptake via the TCblR/CD320 receptor – a mechanism that has been shown to be upregulated in many cancer cell types [1–3].

In this study, the feasibility of conjugating cyanocobalamin with a panel of platinum(II) complexes was examined *in silico* to determine whether such bioconjugation could preserve or enhance transporter recognition while delivering a proven anticancer payload. The complexes selected included the classical cytostatics cisplatin, heptaplatin, and oxaliplatin, as well as the next-generation agent picoplatin, which is currently undergoing clinical evaluation for improved efficacy and reduced off-target toxicity.

Molecular docking simulations were performed using AutoDock 4.0 to predict binding affinities and optimal conformations of both native and modified B_{12} derivatives with the three transport proteins. Key interactions -hydrogen bonds, van der Waals contacts, and π-stacking - were mapped and compared across the series of conjugates. Advanced visualization and interaction analysis were conducted with Discovery Studio Visualizer, which allowed for detailed examination of steric compatibility and interaction energy profiles.

These findings confirmed that cyanocobalamin derivatives could be structurally engineered to carry platinum(II) drugs without significant impairment of transporter recognition. The strategy outlined herein provides a rational framework for the design of targeted B_{12}–drug conjugates and supports the prioritization of lead compounds for subsequent *in vitro* assays and *in vivo* efficacy studies. Subsequent efforts will involve the synthesis of the most promising conjugates, followed by experimental binding studies with the transport proteins and cellular assays using cells overexpressing the TCblR/CD320 receptor.

ACKNOWLEDGMENTS

The study was supported by National Science Centre (Poland) under the grant 2019/35/B/ST4/04266.

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Surface modification of polyethylene by cyclodextrin grafting: application to bioactive cosmetic packaging

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Keywords: microbial contaminations, health risks, preservatives, surface modification, β-cyclodextrin

Microbial contamination remains a major concern in the cosmetics, food, and pharmaceutical industries, potentially compromising product quality and posing risks to human health. Traditionally, manufacturers have relied on preservatives such as parabens to prevent bacterial and fungal growth. However, growing concerns regarding the potential health risks associated with parabens [1, 2] – including their suspected links to endocrine disruption, infertility, and certain cancers – have prompted regulatory bodies to limit their use in consumer products. In response, alternative strategies are being explored to prevent microbial contamination without relying on chemical preservatives.

In this context, our study investigates an innovative approach to develop antimicrobial cosmetic packaging through surface modification of polyethylene (PE). The goal is to endow PE surfaces with antibacterial properties by immobilizing β-cyclodextrin (β-CD), a cyclic oligosaccharide derived from the enzymatic degradation of starch [3], which can encapsulate antibacterial molecules such as thymol, eugenol, and carvacrol.

To achieve this, PE surfaces were first activated via UV/ozone treatment to introduce oxygen-containing functionalities. Subsequent surface functionalization was performed using three strategies: (i) grafting of a phosphonic acid bearing a carboxylic acid group followed by dihydrazide linkage, (ii) use of an aminophosphonic acid, and (iii) application of bifunctional linker molecules. These treatments allowed for the subsequent immobilization of modified β-cyclodextrin.

Surface modification and functionalization were characterized using Toluidine Blue O (TBO) titration, Fourier--Transform Infrared Spectroscopy in Attenuated Total Reflectance mode (ATR-FTIR), Atomic Force Microscopy (AFM), contact angle measurements, and X-ray Photoelectron Spectroscopy (XPS). Successful functionalization was confirmed by the detection of phosphorus on the PE surface via XPS after treatment with 6-aminohexylphosphonic acid. Preliminary results also indicate the feasibility of β-CD immobilization.

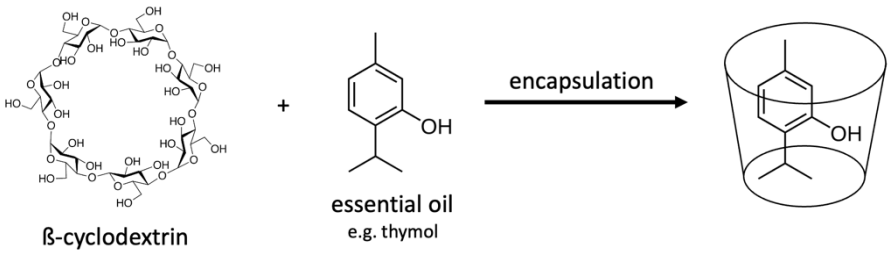

Fig. 1. Surface modification strategy

ACKNOWLEDGMENTS

This work was supported by Normandie Université, the Région Normandie, the Centre National de la Recherche Scientifique, Université de Rouen Normandie and the graduate school for research XL-CHEM.

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Biodegradation of 2-phenylethanol by *Beauveria brongniartii* DSM 6651

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Keywords: biotransformation, biocatalysis, fungi, *Cunninghamella* sp., antioxidants

Fungi of the genus *Beauveria* exhibit a wide range of enzymatic activities. These entomopathogenic fungi are well-known biocatalysts for producing hydroxylated biotransformation products [1]. However, their role is not limited only to the biotransformation processes. Another potential application involves the biodegradation of aromatic compounds found in the environment. The ability to degrade such pollutants is particularly desirable due to the continuously expanding pharmaceutical and chemical industries, which contribute to the increasing release of drug components and chemical waste into ecosystem and leads to environmental contamination [2].

In the present study, *Beauveria brongniartii* DSM 6651 was used to biodegrade the aromatic compound, 2-phenylethanol. During the research, biomass immobilized in agar-agar and calcium alginate was used to carry out 7-day biodegradation processes. The experiments were conducted both on a laboratory scale in flask system and half-preparative scale using a stirred batch bioreactor (capacity of 1 L) and a simplified model of flow bioreactor. Gas chromatography was employed to monitor the progress of the biodegradation process.

At the laboratory scale, depending on the immobilization method, complete degradation of the substrate was achieved within 24 to 48 hours (>99%). The process was subsequently scaled up. At the half-preparative scale, in both systems (simplified flow bioreactor model and a stirred batch bioreactor), complete degradation of 2-phenylethanol was achieved after 48 hours (>99%).

The studies carried out clearly show that, the selected strain, *B. brongniartii* DSM 6651, exhibits high degradation activity and a rapid process rate, achieving a degradation yield of over 99% within 24 hours. This strongly suggests its potential for future applications in the degradation of aromatic compounds. As the biodegradation capabilities of this fungal species have not yet been extensively described in scientific literature [3], further research is crucial. *B. brongniartii* DSM 6651 could prove to be an effective and environmentally safe tool for the bioremediation of aquatic and terrestrial ecosystems, especially for a variety of aromatic compounds.

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Engineering yeast cell factories for high-yield curcumin production: role of YML131W

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Keywords: curcumin biosynthesis, *Saccharomyces cerevisiae*, metabolic engineering, synthetic biology, CRISPR-Cas9

Curcumin, a bioactive compound with antioxidant, anti-inflammatory, and anticarcinogenic properties, is a promising natural product for therapeutic applications. However, its extraction from plant sources is hindered by seasonality, the use of harsh chemicals during purification, and typically low purity yields. As an alternative, microbial production of curcumin via genetically engineered microorganisms has gained attention in recent years. *Escherichia coli* has been widely used as a production host [1], but its non-GRAS (Generally Recognized As Safe) status may limit its use in the pharmaceutical industry. In contrast, the yeast *Saccharomyces cerevisiae*, a GRAS organism with a well-characterized genome and advanced genetic tools, offers a promising platform for curcumin biosynthesis. Hereupon, an engineered *S. cerevisiae* strain capable of producing curcumin from glucose was developed by integrating an artificial biosynthetic pathway [1]. To improve production yields, the copy number of key pathway genes, such as caffeic acid *O*-methyltransferase (COMT) gene, was increased to enhance metabolic flux toward curcumin. During the production experiments, curcumin degradation was observed in the culture medium, suggesting possible autooxidation or degradation by endogenous yeast enzymes. A bioinformatic analysis using the Basic Local Alignment Search Tool identified an uncharacterized yeast protein (YML131W), with sequence similarity to *E. coli* curcumin reductase and potential oxidoreductase activity. To investigate its influence on curcumin synthesis, a CRISPR-Cas9-based strategy was employed to delete *YML131W*. Additionally, a dual-editing strategy was designed to simultaneously delete *YML131W* and integrate an extra copy of the COMT gene. Curcumin production was evaluated in defined media. The parental strain (JQ4) produced 1.7 ± 0.13 mg/L of curcumin. Deletion of YML131W in strain JQ4 (JQ4 ΔYML131W) increased production to 3.0 ± 0.54 mg/L, an approximate 1.8-fold improvement. The double-edited strain (JQ4 YML131W::COMT) also achieved a similar production level (3.1 ± 0.16 mg/L), suggesting that an extra copy of COMT is not beneficial. Concluding, the uncharacterized YML131W deletion had a positive impact in curcumin production in genetically engineered *S. cerevisiae*, possibly preventing curcumin or a curcumin intermediate deviation and enhancing biosynthetic flux. However, the specific mechanism by which this gene affects curcumin biosynthesis remains unclear and requires further investigation. Furthermore, to enhance curcumin yields in yeast cell factories, additional strategies should be explored, particularly those focused on increasing the availability of endogenous key cofactors and precursor molecules, such as nicotinamide adenine dinucleotide (NADH) and malonyl-CoA, that remain unexplored.

ACKNOWLEDGMENTS

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/04469: Centre of Biological Engineering of the University of Minho, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

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Structural assessment of thermally treated Nb_2O_5 catalyst for reuse in biofuel production

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Keywords: niobium pentoxide, catalyst reuse, XRD analysis, drop-in fuel, thermal treatment

This study aimed to evaluate the structural modifications of the Nb_2O_5 catalyst induced by thermal treatment, with the objective to explore its potential for reuse in thermochemical processes for biofuel production. The interest for more efficient and selective catalysts for renewable fuel production has gained increasing relevance in meeting growing net energy demands. However, the reuse of such catalysts remains an underexplored area in current research. Developing strategies that enable the reutilization of these materials are crucial, considering their cost and their critical role in reaction pathways. In this context, the present work investigates the structural behavior of Nb_2O_5 after being subjected to deoxygenation reactions of vegetable oil. The spent catalyst was thermally treated by heating up to 500°C at a rate of 5°C/min. X-ray diffraction (XRD) analyses, conducted within a 2θ range of 5° to 50°, revealed a significant increase in the intensity of crystalline peaks, particularly between 22° and 28°, alongside a reduction in background noise following thermal treatment, as shown in Fig. 1. These results suggested enhanced crystallinity and potential structural purification of Nb_2O_5, with no evidence of phase transformation or collapse in comparison with the original framework. The observed thermal stability indicated that Nb_2O_5 is a structurally robust catalyst, capable of preserving its integrity under severe conditions, thereby supporting its feasibility for reuse in thermochemical biomass conversion routes toward drop-in fuels. Future work will focus on detailed investigation of the catalyst's chemical structure and porosity, aiming to improve the understanding of its physicochemical properties and to optimize its catalytic performance.

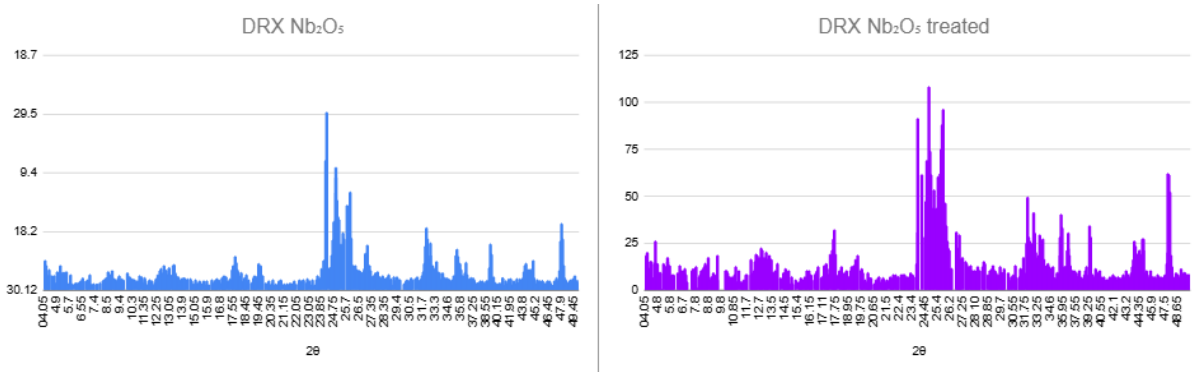

Fig. 1. Comparative X-ray diffraction (XRD) diffractograms of Nb_2O_5 samples:
(a) catalyst before reaction and (b) catalyst after thermal treatment at 500°C

ACKNOWLEDGMENTS

This work was supported by the Research Support Foundation of the State of Rio de Janeiro (FAPERJ) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Finance Code 001). We also acknowledge the Catalysis Laboratory at UFRRJ for providing access to their facilities and technical support during the XRD analyses.

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Production and biochemical characterization of recombinant β-galactosidases 1015 and 1039 from *Paenibacillus* sp. isolated from honey

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Keywords: β-Galactosidase, honey, *Paenibacillus*, oligosaccharides

This study aimed to produce and characterize two recombinant β-galactosidases, 1015 and 1039, derived from *Paenibacillus* sp. isolated from honey. The enzymes were assessed for their hydrolytic properties and transglycosylation potential.

The Bgal_1015 and Bgal_1039 genes were cloned into pBAD vectors and expressed in *E. coli* TOP10. Expression was carried out at 30°C for 22 h followed by cells lysis by sonication. Cell free extracts were purified by ion exchange and gel filtration chromatography. Characterization of the enzyme was performed with ONPG as a substrate and involved determination of optimal pH and temperature, pH and thermal stability, substrate specificity, effects of sugars, metal ions, and reducing agents. Transglycosylation activity was evaluated via HPLC following incubation with lactose and various acceptor sugars.

Based on comparative analysis of conserved sequence motifs across enzymes, both Bgal_1015 and Bgal_1039 were assigned to glycoside hydrolase family 2 (GH2), a group of enzymes known for their β-galactosidase activity and inherent transglycosylation potential in addition to hydrolysis. The domain organization of both proteins includes an N-terminal jelly-roll sugar-binding domain, a catalytic TIM-barrel domain with conserved glutamate residues, and a C-terminal immunoglobulin-like β-sandwich domain. The molecular weight of each enzyme was estimated from its amino acid sequence, and their oligomeric states were determined by size exclusion chromatography. Bgal_1015 was identified as a tetramer with a molecular weight of 425 kDa, while Bgal_1039 formed a dimer of approximately 267 kDa. These structural features are consistent with their enzymatic profiles and support their functional classification within GH2.

Bgal_1015 exhibited maximal activity at 43°C and pH 7.29, and Bgal_1039 at 40°C and pH 7.77. Both enzymes retained high activity in a neutral to slightly alkaline pH range. β-Galactosidase 1015 retaining over 53% of its activity after 2 hours at 40°C and approximately 68% after 2 hours at 35°C, but showed rapid inactivation at 45°C, and after 1 hour, only 18% of its activity remained. The enzyme remained highly stable at pH 7.0–8, maintaining over 80% activity after 24 hours, while stability sharply decreased below pH 6.5. β-Galactosidase 1039 showed moderate thermal stability, maintaining over 85% activity after 2 hours at 30°C and 25% activity after 2 h at 35°C. Enzyme was inactivated rapidly at 40°C. The enzyme was most stable at pH 7.3 and 7.8, retaining over 67% and 92% of its activity, respectively, after 24 hours. The activity dropped significantly below pH 7.0. Both enzymes preferentially hydrolyzed PNP-β-D-galactopyranoside; Bgal_1039 also exhibited activity with PNP-β-D-glucuronide and PNP-β-D-fucopyranoside, while Bgal_1015 showed slight activity with PNP-β-D-fucopyranoside.

Enzymatic activity of both enzymes was enhanced by glucose and galactose, strongly stimulated by Na^+, Mg^{2+} ions, and slightly by K^+, Li^+ ions. Strong inhibition was observed in the presence of Cu^{2+}, Ni^{2+}, and Fe^{3+}. The reducing agents like DTT significantly enhanced Bgal_1039 activity (+87%) but reduced Bgal_1015 activity; TCEP, cysteine, and glutathione inhibited both enzymes.

Both enzymes demonstrated transglycosylation capability. Bgal_1015 produced galactooligosaccharides (GOS) from lactose and synthesized heterooligosaccharides (HOS) in the presence of trehalose, cellobiose, sucrose, and maltose as acceptors. Bgal_1039 also synthesized GOS and HOS in the presence of the same acceptors and melezitose. In both cases, the formation of novel oligosaccharide peaks confirmed enzymatic transglycosylation.

Recombinant *β*-galactosidases 1015 and 1039 from *Paenibacillus* spp. display complementary biochemical properties, including activity at moderate temperatures, pH stability, sugar-induced activation, and transglycosylation ability. Their capacity to synthesize GOS and HOS suggests potential for applications in the production of lactose-free and prebiotic-enriched food products.

Targeting SENPs: substrate profiling as a path to selective chemical tools

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Keywords: proteases, enzymatic activity, peptides, activity-based probes

SUMOylation is a post-translational modification (PTM) involving the covalent attachment of a small ubiquitin-like modifier (SUMO) protein to target proteins [1]. The reversibility and highly dynamic nature of this process result from the activity of SUMO conjugating and deconjugating enzymes [1]. SUMO-deconjugating enzymes (SENPs – sentrin specific proteases) are involved in numerous cellular processes in both normal and pathological states, including cancer and neurodegenerative diseases, making them attractive therapeutic targets [1, 2]. However, the precise role of individual SENPs has not been fully elucidated. Selective chemical tools could facilitate a better understanding of these functions and the mechanisms behind tumorigenesis.

One of the main challenges in assessing the activity of individual SENPs lies in their overlapping substrate specificity. In human, three main SUMO isoforms are targeted by six SENP proteases [2], thus native SUMO-based chemical tools cannot distinguish the activity of specific SENPs. A potential solution to overcome this issue is the C-terminal modification of SUMO using unnatural amino acids. This approach was developed in our team and was successfully applied to create selective chemical tools for assessing the activity of deubiquitinating enzymes (DUBs) [3]. A similar approach appears promising for SENP-related research.

To determine the substrate specificity profiles of individual SENPs Hybrid Combinatorial Substrate Libraries (HyCoSuLs) were designed and synthesized. Based on the obtained SENP substrate preferences, optimal amino acid sequences will be selected for further studies aimed at developing potent and selective tools – fluorogenic substrates and activity-based probes (ABPs) – for the assessment of SENPs activity.

ACKNOWLEDGMENTS

This project is financially supported by the National Science Center grant 2021/41/B/ST4/02789 (OPUS-21) in Poland.

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Exploring morphological links between wild macedonian cannabis and some registered varieties

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Keywords: *Cannabis sativa* L., varieties, UPOV, landraces, machine learning

This study aims to analyze the morphological characteristics of 5 populations of cultivated wild-growing *Cannabis sativa* L. from Macedonia. in comparison to 38 registered commercial varieties and four strains, using UPOV (International Union for the Protection of New Varieties of Plants) descriptors. Access to detailed morphological data on registered cannabis varieties remains highly restricted due to UPOV Convention protections. Within the European Union, only limited datasets, such as that of the Estonian variety Estica, are publicly available. Conversely, Australia maintains transparent varietal documentation through IP (Intellectual property) Australia, providing a unique opportunity for comparative analysis based on UPOV guidelines.

Morphological expression states of registered Cannabis varieties, reported in accordance with UPOV guidelines, were extracted from official journals. These were combined with field-collected data from wild and commercial cannabis samples that were cultivated outdoors. Multivariate statistical analyses were conducted, including Principal Component Analysis (PCA), B.

PCA revealed that two principal components captured 20.20% of variability (R^2X = 0.20, Q^2 = 0.04), while five components increased variance explained to 40.75% but significantly reduced predictive strength (Q^2 = –0.076). The two PCA components were best explained by variables such as seed coat color, plant height, main stem color, 1000-seed weight, leaf anthocyanin coloration, and cotyledon color, whereas the five-component PCA model additionally included anthocyanin coloration of male inflorescences, number of leaflets per leaf, seed marbling, and intensity of green color of leaf. HCA and subsequent PLS-DA modeling identified clear clustering patterns, with wild Macedonian cannabis aligning closely with established varieties such as Kompolti and CHA. Kompolti, a Hungarian sativa landrace and the first registered cannabis variety in Europe (1952), emerged as a key comparator. However, inconsistencies between its UPOV-classified descriptors and recent Australian comparative data suggest genetic drift, loss of varietal stability, or differences in germplasm sources over time. A new draft UPOV guideline (TG/276/2 proj.1, 2022) increases the number of descriptors from 25 to 32 and categorizes varieties by intended use, indicating an evolving standard.

Our findings provide evidence that Macedonian wild-growing cannabis exhibits morphological affinities with historically significant European landraces, positioning it as a valuable, yet underutilized, genetic resource. These results underline the need for better international standardization and accessibility of cannabis varietal data, critical for DUS testing, registration, and the protection of global cannabis biodiversity. Future research may focus on integrating molecular data to reinforce the morphological classification and to support DUS testing procedures for potential new variety registration.

Synthesis and characteristics of metbalamins

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Keywords: cobalamin, antivitamins, transmetalation, new antibiotics

Antivitamins – synthetic analogs of vitamin B_{12} (cobalamin, Cbl) – can be designed to competitively bind to the enzyme or its associated receptor, disrupting bacterial metabolism [1]. Research into antivitamins B_{12} explores compounds that interfere with the biological functions of cobalamin, offering insights into their potential as inhibitors. This study aims to synthesize and characterize metbalamins (Metbls) – group III of vitamin B_{12} analogs with modifications at the metal center – as candidate antivitamin compounds. Metbalamins are synthesized using metal-free ligands, such as Hby (hydrogenobyric acid) and Hbl (hydrogenobalamin) as a starting material, which were primarily obtained only through biotechnological methods [1]. However, it is possible to obtain a metal-free template through purely chemical synthesis routes, resulting in diol derivatives [2].

