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THE VITAL NATURE SIGN

May 15th-16th, 2025

Kaunas, Lithuania

ABSTRACT BOOK

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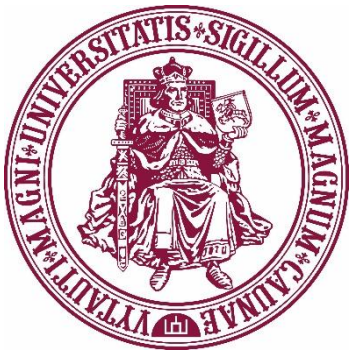

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Editors: Prof. Habil. Dr. Audrius Maruška, Dr. Nicola Tiso, Dr. Vilma Kaškonienė, Dr. Mantas Stankevičius.

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19th International Scientific Conference "The Vital Nature Sign"
Program

Thursday, 15th of May 2025

Microsoft Teams LINK to the conference

9.00-9.15 (Time zone GMT+3)	**Opening of the conference:** ***Rector of Vytautas Magnus University – Prof. Habil Dr. Juozas Augutis*** ***Chairman of the conference – Prof. Habil. Dr. Audrius Maruška***
Oral presentations. ***Chairpersons: Prof. Habil. Dr. Audrius Maruška; Prof. Habil. Dr. Magdalena Ligor***	
9.15-9.30	**Comparative Analysis of Elderberries and Black Chokeberry Extracts Composition by Chromatographic and Spectroscopic Methods** Magdalena Ligor, Kinga Stępkowska, Tomasz Ligor
9.30-9.45	**Exposure of *Spirodela polyrhiza* Fronds to Diclofenac Did Not Alter Biometric and Physiological Parameters While Up Taking the Pharmaceutical Compound** Fabrizio Pietrini, Magdalena Urbaniak, Laura Passatore, Davide Marzi, Massimo Zacchini, Enrica Donati
10.00-10.15	**Induction of Conidiation in *Trichoderma harzianum*** Nicola Tiso
10.15-10.30	**Antioxidant, Antibiofilm and Bactericidal Properties of Six Polyphenol-Rich Mediterranean Wild Edible Plants** Enrica Donati, Valentina Ramundi, Isabella Nicoletti, Laura Righetti, Sara Cimini, Laura De Gara, Francesca Mariani
10.30-11.00	***Coffee break***
11.00-11.15	**Modeling of Wheat Bread Technologies with Buckwheat Flour Sourdough** Jurinta Voisvilo, Ammar Y. Jabr, Elena Bartkiene, Vita Lele
11.15-11.30	**Physicochemical Analysis of Rice Fermented with *Aspergillus oryzae*** Deimantė Karasiovaitė, Kristina Milčiuvienė, Ernesta Trečiokienė, Jurgita Mikašauskaitė-Tiso
11.30-11.45	**The Impact of Spontaneous Sourdoughs on the Quality Characteristics and Acrylamide Formation in Wheat Bread** Skaiste Urbanaviciute, Emilija Vandyte, Ema Rozaityte, Lukas Mikoliunas, Povilas Verpecinskas, Roneta Venckute, Vytaute Starkute, Dovile Klupsaite, Ernestas Mockus, Erika Mozuriene, Darius Cernauskas, Egle Kamarauskiene, Elena Bartkiene
11.45-12.00	**Spectrometric and HPLC UV-VIS Analysis of Medicinal Plant Extracts: Antioxidant Activity and *in Vitro* Screening for Anticancer Activity** Domantas Armonavičius, Audrius Maruška, Baltramiejus Jakštys, Mantas Stankevičius, Tomas Drevinskas, Kristina Bimbiraitė-Survilienė, Modesta Čaplikaitė, Hirotaka Ihara, Makoto Takafuji, Elżbieta Skrzydlewska, Ona Ragažinskienė, Yutaka Kuwahara, Shoji Nagaoka, Vilma Kaškonienė, Saulius Šatkauskas, Arvydas Kanopka
12.00-12.15	**Sustainable Recycling of Automotive Hydrogen Fuel Cells** Matas Bašinskas, Benas Gabrielis Urbonavičius
12.15-12.30	**Ultrafast TADF In Carbazole-Triazine Derivatives for High-Efficiency OLEDs Through Rapid Triplet Harvesting** Melika Ghasemi, Joseph Cameron, Woon Kai Lin, Dmytro Volyniuk, Peter J. Skabara, Juozas V. Gražulevičius, Gjergji Sini
12.30-13.00	***Lunch break***

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Flash presentations I. ***Chairpersons: Assoc. Prof. Dr. Vilma Kaškonienė; Dr. Rūta Mickienė***
(time zone GMT+3)

13.00	**Green Solvent-Based Extraction of Phenolic Compounds from Rose Hips: Exploring γ-Valerolactone, Cyrene, and Ethyl Lactate–Ethanol Mixtures** Hanna Kaczkowska, Marharyta Pestriakova, Aneta Krakowska-Sieprawska, Paweł Fijałkowski, Katarzyna Rafińska, Zbigniew Rafiński
13.07	**Study of Biologically Active Compounds of Frozen and Dried Chamomile (*Matricaria recutita* L.)** Goda Gustevičiūtė, Kristina Bimbiraitė-Survilienė
13.14	**Comparison of the Phytochemical Composition and Biological Activity of Bee Pollen and Flower Pollen** Yelyzaveta Lopatina, Vilma Kaškonienė
13.21	**Protozoan-Based Sustainable Biofilter for Heavy Metal Remediation** Mindaugas Matijūnas, Remigijus Ivanauskas
13.28	**The Content of Phenolic Compounds in Extracts from Different Color Carrot (*Daucus carota* L.) Root** Uladzislau Stuharau, Marina Sidorenko, Saulius Mickevicius, Dalia Urbonaviciene
13.35	**Determination of Qualitative and Quantitative Composition of Phenolic Compounds in Chaga (*Inonotus obliquus* (Ach. ex Pers.) Pilát) Samples** Ernesta Zaksaitė, Mindaugas Liaudanskas, Vaidotas Žvikas, Sonata Trumbeckaitė
13.42	**The Effect of Foliar Biostimulants on Drought Tolerance of Winter Wheat** Irena Januškaitienė, Ugnė Diliūnaitė
13.49	**Effects of Polyethylene Terephthalate (PET) Plastics on Seed Germination in Four Crop Plants: A Short-Term Germination Assay** Irena Januškaitienė, Sanmugavel Saravana
13.56	**Production of Ligninolytic Enzymes by Basidiomycetes Exposed to Thermal Stress** Marta Fernández Camacho, Nicola Tiso
14.03	**Sporulation and Production of Secondary Metabolites in *T. harzianum*** Monika Juselytė, Nicola Tiso
14.10-14.25	***Coffee break***

Flash presentations II. ***Chairpersons: Dr. Mantas Stankevičius; Dr. Nicola Tiso***

14.25	**Additive Manufacturing of PMMA: Advances and Multidisciplinary Applications** Maria R. Rojas, Benas G. Urbonavičius
14.32	**Development of a Microbial Biofuel Cell Using Biocompatible Nanocomposites** Beatričė Kulikauskaitė, Birutė Sapijanskaitė-Banevič, Antanas Zinovičius, Tomas Mockaitis, Arūnas Ramanavičius
14.39	**Derivatives of 2,7-Dibromo-9,9-Dimethyl-9H-Fluorene and 2,7-Dibromo-9,9-Spirobifluorene as Electroactive Materials** Rashad Naghizade, A. Kalatozishvili, R. Keruckiene, J. V. Grazulevicius
14.46	**Imidazole And Fluorene Derivatives as Highly Efficient Fluorescent Light Sources: Synthesis and Investigation** Mohamed Abdella, Guzauskas Matas, Jurate Simokaitiene, Juozas Vidas Grazulevicius
14.53	**Multifunctional Carbazole-Dibenzofuran Derivatives as Emitters and Exciplex-Forming Co-Hosts for Organic LEDs** Dovydas Blazevicius, Raminta Beresneviciute, Saulius Grigalevicius, Zih-Ru Heb, Fong-Yu Yangb, Chih-Hao Chang
15.00	**Carbazole-Based Near-Infrared Absorbing Organic Photodetectors: Design, Synthesis and Characterization** Khushdeep Kaur, Rita Butkutė, Oleksandr Bezvikonnyi, Dmytro Volyniuk, Juozas V. Gražulevičius

15.07 (time zone GMT+3)	**A Shift in Lithuanian Rivers Water Quality During the Past Two Decades** Giedrė Kacienė
15.14	**Isolation and Purification of Enzyme Lysozyme from Chicken Egg White, Evaluation of Antibacterial and Antioxidant Effects** Deividas Grigaliūnas, Mantas Stankevičius, Audrius Maruška, Arvydas Kanopka, Inga Pečiulienė
15.22	**Waste Food Polymers and Microbial Biocontrol Agents as Promising Enhancers of Growth, Development and Quality of Oregano (*Origanum vulgare* L.)** Liliana Kozłowska, Justyna Nawrocka, Kamila Kulbat-Warycha, Urszula Świercz-Pietrasiak, Dorota Żyżelewicz
15.30	**An Overview of Plant-Derived Bioactive Compounds Inhibiting Oral Pathogens: Potential for Natural Oral Care Solutions** Kseniya Hetman, Rūta Mickienė, Gintaras Janužis, Kornelijus Micka
15.37	**Total Peroxidase Enzymatic Activity of *L. edodes*, *I. lacteus* and *P. eryngii* After Heat Shock** Viktorija Sabulytė, Nicola Tiso
15.45	**Closing of the Session**

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Program
Friday, 16th of May 2025

Oral presentations. ***Chairpersons: Prof. Arvydas Kanopka; Prof. Massimo Zacchini***

9.00-9.15 (time zone GMT+3)	**Electroporation-Enhanced Delivery of Bleomycin for Cancer Therapy: An Investigative Study Spanning *In Vitro*, *In Vivo*, and Clinical Trials** Neringa Barauskaitė-Šarkinienė, Greta Chlebopaševienė, Paulius Ruzgys, Saulius Šatkauskas
9.15-9.30	**Utilizing Linear Classifiers for the Multimodal detection of Canine and Feline (Sub) Cutaneous Tumours** Greta Rupšytė, Martynas Maciulevičius, Renaldas Raišutis, Mindaugas Tamošiūnas
9.30-9.45	**Cannabigerol and 3-O-ethyl Ascorbic Acid Combined Effect on Kinase Base Signaling in UVB Irradiated Keratinocytes** Agnieszka Gęgotek, Iwona Jarocka-Karpowicz, Neven Zarkovic, Elżbieta Skrzydlewska
9.45-10.00	**The Role of Caffeine and Other Xanthine Metabolites in the Modulation of Colonic Inflammation** Goda Butaitė, Ugnė Kūlokienė, Rūta Inčiūraitė, Jurgita Skiecevičienė
10.00-10.15	**Assessing Outer Membrane Permeabilization of Gram-Negative Bacteria using Electrochemical Impedance Spectroscopy (EIS): Differentiation from Gram-Positive Bacteria and Evaluation of Pulsed Electric Field (PEF) Treatment Efficacy, With and Without Antibiotics** Simona Gelažunaitė, Mindaugas Visockis, Paulius Ruzgys, Salvijus Vykertas, Saulius Šatkauskas
10.15-10.30	**DNA Methylation Patterns of Behavioural Disorders: A Cross-trait Analysis** Saeed Saado, Yanal Alkuddsi, Marius Aleinikovas, Benas Silinskas, Mohammad Almogdad, Mindaugas Skema, Valda Gudynaite, Manal Almukdad
10.30-11.00	***Coffee break***
11.00-11.15	**The Application of Apple Powder to Enhance Quality in Chicken Meat Products** Akmara Kurmanbekova, Elena Bartkiene, Aigul Tayeva, Erika Mozuriene, Vytaute Starkute, Dovile Klupsaite, Ernestas Mockus, Ernesta Tolpeznikaite, Lina Anskiene, Romas Ruibys, Pranas Viskelis
11.15-11.30	**The Effect of Milk Protein Concentrate and Fermented Grain By-Products on Dough Texture, Physicochemical Properties and Quality of Crackers** Nagoori Khadar Basha, Jūratė Malinauskienė, Loreta Šernienė
11.30-11.45	**Impact of Valorized Milk Protein Production By-Products on the Growth and Health of Neonatal Piglets** Sarunas Badaras, Vytaute Starkute, Ernestas Mockus, Modestas Ruzauskas, Dovile Klupsaite, Erika Mozuriene, Jurgita Dailidaviciene, Agila Dauksiene, Laurynas Vadopalas, Elena Bartkiene
11.45-12.00	**Enhancing Bioactivity in Bee Pollen: An Overview of Treatment Methods** Vilma Kaškonienė, Vaida Damulienė, Paulius Kaškonas, Audrius Maruška
12.00-12.15	**Biological Activity of New Biomaterials** Sylwia Terpilowska, Klaudia Piekarska
12.15-12.30	**Microbiome Profile Alterations During the First Two Months of Live in Very Low-Weight Preterm Newborns** Milena Mirow, Darja Nikitina , Vilma Ivanauskienė, Rokas Lukoševičius , Laura Šauklytė , Vaida Aleksejūnė, Ugnė Krunkaitytė, Jurgita Gailiene, Ilona Aldakauskienė, Aušra Lukošiūtė-Urbonienė, Inga Dekerytė, Jurgita Skieceviciene, Rasa Tamelienė, Juozas Kupcinskas
12.30-13.00	***Lunch break***

