PROGRAMME AND BOOK OF ABSTRACTS

ChemBiotIC

Chemistry & Biotechnology International Conference

June 22–23 2023

Wrocław University of Science and Technology

Wrocław, Poland

ChemBiotIC

Chemistry & Biotechnology
International Conference

PROGRAMME AND BOOK OF ABSTRACTS

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Science is inseparable from the exchange of knowledge and experience.

In 2021, three science enthusiasts: Anna Skorupska-Stasiak Ph.D. student, Agnieszka Śliżewska Ph.D. and Alicja Surowiak Ph.D came up with the idea of creating an international conference that, in its interdisciplinarity, would not only focus on popularising scientific knowledge, but also bring together the worlds of science and business.

The Chemistry & Biotechnology International Conference (ChemBiotIC) is a welcoming space where scientists under 40 years old from all over the world can exchange their experiences and insights directly from their own homes, free of charge.

In addition, the event is honoured by engaging lectures presented by well-respected professors and practical workshops led by representatives of companies that operate at the science- business interface.

ChemBiotIC – Chemistry & Biotechnology International Conference is a completely secure, online event created for science enthusiasts, students, and scientists. We invite you to participate in the conference!

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

Ludwik Hirszfeld

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ChemBiotIC

Chemistry & Biotechnology International Conference

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PP-49	Nikola Sozańska, **Bioinformatics analysis reveals that disordered regions in TCF4 are likely to be responsible for LLPS,** Wrocław University of Science and Technology, Wrocław, POLAND
PP-50	Izabela Krauze, **Neutrophil Serine Proteases activity profile in neutropenia patients**, Wrocław University of Science and Technology, Wrocław, POLAND
PP-51	Pawel Noceń, **Validation of the choice of a miniprotein scaffold used for the obtainment of inhibitors targeted towards PD-1 protein**, Wrocław University of Science and Technology, Wrocław, POLAND
PP-52	Olga Szczepańska, **Ketoprofen pharmacokinetics: Development and evaluation of release soft gelatin capsules,** Wrocław Medical University, Wrocław, POLAND
PP-53	Ilona Nowak, **Changes in the expression profile of ferroptosis -related genes astrocytic series brain tumors,** Medical University of Silesia in Katowice, Katowice, POLAND
PP-54	Anna Wróblewska, **Biofunctionalized boron carbide nanoparticles as promising compounds in boron neutron capture therapy,** Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, POLAND
PP-55	Katarzyna Węgierek-Ciura, **Effect of administration of cellular vaccines based on dendritic cells capable of overproducing IL-12, IL-15 or IL-18 on the inhibition of B16-F0 murine melanoma tumor growth,** Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, PAS, Wrocław, POLAND

PP-56	Edyta Bielec, **Investigation of the role of granzyme A in genetically modified neutrophil-like cells using the CRISPR/Cas9n method,** Wrocław University of Science and Technology, Wrocław, POLAND
PP-57	Aleksandra Korba, **The function of granzyme A in apoptosis and NETosis of neutrophils,** Wrocław University of Science and Technology, Wrocław, POLAND
PP-58	Ignacy Janicki, **New reagents for the highly *Z*-selective Horner-Wadsworth-Emmons olefination**, Polish Academy of Sciences, POLAND
PP-59	Justyna Błaszkiewicz, **Improvement of catalyst activity in hydroisomerization of n-hexadecane towards multibranched hydrocarbons**, Łukasiewicz Network Institute of Heavy Organic Chemistry "Blachownia", Kędzierzyn-Koźle, POLAND
PP-60	Małgorzata Sarad, **Determination of the betalain profile in selected species of *Amaranthus***, Cracow University of Technology, Kraków, POLAND
PP-61	Piotr Kruszyński, ***In silico* analysis of enolase liquid-liquid phase separation**, Wrocław University of Science and Technology, Wrocław, POLAND
PP-62	Weronika Janik, **Influence of Plasticizer on the Physical and Antimicrobial Properties of Sodium Alginate Films**, Łukasiewicz Network Institute of Heavy Organic Chemistry "Blachownia", Kędzierzyn-Koźle, POLAND
PP-63	Magdalena Jankowska, **New photocatalytic systems dedicated to the fabrication of TiO_2 nanocomposites**, Cracow University of Technology, Kraków, POLAND
PP-64	Dominika Krok, **Novel initiating systems enriched with carbon dots for 3D printing applications**, Cracow University of Technology, Kraków, POLAND
PP-65	Krzysztof Legawiec, **Optimization of regioselective oxidation reaction investigated for the obtaining of *n*-alkyl-aminated cellulose nanostructures with controlled hydrophobicity**, Wrocław University of Science and Technology, Wrocław, POLAND
PP-66	Łukasz Dyzma, **Effects of cold plasma treatment on seed germination of *Sinapis alba***, Wrocław University of Science and Technology, Wrocław, POLAND

INVITED SPEAKERS

ChemBiotIC

Chemistry & Biotechnology International Conference

Enhancing Cancer Treatment: Advancing Therapeutic Strategies with Protease-Selective Prodrugs

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Keywords: cancer, anticancer treatment, breast cancer, prodrugs, proteases

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Objective

The objective of this study is to develop new peptide linkers for antibody-drug conjugates (ADCs) that improve targeted drug delivery and controlled release of the active payload at the tumor site. The study aims to leverage protease selective peptides obtained from the HyCoSuL (Hybrid Combinatorial Substrate Library) technology to enhance the efficacy and selectivity of ADCs towards cancer-associated proteases in cancer tissues while reducing toxicity to normal cells.

Methods

We employed the HyCoSuL technology, which utilizes libraries of peptidyl fluorogenic substrates with natural and unnatural amino acids. Initially designed for manufacturing substrates, inhibitors, and activity-based probes for proteases, the HyCoSuL technology was adapted for the development of protease selective linkers in anticancer prodrugs. The study focused on the design, synthesis, and biochemical analysis of prodrugs for cathepsin L, B, and legumain, using highly selective peptides that were previously identified.

Results

The results of this study demonstrate that prodrugs incorporating selective peptides display significantly higher activity and selectivity towards cathepsin L and cathepsin B compared to reference prodrugs. This finding validates the application of unnatural amino acids and protease selective peptides for the further advancement of protease-activated prodrugs. The developed prodrugs show promise in improving targeted drug delivery and reducing off-target toxicity.

Conclusions

By harnessing the capabilities of the HyCoSuL technology, this study successfully developed novel peptide linkers for protease-activated ADCs that enhance their specificity and effectiveness in cancer cells. The use of protease selective peptides containing unnatural amino acids has shown improved activity within the tumor microenvironment while minimizing toxicity to healthy cells. These findings highlight the potential of protease-activated prodrugs as a targeted therapeutic approach in breast cancer and other malignancies.

ACKNOWLEDGMENTS

This project was supported by the OPUS-LAP grant from the National Science Centre in Poland UMO 2020/39/I/NZ5/03104.

Analytical studies of fragrant raw materials. A quest for their odor-active constituents

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Keywords: fragrance chemistry, odorants, perfumery, GC-Olfactometry, frankincense

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Objective
The determination of the most important olfactory contributors of a fragrant natural raw material can be an extremely long and complex task which requires the combination of very efficient analytical techniques. Indeed, the characterization of these components is often difficult since the main contributors are often strongly potent odorants contained only in trace amounts, and therefore, their identification requires an exhaustive analysis of the whole mixture. Consequently, there is still a lack of accurate knowledge about the main odoriferous constituents for many natural raw materials, and this situation is paradoxical when it concerns materials widely used for their odorant properties in the flavor and fragrance industry.

Conclusion
This presentation will describe several examples of analytical investigations based on Gas Chromatography-Olfactometry (GC-O) and focused on the determination of the main odorant contributors of fragrant raw materials such as frankincense.

Algae in biotechnology – utilization and perspectives

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Keywords: algae, cyanobacteria, microalgae, biotechnology, photobioreactor

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Objective

Humankind has used algae since ancient times. The oldest finds are up to 14,000 years old. Algae were most often used as food or for medical purposes. The beginnings of algal biotechnology date back to the end of the 19th century. However, the foundations of modern algal biotechnology were laid in the 1940s. At present, it appears that algae are most useful for the production of high value coumpounds such as pigments, proteins, fatty acids, or bioactive secodary metabolites which are extracted from microalgae to improve the economics of a biorefinery approach.

In this lecture, the history of algal biotechnology will be briefly presented, the possibilities of using algae, principles and types of microalgae cultivation, trophic regimes, different types of photobioreactors and algal representatives suitable for large-scale cultivation will be summarized. Troubleshooting will be discussed. Some specific biotechnology projects and commercial products from algae will also be mentioned. Finally, the future perspectives of algal biotechnology will be outlined.

ACKNOWLEDGMENTS

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Surface modifications of nanoparticles – challenges and applications

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Keywords: Au nanoparticles, Cd-based nanoparticles, ligand exchange, optical filters, sensors

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Objective

A precondition for almost every possible application of nanoparticles (NPs) is the proper functionalization of their surface. The presence of ligands on the surface of NPs influences their size, shape, optical and physicochemical properties. Modification of the surface with different ligands allows controlling the colloidal stability of NPs and their dispersion in polar (e.g., in organic solvents usually used during synthesis of Cd-based NPs) and non-polar (e.g., in buffers) environments[1]. The type of ligands attached to the NPs surface determines the ability of additional biological molecules to conjugate to the NPs surface and, therefore, may have an impact on reducing toxicity, making NPs suitable for biomedical applications [2]. However, there are situations where it is important to modify the surface of NPs with hydrophilic properties (e.g., Au NPs) in order to make them hydrophobic, e.g., for applications in optics (e.g., filters, modulators, emitters) [3].

Methods

According to the literature, there are two strategies for surface modification of NPs: a) The first strategy involves encapsulation of NPs inside amphiphilic polymers, organic dendrons or phospholipid micelles; b) The second strategy is to replace the initial hydrophobic ligands (used during the synthesis of NPs) with various mono- and bifunctional molecules.

Results

Surface modification of cadmium nanoparticles with a variety of geometries (quantum dots, nanorods, nanoplates) was carried out and nanoparticles with hydrophilic properties were obtained. Functionalisation of Au bipyramides was also performed, resulting in nanoparticles with hydrophobic properties.

Conclusions

Surface modification is a process that allows the properties of nanoparticles to be extended, thus enabling their application in a wide range of scientific fields.

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ORAL PRESENTATION

ChemBiotIC

Chemistry & Biotechnology International Conference

OP1: Systemic Inflammation and STING Activation: Implications for Viral Infection Therapeutics

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Keywords: STING, psoriasis, mouse models, innate

Objective

The Stimulator of Interferon Genes (STING) is a protein that plays a crucial role in the innate immune response to viral infections. It recognizes cytosolic DNA and activates a signaling cascade that produces type I IFN and other cytokines that help to fight off viral infections. The study shows that diamidobenzimidazol (diAbZi), a potent non-cyclic dinucleotide STING agonist, is able to reduce viral replication and improve survival in mice infected with SARS-CoV-2 [1]. However other studies show that STING activation may amplify pro-inflammatory responses and potentially worsen the inflammatory damage caused by the virus [2]. Therefore this study was performed to investigate the effects of STING activation under pre-existing systemic inflammation induced by a viral infection.

Methods

Female BALB/cJ mice were treated with a TLR3 agonist poly(IC) followed 24 hours later by subcutaneous injection of diAbZi. The skin at the injection site was evaluated for local inflammation, histopathology, immune cell infiltration, and gene expression. Serum cytokine levels were measured to assess systemic inflammatory responses.

Results

Mice treated with poly(IC) and diAbZi or diAbZi alone showed the highest systemic levels of type I IFNs, IL-6, and TNFα. By day 10, these mice still showed extensive hair loss, erythema, and ulceration. The lesions were self-limiting and resolved within 6 weeks, however persistence of inflammatory cells in the dermis was still observed.

Conclusions

The data suggest that STING activation under systemic inflammatory conditions can lead to severe skin disease. These findings suggest that STING agonists may not be a suitable therapeutic option for controlling viral infections such as SARS-CoV-2.

ACKNOWLEDGMENTS

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OP2: Endotoxin tolerance and its influence on cancer development and behaviour

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Keywords: cancer, endotoxin tolerance, macrophage polarization, immunosuppression, carcinogenic conditions

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Objective
Endotoxin tolerance (ET) is an adaptive phenomenon of the immune system that protects the host from clinical complications due to repeated exposure of the body to endotoxins such as lipopolysaccharide (LPS). Since ET is an immunosuppressive mechanism we hypothesized that it can influence cancer development by modifying the tumour environment. The aim of this study was to evaluate the effect of endotoxin-tolerant macrophages (MOET) on cancer cell behaviour.

Methods
Induction of endotoxin tolerance in macrophages (RAW 264.7) was done by treating cells with two consecutive doses of LPS. In case of ET development the pro-inflammatory cytokine production by these macrophages should be suppressed and this was evaluated by ELISA technique. To characterize breast cancer cell (4T1) and colon cancer cell (CT26) behaviours, the cancer cells were treated with conditioned media derived from the tolerant macrophages and non-treated macrophages. For observing the influence of this conditioned media on the cancer cells, cell viability (MTT assay), clonogenic potential (colony formation assay) and cell motility (scratch assay) were evaluated.

Results
We analysed cell-to-cell crosstalk of MOET with breast cancer cells and examined whether factors released by MOET to conditioned media affect cancer cells. We observed that factors produced by MOET increased the viability and motility of both breast cancer and colon cancer cells. In the colony formation assay, both cancer cells exposed to the conditioned media from MOET showed increased clonogenic potential.

Conclusions
Our results revealed that ET-related reprogramming of macrophages triggers a release of factors that create an environment which is favourable for cancer development. Thus, targeting ET appears to be a new therapeutic option in cancer prevention and treatment.

OP3: Sublethal doses of bifenthrin, chlorpyrifos and their combinations cause oxidative stress and macromolecular damage in the larvae of stored grain pest, *Trogoderma granarium*

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Keywords: Bifenthrin, Chlorpyrifos, Bifenthrin-Chlorpyrifos combinations, Advanced oxidation protein products, Malondialdehyde

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Objective
Fourth and sixth larval instars of A_4 quarantine pest *Trogoderma granarium* are known to be resistant to several groups of insecticides and fumigants. These both life stages have been found susceptible to bifenthrin, chlorpyrifos and combinations of both insecticides (Feroz et al. 2020a). Though the reason behind their susceptibility has not been investigated yet. Mode of action behind toxicity of insecticides is mainly studied at sublethal level hence present study is carried out to investigate the toxicity of LC_{20} of bifenthrin, chlorpyrifos and their combinations in oxidative stress and macromolecular damage in the fourth and sixth larval instars of *T. granarium*.

Methods
In the current study, fourth and sixth larval instars of insecticide susceptible strain (Lab-S) and deltamethrin resistant (GUW) population of *T. granarium* were exposed for 48 h to LC_{20} of bifenthrin, chlorpyrifos as well as 3:1 and 1:3 combinations of both insecticides (bifenthrin: chlorpyrifos) according to Feroz et al. (2020b). Following exposure, in the survived fourth and sixth larval instars the effect of LC_{20} of each treatment was investigated on the hydrogen peroxide (H_2O_2), advanced oxidation protein products (AOPPs), protein carbonylation and malondialdehyde (MDA) contents and DNA damage.

Results
According to Tukey's test, significantly high level of H_2O_2, AOPPs, protein carbonylation and MDA contents indicated oxidative stress and macromolecular damage in comparison to control in the fourth and sixth larval instars of the Lab-S and GUW populations following exposure to LC_{20} of bifenthrin, chlorpyrifos and their combinations (3:1 combination and 1:3 combination) ($p \leq 0.05$). All the treatments caused oxidative damage to DNA, except for chlorpyrifos which did not cause DNA damage in the fourth larval instars of GUW population.

Conclusions
Overall results suggested that the mechanism underlying the toxicity of LC_{20} of bifenthrin, chlorpyrifos and their combinations is linked with its high oxidative stress and damage-causing potential in the fourth and sixth larval instars of both populations of *T. granarium*. It is also recommended that the effect of LC_{20} of bifenthrin, chlorpyrifos and their combinations should be studied on relative fitness, fecundity and longevity in both larval stages of *T. garanarium* for better understanding of mechanisms to develop an efficient pest control strategy.

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OP4: The pivotal role of Pejvakin (DFNB59) protein in hearing loss in humans

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Keywords: cochlear nerve, inner ear, DFNB59, pejvakin, deafness, hair cells, apoptosis

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Objective

The protein pejvakin, also known as DFNB59, is largely expressed in the inner ear, and other organs like testis (highest concentration), liver, brain, lungs, eyes, kidneys, and intestinal tissues. and is essential for hearing [1]. Pejvakin belongs to the gasdermin family of proteins that mediate pyroapoptosis in vertebrates. Gasdermin family comprises conserved affecter N-terminal domain that causes oligomerization, to form channels or pores in plasma membrane [2].

The functional proteins of Gasdermin family have immunoregulatory, cellular growth, and host immune defence [3]. Gasdermin family of protein seem homologous with respect to pore forming N terminal affecter end, this applies to pejvakin protein also [4]. There are many unanswered questions in the pathway, interaction, cellular mechanism of this protein [5].

Methods

This presentation evaluates the role of pejvakin (DFNB59) protein in the development of post-lingual deafness in the light of extant evidence and uses this protein as a case-study protein in molecular mechanisms that lead to deafness in human beings.

Results

My presentation explicates the behavior of ciliary rootlet protein pejvakin (DFNB59) with homology to gasdermin family in the light of available genetic, molecular, biochemical, and computational evidence.

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OP5: One pot synthesis of alkyl analogues of p-amino benzoic acid (PABA) and evaluation of their cytotoxic potential

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Keywords: PABA; cytotoxic screening; PCC; oral squamous carcinoma; cisplatin

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Objective

Cancer has always been the most critical disease all over the world. But lung and oral cancer rank 2nd and 3rd in Pakistan [1]. Several methods have been introduced to treat cancer, Chemotherapy is one of them and considered as the most effective treatment that plays a crucial role in killing or inhibiting the hidden cancer cells [2]. The aim behind the synthesis of mentioned compounds was to add valuable and effective analogues in anticancer drugs for the sake of mankind.

Methods

Under simple and mild reaction conditions, twenty alkyl derivatives of 4-aminobenzoic acid (PABA) in a series from (**2** to **21**) were synthesized by using potassium carbonate and appropriate alkylating agents under mild reaction conditions. Six compounds (**16**-**21**) are found unprecedented.

Results

For the purpose of characterizing these analogues, the spectroscopic methods of proton NMR (^{1}HNMR), Fourier transform infrared spectroscopy (FT-IR), Electron Ionization Mass Spectrometry (EI-MS) have been utilized. In a current analysis, sixteen compounds (**3**, **5** to **11**, **13** and **15** to **21**) were tested for their cytotoxicity likewise accomplished opposed to carcinoma of oral squamous (CAL-27) and lungs (NCI-H460) lines of cell. Lower than the control dose (21.00 µM of cisplatin IC_{50}), compound **20** demonstrated remarkable preventive properties opposed to the cell line of (NCI-H460 of 15.59 µM and 20.04 µM of IC_{50}). Using the statistical package for the social sciences (SPSS), the unwavering quality of the current data was evaluated using the t-test, one-way analysis of variance (ANOVA) and the Pearson correlation coefficient (PCC).

Conclusions

A one-pot synthesis method for alkyl derivatives of 4-aminobenzoic acid (PABA) was developed, resulting in the preparation of twenty analogues, including six new compounds. The method is convenient, single-step, and mild. The synthesized compounds were evaluated for their cytotoxic properties, with compounds **18–21** showing promising activity against the NCI-H460 cell line. Compound **20** exhibited the highest anti-proliferative activity. A significant difference was observed between amino esters and alkylamino esters, and the cytotoxic activity increased with increasing alkyl chain length. Further research on these analogues is recommended.

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OP6: Docking of benzimidazole derivatives as potential anticovid-19 agents

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Keywords: Covid-19, SARS CoV-2 main protease (Mpro), molecular docking, benzimidazole, Anticovid-19 agents

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Objective
Covid-19 was emerged in the late of December 2019 from Wuhan city of China. It was a drastic disaster for humanity causing the death of millions of people in the worldwide. In this work, we are in the search of new inhibitors of the SARS CoV-2 main protease (Mpro), an essential protein for the replication of SARS CoV-2 virus. We use a molecular docking study of a set of benzimidazole derivatives in order to develop new anticovid-19 agents.

Methods
The docking of 754 compounds of benzimidazole derivatives were occurred in the structure of SARS CoV-2 main protease (7VLQ: PDB code). The top ranked compounds those exhibited high affinity with SARS CoV-2 Mpro were characterized by a good ADME-T profile (predicted by Swiss ADME and ADMETLab web servers), were retrieved.

Results
The results of this study revealed two benzimdazole derivatives Pubchem11176685 and Pub-Chem135566361 as potential inhibitors of SARS CoV-2 Mpro. Indeed, they have good pharmacokinetic properties and low toxicity.

Conclusions
In vitro additional works could affirm the antiviral activity of the two obtained compounds against SARS CoV-2.

OP7: Influence of Serum Replacement on the Rate of Proliferation of Bovine Satellite Cells

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Keywords: serum-free medium, satellite cells, cultured meat, proliferation

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Objective
Recreating the process of myogenesis *in vitro*, and thus resembling a structure of muscle tissue, is the base for research focusing on obtaining meat by *in vitro* techniques – cultured meat. However, cell cultivation based on animal-origin serum is opposed to the ideology of cultured meat, raises many ethical controversies and requires high financial investments. Research is still undertaken to find optimal substitutes for animal serum because serum-free cultures are still inefficient. Our recent studies indicate that the serum - bovine serum at a concentration of 20% - is the most significantly important component of the medium which support the efficient proliferation of bovine satellite cell (BSCs). Our study aimed to examine the influence of a commercially available animal serum replacement – KnockOut™ Serum Replacement (Thermo Fisher Scientific) – which ultimately is dedicated to embryonic stem cells and iPSC – on the rate of proliferation of BSCs and the level of expression of genes involved in myogenesis – *Pax7* gene and genes from the family of myogenic regulatory factors (MRF): *Myf5*, *MyoD, Myf6* and *MyoG*.

Methods
To examine the proliferation rate of BSCs, a fluorometric CellTiterBlue (Promega) assay was performed on the third and fifth day of incubation in a medium with 20% addition KnockOut™ Serum Replacement or 20% addition of fetal bovine serum. The results obtained were normalized with the results acquired on the first day. To study the expression of genes involved in myogenesis, BSCs were incubated in a medium with 20% addition KnockOut™ Serum Replacement or 20% addition bovine serum. On the fifth day of incubation, RNA was isolated (PureLink RNA Mini Kit, Thermo Fisher Scientific), reverse transcription was performed (High Capacity cDNA Reverse Transcription Kit, Thermo Fisher Scientific), and gene expression levels were determined using qPCR (HS-PCR Mix SYBR, A&ABiotechnology).

Results
The obtained results indicate that a higher rate of BSCs proliferation was observed in the group with the addition of bovine serum in a medium on the third and fifth day of observation ($p = 1.72 \cdot 10^{-4}$, $p = 2.165 \cdot 10^{-3}$, respectively). However, significantly higher levels of *Myf5* gene expression were observed in the group supplemented with KnockOut™ Serum Replacement ($p = 3.85 \cdot 10^{-4}$). There were no statistical differences in the expression of other genes between the study groups.

Conclusions
In conclusion, a non-animal serum - KnockOut™ Serum Replacement does not support the proliferation process as efficiently as fetal bovine serum. Based on the gene expression analysis, it could be suggested that Serum Replacement keeps cells longer in an undifferentiated state, which is expressed by a higher expression of *Myf5* gene involved in the early stage of myogenesis. It should be noticed that our conclusions are only for BSCs.

OP8: Antibody-Drug Conjugates – use of cancer cells antigens overexpression for targeted cancer therapy

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Keywords: ADC, cancer therapy, monoclonal antibodies, antigens, drug delivery

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Objective
Antibody-Drug Conjugates (ADCs) are an emerging class of targeted cancer therapeutics that combine the specificity of monoclonal antibodies with the potent cytotoxicity of small-molecule drugs. ADCs have the potential to overcome the limitations of conventional chemotherapy by delivering toxic payloads directly to cancer cells while sparing healthy cells, thereby improving therapeutic outcomes and minimizing side effects [1, 2]. A deep understanding of the factors that impact ADCs can greatly benefit scientists in the field.This knowledge can guide the development of better and more efficient ADC for use in medical treatments.

Methods
A review of the current literature on ADCs components, presented in the form of presentation.

Results
A good ADC possesses several key characteristics that contribute to its effectiveness. These essential attributes include: specifity, potent cytotoxic payload, stable linker, low immunogenicity, efficient internalization and low off-target toxicity. Nevertheless, the development of ADCs faces numerous challenges, such as optimizing pharmacokinetics, ensuring site-specific payload release, achieving homogeneous drug distribution within tumors, mitigating undesired side effects, and overcoming drug resistance. Additionally, the complexities of treating various types of cancer and understanding their unique microenvironments further complicate the development process [3].

Conclusions
In my presentation I will try to elucidate what ADC are, what key components characterize an ideal conjugate and what opportunities and challenges lie in front of us with such a technology.

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OP9: The impact of nanosecond pulsed electric field on immune checkpoint receptors in melanoma cells

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Keywords: pulsed electric field, melanoma, PD-1, LAG-3, TIM-3, checkpoint inhibitors

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Objective
Immune checkpoint receptors such as PD-1, LAG-3 or TIM-3 are currently under intense investigation for their role in attenuating the immune response in cancer. Various methods are being used to overcome the problem of examining the unfavorable immune response. This study investigated the effects of nanosecond pulsed electric field (nsPEF) treatment on the expression of immune checkpoint receptors in A375 and C32 melanoma cells.

Methods
Cell viability was assessed using MTT and Presto blue® assays, while the permeability of cell membranes was measured using YoPro-1 dye. Holotomography microscopy was employed to evaluate the effects of nsPEF treatment on cellular morphology. ELISA assay was used to determine the effect of nsPEF on cytokine secretion. The expression of PD-1, LAG-3, and TIM-3 antigens was examined using a Western Blot method. Confocal microscopy imaging were performed to investigate changes in the expression profile of PD-1 and MHC-II in cells.

Results
Our findings revealed that the nsPEF treatment had a high potential to enhance membrane permeabilization and morphological changes in the cell membrane without being cytotoxic. We found that the effect of nsPEF on melanoma leads to induction of vesicles from inside to outside of the cell, cell contraction, and migration of lipids from inside the cell to its periphery. The treatment increased the expression of PD-1 and LAG-3 molecules. Furthermore, we also observed potential co-localization or clustering of MHC class II and PD-1 molecules on the cell surface and the secretion of cytokines such as TNF-α and IL-6.

Conclusions
These findings suggest that nsPEF exposure could be a viable approach to enhance the delivery of therapeutic agents to cancer cells and modulate the tumor microenvironment to promote an antitumor immune response.

ACKNOWLEDGMENTS

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OP10: Optimizing production of human receptor Stabilin-2 in bacterial *Escherichia coli* expression systems

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Keywords: expression system, stabilin-2, bacterial host, optimization of expression system, ligands

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Objective
Human stabilin-2, endocytic scavenger receptor of a size 315 kDa, composed of several epidermal growth factor (EGF), seven fascilin (FAS), one Link and one transmembrane domains, has become an interesting object for research on understanding its role in organisms and its potential application in the drug delivery. Among many others, stabilin-2 can bind hyaluronic acid (HA) and advanced glycation end-products (AGEs). The negative impact of the latter on human health has been proven in many pathologies including diabetes, neurodegenerative and cardiovascular diseases [1]. Up to date, the molecular bases of the interactions between stabilin-2 and its numerous ligands remains unsolved. To investigate the mechanism of stabilin-2 and AGEs interaction, the crucial step is to produce this receptor using an efficient eukaryotic or prokaryotic expression system. So far, experimental structure of only one stabilin-2 domain, FAS1, has been determined [2].