In this research, metbalamins were prepared via three-step transmetalation of vitamin B_{12}. The first step is photooxygenolysis involving cleavage of the corrin macrocycle, which results in the formation of dioxosecocobyrinates. Subsequently, in a one-pot procedure, the cobalt ion was reduced and, in the presence of excess of KCN, the metal center was eliminated with simultaneous coupling of the two carbonyl units. Finally, the insertion of the metal was achieved through a reaction between diol derivative of hydrogenobalamin and an appropriate salt, such as the acetate or sulfate. Structural confirmation and characterization of products was performed with the use of high resolution mass spectrometry (HRMS) and Nuclear Magnetic Resonance (NMR). Moreover, IR, UV-Vis and fluorescence spectroscopy were applied to verify functional groups and electronic features. Further work will involve in vitro enzymatic assays targeting B_{12}-dependent bacterial enzymes such as propanediol dehydratase (PduC) and ethanolamine ammonia-lyase (EAL) to assess binding affinity and inhibitory effects – assuming metbalamins may compete with native cobalamin in enzyme active sites leading to reduced catalytic activity.

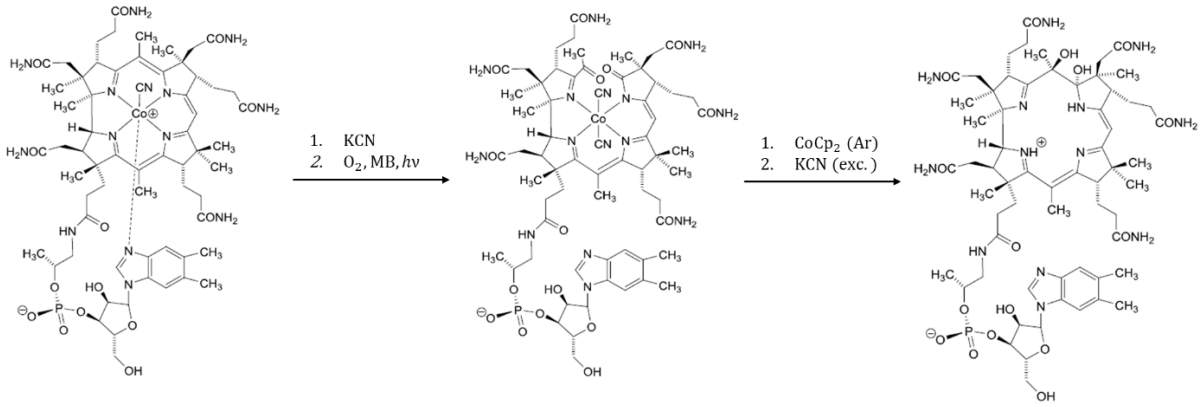

Fig. 1. Chemical demetalation procedure for vitamin B_{12} [2]

ACKNOWLEDGMENTS

This research was funded by grant 2019/35/B/ST4/0426 under NCN project OPUS 18.

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Biomass and cannabinoid production in cannabis' cell suspension cultures: a biotechnological approach

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Keywords: biomass yield, *Cannabis sativa*, cannabinoids, cell suspension cultures, phytohormones

Cannabis sativa L. has gained increasing scientific interest due to its valuable secondary metabolites, particularly cannabinoids, which can be produced in controlled *in vitro* systems. Compared to the traditional field cultivation, *in vitro* culture offers several advantages, including year-round production, precise control over enviromental and nutritional factors, independence from geographic and climatic limitations, and the potential for cannabinoid production under sterile, pathogen-free conditions.

The aim of this study was to investigate the effect of different concentrations of phytohormones on the production of biomass and cannabinoids in *in vitro* cell suspension cultures of Cannabis.

Callus cultures were induced on MS/B_5 medium [1, 2] in the presence of the cytokinin thidiazuron (1.0 $mg \cdot L^{-1}$ TDZ) and auxin 1-naphthaleneacetic acid (0.5 $mg \cdot L^{-1}$ NAA) and subsequently used to initiate cell suspension cultures on medium (without agar) supplemented with the same phytohormone combination. Cultures were maintained on a rotary shaker (120 rpm) under controlled aseptic conditions for 35 days. Lyophilized cell suspensions were extracted, and cannabinoids were quantified using the DAB method for Cannabis flos [3], with results expressed as $\mu g \cdot g^{-1}$ dry extract. According to the obtained results, it was found that highest fresh biomass was obtained on the 28th day of the cultivation cycle (32.79 g), while the highest concentration of cannabidiolic acid (CBDA) and Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), (3.64 and 3.82 $\mu g \cdot g^{-1}$, respectively) were obtained on the 14th day of the cultivation period.

These results demonstrate a temporal discrepancy between peak biomass and maximum cannabinoid accumulation, highlighting the importance of optimizing harvest time to enhance secondary metabolite yield in cannabis cell suspension cultures.

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Utilisation of brewers spent yeasts for the production of vitamin B3 and protein

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Keywords: waste yeast, brewing industry, extraction, hydrolysis, vitamin B3 determination, sustainable brewing

Brewer's yeast is reused or processed into animal feed, fertilizer, biogas, dietary supplements, cosmetics, or food additives – supporting sustainability and circular economy in brewing. Utilizing waste yeast from the brewing industry, chemical compounds that can be extracted through the process of hydrolysis were studied. The yeast used in the research comes from the Wrocław University of Science and Technology brewery. The concentration of proteins and vitamin B3 was determined, with additional comparisons made to assess whether the use of ultrasound enhances these processes. The following analytical techniques were employed in the research: HPLC, FTIR, and protein quantification using the Lowry method. Hydrolysis was conducted over a period of 24 hours at a temperature of 20°C using 0.1 M solutions of the following compounds: sodium hydroxide, acetic acid, phosphoric acid(V), and hydrochloric acid. The objective was to evaluate which of these solutions exhibits the highest efficiency in the isolation of biologically derived materials, and whether yeast cell disintegration via ultrasound improves the extraction yield. The results indicate that the use of ultrasound is beneficial in both increasing the concentration of proteins in solution and the yield of vitamin B3. However, for protein extraction alone, ultrasound is not a significantly enhancing factor. Among the samples not subjected to additional cell disintegration, the most effective was the one hydrolyzed with a 0.1 M solution of hydrochloric acid. The lowest efficiency in this group was observed in the aqueous solution – the control sample. For the samples treated with ultrasound, the highest extraction efficiency was achieved using a 0.1 M sodium hydroxide solution, which also yielded the best results in terms of protein and vitamin B3 concentration. In summary, the conducted research on the use of waste yeast from the brewing industry for the recovery of biologically derived raw materials demonstrates that each of the applied solutions has distinct characteristics. The samples hydrolyzed with phosphoric acid (V), both with and without ultrasound treatment, showed the highest density, although the density across all samples remained relatively similar. The 0.1 M sodium hydroxide solution proved most effective for protein isolation when ultrasound was applied. Without ultrasound, its efficiency decreased. The highest amount of vitamin B3 was obtained through hydrolysis using a 0.1 M solution of phosphoric acid(V). FTIR analysis revealed that, without additional cell disintegration, the most effective chemical compound extraction was achieved using a 0.1 M hydrochloric acid solution. When ultrasound was employed, the 0.1 M sodium hydroxide solution again proved to be the most effective. These findings support further development of methods for the valorization of waste from the brewing industry.

Strategies for improving the electrospinnability of alginate for agricultural applications

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Keywords: alginate, electrospinning, nanofibers

The constantly growing population leads to the intensification of agriculture. Traditional agriculture, which relies on synthetic fertilizers, causes significant environmental problems. Therefore, sustainable solutions to overcome those difficulties are urgently needed. The development of alginate matrices that act as carriers for microorganisms supporting plant growth such as *Wickerhamomyces anomalus* and *Azotobacter vinelandii* can be part of solving this significant problem. These matrices are composed of nanofibers produced via the electrospinning process. Nanofibers, due to their high surface-to-volume ratio and porosity are well-suited for incorporating microorganisms.

Sodium alginate (SA) is a biopolymer derived from brown algae. It is biodegradable, biocompatible, and non-toxic, which makes it a suitable material for agricultural applications [1]. The main challenge is to improve the poor electrospinnability of SA, which is connected to the polyelectrolyte character of SA and its lack of chain entanglements. Various strategies can be employed to address this issue, such as the addition of another polymer, glycerol, non-ionic surfactants or adjusting the storage time of the solutions. Moreover, optimizing electrospinning parameters, such as applied voltage, feed rate, and needle-to-collector distance is crucial for creating fine fibers [2].

The prepared solutions are evaluated in terms of viscosity, conductivity, and surface tension. The produced fibers are analyzed using Scanning Electron Microscopy (SEM) and compared based on their average diameters.

The first applied strategy is the addition of polyethylene oxide (PEO) to the SA solution. By optimizing only the process parameters, it was possible to increase the SA content from 31% to 67% in dried fibers. Moreover, the influence of glycerol and surfactant addition, and storage time of the solutions were also evaluated. The use of biopolymers such as SA has the potential in developing innovative products and technologies, making research in this area crucial for environmental protection and sustainable development.

ACKNOWLEDGMENTS

The work was financed by the National Science Centre (Poland) grant number 2023/49/B/NZ9/00959.

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Unbranched very-long-chain barriers: comparative flower and leaf wax profiles in *Hyacinthella leucophaea* and their early-spring ecological role

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Keywords: *Hyacinthella leucophaea*, wax constituents, alkanes, palmitone, GC-MS analysis

Cuticular waxes form the first physicochemical barrier between plants and their environment. In *Hyacinthella leucophaea* (K.Koch) Schur (Asparagaceae) – a spring-flowering geophyte that must withstand episodic frost, high irradiance, and early-season herbivory-these waxes were characterised from chloroform washings of fresh flowers and leaves collected in the Jelašnička Gorge (SE Serbia, March 2025). Gas chromatography-mass spectrometry revealed a flower wax dominated by unbranched very-long-chain (VLC) alkanes (48.7%, C_{23}, C_{25}–C_{32}) and ketones (41.4%, C_{29}, C_{31}–C_{33}), with 16-hentriacontanone (palmitone, 40.5%) and hentriacontane (26.3%) as signature constituents. By contrast, the leaf wax displayed a broader spectrum (36 identified compounds) in which VLC alkanes (43.7 %, C_{21}–C_{25}, C_{28}–C_{33}) were matched by primary alcohols (38.4%, C_{22}–C_{26}, C_{29}, and C_{30}), chiefly 1-tetracosanol (27.6%). Fatty-acid esters and aldehydes remained minor fractions in both organs, and branched homologues were below the detection limit (<0.1%), confirming the practical absence of methyl-branched chains (Fig. 1). When set against published data for other Asparagaceae geophytes, the palmitone-rich profile of the tepals agrees with that of *Hyacinthus* spp., whereas the alcohol-enriched leaf wax resembles *Muscari foliage*, underscoring organ-specific biosynthetic routing in the family. Ecologically, the high proportion of hydrophobic, crystalline palmitone is consistent with the need for rapid cuticular maturation in early spring, enhancing UV screening and limiting non-stomatal water loss during fluctuating temperatures, while the more flexible alcohol/ester matrix on leaves may facilitate expansion and gas exchange as the season progresses. In conclusion, *H. leucophaea* deploys two distinct but complementary wax chemotypes: a ketone-heavy, entirely unbranched barrier on flowers and an alcohol-augmented shield on leaves. This dichotomy, together with the near-absence of branched VLC lipids, enriches the chemosystematic picture of Asparagaceae and suggests that cuticular fine-tuning is a key adaptive trait for the plant's ephemeral spring lifestyle.

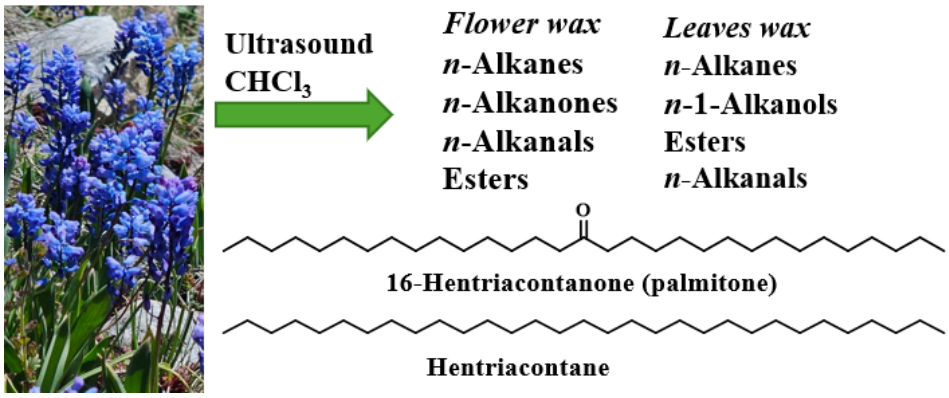

Fig. 1. Chemical composition of flowers' and leaves' washings of *Hyacinthella leucophaea*

ACKNOWLEDGMENTS

This work was supported by the Ministry of Science, Technological Development and Innovation of Serbia, grant numbers 451-03-136/2025-03/200124, 451-03-137/2025-03/200124, and 451-03-137/2025-03/200148 (SDG 15).

Exploring *Bacillus licheniformis* potential for electrospun biodegradable fibers in agricultural biopreparations

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Keywords: *Bacillus licheniformis*, electrospinning, agricultural biopreparations, antagonistic activity

In the era of searching for sustainable solutions in agricultural production, microorganisms with plant growth--promoting properties and the ability to inhibit pathogen development are gaining importance as components of next-generation biopreparation. *Bacillus licheniformis* is a soil bacterium with high biotechnological potential, known for its capacity to produce enzymes, antagonistic compounds, and substances that stimulate plant growth. A promising direction for its application is the immobilization within biodegradable fibers obtained via electrospinning, which enables the controlled delivery of microorganisms to the soil environment.

The aim of this study was to preliminary characterize the *Bacillus licheniformis* CCM 2145 strain in the context of its agricultural application, serving as a foundation for further work on its encapsulation and application in fiber form obtained by electrospinning.

The scope of the work included analyzing the nutritional requirements of the strain and selecting agro-industrial waste carbon sources (including molasses, waste glycerol, DDGS – distillers dried grains with solubles, and potato water) based on growth tests on solid media and flask cultures. Environmental tolerance tests were conducted to assess the effects of varying salinity, temperature, and the presence of heavy metals (Mn, Co, Cu, Cd, Pb) and to determine resistance to stress factors relevant to soil conditions. Antagonistic activity against the pathogenic filamentous fungus *Alternaria alternata* was evaluated, demonstrating significant inhibition of fungal mycelial growth *in vitro*. The final step comprised a preliminary assessment of cell viability following electrospinning with alginate carriers that were subjected to different sterilization methods.

The characterization of *Bacillus licheniformis* CCM 2145 demonstrated its ability to function under variable environmental conditions and its compatibility with carriers produced via electrospinning, which opens the way to its use in modern soil biopreparations.

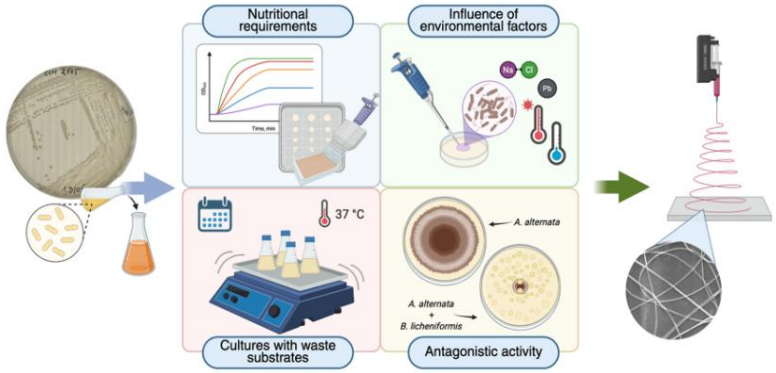

Fig. 1. Overview of the experimental workflow for assessing *Bacillus licheniformis* CCM 2145 as a candidate for biodegradable fiber-based agricultural formulations

ACKNOWLEDGMENTS

This research was funded by the National Science Centre, Poland, OPUS25 Grant Number 2023/49/B/NZ9/00959, *„Designing a versatile and environmentally friendly alginate-based fiber platform and evaluating the impact on the soil environment"*.

POSTER PRESENTATION SESSION

Natural-based hydrogels: synthesizing next-generation enzyme-hydrogel biocatalyst for wastewater treatment

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Keywords: hydrogels, laccase, biocatalyst, immobilization, wastewater treatment

The high susceptibility of free enzymes to harsh conditions like extreme temperatures, pH fluctuations, mechanical stress etc. is a major concern in utilizing enzymes for wastewater treatment. Much efforts have been deployed in terms of research to immobilize enzymes on suitable substrates as a biocatalyst for effective contaminant degradation, yet there is considerable room for improvement for environmental sustainability. Natural-based hydrogels represent a viable and environmentally friendly alternative to synthetic-based materials for immobilizing enzymes dedicated to wastewater treatment applications. In this field, laccase, a multicopper oxidoreductase, has great potential to degrade recalcitrant contaminants such as phenols and azo dyes, pharmaceuticals, endocrine-disrupting chemicals such as bisphenol A, etc. (Fig. 1). The presented research aims to combine the concept of using hydrogel-entrapped laccase as a novel platform to harness the potential of a renewable, environmentally friendly, naturally derived material to produce a high-value, environmentally functional biocatalyst for wastewater treatment.

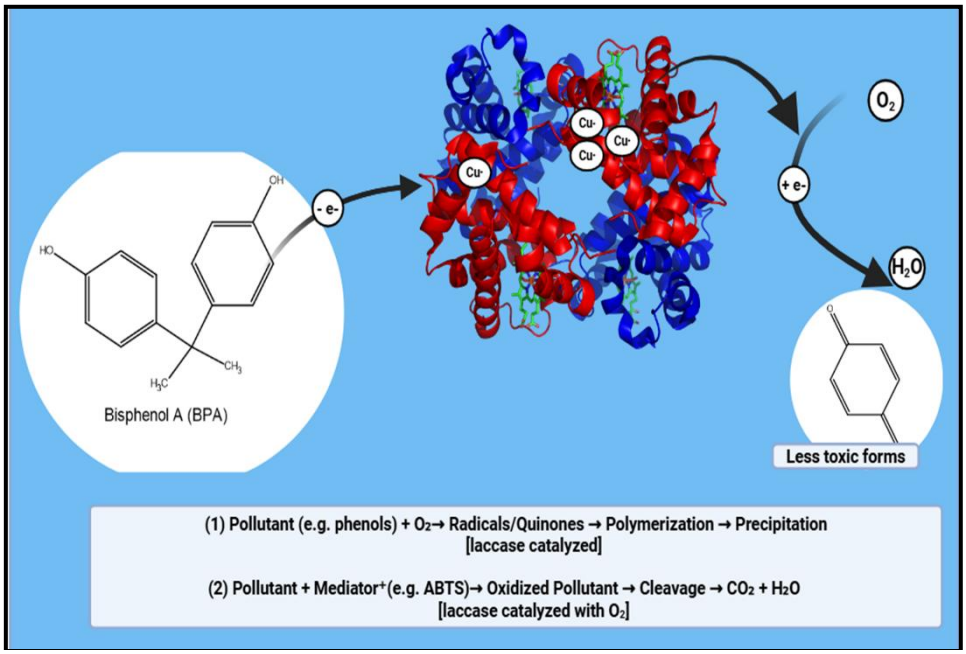

Fig. 1. Laccase degradative action on recalcitrant contaminant (Bisphenol A)

ACKNOWLEDGMENTS

This work is a core part of the master project of Oscar Amponsah under the supervision of Dr. Karolina Labus. Grateful to the Erasmus Mundus Joint Master program, Sustainable Biomass and Bioproducts Engineering, Project 101050789 Sus2BioEng, for financial support for their study and research.

Variation in chemical composition and anticholinesterase activity of the essential oil from *Cryptomeria japonica* foliage at different maturation stages

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Keywords: waste valorisation, *Cryptomeria japonica*, leaf age, chemical composition, cholinesterase inhibition

Cryptomeria japonica (Thunb. ex L.f.) D. Don (Cupressaceae), being widely cultivated in the Azores (Az) for landscaping and timber production, generates large amounts of waste, mainly foliage (Az–CJF). Currently, Az–CJF is the main source for local essential oil (EO) production, with application mainly as household aromatherapy diffusers. The present study aimed to obtain EO from mature leaves (CJML–EO) and shoots (CJS–EO), to investigate the influence of leaf age on the Eos' chemical composition and anticholinesterase activity.

Az–CJF sample was collected in May 2024 and separated into CJML and CJS samples. Essential oils (EOs) were extracted from each sample by hydrodistillation using a Clevenger-type apparatus for 3 hours. The chemical composition of the resulting EO samples was analysed by gas chromatography-mass–mass spectrometry (GC–MS), following the methodology described in [1]. Acetylcholinesterase and butyrylcholinesterase inhibition assays were performed according to [2].

The major compounds ≥2.5% found in CJS–EO and CJML–EO were: (i) the monoterpene hydrocarbons α-pinene (17.8% vs. 15.4%), sabinene (4.3% vs. 7.9%), limonene (5.6% vs. 3.7%), and γ-terpinene (3.1% vs. 1.1%); (ii) the oxygenated monoterpene terpinen-4-ol (9.8% vs. 3.3%); (iii) the oxygenated sesquiterpenes elemol (3.6% vs. 11.3%), and eudesmol isomers (23.0% vs. 10.4%); (iv) the diterpene hydrocarbon phyllocladene (9.8% vs. 15.9%); and (v) the oxygenated diterpene nezukol (0.1% vs. 2.7%). Thus, marked differences in the chemical profiles between the two samples were found, namely, the maturation process revealed a decrease in the component content, except for sabinene, elemol, phyllocladene, and nezukol. Neither of the EOs exhibited inhibitory activity against acetylcholinesterase. However, regarding anti-butyrylcholinesterase activity, the CJS–EO showed a stronger inhibition than CJML, with IC_{50} of 302 and 510 µg/mL, respectively.

This study demonstrates that the developmental stage of Az–CJF significantly influences both the chemical composition and anti-butyrylcholinesterase activity of its EO. The observed variation in biological activity is directly linked to changes in the oil's chemical profile, highlighting the importance of selecting specific leaf maturity stages for targeted applications.