Flash presentations I. ***Chairpersons: Domantas Armonavičius, PhD student; MSc. Kristina Bimbiraitė-Survilienė***

13.00	**Development of an Inflammatory Organoid Model of the Large Intestinal and Assessment of Its Response to Janus Kinase Inhibitors** Monika Žilinskaitė, Ugnė Kūlokienė
13.07	**Electroporation-Induced Sensitization: Identifying Optimal Conditions for CHO and 4T1 Cell Viability and Membrane Permeabilization** Silvija Ambrazevičiūtė, Neringa Barauskaitė-Šarkinienė, Paulius Ruzgys, Saulius Šatkauskas

13.14 (time zone GMT+3)	**Cotton Metal Oxide Nanoparticle-Treated Material Action on Microalgae of the *Prototheca* Genus** Agnė Giedraitienė, Rita Šiugždinienė, Modestas Ružauskas, Raminta Rodaitė, Darius Milčius
13.21	**Towards Safe and Effective Biomedical Nanocoatings: Plasma-Sputtered Magnesium Nanoparticles with Cytoprotective and Antimicrobial Properties** Laura Kairytė, Raminta Rodaitė, Agnė Giedraitienė, Modestas Ružauskas, Rita Šiugždinienė, Šarūnas Varnagiris, Darius Milčius
13.28	**Strain-Dependent Antimicrobial Activity of Lactoferrin Against *Escherichia coli*: an Important Factor That Must Be Taken into Account** Kacper Roszak, Oleksandra Pryshchepa, Michalina Ehlert, Dewi Sartika, Agnieszka Ludwiczak, Kamil Szpotkowski, Ewa Olewnik-Kruszkowska, Joanna Rudnicka, Grzegorz Trykowski, Sylwia Wrotek, Paweł Pomastowski
13.35	**Association Between IL1RAP Polymorphism and Pituitary Adenoma in the Lithuanian Population** Augustė Simanonytė, Meda Norkutė, Kristė Kaikarytė, Rasa Liutkevičienė
13.42	**Cell Electrofusion and Viability Assessment after Electroporation** Erikas Stravinskas, Baltramiejus Jakštys, Saulius Šatkauskas
13.49	**Effect of DNA Methyltransferase 1 Inhibitors on Protein Expression in Cells** Arnas Treimakas, Eglė Jakubauskienė
13.56	**Assessment of Plant Origin, Microbial Contaminants, and Pigment Levels in Bee Pollen** Karina Eidintaitė, Yelyzaveta Lopatina, Vilma Kaškonienė, Rūta Mickienė, Audrius Maruška
14.00-14.15	***Coffee break***
14.15	**Genetic Diversity of Lithuanian Populations of *Impatiens noli-tangere* L.** Rūta Budrevičiūtė, Lina Jocienė, Eugenija Kupčinskienė
14.22	**Phytochemical Analysis and Evaluation of Antimicrobial Activity of *Aegopodium podagraria* L. Aerial Parts** Ugnė Gabrytė, Kseniya Hetman, Rūta Mickienė, Audrius Sigitas Maruška
14.29	**Prevalence of Micromycetes in Strawberries in Relation to Cultivar and Ripening Stage** Jūratė Staveckienė, Rūta Mickienė, Audrius Sigitas Maruška
14.36	**Some Aspects of Genetic Diversity of Lithuanian Populations of *Impatiens parviflora* DC.** Vaida Jasionytė, Lina Jocienė, Eugenija Kupčinskienė
14.43	**Survival of Tardigrades in Low Temperature Conditions** Gintarė Antanavičiūtė, Ingrida Šatkauskienė
14.50	**Spectrophotometric Analysis of *Canabis sativa* L. Extracts and Evaluation of Their Anticancer Activity in vitro** Simona Drackytė, Audrius Sigitas Maruška, Juozas Šiurkus, Domantas Armonavičius, Arvydas Kanopka
14.57	**Perceptions of Ecosystem Services: Evidence from Communities in Lithuania** Bernadeta Laurinaitytė, Gintarė Sujetovienė
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ORAL PRESENTATIONS

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DNA Methylation Patterns of Behavioural Disorders: A Cross-trait Analysis

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Abstract

In the field of epigenetics, DNA methylation attracts the most attention. The analysis of differentially methylated regions (DMRs) is commonly performed using R Bioconductor packages where the analysis pipeline primarily involves preprocessing raw data into Beta values or M values. However, the use of different normalization algorithms can introduce inconsistencies in the identification of DMRs associated with certain disorders. This issue may not be critical when analyzing DMRs of disorders that exhibit global methylation changes (e.g., cancer). On the other hand, disorders with small changes in methylation (e.g., behavioral disorders) are more prone to such inconsistencies. To address this, we re-analyzed a set of experiments that are publicly available on GEO and ArrayExpress using a recent approach shown to achieve higher correlation among replicates compared to traditional methods. The study aimed to explore potential DMRs shared among two or more neurobehavioral disorders. Four experiments comprising nine different phenotypes were selected, and each phenotype was re-analyzed against its respective control group. The preprocessing and differential analysis pipeline was standardized across all nine phenotypes. Finally, a cross-trait analysis was conducted to identify shared DMRs among the nine phenotypes. As expected, the results did not reveal DMRs spanning large genomic distances. Instead, differentiation was confined to a 50 bp region of DNA (the length of a single probe). These findings confirm the absence of global methylation changes in neurobehavioral disorders. However, the cross-trait analysis uncovered shared characteristics among certain phenotypes. Notably, phenotypes such as developmental language disorder and Alzheimer's disease exhibited differentially methylated probes mapped to overlapping gene regions.

Key words: DNA methylation, epigenetics, environmental factors, psychiatric disorders, behavioural disorders.

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Impact of Valorized Milk Protein Production By-Products on the Growth and Health of Neonatal Piglets

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Abstract

Newborn animals are exposed to a variety of microorganisms, including opportunistic pathogens, shortly after birth. This study evaluated the effects of valorized milk protein production by-products (VMP) supplemented with *Pediococcus pentosaceus* (VMPPp) and *P. acidilactici* (VMPPa) on growth, plasma parameters, fecal microbiota, metataxonomic profiles, and other characteristics of feces, including volatile compounds (VC), in neonatal piglets. Thirty-six newborn piglets were assigned to three groups: (A) control, (B) VMPPp group, and (C) VMPPa group. Groups B and C received twenty-five ml of VMP daily, in addition to a traditional commercial diet. After 25 days of the trial, VMPPa piglets showed the highest body weight gain. Also VMPPp piglets had the highest immunoglobulin IgM concentration in blood plasma. Both groups B and C showed higher lactic acid bacteria counts in their feces. Despite the increased numbers of lactic acid bacteria and *Enterobacteria* in the feces, correlation between these microorganisms numbers with the piglets' growth performance were not established. The feces of VMPPa piglets showed a broader variety of VC, including higher domination of butyric acid and indole. The latter VC may be associated with differences in their metataxonomic composition. The VMPPp group displayed a greater diversity of bacterial species compared to both the A and VMPPa groups. At the end of the experiment, the VMPPa group contained the highest numbers of *Terrisporobacter* sp., *Holdemanella* sp., *Lachnospiraceae* sp., and *Parabacteroides* sp. In conclusion, VMPPa enhanced piglets body weight gain and promoted the proliferation of certain bacterial species in piglets feces, along with a unique VC profile in their feces. These findings suggest the need for further research into the underlying metabolic pathways.

Keywords: milk permeate, microbiota, animal health, volatile compounds.

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Sustainable Recycling of Automotive Hydrogen Fuel Cells

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Abstract

When hydrogen fuel cell electric vehicles were first introduced in 2013 [1], they were received with due scepticism and optimism. Despite the undeniable innovation, they came with several glaring issues that discouraged all but the most devout pioneers of sustainable automotive transport. Severe lack of refuelling infrastructure meant that the product could only be offered on select few markets, yet regardless of incentives and reduced prices, their popularity, however miniscule it initially was, had rapidly dwindled, and whatever remained of the relevant infrastructure quickly followed. This has left a relatively significant number of fuel cell electric vehicles (FCEV) available on the market for a significantly reduced price that not only lack the refuelling infrastructure to be useful, but are also nearing their end of usable life due to the designated lifetime of the high-pressure hydrogen tanks, which are unserviceable due to cost. However, the fuel cells contain a non-insignificant amount of platinum, which is in nanoparticle form. This creates an incentive to commit to sustainable recycling, as well as highlights a need to develop a method to efficiently extract the material in question, while retaining its nanoparticle composition. A review [2] of available resources is offered, as well as a few potential methods of nanoparticle extraction are investigated.

Keywords: hydrogen fuel cell, sustainable transport.

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The Role of Caffeine and Other Xanthine Metabolites in the Modulation of Colonic Inflammation

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Abstract

Inflammatory Bowel Disease (IBD) is a chronic autoimmune inflammatory condition that primarily affects the lower gastrointestinal tract. It is pathologically characterized by disruptions in epithelial integrity, immune system dysregulation, and features of an autoinflammatory syndrome [1]. Caffeine, a methylxanthine compound, is among the most commonly consumed bioactive substances globally, and is found not only in coffee but also in tea, soft drinks, and even some medications [2]. Despite the widespread intake of caffeine and its metabolites, their specific effects on intestinal inflammation and epithelial barrier dynamics are still not completely understood [3]. This study aimed to evaluate the influence of caffeine and its major metabolites – paraxanthine, theobromine, and theophylline – on inflammatory signaling and epithelial integrity in gut epithelial models, with the goal of elucidating their potential implications for gastrointestinal health, especially in relation to Inflammatory Bowel Disease.

A concentration-dependent screening was first performed using Caco-2 cells to determine bioactivity. Metabolites selected from this screening were subsequently evaluated in inflamed 3D intestinal epithelial organoids derived from ulcerative colitis patient (n = 1) and non-IBD control (n = 1). Inflammation was induced over a 24-hour period using a combination of TNF-α and IFN-γ cytokines. To assess the impact on inflammatory activity and epithelial barrier integrity, targeted gene expression analysis was performed using RT-PCR. Additionally, in order to test the antioxidant properties of the coffee [3], the production of reactive oxygen species (ROS) was evaluated through flow cytometry and the DHE assay.

The initial analysis revealed that caffeine (affecting *TNF-α, CXCL9*, and *IL18*; $p < 0.05$) and its metabolite paraxanthine (influencing *TNF-α*; $p < 0.05$) significantly modulated the expression of genes related to inflammatory cytokines. Considering expression of tight junction genes, paraxanthine notably increased *OCLN*, *TJP1*, and *CLDN1* levels ($p < 0.05$), while caffeine – only *CLDN1* ($p < 0.05$). These findings have led to more detailed investigation using 3D intestinal organoid models. However, in the inflamed organoid model, neither caffeine nor paraxanthine was able to reduce the expression of genes encoding inflammatory cytokines. Nevertheless, both metabolites significantly reinstated the expression of genes encoding tight junction proteins – *OCLN* and *TJP1* ($p < 0.05$). Additionally, caffeine and paraxanthine lowered the production of reactive oxygen species by 25% and 8%, respectively.