Methods
During the expression of constructs comprising different stabilin-2 domains, four *Escherichia coli* strains and two vectors were tested. Variable conditions that were optimized included the type of medium, inducer concentration, temperature and time of expression. Physical-mechanical, chemical and enzymatic lysis methods were used. The products were analyzed by SDS-PAGE.

Results
The best results for Link domain of stabilin-2 were obtained using the *E. coli* K12 SHuffle strain and pETHSu plasmid. Optimized culture conditions (autoinduction medium, temperature 18°C and duration of 18 h) resulted in the soluble expression. The sonication of the bacterial pellet in buffer with 1% Triton X-100 was found as the optimal method of lysis.

Conclusions
Producing soluble stabilin-2 is a crucial step in elucidation of the roles it plays in the human organism. The prokaryotic expression system offers many possibilities to optimize the production, e.g. the *E. coli* K12 SHuffle strain was designed for proteins rich in disulfide bonds, such as stabilin-2. Further studies will be focused on characterisation of the binding between stabilin-2 and one group of its ligands, AGEs.

ACKNOWLEDGMENTS

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OP11: Biotransformation of 2-phenylethanol to low-molecular-weight polyphenols derivatives by fungi of the genus *Beauveria*

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Keywords: biotransformation, 2-phenylethanol, *Beauveria* sp., fungi, antioxidants

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Objective

Biotransformation can be define as a process of converting substrate to reaction products by isolated enzymes or wholle-cell bacterial/fungal biocatalysts. Whole-cell biocatalysis is a simple, environmental friendly and low-cost method of obtaining desired derivatives. 2-Phenylethanol is an inexpensive and widely available compound that can be the substrate for the preparation of many different compounds with antioxidant activity. These include for example 1-phenylethane-1,2-diol, 4-hydroxyphenylacetic acid and hydroxytyrosol [1]. Fungi of the genus *Beauveria* like *Beauveria bassiana* and *Beauveria brongniartii* show activity towards 2-phenylethanol and were used in biocatalytic processes. To increase the specificity of the reaction, fungal cells were immobilized in calcium alginate, agar-agar and polyurethane foams.

Methods

Biotransformations were carried out in conical flasks (250 ml) containing 50 ml of distilled water as reaction medium (T = 24°C, 135 rpm), fungal biomass and different concentrations of substrate (15 mg, 30 mg, 60 mg). Fungal cells were immobilized in calcium alginate, agar-agar and polyurethane foams.

Analysis of the reaction products was carried out using high-performance liquid chromatography (HPLC).

Results

The tested fungi successfully carried out the biotransformation of 2-phenylethanol. There were observed two main products of the reactions. *Beauveria bassiana* transformed the substrate to 1-phenylethane-1,2-diol but *Beauveria brongniartii* did not show such ability. Some of used immobilizations techniques (calcium alginate, agar-agar) helped direct the reaction towards a specific product, especially in the case of *Beauveria brongniartii*. On the other hand, polyurethane foams did not improve the activity of the biocatalyst.

Conclusions

The success of the reaction was influenced mainly by the amount of the substrate and the form of the biocatalyst (free or immobilized cells). Immobilization in calcium alginate and agar-agar turned out to be a good way to stabilize the activity of the biocatalyst. Selected fungal strains are able to transform 2-phenylethanol into chemical compounds with high-value for among others pharmaceutical industry.

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OP12: Materials of plant origin as antibacterial agents towards dental pathobionts

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Keywords: antibacterial agents, natural materials, oral pathogens, MIC, periodontal disease

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Objective

The aim of this study was to investigate the growth inhibitory effect of pathogenic microorganisms such as *Streptococcus mutans* (ATCC 35668) (MediMark) and *Porphyromonas gingivalis* (ATCC 33277) (Thermo Scientific) by selected natural aroma materials. These species are known as plaque bacteria, which contribute to the progression of caries and the development of systemic diseases [1]. Commonly used oral hygiene products contain chemical antimicrobial agents. Their long-term use has been proven to cause some undesirable side effects, such as gum irritation, altered taste perception and yellow-brown staining on the enamel [2]. Natural materials are a promising alternative to synthetic agents due to their numerous biocidal and antibacterial properties. In addition to combating tartar, natural fragrance materials could be effective preservatives and impart a pleasant scent, taste, and colour in oral hygiene products.

Methods

750 natural materials were pre-screened for inhibitory activity. Minimum inhibitory concentration (MIC) values for the most active materials and synthetic antibacterial agents were determined using a twofold dilution method. The tests were carried out in 96-well plates using alamarBlue® reagent.

Results

Porphyromonas gingivalis was inhibited by 12 natural materials (MIC ≤ 400 μg/mL), while *Streptococcus mutans* was suppressed by 94 materials (MIC ≤ 400 μg/mL). Both species were inhibited by 4 synthetic agents with MIC ≤ 200 μg/mL.

Conclusions

Natural materials exhibited inhibitory activity against chosen species. Some materials showed a comparable or even better suppressive effect than the tested synthetic agents. There is significant potential for plant materials to be used as antibacterial agents in combating tartar.

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OP13: The role of the *Bifidobacterium animalis* ssp. *animalis* CCDM 218 surface antigens in the treatment of allergy diseases

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Keywords: *Bifidobacterium*, surface antigens, allergy, macromolecules, polysaccharides

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Objective
Allergy diseases have become an increasingly common issue, particularly in developed countries. However, current allergy treatments often prove ineffective or may even cause numerous side effects for some patients. The newest attempt focuses on probiotic bacteria and more specifically on their antigens. The most significant advantage is that the determination of a single molecule's structural and functional properties is much easier in comparison to the complex bacteria cell. For this reason, polysaccharides (PSs), lipoteichoic acids (LTAs), surface layer proteins (SLPs), and peptidoglycan (PG) were isolated from *Bifidobacterium animalis* ssp. *animalis* CCDM 218 (Ba218) and their potential role in the prevention/treatment of allergy diseases was evaluated.

Methods
Surface antigens were isolated and tested for their immunomodulatory properties on splenocytes derived from ovalbumin-sensitized mice. Antigens recognition was determined with the use of HEK-Blue™ cells transfected with TLR and NOD receptors. Later, TC-1 (epithelial cells) and JAWS II (dendritic cells) cell lines were used to describe the processing of the selected PS. Finally, immunomodulatory properties of this PS were confirmed in mouse cell lines while chemical and NMR methods were used to determine its structural features.

Results
Cytokine release determined in splenocytes after antigens stimulation allowed the selection of Ba218.3 PS as a molecule with potential anti-allergic properties. It was also found to be well recognized by TLR2 receptor and to be efficiently processed between TC-1 and JAWS II cells. Studies on mouse cell lines indicated pro-inflammatory properties of Bad218.3 while structural analysis revealed presence of galactose, glucose and rhamnose in its structure.

Conclusions
Ba218.3 PS can induce interesting anti-allergic properties while being efficiently recognized and processed by the epithelial and dendritic cells, thus it will be further evaluated in a mouse model of allergy.

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OP14: Characterization of biologically active molecules extracted from microalgae by biosustainable processes

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Keywords: Microalgae, green organic solvent, extractions, antimicrobial activity, extract characterization

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Objective

Microalgae produce a wide range of bioactive metabolites with numerous biological beneficial activities. The factors that influence the extraction yield of the biological compounds from microalgae depend on the extraction method, the pre-treatment of biomass, and the nature of the solvent used, which is the most critical factor. While organic solvents such as methanol, ethanol, and acetone are commonly used, different solvents are required to efficiently recover the compounds produced by given microalgae due to the varied physical and chemical natures of the components present in microalgae biomass. Furthermore, sustainable processes are desirable, and green solvents need to replace conventional ones.
The focus of the present study was to optimize extractions using green organic solvents.

Methods

Freeze-dried biomass of a strain of *Chlorococcum* sp was used for the extractions of metabolites. The biomass was suspended in various organic solvents/water mixtures and sonicated to promote cell rupture, favouring extraction [1]. After removing the solvent, the extract was suspended in an aqueous solution. Subsequently, the extracts were tested against various model microorganisms using the agar well diffusion method and broth dilution method for MIC evaluation [2].

Results

This experiment allowed us to determine whether the extract exhibited bactericidal or bacteriostatic activity. It was found that the extracts obtained with MEK from untreated microalgae cells inhibited the growth of all tested bacteria, particularly exhibiting significant antimicrobial activity against *B. subtilis*. Furthermore, it is noteworthy that there was a notable reduction in the minimum inhibitory concentration when the extracts were treated with sodium hydroxide.

Conclusions

It is possible to optimize the growth conditions of various strains of microalgae, and by using the systems described above (growth in bottles), it is possible to obtain relatively high quantities of biomass for extractions processes.

As far as extraction techniques are concerned, it can be stated that the use of green organic solvents, specifically MEK for the preparation of extracts, allows extraction yields comparable to conventional organic solvents. At present, the extracts obtained showed excellent antimicrobial activities.

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OP15: Uncovering the anti-inflammatory mechanism of *Cinnamomum zeylanicum* essential oil through an integrative approach of network pharmacology and *in vitro* assays

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Keywords: *Cinnamomum zeylanicum*, essential oil, network pharmacology, inflammation

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Objective
Cinnamomum zeylanicum is a medicinal plant that has been used in the Ayurvedic and Chinese medicine system for treating aching joints and pain. However, the potential targets and anti-inflammatory mechanisms of *Cinnamomum zeylanicum* essential oil (CZEO) remain unclear. The current study aims to clarify the anti-inflammatory mechanism of CZEO based on network pharmacology, molecular docking and experimental assays.

Methods
The network pharmacology approach was used to predict the targets of the bioactive constituents of CZEO and the signaling pathway through which it exerts its anti-inflammatory property. Furthermore, the potential molecular mechanisms of CZEO predicted from network pharmacology analysis were validated in *vitro* using the RAW 264.7 inflammation model stimulated with lipopolysaccharides (LPS).

Results
A total of 57 compounds in CZEO were identified by GC-MS, 51 of which passed the drug likeness and bioavailability score and were classified as drug like constituents. A total of 1057 and 526 compound and anti-inflammation-related targets were identified, respectively. 124 compound-disease intersection targets were obtained. Hydrocinnamyl acetate, benzyl benzoate, ethyl cinnamate, benzyl cinnamate, cinnamic acid, α-muurolene and hexadecanoic acid are the core active compounds with degree value >10. The PPI interaction analysis revealed TNF, IL6, TLR4, STAT3, IL8, NFkB as potential hub targets. GO analyses indicated that CZEO regulates biological processes such as the response to lipopolysaccharide, the regulation of the inflammatory response, and the migration of leukocytes. CZEO exerted anti-inflammatory effects by regulating the TNF signaling pathway, the Toll-like receptor signaling pathway and the IL-17 signaling pathway. The molecular docking results showed that benzyl benzoate has higher binding energy with TNF-α and IL-8. The MTT assay revealed that CZEO did not exhibit any cytotoxicity in RAW 264.7 cells. CZEO significantly inhibited the production of NO, PGE_2, TNF-α, IL-6 and IL-1β and promoted the activity of antioxidant enzymes such as SOD, CAT, GPx and GSH in LPS-simulated RAW 264.7 cells. CZEO inhibited intracellular ROS production and attenuated the depletion of mitochondrial membrane potential. CZEO also reduced NF-ϰB translocation and mRNA expression levels of target hubs (TLR4, IL8, IL6, and TNF-α).

Conclusions
Current research comprehensively analysed the potential active compounds, targets, and signalling pathways of *Cinnamomum zeylanicum* essential oil in treating inflammation via the network pharmacology approach and experimental validations, which may support its use as an alternative therapy for treating inflammatory-related diseases.

OP16: Exploring the mechanism of *Eulophia nuda* extract against osteoarthritis by integrating network pharmacology, molecular docking and experimental assays

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Keywords: *Eulophia nuda*, network pharmacology, osteoarthritis

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Objective
Eulophia nuda (Orchidaceae) is a medicinal herb usually known for its ethnomedicinal uses by folklore and local healers in India for treating arthritis. However, the potential targets and the mechanism of Eulophia nuda extract (ENE) against osteoarthritis remain unclear. Therefore the current research aimed to reveal the therapeutic effects and underlying mechanism of ENE in the treatment of osteoarthritis (OA) through network pharmacology, molecular docking and experimental assays.

Methods
Putative therapeutic targets and pharmacological mechanisms of ENE against OA were predicted by a network pharmacology approach. The regulated network of ENE acting on osteoarthritis was constructed using Cytoscape 3.9.2 software. The binding interaction between key compounds and potential targets were analyzed using molecular docking. Anti- osteoarthritic effects of ENE and predicted mechanisms were further validated using IL-1β- induced SW982 human synovial cell model. Furthermore, the role of the IL-17 pathway in the action of ENE was verified by carrying out mRNA expression level of the hub genes.

Results
A total of 26 active compounds were obtained from IMPPAT, KNApSAcK and Pubchem database, 23 of which passed the druglikeness and oral bioavailability parameters. 2344 Compound and 1370 OA-related targets were identified. 81 compound-disease intersection targets were obtained. Compound-disease target network resulted in 6 core compounds (Vanillic acid, Ferulic acid, Protocatechuic acid, Eulophiol, Caffeic acid, 3,4′-Dihydroxy- 3′,5,5′-trimethoxybibenzyl) with degree value > 23. PPI network analysis resulted in 6 hub target genes (CTNNB1, HSP90A1, MMP9, ESR1, MAPK1, and PTGS2). The KEGG and GO analysis suggests that IL-17 pathway, TNF pathway, AGE-RAGE signalling pathways plays a major role in OA by regulating biological processes such as collagen catabolic process, collagen metabolic process and response to lipopolysaccharide. The molecular docking suggests that Eulophiol having highest binding energy (>8.0 kcal/mol) with PTGS2 and MAPK1. MTT assay showed that ENE has no cytotoxic effect on SW982 cells up to a dose of 100 μg/ml. ENE showed significant anti-inflammatory activity by inhibiting PGE2, IL-6 and IL8 levels in IL-1β stimulated SW982 cells. Finally ENE also reduced the mRNA expression level of the hub genes (CTNNB1, HSP90A1, MMP9, ESR1, MAPK1, and PTGS2) that are responsible for OA.

Conclusions
The current study showed ENE to exhibit strong anti-osteoarthritic activity through suppressing IL-17 pathway in activated synovial cells, suggesting its potential as a drug candidate for the development of novel agents for the prevention and treatment of osteoarthritis.

OP17: Biosurfactants – A Novel Generation of Bacterial Biomolecules for Biodegradation of Organic Contaminants

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Keywords: biosurfactant, bioremediation, soil, *Bacillus*

Objective
Several industries and companies use various forms of biosurfactants, including bioremediation, medicines, oil recovery, and detergents. In commercial bioprocess applications, biosurfactants have a number of advantages over chemical surfactants, including (i) lower toxicity, (ii) ease of biodegradation, (iii) stability at higher pH and temperature, and (iv) stronger foaming [1]. This study's main objective was to investigate biosurfactant applications in bioremediation produced by bacteria that break down hydrocarbons. Due to their significant benefits, such as improving bioavailability, removing hydrocarbon contaminants effectively, enhancing biocompatibility, minimizing the use of hazardous chemicals (chemical surfactants), etc., the use of biosurfactants in the bioremediation of hydrocarbon contaminants is gaining attention [2].

Methods
A total of 150 strains from the genus *Bacillus* isolated from different types of soil in North Macedonia were screened with oil spreading technique and emulsification activity to find the most efficient biosurfactant producer.

Results
22 out of 150 isolates showed the ability to produce biosurfactants on different oils. The biodegradation of hydrocarbons can be improved by biosurfactants by increasing the bioavailability of hydrocarbon molecules.

Conclusions
With an emphasis on the discovery of novel strains with potent biosurfactant-producing abilities and the effects of biosurfactants on the degradation of various hydrophobic organic contaminants, this study sought to examine recent developments in this field of inquiry.

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OP18: Microbiological production of Hyaluronic Acid (HA) in synthetic mediums

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Keywords: hyaluronic acid, microbiological production, *Streptococcus zooepidemicus*, synthetic medium, microbial fermentation, HPLC

Objective

Hyaluronic Acid (HA) is one of the most plentiful biopolymer in living organisms, in vertebrates. HA exists naturally and abundantly in neural, connective, and epithelial tissues of humans and animals. Because of its remarkable physio-chemical and biological properties, as well as its low toxicity, pure HA is required for multiple cosmetic, nutritional, pharmaceutical, and medicinal purposes. The HA was commercially produced by extraction from animal tissues such as rooster's comb, but there are risks of contamination with leftover proteins and viruses (endotoxin) and additionally it requires massive purification, so this route of production is considered to be a low yield of production. Currently, as a result of these technical and safety concerns, biotechnological HA synthesis via microbial fermentation is becoming the predominant method of HA production. Therefore, today, research is being done on microbial HA synthesis in an effort to increase yield and quality. In this work HA synthesis via batch mode bacterial fermentation using *Streptococcus zooepidemicus* bacterial strain in synthetic mediums.

Methods

In this work, HA synthesis via batch mode bacterial fermentation using *Streptococcus zooepidemicus* DSM 20727 (DSMZ, Braunschweig. Germany) bacterial strain in sixteen different mediums (M1–M16) was studied. The bacterial strain utilizes and decomposes given nutrients via enzymes aerobically and/or anaerobically. Throughout the whole research, measurement of consumed nutrients concentrations, measurement of protein and biomass concentrations, pH monitoring, and HA molecular weight and quantification via High--Performance Liquid Chromatography (HPLC) all took place. Furthermore, each medium will be consumed at a different rate thus it is critical to stop batch-production as the microorganisms will start to degrade the HA to compensate for the lack of nutrients.

Results

With different medium composition, the HA concentration and molecular weight was changed. Also, the time required to reach the maximum concentration was separate. Therefore, each medium will be consumed at a different rate thus it is critical to stop batch-production as the microorganisms will start to degrade the HA to compensate for the lack of nutrients. The molecular weight of obtained HA was 470 000 +/– 140 000 g/mol, which can be considered as a medium molecular weight of the acid. Finally, the highest yield obtained was 19.049 g/L in 6 days.

Conclusions

In this study, HA production in synthetic medium demonstrated that medium and low MW HA could be obtained, with taking into consideration providing rich mediums for cultivation and pH controlling is crucial for the continuation of fermentation. Furthermore, since the fermentation occurred in batch mode it is necessary to know when to stop the process to achieve the highest collection of HA and to avoid its degradation.

OP19: Comparison of two commercially available *Saccharomycodes ludwigii* and *Torulaspora delbrueckii* strains for the production of beer with reduced ethanol concentration

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Keywords: beer, fermentation, *Saccharomycodes ludwigii*, *Torulaspora delbrueckii*, yeasts

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Objective

Beer is a common beverage across the world. Among modern trends in beer, available technologies allow producing low-alcohol beers and non-alcoholic beers. There are plenty of methods for obtaining such beverages. One of the methods is to utilize non-conventional yeasts for wort fermentation [1, 2]. In this work production of low-alcohol beer using commercially available *Saccharomycodes ludwigii* and *Torulaspora delbrueckii* strains was compared. Results show that *Torulaspora delbrueckii* achieved the lowest level of attenuation, producing beer with an ethanol concentration of 2.58% vol. *Saccharomycodes ludwigii* showed a bit higher level of attenuation. However, alcohol concentration was slightly lower than in case of *Torulaspora delbrueckii* and reached 2.50% vol. Fully fermented beers produced using *Saccharomycodes ludwigii* and *Torulaspora delbrueckii* represented reduced ethanol concentration by 12% and 15%, respectively, in comparison to *Saccharomyces cerevisiae*. Nevertheless in order to produce non-alcoholic beers arrested fermentation is necessary.

Methods

During the experiment three strains were compared during the fermentation of a beer wort with sugar concentration 9,2 °Brix. Reference strain - *Saccharomyces cerevisiae* (Fermentis T-58) Investigated strains – *Saccharomycodes ludwigii* (Fermentum Mobile FM58) and *Torulaspora delbrueckii* (Biodiva TD291). Fermentation took 12 days in 23°C. During the experiment ethanol and sugar concentrations were monitored. Moreover in final beer pH, bitterness and colour were measured.

Results

During the fermentation, it was possible to achieve beer with reduced ethanol content. In comparison to the reference strain, *Saccharomycodes ludwigii* was able to lower the ethanol level by 12%, reaching an average ethanol content of 2.58% vol. *Torulaspora delbrueckii* reduced ethanol concentration by 15%, reaching 2.50% vol. Such results are obtained for uninterrupted fermentation. These results show that both strains are suitable for the production of beer with reduced ethanol content.

Conclusions

Both commercially available strains showed potential for the production of beer with reduced ethanol content. Although with uninterrupted fermentation non-alcoholic beer was not produced in this experiment the further improvements in the mashing regime and fermentation conditions may lead to fully fermented, low-alcohol beer of ethanol content below 2% vol.

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OP20: Chemical characterization of essential oils of *Polyalthia* species using GC-MS coupled with chemometric analysis and assessment of their biological activities

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Keywords: bioactivity studies, chemometrics, essential oil, molecular docking, *Polyalthia* species

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Objective

Polyalthia is the largest and notable genera among the Annonaceae family and is known for its folkloric applications against several ailments. However, the chemical composition and biological activities of *Polyalthia* species have yet to be explored. Therefore, chemometric analysis and biological activities of essential oil of four species of *Polyalthia* (*P. coffeoides*, *P. longifolia, P. cerasoides, P. simiarum*) were carried out.

Methods

GC-MS analysis was performed to characterize the chemical constituents of *Polyalthia* species. The chemometric analysis of the essential oil of *Polyalthia* species was accomplished by performing hierarchical clustering analysis (HCA), principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and partial least squares discriminant analysis (PLS-DA). *In vitro* antioxidant activities were evaluated using DPPH, ABTS and a reducing power assay. In addition, tyrosinase inhibitory activity of essential oils was also carried out. Molecular docking was performed between major components and antioxidant and tyrosinase inhibitory proteins.

Results

The yield of leaf essential oil ranged from 0.15% in *P. cerasoides* to 0.30% (v/w) in *P. longifolia* on a dry weight basis. A total of 56 constituents representing 90.45–93.91% of total leaf oil were identified in *Polyalthia* species. The main compounds were spathulenol (1.00-24.52%), α-zingiberene (2.77–22.35%), *E*-caryophyllene (5.29–21.78%), allo-aromadendrene (1.05–17.85%) and δ-cadinene (1.54–14.33%) in *Polyalthia* species. Sesquiterpene hydrocarbons (42.63–88.91%) were found to be the predominant chemical class in *Polyalthia* species, followed by oxygenated sesquiterpenes (4.87–43.62%). The analysis of HCA and PCA based on essential oil compositions grouped four species of *Polyalthia* into three different clusters. The OPLS-DA analysis achieved a high explanatory power of 97.56% (R^2 = 0.9756) and a predictive capacity of 95.38% (Q^2 = 0.9583). The variable importance projection (VIP) score of the PLS-DA model revealed α-zingiberene and spathulenol as discriminatory markers of *Polyalthia* species with VIP score>2. In terms of bioactivity, the essential oils of *P. coffeoides* exhibited stronger antioxidant activities compared to those of other species of *Polyalthia.* Additionally, *P. longifolia* showed potent inhibitory activity of tyrosinase compared to positive control Kojic acid. The results of molecular docking revealed strong binding affinities between the main constituents of *Polyalthia* species with key antioxidant and tyrosinase enzymes.

Conclusions

The current findings revealed that GC-MS coupled with chemometric techniques could provide a reliable platform for discriminating *Polyalthia* species, which is a benefit for quality control. Bioactivity studies revealed that essential oils from *Polyalthia* could be used as an alternative source of natural antioxidants and cosmetic agents in the food and pharmaceutical industries.

OP21: Network pharmacology-based approach to investigate the pharmacological mechanism of *Cinnamomum zeylanicum* essential oil in the treatment of prostate cancer

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Keywords: *Cinnamomum zeylanicum*, essential oil, molecular docking, network pharmacology, prostate cancer

Objective
Prostate cancer (PC) is one of the leading causes of male mortality worldwide. Current PC treatments are insufficient, necessitating the development of more effective therapies. The therapeutic mechanism of *Cinnamomum zeylanicum* essential oil (CZEO) in PC treatment is unclear. Therefore, a network pharmacology approach was used to explore its pharmacological mechanism.

Methods
GC-MS analysis was carried out to identify the compounds of CZEO. Absorption, distribution, metabolism and excretion filtering was carried out to screen active components of CZEO. The CZEO and PC related targets were recovered from the public database. The compound target-disease target network was constructed using Cytoscape software. STRING was used for the protein-protein interaction (PPI) analysis. GO and KEGG enrichment analysis was performed using ShinyGO server. PyRx software was used to perform molecular docking between the core compounds and hub targets. External validation of the hub targets was carried out using GEPIA, HPA, TIMER and cBio Portal databases, respectively.

Results
A total of 59 compounds in CZEO were identified by GC-MS. Out of the identified compounds, 44 compounds passed the Lipinski rule and the abott bioavailability score and were classified as drug-like compounds. A total of 2849 compound target genes and 2053 cancer-related targets were identified from the public database. 23 compound-disease intersection targets were obtained. The network analysis revealed that camphor, eugenol, nerol, methyl eugenol and trans-farnesyl acetate are the core active compounds with higher-degree values. The PPI interaction analysis revealed CREBBP, TNF, NFKBIA, BCL2, CREB1, SMAD2, ERBB3 and MET as potential hub targets. GO enrichment analyses indicated that CZEO regulates biological processes such as induction of apoptotic processes, proliferation, and programmed cell death. Signaling pathways mainly include the PI3-AKT signalling pathway. GEPIA analysis revealed TNF, ERBB3, CREB1, and SMAD2 were significantly up-regulated in tumor tissues compared to normal tissues. Immunohistochemical staining images from the HPA database showed increased expression of CREB1 levels in prostate cancer tissues. cBioPortal tool showed that 48 of 489 patients with prostate adenocarcinoma (9.82%) had genetic mutations in these targets The molecular docking results showed that trans-farnesyl acetate and eugenol have higher binding affinity with CREBBP gene.

Conclusions
The current study showed that CZEO can treat prostate cancer through a multicomponent, multitarget, multipathway molecular mechanism. However, to clarify the relationship between CZEO and hub genes, along with the specific mechanisms underlying those actions, *in vitro* and *in vivo* experiments are necessary.

OP22: Biotechnological potential of lactobionic acid synthesized using fungal oxidoreductive enzymes

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Keywords: lactobionic acid, enzymatic oxidation, biotechnology, industrial application

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Objective

Lactobionic acid (LBA) is a chemical that belongs to the group of polyhydroxy acids. It is composed of galactose and gluconolactone linked by an ether-like bond. LBA can be obtained using microbiological and enzymatic methods. More and more research teams are deciding to synthesise this compound using enzymes. Lactobionic acid produced by enzymatic pathways is characterised, a.o., by antioxidant and antimicrobial properties, and is useful for applications in biotechnology. The aim of this study was to characterise a novel enzyme system for LBA synthesis consisting of a Phlebia lindtneri cellobiose dehydrogenase (CDH), a *Cerrena unicolo*r (LAC) laccase, a redox mediator (ABTS or DCPIP), and lactose as a substrate for biotechnological applications.

Methods

In this study, fungal oxidoreductases cellobiose dehydrogenase (CDH) from *Phlebia lindtneri* and laccase (LAC) from *Cerrena unicolor* were used. High performance liquid chromatography (HPLC) and thin layer liquid chromatography (TLC) were used to determine the lactobionic acid produced by the enzymatic reaction. The antioxidant properties were determined using a method with 2,2-diphenyl-1-picryl-hydrazyl hydrate (DPPH). The antibacterial properties of LBA were determined using the microdilution method evaluating inhibition of bacterial growth expressed as a percentage and with the TTC method assessing microbial viability.

Results

LBA was obtained in all tested systems. However, the research demonstrated that the optimal temperature for the synthesis of lactobionic acids was 50°C with the addition of ABTS. The LBA mixture synthesised at 50°C with DCPIP exhibited the best antioxidant properties (40% higher than the commercial reagent). Moreover, LBA demonstrated an inhibitory effect on all bacteria tested, with better results observed on Gram-negative bacteria with growth no more than 30% relative to the control bacteria.