ACKNOWLEDGMENTS

Filipe Arruda acknowledges the PhD scholarship (ref. M3.1.a/F/008/2021) awarded by FRCT – Fundo Regional para a Ciência e Tecnologia. This work was supported by DRCT - Direção Regional da Ciência e Tecnologia through the project M1.1.C/PROJ.EXPLORATÓRIOS/003/2022 – PotBioCJap. Additional funding was provided by national funds via FCT – Fundação para a Ciência e a Tecnologia, I.P., under the Scientific Employment Stimulus – Individual Call – 6th Edition (2023.06172.CEECIND). This research was also supported by IITAA – Institute of Agricultural and Environmental Research and Technology (UID/00153/2023).

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The effect of structural modification of lily of the valley alcohol derivatives on sensory properties, microbiological activity and toxicity to skin cells

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Keywords: fragrance compounds, olfactometric analysis, antimicrobial activity, human cell line, cell viability

Despite significant advancements in the fragrance industry, many traditional aromatic compounds are being gradually withdrawn due to issues such as low stability, toxicity, and negative environmental impact. A key challenge remains to recreate the highly valued scent of the lily of the valley flower scent, while ensuring product safety and ecological compatibility [1,2]. This study aimed to synthesize novel derivatives of lily alcohol as safer and more stable alternatives to conventional fragrance ingredients, featuring complex and pleasant olfactory profiles.

The research began with the synthesis of compound mixtures, which were then subjected to comprehensive olfactometric analysis to assess their scent profiles. This analysis revealed a broad and diverse range of fragrance notes. Guided by these results, specific individual compounds exhibiting the most promising fragrance qualities, characterized by pleasantly complex scent profiles, were synthesized separately for further investigation.

These selected compounds were then evaluated for their antimicrobial activity against nine representative skin-associated microorganisms – comprising seven bacterial strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Acinetobacter baumannii*, *Cutibacterium acnes*, *Corynebacterium minutissimum*, *Micrococcus luteus*) and two fungal strains (*Malassezia furfur*, *Candida albicans*) – to assess their potential to inhibit microbial growth. Antimicrobial efficacy was quantified by determining the minimum inhibitory concentration (MIC) according to the procedure described by Bacińska et al. [3]. For some strains, MIC values were greater than 1600 µg/mL, while for others they ranged between 1600 and 3200 µg/mL. Additionally, cytotoxicity was assessed using a human keratinocyte cell line (HaCaT) via the MTS assay, following the manufacturer's protocol (Promega). Cell viability, at a concentration of 200 µM remained high, ranging from 75.11% to 93.79%.

Overall, this approach enabled the identification of novel fragrance molecules with complex and desirable scent profiles, while demonstrating a high level of safety – as evidenced by low cytotoxicity and limited antimicrobial activity – supporting their potential use in skin-friendly fragrance formulations.

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Targeting oral pathogens: anti-biofilm effects of essential oils on *Porphyromonas gingivalis*

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Keywords: *Porphyromonas gingivalis*, biofilm viability, essential oils, natural materials, oral pathogens

Periodontitis is caused by a bacterial biofilm, which is formed by bacteria that adhere to the surface of the teeth and the periodontal pockets. The development of periodontitis is influenced by factors such as poor eating habits, smoking, alcohol consumption, and bad oral hygiene [1]. The key initiator of periodontitis is *Porphyromonas gingivalis*, a pathogen commonly found in oral biofilms [2, 3]. It disrupts commensal microbial communities, inducing dysbiosis [3], and invades epithelial and immune cells, where it can replicate. *P. gingivalis* expresses numerous virulence factors that damage gingival tissues and trigger inflammation. Bleeding facilitates bacterial entry into gingival pockets and the bloodstream, enabling systemic spread and contributing to the development of systemic diseases [3, 4]. Commercial oral care products containing synthetic antibacterial agents may exert cytotoxic effects on human tissues with prolonged use [5]. Therefore, discovering natural alternatives to inhibit the viability of oral biofilm is crucial. Essential oils (EOs) have been proposed for their biological properties and their potential to inhibit oral pathogens [6]. This study evaluated the anti-biofilm activity of four essential oils (EOs) (*Chrysopogon zizanioides*, *Thujopsis dolabrata*, *Leptospermum petersonii*, *Santalum austrocaledonicum*) against *P. gingivalis* ATCC 33277 at a concentration of 40 mg/mL, selected based on their lowest minimum inhibitory concentration (MIC) values in preliminary screening. Additionally, two combinations of these EOs were tested to assess potential synergistic effects. Chlorhexidine digluconate (positive control, 40 mg/mL), a commercially available oral-care product (80 mg/mL), and reference antibiotics – ciprofloxacin (10 µg/mL) and gentamicin (20 µg/mL) – were also examined. Biofilms were formed on the peg lids of specialized 96-well plates by incubating at 37°C for 48 hours in anaerobic conditions. Following exposure to the tested materials, the peg lids were washed with PBS, and viability was assessed using the WST reagent. The results demonstrated that natural materials exhibit strong activity against *P. gingivalis* biofilm. The highest activity was observed for *C. zizanioides* EO with a MIC of 50 µg/mL, followed by *T. dolabrata* EO (MIC = 200 µg/mL), *L. petersonii* EO (MIC = 400 µg/mL), and *S. austrocaledonicum* EO (MIC = 800 µg/mL). The tested EOs combinations showed synergistic effects with a FICI value of 0.25. Chlorhexidine was active at a concentration of 50 µg/mL, while the commercial product demonstrated activity at 800 µg/mL. The tested EOs exhibited notable anti-biofilm activity against *P. gingivalis*. The observed synergistic effects between selected combinations highlight the potential of natural materials as promising alternatives to conventional antimicrobial treatments in oral care.

ACKNOWLEDGMENTS

We would like to thank representatives of the EOs industry and members of IFEAT: A. Fakhry & Co., Berje, Inc., Bordas S.A., Essentially Australia, Bristol Botanicals, Europak, Eucaforest, Hermitage, Lluch Essence, New Zealand Manuka Bioactives, Oshadhi, Ultra International, Van Aroma, and Vessel Essential Oils for the donation of most of the samples of EOs for this study.

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A rapid screening approach for sildenafil adulteration in erectile dysfunction supplements using NIR and MIR spectroscopy with chemometric algorithms

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Keywords: sildenafil, adulteration, NIR, MIR, spectroscopy

Erectile dysfunction supplements (EDS), often marketed as natural, frequently contain undisclosed phosphordiesterase-5 inhibitors, particularly sildenafil [1], posing pharmacological and toxicological risks [2]. This study aimed to develop a rapid, non-destructive screening method using near-infrared (NIR) and mid-infrared (MIR) spectroscopy with chemometric approach to detect sildenafil adulteration in EDS.

A total of 42 sample sets were prepared using 8 EDS matrices, both unspiked and spiked with sildenafil added to the sample matrix in amounts ranging from 0 to 100 mg. NIR spectra (10000–4000 cm^{-1}, 32 scans, 2 cm^{-1} resolution) were recorded using a Spectrum 3 Tri-Range MIR/NIR/FIR Spectrometer (PerkinElmer, USA), while MIR spectra (4000–650 cm^{-1}, 32 scans, 4 cm^{-1} resolution) were obtained using the ATR module of a Carry 600 instrument (Agilent, Germany). Chemometric algorithms involving partial least squares regression-discriminant analysis (PLS-DA) and PLS were applied to process the spectral data.

Sildenafil spectra acquired from both techniques showed distinctive bands that were absent in the unadulterated EDS. Some specific bands appeared in the NIR spectrum (5262 and 4424 cm^{-1}), whereas the MIR spectrum showed larger number of characteristic bands reliable for sildenafil detection (1697, 1578, 1390, and 1172 cm^{-1}). Mathematical modelling of both NIR and MIR spectra was successfully applied for rapid determination of sildenafil adulteration. The correlation coefficients for assessing performance and predictive capability indicate good PLS model accuracy (RMSEE: 2.56, RMSEcv: 2.79 for NIR; RMSEE: 3.71, RMSEcv: 3.84 for MIR).

This study demonstrated that the combination of NIR and MIR spectroscopy coupled by chemometric algorithms represents a reliable and cost-effective tool for screening sildenafil adulteration in diverse EDS.

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Natural origin raw materials as agents inhibiting the growth of *Staphylococcus epidermidis*

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Keywords: *S. epidermidis*, biofilm formation, antimicrobial activity, natural compounds, natural antimicrobials

Staphylococcus epidermidis is commonly found on the surface of the skin and mucous membranes. Although it is usually considered a harmless organism, it can become pathogenic, especially in individuals with weakened immune systems or in the presence of implanted medical devices. The ability of *S. epidermidis* to form biofilms greatly contributes to its pathogenicity, as it allows the bacteria to adhere to surfaces and provides protection against immune responses and antibiotic treatment. Biofilm formation involves a complex interaction of surface structures, such as adhesins, and the production of extracellular polymeric substances (EPS), making its elimination particularly difficult [1,2].

To combat the growth of *S. epidermidis*, various natural compounds have been shown to have promising antimicrobial properties. Natural raw materials derived from plants contain a range of bioactive compounds that inhibit bacterial growth. For example, tea tree oil and lavender oil are known for their antimicrobial activity against various pathogens, including staphylococci. The antimicrobial effectiveness of natural raw materials may be related to their ability to damage bacterial cell membranes, disrupt cellular respiration, or inhibit enzyme activity, leading to bacterial cell lysis.

96 natural raw materials were screened in tests. The results allowed for the selection of 41 raw materials that inhibited the growth of *S. epidermidis* (NCBI 1282) and were subjected to MIC determination. Subsequently, the raw materials with the lowest MIC values were tested for their ability to combat formed biofilms.The alamarBlue® staining method was used in a series of twofold dilutions on 96-well plates. Cultures were grown aerobically at 37°C. For the biofilm inhibition studies, tests were conducted similarly, except that biofilms were first grown in the 96-well plates and then exposed to the selected raw materials.

The results indicate the potential to inhibit *S. epidermidis* growth: Patchoulol natural showed MIC values at 50 µg/ml, while Patchouli oil Sulawesi dark min 29, Patchouli oil Sumatra M.D. Min 32, and Patchouli oil Sumatra Iron Free Min 32 showed MIC values at 100 µg/ml. These compounds were selected for biofilm inhibition studies, which confirmed the bactericidal ability of patchouli oils at 800 µg/ml and Patchoulol natural at 400 µg/ml.

These results suggest that both Patchoulol natural and selected patchouli oils may be promising natural antimicrobial agents, especially in the context of combating *S. epidermidis* biofilms, which are a significant factor complicating the treatment of hospital-acquired infections. The much higher MIC values observed in biofilm studies should not be surprising because: This issue is exacerbated by the formation of biofilms, which can enhance microbial resistance by up to 1000-fold, consequently increasing the risk of infection [3].

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Studies on the mechanism of antifungal activity of polycations in model *Candida albicans* membranes

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Keywords: Langmuir monolayer, polycations, *Candida albicans*

Due to the increasing resistance of fungi, such as *Candida albicans* [1], to commonly used antifungal drugs, there is an urgent need to find new compounds that work through different mechanisms. One promising strategy is the use of polycations - positively charged macromolecules that can interact with components of microbial cell membranes.

Polycations contain amino groups, which give them water solubility and biological activity, including antimicrobial properties. The study specifically focused on two polycations: Poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) and Poly(3-methacrylamido propyl trimethyl ammonium chloride) (PMAPTAC) [2]. Interactions of two polycations (PDMAEMA and PMAPTAC) with model membranes that simulated the composition of *Candida albicans* cell membranes were investigated to better understand their potential antifungal mechanisms.

Langmuir monolayers composed of the phospholipids POPC and POPE (at a 1:1 molar ratio), supplemented with varying amounts of ergosterol (10, 25, 50 mol%), were used to model fungal membranes [3]. By employing systems with different ergosterol contents, it was possible to evaluate the influence of this sterol on polymer–membrane interactions and lipid organization. Analysis of surface pressure versus molecular area per lipid molecule (π–A) isotherms, combined with studies on the polymers ability to incorporate into the monolayer, enabled the identification of differences in polycation behaviour depending on their chemical structure. The results provide a better understanding of how polycations interact with *Candida albicans* membranes. This represents an important step toward developing new materials with potential to treat fungal infections resistant to common antifungal drugs. Further studies are needed to evaluate their activity in more complex biological systems and to explore possible biomedical applications.

ACKNOWLEDGMENTS

This study was partially supported by the National Science Centre, Poland, under the OPUS programme (grant no. UMO-2021/41/B/NZ7/00274).

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Oxidative stress markers in transgenic roots of *Medicago sativa* cultivars differing in resistance to the root lesion nematode

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Keywords: plant–nematode interaction, biotic stress, reactive oxygen species (ROS), malondialdehyde (MDA), *in vitro* root cultures

Phytoparasitic nematodes are among the most destructive pests in agriculture, contributing to substantial reductions in crop yields globally. The root lesion nematode (RLN, *Pratylenchus penetrans*), a migratory endoparasite with a wide host range, invades and feeds on host root cortical cells, causing necrotic lesions, disrupting root function, and increasing plant vulnerability to opportunistic pathogens.

Given the challenges associated with studying nematode infections under greenhouse or field conditions – where environmental variables and biological complexity limit reproducibility – *in vitro* root culture systems offer a reliable and controlled platform for investigating plant–nematode interactions.

In this study, *in vitro* transgenic root cultures of two *Medicago sativa* (alfalfa) cultivars – one resistant and one susceptible to RLNs – were used to assess oxidative stress responses associated with nematode infection. Root extracts were obtained, and protein content was quantified using the Bradford assay. Catalase (CAT) activity and lipid peroxidation, measured *via* malondialdehyde (MDA) content, were evaluated spectrophotometrically as indicators of oxidative stress and membrane damage, respectively.

Catalase activity was 1.2-fold higher in transgenic roots of the susceptible alfalfa genotype, suggesting an elevated demand for detoxification of reactive oxygen species. In contrast, MDA levels were 1.3-fold lower in transgenic roots of the resistant *M. sativa* genotype, indicating a reduced oxidative stress status compared to the susceptible variety.

Overall, this study highlights the value of oxidative stress markers in evaluating plant defence responses and underscores the usefulness of *in vitro* systems for dissecting the physiological mechanisms underlying resistance to root lesion nematodes.

ACKNOWLEDGMENTS

The authors wish to thank Paulo Vieira (USDA) and Cláudia Vicente (NemaLab, Évora University) for sharing the genetic material. This research was partly funded by Fundação para a Ciência e a Tecnologia (FCT/MCTES) through project NemAct ref. 2022.00359.CEECIND (DOI: 10.54499/2022.00359.CEECIND/CP1737/CT0002).

Integrated evaluation of the effectiveness of conventional iron and aluminum coagulants and their impact on microbial communities in post-digestion liquor treatment

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Keywords: coagulation, post-digestion liquor treatment, iron coagulants, aluminum coagulants, microbial communities

The purpose was to study the quality of the digestate from the anaerobic digestion process and to purify the liquid fraction of the digestate. After completion of the anaerobic digestion process, the digestate was separated by centrifugation, and the resulting supernatant was subjected to coagulation processes, in which the effects of initial pH (6.0; 7.5; 9.0) and coagulant dose (200–1600 mg L^{-1}) on the efficiency of removal of: turbidity, total suspended solids, orthophosphate and ammonium nitrogen, as well as on the change in final pH were studied. In parallel, the effect of four commercial Fe(III) and Al(III)-based inorganic coagulants PIX 110S, PIX 113, PAX XL 1910S, PAX 16 on total microbial abundance in the supernatant was evaluated.

Metagenomic sequencing was performed on the supernatant of selected variants, and the results were compared to those of a control post-fermentation liquid sample collected both before and after centrifugation.

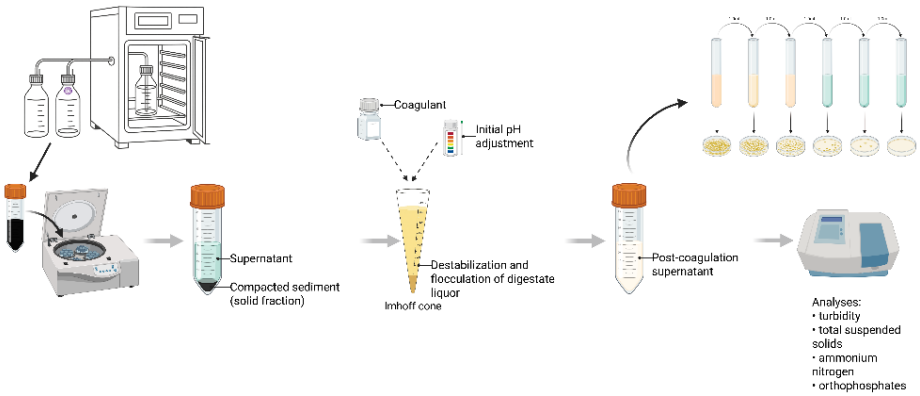

Fig. 1. Schematic workflow of post-digestion liquor treatment

The methane fermentation digestate characterized by a turbidity of 14 300 ± 20 FAU, suspended solids of 2.95×10^4 mg L^{-1}, NH_4-N 2350 ± 26 mg L^{-1}, PO_4-P 680 ± 1 mg L^{-1} (pH 7.31 ± 0,05) and 4.1×10^{11} CFU mL^{-1}, were subjected to a stepwise purification including centrifugation (8000 g, 7 min) and coagulation with Al(III) (PAX 16, PAX XL 1910S) and Fe(III) salts (PIX 110S, PIX 113).

Centrifugation reduced turbidity to 185 FAU (–98.7%), suspended solids to 312 mg L^{-1} (–98.9%), NH_4-N by 98.8%, PO_4-P by 88% and microflora abundance by 3.5 log at unchanged pH, preparing the stream for coagulation.

Coagulation at pH 6.0 provided clarification < 1 NTU and dephosphatation to 0.06 mg L^{-1} PO_4-P (PAX 16 ≥ 375 mg L^{-1}), while iron coagulants required ≥ 1,000 mg L^{-1}, causing strong acidification (pH ≈ 2,3). Microbial reduction increased to 4–6 log, and turbidity < 1 NTU correlated with > 3 log reduction. The combination of centrifugation with PAX 16 750 mg L^{-1} (pH 6.0) simultaneously met sanitary (≥ 3 log) and quality criteria (PO_4-P < 10 mg L^{-1}, turbidity < 1 NTU), confirming the effectiveness of the integrated technology in conditioning the digestate for further management.

The deactivation mechanism was presumably two-stage: (i) adsorption and entrapment of cells in the floc matrix correlated with the final turbidity (< 1 NTU → ≥ 3 $\log_{10}$ reduction) and (ii) chemical stress induced by strong acidification upon Fe^{3+} dosing (pH < 4), which further diminished microbial viability.

Ethosomes as modern carriers of horseradish and carrot extracts

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Keywords: transdermal carriers, surfactants, ethosomes, plant extracts

Ethosomes are modern transdermal carriers composed of lipids, ethanol, and water. The alcohol content affects the penetration of the epidermis and increases the bioavailability of the encapsulated active substances [1, 2]. The aim of the study was to optimize the synthesis of ethosomes modified with plant extracts obtained from native cultivation.

The optimization of the synthesis consisted of comparing two methods: (i) classical cold, (ii) cold with homogenization. The cold method involves the preparation of two phases consisting of: I – phospholipid (2.5% wt.), ethanol (30.0% wt.), ethylene glycol (10.0% wt.), surfactant (1.25% wt.), and extract (1.0% wt.), II – water (up to 100.0% wt.). Both phases were heated to 30 °C, then, while stirring on a magnetic stirrer (300 rpm), the aqueous phase was added in a thin stream to the lipid phase. The mixture was stirred for 15 min on a magnetic stirrer. After this time, the obtained carriers were stored in the refrigerator. The second method involves homogenization (3 min; 15.000 rpm) of the mixture after the phases are combined. In addition, the effect of two different surfactants: Tween 80 and Mirasoft® SL L60 on the stability of the obtained carriers was tested.

The stability of the obtained carriers was determined by measuring the Zeta potential (ZP), average particle size (Z-Ave), and polydispersity index (PDI). Analyses were performed immediately after preparation, after 24 h, and after 14 days of storage in the refrigerator.

Tween 80-based samples showed the smallest average particle sizes in all tests with diameters in the range of 93–186 nm. The synthesis method had no significant effect on these particle sizes. On the other hand, samples based on Mirasoft® SL L60 surfactant showed several times larger particle sizes with diameters in the range of 410–780 nm. However, homogenized samples showed better results. Zeta potential testing after 14 days showed comparable results for each sample (approximately –35 mV). The polydispersity index was more unfavorable for homogenized samples compared to those obtained by the classic cold method.

Based on the results obtained, it can be concluded that the media containing horseradish extract and Tween 80 surfactant, obtained by the classical cold method are the most stable. For samples incorporated with carrot extract, the highest stability was reached for homogenized samples prepared with Mirasoft® SL L60 surfactant.

Further prospects:
Further studies will be focused on increasing the concentration of incorporated extracts and on determination of the release profile of active compounds from the obtained carriers.

ACKNOWLEDGMENTS

Acknowledgements of funds from ID-UB (Initiative for Excellence – Research University) AMU program, grant no 136/13/SNŚ/0002, entitled "Synthesis and characterization of ethosomes – modern carriers of active substances".

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Chemical and molecular profiling of waterlogged ancient ivory

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Keywords: ancient ivory, DNA analysis, infrared spectroscopy, X-ray fluorescence, scanning electron microscopy with X-ray analysis

This research focuses on the chemical and molecular analysis of elephant ivory from two 18th-century British shipwrecks in the Atlantic Ocean: The Princess Louisa, lost off May Island, Cape Verde, in 1743, and the BH-001 wreck, discovered in Horta Bay, Faial Island, Azores. Both shipwrecks yielded substantial ivory collections, providing a unique chance to investigate their ancient routes.

A multi-analytical approach was employed that included X-ray fluorescence (XRF), Fourier-transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR), scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX), and DNA analysis. These methods were used to distinguish elephant species (African vs. Asian), profile the chemical composition, and evaluate preservation states of the waterlogged ancient ivory.

Results showed that ivory from both wrecks originated from African elephants. However, differences in elemental and molecular signatures, as well as varying degrees of structural degradation, suggested distinctions in environmental exposure.