Methylxanthine caffeine and its main metabolite paraxanthine may support gut epithelial barrier function by promoting the restoration of tight junction protein gene expression, leading to the modulation of colonic inflammation.

Keywords: caffeine, inflammatory bowel disease, inflammatory cytokines, paraxanthine, reactive oxygen species, tight junctions.

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Antioxidant, Antibiofilm and Bactericidal Properties of Six Polyphenol-Rich Mediterranean Wild Edible Plants

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Abstract

Plant biodiversity constitutes a valuable source of bioactive compounds to address critical global challenges, including antimicrobial resistance that is a major threat to public health. In Mediterranean countries, Wild Edible Plants (WEPs), also known as phytoalimurgic plants, represent an important regional cultural heritage [1]. In the past, they were an important food resource in times of famine due to their high content of micronutrients essential for human health. Their health-promoting effects are due to their ability to produce biologically active secondary metabolites [2]. Among them, polyphenols are becoming increasingly recognized as a source of solutions to today's health challenges, including antimicrobial resistance.

In this regard, the study aims at bioprospecting the chemical and antioxidant profile as well as the antibiofilm and bactericidal properties of six WEPs, widely distributed in Italy and historically used as food, namely: *Silene alba*, *Silene vulgaris*, *Chenopodium album*, *Sonchus oleraceus*, *Glechoma hederacea* and *Diplotaxis erucoides*. To this end, a multidisciplinary approach including analytical chemistry, plant biochemistry and microbiology was employed. This work could be part of WEPs valorisation strategies, which are essential for the conservation of edible plant biodiversity. Indeed, the results obtained could help improve dietary habits and suggest these species as both a healthy component of daily diet and a valuable additional phytotherapeutic tool to counteract the emergence of antibiotic resistance in pathogens.

Keywords: *Silene alba*, *Silene vulgaris*, *Chenopodium album*, *Sonchus oleraceus*, *Glechoma hederacea*, *Diplotaxis erucoides*, AMR, *S. aureus*, wild edible plants.

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Cannabigerol and 3-O-ethyl Ascorbic Acid Combined Effect on Kinase Base Signaling in UVB Irradiated Keratinocytes

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Abstract

UVB radiation is one of the main harmful physical factors reaching epidermal cells, including keratinocytes. To counteract the induced metabolic effects, it was proposed to use the combined action of two, differing in structure and properties, compounds, such as cannabigerol (CBG) and 3-O-ethyl ascorbic acid (EAA). Therefore, this study aimed to investigate the combined effect of CBG and EAA on changes in human keratinocytes induced by UVB radiation, especially in the context of kinase-dependent signalling based on the modifications of protein structure through adducts formation with lipid peroxidation products, including 4-hydroxynonenal (4-HNE). To achieve the aim, basic cells viability tests (MTT and LDH assays) and proteomic analysis (SDS-PAGE/LC-MS/MS) were used.

Obtained proteomic results indicated differences in the proteome of keratinocytes treated with both used compounds (CBG and EAA, separately as well as together) in non-irradiated and UVB-exposed cells. These changes were visible mainly in the kinases profile, including mitogen activated protein kinases (MAP3K7, MAPK14 and ERK½), but also IKK, RIPK2 and NLK, which UVB-induced expression was decreased by CBG and EAA, particularly used together. Moreover, UVB radiation strongly induced the level of proteins modification by 4-HNE, what was indicated for MAP kinases, such as MAPK11, cJun, ERK1, but also for AKT, Camkk1, IKKα, and PERK. However, used phytocannabinoid (CBG) and ascorbic acid derivative (EAA) significantly reduced the level of 4-HNE-kinases adducts and thus inhibited the pro-inflammatory response and autophagy, as well as the pro-apoptotic response in keratinocytes.

In conclusion, it can be suggested that combined cytoprotective effect of CBG and EAA relies on 4-HNE-based signalization, including a reduction in the effect of 4-HNE on kinases in skin cells especially following their exposure to UVB radiation.

Keywords: 3-O-ethyl ascorbic acid, cannabigerol, kinases structure modification, 4-hydroxynonenal, keratinocytes, UVB radiation.

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Ultrafast TADF In Carbazole-Triazine Derivatives for High-Efficiency OLEDs Through Rapid Triplet Harvesting

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Abstract

Since the discovery of organic light emitting didoes (OLEDs) in 1987, molecular design has played a pivotal role in the development of organic emitters used in OLEDs [1]. Flat emitters, having donor and acceptor moieties, exhibiting thermally activated delayed fluorescence (TADF) have emerged as promising candidates for OLED applications due to their efficient reverse intersystem crossing, resulting in effective triplet harvesting [2, 3].

This study reports on the synthesis and characterization of the new derivative of carbazole and triazine exhibiting ultrafast TADF. Various experimental techniques were employed to analyse the photophysical, thermal, photoelectrical, charge-transporting, electro-optical, and electroluminescent properties of the material in solid state. The films of the compound exhibited green TADF with the average lifetime of 101 ns. The compound showed excellent thermal stability with 5% weight loss temperature of 461 °C. It triplet energy was found to be of 2.39 eV. Good hole and electron transporting properties made it suitable as a host material for OLEDs. Orange TADF and red phosphorescent emitters were used as the guests. The fabricated devices exhibited stable electroluminescence spectra across the different voltages. OLEDs containing the orange emitter were characterized by turn-on voltage and external quantum efficiency of 3.49 V and 8.07%, respectively. Meanwhile, the device with the red emitter showed the values of 3.49 V and 26.48%, respectively. The red phosphorescent OLED demonstrated electroluminescence lifetime of 10.7 μs.

Keywords: thermally activated delayed fluorescence, organic light-emitting layer, fast reverse intersystem crossing, tuning, carbazole, triazine.

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Microbiome Profile Alterations During the First Two Months of Live in Very Low-Weight Preterm Newborns

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Abstract

Over the past decades, bacterial microbiome studies have become increasingly important. The development of the gut microbiome has been shown to play an essential role in human health, and to be especially decisive in infants [1]. It influences nutrient absorption, metabolism regulation, and immune system development. Various factors have been proposed to modulate the development of the microbiome and therefore shape the growth and health of newborns [2]. Premature babies are often low-weight and even more sensitive to health issues. This study aims to analyse the development of the microbiome in preterm neonates over the first two months of life and look for patterns and alterations associated with several factors, like delivery mode, gestation time and necrotizing enterocolitis (NEC).

A total of 78 preterm newborns were included in the study, with stool samples being taken at several timepoints from each baby. In total 515 samples were collected. The timepoints varied between ages of one and 61 days and were grouped in six age groups during the analysis. Genomic DNA was extracted from stool samples, and the bacterial V3–V4 hypervariable region of the 16S rRNA gene was amplified and sequenced on the Illumina MiSeq platform. The data were processed using bioinformatical tools and analysed bioinformatically and statistically to access microbiome composition, abundancies, alpha- and beta-diversities.

The analysis revealed significant differences in the bacterial profiles across different timepoints, gestational ages, delivery modes, and in relation to the development of NEC. Alpha diversity indices increased over time; however, fewer or no significant differences were observed between the later timepoints. Consistent patterns emerged from both PCoA and PERMANOVA analyses. Notably, NEC was associated with distinct bacterial. Additionally, the abundance of several bacterial taxa correlated with gestational age.

The bacterial profile of premature, low-birth-weight newborns varies according to factors such as age, delivery mode, gestational age, and the presence of disease. Comparing these microbiome dynamics in low-birth-weight infants with those in full-term newborns could provide valuable insights into the development of neonatal diseases such as necrotizing enterocolitis.

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The Application of Apple Powder to Enhance Quality in Chicken Meat Products

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Abstract

Recently, manufacturers have been developing new technologies to produce natural meat products, the main idea of which is the enrichment of food products with bioactive compounds obtained from agricultural by-products [1-2]. Apple by-products (A_{pl}-BP) improve the functionality, affordability, and nutritional value of animal origin foods.

This study aims to determine the effect of adding different amounts (5–30%) of A_{pl}-BP to minced chicken meat products (ChMP). The focus is on the influence of ChMP on the main quality parameters and changes in the concentrations of various chemical compounds both before and after heat treatment. In addition, the study investigates the sensory acceptability and emotional expressions of consumers.

We investigated that adding 5–15% A_{pl}-BP maintained ChMP quality: the additive reduced cooking losses and improved color parameters (increased lightness, reduced redness and yellowness) after cooking, as well as increased the amount of free essential amino acids, while non-essential free amino acids were significantly affected by both A_{pl}-BP concentration and processing method ($p \leq 0.05$). The concentration of biogenic amines did not exceed toxic levels, and the addition of A_{pl}-BP reduced the malondialdehyde concentration in ChMP. A broader spectrum of volatile compounds and fatty acid profiles was observed in both thermally treated and untreated ChMP samples.

To conclude, the results showed that 15% A_{pl}-BP is suitable for ChMP enrichment as it improves the overall acceptability of the final product and increases the content of free essential amino acids in it.

Keywords: apple by-products, chicken meat, amino acids, volatile compounds.

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The Effect of Milk Protein Concentrate and Fermented Grain By-Products on Dough Texture, Physicochemical Properties and Quality of Crackers

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Abstract

In the modern age, consumers are increasingly focused on healthy eating and are seeking new products, as they prioritize nutritionally enriched foods. Whereas bakery products have good nutritional profile, sensory characteristics, and texture, due to growing consumer demands for convenience foods, such as crackers, they represent one of the fastest growing segments of bakery products. Milk proteins are among the most popular and valuable ingredients, designed to improve the quality of food products, as they have important nutritional, technological and sensory properties that can be exploited in the development of new nutritious products. This study aims to develop cracker recipes with milk powder concentrate and fermented cereal by-products, to evaluate the texture and physicochemical properties of the dough, and to evaluate the sensory analysis of consumer acceptance and quality of protein-enriched crackers.

Crackers made from fermented and non-fermented composite flours containing oat milk by-products, milk protein concentrate powder, and egg whites or flaxseed gel as a binder. The dough and cracker samples were assessed for physiochemical properties (color coordinates, moisture, pH, total titratable acidity, texture) and sensory properties (overall acceptability, appearance, taste, flavor, smell, crispiness).

Overall, the results showed that fermentation and binder type significantly affected dough properties such as color coordinates, texture, moisture and acidity. Texture profiling showed that fermentation has effect on hardness and elasticity of dough, with egg-based crackers, fermented and non-fermented which exhibiting softer textures than their flaxseed-based counterparts. Total titratable acidity increased in fermented samples, suggesting improved shelf-life stability. Sensory evaluation revealed that both fermented crackers made with flaxseed gel and egg white were the most acceptable, particularly due to their taste and texture, making them a promising, protein-rich alternative. The results indicate that functional crackers with enhanced protein profiles can be successfully developed using composite flours.

Keywords: protein-rich crackers, milk protein concentrate, fermented grain by-products, dough.

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Enhancing Bioactivity in Bee Pollen: An Overview of Treatment Methods

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Abstract

Bee pollen is primarily a food source for bees, serving as a vital component in their nutrition due to its rich content of proteins, lipids, vitamins, and minerals. However, it is also can be used for humans due to its diverse array of bioactive compounds, including antioxidants, phenolic compounds, and essential nutrients that contribute to its potential as a functional food and/or natural supplement. However, its biological value is limited by the strong outer wall called the exine, composed of chemically resistant sporopollenin. This structure protects the pollen's contents but also significantly reduces digestibility and bioavailability in humans. According to the literature only 10-15% of bee pollen nutrients are typically absorbed [1]. Compared to bee bread, which undergoes natural fermentation in the beehive, additional treatment of bee pollen would be recommended to enhance its nutritional and therapeutic properties.

Biotechnological and physical processing methods have been explored in different studies [2-4]. It is believable that both bacterial fermentation and enzymatic hydrolysis weaken the exine, improving nutrient release from the pollen cell. Some physical methods such as ultrasonication, ball-milling, and high-pressure processing would possibly mechanically break down the pollen wall by enhancing bioavailability of bee pollen valuable compounds [5].