Conclusions

The data obtained allowed us to conclude that lactobionic acid produced in a multienzymatic system is a compound with great biotechnological potential.

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OP23: Production of cannabinoid and phenolic compounds in callus cultures of cannabis (*Cannabis sativa* L.) cultivated on medium with various cytokinins

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Keywords: Hemp, THC, CBD, polyphenols, antioxidant, biotechnology

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Objective

The main objective of this study was to determine the influence of various cytokinins on the production of cannabinoids and total phenolics, as well on antioxidant capacity in callus cultures of cannabis (*Cannabis sativa* L.).

Methods

Calli were obtained on MS/B_5 medium [1,2] with various concentrations (0.5, 1.0, 1.5 and 2.0 mg·L^{-1}) of cytokinins N^6-benzyladenine (BA), kinetin (KIN) and thidiazuron (TDZ). The DAB method for Cannabis flos [3] was used for quantification of cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA) and the results were expressed as μg·g^{-1} dry extract (DE). The production of total phenolics (TP) was determined by Folin-Ciocalteu method (gallic acid equivalents per dry weight, mg GA·g^{-1} DW). The results for cupric reducing antioxidant capacity (CUPRAC) were expressed as trolox equivalents per dry weight (mg T·g^{-1} DW).

Results

The highest values for CBDA and THCA (7.7 and 9.7 μg/g DE, respectively) were noticed in calli grown on medium supplemented with 1.5 mg·L^{-1} TDZ. The presence of BA in the medium didn't show significant variation in the production of CBDA (1.76–1.83 μg·g^{-1} DE) and THCA (2.48–6.42 μg·g^{-1} DE). All tested concentrations of KIN showed stimulatory effects on the production of CBDA (0.35–2.15 μg·g^{-1} DE) and THCA (2.2–7.3 μg·g^{-1} DE). Calli obtained on medium with TDZ showed the greatest capacity for TP accumulation (11.1–21.5 mg GAE·g^{-1} DW). The results for CUPRAC assay showed significant positive correlation between antioxidant capacity and TP production ($p < 0.01$) in calli cultured on TDZ (0.5–2.0 mg·L^{-1}).

Conclusions

The results from this study indicated that callus cultures from cannabis cultivated on various cytokinins exhibited capacity for production of CBDA and THCA. The cytokinin TDZ was promoted as the most efficient for cannabinoid and phenolic compounds production. The antioxidant capacity of calli extracts was in positive correlation with total phenolics.

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OP24: Real-time FT-IR monitoring of thick and thin layer curing in radical photopolymerisation systems

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Keywords: radical photopolymerization, thin layer, thick layer, FT-IR, acrylate/methacrylate monomers, thiolene systems

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Objective

Over the past years the free-radical photopolymerization process has been gaining importance in many scientific and industry sectors. Compared to other methods, photopolymerization is characterized by a low cost of conducting and high curing rates, energy efficiency and no solvent requirement. Due to this, it is widely used in many engineering fields, e.g., at coating, composite preparation, solvent-free paints, adhesives or biomedical applications. However, despite its large share in the above areas, currently the most rapidly growing application area of photopolymerization is 3D printing. During the photopolymerization process, rapid and simultaneous functional group transformations occurr which affects the properties of the final product. Therefore, to ensure repeatability of the obtained products, a method that allows monitoring of the synthesis process is necessary.

Methods

During the research, a real-time FT-IR technique was used to monitor the progress of the photopolymerization process. The studied systems were acrylate/methacrylate monomers as well as thiol-ene systems in a thin layer (approx. 25 μm) and in a thick layer (approx. 1.5 mm). The progress of the reaction was observed by monitoring characteristic bands on the FT-IR chromatograph corresponding to the breakdown of the appropriate functional groups of the tested monomers. More precisely, for the thin layer, the observed bands were located at 1630 cm^{-1} corresponding to the tested acrylate/methacrylate (TMPTA – trimethylolpropane triacrylate, and TMPTMA – trimethylolpropane trimethacrylate) monomers and 2570 cm^{-1} corresponding to the tested thiol monomer (MERCAPTO – trimethylolpropane tri(3-mercapto propionate).

Results

Performed studies indicate the possibility of monitoring the photopolymerization process. In the case of thin films, a reduction in the intensity of bands originating from both methacrylate and thiol-ene monomers was observed, from which the degree of monomer conversion in the system can be determined. For the tested thick film systems, only conversions of methacrylate monomers could be observed, as the bands originating from thiol-ene monomers were covered by others.

Conclusions

The real-time FT-IR technique is a useful tool for monitoring the course of photopolymerization and the phenomena occurring within it. An important aspect is the possibility to monitor the process in thick films, which are increasingly used on an industrial scale.

ACKNOWLEDGMENTS

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OP25: Optimization of the mixed fermentation during sour beer production using *Lactobacillus brevis* and *Saccharomyces cerevisiae*

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Keywords: mixed fermentation, lactic acid bacteria, sour beer production, optimization of sour beer production, Box-Behnken Design Method

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Objective

Mixed fermentation (referred to as 'co-fermentation') is a process applied in sour beer production in which two or more microorganisms perform simultaneous fermentation in the same medium. In most cases, two microbes: brewing yeast and lactic acid bacteria are used to produce beer containing both ethanol and lactic acid. However, the concentration of the microorganism's metabolites in the final product is difficult to predict, as their metabolism is strictly dependent on the conditions: concentration of sugars (expressed in Plato) and bitterness (expressed in IBU – International Bitterness Units). Another factor influencing the final product's properties is the rate and order of pitching the microorganisms (either at the same time or one after another, resulting in a particular time when only one of them is present in the wort) [1]. The main objective of this study is to examine the possibility of optimization of the mixed-fermentation process by applying the Box-Behnken Design method (BBD).

Methods

Wort samples were prepared according to BBD Method, applying different initial bitterness and sugar extracts, as well as three different sequences of microorganisms pitching (lactic acid bacteria – Lactobacillus brevis and brewing yeast – Saccharomyces cerevisiae). The microorganisms were either pitched on the same day or one after another with an interval of three days. The Methods applied to measure the fermentation progress and the final samples' properties included gas chromatography and spectrophotometric methods. Optimization was performed using MATLAB with Statistics and Machine Learning Toolbox based on the results. Additionally, organoleptic tests were performed.

Results

It appeared that different initial conditions resulted in a large variety of the final products' properties. Obtained values led to the mathematical prediction of the optimal initial conditions for minimal and maximal concentrations of ethanol and lactic acid in the beer. For example, it predicted that optimized process conditions could lead to approximately 9.5 g/L of lactic acid in the final product.

Conclusions

The BBD method could be an efficient tool for predicting and optimizing the properties of sour beer, especially the outcomes of the mixed fermentation step. Further research might be needed to evaluate the applicability of the BBD method in beer production.

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OP26: Effect of light on the photosensitisation of bacteria

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Keywords: 5-aminolevulinic acid, photodynamic inactivation, *Pseudomonas aeruginosa,* bacteria, laser light

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Objective

Antibiotic resistance is one of the greatest threats to human health. For this reason scientists are focusing on finding novel non-antibiotic strategies to combat pathogens. Photodynamic inactivation of bacteria (PDI) has drawn increasing attention from the scientific society for its potential to effectively kill multidrug-resistant pathogenic bacteria. PDI involves light to excite chemical compound (photosensitiser) in the presence of oxygen, in order to generate reactive oxygen species (ROS). The production of ROS by photosensitiser is essential for photodynamic inactivation. Due to that formation bacterial structures are destroyed by oxidative processes [1]. The purpose of my study was to evaluate the effect of two different light wavelengths on the effectiveness of photodynamic inactivation of bacteria.

Methods

The study was conducted with the bacterial strain *Pseudomonas aeruginosa*. The chemical compound that was used as a precursor of the actual photosensitiser (protoporphyrin IX) was 5-aminolevulinic acid (5-ALA) [2]. In this research two different light wavelengths (635 nm and 405 nm) were applied. In order to obtain information on the viability of *Pseudomonas aeruginosa* the BacTiter-Glo™ Microbial Cell Viability Assay was used as well as culture techniques.

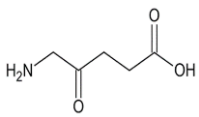

Fig. 1. 5-aminolevulinic acid

Results

The results obtained indicate that blue light (405 nm) mediated photodynamic inactivation has proven to be more effective than PDI with the use of red light (635 nm). Time required to obtain lethal effect is significantly shorter with the use of 405 nm wavelength than with 635 nm.

Conclusions

Photodynamic inactivation is a promising approach to eradicate bacterial pathogens. This study has shown its effectiveness against *Pseudomonas aeruginosa*.

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OP27: A Study of Plants using Medicinal Properties to treat Malaria

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Keywords: malaria, herbal plants, *Plasmodium vivax*, *Plasmodium falciparum*, alkaloids, flavonoids, *Asteraceae*

Objective
Malaria is the most prevalent disease of the haematological system and is brought on by parasite infection [1]. Malaria is a disease that can be fatal and is caused by an abundance of the malaria parasite referred to as *Plasmodium (P) vivax*, *P. falciparum*, and *P. ovale*. It is spread by the female anopheles mosquito biting a person. WHO estimates that India accounts for 3% of the world's malaria cases [2]. Medical plants have recently developed a secure and accessible solution for the treatment of malaria.

Methods
The purpose of this review is to assess the potential value of using herbal natural components to create cures for malaria by examining the research of herbal plants, families, and their metabolites [3]. A variety of sources, including Web of Science, Google Scholar, PubMed, Bentham Science, Elsevier, Springer, Nature, Wolters Kluwer, etc., were used to gather the data.

Results
Following the identification of 300 plants from 90 Families, data on the plant family, portion used, metabolite extract, model use, and parasites were abstracted.

Conclusions
According to the findings, the *Asteraceae, Fabaceae*, and *Rubiaceae* families contained the majority of antimalarial plants [4]. Alkaloids and flavonoids were the most active and prevalent natural plant metabolites with antimalarial action, and the leaves were the component of the plant that received the most scientific attention.

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OP28: Novel cubosomes as relevant nanocarriers for encapsulation of photoactive pigments from seaweed biomass for anticancer drug delivery

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Keywords: seaweed, cubosome, pigments, photoactivity, anticancer

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Objective

Liquid-crystalline nanoparticles characterised by high encapsulation efficiency and high colloidal stability, named cubosomes, are gaining more and more recognition as drug delivery systems [1]. *Ulva Rigida* is a green seaweed valued for its high content of photoactive pigments with anticancer activity achieved with photodynamic therapy (PDT) – chlorophylls, unfortunately limited to instability and insolubility in the body's environment, due to the risk of aggregation or degradation [2, 3]. Pancreatic cancer is considered extremely resistant to therapy due to its complex and obstructive tumour microenvironment [4]. Therefore, it is necessary to find relevant carriers for the solubilisation and protection of the anticancer pigments from *U. Rigida* in order to bypass the pancreatic tumor environment and deliver them to cells in the correct photoactive form.

Methods

In this work, microwave-assisted extraction will be presented as an efficient green method for the isolation of chlorophylls from *U. Rigida* biomass. The self-assembly and sonication approach has been used for the synthesis of two types of cubic formulations and the pigments encapsulation. Their biocompatibility has been evaluated in human pancreatic cancer cell lines with the MTT cytotoxicity test and flow cytometry, as well as the photoactivity by the reactive oxygen assay.

Results

A stable, biocompatible, and photoactive cubic nanoformulations loaded with *U. Rigida* pigments will be presented for potential use in PDT, with the advantages of novel cubosomes, along with their physicochemical characterization.

Conclusions

We believe that this research will contribute to the discovery of an effective treatment for extremely resistant pancreatic cancer.

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OP29: Characterization and Detection of Antibiotic Susceptibility of the Bacteria Isolated from Foot Infection of Diabetic Patients from Diabetic Hospital, Dinajpur

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Keywords: diabetic foot, isolation, identification, *Escherichia coli*

Objective
Diabetic foot infection is the most dreaded complication of diabetes mellitus and the commonest cause of hospitalization and limb amputation. Identification of the causative agent responsible for diabetic foot infection and the earliest initiation of appropriate antimicrobial therapy are vital for the control and prevention of the complication of diabetic foot ulcers. Therefore, we conducted this study to determine the bacteriological profile of diabetic foot ulcers and to detect antibiotic sensitivity pattern from diabetic hospital, in Dinajpur.

Methods
During the study period, samples were collected from the foot ulcers of 30 patients at the Diabetic Hospital Dinajpur. The samples were processed according to the standard laboratory protocol, and bacterial isolates were identified. For molecular characterization of *Escherichia coli* 16E1 and 16E2 primer were applied. Antibiotic susceptibility testing was performed using the modified Kirby-Bauer disk diffusion technique, and results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2016).

Results
A total of 46 bacterial isolates from pus samples of diabetic foot were isolated from 30 positive patients and identified by conventional methods. Out of 46 isolates 59.70% of total isolated organisms were found to be gram-negative while the remaining 41.30% were gram-positive. The gram-positive isolates was most predominant includes *Staphylococcus* spp. (41.30%) whereas gram-negative were *Pseudomonas* spp. (32.61%) and *E. coli* (26.09%), respectively. The highest number of positive bacterial isolates were recorded in 10 (33.33%) at 61–70 years age group patients followed by 8 (26.67%), 51–60 years, 5 (16.67%), 41–50 years, 3 (10%), 71–80 years and 31–40 years, respectively. The antibiotic resistances of identified organisms were carried out by disc-diffusion method with commercially available 10 discs of antibiotics having different modes of action. The gram-positive isolates were highly sensitive to Levofloxacin (100%), Pefloxacin (100%), Ciprofloxacin (100%), whereas resistant to Oxacillin, Gentamicin, and Linezolid (100%), Tetracycline (80%), respectively. The sensitivity rates of isolated gram-negative bacteria were sensitive to Ciprofloxacin (100%), Chloramphenicol (100%), Levofloxacin (100%), and Pefloxacin (100%). The isolates were resistant to Tetracycline (100%), Amikacine (100%) and Cotrimazole (100%), Kanamycin (100%), and Imipenem (100%).

Conclusions
This study revealed that the frequency of *Staphylococcus* spp. increases the probability of diabetic foot infection. It is now very necessary to develop new antimicrobials and therapeutic agents for diabetic foot patients having high effectiveness with no side effects, easy availability, and are less expensive.

ACKNOWLEDGMENTS

The authors want to thank the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh for their full support during the research conduct. The research was financially supported by the National Science and Technology Fellowship, Bangladesh.

OP31: Deciphering the molecular target and mechanism of *Cinnamomum tamala* essential oil in the treatment of inflammation via network pharmacology and molecular docking approach

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Keywords: *Cinnamomum tamala*, essential oil, network pharmacology, inflammation

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Objective
Cinnamomum tamala commonly known for Indian bay leaf has been used in the Ayurveda system of medicine for its medicinal property. However, the potential targets and underlying anti-inflammatory mechanism of *Cinnamomum tamala* leaf essential oil (CTL) still remain unexplored. Therefore, network pharmacological analysis was employed to evaluate its anti-inflammatory mechanism of action.

Methods
GC-MS analysis was carried out to characterize the chemical constituents of CTL. The drug-like compounds of CTL was screened by ADME filtering. CTL and inflammation related targets were obtained using public databases. Venny 2.1 online tool was used to retrieve the genes shared by both CTL and inflammation related targets. Compound-target-disease pharmacological network was visualized using Cytoscape 3.9.1 software. STRING database was used to construct the protein-protein interaction network. GO and KEGG pathway enrichment analysis was performed using ShinyGO 0.77 web tool. Molecular docking between the key targets and hub compounds was performed in PyRx software.

Results
A total of 48 chemical constituents were characterized from GC-MS analysis, of which all passed the ADME screening and were considered as drug-like compounds. A total of 640 compound targets and 772 anti-inflammation targets were obtained. 120 compound-disease overlapping targets were obtained. γ-amorphene (degree-36), γ-cadinene (degree-36), γ-muurolene (degree-36), iso-longifolene (degree-35), α-selinene (degree-34), α-calacorene (degree-34), α-pinene (degree-34), α-ylangene (degree-34) and (2Z,6Z)-farnesol (degree-34) are the core active ingredients. PPI interaction analysis identified MAPK1, MAPK3, MAPK14, JUN, FOS, SRC, AKT1, MMP9, HIF1A and VEGFA as the hub targets. GO enrichment analysis indicated CTL is associated with biological processes including response to lipopolysaccharide and regulation of inflammatory response. KEGG pathway analysis revealed that CTL exerted its anti-inflammatory effect by modulating VEGF signalling pathway and Relaxin signalling pathway. The molecular docking results showed good binding affinities between the core target proteins and active ingredients of CTL.

Conclusions
The current finding revealed that *Cinnamomum tamala* leaf essential oil can treat inflammatory diseases by a multi-target, multi-component and multi-pathway mechanism. However, further *in vitro* and *in vivo* experiments are necessary to validate the specific underlying mechanism behind the anti-inflammatory property of *Cinnamomum tamala* leaf essential oil.

OP32: Halogenated COSAN derivatives: synthesis and antimicrobial activity

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Keywords: metallacarboranes, COSAN, halogenation, antibacterial activity

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Objective

I_2-COSAN, a type of iodinated metallacarborane, has been shown to possess potent antimicrobial activity [1],[2]. Despite this, the antimicrobial activity of other halogenated derivatives has not been investigated so far.

Halogenated derivatives of COSAN were synthesized and evaluated for their antimicrobial activity. A range of mono- and dihalogenated derivatives was tested against Gram-positive (*Staphylococcus aureus* ATCC 27853, *Enterococcus faecium* PCM 2910) and Gram-negative bacteria (*Pseudomonas aeruginosa* PCM 2720, *Escherichia coli* PCM 1630).

The aim of the research was to investigate the effects of halogen substitution on the antimicrobial activity of obtained derivatives.

Methods

High-performance liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) characterized the derivatives, confirming their structure and purity.

The microdilution method was used to determine the minimum inhibitory concentration (MIC). Minimum bactericidal concentration (MBC) was obtained from the microdilution method by subculturing on agar plates without the tested compound.

Results

Our findings demonstrate that halogenated derivatives of COSAN exhibit antimicrobial activity only against Gram-positive bacteria, with MIC ranging from 0,8 to 50 μM. The lowest MIC value was determined for I-COSAN against *Staphylococcus aureus*.

Conclusions

The greater the atomic mass of the halogen present in the derivative structure, the higher its antimicrobial activity.

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OP33: Antimicrobial potential of lily alcohol oxa-derivatives with fragrance properties

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Keywords: lily alcohol, fragrance compounds, MIC, derivatives

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Objective
Many commercially available fragrance compounds have certain limitations related to their use, which causes that functional compounds are searched, which play multiple roles in the product. Derivatives of known fragrances that have antimicrobial potential in addition to interesting profiles may be the answer. The amount of available fragrance materials with the lily of the valley profile is very poor, the most commonly used is lily alcohol. Synthesis of derivatives of lily alcohol, which is an undeveloped research niche and significant commercialization potential, may be the answer for these needs.

Methods
The subject of the research was the synthesis of oxa-derivatives of lily alcohol and the verification of their antimicrobial activity. For this purpose, the synthesis of seven derivatives of phenolic compounds was carried out using the Bargellini reaction. Next step of the study was to determine the MIC (minimum inhibitory concentration) of the microorganisms, i.e. *Staphylococcus aureus* (ATCC 6538), *Kocuria rhizophila* (ATCC 9342), *Enterobacter gergoviae* (ATCC 33028), *Escherichia coli* (ATCC 10536), and *Enterococcus hirae* (ATCC 10541).

Results
The obtained results showed that 2-methyl-2-(4-methylphenoxy)propanoic acid, α-(4-Ethyl-phenoxy)-isobutyric acid and α-(3-methylphenoxy)isobutyric acid, exhibited the best inhibitory effect on the *S. aureus* strain, with a MIC of 300 μg/mL. The lowest MIC value of 1200 μg/mL was obtained for the *K. rhizophila* strain, for two compounds, i.e., 2-(3-fluorophenoxy)-2-methylpropanoic acid and α-(4-Ethyl-phenoxy)-isobutyric acid. For *E. gergoviae*, α-(4-Ethyl-phenoxy)-isobutyric acid showed the lowest MIC value of 600 μg/mL. The mic values obtained for *E. hirae* and *E. coli* were over 2400 μg/mL.

Conclusions
In general, among the seven synthesised compounds, the best results for all tested strains were obtained for the 4-ethylphenol derivative, α-(4-Ethyl-phenoxy)-isobutyric acid. The results justify continuing the chosen path of synthesis, which involves the search for functional fragrance compounds that can ultimately function as an element of the fragrance composition and supporting preservative activity.

OP34: Preparation and evaluation of antioxidant activities of decarboxylated gomphrenin derivatives

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Keywords: betalains, antioxidants, thermal degradation, *Basella alba* L.

Objective

Betalains cover a class of remarkable natural plant pigments that exhibit prospective chemical and biological characteristics. However, their wide-ranging applications may be limited by degradation at elevated temperatures [1]. The aim of our study was to evaluate the antioxidant activities of decarboxylated derivatives formed during thermal treatment of gomphrenin pigments.

Methods

Gomphrenin was extracted from *Basella alba* L. fruits and subjected to isolation by preparative liquid chromatography. Purified pigment was acidified with 10% acetic acid and heated at 85°C. Resulting decarboxylation products were identified by LC-DAD-ESI-MS/MS. A protocol for isolation and purification of mentioned derivatives using anion exchanger and preparative HPLC was also developed. Antioxidant activity of decarboxylated pigments was evaluated using three spectrophotometric tests – ABTS, FRAP, and ORAC.

Results

In this study, three decarboxylated gomphrenin derivatives, named 2-decarboxy-gomphrenin, 17-decarboxy-gomphrenin, and 2,17-decarboxy-gomphrenin with characteristic protonated molecular ions with m/z 507, 507 and 463, respectively, were isolated for the first time in a semi-preparative scale. 2-decarboxy-gomphrenin and 2,17-decarboxy-gomphrenin exhibited improved antioxidant activities ($TEAC_{ABTS}$ values 10.40 ±0.09 mmol/g DW and 10.40±0.06 mmol/g DW respectively) compared to the substrate ($TEAC_{ABTS}$ value 9.87 ±0.15 mmol/g). 17-decarboxy-gomphrenin had lesser action ($TEAC_{ABTS}$ value 6.00 ±0.08 mmol/g DW) compared to the remaining derivatives. All compounds were characterized by a higher ability to scavenge radicals than caffeic acid ($TEAC_{ABTS}$ value 5.55 ±0.21 mmol/g DW).

Conclusions

The results show that all tested pigments are characterized by high antioxidant activities. In addition, heat treatment of gomphrenin-rich products can improve their potential in scavenging reactive oxygen species. Decarboxylation at carbon-17 reduces antioxidant activity compared to another common decarboxylation position at carbon-2. However, biological assays should be performed *in vitro* to confirm potent antioxidant activities of the pigments of interest.

ACKNOWLEDGMENTS

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OP35: New Perspectives for Biology and Innovative Applications of Cold Atmospheric Plasma

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Keywords: cold plasma, food chemistry, microbiology, cytotoxicity

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Objective

Nowadays, methods that do not require disturbing tissues or organisms are becoming a key element in the development of modern technology and scientific progress. In this context, cold atmospheric plasma (CAP) is emerging as a promising tool with the potential to revolutionize biology. CAP offers a non-invasive approach that uses low temperature to generate plasma in the atmosphere and allows experiments and procedures to be performed without compromising their structure. The conclusion is that it is important to pursue the development of cold atmospheric plasma technology in biological areas.

Methods

In order to assess the impact of the CAP treatment on the preservation of processed orange juice (OJ), the standard plate counting method was utilized. The Caco-2 cell line model, representing the human intestinal system, was chosen as the suitable in vitro cellular model for evaluating the bioavailability of metal ions in different food products and beverages (Dzimitrowicz et al. 2021).

Results

The juice obtained in this way was found to have an increased content of polyphenolic compounds (by 6.1%) extended shelf life (up to 26 days after opening), as well as exhibiting cytotoxic properties against colon cancer cell lines (Caco-2). We anticipate that this innovative method based on the use of CAP will soon be implemented in the food industry [1].

Conclusions

In conclusion, the application of CAP treatment demonstrated an extended shelf life of orange juice, estimated to be over 26 days. Furthermore, the absence of cytotoxicity on non-malignant human intestinal epithelial cell lines suggests that the treated juice is safe for consumption. These findings highlight the potential of CAP technology in enhancing the preservation and safety of fruit juices

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OP36: The influence of external factors on the morphology of lipidic mesophases

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Keywords: lyotropic liquid crystals, myelin figures, polarized light microscopy, confocal fluorescence microscopy, two-photon excited fluorescence microscopy

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Objective

Lyotropic mesophases have attracted considerable attention, especially in terms of their essential properties in many biological systems [1, 2]. A well-known example of liquid crystals of biological significance is the myelin sheath, which plays a crucial role in the propagation of the action potential [3]. In this work, we investigated artificial myelin-like structures composed of phospholipids [4]. The important advantage of the single-component lipid structure was the ability to obtain a simplified biomembrane model.

Methods

In all experiments, lipidic mesophases were prepared from saturated phosphatidylcholines. Confocal fluorescence microscopy and two-photon excited fluorescence microscopy were used to visualize the three--dimensional morphology of multilamellar microstructures.

Results

The combination of polarized light microscopy and fluorescence microscopy provided us with detailed information about the effect of pH on the thermal stability of myelin-like structures. Furthermore, different structural morphologies of multilamellar assemblies were also examined using two-photon excited fluorescence microscopy.

Conclusions

Our results showed that the pH of the aqueous phase significantly correlates with the liquid crystalline properties of phospholipid-based structures. Additionally, we demonstrated that one- and two-photon excited fluorescence microscopy can be successfully applied to follow the thermal stability and formation of defects within myelin-like structures.

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OP37: Synthesis and selected catalytic applications of chiral azaaromatic derivatives and their N-oxides

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Keywords: organic synthesis, heteroaromatic N-oxides, organocatalysis

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Objective

Heterocyclic N-oxides are well-known, effective catalysts in nucleophilic substitution and addition reactions. N-oxides of various types can be used in asymmetric synthesis both as organocatalysts and as ligands in reactions catalyzed by metal complexes. In the role of chiral Lewis bases, N-oxides are capable of activating halogenosilanes, e.g., in the allylation reaction of aldehyde with allyltrichlorosilane. The reactions lead to chiral homoallylic alcohols that have significant synthetic value and are often building blocks in the synthesis of pharmaceuticals. However, catalytically efficient heteroaromatic N-oxides usually are quite complex, sophisticated structures that require long and tedious synthesis. Therefore, the development of new structures, readily available and efficient as chiral organocatalysts for the reaction of trichlorosilyl compounds, is still very active [1].

Methods

Within our investigations, several azaaromatic oxazolines, imidazolines, thiazoles and imines and their N-oxides with pyridine, substituted pyridine, 2,2′-bipyridine and isoquinoline fragments were synthesized. Various synthetic pathways have been designed and tested according to the properties and limitations imposed by the target products. Selected compounds were examined in catalytic applications, e.g. allylation of benzaldehyde with allyltrichlorosilane, reduction of ketimine with trichlorosilane and in Henry reaction catalyzed by Cu(II)-complex.

Results

Almost 60 new heteroaromatic compounds and their N-oxides were synthesised in two to five step synthesis with overall yields up to 84%. The best catalytic results reach 90% yield and 79% ee in allylation of benzaldehyde and 85% yield, 57% ee in Henry reaction [2].

Conclusions

The readily available new chiral heteroaromatic N-oxides show catalytic potential both in metal-free and metal catalyzed reactions. The further improvement of such a structures is currently under active investigation in our laboratory.