This comparative research demonstrates that combining chemical and molecular methodologies is effective in reconstructing the historical context of submerged ivory cargoes. Further research may improve the identification of ivory trade centres and support improved conservation strategies for underwater cultural heritage.

ACKNOWLEDGMENTS

The authors would like to thank Jardim Zoológico de Lisboa (Portugal) and Terra Natura Benidorm Zoo (Spain) for kindly providing the samples of elephant faeces from different species.

Development and validation of an HPLC-DAD method for the simultaneous analysis of rutin and verbascoside in herbal mixtures for wound healing

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Keywords: verbascoside, rutin, validation, HPLC

Rutin and verbascoside are quite significant when it comes to treating chronic wounds, due to their antioxidant, anti-inflammatory, and antibacterial activity. To evaluate whether these two compounds can synergistically enhance each other's therapeutic efficacy, it is essential to optimize an HPLC method for their accurate quantification.

Quantification of the components of interest was performed using high-performance liquid chromatography with a diode array detector (HPLC-DAD). The separation of components was done using an Agilent Zorbax Eclipse XDB-C18 (4.6 × 150 mm, 5 µm, Agilent Technologies, USA) column. The injection volume was 10µl, the flow rate 1 mL/min, and the temperature was 25°C. The mobile phase was composed of acetonitrile (A) and a 1% solution of orthophosphoric acid (B). The phase ratio during elution of the components was: 0–3 minutes 15% A and 85% B, 3–10 minutes 25% A and 75% B, 10–14 minutes 40% A and 60% B, 14–17 minutes 60% A and 40% B, 17–21 minutes 25% A and 75% B, 21–23 minutes 15% A and 85% B. In herbal mixtures, verbascoside and rutin were detected at 330 nm and 360 nm, respectively, corresponding to their optimal absorbance wavelengths. Validation of the method used in this study was performed in accordance with the recommendations of the International Council for Harmonization (ICH). Chromatograms obtained after applying the solvent, individual standard solutions of rutin and verbascoside, as well as their mixture, showed complete separation of the components of interest, indicating the specificity and selectivity of the developed method. The resolution value between peaks was greater than 1.5, indicating high selectivity of the method. The linearity of the method was demonstrated by the correlation coefficient (R^2 = 0.999), which indicated a linear relationship between peak area and concentration of standard solutions within the concentration range of 0.02 mg/mL to 3 mg/mL for both components. The obtained values for analytical yield ranged from 99.54% to 100.58% and from 98.25% to 99.22% for rutin and verbascoside, respectively, confirming the accuracy of the method within the range of 50%, 100%, and 150% of the working concentration. The precision of the method was confirmed by the relative standard deviation (RSD) value, which was less than 2%. The robustness of the analytical method was tested under varying chromatographic conditions: flow (±0.2 mL/min), column temperature (–7°C, +2°C), and salt concentration (±0.1%). The results obtained from these changes indicated that the method was robust under these conditions. The limit of detection for rutin was 0.01 mg/mL, while the limit of quantification was 0.02 mg/mL. The limit of detection for verbascoside was 0.02 mg/mL, while the limit of quantification was 0.06 mg/mL. The standard mix remained stable for up to 24 hours at room temperature.

The establishment and validation of a method for the simultaneous quantification of rutin and verbascoside in a single analytical run enables rapid and reliable determination of their content, significantly reducing analysis time. Once validated, this method can be easily implemented in other laboratories, facilitating reproducibility, accelerating further research, and supporting the standardized development of effective herbal mixture for the treatment of chronic wounds.

Antioxidant response of tomato transgenic roots to *Meloidogyne incognita* infection

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Keywords: biotic stress, plant–nematode interaction, oxidative stress, spectrophotometric assay, enzymatic defense response

Root-knot nematodes (RKN, *Meloidogyne* spp.) are destructive phytoparasites that manipulate host root structure to facilitate parasitism, ultimately impairing plant function. Studies investigating the mechanisms of RKN infection and host response are typically performed under field or greenhouse conditions – either to maintain nematode reference cultures or to enable large-scale experimentation. While field studies offer a more realistic environmental context, their outcomes are often heavily influenced by uncontrollable environmental variability. Greenhouse experiments, on the other hand, allow for tighter regulation of environmental parameters and minimize the risk of external contamination or pathogen exposure. However, in genetically diverse plant populations, inherent variability can still affect experimental consistency. For soil-dwelling plant parasitic nematodes like RKNs, *in vitro* cultures of transgenic roots provide a valuable system for controlled investigations under laboratory conditions.

In this study, *in vitro* tomato transgenic roots were co-cultured with *Meloidogyne incognita*, and their redox status was assessed by measuring the activity of the antioxidant enzymes ascorbate peroxidase (APX) and catalase (CAT) using spectrophotometric analysis. Transgenic roots (infected or uninfected) were homogenized in 50 mM potassium phosphate buffer (pH 7.0), and total protein content was quantified using the Bradford assay. RKN infection resulted in a marked increase in APX activity – up to fivefold – while CAT activity was reduced. These findings suggest that infection by *M. incognita* modulates the host's antioxidant enzymatic system, potentially as part of the plant's defense response or as a consequence of the nematode's manipulation of host physiology to create a more favorable environment for parasitism.

ACKNOWLEDGMENTS

The authors wish to thank Leidy Rusinque, at INIAV, for sharing the genetic material. This research was partly funded by Fundação para a Ciência e a Tecnologia (FCT/MCTES) through project NemAct ref. 2022.00359.CEECIND (DOI: 10.54499/2022.00359.CEECIND/CP1737/CT0002).

Comparative evaluation of the volatiles isolated from *Illicium verum* fruits by hydrodistillation, from the hydrolate, and by HS-SPME

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Keywords: volatile compounds, essential oil, aromatic plants, GC-MS, *Illicium verum*

Illicium verum Hook f., commonly known as star anise, belongs to the Schisandraceae family and is widely distributed in Asia and in tropical and subtropical zones. The fruits of *I. verum* are traditionally used in folk medicine for the treatment of diseases and in food flavoring, due to the presence of the various secondary metabolites, including monoterpenoids, phenolic compounds, lignans and sesquiterpenes. These substances are associated with biological activities of interest, such as antimicrobial, antioxidant, insecticidal and anti-inflammatory actions [1, 2]. This study aimed at comparing the chemical profiles of the essential oil (EO), the hydrolate volatiles, and the headspace volatiles from the dried fruits of *I. verum*. The essential oil was obtained by hydrodistillation for 3 h and the hydrolate volatiles by liquid-liquid extraction as detailed in [3]. The headspace volatiles were collected from the plant material before EO isolation, by solid-phase microextraction (HS-SPME) at room temperature as in [4]. The qualitative analysis of the compounds was performed by gas chromatography coupled with mass spectrometry (GC-MS), and quantification by gas chromatography with a flame ionization detector (GC-FID) [3, 4]. The star anise essential oil was obtained in a yield of 3.3% (v/w), with 47 compounds identified, the main ones (≥ 5%) being: *trans*-anethole (74%), *p*-anisaldehyde (12%), and limonene (5%). In the hydrolate, 38 compounds were identified, with emphasis (≥ 5%) on *p*-anisaldehyde (47%), *trans*-anethole (30%). Using the HS-SPME, 45 compounds were detected, with a predominance (≥ 5%) of *trans*-anethole (50%), *p*-anisaldehyde (12%) and β-caryophyllene (5%). The results showed variations in the volatile chemical profile, despite the same main volatile components, with some compounds being identified only in the volatile fraction by HS-SPME, and others only in the essential oil or hydrolate volatile fractions. Such results suggest the importance of understanding the volatile chemical profile of the plant material to better guide its possible applicability.

ACKNOWLEDGMENTS

This work was supported by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG – Project CAG/APQ 02390/2018), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Project CNPQ 311183/2022-0) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance code 001) for the scholarships. Thanks to FCT (Portugal) through national funds, under UID/00329/2025 CE3C & CHANGE.

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Foam systems containing saponins and nanocellulose

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Keywords: cellulose nanocrystals, cellulose nanofibres, *Camelia oleifera*

Plant-based surfactants have long been known to humanity; however, contemporary research is focused on identifying new applications for these compounds. Saponins are currently used, among others, in cosmetic products for skin and hair care. Important applications also include their use as emulsifiers and foaming agents.

Foams are dispersion systems formed by the dispersion of a gas in a liquid. They typically consist of disordered structures in which gas bubbles of various sizes are trapped within the liquid. Foams are thermodynamically unstable entities. Such systems are characterized by a large specific surface area and high internal energy. After gas is dispersed in the liquid in order to create the foam structure, and then the gas flow is stopped, the foam degradation processes begin. A characteristic feature of foams is their ability to change structure over time, resulting from a gradual loss of water content. Low adsorption of the surfactant to the lamellae causes desorption of surface active molecules, which leads to foam coalescence. Small, unstable bubbles break, forming larger ones.

In this study, we present the results of research on the potential to control the stability of foam systems containing saponins, as well as cellulose nanocrystals and nanofibers. The influence of nanocellulose addition on the surface tension of aqueous surfactant solutions and on changes in zeta potential was examined. Foam stability was evaluated using a glass column by measuring the change in height of the gas–liquid dispersion over time. Additionally, the used surfactant (comprising a mixture of flavonoids and saponins) was characterized using hybrid LC-UV-ESI-IT-TOF mass spectrometry.

Nanostructured lipid carriers for delivery of phospholipid-dexibuprofen conjugates with anticancer activity

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Keywords: lipid-drug conjugates, phospholipids, nanostructured lipid carrier, lipid nanoparticles, anticancer properties

Nanoparticles loading lipid-drug conjugates, obtained by linking the drug to a lipid compound (fatty acids, glycerides, or phospholipids), have demonstrated significant potential for improving drug delivery, enhancing tumor targeting, and reducing off-target cytotoxicity [1]. The study focused on the development of nanostructured lipid carriers (NLC) loaded with dexibuprofen (DXI), a non-steroidal anti-inflammatory drug (NSAID), for potential use in cancer therapy. Our approach involves the chemical conjugation of DXI to phospholipids to generate novel drug delivery systems with improved therapeutic performance. We successfully synthesized seven DXI-phospholipid conjugates, four of which are new and have not been previously described in the literature. The structures of the synthesized conjugates were confirmed using appropriate analytical methods. We further evaluated their antiproliferative activity through comparative in vitro assays and employed molecular modelling to investigate their binding and pharmacodynamic potential. NLCs containing either free DXI or DXI-phospholipid conjugates were prepared using the hot high-pressure homogenization method. The resulting nanoparticles were subjected to comprehensive physicochemical characterization, including measurements of particle size (~150 nm), polydispersity index (PDI ~0.15), encapsulation efficiency (EE > 99%), and long-term stability. The drug–carrier interactions, in vitro release profiles, and biological activity in cancer cell lines were evaluated. Based on phospholipid-drug conjugation strategies- binding to the phosphate group [2] or esterification at the *sn*-1 and/or *sn*-2 positions of the glycerol backbone [3–5], synthesized conjugates showed selective anticancer effects, influencing the cell cycle and inducing apoptosis. Heterosubstituted phosphatidylcholines were selected for encapsulation owing to their lipophilic nature, essential for NLC production. Among the two types of synthesized heterosubstituted hybrid molecules, those where the drug was linked to the glycerol backbone of the PC at the *sn*-1 position were selected, whereas at the *sn*-2 position, an acyl fragment from a long-chain fatty acid was present. Under tumor cell conditions characterized by a high concentration of phospholipase A2, this acyl fragment may be hydrolyzed to produce DXI-LPC, which exhibits even higher activity towards cancer cells than the PC forms. Encapsulation led to favorable nanoparticle characteristics and modified release profiles, contributing to enhanced cellular uptake. Antiproliferative assays confirmed superior efficacy of the conjugates compared to free DXI, with evidence of selective accumulation in cancer cells and increased therapeutic activity. In conclusion, this study reports the successful synthesis, characterization, efficient formulation into NLCs and promising anticancer potential of novel DXI-phospholipid conjugates. These multifunctional conjugates when loaded on NLCs offer an efficient and innovative strategy for targeted cancer therapy with improved delivery and reduced systemic toxicity.

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Effect of chemical and enzymatic pretreatment of lignocellulosic biomass on the efficiency of the methane fermentation

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Keywords: anaerobic digestion, biogas, lignocellulosic biomass, chemical pretreatment, enzymatic hydrolysis

Composting 62 000 metric tons of fallen leaves carried out in Berlin in 2016 resulted in emissions of 3 225 t CO_2-eq t^{-1} [1], by contrast model simulations indicate that anaerobic digestion of the same waste stream can achieve a net emission balance of –140 kg CO_2-eq t^{-1} or even –167 kg CO_2-eq t^{-1} when the leaves are pre-ensiled, whereas composting produces +49 kg CO_2-eq t^{-1} [2]. Leaves as a typical lignocellulosic biomass, comprise a compact complex of cellulose, hemicellulose and lignin whose disintegration requires preliminary physical, physicochemical, chemical, biological, or combined pretreatment [3].

The aim of this study was to identify the pretreatment variant that provides the highest methane yield during anaerobic digestion. The feedstock consisted of Norway maple leaves which, after grinding and fractionation (1–2 cm and 0.5–1 cm), were subjected to a combined treatment involving ozonolysis followed by application of an enzymatic preparation (Fig. 1).

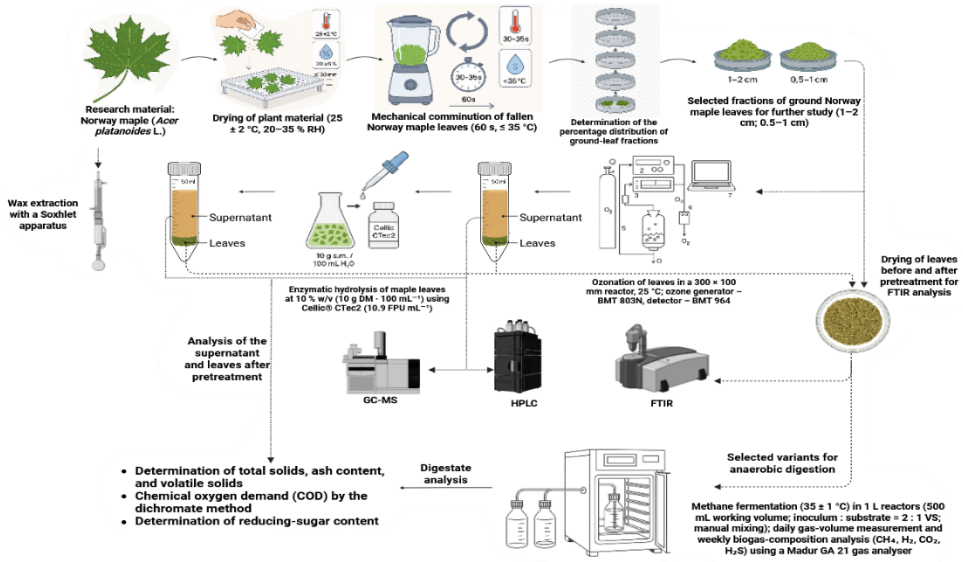

Fig. 1. Schematic workflow of Norway maple (*Acer platanoides* L.) leaf pretreatment and subsequent anaerobic digestion

Gradually reducing the fineness of maple leaves from 1–2 cm to < 0.045 cm resulted in a decrease in the proportion of dry matter and a linear increase in wax extraction efficiency (up to 5.9% in the finest fraction). Pre-ozonolysis (80 mg O_3 L^{-1}; 0.2 L min^{-1}; 20 min) and enzymatic hydrolysis of Cellic CTec2 (100–200 FPU g^{-1} d.m.) transferred more than half of the organic load to the liquid phase, raising the concentration of reducing sugars from ≤ 2 g L^{-1} to 28 g L^{-1} and shortening the latent phase of methanogenesis (lag phase) in the BMP test. The content of reducing sugars in the filtrate correlated strongly positively with the Chemical Oxygen Demand (COD) of the liquid (R = 0.89) and negatively with the COD of the remaining biomass (R = –0.91), confirming the effective translocation of hydrolysis products.

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Study of the antioxidant activity of mixed-ligand copper(II) coordination compounds with *N*-(4-methoxyphenyl)-2-oxopropanamide 4-phenylthiosemicarbazone

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Keywords: thiosemicarbazone, copper(II) coordination compounds, antioxidant activity

Free radicals interact with proteins during normal metabolic processes, which can lead to various types of pathological changes. To protect biomolecules from free radical attacks and/or to suppress the damage they cause, numerous natural and synthetic free radical scavengers and antioxidants have been developed and studied. Among them, thiosemicarbazones and their metal complexes have shown promising results.

The objective of this study is to study the antioxidant activity of the new mixed-ligand copper(II) coordination compounds with *N*-(4-methoxyphenyl)-2-oxopropanamide 4-phenylthiosemicarbazone (HL) towards $ABTS^{\bullet+}$ cation radical. HL (Fig. 1) was synthesized in two steps. The first step was the reaction of synthesis of *N*-(4-methoxyphenyl)-2-oxopropanamide. For this, pyruvic acid, oxalyl chloride, and para-anisidine were reacted in CH_2Cl_2. The second step was the reaction between 4-phenylthiosemicarbazide and *N*-(4-methoxyphenyl)-2-oxopropanamide in ethanol. The structure and purity of HL were confirmed using ^1H and ^{13}C NMR and FTIR spectroscopies. To obtain mixed-ligand copper(II) coordination compounds, the complex $[Cu(L)NO_3]$ was initially synthesized by reaction between HL and copper(II) nitrate trihydrate in a 1:1 molar ratio in ethanol. This complex was then dissolved in ethanol, and a corresponding *N*-heteroaromatic base was added. As a result, three copper(II) complexes were obtained: $[Cu(A)(L)]NO_3$ (A = 1,10-Phen, 3,4-Lut, 3-Pic). The complexes were studied using FTIR spectroscopy, elemental analysis, and molar conductivity measurements.

The antioxidant activity of all synthesized compounds was evaluated against $ABTS^{\bullet+}$ cation radical. The IC_{50} value for HL was 33.89 μM; its coordination to the copper(II) atom resulted in the complex $[Cu(L)NO_3]$ showing no activity, while the introducing of *N*-heteroaromatic bases into the inner coordination sphere of the complex $[Cu(L)NO_3]$ enhanced the antioxidant activity.

Despite the fact that the complexes did not surpass the activity of the obtained 4-phenylthiosemicarbazone, the search for antioxidant agents among thiosemicarbazones and their complexes remains a promising direction. Modifying the structure of thiosemicarbazones can significantly influence their physicochemical properties and the biological activity of both the ligands and their complexes.

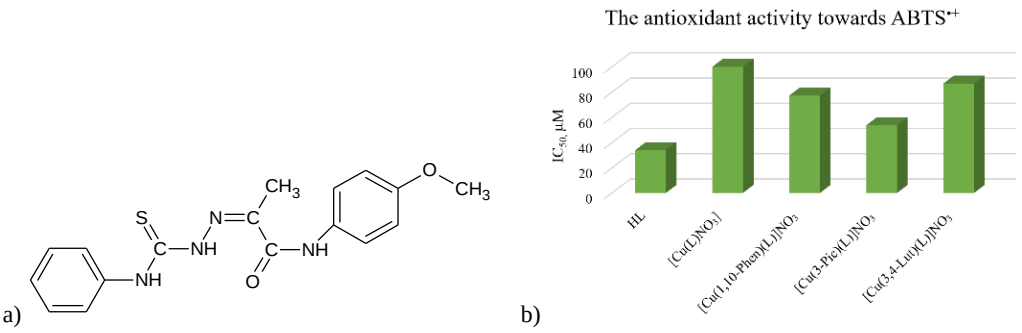

Fig. 1. a) The structure formula of HL, b) The antioxidant activity of the obtained substances

ACKNOWLEDGMENTS

This work was fulfilled with the financial support of the subprogram 010602 of the institutional project.

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Exploring the antibacterial activity of copper(II) complexes with 2-benzoylpyridine 4-norbornylthiosemicarbazone against *S. aureus*

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Keywords: thiosemicarbazone, copper(II) complexes, antibacterial activity

Antibiotic resistance has emerged as a major global challenge, posing serious risks to public health and food safety. This underscores the growing urgency to develop new antibiotics or bioactive compounds capable of combating pathogenic microorganisms. Complexes of transition metals exhibit properties that make them promising candidates for antimicrobial applications. Furthermore, the primary objective of antimicrobial agents is to achieve maximum effectiveness with minimal dosage while preventing the development of resistance.

The aim of this research was the study of antibacterial activity of the new copper(II) coordination compounds with 2-benzoylpyridine 4-norbornylthiosemicarbazone (HL) (Fig. 1). The first step was the synthesis of 2-benzoylpyridine 4-norbornylthiosemicarbazone. HL was obtained in two stages: first the reaction was carried out between 4-norbornylthiosemicarbazide and 2-benzoylpyridine with HCl in ethanol, and then the obtained hydrochloride of HL was dissolved in ethanol and neutralized with Na_2CO_3. The copper(II) coordination compounds were synthesized during the reaction between HL and the corresponding copper(II) salt in 1:1 molar ratio in ethanol. The obtained complexes were studied using FTIR spectroscopy, X-ray diffraction, elemental analysis and molar electrical conductivity. The following complexes were synthesized: $[Cu(L)NO_3]$, $[Cu(L)Cl]$ and $[Cu(L)CHCl_2COO]$.

The antibacterial activity of the HL and copper(II) complexes was studied towards Gram-positive microorganism *Staphylococcus aureus* (ATCC 25923). The HL itself is not active, but its coordination to the copper(II) atom leads to an increase in antibacterial activity. The activity of the complexes is affected by the nature of the acid anion, the activity decreases in the following order: $Cl^- > NO_3^- > CHCl_2COO^-$. The minimum inhibitory concentration (MIC) for the most active complex, [Cu(L)Cl], is 0.24 μg/mL, and the minimum bactericidal concentration (MBC) is 0.49 μg/mL.

2-Benzoylpyridine thiosemicarbazones often exhibit various types of biological activity. The presence of a natural fragment derivative in the structure of the synthesized thiosemicarbazone makes it a promising antibacterial agent. Therefore, it is of interest to study its antibacterial activity against a broader range of microorganisms.