Different treatments may also enhance the pharmacological potential of bee pollen. Treated bee pollen exhibits stronger antioxidant, anti-inflammatory, and antibacterial properties [6]. Fermented or hydrolysed pollen even shown synergistic effect when used with antibiotics, suggesting broader applications in health and medicine [7].

Therefore, further research into bee pollen processing methods is encouraged; however, optimizing each process is crucial, as the effectiveness of the positive changes largely depends on the botanical origin of the pollen.

Keywords: bee pollen, enzymatic hydrolysis, fermentation, physical treatment.

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Utilizing Linear Classifiers for the Multimodal detection of Canine and Feline (Sub-)Cutaneous Tumours

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Abstract

Accurate diagnosis of canine and feline (sub-)cutaneous tumours remains a clinical challenge, mainly due to the limitations of conventional imaging techniques. Researchers suggest that ultrasound (US) imaging, although offering valuable real-time findings, usually has limited resolution. In contrast, white light (WL) imaging allows high-resolution imaging of cellular and tissue morphology, but penetrates only to a limited depth [1–2]. Fluorescence (FL) imaging enhances contrast at the level of the extracellular matrix, but similarly remains constrained to superficial assessments [3]. Studies have shown that reliance on a single imaging modality often results in ineffective classification performance [4]. Consequently, multimodal strategies have been proposed to combine complementary strengths of different imaging techniques and overcome individual weaknesses [5–6].

In this study, we propose an integrated diagnostic framework that leverages the multimodal potential of US, WL, and FL imaging for the classification of canine and feline subcutaneous mast cell tumours (MCT) and soft tissue sarcomas (STS) tumours. High-frequency (~350 MHz) ultrasound was used to capture deeper tissue properties, WL imaging provided surface-level morphological features, and FL imaging offered stromal differentiation. Quantitative analysis was performed to extract textural and morphological features. Feature sets were supplied to ML classifiers: Support Vector Machine (SVM) and Random Forest (RF) - pursued with sequential feature selection, to improve classification robustness and reduce overfitting.

Results showed that combined imaging modes consistently outperformed single-mode diagnostics The US+FL combination achieved 75–80% classification efficiency, US+WL reached 85–90%, and the combination of US, WL, and FL (OPT) delivered the highest accuracy, ranging from ~90–95%, while the single mode classification efficiency ranged between 65-85% in differentiating MCT and STS. These improvements were statistically significant ($p<0.05$) across different training/testing splits through 10-fold cross-validation.

In conclusion, this multimodal framework demonstrates strong potential as a decision-support tool in veterinary oncology. By improving classification accuracy in distinguishing MCT and STS from benign lipomas, it aids pathologists in real-time diagnosis and may help reduce misdiagnosis rates.

Keywords: machine learning, multimodal, diagnostics, cancer, imaging, veterinary.

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The Impact of Spontaneous Sourdoughs on the Quality Characteristics and Acrylamide Formation in Wheat Bread

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Abstract

This study evaluated the impact of different types and amounts of spontaneous sourdoughs – rye, rye/wheat blend, and wheat – on the quality parameters, acrylamide formation, and volatile compound profiles (VC) in wheat bread produced without commercial additives. To evaluate their effects on bread characteristics, sourdoughs were added to bread formulations at amounts from 5 to 15%. Bread characteristics varied significantly based on sourdough type. Wheat sourdough had the highest acidity, while rye/wheat blend sourdough the highest lactic acid bacteria (LAB) counts. Both sourdough type and quantity significantly affected bread porosity, and moisture content ($p < 0.001$). Sourdough addition, in all bread formulations, decreased bread shape coefficient and increased acrylamide. Breads with rye/wheat blend and 15% wheat sourdough exhibited the highest acrylamide levels. Significant correlations ($p < 0.001$) were observed between acrylamide and certain VCs. Despite enhanced acrylamide formation from sourdough, particularly wheat or rye/wheat, levels remained within European Commission guidelines. This study showed that spontaneous sourdough fermentation significantly influences the quality parameters and chemical composition of commercial additive-free wheat bread. Although sourdough in wheat bread increases acrylamide formation, it contributes to desirable characteristics such as increased LAB counts. The strong correlations between acrylamide and several VCs highlight the intricate chemical processes involved in sourdough spontaneous fermentation. Therefore, further research is necessary to optimize bread production by using spontaneous sourdoughs, to achieve the desired sensory properties with minimal acrylamide formation, thus ensuring the production of safe and high-quality bread.

Keywords: bread quality, fermentation, spontaneous sourdough, acrylamide.

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Biological Activity of New Biomaterials

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Abstract
Tissue engineering uses innovative methods of producing biomaterials like scaffolds. Three-dimensional (3D) biomaterials are promising options for *in vitro* research on cancer development of various origins. Currently, practice is moving away from two-dimensional (2D) scaffold models in cell cultures, which are too simplified to study the basic pathological mechanisms and lack the structural architecture necessary for cell-cell interactions. The biomaterials can be obtained using many natural and synthetic substances. Promising substances used in biomaterial construction are alginate, obtained from marine algae (micro and macroalgae) products. Alginate has excellent biocompatibility, relatively low cost, and simple gelation behaviour with divalent cations such as Ca^{2+}, Mg^{2+}.
All new biomaterials used as scaffolds have to be non-toxic, not induce oxidative stress and production of proinflammatory cytokines. This study investigated the biocompatibility of new modifications of alginate foams as biomaterials that mimic the structure of the extracellular matrix as potential 3D scaffolds for an *in vitro* cancer model.
The newly synthesized biomaterials were cultivated with BJ fibroblasts. New biomaterials were evaluated with the use of with the use of ISO-10993 standard entitled “Biological evaluation of medical devices”. The cytotoxicity and genotoxicity of the newly synthesized alginate biomaterials were investigated. Moreover, the concentration of reactive oxygen species, antioxidant enzyme production, and IL-1 and IL-6 concentration were investigated.
The study shows that biomaterials were non-toxic, and did not induce reactive oxygen species and IL-1 and IL-6. However, an increase in the activity of antioxidant enzymes was observed. It can suggest that new biomaterials can act as antioxidants.

Keywords: biomaterials, cytotoxicity, genotoxicity, biocompatibility.

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Modeling of Wheat Bread Technologies with Buckwheat Flour Sourdough

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Abstract

The aim of the study was to optimize the technological modeling of wheat-bread with buckwheat (*Fagopyrum esculentum* L.) flour sourdoughs. During the scientific research, two types of buckwheat flour sourdoughs were prepared using starter cultures: (1) *Lactilactobacillus sakei (L. sakei*) and (2) *Lacticaseibacillus paracasei (L. paracasei*). The produced sourdoughs were evaluated based on the following quality parameters: pH, texture, moisture content, total titratable acidity (TTA), color coordinates, sensory properties, and overall acceptability. It is important to note that although buckwheat is nutritionally valuable, its use in wheat-bread is limited due to the natural absence of gluten [1-2]. The most acceptable sourdough was identified as the one fermented with *L. sakei* (Sourdough 1). Using the selected Sourdough 1, six recipes of bread were baked along with one control recipe (without sourdough). The total titratable acidity (TTA) of Sourdough 1 increased to 10.03 ± 0.42°N after 24 hours of fermentation. The moisture content reached 38.53 ± 1.53%, and the texture was 0.9 ± 0.12 mJ. The bread formulations were prepared with varying amounts of sourdough: 5, 10, 15, 20, 25, and 30%. The following bread quality parameters were evaluated: color coordinates, texture, total titratable acidity (TTA), moisture content, specific volume, mass loss after thermal processing, porosity, microbiological spoilage, shape retention coefficient, sensory properties, and overall acceptability. Different sourdough amounts had a significant impact ($p \leq 0.05$) on bread quality parameters. The highest bread porosity (71.5 ± 1.2%) and shape retention coefficient (3.4 ± 0.8%) were observed with 15% sourdough. The highest specific volume (3.84 ± 0.07 cm^3/g) was recorded in the sample with 20% sourdough, while the lowest specific volume and shape retention values were found in the sample with 30% sourdough, at 2.91 ± 0.01 cm^3/g and 2.17 ± 0.12 cm^3/g, respectively. Signs of microbiological spoilage were detected after 72 hours in bread with 5% and 10% sourdough, whereas no spoilage was observed in bread with 15% or more sourdough for up to 96 hours. Based on sensory analysis results, bread with 15% sourdough was the most acceptable (106.3 ± 12.5 mm). Considering the sensory evaluation, quality, and safety assessment results, it is recommended to produce bread with 15% *Fagopyrum esculentum* L. sourdough.

Keywords: wheat bread, buckwheat (*Fagopyrum esculentum* L.), sourdough, lactic acid bacteria, wheat bread quality.

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Exposure of *Spirodela polyrhiza* Fronds to Diclofenac Did Not Alter Biometric and Physiological Parameters While Up Taking the Pharmaceutical Compound

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Abstract

Pharmaceutically active compounds (PhACs) found in ecosystems are a growing health concern. Aquatic environments are particularly vulnerable [1]. Massive use of PhACs leads to wastewater enrichment and surface water contamination due to incomplete removal. The non-steroidal anti-inflammatory drug (NSAID) diclofenac (2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid, DCF) is widely distributed in surface waters with concentrations in the ng/l range. Among the NSAIDs, diclofenac is the most toxic and is the most frequently detected [2]. In this context, adverse effects of DCF on terrestrial, marine and freshwater biota have been reported. The literature on DCF-plant interactions highlighting species-specific toxicity responses is extensive, but few studies focus on duckweed plant responses to DCF. To expand the knowledge on the effects of DCF on a model plant of the aquatic ecosystem, *Spirodela polyrhiza* (L.) Schleid fronds were treated in a 7-day assay with DCF concentrations up to 100 µg/l, analysing standard and non-standard endpoints [3] under laboratory conditions. Results showed that no effect was exerted by DCF, at each concentration tested, on frond growth evaluated by several biometric parameters. Consistently, at physiological level, no toxic action was observed on pigment-associated reflectance indexes and on photosynthetic performances evaluated by chlorophyll fluorescence values and imaging parameters. UHPLC-MS/MS analyses showed that *S. polyrhiza* fronds absorbed the DCF according to its concentration in the medium. To the best of our knowledge, this is the first report highlighting the uptake and the effects of DCF in *S. polyrhiza*, a plant species that has previously been investigated for characterising the plant responses to xenobiotic toxicity [4].

Keywords: aquatic plants, duckweed, pharmaceuticals, photosynthesis, UHPLC-MS/MS.

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Physicochemical Analysis of Rice Fermented with *Aspergillus oryzae*

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Abstract

The filamentous fungus *Aspergillus oryzae* is widely utilized in Asia, particularly in Japan, for the production of traditional fermented foods such as soy sauce, rice wine (sake), and soybean paste (miso). It is considered an edible and safe microorganism, as it does not produce mycotoxins during the fermentation process. *A. oryzae* can be cultivated in both liquid and solid-state media; however, it exhibits higher enzyme production under solid-state fermentation conditions [1].

This fungus is also known for synthesizing bioactive compounds with antioxidant, antimicrobial, and immunomodulatory properties [2]. Despite its high industrial potential, the optimization of fermentation conditions and the efficient extraction of its active metabolites remain underexplored.

In this study, sensorial and physicochemical parameters—including moisture content, color, caloric value, pH, water activity, and texture—as well as microbiological safety, were evaluated in rice fermented with *A. oryzae* over cultivation periods of 2, 5, and 7 days.

The results indicate that the texture, pH, and caloric content of the fermented rice change significantly during fermentation. Microbiological analysis confirmed the safety of the fermented rice for consumption. Sensorial evaluation revealed that samples fermented for 2 days received the highest consumer acceptance. Physicochemical analysis showed a significant decrease in caloric value and pH after 7 days of fermentation. Notably, dry matter content began to change significantly after 48 hours. The colour of the samples shows a significant change after 5 days of fermentation. Water activity remained relatively constant throughout the fermentation period.

Keywords: *Aspergillus oryzae*, solid-state fermentation, rice, texture.