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OP39: Poly(glycerol sebacate)/hydroxyapatite scaffolds: a promising matrix for bone tissue engineering application

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Keywords: poly(glycerol sebacate), PGS, tissue engineering, composites, biomaterials

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Objective
The aim of the presented study was to validate whether poly(glycerol sebacate) (PGS) scaffolds with addition of hydroxyapatite are promising candidates for bone tissue engineering application.

Methods
Poly(glycerol sebacate) (PGS) prepolymer was synthesized using solventless thermal polycondensation method using sebacic acid and glycerol as monomers. Hydroxyapatite (HAp) was obtained using wet precipitation technique. PGS-based scaffolds were manufactured using thermally induced phase separation followed by thermal cross-linking and salt-leaching (TIPS-TCL-SL technique). In the course of the experiments polymer PGS scaffolds were obtained in addition to the composite foams containing 10, 20 and 30 wt. % of apatite filler. Physico-chemical characterization consisted of FT-IR, NMR, DSC, TGA, water contact angle and gel-sol content determination. Scaffolds morphology was observed under SEM microscope. Mechanical characterization was performed using static compression and dynamic DMTA measurement. Cytotoxicity of the biomaterials was assessed against L929 fibroblasts using MTT reduction assay under procedure recommended by ISO 10993-5:2009 standard.

Results and conclusion
SEM images showed alterations in morphology of the scaffolds after increasing addition of HAp. Addition of apatite filler did not affect the degree of cross-linking and the presence of HAp was confirmed by FT-IR and EDS measurement as well as in TGA. Results indicated the superior mechanical properties for the sample containing 20 wt. % of HAp. Finally, the research revealed that all evaluated specimens were not cytotoxic against L929 fibroblasts and the cell cultures populated the foams. The presented results are a part of the research published in peer-reviewed journals [1].

ACKNOWLEDGMENTS

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OP40: Benzylidene iodonium salts as photoinitiators for cationic 3D-VAT printing

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Keywords: iodonium salts, cationic photopolymerization, epoxides, 3D-VAT printing

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Objective

The application of cationic 3D-VAT printing is still limited by the lack of photoinitiators absorbing the light emitted by printers (about 405 nm). Diaryliodonium salts absorb poorly above 300 nm and need photosensitizers to initiate polymerization efficiently [1]. Therefore, the development of advanced iodonium salts that absorb in the longer wavelengths is essential.

Methods

Benzylidene chromophores were synthesized using Knoevenagel condensation. Iodonium salts were synthesized using diacetoxyiodobenzene (asymmetric) or iodosyl sulfate (symmetric). Absorption and photolytic properties as well as quantum yield of photoacid generation were determined using UV-Vis absorption spectroscopy combined with tungsten lamp and LEDs. Photoinitiating activity was investigated using real time FTIR. 3D-VAT printing experiments were performed using LCD printer Anycubic Photon Mono X with a 405 nm light source (Anycubic, Shenzhen, China).

Results

Benzylidene chromophore designs allow iodonium salts to be obtained in a selective manner which was previously impossible for the more sophisticated chromophores [2]. Moreover, the first symmetric iodonium salts containing a double bonds were prepared [3]. Such advances have produced compounds with excellent absorption properties reaching into the visible light. They can photolyse efficiently at 365 nm and 405 nm LED photoinitiating cationic polymerization of epoxides and vinyl ethers.

Conclusions

New design leads to a great improvement in absorbing and photoinitiating properties of iodonium salts and they can be used successfully in such advanced applications as cationic 3D-VAT printing.

ACKNOWLEDGMENTS

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OP42: Evaluation of the activity of peptidoglycan isolated and purified from bifidobacteria

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Keywords: *Bifidobacterium*, peptidoglycan, isolation, purification

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Objective
Peptidoglycan is a major component of the cell wall of gram-positive bacteria, including bifidobacteria. Probiotic strains of bifidobacteria have been shown to modulate the immune system through various components of the cell wall (i.e., peptidoglycan, polysaccharides, and surface proteins). To study the immunomodulatory properties of bifidobacteria peptidoglycans, it is necessary to adapt isolation and purification methods to obtain an uncontaminated form of the antigen. However, peptidoglycan isolation methods do not rule out the simultaneous co-isolation of lipoteichoic acids and lipoproteins, which may cause a false signal for the TLR2 receptor. Therefore, it is essential to have a reliable method for isolating and purifying peptidoglycan from *Bifidobacterium* strains to study its effects on the immune system. The appropriate method for isolating and purifying peptidoglycan from *Bifidobacterium* strains was adjusted.

Methods
To isolate the peptidoglycan, bacterial dry mass was boiled in various concentrations of SDS. Different enzymes were used to purify PG: DNase, mutanolysin, etc. The efficacy of the peptidoglycan purification methods was tested using HEK-293 cell lines stably transfected with TLR2 or NOD2 receptor genes (HEK-BueTM-hTLR2 and HEK-BlueTM-hNOD2). To analyse the activation of receptors in individual cell lines, two types of HEK-293 cell lines were used: (1) HEK-293 cell lines stimulated in a Hek-Blue Detection medium, which allowed to observe of the real-time receptor activation through colourimetric reaction (2) HEK-293 cell lines that produced IL-8 after receptors activation and required an ELISA test.

Results
The most efficient method of isolating and purifying peptidoglycan of *Bifidobacterium* strains was determined. The appropriate isolation and purification method eliminates the partial signal from the TLR2 receptors. Peptidoglycans isolated from *Bifidobacterium* strains are recognized by NOD2 – common peptidoglycan receptor, however any tested methods do not eliminate signals from TLR2 receptors.

Conclusions
It is important to note that the isolation and purification methods of antigens affect their purity as well as their subsequent activity.

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OP43: Revealing the Influence of Micellar Systems on the Solubilization Behavior of Solvatochromic-Origin Dyes: A UV-Visible Study

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Keywords: disperse Orange 3, 2,6-dichloro-4-(2,4,6-triphenyl-1-pyridinio) phenolate, UV-Vis spectroscopy, surfactant micelles, polarity indicators, polarity scale

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Objective

In this study, we investigate the interactions between surfactants and dyes in supramolecular micellar structures. The study aimed to analyze the influence of surfactant properties, such as surfactant head group charge and choice of solvatochromic probe on the localization of dyes within micelles. The use of surfactants with diverse structures and charges was expected to affect the surfactant-dye interaction and the solubilization process [1]. The study demonstrated that surfactant properties and the microenvironment within micelles have a significant impact on the solubilization of dyes. The integration of cutting-edge techniques such as use of surfactant nanocarriers and polarity indicators, along with the development of a comprehensive polarity scale, ensures wide applicability of these breakthroughs across the biotechnology and chemistry industries.

Methods

UV-Vis spectroscopic studies were conducted using different surfactants to understand the solubilization of selected dyes. Spectrophotometric measurements estimated the approximate location of the dye in the micelle, indicating that both dyes were located in the outer layers of the micelles. Additional dynamic light scattering (DLS) and electrophoretic light scattering (ELS) studies supported the hypothesis that the dyes were located in the outer layers of the micelles.

Results

The transition of the dye from the solvent to the micelle led to spectral changes due to the polarity gradient within the micelle. The results showed that surfactant characteristics and the dye's structure play significant roles in solubilization within micellar systems. Changes in absorption bands were observed due to alterations in the microenvironment surrounding the dye. Significantly, the findings revealed that both model molecules were located in the outer part of the micelle rather than the core.

Conclusions

Overall, the results provided insights into the solubilization behavior of the selected dyes in micellar systems. The spectral changes suggested a transition of the dye from a more polar water environment to the less polar interior of the micelle. This understanding of surfactant-dye interactions has tremendous potential in the field of chemistry and biotechnology, particularly in the design and optimization of drug delivery systems and also in determining solvent parameters in chemical technology.

ACKNOWLEDGMENTS

The research project was supported by the Department of Physical and Quantum Chemistry at Wrocław University of Science and Technology.

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OP44: The Influence of a Fire at an Illegal Landfill in Southern Poland on the Formation of Toxic Compounds and Their Impact on the Natural Environment

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Keywords: landfill; fire; contamination; PAHs; leachate; gas chromatography (GC-MS)

Objective
Landfill fires pose a real threat to the environment as they cause the migration of pollutants to the atmosphere and water sources. A greater risk is observed in the case of wild landfills, which do not have adequate isolation from the ground. The purpose of this study is to investigate the toxic substances generated during a wild landfill fire. Such research has not yet been performed in Poland. Moreover, in the world's literature, the problem has not been sufficiently addressed, with such key information missing as the level of contaminants produced, their migration extent, and time present in the soil.

Methods
A total of 32 samples of incinerated waste and soil were collected. The samples soil and burn waste were analyzed using the GC-MS method. In the leachates - chlorides were analyzed using the colorimetric method using a Discrete Analyzer, while the other ordered compounds were analyzed using the ICP-OES technique.

Results
The organic compounds included naphthalene, fluorene, phenanthrene, anthracene, acenaphthene, acenaphthylene, fluoranthene, pyrene, benzo (c) phenanthrene, benzo (a) anthracene, chrysene, benzo (ghi) fluoranthene, benzo (b + k) fluoranthene, benzo (a) fluoranthene, benzo (c) fluoranthene, benzo (a) pyrene, benzo (e) pyrene, perylene, indeno[1,2,3-cd] pyrene, benzo (ghi) perylene, and dibenzo (a + h) anthracene. Among the inorganic parameters, sulfates, chlorides, arsenic, boron, cadmium, copper, lead, and zinc were taken into account. Fluoranthene dominated in most of the samples. Sulfates and chlorides were present in the samples.

Conclusions
The most significant finding of the research is that the currently deposited burnt solid waste in the wild landfill poses a potentially permanent hazard to the environment. Samples taken even a long time after the fire started in the study area contain significant concentrations of hazardous organic compounds, in particular PAHs. No significant spikes in heavy metal content were observed in leachate collected from the incinerated waste samples. This may be justified because, during the incineration of solid waste such as tires and black rubber, more organic compounds are formed by high temperature and oxidation. Research of this type must be carried out in conjunction with environmental monitoring to identify potential threats to the environment and human health early.

OP45: A Sustainable Approach to Mitigate Environmental Pollution, Global Warming and Climate Change

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Keywords: indoor air quality, button up mode, CO_2 removal, oxygen replenishment, positive pressure

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Objective

The present research describes an indigenously developed system, which removes CO_2, Total Volatile Organic Compounds (TVOC), other toxic gases, particulate matters and other pollutants from the air. The system is so designed as to collect the pollutants from the outdoor and indoor air by absorption and adsorption into the filter materials till their saturation capacity. Due to chemistry of the filters, their saturated material gets enriched with carbonates and silicates of Calcium, Sodium, Lithium and other useful minerals like Magnesium, Sulphur, Carbon, etc; which are of high nutritional value for agricultural crops. After the filters get saturated, these are replaced by the new filters and the saturated material of these filters are sent to an organic manure manufacturing plant and use them to produce **"Minerals Rich Organic Manure (MROM)"**. The complete cycle of movement of various components and the stages form a Sustainable Eco System. In this system the trees and plants provide us the food and oxygen and whatever CO_2, TVOC and other pollutants we (human and live stock) produce, these are collected by the filtration system and returned back to the trees and plants in the form of MROM as their nutrient. So it provides a very good self-sustaining eco system of mutual survival of human beings and plants together in nature.

Methods

The CO_2/TVOC and Pollutant Removal Systems are so designed that these can be easily used in a house, office, hospitals, Malls etc. Even smaller units which can be fitted to cars and the mobile units which can be carried by the individuals as part of their personal kit or in their hand bags, brief cases etc are also developed. Even the smallest system installed in a room or on top of a car absorbs the quantity of CO_2 from the environment which is equivalent to two numbers of fully grown trees of 5 to 10 years of age. Therefore, when such a system is installed in houses and cars in a big city, then you may well imagine that the so called **"Concrete Jungles"** of the big cities will start working as the **"Natural Forests"** to clean the air and the moving traffic on the roads will start working as the **"Mobile Forests on Wheels" moving** on the roads of the city.

Results

With additional provisions of **Oxygen Replenishment System, Positive Pressure Maintenance System** and **"Facility Management System"** integrated with the above **CO_2/TVOC and Pollution Removal System**, any house can be converted into an **"Emergency Home"**, which can be used for safe stay of a family for the desired **"Button Up Period"** in case of any emergency or under a threat of a biological or chemical disaster or corona type pandemic.

Conclusions

The proposed System does not only provide a clean and healthy living environment both indoors and outdoors, to the public, but it helps in eradicating the problem of rising pollution, climate change and global warming from the entire world and make this planet earth liveable for our present and future generations.

OP46: Computational Tools for the Design of Fluorescent Dyes

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Keywords: electronic absorption spectra, fluorescent dyes, machine learning

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Objective

Fluorescent dyes are everywhere: from entertaining glowing paints for children to complicated medical equipment to observe processes in cells. Uncountable syntheses and analyses of new fluorophores have to be performed to fulfill the requirements of nowadays market. However, theoretical chemistry is a promising solution for pre-screening of optical properties of dyes and the aim of the talk is to show the potential of computational techniques for efficient and accurate simulations of vibrationally-resolved absorption spectra.

Methods

20 fluorescent dyes representing a wide palette of both core/substituent modifications have been selected for the present study. Vibronic spectra simulations have been optimized in terms of density functional approximation for hessian calculations and different harmonic approximations have been tested for simulations of vibronic structure. For the estimation of inhomogeneous broadening of absorption bands, a new machine learning approach has been proposed based on molecular dynamics trajectory involving calculations of vertical excitation energies at a set of solute-solvent configurations.

Results

It has been shown that a computationally cheap combination of electronic-structure theory and vertical gradient approximation is suitable for a very accurate prediction of vibronic structure. A new machine learning approach enabled the calculation of inhomogeneous broadening parameters with errors as low as 2 cm^{-1} at a small fraction of the computational cost of the conventional approaches. Final vibrationally-resolved spectra have demonstrated excellent agreement with the band profiles obtained experimentally.

Conclusions

The proposed cost-efficient computational protocol demonstrates a good agreement with the experiment and can be used as a helpful tool for the preselection of fluorescent dyes.

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OP47: Bioaccumulation of copper in the above-ground part of mustard seeds (*Sinapis alba*) after application of organic matter as a soil additive in contaminated soil

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Keywords: copper, soil additives, phytoremediation, bioimmobilization, ICP-OES analysis

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Objective
The content of toxic metal ions in the soil is increasing as a result of the environment's growing pollution caused by urban agglomerations, constantly developing transport, and the increased activity of industrial plants. One of these metals is copper, which is also a microelement necessary for the proper growth and functioning of plants. A lack of this element, and in particular its excess, can have a harmful effect on the plant [1]. The aim of this study was to compare the bioaccumulation of copper in the above-ground part of plants after the application of phytoremediation and bioimmobilization of copper ions in the soil with the use of organic additives (leaves biomass: oak, maple, beech).

Methods
Pot experiments were carried out on mustard seeds, in which the multielemental composition (ICP-OES technique) in the above-ground parts of plants grown in 4 groups was examined: control soil (uncontaminated) with (1) 3% soil supplement (oak, maple, beech) and (2) without this supplement, and soil contaminated with copper (1251 mg Cu/kg) with (3) 3% soil additive and (4) without this addition. In addition, the biomass of leaves used as a soil additive was subjected to a multielemental and elemental (CN) analysis, as well as for mercury and ash content.

Results
When comparing the control group with the contaminated, a three-fold increase in the copper content in the above-ground parts of plants was detected for each of the tested group. The exception was the addition of beech, which resulted in a two-fold increase in content (25.8 mg/kg for the control soil with beech and 53.5 mg/kg for the contaminated soil with beech additive). The greatest copper bioimmobilization was shown by the addition of maple biomass to the contaminated soil in comparison to the contaminated soil without additives (content decrease by 24.4%).

Conclusions
Further research using maple as a soil additive is recommended, as it caused the lowest copper accumulation in the above-ground parts of plants. In addition, the maple biomass was distinguished from the other soil additives by a higher content of macroelements.

ACKNOWLEDGMENTS
Special thanks to the students of Biotechnology (Wrocław University of Science and Technology) who conducted research with us.

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OP48: Betacyanin profile of a herbaceous succulent plants *Tallinum paniculatum* (Jacq.) Gaertn.

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Keywords: betecyanins, acylated betacyanins, succulent subshrub, mass spectrometry, chromatography

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Objective
Betacyanins together with betaxanthins form a group of betalain plant pigments which attracted a lot of attention from scientists in recent decades. Their importance has increased in the industry by focusing the market on natural colorants but their valuable health-promoting properties are increasingly being highlighted [1]. Betacyanins are attributed to a beneficial pro-health potential, including antioxidant, anti-cancer and anti-inflammatory properties [2]. The main known sources of betalains include species from genera such as *Bougainvillea*, *Beta*, *Amaranthus*, *Gomphrena*, *Basella*, and genera of the *Cactaceae* family including *Opuntia*, *Hylocereus*, *Melocactus*, *Mammillaria*. In addition, these pigments have also been identified, e.g., in a lesser known source – genus *Talinum*. Due to the continuous search for new sources of these valuable chemical entities, the aim of the present study was to determine the profile of betacyanins in *Tallinum paniculatum* (Jacq.) Gaertn.

Methods
Samples of *T. paniculatum* plant were extracted with water, filtered through silica gel to remove hydrocolloids and proteins, and then concentrated. The pigment extract was purified by flash chromatography and preparative high-performance liquid chromatography. The basic betacyanin profile was determined by LC-DAD-ESI-MS/MS system. The high resolution mass spectra (HRMS) of the unknown betacyanins, as well as their HRMS fragmentation patterns were analyzed by LCMS-IT-TOF mass spectrometry.

Results
In addition to common betacyanins such as betanin, found in other plants, the presence of very rare, doubly acylated betacyanins has been tentatively reported in *T. paniculatum* plants.

Conclusions
Performed studies enabled detection of doubly acylated betacyanins in the *T. paniculatum* extracts. The properties of these pigments are unexplored, indicating the need for further research in this direction. In addition, it confirms the need to search for new sources of betacyanins with profiles different from those known so far.

ACKNOWLEDGMENTS

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OP49: Use of waste materials and microbial agents in producing microbial granular fertilizers

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Keywords: sewage sludge ash, fish meal, bacterial lyophilizate, solublization, granulation

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Objective

Recycling waste from farms and sewage plants is an excellent way to create fertilizer that benefits the environment. However, the availability of waste-derived nutrients to plants is limited. An environmentally friendly method of increasing their availability is microbial solubilization. It can be carried out in a liquid suspension, resulting in a production of large quantities of fertilizers with low nutrients density. However, it is possible to produce granular fertilizers characterized by a smaller volume and higher nutrient density, into which solubilizing microorganisms can be introduced as lyophilizates [1].

Methods

This research used a combination of sewage sludge ash, fish meal, dried hemoglobin, and lyophilized bacteria. These materials were pelletized in a laboratory plate granulator, with water as the binding agent, and dried afterwards. The granules were tested for their hardness [N]. The bacteria's viability [cfu/g] in the final product was determined by suspending the granules in a liquid medium, spreading them onto agar media, and observing the growth of colonies.

Results

Solid granules, characterized with good mechanical properties and containing active bacterial cells essential for releasing nutrients to plants were produced.

Conclusions

Combining secondary raw materials, containing nutrients, with lyophilized bacterial cells can create high-quality, environmentally friendly granular fertilizers that plants can easily absorb.

ACKNOWLEDGMENTS

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OP50: ATR-IR spectroscopy and chemometrics as tool for classification of seeds of Cannabis strains and wild-types

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Keywords: hemp seeds, macronutrients, infrared spectroscopy, PCA, PLS-DA

Objective

Many cannabis seed companies offer seeds of different cannabis strains with varying degrees of genetic similarity, which creates the need for appropiate seed sorting methods [1]. IR spectroscopy is a simple and rapid technique that requires no sample preparation and provides comprehensive data on the chemical properties of the sample, which can be further analyzed using multivariate statistical tools. The aim of this study was to evaluate the potential of IR spectroscopy in conjunction with multivariate analysis to for the classification of cannabis seeds.

Methods

Five seeds from nine wild-type cannabis strains and three seeds from commercial cannabis strains were selected and each seed was crushed with a mortar and pestle. ATR-IR spectra were collected by placing the sample directly on a diamond ATR crystal and recorded in the mid-IR region from 4000 to 370 cm^{-1} region. Principal component analysis (PCA), hierarchical cluster analysis (HCA) and partial least square-discriminatory analysis (PLS-DA) were performed using SIMCA v14.1 to analyse the independent variables and cluster affiliation (dependent variables).

Results

In contrast to the expected classification based on secondary metabolites (cannabinoids) in the seeds, classification was based on the macronutrient profile. The bands at 3275 cm^{-1} (H_2O), 2921 cm^{-1}, 2852 cm^{-1} (lipids), 1743 cm^{-1} (lipids and/or acetylated glucomannan from hemicellulose), 1630 cm^{-1}, 1532 cm^{-1} (amide I and amide II from proteins), 1459 cm^{-1}, 1239 cm^{-1}, 1157 cm^{-1} 1094 cm^{-1}, 1018 cm^{-1} (carbohydrates) were identified as the most distinctive and prominent spectral features. The PCA model (R2X = 0.88 and Q2 = 85) was composed of 5 main components explaining 88% of the spectral variations. Further, a HCA was performed based on the ward model. The HCA dendogram resulted in two large groups (Group 1 and 2), based on which a subsequent PLS-DA model was built. The loading plot of the PLS-DA model reveals the distinctive spectral features for both groups (lipid and carbohydrate bands – group 2 samples, protein and water content - group 1 samples).

Conclusions

The presented method enabled simple and rapid classification of cannabis seeds based on macronutrients utilizing IR spectroscopy and multivariate data analysis. This method could be further developed for predicting seed longevity, vigor, plant yield and/or facilitate the process to select the most appropriate germination protocol.

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POSTER SESSION

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PP-1: The effect of essential oils on the inhibition of the growth of *Cutibacterium acnes*

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Keywords: microbiological activity, inhibition, essential oils, pathogenic microorganisms

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Objective
The important natural products in cosmetics are produced mainly by plants. Current global trends related to the increase in human population strive to produce cosmetics and hygiene products based on ingredients of natural origin. This is related to the development of the global market for natural cosmetics. One of the proposals for using natural substances are essential oils. The study determined the MIC value of 12 essential oils against *Cutibacterium acnes* (ATCC 11827) (bacteria responsible for the production of acne in people between 11–30 years old).

Methods
The method of staining with the alamarBlue® reagent was used for the study, in a series of twofold dilutions on 96-well plates. Culturing was carried out under anaerobic conditions in a CO_2 incubator.

Results
The research confirmed the assumptions that the essential oils used in the research show various properties that inhibit the development of *C. acnes*. The lowest MIC value was for Hiba 12.5 μg/mL. Essential oils from angelica root (*Angelica archangelica,* France) and cubeb (*Piper cubeba* Lf, Singapore) showed a MIC value of 25.0 μg/mL The highest MIC value was shown by cistus (*Cistus ladanifer*, Spain) which was >800 μg/mL.

Conclusions
The MIC values obtained against *C. acnes* are promising. Tested essential oils could in the future be used as an ingredient in creams or tonics that fight acne.

PP-2: Necrotic cell death of *Candida albicans* as a consequence of the action of a new halogenomethylphenol sulfone derivative

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Keywords: halogenomethylphenyl sulfone, *Candida albicans*, necrosis, ergosterol

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Objective
The emergence of drug-resistant *Candida albicans* strains requires the identification of new antimycotic agents and the study of their mode of action. *C. albicans* is the most common species involved in candidiasis and accounts for more than half of cases. For these reasons, new compounds acting against this fungus are highly sought after. Organic compounds with a sulfonyl moiety in their structure are among the most important organosulfur compounds. Halogenated methyl sulfones belong to biologically active compounds, and their antifungal activity depends on the type and amount of halogen atoms in the halogenomethylsulfonyl groups.

Methods
To determine antifungal activity: the microdilution method M27-A3, MFC index, cytotoxicity: ATCC XTT Cell Proliferation Assay. Investigation of action mode: HSP90 expression by RT-PCR, ROS flow cytometry analysis, cell cycle and cell death, CLSM, action against fungi with exogenous ergosterol (spectrophotometric measurements).

Results
2-[(4-chlorophenyl)sulfonyl]-2,2-dibromoethyl-N-(4-fluoromethylphenyl) carbamate (AK-55) at 8 μg/mL (fungicidal concentration) was considered non-toxic. The adverse effects of AK-55 against *G. mellonella were not assessed in vivo*. The flow cytometry analysis showed that the sulfone derivative induces a nonspecific type of death (necrosis) in cells (88.43%) and protoplasts (96.25%) of *C. albicans* and cells arrested in the G_0 phase at the level of 99%. The value of accumulated ROS (31.17%) is characteristic of necrotic cells. CLSM observations suggest that **AK-55** inhibited filamentation in the conglomerate biofilm. The presence of extra ergosterol supports cells of C. albicans alive against **AK-55**.

Conclusions
The new synthesised sulfone derivative AK-55 showed activity comparable to that of commercially available drugs, eg, amphotericin B. AK-55 as a promising contender for the development of a new antifungal agent can be used to treat local and systemic forms of candidiasis.

PP-3: Structure and biological activity of indole-imidazole hybrids complexes with $ZnCl_2$

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Keywords: indole, imidazole, hybrids, complexes, zinc, hemolytic activity, cytoprotective activity, antibacterial activity, fungicidal activity

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Objective
One way of searching new biological active compounds is complexing already active ligands, such as imidazole or indole derivatives, with biometals, like zinc. Such complexes may have better properties than their ligands. The aim of this study was to obtain six new biological active complexes with zinc and indole-imidazole hybrids.

Methods
Complexes were obtained by mixing solutions of zinc chloride in MeOH with solutions of ligands in MeOH (**1**–**5**) or CH_3CN:MeOH 2:1 (**6**) and letting them to crystallize on air. Hemolytic and cytoprotective activity of compounds were tested by spectrophotometric method, using human erythrocytes. Antimicrobial tests were performed on selected four bacteria and five fungi strains.

Results
Six complexes of indole-imidazole hybrids with $ZnCl_2$ in a 2:1 ratio (ligand: $ZnCl_2$) were obtained. They were characterized by spectral and elemental analysis, and for **1**–**5** the crystal structures were determined. All complexes were tested for their cytoprotective and hemolytic activity and results were compared to these of ligands. Antimicrobial tests showed they inhibit growth of four bacterial and four fungi strains, while they also stimulated growth of one fungi.

Conclusions
This research confirmed that complexing bioactive molecules with zinc can enhance their properties. Complexes **1**, **2** and **4** were hemocompatible and showed high cytoprotection, thus are good candidates for further evaluation.

ACKNOWLEDGMENTS

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PP-4: Network pharmacology and bioinformatic methods reveal the underlying mechanism of *Cinnamomum tamala* essential oil against non-small cell lung cancer

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Keywords: *Cinnamomum tamala*, network pharmacology, non-small cell lung cancer

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Objective
Cinnamomum tamala has demonstrated anticancer properties against various solid tumors, however the therapeutic mechanism of its essential oil against nonsmall cell lung cancer **(**NSCLC) remains unclear. Therefore, network pharmacology and bioinformatics approaches were used to explore the molecular mechanisms of *Cinnamomum tamala* essential oil (CTEO) against NSCLC.

Methods
GC-MS was used to identify the chemical constituents of CTEO. Lipinski, Veber, Egan rule and Abott bioavailability score was used to screen active components of CTEO. CTEO and NSCLC related targets were recovered from the public database. Compound target-disease target network was constructed using the Cytoscape software. STRING was used for the protein-protein interaction (PPI) analysis. GO and KEGG enrichment analysis was performed using the ShinyGO server. PyRx software was used to perform molecular docking between the core compounds and hub targets. External validation of the hub targets was carried out using GEPIA, HPA, TIMER, and cBio Portal databases, respectively.