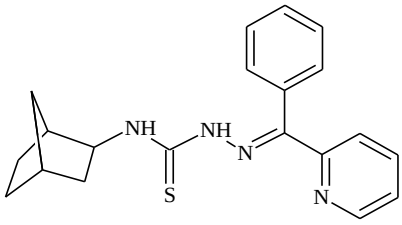

Fig. 1. The structure formula of HL

ACKNOWLEDGMENTS

This work was fulfilled with the financial support of the ANCD project 24.80012.5007.14TC.

Comprehensive chemical profiling of *Melaleuca alternifolia*: uncovering the hidden complexity of tea tree oil

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Keywords: *Melaleuca alternifolia*, tea tree oil, monoterpenoids, essential oil

Tea tree oil, the essential oil derived from *Melaleuca alternifolia* (Myrtaceae), is widely recognized for its therapeutic applications due to its established antimicrobial, antiviral, and anti-inflammatory properties. These bioactive characteristics make it a valuable ingredient in formulations targeting dermatological and oral health conditions, such as acne, dandruff, and infections within the oral cavity [1, 2]. A detailed chemical investigation was carried out on a commercially sourced sample of *M. alternifolia* oil using gas chromatography-mass spectrometry (GC-MS) and preparative chromatographic separation. The analysis revealed a complex composition dominated by oxygenated monoterpenes (64.2%) and monoterpene hydrocarbons (24.6%), with major constituents identified as terpinen-4-ol (35.8%), 1,8-cineole (19.8%), *p*-cymene (19.5%), and α-terpineol (3.4%). Significantly, the investigation uncovered that more than 50% of the constituents had not been previously reported in *M. alternifolia* essential oil. Among these were polyoxygenated monoterpenoids, a class of metabolites seldom encountered in plant-derived essential oils. The discovery of these constituents expands the known chemical diversity of tea tree oil and highlights the need for further exploration of their potential bioactivities.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant Nos. 451-03-136/2025-03/200124 and 451-03-137/2025-03/200124).

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Invisible evidence – chemistry in the fight against crime

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Keywords: forensics, trace, evidence, crime

In modern forensic investigations, collaboration between law enforcement agencies, such as the Police, and the scientific community is essential for effective case resolution. Particular emphasis is placed on physicochemical methods, which are crucial for the identification of trace evidence left at crime scenes. These techniques play a key role in detecting and analyzing evidence that is often invisible to the naked eye.

The purpose of this presentation is to outline selected analytical methods employed in forensic examinations. The focus will be on fiber analysis and the identification of biological traces, including blood.

Fibers transferred onto clothing frequently enter forensic laboratories as evidence indicating a suspect's presence at the crime scene. Through the application of advanced technologies, it is possible to analyze their chemical composition, structural properties, and the types of dyes used, enabling their association with specific sources. Additionally, the analysis of volatile organic compounds, such as trace amounts of perfumes present on textiles, can be performed using Headspace Solid-Phase Microextraction (HS-SPME), which aids in event reconstruction [1, 2].

The analysis of biological traces, especially blood, is of paramount importance. Even aged or visually undetectable bloodstains can be located through chemiluminescent reactions, for example using luminol. Following detection, further investigations are conducted to establish genetic profiles of suspects or victims [3].

The introduction of diverse analytical instruments allows for precise examination while minimizing sample consumption. This capability ensures that delicate evidence can be analyzed without destruction, which is critically important for subsequent judicial proceedings.

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Evaluation of sucrose fatty acid esters as novel agents for controlling microbial biofilms and virulence in the human gastric system

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Keywords: biofilm inhibition, antimicrobial agents, glycolipids, anti-inflammatory properties, gene regulation

Biofilm-associated infections present a considerable challenge in both clinical and industrial contexts due to their heightened resistance to antimicrobial agents and host immune responses. Consequently, the development of novel strategies to prevent biofilm formation and microbial adhesion is of critical importance. Sucrose fatty acid esters, such as Sisterna's SP70 and SP50, are commonly used as surfactants and emulsifiers in the food and pharmaceutical industries. However, the full potential of these compounds in terms of their antimicrobial and anti-biofilm properties remains to be fully explored.

In this study, the effects of SP70 and SP50 on the survival, virulence gene expression, and biofilm-forming capacity of key pathogenic microorganisms associated with gastrointestinal and nosocomial infections were investigated. Quantitative real-time PCR analysis indicated that both compounds significantly downregulated the expression of virulence genes in *Enterococcus faecalis* (*cstR*, *efaA*, *esp*) and *Klebsiella pneumoniae* (*ompK35*, *mrkA*, *arcB*), suggesting a potential role in attenuating bacterial pathogenicity.

Furthermore, exposure to SP70 and SP50 led to a significant decrease in the mRNA levels of hyphal-specific genes in *Candida albicans*, including *HWP1*, *EAP1*, *EGF1*, *ECE1*, and *SAP5*, indicating a disruption of morphological transitions critical for fungal virulence. However, no substantial inhibition of overall growth was observed.

Subsequent experiments evaluated the impact of these compounds on biofilm formation using human cell lines. SP70 and SP50 effectively inhibited *C. albicans* biofilm development on both normal human dermal fibroblasts (NHDF) and HT-29 intestinal epithelial cells without inducing cytotoxic effects, as confirmed by standard viability assays. Moreover, the compounds exhibited anti-inflammatory properties by reducing the inflammatory response induced by *C. albicans* infection in the host.

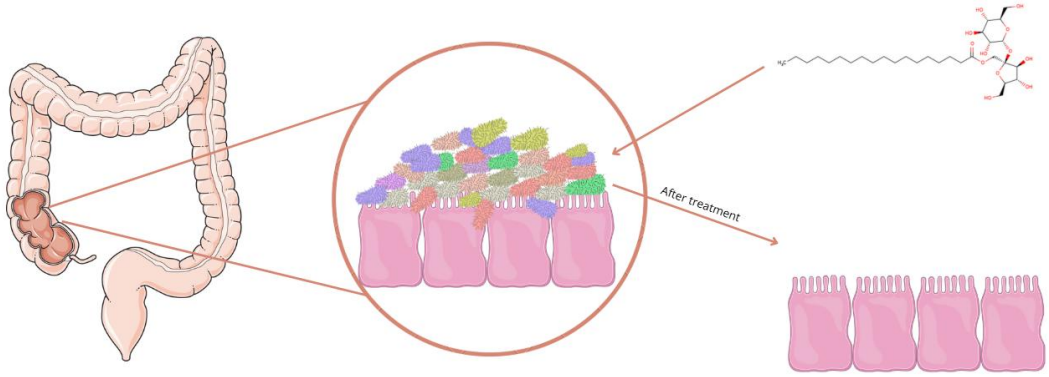

Fig. 1. Effects of sucrose fatty acid esters against pathogens associated with gastrointestinal infections

ACKNOWLEDGMENTS

This research was funded by the Wrocław University of Environmental and Life Sciences (Poland) as the Ph.D. research program "Bon doktoranta SD UPWr" no. N020/0002/24.

Evaluation of the bioactive properties of peach (*Prunus persica*) kernel extracts

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Keywords: peach kernel extracts, antioxidant activity, phenolic compounds, antimicrobial potential, bioactive compounds

This research aimed to evaluate the biological potential of peach kernel (*Prunus persica*) extracts, focusing on their antioxidant and antimicrobial properties, as well as their chemical composition, to assess their suitability for use in food preservation, pharmaceuticals, and other industrial applications.

Peach kernels were analyzed for proximate composition, including moisture, dry matter, protein, and minerals. Moisture and dry matter were determined gravimetrically by oven-drying at 105 °C to constant weight. Ash content was assessed by incineration in a muffle furnace at 550 °C, and organic matter was calculated as the difference between dry matter and ash. Nitrogen content was determined by the Kjeldahl method, with protein content calculated using a 6.25 conversion factor. Potassium (K_2O) was measured using a flame photometer, while calcium (Ca) was determined by ICP-AES [1]. Two extraction methods were used to obtain aqueous and ethanol extracts from 10 g of dried, finely powdered kernels. The aqueous extract was prepared by boiling in distilled water (200 mL) for 1 hour [2], while the ethanol extract was obtained by ultrasound-assisted extraction in 50% ethanol (100 mL) for 40 minutes at 45 °C [2]. Extracts were analyzed for total phenolic and flavonoid content, antioxidant activity (DPPH radical scavenging and iron chelation) [3–5], and antimicrobial activity using the disc diffusion method against selected pathogenic bacteria [6].

Proximate analysis of dried peach kernels revealed 4.10% moisture and 95.90% dry matter, with 22.54% protein and 5.42% potassium. The aqueous extract showed significantly higher ($p < 0.05$) total phenolic content (61.27 mg GAE/100 g) than the ethanol extract (50.81 mg GAE/100 g), while the ethanol extract had higher flavonoid content (39.07 mg QE/100 g vs. 33.34 mg QE/100 g).

In terms of antioxidant activity, the ethanol extract exhibited greater DPPH radical scavenging (68.05% at 10 mg/mL) than the aqueous extract (65.29%), whereas the aqueous extract had stronger iron chelation (57.09% vs. 54.36%).

Both extracts showed strong antimicrobial activity. The aqueous extract was more effective against *Bacillus cereus* (10.02 mm vs. 9.9 mm for the ethanol extract) and *Enterococcus faecalis* (10.3 mm vs. 8.5 mm), while the ethanol extract was more active against *Staphylococcus aureus* (16.2 mm vs. 13.7 mm for the aqueous extract), *Listeria monocytogenes* (17.3 mm vs. 14.6 mm), *Yersinia enterocolitica* (21.1 mm vs. 18.7 mm), and *Proteus vulgaris* (15.0 mm vs. 13.4 mm).

This research demonstrated that peach kernel extracts, particularly those obtained using aqueous and ethanol solvents, possess promising antioxidant and antimicrobial properties, which are primarily attributed to their phenolic and flavonoid content. These findings suggest that peach kernels may be a valuable source of bioactive compounds with potential applications in the food, pharmaceutical, and cosmetic industries. Future research should focus on isolating and characterizing the specific active compounds within peach kernel extracts and investigating their mechanism of action, safety, and potential for large-scale application.

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Evaluation of the apoptosis-inducing properties of lipopeptide biosurfactants in cancer cells

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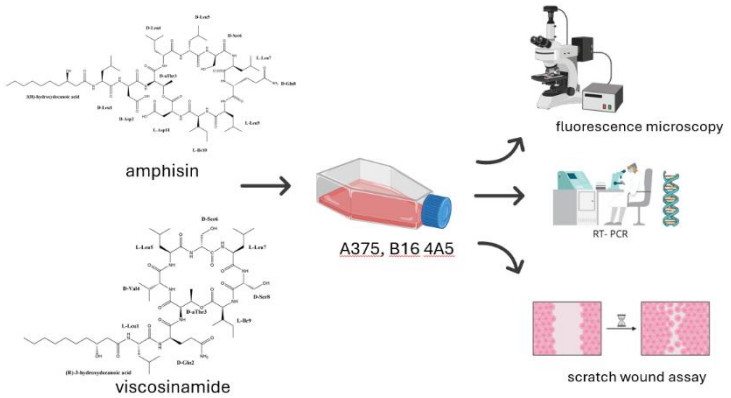
Keywords: lipopeptide biosurfactant, melanoma, apoptosis, scratch assay

In the case of melanoma, advances in therapies remain limited, underscoring the urgent need for new, effective therapeutic agents, including those derived from microbial production. In this study, the anticancer activity of two cyclic lipopeptide biosurfactants, viscosinamide and amphisin, was evaluated against A375 (Human melanoma) cells and B16 4A5 (Mouse melanoma) cells. The aim was to assess their ability to induce cell death and explain potential apoptotic mechanisms.

Cell viability and apoptosis were assessed using Hoechst 33342 nuclear staining, Annexin V-FITC visualization, and RT-qPCR analysis of apoptosis-related genes BAX and BCL-2. Additionally, the scratch wound assay was performed to evaluate the influence of the biosurfactants on cell migration.

Both viscosinamide and amphisin demonstrated significant pro-apoptotic effects, with nuclear condensation and increased Annexin V binding observed in treated melanoma cells. Gene expression analysis revealed an upregulation of BAX and downregulation of BCL-2, indicating activation of the intrinsic apoptotic pathway. Furthermore, both biosurfactants effectively inhibited cell migration in the scratch assay.

These findings suggest that viscosinamide and amphisin could be potential candidates for further development as anti-melanoma agents due to their selective cytotoxic and anti-migratory properties against melanoma cells.



ACKNOWLEDGMENTS

This work was supported by the Wrocław University of Environmental and Life Sciences (Poland) as part of the research project no. N070/0010/23.

Physicochemical landscape of α-iminoamidines: evaluating key parameters for bioactivity prediction

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Keywords: *α*-iminoamidines, basicity, lipophilicity, UV–Vis

The design of molecules with tailored physicochemical properties is critical in the development of bioactive compounds, particularly antiparasitic agents. In this study, we investigated the acidity and lipophilicity of a series of protonated *α*-iminoamidines synthesized from various aliphatic and aromatic amines [1]. Volumetric titrations monitored by UV-Vis spectroscopy revealed that *α*-iminoamidinium ions derived from aliphatic amines exhibited pKa values around 10 (Fig. 1), while the aniline-derived analogue showed significantly higher acidity (pKa = 3.5). These findings indicate that under physiological conditions, *α*-iminoamidines derived from aliphatic amines predominantly exist in their protonated form. Water solubility varied depending on the alkyl chain length, with longer chains leading to lower aqueous solubility and increased lipophilicity. LogP_{ow} and logP_{oPBS} values (up to ~6.0 and ~2.8, respectively) confirmed high membrane permeability potential. The observed combination of adjustable basicity and lipophilicity, governed by simple structural modification of the amine component, positions *α*-iminoamidines as highly promising scaffolds for further biological evaluation. This study provides a physicochemical framework that may guide other researchers in the rational design of structurally related compounds with optimized bioavailability and pharmacokinetic properties.

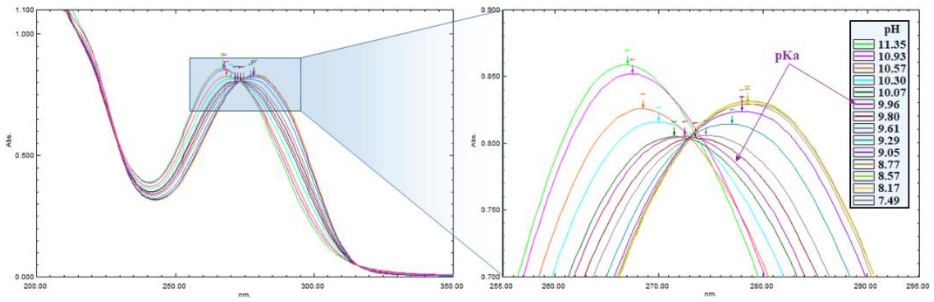

Fig. 1. UV-Vis spectra of (1*E*,2*Z*)-*N*,*N*'-dipropyl-2-(propylimino)-2-(4-iodophenyl)acetamidine in pH range 7.49–11.35

ACKNOWLEDGMENTS

This work was supported by the Ministry of Science, 404 Technological Development and Innovation of Serbia [Grant 405 Nos. 451-03-137/2025-03/200124, 451-03-136/2025-03/200124].

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M13 phage-modified titania nanotubes for *E. coli* detection

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Keywords: bacteriophage, titania nanotubes, surface modification, bacteria detection, electrochemistry

M13 bacteriophages (in short, phages) possess intrinsic ability to infect *Escherichia coli* (*E. coli*) bacteria. These viruses can be genetically modified to display specific peptides on their surface, allowing them to selectively bind to various target molecules [1]. As a result, both engineered and unmodified phages can serve as recognition elements in biosensors. This study focuses on evaluating the performance of sponge-like, laterally spaced, hydrogenated titania nanotubes (S-TiO_2-NTs) as a platform for immobilizing M13 phages in electrochemical systems. The electrodes were prepared through anodization followed by calcination in hydrogen atmosphere [2].

Electrochemical techniques were employed to investigate the capacitive and Faradaic responses of the S-TiO_2-NTs electrodes exposed to different concentrations of M13 phage lysate, immobilized by physical adsorption. The data indicated successful attachment of the phages to the electrode surface, a conclusion supported by scanning electron microscopy imaging. Additional experiments were carried out at 37°C and in the human serum to assess the thermal and biological stability of the electrodes.

Finally, S-TiO_2-NTs electrodes functionalized with wild-type M13 phages were utilized in the detection of *E. coli* bacteria. The electrodes demonstrated a detection limit of 3 cells/ml and a linear detection range spanning from 10 to 10^4 cells/ml. Further tests involving bovine serum albumin as a blocking agent were also conducted. The findings suggest that M13 phages can be effectively immobilized via physisorption on S-TiO_2-NTs electrodes, highlighting their potential application in biosensors [3].

ACKNOWLEDGMENTS

The financial support was given from National Science Centre via project OPUS LAP 2020/39I/ST5/01781 for Katarzyna Siuzdak and project SONATA 2017/26/D/ST5/00980 for Katarzyna Szot-Karpińska.

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Surface modification of quartz using an ionic surfactant mixture

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Keywords: anionic surfactant, cationic surfactant, surfactant synergy, zeta potential, hydrophobicity

Surface-active agents (surfactants) are widely used in various industrial processes, particularly where modification of surface physicochemical properties is desired. One notable application is mineral flotation, where surfactants enhance particle–bubble attachment. In this process, reagents such as collectors, regulators, and frothers are used. Collectors, in particular, play a key role by selectively adsorbing onto mineral surfaces and increasing their hydrophobicity. While conventional collectors are typically anionic or cationic surfactants, recent research has highlighted the potential of mixed anionic–cationic surfactant systems to improve flotation efficiency and selectivity their synergistic effects. When both types of surfactants are mixed, precipitation can occur. Careful adjustment of the surfactant ratio can prevent this phenomenon. On the other hand, if precipitation occurs on the surface of a solid, it can lead to unexpected interfacial properties.

In this study, we investigated the interaction of an anionic, sodium dodecyl sulfate (SDS), and a cationic, dodecylamine hydrochloride (DDA) surfactants, both obtained from Alfa Aesar and further purified by recrystallisation. Fine quartz particles (<40 μm) were treated with surfactant mixtures at different SDS:DDA ratios.

To examine surface modification, two analytical techniques were employed. First, zeta potential of quartz after adsorption of surfactants was determined to assess changes in the electrical double layer. Then, to evaluate modifications in surface hydrophobicity, dried quartz particles were pressed into pellets, and water contact angle measurements were performed.

This topic has not been thoroughly investigated so far. The findings will enable the identification of mixed surfactant systems that exhibit notable synergistic effects.

ACKNOWLEDGMENTS

This work was supported by the National Science Centre, Poland (project number 2023/49/N/ST8/04259, Surfactant synergy in flotation process: Exploring the impact of anionic/cationic surfactants mixtures on thin liquid film stability.

Investigating the role of NPAS4 in endoplasmic reticulum stress under hypoxic and oxidative conditions in N2a cells

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Keywords: hypoxia, endoplasmic reticulum stress, unfolded protein response, NPAS4, neutroprotection

Introduction: NPAS4 (Neuronal PAS domain containing protein 4) belongs to the bHLH-PAS family of transcription factors (TFs). Though NPAS4 was discovered in neuronal cells, later studies showed that protective role fulfilled by NPAS4 was important also in other organs/systems like pancreas, liver, and hematopoietic system [1]–[3]. It has been shown, that increased expression of NPAS4 protects neuronal cells during ischemia and pancreas beta-cells in type II diabetes. The potential cytoprotective mechanism is probably by modulation of ER stress activated both in hypoxic and oxidative stress conditions [4]. Objectives: The mechanism of NPAS4 functioning in ER stress regulation is largely unknown, thus we analyzed expression level and localization of NPAS4 in neuroblastoma cells (N2a), previously successfully used as cell model natively expressing NPAS4. The main goal is to understand the cytoprotective function of the NPAS4 protein and its influence on ER stress under conditions of hypoxia and oxidative stress.It is worth mentioning, that expression of the bHLH-PAS TFs, including NPAS4, is typically very low under homeostatic conditions. It results in difficulties with protein detection. TFs activation occurs in response to specific environmental signals and is tightly regulated over time. Moreover, proteins from this family have a short half-life, which further complicates functional analysis. Therefore, in addition to the studies of endogenous protein, we to perform overexpressed NPAS4 tagged by YFP, which enabled tracking of NPAS4 cellular localization. Hypoxic conditions were induced in two ways:, chemically (specific for HIF - Hypoxic Inducible Factor pathway) using deferoxamine (DFO) or cobalt chloride ($CoCl_2$) and the second physical method using hypoxia chamber in which the oxygen level in very low. Oxidative stress was induced by treatment of the cells with hydrogen peroxide (H_2O_2). **Methodology: RT-qPCR:** Comparison of endogenous *Npas4* expression levels under ER stress induced by hypoxia and oxidative stress in relation to normoxic conditions. We assess expression of ER stress markers (*Perk, Ire1, Atf6, Grp94, Bax, Bcl2*), hypoxia (*Hif1α, Vegfa*), and oxidative stress (*Sod1, Nrf2*); **Western blot**: Comparison of recombinant YFP-NPAS4 protein overexpression under ER stress induced by hypoxia and oxidative stress relative to normoxic conditions; **Confocal microscopy:** Comparison of YFP-NPAS4 protein localization in cell under ER stress induced by hypoxia and oxidative stress relative to normoxic conditions. **Results and conclusions:** We observed increased *Npas4* expression under physiological hypoxia, but not with chemically induced hypoxia, which was marked by unstable, low *Hif1α* and elevated *Vegfa* expression. This aligns with previous reports of *NPAS4* inhibiting *HIF1α* activity in pancreatic β-cells [5]. Notably, *NPAS4* has been identified as an angiogenic factor activated by hypoxia [6]. Our findings suggest that *NPAS4* is induced via HIF1α-independent pathways. Additionally, *NPAS4* may alleviate ER stress by modulating UPR markers. Preliminary imaging revealed a punctate cytoplasmic distribution of YFP-NPAS4 in N2a cells during hypoxia (Fig. 1), indicating potential involvement in stress granule formation [7] and a role in cellular stress responses.