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Spectrometric and HPLC UV-VIS Analysis of Medicinal Plant Extracts: Antioxidant Activity and *In Vitro* Screening for Anticancer Activity

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Abstract

This study systematically evaluates the capacity of selected medicinal plants to biosynthesize secondary metabolites with cytotoxic activity against cancer cell lines. Plant extracts were obtained via solvent-based extraction and subsequently analysed using ultraviolet-visible (UV-VIS) spectrophotometry [1] and high-performance liquid chromatography coupled with ultraviolet-visible detection (HPLC UV-VIS) to quantify phenolic compounds, flavonoids, and identify key antioxidants [2]. The antiproliferative effects of the extracts were assessed using the MTS assay [3] on a panel of cancer cell lines (4T1, A549, Caki-1, HCT116 and MCF7), while potential cytotoxicity towards non-malignant cells was evaluated using the HEK-293 cell line.

Quantitative analysis revealed that *Juglans nigra* L. (black walnut), *Chamaenerion angustifolium* L. (fireweed), and *Quercus robur* L. (pedunculate oak) exhibited the highest concentrations of phenolic compounds and antioxidant constituents. HPLC UV-VIS profiling identified several bioactive compounds, including oenothein B, chlorogenic acid, rutin, ferulic acid and other compounds across different species. Notably, extracts from *Chamaenerion angustifolium* L., *Solidago canadensis* L. (Canadian goldenrod), and *Juglans nigra* L. demonstrated significant antiproliferative activity, as evidenced by low IC50 values across multiple cancer cell lines, with pronounced efficacy against MCF7-WT and HCT116 cells.

These findings underscore the potential of diverse medicinal plant species as sources of bioactive compounds with selective anticancer properties *in vitro*, while exhibiting negligible cytotoxicity towards healthy mammalian cells.

Keywords: antioxidants, phenolic compounds, ellagitannins, oenothein B, HPLC UV-VIS, MTS assay.

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Comparative Analysis of Elderberries and Black Chokeberry Extracts Composition by Chromatographic and Spectroscopic Methods

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Abstract

The occurrence, varieties, medicinal properties and industrial use of plants like *Sambucus nigra* L. and *Aronia melanocarpa* (Michx.) Elliott are still a significant research problem. Valuable ingredients and toxic substances (sambunigrin, applies only to black elderberry) that characterize mentioned plants were discussed, as well as contaminants that may occur in the form of residues on the fruit-peel, both black chokeberry and black elderberry. In addition, the most frequently used methods for isolating biologically active compounds from plant raw materials, i.e. polyphenols, anthocyanins, phenolic acids, terpenes (found in flowers) were discussed, also qualitative and quantitative analysis has been presented.

The main aim of this study was to develop a useful method for the obtaining extracts from black chokeberry and elderberry fruit samples, freeze-dried black chokeberry fruit, dried elderberry flowers and elderberry flower syrup. In the extraction process of individual groups of analytes, the classic method was used - maceration and Folch's extraction. The extraction efficiency was presented and compared. Additionally, methodologies for the qualitative and quantitative determination of phytonutrients, such as polyphenols, in the obtained plant extracts were developed. The last stage was the analysis of the qualitative and quantitative composition of the tested samples and "screening" for foreign substances of unknown origin (PAHs, pesticides), using TLC, TLC-IR and HPLC techniques.

Results obtained after analysis of extracts of *Sambucus nigra* L. and *Aronia melanocarpa* (Michx.) Elliott allow us to conclude that they have high antioxidant capacity due to their composition rich in polyphenols (anthocyanins, gallic acid, caffeic acid, chlorogenic acid, tannin, rutin). The obtained plant extracts are free from toxic substances due to the ripeness of these fruits that could pose a threat to the health of consumers.

Keywords: elderberries, black chokeberry, extraction, chromatography, spectroscopy

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Electroporation-Enhanced Delivery of Bleomycin for Cancer Therapy: An Investigative Study Spanning *In Vitro, In Vivo,* and Clinical Trials

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Abstract

Electrochemotherapy (ECT) is an emerging, clinically applied method combining cytotoxic drugs (like bleomycin) with local electroporation – the application of electric fields to transiently increase cell membrane permeability [1]. This significantly enhances intracellular drug uptake, particularly for poorly permeating agents like bleomycin, increasing local effectiveness dramatically. ECT is internationally approved and used for both curative and palliative purposes, most effectively for small, superficial lesions [2]. It can improve quality of life by reducing pain, bleeding, preserving function, and offering cosmetic benefits, even achieving remission in selected cases. A key advantage is its predominantly local effect, making it potentially applicable in patients with organ dysfunction, as administered drug concentrations can be lower [3]. Here, we present preclinical (*in vitro* and *in vivo*) experiments and a clinical case study demonstrating the efficacy of intracellular bleomycin electrotransfer for cutaneous metastatic melanoma.

In vitro studies: Chinese hamster ovary (CHO) and murine breast cancer (4T1) cells were used. Electroporation parameters 1 pulse 1000 – 1800 V/cm, 1 Hz, 100µs and bleomycin concentrations were from 5 to 20 nM. Cell viability after treatment was assessed using colony formation assays.

In vivo studies: Female BALB/c mice were inoculated with 4T1 cells to induce tumors. Once tumors were established, mice were treated with intravenous bleomycin followed by electrical pulses applied directly to the tumor area according to the ESOPE protocol. Tumor volume was measured daily to monitor treatment response.

Clinical Case Study: A 68-year-old male with extensively pre-treated, progressive cutaneous metastatic melanoma was treated with palliative ECT. The primary goal was symptom control and quality of life improvement.

Preclinical findings (*in vitro* and *in vivo*) demonstrate the efficacy of bleomycin electrotransfer in tumour cell killing and tumor growth control. A clinical case study illustrates that ECT can be a valuable palliative option for patients with superficial melanoma metastases when conventional treatments are exhausted. It offers good local control, symptomatic relief, and is well tolerated, making it a relevant therapeutic consideration even in advanced disease stages.

Keywords: electroporation, electrochemotherapy, bleomycin, *in vitro*, *in vivo*, clinical case study.

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Assessing Outer Membrane Permeabilization of Gram-Negative Bacteria using Electrochemical Impedance Spectroscopy (EIS): Differentiation from Gram-Positive Bacteria and Evaluation of Pulsed Electric Field (PEF) Treatment Efficacy, With and Without Antibiotics

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Abstract

Understanding and manipulating bacterial membrane permeability is essential for developing non-chemical antimicrobial strategies and optimizing antibiotic efficacy. Electrochemical impedance spectroscopy (EIS) is a label-free, real-time, and non-destructive method that provides valuable insights into the dynamic changes in bacterial membranes, even in turbid or opaque media. This study examines the application of EIS to assess the outer membrane permeability of Gram-negative bacteria, distinguishing their response from that of Gram-positive bacteria, under Pulsed Electric Field (PEF) treatment and evaluating the synergistic effects of combining PEF with antibiotics.

Utilizing *Escherichia coli* (Gram-negative) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Gram-positive) as model organisms, PEF treatments ranging from 4 to 16 kV/cm with pulse durations of 10 × 10 µs and 10 × 100 µs were applied. EIS measurements revealed that E. coli exhibited greater susceptibility to membrane permeabilization compared to *L. bulgaricus*, particularly at longer pulse durations, as indicated by significant changes in admittance magnitude and phase angle. These findings underscore EIS capability to effectively differentiate bacterial responses based on cell wall structure and PEF parameters [1].

In addition, we investigated the synergistic potential of combining PEF with the aminoglycoside antibiotics kanamycin and gentamicin. EIS data correlated with bacterial growth inhibition, showing that PEF significantly potentiated the effect of kanamycin against *E. coli*, especially during the first 3 h after treatment [2]. This suggests that PEF can potentiate antibiotic action, potentially allowing for lower antibiotic dosages while maintaining therapeutic effectiveness.

Bacterial detection in samples and the separation of Gram-negative and Gram-positive species remain a major analytical challenge, as traditional Gram staining is a labor-intensive and time-consuming method. Gnaim R. et al. [3] aimed to evaluate the use of electrical bioimpedance spectroscopy (EBIS) as a low-cost, practical alternative for real-time bacterial detection and classification. The results showed that significant differentiation of bacterial types could be achieved by analyzing the magnitude and phase of the impedance at frequencies of 158,489 Hz and 5,248 Hz, respectively, at a bacterial concentration of 1 µg/µl. Further analysis showed that the sensitivity of this method varied depending on the bacterial concentration. Overall, the results indicate that EBIS is a promising method for detecting the presence of bacteria and distinguishing between Gram-positive and Gram-negative species [3].

EIS has proven to be a sensitive tool for distinguishing between Gram-negative and Gram-positive bacterial responses to membrane-targeting interventions. Furthermore, the combination of PEF with aminoglycoside antibiotics significantly improves antibacterial efficacy, particularly with compounds like Kanamycin. These insights not only validate the utility of EIS in real-time assessment of bacterial membrane dynamics but also highlight the potential of combining physical and pharmacological treatments to enhance antimicrobial strategies.

Keywords: Electrochemical admittance spectroscopy (EIS), pulsed electric field (PEF), aminoglycosides, bacteria.

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Induction of Conidiation in *Trichoderma harzianum*

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Abstract

Filamentous fungi, aside from their normal sexual cycle, can also reproduce asexually from spores produced through a biological process called conidiation.

Spores are designed by nature to survive unfavourable environmental conditions and serve a similar purpose to that of seeds in the plant kingdom. They are widely used for industrial applications [1-2] including development of probiotics, biosensors and biopolymers, in the fields of biocontrol and biocatalysis, in biosorption and bioremediation, and for pharmaceutical applications such as the production of oral vaccines, antibody and drug delivery systems.

This preliminary study investigates the mechanisms of induction of conidiation in *T. harzianum*, used as model microorganism, triggered by physical and chemical external stimuli such as mechanical injury, photoinduction, thermal stress, nutritional factors, pH modulations, availability of alkaline earth metal ions, influence of volatile organic compounds and oxygen level.

The results obtained when *T. harzianum* was subject to cold shock appear to be the most interesting for further studies among the results obtained from all the investigated stimuli applied to induce conidiation. In particular, a cycle of 30’ or 60’ at 4 °C increased significantly the sporulation in the tested samples.

This study will serve as part of a wider investigation on the development of drug delivery systems using inactivated spores as drug carriers.

Keywords: conidiation, sporulation, *Trichoderma harzianum*, photoinduction, cold shock.

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Imidazole And Fluorene Derivatives as Highly Efficient Fluorescent Light Sources: Synthesis and Investigation

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Abstract

The application of organic light-emitting diodes (OLEDs) in various flat-panel display technologies, such as smartphones and large-screen televisions, has garnered significant interest [1]. OLEDs offer numerous advantages, including streamlined development, swift response times, wide viewing angles, minimal energy consumption, compatibility with flexible plastic substrates, and diverse form factors suitable for various display types [2]. The integration of imidazole derivatives as acceptors with fluorene as a donor may promote efficient charge transfer, potentially increasing the singlet exciton yield. The imidazole and fluorene compounds are expected to function as efficient deep blue luminophores. The introduction of sterically bulky substituents at the C-9 position of the fluorene moiety reduces the overlap of frontier molecular orbitals and electron exchange with neighbouring molecules, thus promoting a high photoluminescence quantum yield in the solid state [1, 3]. This study involved the synthesis and analysis of various derivatives of imidazole and fluorene-based compounds as potential materials for blue OLEDs. The thermal and photophysical evaluation of these materials revealed that the synthesized compounds demonstrated high-intensity emission with short emission lifetimes between 1.54 and 7.63 ns, making them promising candidates for rapid-response OLEDs. Moreover, they demonstrated considerable thermal stability and produced molecular glasses with high glass transition temperatures of 130 ℃.

Keywords: fluorene, short emission lifetime, photophysical properties, OLED.

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Electroporation-Induced Sensitization: Identifying Optimal Conditions for CHO and 4T1 Cell Viability and Membrane Permeabilization

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Abstract

Electroporation is a technique that uses high-intensity electric fields to increase plasma membrane permeability, with effects that can be either reversible or irreversible. Reversible electroporation allows for membrane permeability recovery and is commonly used in electrochemotherapy to introduce drugs into cells [1].