Results
A total of 49 compounds in CTEO were identified by GC-MS, 44 of which passed the Lipinski, Veber, Egan rule and Abott bioavailability score and were classified as drug like compounds. A total of 3961 and 4855 compound and cancer-related targets were identified, respectively. 62 compound disease intersection targets were obtained. E-cinnamaldehyde, Z-ethyl cinnamate, acetophenone, borneol, epi-cedrol, α-muurolol, γ-amorphene, benzaldehyde, and γ-cadinene are the core active compounds of higher degree. PPI analysis revealed JUN, P53, IL6, MAPK3, HIF1A, and CASP3 as the hub targets. GO analyses indicated that CTEO regulates biological processes such as proliferation, apoptosis, and programmed cell death. The MAPK and TNF signaling pathways were the main signaling pathways. JUN and IL6 were significantly upregulated in tumor tissues. HIF1A and CASP3 were associated with a worse overall survival in patients with lung adenocarcinoma. Molecular docking analysis revealed γ-cadinene and amorpha-4,7(11)-diene have a higher binding affinity to MAPK3.

Conclusions
Overall, the study showed that *Cinnamomum tamala* essential oil can treat non-small cell lung cancer through a multi-component, multi-target, multi-pathway molecular mechanism.

PP-5: Biocatalytic activity of cyanobacteria towards vinylphosphonate and epoxyphosphonate

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Keywords: biotransformation, cyanobacteria, phosphonate

Objective

Cyanobacteria are photosynthesizing prokaryotes with an extraordinary ability to adapt to changing environmental conditions, which has enabled them to colonize almost all aquatic and terrestrial environments. Cyanobacteria are also of great value for biotechnology, due to their ability to convert structurally different substrates into valuable products, which has a wide range of applications in various fields of industry, such as food, cosmetics, pharmaceutical and fuel. Cyanobacteria of the genera *Leptolyngbya* and *Nodularia* are known as a rich source of secondary metabolites (lipopeptide, glicolipids, carotenoids) with antibacterial, anti-inflammatory and anticancer activity [1] and enzymes, e.g., capable of degrading organophosphorus pesticides such as glyphosate [2].

Methods

Leptolyngbya foveolarum CCALA 76 and *Nodularia sphaerocarpa* CCALA 114 cultures were used as a biocatalyst in a biotransformation of two vinylphosphonate (dimethyl vinylphosphonate, diethyl vinylphosphonate) and one epoxyphosphonate (epoxymethyl dimethyl phosphonate). Process was carried out for 7 days at 29°C (±1) under continuous illumination and under stationary conditions. Products were extracted with ethyl acetate and analyzed by ^{31}P NMR.

Results and Conclusions

After 7 days of the process *Leptolyngbya foveolarum* and *Nodularia sphaerocarpa* were able to transform epoxyphosphonate with high conversion degree (96% and 76%, respectively). In biotransformation with vinylphosphonates biocatalysts showed no activity towards tested compounds. Previous studies have shown *Nodularia sphaerocarpa* stability under high substrate concentrations, which indicated the possibility of increasing the scale of the process [3].

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PP-6: Densitometry in the standardisation of conditions of yeast *Rhodotorula mucilaginosa* cultivation

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Keywords: densitometry, optical density, *Rhodotorula mucilaginosa*, yeasts

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Objective

Rhodotorula mucilaginosa is a commonly found species of yeasts in nature. These yeasts have characteristically salmon-colored cells, which is due to the production of carotenoids like β-carotene, torulene and torularhodin. They are being studied in biotechnology for carotenoid production, bioremediation and biodiesel production. Having a rapid and effective approach to determine yeast populations in studies is essential. There is a standardisation method of measuring optical density using a densitometer in McFarland units, yet it needs to be correlated to a researched microorganism that has the right turbidity in liquid culture. The purpose of the research was to establish the correlation between McFarland units and the number of cells of *R. mucilaginosa*, as well as to determine the optimal temperature for their cultivation.

Methods

Rhodotorula mucilaginosa was cultivated on PDB (Potato Dextrose Broth) culture medium and shaken at 130 rpm for five days. 1 mL of culture was taken each day, from which a series of dilutions were made, where from each of 10^{-5} and 10^{-6} dilutions a spread plate technique was made on PDA (Potato Dextrose Agar) solid medium. Each day, measurements were taken with a densitometer from appropriate dilutions from 1 mL liquid cultures of *R. mucilaginosa*. After three days of growth on solid PDA medium, the grown colonies of *R. mucilaginosa* were counted to correlate the number of McFarland units from the densitometer measurements with the number of yeast cells of *R. mucilaginosa*. The experiments were conducted at three different temperatures of 21°C, 25°C and 30°C.

Results

Performed experiments allowed establishing the precise correlation between the number of the cells of yeast and the McFarland Optical density for different temperatures. Also, the study found that a McFarland optical density of 0.5 corresponds to $1.6 \cdot 10^6$ cells of *R. mucilaginosa*, and the optimal temperature for cultivation is 25°C.

Conclusions

R. mucilaginosa is an organism with enormous scientific potential. For research, it is important to have a fast and efficient method to determine yeast populations based on standardisation methods, such as the use of the densitometer used in this study. In the literature there is a wide variation in the temperatures at which *R. mucilaginosa* was cultured for testing. It should be noted that, depending on the strain, its place of origin and the purpose of the work, it is important to optimise the culture of the microorganism.

PP-7: Analysis of stress markers induced by Zearalenone in *Saccharomyces cerevisiae*

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Keywords: *Saccharomyces cerevisiae*, Zearalenone, mycotoxin, oxidative stress, HSP

Objective
Zearalenone (ZEN) is a mycotoxin, which causes protein denaturation and oxidative stress in cells. It is responsible for contamination of grain raw materials in production of alcohol, biomass of yeast or in baking. The aim of the study was to investigate the metabolic response of yeasts to stress induced by ZEN and understanding their adaptive mechanisms to stress conditions caused by the action of mycotoxin.

Methods
The culture of yeasts was conducted in aerobic and fermentation conditions in the presence of ZEN in two different doses selected on the basis of literature data. Concentrations of biomass, malondialdehyde (MDA), activity of superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione peroxidase (GPx) were determined using microplate reader. Expression of HSP31 and HSP104 proteins was tested by SDS-PAGE electrophoresis, semi-dry transfer and chemiluminescence visualization.

Results
Biomass reduction was found after application of ZEN in aerobic conditions. Concentration of MDA was higher in samples with ZEN compared to the control, regardless of the cultivation conditions. Higher SOD and GPx activity was found in the cultures treated with the lower dose of ZEN in both cultures. In aerobic conditions, reduced expression of HSP31 was shown, regardless of the dose of ZEN. In fermentation conditions, the higher dose induced increase of production of those proteins. Expression of HSP104 was higher in samples with lower dose of mycotoxin under aerobic conditions, whereas in fermentation conditions increase occurred at both concentrations.

Conclusions
Supplementation of culture with ZEN results in activation of antioxidant mechanisms regardless of culture conditions. However, under fermentation conditions, this mycotoxin engages heat shock proteins to the greatest extent to maintain proper metabolism under the conditions of toxic stress.

ACKNOWLEDGMENTS

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PP-8: Biotransformation of oximes by bacteria of the *Pseudomonas* genus

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Keywords: biotransformation, oximes, *Pseudomonas*, gas chromatography

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Objective

Oximes are known for their anti-inflammatory, relaxing and diastolic properties. Their potential, as inhibitors of elastase and tyrosine, has recently been discovered. Tyrosine and elastase are the enzymes responsible for skin aging. Therefore, they are often used in the cosmetics industry[1]. Bacteria of the genus *Pseudomonas* living on human skin may have the ability to oximes biotransformation. As a result, irritating products for the skin could be formed, causing an allergic reaction. The aim of this study was to test the ability of *Pseudomonas fluorescens* (ATCC 13525), *Pseudomonas putida* (ATCC 49128) and *Pseudomonas aeruginosa* (ATCC 15442) bacteria to biotransform the following compounds: *p*-anisaldehyde oxime, carvone oxime, *o*-tolualdehyde oxime and (−)-verbenone oxime.

Methods

Synthesizing methodology of oximes was shown by Balcerzak et al. (2019)[2]. A 24h culture of each of the bacteria was used for the biotransformation. Tested compounds were used at a final concentration of 1 mg/ml. Samples were taken every 2-3 days and the experiment lasted a week. After extraction, samples were analyzed by GC on a Shimadzu GC-2010 Plus.

Results

The obtained results indicate the lack of ability of *P. fluorescens*, *P. putida* and *P. aeruginosa* to biotransform *p*-anisaldehyde oxime, carvone oxime, *o*-tolualdehyde oxime and (−)-verbenone oxime. The conducted GC analysis did not show the presence of any biotransformation products. In addition, the control samples (the test compound placed in the microbiological medium) did not show the ability of the microbiological medium to degrade the substrates by itself.

Conclusions

It has been shown that the above-mentioned bacterial strains do not have the ability to biotransformation selected compounds from the oxime group. This is a reproducible result. Increasing the process duration does not affect the results. Thanks to the above research, studied oximes can be safely used in the cosmetics industry, because bacteria of the *Pseudomonas* genus living on human skin will not biotransform them. There will be no new products that could potentially cause allergic reactions on the skin.

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PP-9: Representative of the genus *Cunninghamella* as a biocatalyst in the synthesis of hydroxylated derivative of 2-phenylethanol

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Keywords: biocatalysis, biotransformation, fungi, 2-phenylethanol, *Cunninghamella blakesleeana*

Objective
Biotransformations play an important role in obtaining derivatives with valuable features. The experimental protocols that have already been developed are characterized by a harmless impact on the environment and simplicity of implementation [1]. Fungi of the genus *Cunninghamella* have known predispositions to carry out modifications of substances introduced artificially to the environment, due to the activity of the enzyme systems such as the cytochrome P450 superfamily [2]. Phenylethyl alcohol is a cheap and widely avilable substrate and can be used to obtain derivatives with added functional value for applications in the pharmaceutical and cosmetic industries. *Cunninghamella blakesleeana* (DSM 1906) shows the activity towards 2-phenylethanol and can transform tested substrate to high-value chemical compounds.

Methods
High-performance liquid chromatography (HPLC) was used in the study to identify individual products, substrate and determine the reaction yield.

Results
The biotransformation of 2-phenylethanol produces 3 derivatives, one of which is 1-phenyl-1,2-ethanediol.

Conclusions
The identified derivative is 1-phenyl-1,2-ethanediol. It is an optically active compound. This chiral alcohol can be used to synthesize pharmaceuticals used in the treatment of mental disorders [3]. The other two derivatives will be identified by different analytical methods.

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PP-10: Biotransformation of 2-phenylethanol by cyanobacteria *Synechococcus bigranulatus*

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Keywords: biotransformation, cyanobacteria, 2-phenylethanol, microorganisms, chromatography

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Objective

Cyanobacteria are photosynthetic aerobic bacteria. Biotransformations based on the use of cyanobacteria have many advantages: they allow to reduce the number of product synthesis steps, usually have higher selectivity compared to chemical synthesis, and use less solvents [1]. These organisms are able to photosynthetically split water and regenerate the cofactor, which enables the biotransformation reaction to be carried out with high efficiency [2]. *Synechococcus bigranulatus* is non-diazotrophic cyanobacteria. Their small size and sufficiently large surface area make these microorganisms carry out very efficient metabolic processes in their cells. They also have good resistance to changes in environmental conditions, which is why they are so widespread in the biosphere [3].The aim of the study was to demonstrate the biotransformation capacity of cyanobacteria *Synechococcus bigranulatus* (CCALA 187) towards 2-phenylethanol.

Methods

Photobiocatalyst was cultured for 3 weeks on BG-11 medium. After this time, 12 or 40 µl of 2-phenylethanol were added to the culture and the biotransformation process was carried out for 1 to 6 days. Samples after biotransformation were analyzed by HPLC chromatography.

Results

After 4-6 days of biotransformation, the presence of an unexpected hydrophilic product was detected.

Conclusions

The experiments carried out demonstrated the ability of the cyanobacteria strain tested to convert 2-phenylethanol. The resulting product, after isolation and purification, will be analysed to determine the structure.

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PP-11: Exploring Bioluminescent Fungi: Illuminating the Mysteries of Mycelium Growth and Environmental Applications

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Keywords: bioluminescence, mushroom, luciferin, environmental protection

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Objective

Bioluminescent fungi belongs to *Basidiomycota* division and are able to emit light by enzymatic oxidation reactions. They primarily grow on dying trees and exhibit bioluminescence at various stages of growth, including mycelium, rhizomorph, and fruit body formation. Our research is focused on studying light emission during different stages of mycelium formation under the influence of selected factors. Three different speciesof the fungi were chosen for the study.. Importantly, the bioluminescent fungi are not widely known in the scientific world, despite a wide range of their potential applications. For example the dependence of fungal bioluminescence pathway from various factors, could be used for creating bioassays detecting environmental pollution. The pathway of bioluminecence coulod be also introduced to the lignin pathway in plants, that could lead in close future to the replacement of artificial electrical lighting with glowing plants.

Methods

For the study *Armillaria gallica, Panellus stipticus* and *Mycena chlorophos* were chosen. The fungi were grown on agar plates. We tested an ability of *Armillaria* light emission and rhizomorph structure appearance on MEA, PDA, MEYA, YEPD media. The BioRad system was used to detect the luminescence.

Results

All tested fungi exhibited emission of light. *Armillaria gallica* bioluminescence is related to the rhizomorph appearance and is located only in young areas of mycelium. *Panalleus* intensity of light emission was lower than in *Armillaria* and was located only in the oldest part of dense mycelium. *Mycena chlorophos* showed the lowest light emission, which could be observed only for few days, and only for mycelium in a specific age. In the case of *Armillaria galica*, no major changes in the bioluminescence tendency were observed on different media. However, the best media for the formation of the rhizomorphic mycelial structure were the media enriched with yeasts. In the case of *Panellus stipticus*, no bioluminescence was observed on the MEA medium while most intensive appeared on the MEYA medium. *Mycena chlorophos* was able to glow only on the yeast medium. Interestingly, on the MEYA medium a blue spot were observed in the centrum of this fungus mycelia, indicating probably the formation of secondary metabolites.

Conclusions

The mycelium emitting the highest glowing light was *Armillaria galica*. This is probably because only the mycelium of this mushroom glows, unlike the rest of the mushrooms, where the glow can also be noticed in the fruit body, which makes their mycelium glowing with lower intensity, probably because the greatest expression of bioluminescent genes occurs during the formation of the fruit body.

PP-13: Sustainable Agriculture: Bioprocess Engineering of Waste to Nutrients

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Keywords: bioprocess, microorganisms, plant growth promoting, agriculture, nutrients

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Objective

Total fertilizer nutrient demand (nitrogen, phosphorus, and potassium) is expected to grow at a 1.9% annual rate, reaching 210 million tons (expected) by the end of 2025. However, nutrient utilization is not consistent. Only a minority of farmers in developing countries use synthetic fertilizers, while the majority produce at a subsistence level using crop rotation and recycling of crop residues, animal excreta, and organic waste. When dealing with nutrients, there are several factors to consider. For example, to feed 8 billion people, the world's sustainability depends on nutrients. Humans have nearly tripled global nitrogen (N) and phosphorus (P) cycling on land. This has led to an imbalance in the N and P cycles, which is causing severe issues for the environment, economy, and health. One promising solution to address this problem is the use of beneficial microbes to recycle waste and biomass substances in a sustainable and eco-friendly way.

Methods

Our current research aims to assess how waste compounds can be broken down by microorganisms to provide nutrients for plants, thus improving their growth and health. At the moment, we are in the early stages of evaluating isolated microorganisms *in vitro* for their plant growth promoting (PGP) properties. All the isolates were screened for the PGP traits and based on the preliminary results the most prospective strains will be further assessed on plants.

Results

We have identified a range of beneficial bacterial and fungal microorganisms from soil, plants, and waste compounds. A total of 82 isolates belonging to *Pseudomonas* spp., *Streptomyces* spp. *Azotobacter* spp. *Bacillus* spp. was tested for the PGP traits like Ammonia production, Phosphate solubilization, Potassium solubilization, Zinc solubilization, Hydrogen Cyanide production, Siderophore production, phytohormones production. The most prospective strains were further infused with waste compounds to observe how these compounds degraded and released nutrients.

Conclusions

In the context of sustainable agriculture practices, using of beneficial microbes and waste compounds is a promising biotechnological approach in decreasing waste compounds residue and utilizing them for the release of nutrients which can be beneficial to plants in various ways. Our approach is to develop commercial product with utilization of beneficial microbe with waste compounds.

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PP-14: MALDI-TOF/MS in the microbiome identification of honey samples from different regions of Poland

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Keywords: bacteria identification, MALDI-TOF/MS, honey microbiome, antibiotic resistance

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Objective
Bacteria are single-celled organisms that play a huge role in all ecosystems. Honey, a supersaturated solution of sugars, produced by the honeybee (*Apis mellifera*), is the first sweetener used by humans [1]. The main sources of microbial contamination of honey are pollen, the digestive tract of honey bees, or plant raw materials. The microorganisms present in honey may have antibacterial properties due to their ability to produce metabolites with antimicrobial activity. A thorough understanding of the microbial composition of honeys is important in order to obtain detailed information on the antimicrobial properties of the microbiome [2, 3]. The purpose of this study is to determine and compare the diversity of bacterial species isolated from honey samples from different regions of Poland. The study was carried out using MALDI-TOF/MS technique.

Methods
Matrix-assisted laser desorption/ionization - time-of-flight mass spectrometry (MALDI-TOF/MS) technique was used to identify bacteria isolated from honey samples from different regions of Poland. The technique is a spectrometric approach based on ionization of a compound. Identification of honey microbiome was carried out using a whole-cell approach with 70% formic acid for bacterial protein extraction and a HCCA matrix of 10 mg/ml.

Results
MALDI-TOF/MS analysis identified various bacterial species present in the honey samples. The most commonly identified bacterial families in honey included: *Bacillaceae, Pseudomonadaceae*, *Paenibacillaceae, Staphylococcaceae*. *Paenibacillus larvae* strains were also identified. The composition of the microbiome varied according to both the type of honey and the region it came from.

Conclusions
Identification of bacterial species in honey can help to monitor the quality and detect undesirable changes in its composition, as well as the presence of dangerous pathogens. It also enable the prevention of diseases, including American Bee Foulbrood (ABF). The presence of *Paenibacillus larvae* in one of the analyzed honey samples could indicate the potential contamination of the honey with the ABF.

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PP-15: Identification of bacteria associated with post-operative wounds of patients with the use of MALDI-TOF MS approach

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Keywords: antibiotic influence, bacterial strain, MALDI-TOF MS, post-operative wound

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Objective
The bacterial infection of post-operative wounds is a common health problem [1]. Therefore, it is important to look for fast and accurate methods of identifying bacteria in clinical samples [2]. The aim of the study was to analyze the use of the MALDI-TOF MS technique to identify microorganisms wounds that are hard to heal.

Methods
Various solid culture media were used in the study: Brain Heart Infusion Agar, Mueller-Hilton Agar, Glucose Bromocresol Purple Agar, Vancomycin Resistance Enterococci Agar Base and liquid: Tryptic Soy Broth, BACTEC Lytic/10 anaerobic/F, as well as different incubation times (4, 6, 24 h) in order to select the best conditions for isolated microorganisms. Then the protein profiles of bacteria from patients not treated with antibiotics were compared to those treated with antibiotics.

Results
Identification of microorganisms has shown that the most common bacteria are *Escherichia coli*, *Staphylococcus* spp. and *Enterococcus* spp. The following study also demonstrated the effect of different culture conditions and method of the preparation of bacterial protein extracts to identify factors and the quality of the obtained mass spectra. By comparing the protein profiles of bacteria from patients not treated with antibiotics to those treated with antibiotics, based on the presence/absence of specific signals and using the UniProt platform, it is possible to predict the mechanism of the action of the antibiotic used and the mechanism of the drug resistance. One of the proteins identified in this way is the phenol-soluble PSM-α-3 modulin, a vital factor in bacterial virulence and anti-antibiotic resistance.

Conclusions
The obtained results of the studies show that MALDI-TOF MS can be a relevant tool for the detection of antibiotic resistance. In addition, results of our research may enable to verify the medical diagnosis; they bring hope for the development of methods enabling a faster diagnosis, the detection of disease changes at the cellular level prior to the occurrence of clinical changes.

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PP-16: Effect of organic additives on the betalain profiles of extracts of *Hylocereus polyrhizus* (Weber) Britton & Rose fruit pulp and pericarp

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Keywords: extraction, natural dyes, betalains, *Hylocereus polyrhizus*, mass spectrometry

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Objective

Betalains are pigments attributed with antioxidant and anti-inflammatory properties [1] and their colouring properties are competitive with synthetic dyes [2]. For this reason, they are becoming increasingly important, and current trends are focussing on finding their new plant sources.

Therefore, the objective of the research was to test and select a suitable extractant for the extraction of betalains from *Hylocereus polyrhizus* (Weber) Britton & Rose fruits. The extraction was carried out separately for the pulp of the fruit and the pericarp and the results were compared to each other.

Methods

The raw material was extracted with a mixture of formic acid, acetone and water in the following volume ratios: 0:0:1; 0:0.67:1; 0:4:1; 0.01:0:1; 0.02:0.68:1; 0.05:4.2:1 or analogously with a mixture of acetic acid, ethanol and water (v/v/v). UV-Vis spectra in the range 380 – 650 nm were investigated for the extracts using a 96-well thermostated microplate reader. In addition, extracts were analyzed using the LC-DAD-ESI-MS technique (liquid chromatography combined with a diode detector and tandem mass spectrometry with electrospray ionization). On the basis of the conducted research, the total content of betalains per 1 g of raw material was determined.

Results

The highest amount of extracted betalains was obtained for the pulp (1.62 mg) for which the extractant was demineralised water. For the pericarp (1.53 mg), it was a mixture of ethanol/acetone and water in a ratio of 0.67:1.

Conclusions

The highest extraction efficiency of betalains from *H. polyrhizus* fruits was obtained with demineralised water as the extractant. Similar results were obtained with 40% acetone or 40% ethanol solutions. On the other hand, it was observed that the addition of organic acids (formic or acetic) deteriorates the extraction efficiency of betalains. This indicates the need for a continuous search for extraction systems to obtain extracts with high betalain content.

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PP-17: Effect of a stabilized F8 bacteriophage preparation on the reduction of *Pseudomonas aeruginosa* biofilm

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Keywords: bacteriophage, biofilm, *Pseudomonas aeruginosa*, antibiofilm activity

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Objective

The aim was to study the differences in the reduction of *Pseudomonas aeruginosa* biofilm by different forms of the bacteriophage preparations such as pure lysate, purified phage, and purified phage with 1-octanol addition.

Methods

Bacteria and bacteriophage: *Pseudomonas aeruginosa* bacteriophage F8 and host bacteria *P. aeruginosa* PCM 2720 from Polish Collection of Microorganisms, Hirszfeld Institute of Immunology and Experimental Therapy.

Bacteriophage preparation: F8 phage preparation was performed according to the procedure by Szermer-Olearnik and Boratyński (1). Additionally, the purified F8 phage preparation was enriched with the addition of 1-octanol for stabilization.

Measuring the effect on biofilm degradation: 24 h old *P. aeruginosa* biofilm was treated with F8 phage lysate, purified preparation of phage F8, and purified preparation of phage F8 with 1-octanol for 6 hours. In the next step, biofilm was collected and plated onto agar plates to estimate the CFU/ml. In parallel, the same procedure on mature biofilm was performed using a light microscope slide Lab-Tek® Chamber SlideTM System.

Results

Among the tested phage preparations, the preparation purified with the octanol addition turned out to have the highest activity against biofilm. Differences in the bacteria number after treatment of the biofilm with the purified preparation and purified preparation with the octanol addition were statistically significant compared to the untreated control. It was also confirmed that octanol alone does not affect the number of bacteria.

Conclusions

Pseudomonas aeruginosa is a bacterial species responsible for a high number of hospital-acquired and chronic infections. This species is considered critical for its virulence factors, mechanisms of multi-drug resistance, and biofilm production. The bacteria in biofilms often exhibit increased virulence and tolerance against antimicrobials. In our research, we demonstrate that phage F8 with 1-octanol is stable and eliminates *P. aeruginosa* biofilm with high efficacy.

ACKNOWLEDGMENTS

This study was supported by National Science Center, Poland (Grant Numbers UMO-2019/03/X/NZ6/01710).

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PP-18: Antimicrobial activity of novel fragrance compounds towards Gram-positive bacteria

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Keywords: oxime ethers, olfactory compounds, antimicrobial activity

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Objective
Fragrance compounds are part of many different industries. New fragrances with new fragrance notes, a better safety profile and intended for various products are still being sought. An important trend in the synthesis of fragrance compounds is the search for functional substances, i.e. substances that play many roles in the product. An example of such a combination may be having fragrance and antimicrobial properties. Such substances can be used in the compositions of products intended for disinfection, enhancing their additional effect, or in cosmetic products supporting the preservative effect.

Methods
Oxime ethers were diluted to a concentration of 30 mg/mL in DMSO. The antimicrobial potential was evaluated by determining the MIC parameter (Minimal Inhibitory Concentration) towards: *Bacillus cereus* (ATCC 10876) (TCS), *Staphylococcus aureus* (ATCC 6538), *Kocuria rhizophila* (ATCC 9341), *Staphylococcus epidermidis* (ATCC 12228). The research was carried out in two stages, in the first place, a screening test was carried out for one concentration using the Alamar blue® dye. Fragrances that showed activity in the screening test were implemented in the appropriate tests to determine the MIC parameters by the serial microdilution method on a 96-well plate using the same dye.

Results
The oxime ethers were tested in the concentration range of 2400 – 9.38 μg/mL. The best activity possessed dihydrocinnamaldehyde oxime *O*-allyl ether against *K. rhizophila* the MIC value of 37.5 μg/mL. Then *trans*-cinnamaldehyde oxime *O*-methyl ether, *trans*-cinnamaldehyde oxime *O*-allyl ether, dihydrocinnamaldehyde oxime *O*-methyl ether, dihydrocinnamaldehyde oxime *O*-allyl ether, phenylacetaldehyde oxime *O*-*n*-propyl ether presented significant (MIC = 150 μg/mL) activity towards *S. epidermidis*. Similar activity showed dihydrocinnamaldehyde oxime *O*-allyl ether against *B. cereus*, dihydro-β-ionone oxime *O*-*n*-propyl ether and α-ionone oxime *O*-methyl ether against both *B. cereus* and *K. rhizophila.*

Conclusions
In conclusion, oxime ethers show satisfactory antimicrobial activity against gram-positive bacteria. Further studies are necessary to confirm its effectiveness as preservatives.

PP-19: Synthesis and spectral characterization of new chalcone-type A-substituted estra-1,3,5(10)-triene–ferrocene conjugates

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Keywords: ferrocene, ferrocene-steroid conjugates, 2-formylestradiol, acetylferrocene, esterification

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Objective

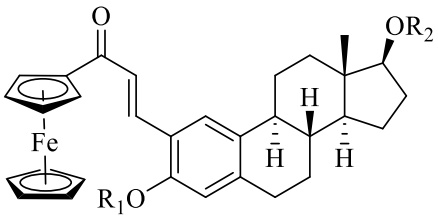
Ferrocene–steroid conjugates have been explored as molecules with the potential to exhibit a selective antiproliferative effect toward hormone-dependent tumors [1]. With this in mind, we sought to expand the structural diversity in this compound class [2], on this occasion by tethering ferrocene to the A ring of estradiol via carbon-carbon bonds.

Methods

A Claisen–Schmidt reaction of 2-formylestradiol and acetylferrocene in refluxing ethanol in the presence of sodium ethoxide was performed. The condensation product (**1**, Fig. 1) was esterified upon chromatographic separation to the corresponding mono- (**2**) and diacetate (**3**).

Results

New steroid–ferrocene conjugates **1–3** were prepared. Conjugate **1** rapidly decomposed upon attempts to isolate it from nonpolar solvent solutions, but esters **2** and **3** showed no such behavior.



1, R_1 = H, R_2 = H
2, R_1 = Ac, R_2 = H
3, R_1 = Ac, R_2 = Ac

Fig. 1. Ferrocene–steroid conjugates **1–3**

Conclusions

Three chalcone-type ferrocene–steroid conjugates were synthesized. It was shown that the presence of an unprotected phenol group renders such molecules prone to decomposition, making it more convenient to prepare and work with their esterified derivatives. Future antiproliferative activity assays will determine whether these compounds possess favorable biological activities.