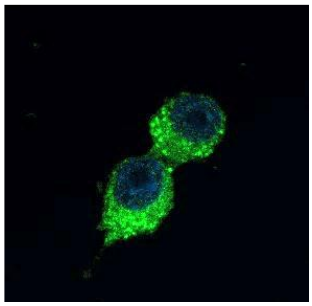

Fig. 1. Representative image of YFP-NPAS4 expressed in cells in hypoxia generating chamber

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BV6 – A SMAC mimetic that sensitizes cancer cells to apoptosis and chemotherapy

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Keywords: apoptosis, BV6, cancer, IAP degradation, Smac mimetics

SMAC mimetics represent a promising class of compounds with potent anticancer activity by mimicking the function of the endogenous SMAC/DIABLO protein in regulating apoptosis. They act by binding to inhibitor of apoptosis proteins (IAPs), such as cIAP1, cIAP2, and XIAP, and promoting their proteasomal degradation. This, in turn, relieves the IAP-mediated caspase inhibition and effectively restores the ability of tumor cells to undergo apoptosis. The bivalent SMAC mimetic BV6 has demonstrated potent pro-apoptotic effects by inducing the proteasomal breakdown of cIAP1 and cIAP2, and in many cases, XIAP, thereby triggering caspase-dependent cell death across numerous human tumor cell lines. In in vitro studies using a panel of 10 cell lines (PCI-1, PCI-9, PCI-13, PCI-52, PCI-68, Detroit 562, FaDu, SCC-9, SCC-25, HaCaT), BV6 reduced cell viability in a concentration-dependent manner (IC50 range: 1.2–8.7 μM) and significantly raised the population of late apoptotic cells. Furthermore, this compound effectively degraded cIAP1 and, in most cases, cIAP2 and XIAP, although HaCaT and SCC-9 were less susceptible to these effects.

Synergistic effects were observed when BV6 was combined with FasL or cisplatin. This combination resulted in a marked increase in cytotoxic effects and successfully overcame resistance to monotherapy in certain cell lines, such as SCC-9 and PCI-52. Importantly, BV6 was able to sensitize previously resistant cells to the extrinsic apoptosis inducer TRAIL, adding a powerful dimension to its potential therapeutic application.

Furthermore, in cisplatin-resistant SCC4 (SCC4cisR) cells, BV6 demonstrated potent monotherapy effects, reducing cIAP1, XIAP, and livin levels and sensitizing these cells to cisplatin-induced apoptosis. Importantly, this highlights the ability of SMAC mimetics to circumvent resistance mechanisms and enhance the efficacy of conventional chemotherapy and death receptor-directed therapies.

These results collectively underscore the potential of BV6 as a novel therapeutic agent to treat a range of human malignancies, including those that are resistant to standard treatment modalities. Nevertheless, further investigations are required to fully elucidate the mechanisms of action of BV6, to assess its in vivo efficacy, and to identify patient subsets most likely to benefit from combination strategies involving SMAC mimetics. Such knowledge may help guide the future design of personalized therapeutic approaches for challenging and drug-resistant cancers.

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Modern organic matrices as carriers of fragrance compounds

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Keywords: carriers, ethosomes, fragrance, limonene

Traditional methods of delivering active substances have proven inefficient, as the substances typically affect the entire organism rather than the intended target site. Modern carriers, such as ethosomes (vesicular structures based primarily on ethanol), have been developed to enable selective delivery to specific locations in the body, thereby enhancing efficacy and reducing side effects [1–5].

The aim of the research was to synthesize and characterize modern carriers based on an organic matrix, such as ethosomes, and to test their potential for use as carriers of selected substances, e.g., limonene, a compound commonly found in cosmetic products.

The synthesis of ethosomes was based on preparing two phases, the first of which consisted of phospholipids (2.5% by weight), ethanol (30.0% wt.), ethylene glycol (10.0% wt.), surfactant (1.25% wt.), and limonene (1.0% wt.), while the second consisted of trehalose (1.0% wt.) and water (up to 100.0% wt.). Both phases were heated to 30°C and then, while stirring on a magnetic stirrer (300 rpm), phase II was mixed with phase I. The mixture was stirred for 15 minutes on a magnetic stirrer and then homogenized for 1 minute (15.000 rpm). The stability of the obtained carriers was determined by measuring the zeta potential (ZP), average particle size (Z-Ave), and polydispersity index (PDI). The analyses were performed immediately after preparation of samples, after 24 hours, and after 14 days of storage in a refrigerator.

To assess sample stability, zeta potential (ZP) was measured using the electrophoretic light scattering technique. In addition, average particle size (Z-Ave) and polydispersity index (PDI) were determined using dynamic light scattering (DLS). All synthesised samples exhibited ZP values above 20 mV, average particle sizes around 150 nm, and PDI values between 0.2 and 0.5, indicating good stability.

The results confirmed the possibility of obtaining organic matrices with favorable physicochemical parameters. Future work will focus on evaluating the limonene release profile and further optimizing the etosomal systems for transdermal and topical applications.

ACKNOWLEDGMENTS

Acknowledgements of funds from ID-UB (Initiative for Excellence – Research University) AMU program, grant no 155/34/UAM/0024.

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Upcycling of brewing byproducts into the beer production process: a circular and functional brewing approach

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Keywords: beer, brewers' spent grains, spent hops, byproduct valorisation, sustainable brewing

This study explored the feasibility of incorporating brewers' spent grain (BSG) and spent hops (SH) – the two main byproducts of the brewing industry – into the production of a novel beer, aligning with circular economy principles and sustainable process design. The study aimed to evaluate the brewing potential of these materials, optimize mashing conditions for improved fermentable sugar extraction, and assess the physicochemical and sensory characteristics of the resulting beer.

Preliminary testing involved congress mashing and hydrolysis methods to assess fermentable sugar potential from BSG and bitter compound retention in SH. Preliminary trials demonstrated that SH retained significant bittering potential and that BSG, though partially depleted, still contained usable fermentable sugars. While acid and enzymatic pre-treatments were tested, they did not outperform conventional mashing. A Box-Behnken experimental design was used to optimize mashing conditions (rest time and temperature) for a mixture of BSG and malt. FTIR and HPLC characterized BSG and SH, while density and bitterness measurements assessed wort and beer properties. A semi-industrial brewing trial (70 L) was conducted under optimized conditions, and the resulting beer was evaluated through physicochemical analysis and sensory testing (n = 39).

The optimized mashing regime (60 min at 62.5°C followed by 22.5 min at 72.5°C) produced wort with sufficient fermentable sugars. Beer produced at pilot scale using BSG, SH, and recycled yeast showed high bitterness (68 IBU), dark color (27 EBC), and elevated phenolic content (472 mg GAE/L), aligning with craft beer profiles. Consumer testing showed 82% acceptability with no significant difference compared to a commercial reference, though overall taste scores were slightly lower (average 6/10), with bitterness and herbal aroma noted as defining features.

The study confirms the feasibility of reintegrating BSG and SH into the brewing process without compromising beer quality or consumer acceptance. Mashing optimization proved more effective than chemical or enzymatic pre-treatments, and the final product exhibited characteristics suitable for a niche craft beer market. This work demonstrates that BSG and SH can be effectively upcycled to produce a beer with unique sensory qualities and market potential. The approach offers not only an innovative brewing concept but also a scalable and sustainable application for industry byproducts.

Future work should explore recipe adjustments – particularly bitterness reduction through spent hops dosage optimization – to increase appeal among a broader consumer base. Additional investigation into shelf-life stability, nutritional labeling potential (e.g., antioxidant and fiber content), and the behavior of different yeast strains could further improve the product. Given the successful scale-up and consumer acceptance, the process holds strong potential for commercial application, including patent submission and integration into small- to medium-scale brewery operations seeking sustainable and innovative product lines.

Fig. 1. Graphical abstract

ACKNOWLEDGMENTS

Financial support of this research from the Erasmus Mundus program of the European Union is gratefully acknowledged.

Lupulin in the industrial field as an alternative source of Xanthohumol

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Keywords: flavonoid, purification, chromatographic techniques

Xanthohumol is a natural compound, flavonoid, which can be found almost exclusively in the female inflorescence of hops *(Humulus lupulus)*. This prenylated chalcone has diverse biological activities, such as antitumor, antioxidant, antibacterial, antiviral, and anti-inflammatory properties, which results in its growing interest in functional food, cosmetics and pharmaceutical areas. However, the daily intake of Xanthohumol is limited, as the main source of it in the human diet is – beer. Moreover, during the brewing process a large part of Xanthohumol can easily be isomerized to less biologically active Isoxanthohumol [1, 2].

Main industrial source of Xanthohumol are spent hops, a waste product after *sc*CO_2 extraction in production of hop extract. In our research, we used lupulin and lupulin after *sc*CO_2 extraction, unusual materials, not subjected to any thorough evaluation as an alternative source of Xanthohumol. Lupulin is a yellow powder from hop female glands, also used in beer brewing, which mainly contains lots of resin compounds and essential oils, which might lead to more complicated purification processes [3]. Analysed Xanthohumol concentrations were compared with values obtained for two different types of spent hops and hop granulate. Extraction experiments were conducted for all five materials with different solvents (acetone, methanol and ethanol), resulting in selection of the best conditions for extraction. Extracts were split and subjected to chromatography and acid-base precipitation. The final purity of Xanthohumol using a combination of these techniques was determined by HPLC analysis and resulted in 91% purity and the total process yield of 40%.

In future research, obtained purified Xanthohumol will be used for biotransformations and biocatalysis.

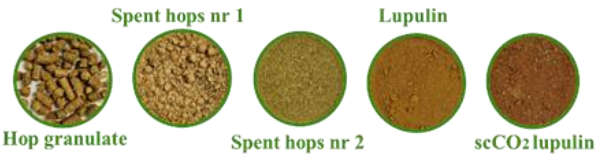

Fig. 1. Hop waste materials for Xanthohumol extraction used for concentration comparisons in this research

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New ferrocene-based hydroxamic acids as urease inhibitors

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Keywords: urease inhibition, hydroxamic acids, ferrocene derivatives

Urease is a nickel-dependent enzyme that hydrolyzes urea into ammonia and carbon dioxide, raising the local pH and promoting the pathogenicity of bacteria like *Helicobacter pylori* and *Proteus* spp. This activity contributes to conditions such as gastric ulcers and urinary stones, making urease a key therapeutic target. Among its most potent inhibitors, hydroxamic acids are particularly effective due to their strong metal-chelating ability, which allows them to inactivate the enzyme by binding to its nickel-containing active site [1].

Bioisosteric replacement of aryl groups with ferrocene (Fc) in biologically active molecules is known to enhance both biological activity and pharmacokinetic properties [2]. In this study, several novel Fc-substituted hydroxamic acids were synthesized and their urease inhibitory activity compared to phenyl-based analogues (Fig. 1). *In vitro* inhibition was assessed against Jack bean urease using the standard indophenol-based spectrophotometric assay at 100 μM. Among the Fc derivatives, 3-ferrocenylpropanohydroxamic acid (**2**, 84%) and 4-ferrocenylbutanohydroxamic acid (**3**, 85%) showed significantly higher inhibition than the standard acetohydroxamic acid (AHA; 66%). 5-Ferrocenylpentanohydroxamic acid (**4**, 68%) showed comparable activity to AHA, while ferrocenecarbohydroxamic acid (**1**, 53%) was less active. Phenyl-substituted analogues also showed notable activity, with 4-phenylbutanohydroxamic acid (**3a**, 72%) and 5-phenylpentanohydroxamic acid (**4a**, 67%) outperforming benzohydroxamic acid (**1a**, 55%), though still less potent than the top-performing Fc derivatives. These results suggest that the incorporation of a lipophilic, redox-active Fc moiety, especially at optimal distances from the hydroxamic group, enhances interaction with the urease active site, leading to improved inhibition. The superior activity of Fc-based inhibitors highlights the need for further structural optimization and mechanistic studies, including metal-binding assessments and molecular docking, to better understand their mode of action.

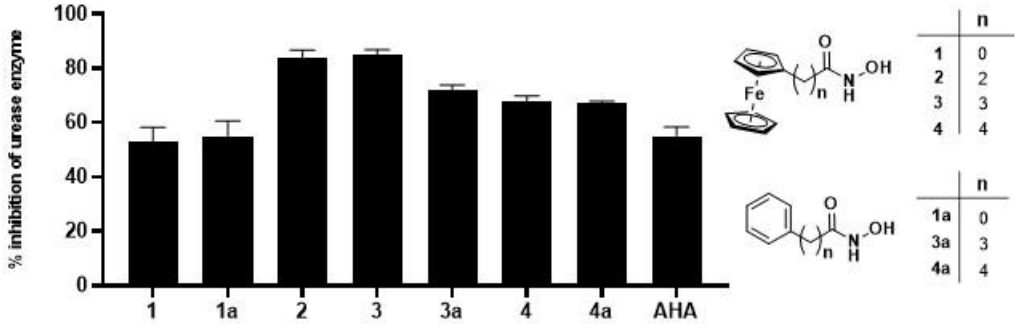

Fig. 1. Structures and anti-urease activity of phenyl- and ferrocenyl-analogs

ACKNOWLEDGMENTS

The work was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number: 451-03-136/2025-03/200124 and 451-03-137/2025-03/ 200124).

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Application of additive technologies for the fabrication of polymer composite ceramic objects imitating bone structure from light-curing resins using zirconium oxide

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Keywords: nanocomposites, photopolymerization, 3D printing, bones

Photopolymerization, as a fast-growing technology based on the process of curing light-sensitive resins, is getting more popular in materials engineering, including advanced ceramic component production. This method involves the selective curing of liquid resins using light of a specific wavelength, which enables the precise shaping of microstructures with high resolution. It is particularly important in the context of additive manufacturing techniques, such as stereolithography (SLA) or digital light projection (DLP), where it is possible to reproduce complex spatial geometries using composite resins containing highly packed ceramic fillers. Among the structures of particular importance in biomedical engineering are porous gyroidal systems. The combination of an organic photopolymer matrix with an inorganic filler, e.g., zirconium oxide, allows for the production of composites that are then subjected to a controlled firing process. As a result of the removal of the organic fraction and the densification of the ceramic skeleton, a highly porous material with characteristics typical of functional ceramics is formed. Maintaining the right ratio of porosity to mechanical integrity is important for later applications as bone replacements. The three-dimensional structures produced in this way can be successfully used in biomedicine, especially in the fields of tissue engineering and implantology. Their high porosity, similar to that of natural bone tissue, promotes osteointegration processes, and a carefully selected chemical composition – e.g., stabilized zirconium oxide – ensures biocompatibility and long-term stability of the material in a physiological environment. Thanks to the possibility of controlled design of the internal architecture, such structures can also be used as in vitro models for research on the proliferation and differentiation of bone cells (osteoblasts), enabling the reproduction of conditions prevailing in the actual extracellular matrix of bone [1].
In this work, three-dimensional polymer composite objects in the form of gyroidal prints were made using zirconium oxide as an inorganic filler, manufactured using the 3D VPP method, and the effect of the filler on photopolymerization processes and the behavior of the object after firing was investigated. It has been observed that a higher weight concentration of the filler used leads to an increase in the viscosity of the composition, but results in less polymerization shrinkage after the process. After the firing process, a significant change in the dimensions of the obtained objects was observed. Due to the evaporation of the polymer, the object consisted only of a ceramic skeleton and underwent significant shrinkage.

ACKNOWLEDGMENTS

Research funding from the National Center for Research and Development – LIDER PROJECT13/0081/2022 “Innovative porous ceramic materials printed by DLP technique using high-performance photochemical initiators dedicated to bone tissue integration”

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microRNAs in Pancreatic Cancer – emerging therapeutic strategies – review

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Keywords: microRNA, pancreatic cancer, therapeutic strategies

Pancreatic cancer (PC) is among the most lethal malignancies due to its late diagnosis and limited treatment options [1]. This review aimed to summarize recent findings regarding the role of microRNAs (miRNAs) in PC progression, diagnosis, and therapy, with a focus on their potential as therapeutic targets and drug sensitizers.

A comprehensive review of current literature was conducted to identify key miRNAs involved in pancreatic cancer pathogenesis. Studies exploring oncogenic and tumor-suppressive miRNAs, their therapeutic inhibition or restoration, and emerging drug delivery strategies were examined.

miRNAs have been shown to act as either tumor suppressors or oncogenes, regulating processes such as proliferation, apoptosis, angiogenesis, and metastasis [2, 3]. Oncogenic miRNAs (e.g., miR-21, miR-155, miR-196a, miR-210, miR-221) were frequently upregulated in PC and implicated in tumor progression, chemotherapy resistance, and survival pathway activation [3, 4]. Inhibition of these miRNAs using antisense oligonucleotides (ASOs) or antagomirs significantly reduced cancer cell proliferation and improved sensitivity to gemcitabine in preclinical models [5, 6]. Combination therapies with ASOs and gemcitabine resulted in enhanced tumor suppression [6]. Furthermore, restoration of tumor-suppressive miRNAs such as miR-34a, miR-146a, and miR-145 – through demethylating agents or natural compounds (e.g., isoflavones, curcumin analogues) – was effective in limiting epithelial-to-mesenchymal transition (EMT) and metastatic potential [7, 8].

Nanoparticle-based delivery platforms were also reported to improve miRNA stability, circulation time, and therapeutic efficacy. Co-delivery of miRNAs with chemotherapeutic agents, such as miR-205 with gemcitabine, has shown potential in overcoming drug resistance and enhancing treatment outcomes [9, 10].

miRNAs represent promising biomarkers and therapeutic targets in pancreatic cancer. Their modulation through inhibition or restoration – especially when combined with chemotherapeutic agents and advanced delivery systems – may improve clinical outcomes. Future research should focus on personalized miRNA expression profiling and further clinical trials to validate the safety and effectiveness of miRNA-based therapies in PC treatment.

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Comparison of the volatiles emitted by *in vitro* shoot cultures *Pinus pinaster* with different susceptibility to Pine Wilt Disease

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Keywords: *Bursaphelenchus xylophilus*, *Pinus pinaster*, in vitro cultures, Volatile Organic Compounds (VOCs), Pine wilt disease (PWD)

The pinewood nematode (PWN) *Bursaphelenchus xylophilus*, is the causal agent of Pine Wilt Disease (PWD), a devastating threat to conifer forests. *Pinus pinaster* displays variable susceptibility to the PWN, yet the underlying biochemical basis of resistance remains poorly understood. In this study, *in vitro* shoot cultures of *P. pinaster*, with different susceptibility to PWD, were established and characterized through volatile organic compound (VOC) profiling to investigate potential metabolomic markers of resistance. Explants were inoculated with the PWN and monitored over a period of 1 month, with PWD symptomatology stages recorded to assess disease progression. Notably, the genotype with higher susceptibility reached a higher degree of symptomatology, while the genotype with lower susceptibility showed delayed disease progression. Volatile emissions were analysed, over the course of infection, via headspace extraction followed by Thermal Desorption coupled to GC-MS. Under control (non-inoculated) conditions, both genotypes emitted similar proportions of α- and β-pinene. However, the genotype with lower susceptibility exhibited higher levels of limonene. During PWN infection, the genotype with higher susceptibility showed a progressive increase in α-pinene, β-pinene and limonene emissions as symptom severity advanced. In contrast, the genotype with lower susceptibility exhibited a decrease in these compounds over the progressive symptom stages. These findings suggest that volatile terpene emissions may play a role, either directly or indirectly, in the defence strategy of *P. pinaster* genotypes. The use of *in vitro* culture systems offers a reproducible platform to explore these biochemical traits and their functional relevance in host-pathogen interactions. This study highlights the potential of VOC profiling as a tool to identify early biomarkers of resistance in an effort to develop more sustainable strategies, aimed at improving PWN control and management.

ACKNOWLEDGMENTS

The authors wish to thank Isabel Carrasquinho at INIAV for providing the seeds for these genotypes. This research was partly funded by the EU under the PurPest project through grant agreement 101060634.

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New pyridine-3-carboxamide derivatives as collagenase-3 inhibitor with anticancer activity

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Keywords: MMP-13 inhibitors, CADD, molecular modeling, anticancer drugs, dimethylpyridine derivatives

Matrix metalloproteinase-13 (MMP-13 also known as collagenase-3) is a zinc-dependent proteolytic enzyme that plays a role in degrading and remodelling extracellular matrix components, particularly type II collagen. Although MMP-13 plays a physiological role in bone development and cartilage remodelling, its overproduction has been associated with pathological conditions such as rheumatoid arthritis, cardiovascular disorders and the progression of various type of cancers. MMP-13 promotes tumour cell invasion by degrading the extracellular matrix and inducing angiogenesis via the upregulation of VEGF and VEGFR-2. It also modulates the tumour microenvironment, enhancing inflammation and cancer cell invasion. Elevated levels of MMP-13 have been observed in advanced melanoma, breast and colorectal cancers, making it an attractive target for the development of anticancer drugs. This study involved designing, synthesis and evaluating a series of novel pyridine-3-carboxamide derivatives as potential MMP-13 inhibitors with anticancer properties. A hybrid drug design approach combining ligand- and structure-based methods was applied. The physicochemical properties of the synthesised compounds were characterised, and their binding affinity to MMP-13 was assessed using molecular modeling methods. The biological evaluation of the compounds was performed using MTT assays on two cell lines: human colorectal cancer cells (HT-29) and melanoma cells (A375). Normal Human Dermal Fibroblasts (NHDF) were also included in the study to assess selectivity and potential cytotoxicity towards non-malignant cells. Several derivatives demonstrated notable cytotoxicity, with selected compounds showing IC_{50} values in the low micromolar range. Docking studies supported their high affinity for the MMP-13 active site, indicating that these compounds may exert anti-cancer activity through MMP-13 inhibition.