Cancer remains one of the leading causes of death globally, with chemotherapy being a standard treatment [2]. However, chemotherapy is often associated with significant side effects, including toxicity to healthy tissues. Electrochemotherapy addresses these issues by reducing the dose of cytotoxic drugs while enhancing their efficacy specifically in tumor cells. Despite progress, challenges remain, such as the survival of resistant tumor cells. Recent efforts are focused on optimizing electrochemotherapy protocols and improving drug delivery.

Electroporation-based cell sensitization enhances cellular response to electric pulses by increasing membrane permeability, which facilitates more effective molecular transport into cells [3]. Recent studies have explored electroporation protocols for Chinese hamster ovary (CHO) and murine mammary cancer (4T1) cell lines. These experiments used a laboratory-prepared electroporation medium and stainless-steel electrodes with a 2 mm gap, delivering one or eight high-voltage (HV) pulses of 100 µs at 1 Hz, with additional pulses given after various time intervals. Cell viability was assessed via clonogenic assays, and electropermeabilization was measured by propidium iodide (PI) uptake.

Results showed that CHO cells were more sensitive to repetitive pulses, maintaining over 90% viability after a single HV pulse with an additional pulse after 5 minutes, or after eight HV pulses with an additional pulse after 1 minute. The lowest viability was observed after eight HV pulses followed by an additional pulse after 15 minutes. For 4T1 viability remained high for most conditions, except after eight HV pulses with an additional pulse after 10 minutes.

PI uptake, a measure of membrane permeabilization, was analyzed to correlate with sensitization. For CHO cells, the optimal conditions were one HV pulse followed by another pulse after five minutes, achieving 89.3% PI uptake. In contrast, the best conditions for 4T1 cells were eight HV pulses followed by a pulse after one minute, resulting in 99.2% PI uptake.

In conclusion, CHO and 4T1 cell lines require different electroporation conditions for optimal sensitization and effective uptake of external molecules without compromising viability. The 4T1 cells demonstrated greater resistance to electric pulses, able to endure harsher conditions while maintaining viability.

Keywords: Electroporation, cell sensitization, membrane permeability, tumor cells, CHO cells, 4T1 cells, high-voltage pulses, cell viability, clonogenic assay, electropermeabilization, PI uptake, molecular transport.

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Survival of Tardigrades in Low Temperature Conditions

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Abstract

Tardigrades are one of the few organisms that can survive a range of extreme conditions by going into a quiescent state - cryptobiosis - in which the organism shows no obvious signs of life, and its metabolic activity becomes difficult to detect or temporarily stops [1]. Several types of cryptobiosis have been identified, resulting from different environmental and physiological factors, including freezing (cryobiosis), drying out (anhydrobiosis) etc. [2]. During these quiescent states, mechanisms are activated to protect cells and organisms change their body shape to a static state, called tun. In this way, tardigrades survive extreme cold or water scarcity, and the processes that occur in these organisms during dormancy can be applied to the human environment. However, few studies have been carried out on anhydrobiotic or cryptobiotic organisms, especially tardigrades, and these are crucial to understanding how and why these individuals survive in conditions that would kill others. Understanding the characteristics of slow-moving animals' survival in the cold, the processes that occur in their bodies and the appropriate adaptations to these processes could open new opportunities in the life sciences.

The aim of my study was therefore to investigate the survival of tardigrade *Ramazzottius oberhaeuseri* in low temperature conditions. The experiment consisted of freezing tardigrades in different subzero temperatures (-20 ℃ and -80 ℃) for 6, 9 and 20 days long. Lichen *Xanthoria parietina* was collected from trees, submerged in distilled water for 2 hours and tardigrades were extracted under a microscope using a micropipette and kept at 5 ℃ temperature for 24 hours prior to freezing. Two plates with 15 individuals each were then placed either in -20 ℃ or -80 ℃ freezers or kept as controls in a 5 ℃ fridge (30 individuals for one temperature) for the selected period of time. After that tardigrades were thawed at 5 ℃ temperature and their vitality was assessed using a microscope. Tardigrades with no movement shown after 24 hours were considered dead. The results showed that when comparing temperatures, survival of tardigrades kept at -20 ℃ freezer was slightly better than those kept at -80 ℃. There was no difference in survival rate based on freezing duration. It was also observed that survival increased when tardigrades formed tuns.

Keywords: tardigrades, cryptobiosis, freezing survival.

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Multifunctional Carbazole-Dibenzofuran derivatives as emitters and exciplex-forming co-hosts for organic LEDs

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Abstract

Organic light-emitting diodes (OLEDs) have garnered considerable interest in recent decades due to their numerous advantages, including high efficiency and versatility [1]. While OLEDs are already incorporated into a variety of commercial products, challenges related to stability and efficiency remain [2,3], prompting the development of two new multifunctional compounds, 3,3'-[(2-Methoxy-dibenzofuran-3-yl)imino]bis(9-ethyl-9H-carbazole) BeCzMFI and 3,3'-[(2-methoxy-dibenzofuran-3-yl)imino]bis(9-butyl-9H-carbazole) BbCzMFI, that were synthesized during a three-step procedure. These compounds feature a methoxydibenzofuran–carbazole electron-donating core, enhanced with aliphatic chains to improve film-forming properties. They demonstrate exceptional thermal and morphological stability with glass transition temperature exceeding 100 °C, as well as favourable photophysical characteristics, positioning them as promising candidates for OLED applications. The materials function both as emitters and host materials, either independently or as part of exciplex host systems when paired with an acceptor material (CN-T2T). OLEDs using mCBP-CN as the host material and BbCzMFI as the emitter exhibited superior efficiency, driven by strong exciplex emission. These devices achieved an external quantum efficiency of 10.4%, current efficiency of 21.4 cd/A, and power efficiency of 28.0 lm/W, making them suitable for lighting applications. Additionally, deep-blue emitting devices with BbCzMFI as the emitter demonstrated balanced carrier injection, achieving efficiencies greater than 5%, highlighting their potential for display applications. Furthermore, BeCzMFI and BbCzMFI were shown to function effectively as traditional host materials, carrier transport components in co-host systems, and exciplex-forming components in OLEDs. Their roles as donors in exciplex systems or electron transport molecules in co-host systems facilitate improved exciton management, enhancing both efficiency and luminance in electroluminescent (EL) applications. Notably, red phosphorescent OLEDs incorporating these materials in exciplex configurations displayed excellent performance, with the top device achieving an external quantum efficiency of 13.6%, current efficiency of 9.5 cd/A, power efficiency of 13.8 lm/W, and a maximum luminance of 30,000 cd/m². These results highlight the potential of these versatile materials for advancing next-generation OLED technologies, offering a flexible approach to optimizing charge transport and energy transfer.

Keywords: OLED, organic semiconductor, blue emitter, host material, co-host, exciplex.

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Genetic Diversity of Lithuanian Populations of *Impatiens noli-tangere* L.

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Abstract

Touch-me-not balsam (*Impatiens noli-tangere*) is an annual herbaceous plant of the *Impatiens* genus in the *Balsaminaceae* family. *I. noli-tangere* grows naturally in Asia, North America and Europe, it is common in Lithuania, in moist forests, schrublands, besides ditches [1]. Some molecular studies on the population diversity of *I. noli-tangere* have been carried out with the application of nuclear DNA markers [2-4]. In comparison to nuclear and mitochondrial genomes, the chloroplast genome is capable of self-replication, has relatively independent evolution, low mutation rate and unique maternal inheritance [5]. Thus, the chloroplast genome has become a key molecular technology for germplasm identification, phylogenetic studies, species evolution, and diversity analysis [6, 7]. Chloroplast DNA has been used as phylogeographical marker for analysing weed invasions [8, 9]. The comparisons of genetic diversity of native species with their invasive congeners might be a convenient tool for identifying mechanisms linked to plant invasiveness [3, 10]. In recent years, chloroplast genome sequencing has been completed in several *Impatiens* species [9, 11, 12]. Compared to the other *Impatiens* congeners, Compared to the other *Impatiens* s., studies on the chloroplast DNA of *Impatiens noli-tangere* are very scarce. [13, 14]. The aim of this study is to assess the molecular diversity of Lithuanian populations of *Impatiens noli-tangere* according to chloroplast DNA markers. DNA was extracted from plants collected from different areas of Lithuania and its concentration and quality were checked. Selected individuals from some populations were evaluated according to *rpl32-trnL*, *rps16*, *trnG* and *psbA-trnH* DNA markers. These chloroplast DNA regions were selected due to high variability in *I. glandulifera* [15]. When individuals from different populations were compared, single nucleotide polymorphisms were detected at the *rpl32-trnL* and *rps16* loci. These two markers allowed the detection of single nucleotide changes between populations of *Impatiens noli-tangere*. According to the *psbA-trnH* and *trnG* markers, there was no sequence variation between the populations. We searched for and compared the *psbA-trnH* and *trnG* marker sequences of *I. noli-tangere* with corresponding sequences from congeneric species [15-17]. The phylogenetic tree revealed that *I. noli-tangere* is more related to *I. capensis* and *I. parviflora*, rather than to *I. glandulifera*. Our results show that it is expedient to extend study of the genetic diversity of populations of *Impatiens noli-tangere* according to chloroplast DNA markers using more individuals from a larger number of populations.

Keywords: *Balsaminaceae*, native species, DNA markers.

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The Effect of Foliar Biostimulants on Drought Tolerance of Winter Wheat

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Abstract

Drought is a problem that, depending on its intensity and duration, can pose a serious threat to humanity and the environment, with significant social, economic and ecological impacts. Due to climate change-induced erratic rainfall and rising temperatures, drought events are likely to occur more frequently in many areas, further straining freshwater supplies and posing serious challenges to agriculture. During drought, plants experience stress, which can significantly reduce their productivity and recovery capabilities. It is relevant for the agricultural sector to look for more and more new solutions to increase plant productivity while using less and less chemical fertilizers and pesticides. This study aims to determine the effect of foliar biostimulants on plant resistance to drought stress. Experiment was conducted in a controlled environment: ambient 400 ppm CO_2 concentration and 21/14 °C of day/night temperature, relative air humidity was 60/70% and 14/10 h light/dark regime. Winter wheat (*Triticum aestivum* L. cv. 'Informer') (16 seeds per pot) were sown in a neutral (pH 6.0–6.5) peat and sand (3:1) substrate. When the plants had the third true leaf (BBCH 13), 50% of the experimental pots were stopped watering. The experimental drought exposure lasted for 14 days. In the midst of drought stress exposure, plants were sprayed with biostimulants "Terra-sorb", "Millerplex" and their mixture based on manufacturers' recommendations. On the last day of drought stress, plant morphological and physiological indicators were measured. Physiological parameters stomatal conductance, photosynthetic rate, transpiration rate, intercellular CO_2 concentration, and water use efficiency were evaluated using the Li-cor device. The results showed, both biomass and physiological indicators of plants sprayed with biostimulants were slightly higher than those not sprayed. Drought exposure reduced plant height from 50 to 25% ($p<0.05$ compared to control) and the reduction was lower in plants sprayed with biostimulants. The most effective effect was achieved when a mixture of the tested biostimulants was applied. The pattern of changes in plant dry biomass was similar, with wheat biomass decreasing from 52 to 35 % due to drought stress compared to control plants ($p<0.05$). Plant biomass losses in plants sprayed with a biostimulants mixture in the middle of drought stress were 20 % lower than in unsprayed ones. On the last day of drought stress, the photosynthetic rate of wheat was about 80 % ($p<0.05$) lower compared to the control. The losses in photosynthetic rate of plants sprayed with biostimulants were smaller compared to those not sprayed. The transpiration rate of wheat not sprayed with biostimulants decreased by about 85%, while after spraying the plants with "Terra-sorb", "Millerplex", and their mixture, the transpiration rate decreased by 78, 82, and 65%, respectively ($p<0.05$). Changes in stomatal conductance due to both drought and biostimulant effect were analogous to transpiration rate. Again, changes in stomatal conductance of sprayed plants were milder and smallest under the effect of the mixture of investigated biostimulant. Thus, this study emphasizes that biostimulants used in agriculture today reduce plant stress to drought effects, but applying mixtures of different biostimulants can increase plant drought resistance.