ACKNOWLEDGMENTS

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PP-20: Synthesis and analysis of porphyrins and porphyrin complexe

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Keywords: porphyrin, porphyrin complexes, organic synthesis

Objective

Porphyrins are an important group of chemical compounds that is widely found in nature. Their synthesis and potential applications have become significant topics in scientific research in recent years. Porphyrins form coordination compounds with cations of many metals, and such connections show extremely high potential for various practical applications. Recent studies demonstrate the potential use of such complexes in cancer therapy [1, 2], porphyrins have also played a significant role in the development of supramolecular chemistry. Furthermore, research suggests that porphyrin complexes with gadolinium(III) cations could potentially have additional applications in medicine, serving as a contrast agent used in nuclear magnetic resonance imaging [3]. Porphyrins also demonstrate potential as chemical sensors, detecting heavy metal cations [4]. The main challenge in research on porphyrin applications is the low yield of the porphyrin synthesis reaction. The aim of this study was to find an efficient method for synthesising two porphyrins: 5,10,15,20-tetrakis(4-methoxyphenyl)porphyrin and 5,10,15,20-tetraphenylporphyrin and to analyse their coordinational and physicochemical properties.

Methods

In this study, the synthesis of the porphyrins mentioned above was carried out using ten different methods, using pyrrole and the appropriate aldehyde as substrates. The obtained products were subjected to analysis using NMR, IR, UV-Vis, and fluorescence spectroscopy.

Results

Among the synthesis methods applied, the one with the highest yield in producing the desired products was selected. The obtained products are being used for further research, focussing on the complexing properties of the synthesised porphyrins.

Conclusions

More research is needed to achieve satisfactory yields in the synthesising of various porphyrins. The synthesis methods investigated so far have allowed for the production of small amounts of the desired product.

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PP-21: Preparation of an innovative bifunctional CuO,ZnO/Al_2O_3 catalyst for the hydrogenolysis of glycerol

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Keywords: catalyst, hydrogenolysis, glycerol, 1,2-propanediol, hydrogen

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Objective
The production of 1,2-propanediol is a significant challenge for the chemical industry due to the increasing demand for this versatile product and the pro-ecological regulatory actions. The traditional method of producing this compound through the hydrolysis of petroleum-derived 1,2-epoxypropane (propylene oxide) is energy intensive, and sustainable routes based on renewable raw materials are becoming more attractive. One of the possibility is hydrogenolysis of glycerol to 1,2-propanediol. The key aspect is the selection of a catalyst that will ensure the appropriate level of selectivity and efficiency, so that the production turns out to be profitable. The main aspect of the research was to determine the selectivity and activity of the doped catalyst based on aluminum oxyhydroxide as a carrier and the active phase based on copper and zinc in the reaction of hydroconversion propylene glycol from glycerol.

Methods
The synthesized doped catalysts, is prepared by direct mixing of precursors of the active phase and the appropriate selected carrier into precatalyst paste, which is then extruded into catalyst pellets. The resulting catalyst was characterized by temperature-programmed hydrogen reduction (TPR-H2), evaluation of BET surface using nitrogen adsorption at 77K and by optical microscopy. Catalyst tests were carried out in a trickle bed reactor with a stationary catalyst bed. The parameters of the glycerol hydrogenolysis process, such as conversion and selectivity, were determined basing on gas chromatography results.

Results
The specific surface area of the catalyst is 204 m^2/g, the total pore volume is 0,57 m^3/g, optical photography shows a good dispersion of the active phase on the surface of the catalyst The selectivity towards the production of 1,2-propanediol in optimal conditions reached 94%, while the conversion was 80% in the 1000-hour test.

Conclusions
The CuO,ZnO/Al_2O_3 catalyst is a doped catalyst with a high content of mesoporous structures. The test has confirmed very high resistance of the catalytic system to water, which often affects the mechanical strength of catalysts

ACKNOWLEDGMENTS

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PP-23: Differentiation of bacteria based on metabolic profiles obtained by MALDI and NALDI mass spectrometry methods

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Keywords: MALDI, mass spectrometry, metabolites, microorganisms, nanoparticles

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Objective

Rapid and reliable identification of microorganisms is one of the most effective ways to reduce the damage caused by infections, assessing the microbiological safety of food and production lines and the search for new strains for commercial applications. In recent years, increasing emphasis has been placed on the search for modern, reliable and rapid methods of detecting microorganisms. In our research, we propose to use metabolomic profiling to distinguish microorganisms using matrix-assisted laser desorption/ionization (MALDI) and nanoparticle-assisted laser desorption/ionization (NALDI) mass spectrometry (MS) techniques.

Methods

The studies used bacterial strains isolated from the urine of patients with diagnosed prostate cancer. Metabolites were extracted from fresh bacterial cultures using Bligh & Dyer [1] extraction protocol. Bacterial metabolite extracts were analyzed using a commonly used 2,5-dihydroxybenzoic acid (DHB) matrix in MALDI and a silver nanoparticle-coated target using the chemical vapor deposition technique (CVD) in NALDI MS method.

Results

Statistical analysis of the obtained spectra of metabolic extracts by the PLS-DA method allowed complete separation of the studied groups of microorganisms.

Conclusions

On the basis of metabolic profiles, it is possible to differentiate bacteria in both MALDI and NALDI mass spectrometry techniques.

ACKNOWLEDGMENTS

This research was funded by the National Science Centre, Poland, grant number 2020/39/B/NZ7/02733 (Opus-20).

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PP-24: Printed collagen hydrogel as wound dressing material

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Keywords: chronic wound, hydrogel, printing 3d, in vivo, drug delivery

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Objective
Wounds that do not heal for more than six weeks are called chronic wounds. These wounds require frequent dressing changes and numerous hospital stays. Approximately 20 million patients worldwide suffer from chronic wounds, in Poland the problem affects more than 500,000 people a year. Hydrogel-based dressings are among the most promising materials in wound healing. In recent years, there has been a development in the direction of personalized dressings, which contain the right amount of active substances for the patient and also take into consideration the shape and depth of the wound. 3D printing technology allows all of these factors to be taken into account, creating various types of complex structures, layer by layer. The goal of the research was to develop a well-printable collagen hydrogel with an active ingredient that will contribute to the treatment of difficult-to-heal wounds.

Methods
The hydrogel was characterized by scanning electron microscopy (SEM) and rheology. Finally, an implant was printed using a Cellink Bio X 3D printer, which was tested in vitro and then in vivo test.

Results
The hydrogel showed a porous structure and very good rheological properties, which allowed printing and testing the release of the active substance.

Conclusions
Through the research, a highly printable hydrogel with low cytotoxicity was developed. The non-existence of skin inflammation around the printed implant, as well as the lack of a drastic decrease in the weight of the mouse, confirmed that the material could be used as a wound dressing.

ACKNOWLEDGMENTS

The research was realized under the project: „Badanie możliwości zastosowania w przyszłości biodrukowanych 3d hydrożelowych opatrunków kolagenowych i alginianowych na trudno gojące się rany, Ocena ich biokompatybilności w testach in vitro i in vivo", number: SUBK.C100.22.068 funded by the Wroclaw Medical University.

PP-25: The optimization of the surface activation process for 3D printed working electrodes

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Keywords: electrochemistry, electroanalysis, 3D printing, printed electrodes, surface activation

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Objective
The main objective of the research was to carry out the activation of 3D printed electrodes with several organic solvents, i.e. tetrahydrofuran (THF), dichloromethane (DCM), dichloroethane (DCE), acetonitrile (ACN) and acetone (AC), and to perform a detailed characterization of their properties. An additional objective was to test the applicability of the activated electrodes to the electroanalysis of salicylic acid.

Methods
Extensive characterization of the activated electrodes was carried out using an optical tensiometer, scanning electron microscope, and voltammetric techniques (cyclic voltammetry (CV) and differential pulse voltammetry (DPV)).

Results
The effect of the type of solvent used on each of the three conductive filaments used to print the electrodes was examined, and the effect of solvent-print interaction time was analyzed. The extensive characterization of the activated electrodes allowed the selection of electrodes defined by the best properties for use in electroanalysis.

Conclusions
In the present work, a comprehensive study was conducted to evaluate the effects of five different solvents on the electrochemical performance of 3D printed electrodes using PLA-based conductive filaments with carbon additives. The selected solvents have different solubilities for PLA. The characterization performed allowed to observe changes in the physicochemical properties of the surface depending on the type of solvent, its contact time with the electrode and the type of conductive filament used in the printing process. The electrochemical properties of all the printed electrodes were evaluated by the redox reaction of the reversible probe ferrocenemethanol. The data obtained were correlated with contact angle measurements and surface appearance derived from microscopic studies. Finally, an activation method with satisfactory electrochemical properties was selected and the 3D printed electrode was used for the determination of salicylic acid.

ACKNOWLEDGMENTS

The research was financed from the funds of the 7th edition of Student Research Grants of the University of Lodz.

PP-27: *De novo* design and synthesis of miniproteins that contain non-native helices

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Keywords: miniproteins, β-amino acids, solid-phase peptide synthesis

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Objective
The aim of the study is to *de novo* design and synthesise conformationally stable miniproteins that fold co-operatively without the presence of cross-links or a binding metal, using rational and computational methods. The proposed oligomers are expected to be accessible through the solid-phase protein synthesis, facilitating reachable analysis.

The synthesised miniproteins are intended to serve as rigid scaffolds that can be freely modified, making them promising candidates carrying biological activities, including inhibition of protein-protein interactions or peptide-based catalysis.

Methods
The structurally extended miniproteins were designed *de novo* using both rational and computer-aided methods, synthesised by microwave-assisted automated peptide synthesis (SPPS), and finally the three-dimensional structure and stability were analysed with spectroscopic methods (CD, NMR), fluorimetry (nanoDSF), and crystallography.

Results
The series of peptides composed of three helices was synthesised. Subsequent analysis showed that the received miniproteins hold a stable tertiary structure, despite being supported only by the packing of the hydrophobic core. The rigidity of the miniproteins was induced by incorporation of synthetic β-amino acid residues (*trans*-(1*S*,2*S*)-aminocyclopentanecarboxylic acid [*trans*-(1*S*,2*S*)-ACPC]) into each helical secondary structure in a position consistent with the motif αβααβαααβ. Additionally α-residues were selected using the Rosetta FastDesign protocol in a way to optimise the packing of the hydrophobic core. All of the received miniproteins indicated cooperative folding and good conformational stability. To improve physicochemical properties of the peptides, particular trans-ACPC residues were replaced with the corresponding self-prepared *trans*-(3*S*,4*R*)-APC units (*trans*-(3*S*,4*R*)-1-*N*-Boc-4-Fmoc-aminopyrrolidine-3-carboxylic acid).

Conclusions
The series of stable miniproteins that fold cooperatively was successfully designed and synthesised. Due to the possibility of controlling the folding process of the synthesised miniproteins, as well as their rigidity and specific physicochemical, and pharmacokinetic properties, such as high proteolytic stability and biocompatibility, the obtained oligomers can become the essential scaffolds for drug design and other biomedical applications.

PP-28: Optimization of the catalytic activity of a MvaT-based mini-protein

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Keywords: enzyme design, catalysis, mini-protein

Objective

The ability to create catalysts perfectly suited for the chosen reaction and the desired conditions is one of the main goals of modern chemistry. Enzymes exhibit remarkably high affinity, selectivity, and specificity towards their substrates; however, the rational method of enzyme design is still in need of optimization. In this work, a published retro-aldolase based on the MvaT domain has been tested for activity towards different substrates and in different conditions [1]. The main objective is the development of a straightforward protocol for enzyme customization by the introduction of rationally chosen mutations.

Methods

Changes to the sequence have been chosen based on rational design methods with the help of Rosetta FastDesign protocol and utilizing the knowledge of the reaction mechanism [2], [3]. The mini-proteins have been synthetized on a solid support, while the catalytic activity has been measured by chromatography of the reaction mixture at different time intervals.

Results

The retro-aldolase has been found to exhibit activity towards the aldol condensation of benzaldehyde and acetone, and different substituents of the substrate's benzene ring have been considered with varying effects on the catalysis. A set of MvaT mutants has been proposed and the influence of a single mutation in the proximity of the active site has been investigated: the substitution of Phe-19 into tyrosine, O-methyltyrosine and 4-nitrophenylalanine increased enzyme's affinity towards different substrates.

Conclusions

Enzyme specificity and activity can be re-engineered without the need for laborious testing of random mutations and can be done more deliberately. Mini-protein-based catalysts are an excellent choice for this task due to their exceptional stability, necessary for the introduction of the various mutations.

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PP-29: Structural characteristics and potential immunomodulatory effects of polysaccharide produced by cyanobacterium *Nostoc* spp.

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Keywords: polysaccharides, RAW264.7 murine macrophages, HDF, HaCaT, cytokine expression

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Objective
The blue-green algae, also known as Cyanobacteria (CB), are among the oldest photosynthetic organisms that inhabit aquatic and terrestrial environments. Secondary metabolites from *Nostoc* spp. may contain novel chemical structures and be developed as potential wound-healing drugs.

Methods
Polysaccharides were extracted by cold and hot extraction methods and characterized by NMR (1H and 13C), HPLC-SEC, and FTIR spectroscopy to evaluate their immunomodulatory properties. MTT and AlamarBlue assays were used to assess cytotoxicity and proliferation effect of polysaccharides. A nitric oxide production and phagocytosis activity were conducted to determine the anti-inflammatory effects of RAW264.7 murine macrophages. In order to evaluate cell migration, scratch wound healing assays were performed on HaCaT cells at 24 and 48 hrs. Moreover, the cytokine expression of TNF-α, TGF-β, IL-8, and MMP-1 was measured to confirm their bioactivity effects on cells.

Results
This study showed that three different cell lines (i.e., RAW264.7, HDF, HaCaT) were tested presenting no cytotoxic effects after 24 hrs. Extracted polysaccharide concentrations of 0.5 µg/µL significantly increased HDF cell proliferation and migration after 24 hrs. In addition, Cold extracts of the Nostoc polysaccharides did not cause any toxicity to RAW264.7 within 24 hrs and enhanced nitric oxide production. NI1B and NO1B polysaccharides significantly enhanced cell migration on HaCaT ($p < 0.05$). The TNF-α and IL-8 expression was increased in HaCaT cells treated with NO1B whereas it was lower in control groups. TGF-β1 suppressed significantly higher in NO1B and NI1B treated HaCaT cells at 24 and 72 hrs, which were responsible for controlling cellular behaviours (i.e., cell proliferation, migration, nitric oxide production, phagocytosis).

Conclusions
A bioactive component of Nostoc is nonsulfated polysaccharides and a lower Mw, which may reduce inflammation, speed up wound healing, and promote skin health, and did not exhibit any cytotoxic effects on immune cells or skin cells. There was no evidence that *Nostoc* spp. polysaccharides (NPs) promoted the proliferation of RAW264.7 murine macrophages. However, this study found that NPs significantly accelerated the proliferation and migration of HDF and HaCaT cells. In the near future, we believe there is a high probability that novel wound healing therapeutics could be developed based on isolating the principal active compounds from nostoc spp. polysaccharides.

ACKNOWLEDGMENTS

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PP-30: Synthesis of Miniproteins Interacting with PD-L1

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Keywords: miniproteins, PD-L1, immune checkpoint inhibitors, cancer immunotherapy

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Objective
Immune checkpoint inhibitors target the PD-1/PD-L1 axis and prevent cancer cells from evading immune response. However, commercial inhibitors based on monoclonal antibodies are limited by insufficient tumour specificity, structural complexity, and adverse immune response. Here, we demonstrate the potential of *de novo* designed miniproteins as an alternative class of immune checkpoint inhibitors of the PD-1/PD-L1 axis. Miniproteins are characterized by their small size, ease of synthesis, and high tendency to form compact conformations in solution. Because of their size, they can be easily modified to include functional groups that confer biological activity, added stability, and high selectivity. This study focused on the synthesis and characterization of computationally-designed miniproteins that bind to PD-L1.

Methods
The inhibitors were developed by incorporating noncanonical amino acids into miniprotein scaffolds, which were previously shown to have high stability. Three miniproteins were synthesized using Fmoc chemistry in a microwave-assisted automated solid-phase peptide synthesizer. The peptides were analyzed using ESI MS and purified using HPLC. CD spectroscopy was used to determine the secondary structure and the conformational stability of the miniproteins. Further analysis using nano differential scanning fluorimetry was done for miniproteins with inconclusive temperature scans. Finally, the affinity of the miniproteins to PD-L1 were measured using biolayer interferometry.

Results
Miniproteins with 41-residue-log sequence were synthesized using a microwave-assisted automated solid-phase peptide synthesizer by applying Fmoc-based chemistry. CD analysis showed that all peptides have CD spectra characteristic of alpha-like helices in peptides containing β-amino acids. Further analysis using nano differential scanning fluorimetry showed melting points of 39.2^{o}C, 49.3^{o}C, 57.6^{o}C, respectively, indicating that a stable miniprotein with a well-defined three-dimensional structure has been obtained. Biolayer interferometry analysis shows that the dissociation constant of 219_735 remains constant at 2.12 μM, while 219_735b achieved the best dissociation constant at 0.025 μM, at 26.9 nM and 8.14 pM, respectively, indicating that the synthesized miniproteins bind to PD-L1.

Conclusions
Miniproteins incorporating noncanonical amino acids were successfully synthesized and shown to have potent inhibitory activity against the interaction between PD-1 and PD-L1. It was demonstrated that miniprotein scaffolds can be modified to have potent biological activity, while retaining their original structure, and maintaining stability in solution. Future studies could explore other scaffold designs and incorporate other functional groups that could improve the activity of miniproteins against the PD-1/PD-L1 axis.

ACKNOWLEDGMENTS

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PP-31: SENP1 protease activity towards fluorogenic peptides of various chain lengths

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Keywords: SUMO, SENP proteases, enzyme activity

Objective
Sentrin-specific proteases (SENPs) are responsible for activating preSUMO proteins as well as for removing a SUMO protein from its peptide conjugates. Studies have shown that SENPs can be connected with a number of diseases including cancer, neurodegenerative diseases and cardiac diseases. Thus SENPs appear as attractive therapeutic targets. Determination SENPs activity in both normal and pathological states is important to understand their role. Therefore, selective chemical tools for individual SENPs are needed. The aim of this study was to evaluate the influence of peptide chain length on the SENP1 activity. This knowledge can be applied in activity-based probes and SENP-inhibitors synthesis.

Methods
SENP1 activity was evaluated towards fluorogenic substrates of various peptide chain lengths (4 to 15 amino acids). Substrates were synthesized using the Fmoc strategy of Solid Phase Peptide Synthesis and contained the C-terminal motif of natural substrate of the SENP1 protease – SUMO-1 protein.

Results
Study has shown that all evaluated substrates were hydrolysed with a similar rate, therefore shortest sequences should be chosen for further study. Peptide chain elongation does not increase SENP1 activity, and results in a substrate solubility problem due to the hydrophobicity of the sequence.

Conclusions
Tetrapeptide substrate possesses peptide chain of appropriate length upon which SENP1 is reasonably active and it can be utilized to explore SENP1 substrate specificity. Development of specific chemical tools for the investigation of SENPs will allow for better understanding of their role in normal and pathological states including cancer diseases.

ACKNOWLEDGMENTS

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PP-32: Escaping from phagocytosis - phosphatidylserine in cancer research

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Keywords: cancer, metastasis, phosphatidylserine, phospholipids, apoptosis

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Objective

Phosphatidylserine (PS) is an anionic phosholipid, primarly located in the inner leaflet of the cell membrane. Its transfer to the cell surface in healthy subjects causes apoptosis. However, in the tumor microenvironment PS-related signaling is highly dysregulated and does not necessarily trigger cell death. Exposure of non-apoptic PS and its binding to phosphatidylserine receptors (PSRs) plays an important role in cancer metastasis. Therefore, understanding PS signalling pathways may be crucial for creating new cancer treatments and extending patients' lives. PS plays the role of an "eat me" signal for macrophages. Its exposure on cell surface stimulates efferocytosis [1]. However, in the tumor microenvironment, the binding of PS to PSRs can activate immunosuppressive pathways. This feature is exploited by cancer cells as a mean of immune evasion [2]. As PS exposure alone may be insufficient to trigger the uptake of cells, anti-phagocytic ligands have to be blocked or removed from the target cell before efficient uptake takes place. These "Don't-eat-me" signals include CD47, CD24, CD31 and CD300a. Engagment of PS receptors during efferocytosis triggers immune tolerance, which includes the release of anti-inflammatory cytokines and inhibition of release of pro-inflammatory cytokines [3]. In the poster I focus on phosphatidylserine-targetting treatments. These include change the expression phenotype from ATP11Blo PTDSS2hi to ATP11Bhi PTDSS2lo through a combination of anti-PS antibody with paclitaxel or docetaxel [4]. I also shed a light on radioiodinated with 131I Saposin C-dioleoylphosphatidylserine nanovesicles, which selectively target cancer cells by honing in on their surface PS and extend the life span of mice suffering from glioblastoma multiforme [5].

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PP-33: General characteristic of bovine lactoferrin: molecular mass, isoelectric point, level of glycosylation

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Keywords: bovine lactoferrin, glycosylation, ultrafiltration, MALDI-TOF MS, trypsin digestion

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Objective
Bovine lactoferrin is considered as a valuable iron-binding glycoprotein which is naturally found in milk and other biological fluids, such as colostrum, tears, saliva. The presence of glycan chains has a significant effect on the biological activity, e.g., folding, immunogenicity, antibacterial and antiviral activity. Importantly, the protein glycosylation is also highly influenced by the selected method of isolation. The conventional isolation methods do not contribute to a sufficient purity of the final product. Thus, smaller particles (salts, peptides) remain adsorbed on the protein surface during isolation stages. In case of bovine lactoferrin, the use of ultrafiltration enables to obtain the product at a higher purity level. The aim of present work relies on the physicochemical characteristic of bovine lactoferrin (molecular mass, isoelectric point). In addition, the effect of ultrafiltration process on the glycosylation level in lactoferrin protein also was examined.

Methods
Firstly, the protein ultrafiltration was performed by application of Amicon Ultra-15 Centrifugal Filter Unit with cut-off of 50 kDa to obtain the lactoferrin at the highest purity. The isoelectric point of the filtrated protein was determined by DLS technique. The molecular profiles of bovine lactoferrin before and after ultrafiltration was compared by SDS-PAGE analysis in both, reduced and non-reduced modes. The characteristic lactoferrin bands were cut out and subjected to in-gel trypsin digestion. The primary structure (sequence) of lactoferrin as well as changes in glycosylation level were analyzed by advanced MALDI-TOF MS technique.

Results
According to MALDI-TOF MS analysis, the molecular mass of lactoferrin before and after ultrafiltration did not differ significantly and was at about 83 kDa. However, the increase of ionization intensity on MALDI-TOF-MS spectra of bovine lactoferrin after ultrafiltration was observed. SDS-PAGE analysis of protein filtrate confirmed an effective removal of peptides with molecular mass at about 15–20 kDa. Importantly, the protein fragment observed at 25 kDa on MALDI-TOF-MS spectra wasn't identified after ultrafiltration. The determined isoelectric point of the filtrated protein was 3.7, 8.2 and 9.8 at the concentrations 0.9, 0.09, and 0.009% of NaCl, respectively. According to sequencing results, it was shown that the ultrafiltration of lactoferrin led to carbohydrate rearrangements and the increase of the number of free sugars (deglycosylation).

Conclusions
Based on present work, it worth to conclude that ultrafiltration has significant effect on the protein post-translational modifications. Although the purity of lactoferrin increased after the ultrafiltration, but structural changes, particularly in degree of glycosylation, also occurred.

ACKNOWLEDGMENTS

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PP-34: Multinutrient coated fertilizers for sustainable agriculture

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Keywords: coated fertilizer, biodegradation, biopolymers

Objective

Continously growing population will need a significantly more food. Additionally, Regulation (EU) 2019/1009 of the European Parliament and of the Council lays down rules on the making available on the market of EU fertilising products, makes fertilizer producers to use only biodegradable polymers in their products. The desire to increase the efficiency of using fertilizers, as well as legislative changes, forced the researcher to invent the new biodegradable materials for the production of speciality fertilizers characterized by slowed released nutrients.

The aim of this study was to prepare an environmentally friendly controlled release fertiliser (CRF), which were containg a biodegradable, biobased raw materials. In this work, the natural-based polymers (lignin) were used as a raw material for modification via acylation reacion. Thus, the material obtained was used as a coating for multicomponent, granural, and mineral fertilizer.

Methods

The coated fertilizer was obtained by spraying polymer solution on granular multicomponent fertilizer. The coating process was carried out in a slow-running drum with an adjustable angle of inclination and the possibility of heating. The prepared ferilizer was evaluated in accordance with EN 13266. The nitrogen released was determined by elemental analysis, and the rate of release was estimated on its amount. Biodegradation of the used materials was arsessed. The manometric respirometry test was performed on the basis of the OECD 301F "Manometric Respiromtery" guidelines and the C.4-D.

Results

Prepared fertilizer contains 10% by weight of modified lignin. Polymer solution was used to obtain the coated fertilizer by spray coating method. The nutrients released in the water test, according to EN 13266 were, respectively, 4.3%, 16,1%, and 65,4% for the first, seventh and 28th day. The results of the biodegradation test were 10,2% over 28 day test according to OECD 301F.

Conclusions

The spray coating method is appropriate to produce coated granular fertilizer. The coated fertilizer materials meet the criteria for controlled released fertilizers EN 13266. The amount of nitrogen released after the first day does not exceed 15% (4,3%), and after 28 days of trial was lower than 75% (65,4%). The results of biodegradation test allow to conclude that used material will biodegrade in the time specified by the EU regulations.

ACKNOWLEDGMENTS

This research was co-financed by the Ministry of Science and Higher Education of Poland under Grant No. DWD/4/21/2020—06/003.

PP-35: Waste materials applied as soil amendments for the immobilization of metals and metalloids in contaminated soils

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Keywords: antimony and arsenic, iron-rich refuse, sludge-char, sorbents, sustainability

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Objective
Soil pollution by non-biodegradable metal(loid)s is a worldwide problem, posing risk to living organisms. Chemical immobilization is an *in situ*, environmentally friendly and cost-effective soil remediation technique for such elements. We studied the immobilization potential of various waste-materials towards several metal(loid)s in contaminated soils to reduce their leachability and thus potential environmental toxicity in a sustainable way.

Methods
We tested sludge-char, iron-scrap, iron-mud, and compost separately or in combination to remediate three distinct soils polluted by 1) Cr and Pb, 2) Zn and Pb, and 3) Sb and As. Amended and control soil samples were incubated in plastic pots for 1 month under laboratory conditions. After the incubation, the samples were immediately (i.e., in the wet state) extracted by demineralized water to determine the immobilization potential of the amendments. Total dissolved pollutant concentrations in the soil extracts were measured by ICP-OES/MS, while changes in the solid phases were inspected by SEM/EDS.

Results
The immobilization potential of the amendments was significantly influenced by soil and element type. In general, the iron-rich waste materials showed high immobilization potential towards the studied metal(loid)s. Chromium extractability was reduced the most by the iron-scrap treatments (alone or in combination with the organic materials). For Pb, the iron-scrap treatments had the opposite effect on its extractability in the Cr-soil and in the Zn-soil, similarly to the sludge-char treatment. Zinc concentration was the lowest in the samples treated with the combination of iron and organic materials, while the lowest Sb and As concentrations were found in samples treated with the iron-rich waste materials.

Conclusions
In conclusion, it is well known that trace metal(loid)s have high affinity toward iron (hydr)oxides, and our study confirmed that even iron-rich waste materials can effectively immobilize metal(loid)s in soil. However, some of our amendments increased pollutant extractability in some cases, as their performance strongly depends on, e.g., soil and pollutant characteristics. Future research work should focus on detailed testing and improvement of the tested amendments to increase their immobilization potential.