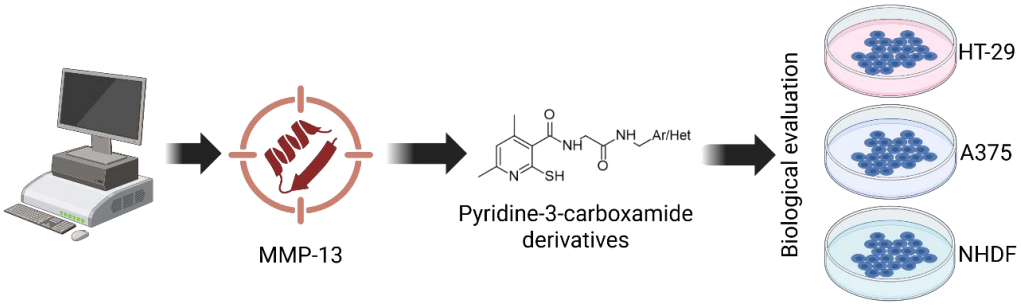

Fig. 1. In silico design, synthesis, and biological evaluation of novel derivatives of dimethylpyridine

ACKNOWLEDGMENTS

The authors gratefully acknowledge the allotment of the CPU time for calculations in the Wroclaw Centre of Networking and Supercomputing (WCSS); This research was funded by Wroclaw Medical University, grant number SUBZ.D290.25.066.

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Crystal structure of the novel silver(I) complex with an adamantane derivative

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Keywords: dihydrazones, 2,6-diacetylpyridine, synthesis, structural characterization

For the last few decades, adamantane (Ad) derivatives have represented a widely studied group of compounds due to their versatile biological activity, i.e., antiviral, antibacterial, antidiabetic, antimalarial, anticancer, and antiinflammatory [1]. To obtain better insights into their potential applications in drug design, it is of high interest to structurally characterize not only adamantane derivatives but also their metal complexes. In this work, the synthesis and structure of the new silver(I) complex with the symmetric derivative of adamantane-1-carbohydrazide and 2,6-diacetylpyridine (L), of the formula $[Ag(L)]ClO_4$, is presented. White needle-like crystals were obtained by evaporating the solution formed by mixing a warm suspension of L in EtOH and a warm EtOH solution of $AgClO_4$, in a molar ratio of 1:1. The complex shows good solubility in acetonitrile and dimetylformamide, while it is sparingly soluble in acetone, and almost insoluble in water and alcohols. The coordination mode was assumed by FTIR spectroscopy, and the SC-XRD analysis confirmed the N3O2 pentadentate coordination of the ligand molecule, resulting in the formation of four five-membered chelating rings (Fig. 1). The geometrical parameters of the obtained complex were compared with those of the ligand and earlier characterized Co(II) complexes [3], so the effects of coordination and metal center were studied. To investigate the pentagonal-planar coordination environment of Ag(I) found in this complex, a search of the CSD was conducted. Among 26 structures, there are 16 clusters and 7 macrocyclic compounds. So, this coordination mode was found in only three silver(I) complexes (Ref. codes JACVIS, DEMKEO, and DEMKAK) besides the one presented here. This could enable interesting spectroscopic and fluorescent properties of the complex, which will be examined in the future.

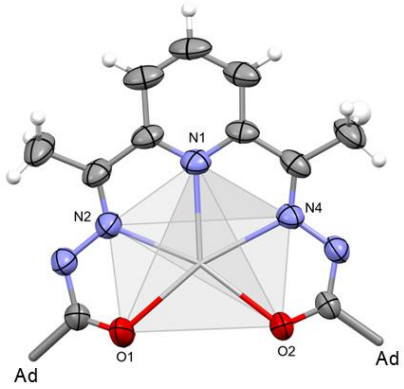

Fig. 1. The structure of $[Ag(L)]^+$ and coordination environment of the metal center (adamantane moieties are omitted for clarity)

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/200125).

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Traditional plants from Madeira with digestive properties: a literature review on fennel, marcela and peppermint

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Keywords: fennel; marcela; peppermint; Madeira island; digestive properties

On Madeira Island, the use of plants with therapeutic properties is a common practice in folk wisdom, passed down from generation to generation, like fennel (*Foeniculum vulgare*), peppermint (*Mentha × piperita*) and marcela (*Santolina chamaecyparissus*), widely used by the Madeiran population to relieve gastrointestinal discomfort, in the form of infusions.

The aim of this work is to explore the phytochemical composition and therapeutic properties related to the digestive system, of this three species.

A literature review was carried out in the PubMed® database, analysing several scientific articles dating from the last five years that focused their themes on the terms "mentha piperita gastrointestinal effects", "fennel", "Santolina chamaecyparissus" and "Medicinal plants digestive health". The review shows that these plants contain compounds such as anethole, flavonoids, and menthol, with carminative, spasmolytic, and anti-inflammatory properties. Although the results are promising, the methodological diversity and the limitations of clinical trials in humans reflect the need for additional research to validate the safety and efficacy of these species. Even so, the studies indicate consistency between traditional use and current scientific data, reinforcing the potential use of these species as adjuvants in the treatment of mild gastrointestinal disorders.

It is concluded that future studies are needed to validate therapeutic efficacy and establish reliable and safe recommendations for clinical practice.

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Binding affinity of indole ligands to G4 DNA – preliminary study

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Keywords: Indole derivatives, heterocyclic compounds, guanine quadruplex (G4 DNA), ligand-DNA interaction, spectroscopic characterization

Heterocyclic compounds play a crucial role in medicinal chemistry and pharmacy. Their diverse biological activities and specific reactivity make them essential in drug development. Indole, a weakly basic heterocyclic compound, features a unique structure composed of fused six-membered benzene and five-membered pyrrole rings. Due to its distinctive physicochemical and biological properties, including the ability to bind to various receptors and enzymes, indole serves as a privileged scaffold in the design of new biomedicinal compounds [1, 2].

Guanine quadruplexes (G4 DNA) are unique, non-canonical, folded single- or multistranded nucleic acid structures formed in G-rich DNA sequences. They are formed by multiple vertically stacked guanine quartets (G-tetrads), which are stabilized by Hoogsteen hydrogen bonds in the presence of selected metal cations (e.g., Na^+, K^+) and can be stabilized by small organic ligands [3]. The therapeutic potential of these ligands in cancer treatment is linked to their ability to inhibit or poison DNA enzymes, particularly topoisomerases and telomerase, as well as the regulation of gene expression at transcriptional or translational levels [4]. Ligands that specifically interact with DNA are utilized in bioanalytics (for detecting and diagnosing gene sequences or their mutated forms) and biomedicine (developing new drugs and therapies for cancer, viral, and genetic diseases) [5].

In this study, the library of indole derivatives was expanded to include new compounds. The binding affinity of indole ligands to the four-stranded DNA structure formed on the oligonucleotide sequence of the human proto-oncogene c-MYC was investigated, using UV-Vis, fluorescence, and CD spectroscopic techniques. The study of DNA melting in the presence of ligands has been extended to include thermodynamic analysis of denaturation profiles.

ACKNOWLEDGMENTS

This work was partly financed by the ID-UB 161 grant number 161/34/UAM/0004.

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How does bioelectrostimulation enhance Thiabendazole degradation in microbial electrochemical systems?

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Keywords: bioelectrodegradation, bacterial shifts, biostimulation, wastewater treatment, recalcitrant fungicide

Bioelectrochemical systems (BES) have demonstrated a promising potential in treating wastewater containing organic pollutants that are challenging to eliminate using conventional approaches. For this purpose, this study investigated the potential of BES inoculated with Tunisian saline sediments (TSS) for the degradation of the persistent fungicide thiabendazole (TBZ). TSS underwent different stages of biostimulation with increasing doses of TBZ (0, 10, 100, and 300 mg Kg^{-1}). Each reactor was fed with a synthetic medium (80%) containing thiabendazole solution (150 ppm) and glucose as a co-substrate (30 ppm), and 20% TSS. The reactors operated for 35 days using carbon felt as a working electrode (72 cm^2 L^{-1}) polarized at 0.1 V/SCE and maintained at 25°C. While current production was low, the pretreated sediments with 100 mg kg^{-1} of TBZ generated the highest current density (3.2 mA m^{-2}), a fivefold increase over untreated sediments (0.6 mA m^{-2}). GC-FID analysis showed over 99% TBZ degradation in all reactors. The TBZ half-elimination time decreased from 27 days with biological treatment alone, to 19 days in BES, and further to 6 days following biostimulation. Confocal Laser microscopy revealed great modifications in the biofilm's extracellular polymeric matrix, specifically in protein and polysaccharide content, correlated with enhanced current generation.

Metataxonomic analysis indicated a marked shift in bacterial community composition after biostimulation, with a reduction in Bacillota (−64%) and an increase in Proteobacteria (+62%), dominated by *Pseudomonas* (45%) and *Marinobacter* (16%). These findings highlight the potential of biostimulation to selectively enrich electroactive microbial communities and enhance bioelectrogeradation kinetics for TBZ wastewater treatment.

Photobiocatalytic synthesis of dimethyl-3-hydroxyprop-1-enylphosphonate

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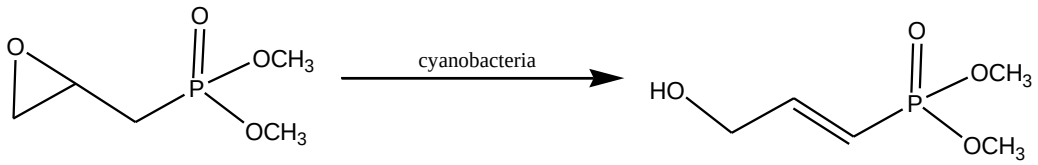
Keywords: biotransformation, cyanobacteria, phosphonate, bioreactor

Cyanobacteria are the largest and most diverse photosynthetic prokaryotes capable of growing in extreme habitats and restoring nitrogen in the atmosphere. As a rich source of biologically active compounds, they are applied to many industries, such as pharmaceuticals, cosmetics, and food. Cyanobacteria hold great promise as biocatalysts because they convert CO_2 into fuels, chemicals, and other value-added products during photosynthesis. This ability makes cyanobacteria attractive hosts for sustainable industrial processes [1, 2]. Biotransformation is an environmentally friendly method that allows for the production of pure isomers, but the problem with this approach is low efficiency and the negative impact of high substrate concentrations on the catalytic activity of biocatalysts.

Synechococcus bigranulatus, *Nostoc cf-muscorum*, *Limnospira indica*, *Limnospira maxima*, *Leptolyngbya foveolarum*, and *Nodularia moravica* cultures were used as the biocatalyst in a biotransformation of epoxymethyl dimethyl phosphonate. The process was carried out for 7 days at 29°C (±1) under continuous illumination and stationary conditions. The products were extracted with ethyl acetate and analysed by ^{31}P NMR. Selected strains of cyanobacteria were used to scale up the process in the 1 L bioreactor. Substrate toxicity tests were also performed due to the inhibitory properties of phosphonates.

Tested cyanobacteria strains were able to transform 1 mM epoxyphosphonate with high conversion degree, reaching over 99% after 1 and 3 days of bioconversion for *Limnospira* sp. and *S. bigranulatus* respectively. The use of *S. bigranulatus* in a bioreactor demonstrated high activity of this strain and resistance to 5 mM substrate concentration. After 4 days of biotransformation, the conversion degree reached 97%. Conducted experiments have shown the stability of cyanobacteria cells under high substrate concentrations, which indicated the possibility of increasing the scale of the process.



epoxymethyl dimethyl phosphonate

dimethyl
(1*E*)-3-hydroxyprop-1-enylphosphonate

Fig. 1. Biotransformation of epoxymethyl dimethyl phosphonate with the use of cyanobacteria cells as biocatalyst

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Insights into the solution-state structural behavior of Marubiin via ^{1}H NMR analysis

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Keywords: Marrubiin, acetone-d_6, spin simulation, conformation

Marubiin, a labdane-type diterpenoid isolated from *Marrubium peregrinum*, exhibits various pharmacological activities, including antispasmodic, anti-inflammatory, and antinociceptive effects. To elucidate its structural and conformational properties in solution, ^{1}H NMR spectra were recorded in several solvents. Notably, the signals in the spectrum obtained in acetone-d_6 were the most resolved, and the spin–spin coupling patterns were well-defined, even though they appeared as higher-order multiplets. Manual ^{1}H NMR–spectra spin simulation [1] and fine-tuning of coupling constants revealed that marubiin adopts a single, well-defined dominant conformation in this solvent (Fig. 1). This conformational rigidity is particularly intriguing, given the presence of a flexible aliphatic chain between the cyclic parts of the structure, as well as within a six-membered ring that contains three additional methylene groups. The observed structural constraint may be the reason of the marubiin's enhanced interaction with biological targets, therefore contributing to its pharmacological efficacy. These findings provide a foundation for future structure–activity relationship studies and molecular docking simulations aimed at exploring marubiin's bioactivity.

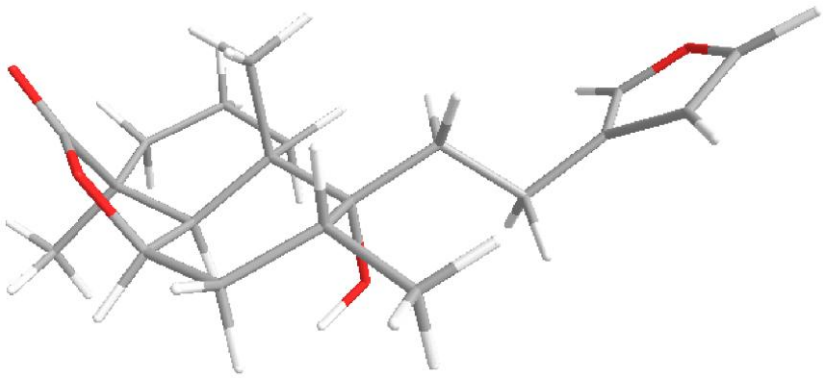

Fig. 1. 3D structure of marubiin

ACKNOWLEDGMENTS

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Aminated cellulose nanocrystals as flocculants for fine quartz and chalcocite particles

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Keywords: nanocellulose, flocculation, flotation, fine particles

Fine solid particles have long posed a problem in many industrial processes. They form stable suspensions in solutions and do not undergo efficient separation, for example, in the flotation process [1].

One known solution to this problem is particle aggregation using the process of flocculation, which is conventionally carried out with ionic flocculants. Ionic flocculants are most often of synthetic origin, making them less biodegradable and potentially harmful to living organisms. Therefore, the aim of this study was to investigate new reagents capable of aggregating fine solid particles, but based on the most abundant biopolymer – cellulose.

In our research, the cellulose was first oxidized to 2,3-dialdehyde cellulose using sodium periodate (Fig. 1A). In the next step, the reductive amination with ethyl-, *n*-butyl-, and *n*-hexylamine in presence of 2-picoline-borane was performed (Fig. 1B and C). The modified microfibers were then subjected to high-pressure homogenization to obtain aminated cellulose nanocrystals.

The efficiency of the aggregation processes was evaluated using the static multiple light scattering (SMLS) technique, measuring changes in transmittance (T) and backscattering (BS) over time. Analysis of the backscattering (BS) and transmittance (T) profiles enabled the determination of the sedimentation rate index. The influence of parameters such as pH (3, 6, and 9), as well as the concentration of aminated nanocrystals, was investigated. To explain the nature of the interactions, zeta potential was measured.

The obtained results indicate that aminated cellulose nanostructures can effectively destabilize both chalcocite and quartz suspensions, although this effect is highly dependent on the pH of the environment.

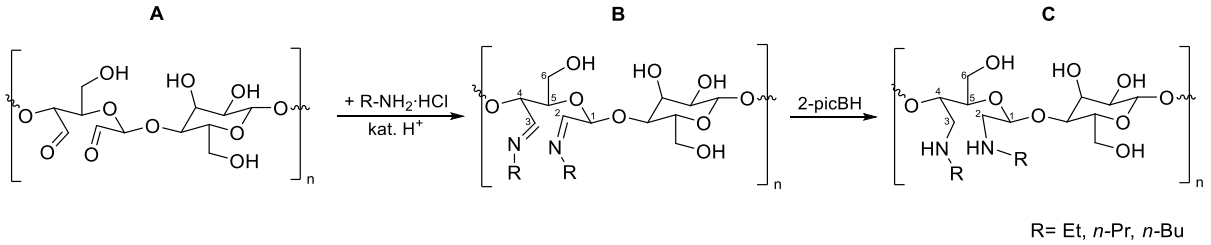

Fig. 1. Indirect amination reaction of 2,3-dialdehyde cellulose

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Antioxidant activity of polyphenolic extracts from the leaves and fruits of *Elaeagnus multiflora* Thunb.

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Keywords: plant extracts, polyfloral oleaster, free radical, UVC, AAPH

Plants rich in polyphenols play a significant role in the prevention of numerous diseases associated with oxidative stress. Elaeagnus multiflora Thunb. (commonly known as the cherry silverberry) is recognized for its high content of bioactive compounds. The aim of this study was to evaluate the antioxidant activity of polyphenolic extracts derived from the leaves (EML) and fruits (EMF) of *Elaeagnus multiflora*, with particular focus on their free radical scavenging capacity.

Polyphenol and isoprenoid profiles were analyzed using UPLC-PDA-MS/MS and RRLC-MS/MS, while organic acids were identified using HPLC-RID. All analyses were conducted at the Department of Fermentation and Cereals Technology, Wrocław University of Environmental and Life Sciences [1]. The total polyphenol content (TPC) was determined spectrophotometrically using the Folin–Ciocalteu method. Antiradical activity was assessed via the DPPH assay and compared to the standard antioxidant L-(+)-ascorbic acid. Additionally, the antioxidant potential of Elaeagnus multiflora extracts was evaluated against erythrocyte "ghost" membranes and model lipid membranes (RBCL), using both spectrophotometric and fluorometric methods. Two oxidation inducers – UVC radiation and AAPH – were employed in these assays.

The results indicate that the analyzed extracts from *Elaeagnus multiflora* (cherry silverberry) are rich in polyphenolic compounds, particularly quercetin and kaempferol derivatives. The total polyphenol content (TPC), expressed as gallic acid equivalents (GAE), was determined to be 180.46 ± 2.63 mg GAE/g for the leaf extract and 271.92 ± 8.34 mg GAE/g for the fruit extract. The fruit extract demonstrated higher free radical scavenging activity, with an IC_{50} value of 13.23 ± 1.24 µg/mL, comparable to that of the reference antioxidant L-(+)-ascorbic acid (IC_{50} = 12.82 ± 1.23 µg/mL). In contrast, the leaf extract exhibited lower activity (IC_{50} = 61.21 ± 3.70 µg/mL).

Furthermore, the antioxidant activity of the extracts was assessed using two complementary membrane systems: erythrocyte “ghosts” and liposomes composed of lipids extracted from erythrocyte membranes, serving as a biomimetic model of the red blood cell membrane. Oxidative damage was induced by two different pro-oxidants: UVC radiation and AAPH. Under UVC-induced oxidation, the leaf extract exhibited stronger protective effects on erythrocyte ghosts (IC_{50} = 7.55 ± 0.05 µg/mL) than the fruit extract (IC_{50} = 13.42 ± 1.88 µg/mL). In contrast, during AAPH-induced peroxidation of erythrocyte-lipid-based liposomes (RBCL), the fruit extract showed greater antioxidant efficacy (IC_{50} = 3.15 ± 0.26 µg/mL) than the leaf extract (IC_{50} = 10.03 ± 0.50 µg/mL). These findings indicate that the antioxidant effectiveness of *Elaeagnus multiflora* extracts depends not only on their polyphenolic composition but also on the type of oxidative agent and the membrane model used.

The studies confirm the antioxidant potential of *Elaeagnus multiflora* leaf and fruit extracts, with activity dependent on the type of oxidant and membrane model used. The results highlight their potential application as functional ingredients in nutraceuticals or functional foods aimed at protecting cellular structures from oxidative damage.

ACKNOWLEDGMENTS

This work was financially supported by funds for statutory activities of the Department of Physics and Biophysics of Wrocław University of Environmental and Life Sciences, B010/0014/25.

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Optimization of phenolics extraction in potato roots infected with the root-lesion nematode *Pratylenchus penetrans* by UPLC-QTOF-MS

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Keywords: metabolome, phenolics, potato cultivars, root-lesion nematode, UPLC-QTOF-MS

Pratylenchus penetrans is one of the most severe root-lesion nematode (RLN) species affecting a wide range of food crops (e.g., potato, carrot, soybean, maize) [1]. Previous studies indicate that the potato cultivar Laura presented a lower susceptibility index to this RLN, comparatively to other cultivars, such as Agria and Kennebec [2], and that phenolic secondary metabolites are likely involved in the defence mechanisms [3]. To compare the metabolomic profile of the three potato cultivars (Laura, Agria and Kennebec), two extraction methods were compared, an ultrasonic method and a double extraction method with methanol, using ultra-pressure high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS). The analysis was run on target screening mode to evaluate the recovery rate of the phenolics quercetin and azelaic acid. The analysis showed that the double methanol extraction method presented a higher recovery rate than the ultrasound-based extraction method. These results will allow a more precise metabolome characterization of the different potato cultivars and identification of natural phenolic compounds of interest for the development of novel RLN control measures.

ACKNOWLEDGEMENTS

This work is funded by National Funds through FCT – Foundation for Science and Technology under the projects PratyOmics (PTDC/ASP PLA/0197/2020) (10.54499/PTDC/ASP-PLA/0197/2020) and UIDP/04449/2020.

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Decomposition of the simulation task for a chlorobenzene production plant: mass balance

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Keywords: Aspen Plus, process simulation, chlorobenzene plant, mass balance

Process simulators are widely used in industry to design new installations and to optimize existing ones. When a new process system is being designed, typically, only the reaction pathways and the separation sequence are known. The simulation begins with a mass balance. At this point, limited information is available about the reaction conditions and the feed-stream properties. Therefore, simplifying assumptions are necessary – such as ideal separation and a limited number of recycle streams. Energy balances are usually deferred at this stage, as they are not yet essential to the analysis.

In this study, the Aspen Plus (v14) simulator was used to model a chlorobenzene-production plant based on the process-flow diagram shown in Figure 1. The feed streams consisted of chlorine and benzene. A stoichiometric reactor (RStoic) block was employed to represent the chemical conversions, while the separation steps were simulated with Flash, Sep, and Sep2 blocks. For the simulation, an equimolar ratio of chlorine to benzene was assumed. Both feed streams entered at 25°C and 1 atm.