Keywords: drought stress, *Triticum aestivum*, foliar biostimulants, dry weight, gas exchange.

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Assessment of Plant Origin, Microbial Contaminants, and Pigment Levels in Bee Pollen

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Abstract

Bee pollen is collected by honeybees from the flowers of various plants. It is primarily used as food for bees – especially to produce royal jelly and bee bread – but it is also can be consumed by humans as a functional food. For this purpose, the pollen is collected in the form of granules when the bees return to the hive [1]. Bee pollen is known to possess antioxidant, antibacterial, anti-inflammatory, and anticancer properties [2]. It is also considered a promising remedy for individuals suffering from allergies. While airborne pollen is known to trigger allergic reactions, the flavonoid myricetin found in bee pollen, which helps suppress allergic responses, reduces the effect of specific antigens that cause allergies [3].

The aim of this study was to determine the plant sources of the tested bee pollen, evaluate microbiological contamination, and measure the levels of chlorophylls and carotenoids.

The pollen was examined both as a mixture and in individual colour fractions. The bee pollen was classified into green, brown, orange, and yellow colour fractions. To assess microbiological contamination, the pollen was placed in Petri dishes on agar media. For melissopalynological analysis, pollen structures were observed under a microscope and compared with reference databases [4,5]. The amount of chlorophyll a, chlorophyll b, and carotenoids content were evaluated in acetone extracts by spectrophotometric tests.

Melissopalynological analysis revealed that rapeseed (*Brassica napus*), raspberry (*Rubus idaeus*), buckwheat (*Fagopyrum esculentum*), pea (*Pisum sativum*), chamomile (*Matricaria chamomilla*), and maple (*Acer platanoides*) were the main bee pollen plants. Study showed that inappropriate storage of bee pollen leads to the growth of microscopic fungi, such as *Trichoderma* sp., *Aspergillus niger*, and *Penicillium* sp. The highest chlorophyll content was found in the green-coloured pollen (chlorophyll a – 14.23 mg/g; chlorophyll b – 22.59 mg/g), while the highest carotenoid content was found in the orange-coloured pollen (223.18 mg/g).

Keywords: bee pollen, melissopalynological analysis, microbiological contamination, chlorophylls, carotenoids.

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Cotton Metal Oxide Nanoparticle-Treated Material Action on Microalgae of the *Prototheca* Genus

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Abstract

Prototheca spp., chlorophyll-free unicellular algae, is identified as an opportunistic pathogen in vertebrates [1]. It can cause a rare but mostly resulting in chronic infectious disease named protothecosis in both humans and animals. Widespread resistance to the available drugs, including antibiotics, antifungals and antialgals of *Prototheca* species was observed [2]. The nanoparticles' ability to damage cell membranes and inhibit metabolic activities may be a promising tool to combat protothecosis [3]. Applying antimicrobial textiles may prevent or inhibit the growth of microbes or eliminate them [4]. Our study was conducted to evaluate the activity of cotton material coated with metal nanoparticles (NPs) against microalgae of the *Prototheca* genus. The metal oxide CuO, WO_3, AgO, Fe_2O_3, ZnO, SiO_2, CeO_2 or Mg and Fe NPs were deposited using low-temperature plasma technology in a pure oxygen atmosphere on one or both sides of the cotton material. For determining the antimicrobial activity of metal oxide NPs on clinical *Prototheca spp*. culture, the broth microdilution method was used. Cotton AgONPs (P=200 W, t=2x2 h) and MgNPs (P=200 W, t=30 min and 2 h) coated material showed a strong killing activity towards yeasts-like microalgae *Prototheca spp*. Less effective against algae cells were CuONPs (P=200 W, t=2x2h) and ZnONPs (P=150 W, t=2 h). NPs such as Fe_2O_3 (P=150 W, t=2x2 h), SiO_2 (P=150 W, t=2x2 h) and WO_3 (P=200 W, t=2x2 h) possibly acted as a co factors that facilitate the growth of *Prototheca spp.*as they boosted the growth of cells. The results suggest that nanoparticle-treated materials in the nearest future might become a promising alternative for managing microalgae infections, including those caused by *Prototheca spp.*

Keywords: metal nanoparticles, antimicrobial coatings, *Prototheca spp*., cotton textiles, plasma technology.

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Study of Biologically Active Compounds of Frozen and Dried Chamomile *(Matricaria recutita L.)*

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Abstract

Chamomile (*Matricaria recutita* L.) is a widely spread plant throughout the world. It is used in traditional medicine to treat various diseases, such as digestive and liver disorders, respiratory disorders, and infections. The pharmacological properties of the plant depend on the biologically active compounds [1]. This study aims to investigate the effect of freezing at -18 ℃ and drying at room temperature of 22 ℃ on the content of biologically active compounds of the plant. The plant raw material was separated into three parts: blossoms, stems and roots. Three different methanol concentration (50% (v/v), 60% (v/v), and 70% (v/v)) were used of extracts preparation. The total amount of phenolic compounds is determined Folin-Ciocalteu method. The total amount of flavonoids is determined aluminium chloride method. The radical scavenging activity of extracts was evaluated using the DPPH radical scavenging method [2]. Results were expressed as milligrams of rutin equivalents (RE) per one gram of dry raw material. The highest total phenolic content was determined in dried chamomile flowers extracted with 70% (v/v) methanol (87.08 mg/g). The lowest phenolic content was determined in the 50% (v/v) methanol extract of frozen stems (6.78 mg/g). Flavonoid content was also highest in the 70% (v/v) methanol extract (22.12 mg/g), while the lowest amount was found in the 60% (v/v) methanol extract of frozen stems (1.01 mg/g). The highest radical scavenging activity was determined in dried chamomile flowers extracted with 70% (v/v) methanol (63.09 mg/g), whereas the lowest activity was found in the 50% (v/v) extract of frozen stems (3.87 mg/g). Drying and freezing as raw material preparation methods affect the levels of biologically active compounds differently. The methanol extracts of dried chamomile roots, stems, and flowers contained higher amounts of biologically active compounds compared to those of frozen samples. The results of the study show that most effect for extraction of biological compounds were observed using 70% (v/v) methanol extracts.

Keywords: biologically active compounds, chamomile (*Matricaria recutita* L.), extraction efficiency.

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Green Solvent-Based Extraction of Phenolic Compounds from Rose Hips: Exploring γ-Valerolactone, Cyrene, and Ethyl Lactate–Ethanol Mixtures

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Abstract

The growing demand for environmentally friendly and sustainable extraction methods has led to increasing interest in the use of green solvents as alternatives to traditional organic solvents. Among them, γ-valerolactone (GVL), Cyrene™ (dihydrolevoglucosenone), and ethyl lactate have emerged as promising bio-based solvents due to their low toxicity, biodegradability, and efficient solvation properties. These solvents, used alone or in combination with ethanol, offer a viable approach for the selective extraction of valuable phytochemicals from plant materials.

This study investigates the extraction of bioactive compounds from rose hips (*Rosa* spp.) using GVL, Cyrene, ethyl lactate, and their mixtures with ethanol in various volume ratios. The extraction efficiency, total phenolic content, and antioxidant potential of the resulting extracts were evaluated to assess the suitability of these green solvents for recovering natural antioxidants. The findings contribute to the development of sustainable extraction strategies for cosmetic, nutraceutical, and functional food applications.

Keywords: green solvents, γ-valerolactone (GVL), Cyrene™, ethyl lactate, rose hips, phenolic compounds, antioxidant activity, sustainable extraction, bioactive compounds.

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Some Aspects of Genetic Diversity of Lithuanian Populations of *Impatiens parviflora* DC.

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Abstract

Impatiens parviflora DC. known as small balsam, is an invasive plant species native to Central Asia. It has rapidly spread throughout Europe due to its ability to adapt to various climatic and soil conditions. In Lithuania, the species is widespread in shaded and moist habitats, where it threatens native biodiversity and alters plant community structure [1]. The aim of this study was to assess the genetic diversity of *I. parviflora* populations in Lithuania using chloroplast DNA (cpDNA) markers. Plant material was collected from geographically different regions of the country. DNA was extracted from leaf tissues preserved at low temperature until analysis. DNA quality and concentration were evaluated, and individuals were selected for sequencing using four cpDNA markers: rps16, trnG, rpl32-trnL, and psbA-trnH. These markers are commonly used in phylogenetic and population-level studies due to their moderate sequence variation and localization within non-coding regions of the chloroplast genome. The amplified fragment sizes generally range between 350 and 900 base pairs, depending on the marker [2, 3]. Studies on chloroplast genomes in the Impatiens genus further support the suitability of these markers for understanding evolutionary relationships [4, 5]. The selected markers are widely used in plant phylogenetic and population-level studies and were selected for their effectiveness in resolving genetic relationships among individuals [3]. In this study, no polymorphism was detected among individuals from different populations. This suggests low cpDNA diversity, which could be explained by a narrow maternal origin. The introduction of *I. parviflora* into Western Europe occurred in the early 19th century, while first records in Lithuania date to the second half of the 20th century [4]. To determine genetic relationships with other European populations, Lithuanian sequences of *I. parviflora* were compared with GenBank data obtained by the other researchers. The psbA-trnH region of Lithuanian *I. parviflora* showed highest similarity with sequences from Sweden and Switzerland sites, while rps16 and rpl32-trnL matched populations from the United Kingdom. These results support the hypothesis that Lithuanian populations may have originated through secondary dispersal from the other parts of Europe [5]. Comparative studies with other *Impatiens* species confirm the utility of cpDNA in tracking geographic patterns of spread [7-10]. Future research could benefit from expanding the range of cpDNA markers and increasing the number of studied populations and individuals.

Keywords: *Impatiens*, non-native species, DNA markers, plant invasions.

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Towards Safe and Effective Biomedical Nanocoatings: Plasma-Sputtered Magnesium Nanoparticles with Cytoprotective and Antimicrobial Properties

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Abstract

The increasing prevalence of antimicrobial resistance and the need for biocompatible, multifunctional surfaces in healthcare have driven the development of innovative nanocoating strategies [1]. Among these, magnesium (Mg) nanoparticles are gaining attention due to their intrinsic antimicrobial properties, biological relevance, and low cytotoxicity. Beyond antibacterial textiles, Mg-based nanocoatings hold promise in agriculture, enhancing seed germination and nutrient absorption, as well as in biodegradable packaging, wound healing applications, and eco-compatible biomedical implants [2]. Their dual functionality—antimicrobial activity combined with cytocompatibility—positions plasma-sputtered Mg particles as a versatile and sustainable platform for next-generation materials [3]. In this study, magnesium nanoparticles were applied to textile substrates via low-temperature glow discharge plasma sputtering. This solvent-free, eco-friendly deposition method ensures excellent coating adhesion, uniform coverage, and precise control of layer thickness. SEM, EDS, and XPS analyses confirmed the formation of homogeneous Mg layers modifying the fiber surface morphology. An antimicrobial activity against both Gram-positive and Gram-negative strains and fungi *Candida spp.* was determined. Cytocompatibility was evaluated using human endothelial HUVEC cells and MTT/PrestoBlue assays. Notably, 120-minute coatings not only maintained cell viability but enhanced it by ~20%, suggesting a cytoprotective effect. These findings highlight plasma-sputtered Mg nanoparticles as a promising solution for multifunctional, eco-conscious, and biocompatible surfaces in medicine and beyond.

Keywords: plasma sputtering, magnesium nanoparticles, antimicrobial coatings, cytocompatibility, eco-friendly nanotechnology, biomedical textiles.