PP-36: Development of enzyme based electrochemical biosensor for detection of chromium

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Keywords: chromium, biosensor, bioremediation, pollutants, heavy metals

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Objective

Detection of water contamination is vital for the protection of the climate and avoidance of adverse consequences that it can have on human wellbeing. Heavy metals are especially perilous due to their capacity to collect over the long run into the plants and creatures, just as in water. Biosensors address a straight forward, dependable, and quick answer for observing water and soil contamination brought about by different heavy metals.

Biosensors permit not just decides the presence and in general natural accessible convergences of heavy metals in water and soil yet additionally evaluating their organic impacts. In this regard, detection of pollutants such as heavy metals (Chromium) by using enzymes such as invertase and acid phosphatase as recognition elements in biosensors is a versatile field. By this technology we will be able to use the enzymes which are effective and this could save our time and money to make new treatment.

Methods

Detection of pollutants such as heavy metals (Chromium) by using enzymes such as invertase and acid phosphatase as recognition elements in biosensor field has been determined, The cellular presence of invertase is being analysed through various biochemical assays.

Results

The positive results were obtained for the invertase enzyme activity in presence of chromium, The bacterial characterization revealed the bacteria as *Klebsiella oxytoca*.

Conclusions

Presence of invertase enzyme in the cellular component of bacteria confirms the utilization of the enzyme for the fabrication of electrochemical based biosensor for detection of chromium.

PP-37: Role of microbial peptide as biocontrol agent

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Keywords: pesticides, agriculture, peptides, antimicrobials, probiotics, pest management

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Objective
Due to harmful effects of chemical pesticides utilized in agriculture, rise of new concept of microbial peptides proves to be a positive evolving candidate to be used in place of chemical pesticides. Microbial peptides being natural compounds with low toxicity aids in integrated pest management for field of agriculture. Aim of the research is to evaluate the potential suppressive activity of antimicrobial peptide produced by *Lactiplantibacillus argentoratensis* probiotic strain against phytopathogens of solanaceous family. Antimicrobial peptides, which are considered to be safe since they can be easily degraded by proteolytic enzymes of the mammalian gastrointestinal tract. Since they pose no health risk concerns, microbial peptides, either purified or excreted by microbial peptide producing strains, are a great alternative to the use of chemical pesticides in agriculture

Methods
The presence of suppressive activity of antimicrobial peptide was primarily confirmed through agar well diffusion method as well as biuret test and was characterised by SDS PAGE and MALDI TOF.

Results
The sequence obtained for the peptide was PRKGSVAKDVLPDPVYNSKLVTRLINHLMIDGKRGK, which matched the peaks at 842.5 and 2866.4 m/z ratio, respectively, and had a molecular weight of about 5 kDa according to a tricine SDS-PAGE analysis.

Conclusions
This poster presents the identification and characterization of a novel AMP produced by *Lactiplantibacillus argentoratensis* IT, isolated from goat milk. The AMP demonstrated potent antibacterial activity against several microorganisms, including the Gram-negative Ralstonia solanacearum. The study highlights the potential of *Lactiplantibacillus* spp. IT as a source of novel AMPs for treating bacterial infectious diseases in plants.

PP-38: Polyolefin vitrimers as a cross-linked, recyclable material

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Keywords: vitrimer, polyolefins, recycling, cross-linking of polyolefins, polyolefin vitrimers

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Objective

The currently used methods of cross-linking polyolefins lead to the formation of permanent covalent bonds in their structure. This causes the material not to flow when exposed to heat, so it is not possible to recycle it. Therefore, most cross-linked plastics are burned or landfilled [1]. A solution to this problem may be vitrimers, which are materials characterised by reversible cross-linking [2]. The aim of this research was to develop an effective and economically justified method of polyolefin vitrimerization.

Methods

Vitrimerization of polyethylene by reactive processing was carried out in a periodic mixer. Polyethylene was processed with the addition of functional agents (initiator, functionalizing agent, cross-linking agent, catalyst). Dicumyl peroxide was used as the initiator, and maleic anhydride, known from the literature, was used as the functionalizing agent [2, 3]. Compounds containing ether groups in the structure were tested as a cross-linking agent. Transesterification catalysts were used.

Results

The results showed that effective vitrimerization of polyethylene is possible. Torque changes during the process were observed. Changes in the FTIR spectrum were also observed, confirming the incorporation of maleic anhydride into the polyethylene chain.

Conclusions

The development of an effective method of polyolefin vitrimerization may prove to be an economical way to obtain recyclable cross-linked plastics and thus reduce the consumption of raw materials used in their production.

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PP-39: Synthesis of linear benzothiadiazole derivatives for use in optoelectronics

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Keywords: benzothiadiazole, optoelectronics, synthesis, biosensors, photovoltaic

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Objective

This present work focuses on the synthesis of linear benzothiadiazole derivatives for use in optoelectronics.The specific compound *(E)*-4-(3,4-ethylenedioxythiophen-5-yl)-7-(7-(2-(thiophen-2-yl)vinyl))-3,4-ethylenedioxythiophen-5-yl)benzo[c][1,2,5]thiadiazole will be obtained as a result of a four-step synthesis. The derivative's acceptor properties suggest potential applications in medical diagnostics, optoelectronics (e.g., photovoltaic cells, light-emitting diodes, and sensors), and medical imaging [1].

Methods

The synthesis process involves the substitution of bromine atoms into the benzothiadiazole unit in the first stage, using the bromine solution (Br_2) together with hydrobromic acid (HBr), followed by C-C coupling reaction using palladium catalyst to expand the molecule with 3,4-ethylenedioxythiophene rings. The Vilsmeier–Haack reaction was then used to synthesize the aldehyde using N,N-dimethylformamide and phosphorus(V) oxychloride, and the final product was obtained using the McMurry reaction to allow the reaction between two carbonyl compounds. The research is ongoing to optimize the reaction and choose between the Stille and Suzuki reactions for the best performance.

The next stages of the project will involve testing the semiconducting and optical properties (absorbance and fluorescence) of the monomer and subjecting the compound to electro polymerization, creating a polymer film, to the potential use as a matrix in biosensors.

Results

We are still trying to obtain our derivative and we are also working on obtaining the derivative in the highest yield. At this point we are performing an analysis of ^{1}HNMR, ^{13}CNMR and MS after each step to confirm the structure of the compound. The next stages of the project will involve testing the semiconducting and optical properties (absorbance and fluorescence) of the monomer and subjecting the compound to electropolymerization, creating a polymer film, to the potential use as a matrix in biosensors.

Conclusions

Based on previous research we assume that it is possible to obtain expected derivative. We want to optimize the synthesis by choosing Stille's or Suzuki reaction to perform the best yield. In the future we will test the derivative as a potential use in biosensors.

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PP-40: Low-temperature plasma as an alternative to other sterilization methods

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Keywords: cold plasma, phytopathogenic fungi, crop loss, plant diseases

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Objective

Providing sustainable, secure, and nutritious food supply for the growing population is currently one of the key challenges facing humanity. Research on the impact of fungi on food security constitutes a significant part of these efforts [1]. The presence of phytopathogenic fungi contributes to the loss of up to 30% of global crops, which would be enough to feed 600 million people a year [2]. It is widely believed that low-temperature plasma has the potential to eliminate various disadvantages associated with conventional fungicides, making it one of the most promising methods of sterilization.

Methods

The effectiveness of low-temperature plasma on the eradication of phytopathogenic fungi (*Botrytis cinerea*) was compared to other commonly used sterilization methods, such as UV and UVC radiation. For this purpose, wooden discs were artificially contaminated with a suspension of fungi. These discs were then placed 25 cm from the radiation source (UV and UVC, respectively). Low-temperature plasma treatments were performed in a dielectric barrier discharge (DBD) reactor. The number of viable cells was assessed by plating on a solid medium. In order to compare the effectiveness of the sterilization methods, the D-value was determined. This parameter is defined as the dose or time required to destroy or inactivate 90% of the microbial population present in the sample. It is a measure of the effectiveness of a given disinfection or inactivation process and allows you to assess how effectively microorganisms are destroyed or eliminated.

Results

The D-value for low-temperature plasma was less than 10 minutes, while for UV and UVC radiation treatment, this parameter was 55 and 43 minutes, respectively.

Conclusions

Low-temperature plasma is a very effective sterilization method. In terms of efficacy, the duration required to eliminate 90% of phytopathogenic fungi cells is significantly reduced when employing low-temperature plasma in contrast to treatments involving UV or UVC radiation. Considering the fact that phytopathogenic fungi cause significant losses in food production, cold plasma should be considered as a method to combat these contaminants.

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PP-41: Comparison of the bioconversion degree of selected organic wastes with their biochemical methane potential

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Keywords: wastes management, anaerobic digestion, bioconversion, correction coefficient

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Objective
The use of waste in the anaerobic digestion process enables the production of biogas and its conversion into electricity from renewable raw materials [1]. The main objective of the research was to determine the energy potential of biomass used in biogas production. Based on the obtained results, the degree of biomass conversion was determined.

Methods
In the analyzed biogas plant, waste from vegetable processing and maize silage were used. In addition, the installation was fed with pig slurry, the purpose of which was to hydrate the feedstock. Physicochemical analyzes and quantitative determination of the chemical composition of the tested materials were performed [2]. The biochemical methanogenic potential was determined for the periodic mode of operation of micro fermentation chambers in mesophilic conditions.

Results
The most caloric input was maize silage. The daily value of calories served was 33.819 MWh·day^{-1}, followed by potatoes, whose energy value was 7.708 MWh·day^{-1}. Celery 0.868 MWh·day^{-1} and leek 0.672 MWh·day^{-1} turned out to be the least energetic. The daily production of biogas for liquid manure and used waste amounted to 8160 m^3·day^{-1}. Conversion of energy accumulated in the input materials amounted to 17,760 MWh·year^{-1} in relation to the chemical energy obtained in the produced methane (12,031 MWh·year^{-1}).

Conclusions
Anaerobic digestion is a process that efficiently converts the primary chemical energy contained in waste into chemical energy contained in biogas. With the content of methane in biogas at the level of 52%, the obtained chemical energy concentrated in methane at the level of 59.93% of the entire stream of chemical energy contained in the waste.

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PP-42: Heavy metal accumulation by the macrophyte *Sparganium erectum*

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Keywords: *Sparganium erectum*, macrophytes, heavy metals, phytoremediation, bioaccumulation

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Objective
Phytoremediation is widely used in cleaning techniques of degraded industrial areas. New plants that can support the remediation process are constantly being sought. The aim of the work was to assess the possibility of using the macrophyte *Spraganium erectum* for phytoremediation. The choice of the plant for the study was determined by the fact that it is a common plant in Poland and around the world in water reservoirs, on the banks of rivers and lakes.

Methods
The level of selected heavy metals Cr, Pb, Ni, Zn and Cu in *Spraganium erectum* and bottom sediments in mineralized samples was measured using the ICP-OES spectrometer. Samples were collected twice at the beginning and end of the growing season of the plant together with bottom sediments from the Rzeszów reservoir (Podkarpackie voivodeship) at three points. The removal of excess heavy metals from the bottom sediments enables their mangage after extraction from the bottom of the reservoir. The bioaccumulation factor (BAF) and translocation factor (TF) were calculated. The BAF was calculated as the ratio of a metal concentration in a plant to the concentration in the bottom sediment. The TF is the ratio of a metal concentration in the tissues of organs above ground plantand to the concentration in the roots.

Results
The calculated BAF indicates the intensive degree of accumulation of all five metals from the bottom sediments to the plant. For samples collected at the beginning of the growing season, bioaccumulation was more intensive at the second and third points than at the first point (located near the dam). The opposite was true at the end of the vegetative season, the strongest accumulation was at point 1. The BAF factor for individual metals was in the range: Cu – 0.39 – 1.95, Zn – 0.33-1.67, Pb – 0.46 – 1.21, Cr – 0.11 – 4.36 and Ni – 0.45 – 2.71. The translocation factor indicates the most intensive movement of Ni in *Sparganium erectum,* for 12 of 18 samples received an amount greater than 1. By analysing the values of TF from the roots to the above-ground parts for individual metals the relationship Cr < Pb< Zn < Cu < Ni was obtained.

Conclusions
The analysis of the test results indicated trends for the uptake of heavy metals in *Sparganium erectum* in the descending order of root > stem > leaf. On the basis of the bioaccumulation factor, it can be concluded that *Sparganium erectum* can accumulate the heavy metal in its roots and in either part of plants, and the same clean the sediment. The calculated translocation factor for most cases indicates the retention of the marked metals in the root part; for them the translocation factor was TF > 1. The plant can be used in phytoremediation, but for the best results, mixed plants of the other species are proposed.

PP-43: Chemical composition and cytotoxic activity of commercially available *Melaleucae aetheroleum*

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Keywords: *Melaleucae aetheroleum,* chemical composition, cytotoxic activity, BSLA, LC_{50}

Objective

Tea tree essential oil (*Melaleucae aetheroleum*) has antibacterial and antifungal properties against a large number of pathogenic microorganisms. It is used as a traditional herbal remedy for minor superficial wounds and acne, as well as for the treatment of milder mucosal inflammations [1]. The objective of this study is to determine the chemical composition of commercially available *Melaleucae aetheroleum* and its cytotoxic activity.

Methods

GC/MS analysis was used to investigate the chemical composition of the studied essential oil, while for determining the cytotoxic activity, brine shrimp lethality assay (BSLA) was used [2]. Meyer's and Clarkson's scales of toxicity were used to categorize the essential oil based on the obtained LC_{50} values [2], [3].

Results

The predominant components identified in *Melaleucae aetheroleum* were terpinene-4-ol (39,06%), *γ*-terpinene (21,03%), Δ3-carene (9,89%), *o*-cymene (4,76%), *α*-terpineol (4,31%), terpinolene (4,07%), *α*-pinene (3,98%), limonene (2,43%), viridiflorene (1,40%) and *β*-pinene (1,27%). According to the results from BSLA, this essential oil displayed toxic activity in accordance with the Meyer's scale and highly toxic activity in accordance with the Clarkson's scale, with the peak toxic potential occurring after 24 hours of exposure with LC_{50} value of 34.02 μg/mL.

Conclusions

This study revealed the chemical composition and cytotoxic properties of *Melaleucae aetheroleum*. Further examination is required to determine more closely the possible mechanism of cytotoxic activity.

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PP-44: Long-term storage and stability of different *Cannabis* crude oils

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Keywords: cannabis oil, stability, potency, CBD, THC

Objective

It has long been recognised that *Cannabis* resin and flower lose potency over time due to the content variability of the major cannabinoids. This instability must be taken into account as *Cannabis* is used for medicinal purposes [1]. Products containing less stable drug substances, such as cannabinoids, should be tested reasonably more frequently than those for which stability data are available. To determine the stability of cannabis components during the year, different crude oils of *Cannabis* were tested under controlled environmental conditions.

Methods

In this study, samples of CBD and THC cannabis crude oil were analyzed. The samples were stored under controlled environmental conditions, a temperature of 25°C, and a humidity of 60% for 12 months.
Sample preparations and HPLC analysis on cannabis oils were performed according to the UNDOC method 2009 (Recommended methods for the identification and analysis of cannabis and cannabis products). HPLC analysis was performed at zero time point and after 12 months.

Results

The results of the CBD *cannabis* crude oil stability study showed a decreasing effect in less than 13% of the CBD content, from 72.8% to 64.7%, 73.6% to 71.2% and from 80.8 to 75.4%, after 12 months of storage. On the other hand, HPLC analysis of THC cannabis crude oil showed an increasing effect on THC content in less than 10%. The THC content in the oil increased from 70.8% to 71.5%, 72.6% to 80.4% and from 71.2% to 77.7%.

Conclusions

Regarding the stability study of the main cannabinoids content in the *cannabis* crude oils, variability in the CBD and THC content was established after 12 months, as a function of storage form and storage conditions. Due to this, potency retesting is requested after a prolonged period of time, and care should be taken when comparing sample concentrations.

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PP-45: Determination of the thermodynamic parameters of copper and zinc ion binding as well as antifungal and cytotoxic properties of four bioactive peptides.

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Keywords: bioactive peptides, *Candida albicans*, metal-peptide interaction, cytotoxicity

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Objective

Bioactive peptides can have various positive effects, such as preventing diseases or modulating physiological systems. The aim of our work was to investigate the interactions of peptides with potential biological activity with Cu(II) and Zn(II) ions. We selected four peptides identified in kombucha-treated milk extracts, which have been shown to have a strong antihypertensive effect [1].

Methods

The affinity of the metal-peptide interaction and the changes in enthalpy and entropy were studied using the ITC technique by fitting the theoretical curve to the measurement points with the nonlinear least squares method. The UV-Vis technique was used to characterize the formation of Cu (II) complexes with each of the peptides studied. The microbiological studies were performed using the dilution susceptibility testing method in Sabouraud dextrose liquid medium. The cytotoxicity effect of peptides on carcinoma cell line in vitro was carried out using lactate dehydrogenase (LDH) assay. In addition, the resazurin reduction cell viability and metabolism assay was conducted [2].

Results

All peptides: **AVPQEVLNENLLR, YLQGSNLVVPLTDD, KFKGFVEPFPAVE and FVAPEP-FVFGKEK** (named by us Pep1-Pep4, respectively) have shown a very weak cytotoxicity against breast carcinoma cell line (MDA MB 231) and human pancreatic carcinoma cell line (PANC-1) with Pep1 surpassing the 3 others. Compared with the control group, the cell inhibitory ratio (IC50) of Pep1 was 1.02 mM for MDA MB 231cell line and 1.48 mM for PANC-1 cell line. All of them bind Cu(II) ions with moderate affinity (Kd in the range of 35-92μM) and Zn (II) with much lower affinityAntifungal susceptibility testing of *Candida albicans* has shown that only Pep1 has a weak antifungal effect and the presence of copper ions does not support this effect.

Conclusions

All of the studied peptides have similar binding modes of Cu(II) ions. The antifungal tests should be repeated for Pep1 with a higher concentration of the peptide.

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PP-46: *Hylocereus polyrhizus* (Weber) Britton & Rose fruits extract profiles – the influence of ultrasound and versenic acid on the betacyanis content

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Keywords: ultrasound extraction, EDTA, betacyanins, *Hylocereus polyrhizus*

Objective
The studies included determining the profile of dominant betacyanins in pulp and pericarp extracts of freeze-dried *Hylocereus polyrhizus* (Weber) Britton & Rose fruits depending on the effect of ultrasound and EDTA. Further research will allow us to learn more about the properties of plant substances and use them in the production of food, dietary supplements, or cosmetics.

Methods
Freeze dried fruits (separately pericarp and pulp) were mixed with extractant: 40% vol. ethanol. Some of the samples were supplemented with EDTA and/or subjected to ultrasounds. The absorbance of the extracts was measured using a thermostated microplate reader. They were also analysed by the LC-DAD-ESI-MS technique (liquid chromatography combined with a diode detector and tandem mass spectrometry with electrospray ionisation). The precipitate remaining after the first extraction was subjected to another extraction in the same way.

Results
Analyses revealed that the main betacyanins were present in both the peel and pericarp of dragon fruits (*H. polyrhizus*)with betanin, phyllocactin, and hylocerenin as the dominant pigments. The use of ultrasound resulted in a reduction of the extraction yield for both sample variants by 2–5%. On the basis of the areas of the LC-MS chromatographic peaks, it was determined that the phyllocactin content in the pericarp is higher than in the pulp. The amount of betanin remains similar in both pitaya pericarp and pulp, whereas the amount of hylocerenin is lower in pericarp. The presence of EDTA in the extraction mixture increased the extraction yield by 1–6%.

Conclusions
Analysis of *H. polyrhizus* fruit extract samples showed that the content of individual betacyanins changed depending on the use of EDTA or ultrasound. Lower extraction yields for *H. polyrhizus* fruit pericarp (peel) samples may result from the influence of higher amounts of sticky mucous substances and pectins than in fruit pulp.

The addition of EDTA to the samples increases the extraction efficiency by 1–6%. This may be due to the fact that EDTA reduces the rate of degradation of betacyanins, which have limited stability. The use of ultrasound reduced the yield from 2–5% for both pulp and peel samples. The structure of the plant can influence the retention of phytochemicals in the plant. Despite this, ultrasound extraction is a cheap, quick and simple extraction method, which gives a wide range of solvents to be used.

ACKNOWLEDGMENTS

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PP-47: *Monascus* sp. and its health-beneficial products

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Keywords: Monascus purpureus, lowastatin, fermentation, health food

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Objective
The Monascus strain belongs to the saprophytic fungi group. During its growth, Monascus breaks down starchy substrates into several metabolites, including pigments produced as secondary metabolites pigments, i.e. yellow (monascinand ankaflavin), orange (rubropunctain and monascorubrin) and red (monascorubramine, rubropunctamine) [1]. It has been used for many centuries in Asian countries as a food colouring, preservative, dietary supplement and in traditional medicine. A common food product in which the Monascus species is used is Angkak (red mould rice), which is obtained by fermentation using the aforementioned mould fungus.

Methods
Literature review using web of science, Scopus and Google Scholar databases.

Results
– Natural extracts of red rice have been shown to have potent anti-cancer activities, making them potential current chemo preventive agents against breast cancer [3].

– Purple rice varieties have been found to be a good source of nutrients, minerals, phytochemicals and antioxidant compounds. In particular, a high content of anthocyanins and the antioxidant gamma oryzanol was found in the hulls of the grains. These substances allow purple rice to be used as traditional medicines to lower plasma cholesterol levels, cholesterol absorption and reduce early arteriosclerosis [4].

Conclusions
Because of the above properties of the fungus used and the substations secreted by it, it allows interesting health-promoting food products and medicines of natural origin to be obtained.

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PP-49: Bioinformatics analysis reveals that disordered regions in TCF4 are likely to be responsible for LLPS

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Keywords: TCF4, LLPS, IDRs

Objective

Liquid-liquid phase separation (LLPS), is an essential process for cell life, resulting in the formation of membraneless organelles (MLOs). It involves the spontaneous separation of liquid droplets stably existing in another liquid, from an initially homogeneous solution of macromolecules. The separation of light and dense phases occurs, due to changes in environmental conditions (e.g. pH, ionic strength, temperature) and is reversible. Condensates can contain a large number of molecules, among which a significant role is played by nucleic acids and proteins containing intrinsically disordered regions (IDRs), of which only a part, defined as scaffold molecules, is necessary to maintain the integrity of condensate. Condensates owe their stability to the sum of many multivalent interactions within the complexes.

For many years, researchers have wondered how molecules find their way in a crowded cellular environment at a particular time. It turns out that many cellular processes take place in liquid condensates, and gene expression, including transcription, is no exception. One of the eukaryotic transcription factors is TCF4 that belongs to a family of proteins containing a helix-loop-helix motif preceded by a basic domain (bHLH). The short bHLH domain is responsible for the binding of the E-box sequence. To date, it is the only structurally characterized domain of TCF4. The lack of broader knowledge of the structure of bHLH family members inspired us to carry out bioinformatics analyses of TCF4 – prediction of disorder, backbone dynamics and tendency to LLPS.

Methods

Analysis of the TCF4 I- sequence was performed using bioinformatics tools with default settings. Disorder prediction was made using the PONDR server, IUpred3 and NetSurfP-2.0. Protein backbone dynamics were predicted using DynaMine. The propensity of TCF4 I- to undergo LLPS was predicted using FuzDrop, which additionally provides information on aggregation hot spots.

Results

The results of disorder obtained with all the algorithms are consistent. About 80% of the analysed sequence seems to be disordered. The remaining 20% of the sequence mainly cover the bHLH domain (residues 348–401). DynaMine analysis revealed that TCF4 I- sequence is mostly identified as flexible/disordered. Context dependent and rigid/ordered regions mainly cover the bHLH domain. Moreover, disordered regions of TCF4 are likely to be responsible for potential LLPS, as shown by analysis using the FuzDrop tool.

Conclusions

Bioinformatics analysis showed that human TCF4 I- except for the bHLH domain is likely disordered. The IDRs of the protein probably can cause the protein to undergo LLPS, which may have a role in the transcription process. However, these conclusions need to be confirmed experimentally.

PP-50: Neutrophil Serine Proteases activity profile in neutropenia patients

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Keywords: neutrophiles, neutropenia, neutrophil serine proteases, chemical markers, confocal microscopy

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Objective

Polymorphonuclear leukocytes (PMNs, neutrophils) are the main component of the innate immune system and host defense. These cells protect host against many different pathogens using a set of strategies, including phagocytosis, degranulation or releasing neutrophil extracellular traps (NETs) in a process called netosis. In each of these mechanisms PMNs involve Neutrophil Serine Proteases (NSPs including: NE (neutrophil elastase), CatG (cathepsin G), PR3 (proteinase 3) and the recently discovered neutrophil serine proteinase 4 (NSP4), but also Granzyme A (GrA) or Granzyme B (GrB). Among others these enzymes play a regulatory role in noninfectious inflammatory diseases like neutropenia, which is a hematological disorder characterized by an abnormally low number of neutrophils. Theare are two main type of hereditary neutropenia: cyclic and severe cognital and it was described that NSPs mediate the development of these conditions. Both severe congenital neutropenia and cyclic neutropenia are characterized by inhibition of neutrophil maturation at the stage of myelopoiesis. Among other factors, from the NSPs, so far most attention has been devoted to neutrophil elastase. The molecular basis of severe and cyclic neutropenia has been identified in patients with mutations in the *ELANE2* gene encoding NE. Research shows that *ELANE2* mutations interfere with normal intracellular NE transport. The main goal of our research is to develop the first subcellular map of PR3 activity in neutrophils from neutropenia patients and healthy donors using confocal microscopy.

Methods

Confocal microscopy: analysis of glass slides with neutrophils from neutropenia patients and healthy donors Neutrophils isolated from blood are stained with ABP (Activity-Based Probes) for PR3 to detect active enzymes and with antibodies to determine the amount of expressed PR3. **Flow cytometry:** After neutrophils isolation the phenotype of neutrophils is performed with Flow cytometry.

Results

The obtained results indicate that neutrophils are characterized by a diverse amount and also activity of neutrophil serine protease PR3 in neutropenia patients with different mutation.

Conclusions

NSPs are extremally important in contexts of neutrophil functioning so mutations in the genes that encode NSPs often lead to neutrophil defects. Identification of the NSPs activity in patients with neutropenia may help in formation of a unique diagnostic method and our result suggest that PR3 may be interesting diagnostic purpose.

ACKNOWLEDGMENTS

The "Fix Neutropenia (FIXNET): focusing on neutrophil proteases defects which serve as novel diagnostic and therapeutic options" project is carried out within the TEAM-NET programme of the Foundation for Polish Science (POIR.04.04.00-00-1603/18) financed by the European Union under the European Regional Development Fund.

PP-51: Validation of the choice of a miniprotein scaffold used for the obtainment of inhibitors targeted towards PD-1 protein

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Keywords: miniprotein scaffolds, inhibitors, potential anti-cancer drugs, rational drug design, solid-phase peptide synthesis

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Objective

The PD-1/PD-L1 interaction and the way it modulates the tumor cell microenvironment [1] opened new possibilities in terms of designing anti-cancer drugs. The interaction between the ligand and the receptor causes lack of the apoptotic response of the immune system [1, 2]. A large group of miniproteins that may be considered as potential inhibitors of the PD-1/PD-L1 interaction was synthesized. The spatial arrangement of amino acid residues and the physicochemical characteristics of those which are exposed towards cavities on the surface of the target protein were the key points in optimization of models that would lead to obtain potentially effective inhibitors.

Methods

Rational drug design and the Rosetta Protein Design software[3] were implemented to suggest new sequences that could potentially build active inhibitors. Solid-phase peptide synthesis was used to obtain the products. Various methods, such as preparative HPLC, mass spectrometry, analytical HPLC, circular dichroism, and biolayer interferometry (BLI) were used for purification, product identification and analyses.

Results

MvaT and ENH, scaffolds of well described topologies, were applied to the Rosetta Protein Design software protocol and optimized to better fit the PD-1 protein cavity which is known to bind PD-L1. Further modifications were suggested using rational drug design. A total of 69 miniproteins were obtained and investigated in terms of folding, thermal stability, and affinity towards the target molecule. Using the BLI method, it was demonstrated that miniproteins based on the MvaT scaffold showed greater suitability as potential interaction partners compared to those based on the ENH scaffold.