Monochlorobenzene was the main product, accounting for approximately 63% of the feed mass. Dichlorobenzenes were minor by-products (about 2% of the feed mass). All HCl formed was captured in the water stream (5,757.7 $kg \cdot h^{-1}$ of aqueous HCl). Unreacted benzene (3,029.8 $kg \cdot h^{-1}$) and chlorine (276.8 $kg \cdot h^{-1}$) were efficiently recycled. These preliminary studies produced a mass balance that will serve as the basis for the detailed design of the reactor and of the mass- and energy-transfer equipment.

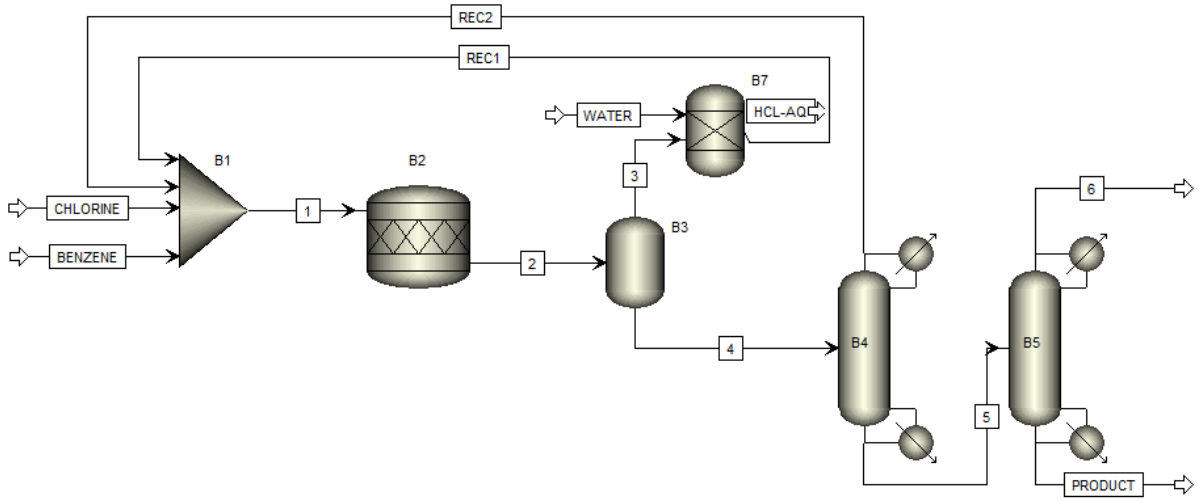

Fig. 1 Process flow diagram for mass balance simulation

High-resolution mass spectrometry for quantitative analysis of drugs, biologicals and biomarkers in clinical and preclinical research

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Keywords: LC-MS/MS, biotherapeutics, biomarkers, PK/PD, bioanalytics

The quantification of therapeutics and associated biomarkers is crucial for understanding drug efficacy and patient response in personalized medicine, and in clinical studies. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) offers unique advantages for high resolution quantitative analysis of small-molecules, peptides, and even proteins that are used as therapeutics (including monoclonal antibodies, bispecific antibodies, and antibody-drug conjugates).

We have developed and validated a broad spectrum of LC-MS/MS methods for quantitative analyses of drugs and metabolites as well as biomarkers, with high standards needed for pharmacokinetic (PK) and pharmacodynamic (PD) studies. We enable analyses in various matrices such as in plasma, urine, tissue, cultured cells, organoids, and dried blood spots (DBS). Our workflow incorporates advanced sample preparation techniques and targeted proteomics approaches, ensuring full compliance with key validation parameters such as accuracy, precision, selectivity, linearity, recovery, matrix effect, carry-over, and stability [1].

In various clinical and in vitro research projects, we have developed analytical protocols for a wide range of small molecule drugs, including tyrosine kinase inhibitors (TKIs), phosphodiesterase inhibitors, ion channel inhibitors, immunomodulators, neuropsychiatric agents, and MDM2 inhibitors [2–4]. We have expanded our methods for analysis of peptides and proteins for quantification of biotherapeutics such as monoclonal antibodies [5].

Our current focus extends to the simultaneous quantification of biotherapeutics while monitoring relevant biomarkers, providing comprehensive molecular-level insights into individual bioavailability and treatment response. This integrated approach enables the study of efficacy and safety of biological therapeutics, as well as the identification of novel protein biomarkers associated with treatment response and disease progression.

ACKNOWLEDGMENTS

This research was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project-ID 404521405 – SFB 1389, UNITE Glioblastoma, Work Package C01.

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Analysis of the potential anti-inflammatory properties of novel phthalic acid imide derivatives – in silico studies and ADMET profile evaluation

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Keywords: phthalimide derivatives, ADMET, molecular modeling, COX, LOX

The aim of the present study is to evaluate the potential anti-inflammatory properties of three newly designed derivatives of phthalic acid imide using *in silico* approaches, including molecular docking and computational prediction of pharmacokinetic parameters. Phthalic acid imide derivatives represent a chemically versatile class of compounds with a broad range of reported biological activities, including anti-inflammatory, antitumor, antimicrobial, and anticonvulsant effects [1]. Their rigid aromatic-imide framework allows for structural modifications that may enhance interaction with specific molecular targets, making them attractive candidates for drug development [2].

The study involves molecular docking simulations to investigate the potential binding affinity of the selected compounds towards key targets involved in the inflammatory response, including cyclooxygenase (COX-1 and COX-2), and lipooxygenase (LOX). The docking analyses are carried out using widely recognized molecular modeling tools to assess binding energy, interaction types, and potential ligand orientation within the active sites. Additionally, *in silico* pharmacokinetic and toxicity assessments are performed using ADMETlab 3.0 and SwissADME, which allow for a comprehensive evaluation of absorption, distribution, metabolism, excretion, and toxicity parameters, as well as drug-likeness and medicinal chemistry friendliness of the investigated structures.

Preliminary docking simulations indicate the ability of the investigated compounds to interact with selected molecular targets involved in inflammation. Specific binding interactions such as hydrogen bonding, π–π stacking, and hydrophobic contacts are evaluated in relation to their potential contribution to anti-inflammatory activity. The ADMET analyses provide predictive data regarding oral bioavailability, blood-brain barrier permeability, metabolic stability, and potential toxicity risks, offering an initial insight into the pharmacological suitability of the studied molecules.

The initial *in silico* results suggest that the newly synthesized phthalic acid imide derivatives may exhibit promising anti-inflammatory activity, mediated by interactions with multiple molecular targets relevant to inflammatory signalling pathways. Furthermore, the compounds display pharmacokinetic characteristics that may support their further development as drug candidates. The findings justify the need for subsequent experimental validation through *in vitro* and *in vivo* assays. The structural scaffold used in this study may also serve as a basis for the development of analogues with broader pharmacological potential, warranting future structure-activity relationship (SAR) studies.

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In silico analysis of the interaction between salivary α-amylase and bioactive compounds from grape pomace flour

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Keywords: grame pomace flour, amylase, type 2 diabetes, molecular docking, ligands

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by persistent hyperglycemia resulting from insulin resistance, impaired insulin secretion, or both. A promising strategy to mitigate the effects of T2DM involves the inhibition of digestive enzymes responsible for the breakdown of complex carbohydrates, such as salivary α-amylase, which catalyzes the initial hydrolysis of starch into maltose and smaller dextrins. In this context, phenolic compounds found in agro-industrial byproducts have been investigated as natural enzyme inhibitors. Grape pomace flour (GPF), rich in flavonoids and phenolic acids, stands out as a potential source of hypoglycemic bioactive compounds. Accordingly, this study aimed to evaluate, through *in silico* simulations, the interaction between major compounds found in GPF and human salivary α-amylase, in order to identify potential natural enzyme inhibitors. For this purpose, the crystallographic structure of human salivary α-amylase was retrieved from the Protein Data Bank (PDB ID: 1SMD) and prepared using AutoDock Tools 1.5.6 by removing water molecules, adding nonpolar hydrogens, and assigning Gasteiger charges. Ligands (chlorogenic acid, rutin, myricetin, malvidin, peonidin, delphinidin, catechin, and epicatechin) were obtained from the PubChem database. Molecular docking simulations were performed using the Lamarckian Genetic Algorithm with 100 runs, 2,500,000 energy evaluations, and 27,000 generations per run. The docking grid box was centered on the catalytic site, with dimensions of 60 × 74 × 104 Å and a spacing of 0.375 Å. Complex analyses were carried out using BIOVIA Discovery Studio Visualizer. The results showed that rutin exhibited the highest binding affinity (–14.43 kcal/mol), establishing multiple hydrogen bonds with key catalytic residues, such as ASP197, GLU233, ASP300, and GLU240. Myricetin (–12.41 kcal/mol) and chlorogenic acid (–12.36 kcal/mol) also showed strong interactions, particularly with HIS299 and ILE235. Delphinidin, epicatechin, peonidin, and catechin exhibited intermediate affinities, with notable hydrogen bonds involving ASP197, GLU233, and TYR62. Malvidin showed the lowest binding affinity (–5.78 kcal/mol), with peripheral interactions located outside the catalytic site. These findings suggest that rutin, myricetin, and chlorogenic acid have the highest potential as natural inhibitors of salivary α-amylase, reinforcing the value of grape pomace flour as a source of functional compounds for glycemic control.

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Synthesis and spectroscopic analysis of four-helix miniproteins

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Keywords: miniprotein, solid-phase synthesis, spectroscopic techniques

The term miniprotein refers to polypeptide chains with a molecular weight not exceeding 10 kDa, which are characterized by a defined three-dimensional structure [1, 2]. In research on the synthesis of miniproteins, the solid-phase synthesis method (SPPS) is often used. This approach allows for designing protein from scratch, enabling the creation of entirely new and previously undescribed protein structures. The structures of miniproteins can be precisely analyzed using various spectroscopic techniques, such as nuclear magnetic resonance (NMR), circular dichroism (CD), and fluorescence spectroscopy [1].

In the presented work, a *de novo* designed miniprotein with the molecular formula $C_{349}H_{565}N_{95}O_{106}S_1$ and the amino acid sequence:

SFEEYLLTRVRLTEDIRDSDEEIKKLLKDIKELKNAPKQLLEIPKDYNEGERWKKMAQEAREWL

has been synthesized (spatial structure is shown on Fig. 1). The miniprotein was designed using computational methods and a *de novo* approach. Its secondary and tertiary structure consists of four alpha helices arranged perpendicularly to one another. The synthesis of the miniprotein was carried out by the solid-phase synthesis method (SPPS) using a modern automatic peptide synthesizer. The obtained crude product was analyzed for identification by mass spectrometry (MS) and analytical high-performance liquid chromatography (analytical HPLC), then purified by preparative high-performance liquid chromatography (HPLC) and freeze-dried to remove solvents. The pure compound was obtained as a dry, white powder with a mass of 4 mg, which was then analyzed using spectroscopic techniques. A circular dichroism (CD) spectrum was recorded to confirm the expected secondary structure of the protein. Thermal analysis confirmed its thermal stability, with a melting temperature (Tm) of 49.25°C. This temperature corresponds to the point at which the structured arrangement of the miniprotein unfolds. These results were confirmed using nano-differential scanning fluorimetry (NanoDSF), yielding a similar Tm value of 44.2°C. The difference between Tm values may result from method-specific sensitivity. The agreement between the results obtained from both methods indicates comparable thermal stability of the protein.

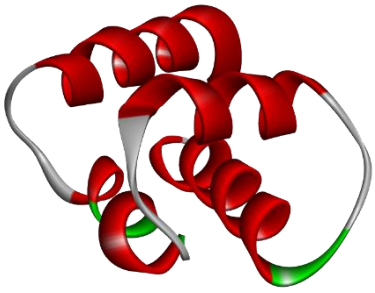

Fig. 1. Spatial structure of synthesized miniprotein

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Bacillus amyloliquefaciens – biohydrogen producing bacteria in beer brewing

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Keywords: *Bacillus amyloliquefaciens*, biohydrogen, brewing, fermentation, hydrogen-production

Bacillus amyloliquefaciens is known for its ability to produce biohydrogen during fermentation [1]. This study aimed to evaluate whether the bacterium can coexist in wort with the yeast Saccharomyces cerevisiae and its fermentation products. The goal was to assess the potential for creating a novel type of beer with acceptable quality parameters. In addition, fermentations carried out solely with the bacterial strain were analyzed in terms of alcohol content, color, and density of the final product.

The bacteria were cultured on a nutrient medium for five days, with optical density measured prior to use. Wort with a sugar concentration of 16° Blg was prepared and distributed into 1 L sterilized flasks. The experimental design followed the Box-Behnken method, resulting in seventeen fermentation trials with varying concentrations of sugars (8, 11, and 14° Blg) and hop extract (approx. 20, 60, and 100 IBU). The aim was to examine how these factors influence ethanol production, color intensity, polyphenol content, and overall sensory characteristics. Three microorganism addition strategies were tested: (−1) yeast on day 1 and *Bacillus amyloliquefaciens* on day 5; (0) yeast and *Bacillus amyloliquefaciens* added on day 1; and (1) *Bacillus amyloliquefaciens* on day 1 and yeast on day 5. Two control fermentations were also prepared: one with yeast only and one with *Bacillus amyloliquefaciens* only. Fermentation lasted for four weeks, after which samples were analyzed for ethanol content, residual sugars, polyphenols, bitterness, color, and presence of off-flavor compounds. Pasteurized samples were also subjected to sensory evaluation, assessing sweetness, sourness, bitterness, sulfuric, fruity, and herbal aromas, general taste, and overall acceptability.

The product was a reddish-brown beer, which was sour but, in most cases, acceptable for drinking. The alcohol content was lower than in samples where only yeast was used. During fermentation, hydrogen gas was produced, as confirmed by the spontaneous ignition of gas upon opening and flaming the flasks during sampling. Due to competition between bacteria and yeast, this method could be used for producing lower-alcohol beverages. Since bacteria produce hydrogen, less CO_2 is generated in this process, making it more environmentally friendly. There is potential for larger-scale hydrogen production using this method, but further research is required.

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Ferrocenyl analogs of *N*,4-diaryl-4-oxobutanamides – synthesis, spectral characterization and anti-acetylcholinesterase activity

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Keywords: *N*,4-diaryl-4-oxobutanamides, ferrocene, synthesis, spectral characterization, acetylcholinesterase inhibition

Using fragment-based drug design, 4-oxo-*N*,4-diarylbutanamides have recently been identified as dual selective inhibitors of monoamine oxidase B and acetylcholinesterase (AChE). Among 19 synthesized compounds, the amide derived from 4-oxo-4-phenylbutanoic acid and 4-aminoacetophenone emerged as a promising lead structure for developing therapeutic agents targeting Alzheimer's and Parkinson's diseases [1]. It is well established that introducing a ferrocene moiety into biologically active molecules can enhance their biological activity and/or pharmacokinetic properties [2]. Based on this strategy, five ferrocenyl analogs (**1**–**5**) of 4-oxo-*N*,4-diarylbutanamides were synthesized by coupling 4-ferrocenyl-4-oxobutanoic acid with *para*-substituted anilines (–H, –Me, –Ac, –Cl, or –OMe), selected to explore the effects of substituent lipophilicity and electronic properties on biological activity. All synthesized compounds represent novel organometallic entities and were fully characterized by mass spectrometry, infrared, and nuclear magnetic resonance spectroscopy. Their AChE inhibitory activity was assessed at three concentrations (37.5, 15, and 3 μM) using the standard Ellman spectrophotometric method**.** Although none of the ferrocenyl amides outperformed the positive control, rivastigmine (Fig. 1), the results indicate that anti-AChE activity correlates with the electronic nature of the *para*-substituent on the aniline ring. Notably, the acetyl-substituted analog **3** exhibited the highest inhibitory effect (26%) at 37.5 μM, whereas analog **5**, bearing an electron-donating methoxy group, was the least active. Furthermore, compound **3** was the only analog to demonstrate measurable inhibition at both lower concentrations (approximately 19% and 14% at 15 and 3 μM, respectively). Interestingly, no significant difference in AChE inhibitory activity was observed between the ferrocenyl analog **1** and its parent phenyl derivative **6**. The focus of future research will be to determine whether this bioisosteric replacement exerts a more meaningful effect on inhibitory activity against monoamine oxidases A and B.

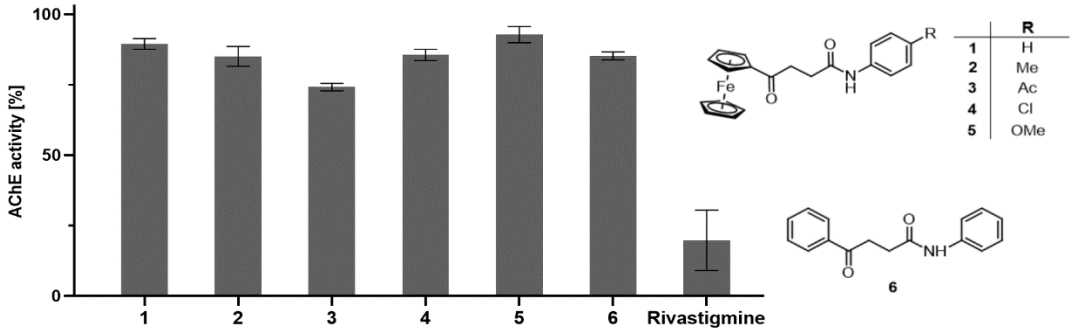

Fig. 1. The effect of compounds **1**–**6** and rivastigmine on AChE activity at concentration of 37.5 μM

ACKNOWLEDGMENTS

The work was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number: 451-03-136/2025-03/200124 and 451-03-137/2025-03/200124).

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Assessment of the toxicity of titanium dioxide nanoparticles using the brine shrimp lethality assay

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Keywords: TiO_2, BSLA, nanoparticles, toxicity, LC_{50}

Titanium dioxide (TiO_2) is a widely used compound found in numerous consumer products, including food, cosmetics, and pharmaceuticals. Despite its broad application, concerns have been raised regarding its potential toxicity and environmental impact [1]. The objective of this study was to assess TiO_2 nanoparticles (TiO_2 NPs) toxicity using the Brine Shrimp Lethality Assay (BSLA).

Acute toxicity testing was conducted on two batches of TiO_2 NPs as UV filters for sun care products. Brine shrimp (*Artemia salina*) eggs were hatched in artificial seawater for 24–48 h under constant aeration and light. The nauplii were then exposed to a concentration range of 0.01–10 mg/mL to each TiO_2 NP sample for 24 h. The number of dead nauplii was recorded, and a probit regression analysis was used to determine the LC_{50} values. The obtained LC_{50} values were evaluated according to the classification criteria proposed by Meyer and Clarkson [2, 3] to interpret the toxicity level of TiO_2 NPs.

According to the results from BSLA, TiO_2 NPs displayed toxic activity consistent with Meyer's toxicity scale and were classified as highly toxic based on Clarkson's toxicity scale. LC_{50} values of 65.6 µg/mL and 49.9 µg/mL were observed after 24 h of exposure, respectively.

The BSLA proved useful as a rapid and cost-effective screening tool for the toxicity of TiO_2 NPs. The obtained results within the investigated concentration range suggest that TiO_2 NPs pose potential biological risks, underscoring the need for further *in vivo*, ecotoxicological, and human health studies.

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A Virtual Screening Approach for Sustainable Pest Control Targeting Odorant-Binding Proteins

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Keywords: odorant-binding proteins, virtual screening, molecular docking, *Drosophila Melanogaster*, crop protection

The global agricultural sector is facing multifaceted challenges due to the increasing impact of pests. The widespread use of chemical pesticides as a primary means for pest control has led to serious concerns regarding environmental pollution, food safety, and human health [1]. The main objective of our research is the application of *in silico* screening to identify natural compounds from agricultural crop and food production residues (ACFPR) as environmentally friendly alternatives to chemical pesticides, targeting odour binding proteins in *Drosophila* species. As an initial test of our hypothesis that components of ACFPRs could act as modulators of odour binding proteins, their potential for interaction with residues of the binding site within the crystal structure of LUSH odour binding protein was explored by docking using DockThor software [2]. Odour binding proteins in *D. melanogaster* were identified via UniProt database, while compound SMILES were retrieved from PubChem and converted to 3D structures using Open Babel. The crystal structure of the LUSH protein (PDB ID: 2GTE) [3] was prepared for docking using VegaZZ software. The predicted binding affinities for 164 components of ACFPR from different sources were in the range –1.6 to –6.2 kcal/mol. This range, including comparison of the docking scores of top five compounds from different classes (peonidin 3-rutinoside, cyanidin 3-glucoside, dehydrotomatine, α-tocopherol and rutin), suggest that the components of ACFPRs may have a potential for modulating the function of the LUSH odour binding protein through favourable interactions with its binding site residues.

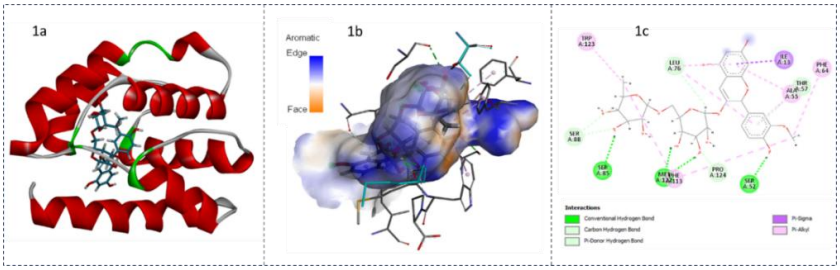

Fig. 1 Visualization of protein-ligand (LUSH odour binding protein – peonidin 3-rutinoside) complex by Discovery Studio Visualizer (a) interactions of peonidin 3-rutinoside with 3D structure of LUSH protein shown as (b) 3D and (c) 2D protein – ligand plot

The top-scoring ACFPR compound (peonidin 3-rutinoside, binding affinity –11.6 kcal/mol) interacts more extensively with the polar regions of the LUSH binding site, while still incorporating non-polar interactions (π–alkyl) to stabilize the overall complex (Fig. 1a–c). As part of the comparative analysis, known Drosophila-active compounds from *Melaleuca alternifolia* essential oil [4], such as α-gurjunene, isoledene, cubenol and β-caryophyllene showed binding affinities up to –9.3 kcal/mol. These results support the potential of ACFPR components to modulate LUSH protein function and provide a basis for further research into structure–activity relationships and olfactory responses in species of *Drosophila*.

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ISBN 978-83-8134-005-2
https://doi.org/10.37190/ChemBiotIC_2025