Conclusions

Based on the collected data, it can be concluded that the ENH scaffold is not suitable for constructing PD-1 inhibitors. None of the modifications applied to the ENH-based sequences resulted in improved affinity of this set of miniproteins towards the target molecule. In contrast, there are a few examples of MvaT-based miniproteins that exhibit a moderately high level of affinity towards PD-1.

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PP-52: Ketoprofen pharmacokinetics: Development and evaluation of release soft gelatin capsules

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Keywords: ketoprofen, release profiles, release kinetics, drug form, formulation

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Objective
Ketoprofen belongs to a group of widely used non-steroidal anti-inflammatory drugs (NSAIDs). It is found in several doses and various forms of medications. Therapeutic efficacy is related to the rate of release of the active substances, which depends on the formulation, the quality of the constituent substances and solubility. Evaluation of efficacy, on the other hand, allows to choose pharmaceutical preparations consciously.

Methods
In order to determine the efficacy of drug products containing ketoprofen available on the pharmaceutical market in Poland, studies comparing the release profiles of the active substance contained in specific formulation and doses, Ketokaps MAX, 50 mg, soft capsules, Ketonal Active, 50 mg, hard capsules, Ketokaps MED, 100 mg, soft capsules, Refastin, 100 mg, film-coated tablets and Ketonal Forte, 100 mg, film-coated tablets have been conducted. Drug release has been tested using FaSSGF and FaSSIF bioequivalent media in a type IV flow apparatus.

Results
The results indicate that the product Ketokaps MAX, 50 mg has a shorter release time of ketoprofen and a faster reached maximum concentration of the released active substance than the market product Ketonal Active, 50 mg. The same outcomes will be achieved by the product Ketokaps MED, 100 mg compared to the market products Refastin, 100 mg and Ketonal Forte, 100 mg.

Conclusions
In vitro studies confirm that the tested products differ noticeably in the kinetics of release of the active substance. Ketokaps MAX, 50 mg and Ketokaps MED, 100 mg reach the therapeutic effect the fastest, making them the most effective products.

PP-53: Changes in the expression profile of ferroptosis-related genes astrocytic series brain tumors

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Keywords: ferroptosis, glioma, diagnostic targets, microarrays

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Objective

Glioblastoma multiforme (GBM), classified as a grade IV astrocytoma, is the most common and aggressive brain tumor. It is characterized by high malignancy and often an unfavorable prognosis. Many studies have linked ferroptosis to metabolic changes in tumor cells, which may have potential applications in therapy. Ferroptosis is a type of programmed death caused by the accumulation of excess iron in cells. It is confirmed to play an important role in cancer biology [1, 2].

The aim of our work was to identify ferroptosis-related genes whose expression changes in astrocytomas depending on the degree of malignacy.

Methods

The study involved extraction of total RNA from 43 sections of resected brain tumours of the astrocytic series with varying degrees of differentiation. Histopathological classification determining the degree of malignancy of the tumours according to the WHO scale was carried out in the Department of Neurosurgery and Clinical Division of St. Barbara Regional Hospital in Sosnowiec. Determination of the transcriptional activity of genes associated with ferroptosis was carried out using the HGU-133A 2.0 oligonucleotide microarray technique - Affymetrix.

Results

Using an oligonucleotide microarray method, we identified 12 mRNA IDs of ferroptosis-related genes whose expression was upregulated (*STEAP3*, *HSPA5*, *HSPB1*) or downregulated (*DDIT4*, *SAT1*, *AKR1C1*, *GABARAPL1*, *ZEB1*, *SLC1A4*, *FTH1*, *ATP6V1G2*, *VDAC3*) in G3/G4 stage gliomas relative to G2. STRING database analysis showed that the proteins encoded by the analyzed genes form a strong interaction network ($p < 0.001$), and a significant number of proteins are involved in carcinogenesis.

Conclusions

Differences in the expression pattern of ferroptosis-related genes confirm that they may have a significant impact on astrocytic transformation of brain tumors. However, further studies on a larger group are needed to accurately characterize the marker methodology in brain tumors, but this study may provide a preliminary basis.

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PP-54: Biofunctionalized boron carbide nanoparticles as promising compounds in boron neutron capture therapy

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Keywords: nanoparticles, boron carbide, biofunctionalization, anticancer therapies, BNCT

Objective

Boron neutron capture therapy (BNCT) is a targeted radiotherapy dedicated to the treatment of patients with tumors located in hard-to-reach areas. BNCT is based on the selective delivery of boron-10 isotope to cancer cells and irradiation of the tumor site with a neutron beam, which generates high energy that destroys cancer cells. BNCT is still at the stage of clinical trials, primarily due to the search for compounds that selectively deliver boron to cancer cells. One promising compound is boron carbide, which has a high boron content. The aim of our research was the functionalization of two boron carbide preparations with antibodies against LDL receptors overexpressed in cancer cells, to increase the selectivity of delivery to tumor cells. Their toxicity and uptake by cancer cells were also determined.

Methods

In the first stage of the research, the dynamic light scattering (DLS) technique was used to characterize the physicochemical changes during the adsorption of anti-LDLR antibodies on the surface of boron carbide nanoparticles and the stability of the obtained compound-antibody complex was measured. In addition, the expression of LDLR on the surface of MC38, TRAMP-C1 and B16 F0 cancer cells was determined by the flow cytometry method. In the next stage, the toxicity of native and functionalized boron carbide preparations on the tested cancer cells was assessed using the MTT assay and the uptake of functionalized boron carbide by cancer cells was determined by the flow cytometry method.

Results

The research showed changes in the zeta potential along with the adsorption of anti-LDLR antibodies on the boron carbide surface. In addition, the stability of the complex during storage was demonstrated. Moreover, the functionalization of boron carbide did not increase its toxicity compared to the native compound on MC38, TRAMP-C1 and B16 F0 cancer cells, all of which had LDLRs on their surface. Whereas, the uptake of functionalized boron carbide increased with the time of incubation with cancer cells and was highest for TRAMP-C1 cells that had the most LDLRs on their surface.

Conclusions

The research proves that the functionalization of boron carbide increases its selectivity towards cancer cells with an abundance of LDLRs on their surface, which makes it a promising compound for BNCT.

ACKNOWLEDGMENTS

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PP-55: Effect of administration of cellular vaccines based on dendritic cells capable of overproducing IL-12, IL-15 or IL-18 on the inhibition of B16-F0 murine melanoma tumor growth

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Keywords: dendritic cells, IL-12, IL-15, IL-18, immunotherapy, mouse melanoma

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Objective

For many years, research on the use of dendritic cells (DCs) has focused on their use as vaccines against cancer to initiate and shape an anti-tumor-specific immune response. An attractive approach is the use of dendritic cells not only as antigen-presenting cells but also as carriers of pro-inflammatory cytokines such as IL-12, IL-15 or IL-18. This form of cytokine delivery by modified dendritic cells secreting the cytokines for a long time but in smaller amounts is intended to circumvent the limitations of recombinant cytokines administration. IL-12 administered as a recombinant agent caused high toxicity, while IL-15 and IL-18 have a short half-life. These cytokines, secreted for a longer time, can have a beneficial effect on the enhancement of the antitumor response.

Methods

Bone marrow-derived dendritic cells (DCs) were transduced with lentiviral vectors carrying IL-12, IL-15, or IL-18 genes and stimulated with tumor antigens (TAg). The effectiveness of transduction was verified based on the expression of marker proteins and cytokine production. To determine the maturation status of DCs the expression of surface markers was examined. The obtained cellular vaccines were administrated peritumorally (p.t.) on the 11^{th} and 16^{th} days to C57BL/6 female mice with established B16-F0 tumors. Tumor growth was monitored every 2-3 days and was used to determine tumor growth curves and inhibition (TGI).

Results

Genetically modified dendritic cells were able to express marker proteins and produce cytokines whose genes were introduced. The highest expression of co-stimulatory molecules CD40, CD80 and CD86 as well as MHC II molecules was found on cells capable of overproducing IL-15. This was also reflected in their effectiveness in inhibiting tumor growth, which amounted to 50.1%. Other transductants also showed therapeutic effectiveness but at a lower level.

Conclusions

The obtained results reveal that the enhanced production of IL-12, IL-15 or IL-18 by DCs increases their maturation status and effectively inhibits the growth of the B16-F0 tumor. However, an administration of DC/IL-15/TAg vaccine caused the greatest potential for inhibition of tumor growth. We postulate that DC-based cytokine-producing vaccines can be helpful for immunotherapy in the generation of anti-tumor response.

ACKNOWLEDGMENTS

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PP-56: Investigation of the role of granzyme A in genetically modified neutrophil-like cells using the CRISPR/Cas9n method

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Keywords: CRISPR/Cas9, knockout, neutrophils, phagocytosis

Objective

Granzymes are highly conservative serine proteases, and in humans, this group includes 5 enzymes [1]. Granzyme A (GrA), along with granzyme B, is present in the granules of NK cells and cytotoxic T lymphocytes. There is much controversy regarding the cytotoxic properties of granzyme A and its expression in human polymorphonuclear leukocytes (PMNs, neutrophils). Neutrophils are terminal phagocytic cells responsible, among others, for pathogen neutralization. They are recruited to sites of inflammation or infection in response to inflammatory factors. Isolated circulating in human blood neutrophils are characterized by a short lifespan (6–8 hours), which makes genetic modifications impossible to perform [2]. The role of neutrophilic GrA is particularly poorly understood. It is speculated that it may mediate tumor progression and metastasis through neutrophils recruited to the tumor microenvironment [3]. Also it is possible that GrA plays role in pathogen infection for example in phagocytosis, chemotaxis or neutrophils migration [4]. Therefore, this project focusses on the investigation of the role of neutrophil GrA for physiological PMNs functions using a few neutrophil-like cells as models.

Methods

To obtain genetically modified neutrophil-like cells CRISPR/Cas9n vectors are introduced with electroporation to myeloid progenitor cell lines and induced pluripotent stem cells (iPSCs) to generate granzyme A knockout.

Results

This study presents a new genetically modified cellular model that can be used for the functional analysis of granzyme A in neutrophils under in vitro conditions. Such a model will allow independent research and eliminate variability that could interfere with data interpretation, but also facilitate functional assays to study the role of GrA in PMNs.

Conclusions

The results obtained using this model may contribute to a better understanding of the role of one of serine proteases, particularly neutrophilic granzyme A, especially in defence against pathogenic microorganisms.

ACKNOWLEDGMENTS

The research is funded by National Science Center Poland Opus grant entitled "Study of the role of granzyme A in neutrophils with the use of induced pluripotent stem cells" (2020/39/B/NZ1/03027)

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PP-57: The function of granzyme A in apoptosis and NETosis of neutrophils

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Keywords: cell death, apoptosis, NETosis, neutrophil, inflammation, neutropenia

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Objective
Neutrophils are white blood cells also called neutrophil granulocytes or polymorphonuclear leukocytes and are the most abundant cells of the immune system. The main task of neutrophils is to defend human body against pathogens. For this purpose, these cells use a number of methods and factors, among which the most commonly used are antimicrobial peptides, proteolytic enzymes and reactive oxygen species. Neutrophils enter the circulation in the form of terminal cells that lack the ability to proliferateThere, depending on the factor causing inflammation, neutrophils use different techniques to eradicate pathogens, including phagocytosis, degranulation, and netosis. Granzyme A (GrA) belongs to the neutrophils' serine proteases (NSPs), the most important proteolytic enzymes found in azurophilic granules where they are stored in the active form to perform effector functions in the host defense. To date, only a few studies providing information on the enzyme presence in neutrophils have been publisched, while there is no information on the enzyme functions performed in neutrophils avalaible [1]. In other cells of the immune system, including but not limited to NK cells, granzymes play a major role in cell death leading to changes in the cell nucleus and chromatin decondensation that results in cell lysis.

Methods
In this study we used various techniques to demonstrate the function of GrA in cell death of neutrophils including. Western Blotting was used to show the cleavage of various proteins, while activity-based probes visualised the active form of the enzyme. Flow cytometry was performed to quantify the changes in cell death. Confocal microscopy was applied for classical slide preparation as well as live-imaging of cells.

Results
In the research we have performed preliminary experiments to show that granzyme A may participate in apoptosis of neutrophils through the TNF-alpha induced pathway, however not in NETosis and necrosis. More experiments need to be performed to varify other pathways of the different types of cell death.

Conclusions
The project provide new insights into GrA biology in neutrophils and is highly innovative. The use of state-of-the-art technologies such as confocal microscopy, flow cytometry, and the use of selective chemical markers significantly increases the chances of achieving the proposed goals. In the future, the study of this enzyme may contribute to better and faster medical diagnosis in cancer and other diseases, improving the probability of patients survival and quality of their life.

ACKNOWLEDGMENTS

The project is financed by the grant by the grant Opus, National Science Center (NCN) entitled "Study of the role of granzyme A in neutrophils with the use of pluripotent stem cells" (2020/39/B/NZ1/03027).

PP-59: Improvement of catalyst activity in hydroisomerization of n-hexadecane towards multibranched hydrocarbons.

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Keywords: catalyst, hydroisomerization, hexadecane, multi-branched hydrocarbons

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Objective

In recent years, the need to produce low-emission renewable fuels isomerization due to linear alkanes has attracted much interest in the chemical industry. It's known that hydroisomerization of n-alkanes can significantly increase the octane number of gasoline, it can also positively affect the flow properties at low temperatures of diesel fuel, and lower the viscosity and solidification point of the fuel. Available knowledge suggests that most active catalysts used for hydroisomerization have bifunctional active centers responsible for the function of hydrogenation-dehydrogenation (metallic active sites) and rearrangement of the carbon skeleton (zeolitic support).

These challenges have led to conduct studies, in which the effect of the amount of platinum deposited on the Al_2O_3 + SAPO-11 carrier on the formation of mono and multi-branched hydrocarbons was examined.

Methods

The active phase of the catalyst was applied on the extruded Al_2O_3+ SAPO-11 carrier by the first moisture impregnation method. The size and pore volume of the catalysts were determined using the Micromeritics ASAP 2020 analyzer. The activity of the catalysts was determined by the hydroisomerization of n-hexadecane in a high-pressure trickle-bed reactor. The selectivity and yield of mono- and multi-branched hydrocarbons were determined using GC-MS analysis.

Results

The influence of different amounts of platinum (0%, 0,2%, 0,5%, 1%) on reaction selectivity was compared. Catalyst have a specific surface area of 190–240 g/m2 and a pore volume of 0.365-0.396 cm^3/g. The highest conversion of n-hexadecane (92% wt.) was obtained for 0.2% Pt, with a high proportion of multi- to mono-branched hydrocarbons.

Conclusions

The synergy between metal and acid active sites as well as the overall availability of active sites results in obtaining a satisfactory ratio of multi-branched hydrocarbons.

ACKNOWLEDGMENTS

This research was co-financed by The National Centre for Research and Development of Poland under Grant No. LIDER/33/0171/L-12/20/NCBR/2021.

PP-60: Determination of the betalain profile in selected species of *Amaranthus*

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Keywords: betacyanins, betalains, extraction, chromatography, spectroscopy

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Objective

Plants *Amaranthus hybridus* L. and *Amaranthus lividu*s L. are native species from North America containing betalains in their flowers known for their antioxidant and anti-inflammatory properties. The conducted research aimed to determine the betalain profiles in the studied plants which can be utilized in the pharmaceutical and food industries.

Methods

The basis of the study was the preparation of extracts from flowers of *A. hybridus and A. lividus* plants. To separate the components of the extract, column chromatography with a bed of ODS (octadecyl modified silica) was used. The purified compounds were analysed by LC-DAD-MS.

Results

The results of the analysis indicated the presence of several betalain group in the both plants studied. In the case of *A. hybridus*, the highest concentrations of amaranthin and its isoform were found, while the lowest was that of 17-decarboxy-amaranthine. In the profile of *A. lividus*, the concentrations of amaranthin and 2-decarboxy-amaranthin and their isoforms dominated over other pigments, particularly betanin, which occurred in the smallest amounts. The intensities of the analysed pigment ion signals were significantly higher in *A. hybridus* than in *A. lividus* samples.

Conclusions

The results of this research have potential practical uses in the manufacturing of natural colorants for food products or dietary supplements that exhibit antioxidant and anti-inflammatory properties.

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PP-61: *In silico* analysis of enolase liquid-liquid phase separation

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Keywords: liquid-liquid phase separation, sequence-based predictors, enolase, *Klebsiella pneumoniae*

Objective

Enolases, are highly conserved enzymes associated with catalytic conversion of 2-phosphoglyceric acid to phosphoenolpyruvic acid in the glycolytic pathway. However, in addition to the main function, human enolases perform many additional cell-type and development specific functions and can be linked to a wide spectrum of diseases. Some of the enolases, including human and *Klebsiella pneumoniae* enolase, can exist in a cell surface protein form. In this form, enolase can interact with plasminogen and activate it. In the case of *K. Pneumoniae*, activated plasminogen degrades the extracellular matrix, promoting bacterial infection [1, 2]. Liquid-liquid phase separation (LLPS) is a process in which proteins spontaneously condense, creating a separated phase. Such separation is made possible by intrinsically disordered regions (IDRs) of proteins [3].

Methods

Homo sapiens α-enolase (UniProt: P06733), β-enolase (UniProt: P13929), γ-enoalse (UniProt: P09104) and *K. pneumoniae* enolase (UniProt: A6TD53) enolase sequences alignment was performed with ClustalX, while for LLPS propensity prediction PSPer, PSPredictor, PScore, DeePhase and ParSe v2 were used.

Results

In the case of human enolases no predictor indicated propensity to LLPS. In contrast, *K. pneumoniae* results were not consistent. PSPredictor indicated that *K. pneumonia* enolase can undergo phase separation event, while PSPer and PScore excluded such possibility of phase separation. The probability of *K. pneumoniae* to undergoing LLPS was supported by DeePhase and ParSe v2, indicating the regions prone to undergo phase separation.

Conclusions

We conclude that human and *K. pneumoniae* enolases differ in the probability to undergo LLPS. We presume that *K. Pneumoniae* enolase ability to undergo LLPS could determine the specific way of interaction with plasminogen resulting in the latter degradation. Experimental verification of obtained results is in progress.

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PP-62: Influence of Plasticizer on the Physical and Antimicrobial Properties of Sodium Alginate Films

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Keywords: sodium alginate, polysaccharide, plasticizer, antibacterial, active film

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Objective

With the growing demand for sustainable and environmentally friendly antimicrobial packaging materials, biopolymers have recently become a focus of research [1]. Biodegradable biopolymers, especially polysaccharides, typically lack mechanical properties, making them unavoidable to be blended with plasticizers [2]. The aim of this work was to investigate the plasticization efficiency of alternative bio-based plasticizers incorporated into sodium alginate films.

Methods

The films were prepared by casting, and sodium alginate was cross-linked with calcium chloride. Six different plasticizers were used to compare their effects on film properties. The mechanical (i.e., tensile strength and elongation at break), water barrier (i.e., water vapor transmission rate, contact angle), and antibacterial properties (i.e., against *Escherichia coli* ATCC25922, *Staphylococcus epidermidis* ATCC12228, and *Candida albicans* ATCC18804) were determined. The morphology of the sodium alginate films was examined by scanning electron microscopy.

Results

The results showed that the sodium alginate films prepared with the plasticizers proposed in this study showed better mechanical and antimicrobial properties than the films obtained with commercially available plasticizers. All the obtained films exhibit a high barrier effect to water vapor.

Conclusions

As the sodium alginate-based films plasticized with the synthesized plasticizers showed better elongation at break and significantly better antibacterial properties compared to glycerol, further work is planned with these plasticizers to improve the mechanical properties of the films even more.

ACKNOWLEDGMENTS

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PP-63: New photocatalytic systems dedicated to the fabrication of TiO_2 nanocomposites

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Keywords: photopolymerization, photocurable resins, nanocomposites, DLP 3D printing, initiating systems

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Objective
Photopolymerization processes are widely used in many industries including biomedical engineering, automotive and dentistry. These processes are currently rapidly expanding with technologies related to forming 3D models using light-initiated 3D printing. Photopolymerization is also frequently used in the polygraphic industry to obtain photo-cure UV varnishes and inks. In this paper, the suitability of 9 compounds from the anthracene group varied in terms of the type of attached substituent to act as photosensitizers of iodonium salts, was investigated. The research studied the effects of different nanoparticles on the kinetic parameters of photo-curable resins, as well as on 3D printing parameters (such as critical energy).

Methods
Fourier transform infrared spectroscopy was applied to study the kinetics of the photopolymerization process. NICOLET™ iS™ 10 spectrometer from Thermo Fisher Scientific, which was equipped with a horizontal attachment, was used to study the kinetics of the photopolymerization process. As the light source was used Vis Led diode λ_{max} = 405 nm (M405L3 Thorlabs; light intensity on the sample surface: 26.50 mW/cm^2). The distance from the end of the optical fibre to the surface of the sample was 2.1 cm. Anycubic Photon Mono X printer (Anycubic, China) with an averaged light output of 15.63 mW/cm^2 was employed to obtain nanocomposite prints by DLP (Digital Light Processing).

Results
The research started by checking the spectroscopic properties of the tested anthracene derivatives. The conducted tests showed that the investigated compounds absorb radiation reaching the visible range up to 450 nm. Next, the photosensitizing properties of the anthracene derivatives were checked. The possibility of initiating the photopolymerization of: cationic epoxy monomer CADE, radical acrylic monomer TMPTA and hybrid photopolymerization by means of two-component photoinitiating systems was verified. The next stage of the conducted research was to examine the effect of nanoparticles on the kinetics of the photopolymerization process, on the parameters of 3D printing, as well as the possibility of obtaining photo-curable polymer composites. It was found that the amount of each nano-additive needs to be optimized. The selection of the right amount of nano-additive, resin composition, as well as 3D printing parameters is essential for the correct printing process of nanocomposites, as well as for obtaining a print with high quality and very good resolution.

Conclusions
The examined initiator systems showed versatile performance, and can be successfully applied as photoinitiators for radical, cationic and hybrid photopolymerization. The innovative application of the new high-performance initiator systems is their usage for obtaining photo-curable nanocomposites from radical resins, as well as hybrid resins, which has been confirmed by kinetic studies, as well as DLP 3D printing experiments using low-cost equipment.

ACKNOWLEDGMENTS

The present work was funded by the OPUS LAP project contract number 2020/39/I/ST5/03556 "Advanced photopolymerized nanocomposite materials processed by additive manufacturing".

PP-64: Novel initiating systems enriched with carbon dots for 3D printing applications

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Keywords: carbon dots, light, photoinitiating systems, photopolymerization

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Objective
Carbon dots currently represent a rising star among nanomaterials. Many researchers are undertaking the synthesis of these materials and new applications for carbon dots are being sought. In this research work, various types of carbon dots were synthesized using citric acid as a precursor. In addition, a procedure was used that effectively purified the synthesized materials. The next step was to study the effect of the synthesized carbon dots on the radical photopolymerization processes of the acrylic monomer. The use of carbon dots (CDs) as components of initiating systems for hydrogel production was also investigated.

Methods
A real-time FTIR method was used to determine the performance of the developed photoinitiating systems, which allows for the determination of monomer conversion rates during the photopolymerization process. The DLP (Digital Light Processing) method was used to obtain polymer hydrogels. DLP is a 3D printing technology based on the curing of photosensitive materials (photopolymers) using projector light.

Results
In the radical photopolymerization studies carried out using the synthesised carbon dots, their effective performance as photosensitisers of iodonium salts was demonstrated.

The work also revealed the application of the developed initiating systems based on new types of carbon dots in 3D printing.

Conclusions
The present study confirms the feasibility of using carbon dots in systems that initiate radical photopolymerization processes of acrylate monomers. In addition, 3D printing experiments using formulations consisting of initiator systems with carbon dots have been successfully carried out. This has undoubtedly created new opportunities in the application of carbon nanomaterials. Systems based on carbon dots doped with heteroatoms also have great potential for biomedical applications, including the fabrication of hydrogel materials both in situ and through 3D printing using safe light sources in the visible range.

These discoveries will help researchers to move forward and uncover the latent potential of CDs in the development of new photoinitiating systems and in other areas of photochemistry, including interdisciplinary research into 3D printing, which is one of the key pillars of Industry 4.0.

ACKNOWLEDGMENTS

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PP-65: Optimization of regioselective oxidation reaction investigated for the obtaining of *n*-alkyl-aminated cellulose nanostructures with controlled hydrophobicity

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Keywords: DOE, sodium metaperiodate, amphiphilic nanostructures, contact angle

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Objective

During the reaction of cellulose fibers with sodium periodate, highly selective oxidation of hydroxyl groups occurs at the C2 and C3 carbon atoms in the anhydroglucose unit of the biopolymer, resulting in the formation of a pair of aldehyde groups while simultaneously breaking the carbon-carbon bond between these atoms. The obtained dialdehyde cellulose, produced in this manner, exhibits a wide range of technological applications and, importantly from an engineering standpoint, can undergo further modifications towards the development of functional materials.

This study aimed to optimize the reaction by employing the Design of Experiments (DOE) approach. Understanding the reaction model will enable a more controlled and efficient execution of the amination process. Reductive amination offers the possibility to obtain materials with targeted hydrophobicity.

Methods

Commercial Arbocel bleached cellulose was utilized as the raw material, and levels of independent variables were established to optimize the reaction conditions. The conditions differed from conventional reaction methods in that they employed higher temperatures (65–85°C) and shorter reaction times (1–3 hours), while the third independent variable was the molar ratio of sodium periodate to cellulose fibers. The system response was evaluated based on the reaction yield (as a percentage) and aldehyde content (in mmol/g of fiber). The experimental data was utilized to construct a model using the Response Surface Method (RSM), which was subsequently tested for statistical significance using Analysis of Variance (ANOVA). The next step involved generating two optimization scenarios with different objective functions. The first scenario aimed to minimize energy consumption by minimizing the temperature and reaction time. The second scenario aimed to maximize the reaction yield and aldehyde group content.

Results

The optimum levels of reaction parameters, calculated to achieve the objectives of each scenario, were verified experimentally. The resulting fibers were then subjected to reductive amination (using *n*-ethylamine, *n*-butylamine, and *n*-hexylamine) to produce materials with controlled hydrophobicity. The water contact angles of the films made from the resulting functionalized nanofibers were measured to be 68, 78, and 115 degrees, respectively.

Conclusions

By understanding the reaction model, it is possible to control the degree of oxidation (aldehyde content) in cellulose fibers by adjusting independent variables such as temperature, time, and the ratio of oxidant to cellulose fibers. Optimization of this process can minimize the consumption of chemical raw materials and energy.

PP-66: Effects of cold plasma treatment on seed germination of *Sinapis alba*

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Keywords: cold plasma, germination, dielectric barrier discharge, *Sinapis alba*

Objective
Sinapis alba (mustard seed) is a plant commonly used as the vegetetive cover to reduce the rate of soil erosion [1]. The purpose of the study was to evaluate the effect of cold plasma treatment of *S. alba* seeds for germination enhancement.

Methods
In this study the reactor used for cold plasma treatment was a Dielectric Barrier Discharge (DBD) type. Medium in which the seeds were treated was air. The treatment of *S. alba* seeds was conducted using constant discharge power of 4 Watts. Energy dosage delivered to seed was regulated using varying treatment times. Seeds were planted in a seeding tray and collected after fourteen days. The effectiveness of treatment was determined using germination rate and seedling dry weight. The germination rate was tested as a ratio between germinated seed and number of total seeds. Dry weight of seedlings was measured using laboratory scale after a week of air drying.

Results
Seedlings germinated from seeds after plasma treatment had improved germination rate and higher dry weight. The enhancement rate was directly proportional with the treatment time of the seeds.

Conclusions
The plasma treatment of *S. alba* seed has shown positive impact on germination parameters. The highest rate of improvement was observed for the longest treatment time. That suggests possibility of increasing treatment time for further improvement.

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