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Carbazole-Based Near-Infrared Absorbing Organic Photodetectors: Design, Synthesis and Characterization

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Abstract

The unique ability of organic semiconductors exhibiting absorption in long-wavelength regions of visible spectrum to capture low-energy photons make them crucial in advancement of next-generation optoelectronic devices such as high-efficiency solar cells, photodetectors, biomedical imaging, and optical communication devices [1]. Additionally, such materials are suitable for night vision in security and surveillance which helps to improve sensitivity towards low light conditions. Carbazole moiety is frequently used as an electron-donating group in the design of π-conjugated molecules owing to the hole-transporting capabilities, optical properties, thermal and chemical stability, and easy structural modification of carbazole derivatives [2]. Also, the strong electron acceptors, 1,1,2,2-tetracyanoethylene (TCNE) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) are highly reactive toward electron-rich alkynes [2,3]. Owing to the increased demand for the development of organic semiconductors exhibiting absorption in long-wavelength regions of the visible spectrum, we designed novel materials containing 9-ethyl carbazole as donor unit appended with strong acceptor units i.e. TCNQ and TCNE. The synthesis of the designed compounds was successfully furnished using two step synthetic schemes followed by structural characterization using NMR and mass spectrometry. The photophysical, electrochemical and theoretical analysis of the synthesized compounds was carried out and the structure-property relationship was established. Conclusively, the newly synthesized materials were found to be suitable for photonic and optical devices particularly for organic photodetectors.

Keywords: carbazole, tetracyanoquinodimethane, tetracyanoethylene, organic photodetectors.

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Development of a Microbial Biofuel Cell Using Biocompatible Nanocomposites

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Abstract

Currently, the production of sustainable and environmentally clean energy from renewable sources is becoming increasingly important. Microorganism-based biofuel cells (MBFCs) have attracted significant interest due to their unique ability to convert chemical energy stored in biodegradable materials into electrical energy. MBFCs utilize substrates derived from renewable sources and therefore exhibit high conversion efficiency [1–3]. Most MBFCs operate at ambient temperature, which, compared to conventional fuel cells, means they are environmentally friendly since they do not require high operating temperatures to function effectively [2].

Microorganisms used in MBFCs have several important advantages over enzymatic systems: regeneration capability, broad catalytic properties, and lower sensitivity to toxic compounds and environmental changes [4]. The power generated by MBFCs strongly depends on the efficiency of charge transfer from microorganisms to the electrodes; therefore, electrode modification is a critical consideration in the design of microbial biofuel cells [5].

In this study, a biocompatible composite consisting of a polypyrrole matrix and nanomaterials was formed on the electrode surface. The modified anode was applied in the development of a microbial biofuel cell prototype.

In the prototype system modeling, a glucose concentration of 25 mmol/l was used. Further measurements were conducted to determine which modified electrode generates the highest current under favorable conditions. Measurements were performed with five electrodes, one of which served as a control. The surfaces of the other electrodes were modified with a polypyrrole and nanomaterial layer to improve conductivity and enhance electron transfer between yeast and the electrodes. The surface morphology of the modified electrodes was investigated using a scanning electron microscope.

It was determined that all modified anode surfaces improved electron transfer. In the system where the electrode was modified with PPy/rGO/yeast/PQ, the generated power reached 910.75 mW/m². The electrode modified with PPy/2 nm AuNP/yeast/PQ generated a power of 30.66 mW/m². The anode coated with PPy/yeast/PQ generated the lowest power among all modified electrodes—11.7 mW/m². It was found that the biofuel cell with the anode modified with PPy/10 nm AuNP/yeast/PQ generated the highest power among all systems, with a maximum power output of 1172.46 mW/m², which is 677.72 times higher than the power output of the control system (1.73 mW/m²).

Keywords: microbial biofuel cell, anode, yeast, polypyrrole, nanomaterial bioenergy.

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Perceptions of ecosystem services: Evidence from communities in Lithuania

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Abstract

21st century facing a problem well known to society, when massive and destructive land cover changes have a negative impact on the environment. Every year, larger areas of land are needed for growing food, extracting materials, industrial and economic zones, settlements, and other human needs [1]. Natural land areas are disturbed by anthropogenic activities, which negatively affects the quality and quantity of ecosystem services (ES) and can lead to ecosystem degradation [2]. The aim of this study is to analyse Lithuanian residents' attitudes about ES. Quantitative survey assessment of Lithuanian residents' attitudes about ES. The conducted quantitative survey showed that a large part of the Lithuanian population is not well acquainted with ecosystem services. Most of the respondents, 42% have only heard about ES. However, residents unequivocally say that ES is an important component of ensuring the well-being of mankind and maintaining the Earth's balance. From the obtained results, it can be concluded that even in a short period of time, changes in land areas are significant. It is important to conduct a detailed and accurate assessment of the land fund and ecosystem services at the state level every year. This would help to find out how the ecosystems affected by anthropogenic activities change, the services they provide, how it can be controlled and maintain the balance between humanity and the prosperity of nature. A large part of the Lithuanian population is not well acquainted with ES, so it is important to educate the public on the topics of environmental protection and ecology. The level of education is significant for the level of conception about ES.

Keywords: ecosystem, ecosystem services, land use.

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Protozoan-Based Sustainable Biofilter for Heavy Metal Remediation

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Abstract

Dissolved heavy metals in wastewater represent a persistent threat to environmental and public health due to their prolonged toxicity [1]. Conventional treatment methods, such as chemical precipitation and membrane ultrafiltration, are often energy-intensive and may lack long-term sustainability, especially in lower concentrations [2]. In contrast, biological processes offer an eco-friendly alternative by harnessing microorganisms' natural capabilities for heavy metal sequestration via bioprecipitation, biosorption, or bioaccumulation [3].

In this study, the bioaccumulation potential of the protozoan *Tetrahymena thermophila* was evaluated, and exploited by design a novel filter (2.5 cm in height × 5.0 cm in length × 0.25 cm in width). The filter matrix was fabricated from recycled, compressed scrap textile material, into which a concentrated suspension of *T. thermophila* was inlaid. Spirulina was employed as a renewable nutrient source in place of conventional peptone. The optimized biofilter achieved cell densities ranging from 20 000 to 32 000 cells/cm^3 and bioaccumulated approximately 90% (47.02 μM) of Zn^{2+} ions from the wastewater sample over a three-day period. Post-treatment, incineration was employed for heavy metal reclamation. The higher heating value was determined to be carbon neutral or carbon positive. These results demonstrate that integrating biological and sustainable engineering approaches can yield effective strategies for the remediation of heavy metal–contaminated wastewater.

Keywords: heavy metals, *Tetrahymena thermophila*, bioremediation.

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Derivatives of 2,7-Dibromo-9,9-Dimethyl-9H-Fluorene and 2,7-Dibromo-9,9-Spirobifluorene as Electroactive Materials

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Abstract

Low-molar-mass and polymer organic light emitting diodes have attracted considerable attention due to their great application potential in large area flat panel displays and solid state lighting [1]. Such devices tend to have multilayer device configuration with a hole transport layer, an electron transport layer and an emissive layer, and some of them also have a hole injection and an electron injection layer [2].

The selection of appropriate materials for each layer is of great importance. Generally, the molecular structures of hole transport materials usually contain electron-donating moieties, such as carbazole, triarylamine, diphenylamine, N-phenyl-1-naphthylamine etc. [3]. However, traditional methods for synthesizing electroactive materials have notable drawbacks such as low reaction yields, longer reaction times, dependence on hazardous, expensive and moisture-sensitive reagents, as well as involving large amounts of reactants. Additionally, these methods often require intense reaction conditions, longer workup procedures, and difficulties in catalyst regeneration.

In this work, we employed efficient Suzuki coupling reactions of 2,7-dibromo-9,9-dimethyl-9H-fluorene and 2,7-dibromo-9,9'-spirobifluorene with (B-(9-phenyl-9H-carbazol-3-yl)-boronic acid and (6-Phenyldibenzo[b,d]furan-4-yl)boronic acid. Their thermal, electrochemical, and photophysical properties will be presented.

Keywords: 2,7-dibromo-9,9'-spirobifluorene, 2,7-dibromo-9,9-dimethyl-9H-fluorene, B-(9-phenyl-9H-carbazol-3-yl)-boronic acid, (6-Phenyldibenzo[b,d]furan-4-yl)boronic acid, OLED.

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Additive Manufacturing of PMMA: Advances and Multidisciplinary Applications

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Abstract

3D printing technology is an emerging technique that has already shown its potential in healthcare, automotive, and aviation industry [1]. Also known as Additive Manufacturing, 3D printing allows for layer-by-layer production of models through computer-aided design. Traditional manufacturing, on the other hand, usually refers to subtractive methods in which the use of a mould is required, and the final object is created by removing residuals of raw material [2]. An outstanding Fused Deposition Modelling (FDM) thermoplastic material is polymethyl methacrylate (PMMA), which major application took place in WWII in aircraft and gun turrets [3] and currently shows a relevant presence in medicine and dentistry [4] because of its mechanical and physical properties [5]. Despite its recurrent use, there is currently no detailed description of the printing settings and achievable results. This study evaluates the feasibility of 3D printing PMMA for medical devices and broader applicability in biomedical engineering. The primary goal was to develop a reliable protocol for FDM 3D printing of PMMA filament using the Zortrax M300. To that end, initial printing testing was performed, involving printing simple cube geometries at 10%, 50%, and 100% infill densities. While the lower infill densities were printed successfully using standard setting parameters, the 100% infill presented significant challenges including warping, nozzle clogging, and adhesion issues. Therefore, multiple adjustments to temperature and speed were required. The final protocol for successful printing involved 0.4 mm nozzle diameter, extrusion temperature of 245 ℃, platform temperature of 60 ℃, and layer thickness 0.14 mm. To assess the model applicability in the medical physics field, selected prints were scanned using the Halcyon LINAC CBCT system and its attenuation properties were assessed by its Hounsfield Unit (HU) values evaluating models' suitability for CT imaging. The 10% and 50% infill samples showed low attenuation characteristics, indicating poor suitability for tissue mimicking standards. In contrast, 100% infill samples achieved values between +20 and +80 HU that, though still below accepted parameters (+100 to +140 HU), suggests potential for further optimization and use in medical physics applications.

Keywords: 3D printing, PMMA, additive manufacturing, biomedical applications, fused deposition modelling, CT phantoms, Hounsfield units.

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Strain-Dependent Antimicrobial Activity of Lactoferrin Against *Escherichia coli*: an Important Factor That Must Be Taken into Account

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Abstract

Lactoferrin is an iron-binding glycoprotein produced by the epithelial cells, which has been identified in various biological fluids including in milk in which it has been identified for the first time [1]. This protein functions as a multifunctional molecule with anti-inflammatory, immunomodulatory, antioxidant, antitumor and antimicrobial properties. Due to its diverse biological functions, low cytotoxicity and ease of obtaining, lactoferrin became a promising target of pharmaceutical and food application researches [1,2]. The lactoferrin antimicrobial property is mainly based on two mechanisms: regulation of extracellular iron availability dependent on protein iron saturation and protein-bacterial cell wall interaction resulting from lactoferrin positive charge [3]. Goal of this study was to compare quality and antimicrobial activity of three lactoferrins from different manufactures (LF1, LF2, and LF3) from which two were produced via freeze-drying and third through spray-drying methods. As a target bacteria, 65 *E. coli* strains isolated from milk (n=50) and hospital specimens (n=15) were used. Physicochemical analyses of all three lactoferrin samples showed their comparable quality. The iron saturation ranged from 10% to 20% and similar level of proteins denaturation. Based on obtained results, *E. coli* strains have varying sensitivity to lactoferrin. LF1 exhibited the most potent antibacterial effect (10% cell viability for 5 mg/ml variant). In contrast, LF3 was the least effective (20% viability at a concentration of 20 mg/ml for majority of strains). However, in the case of several *E. coli* strains, LF3 showed comparable antibacterial activity to LF1. In addition, all hospital *E. coli* strains had greater resistance to lactoferrin compared to milk ones, with highest differences for LF1 for both 5 and 20 mg/mL concentrations. The variations in results among lactoferrin samples could not be explained according to physicochemical features. Thus, those results indicate complex nature of LF's activity, suggesting different bacterial susceptibility to lactoferrin.

Keywords: lactoferrin, *Escherichia coli*, bacterial susceptibility, milk, antimicrobial.

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Effects of Polyethylene Terephthalate (PET) Plastics on Seed Germination in Four Crop Plants: A Short-Term Germination Assay

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Abstract

Plastic pollution has emerged as a critical environmental issue, with increasing concern about its impact on terrestrial ecosystems, particularly seed germination and early plant development. This study aimed to assess whether plastic presence influences the germination of different crop seeds by conducting a short-term germination assay. Experiment was conducted in a controlled environment: ambient 400 ppm CO_2 concentration and 21/14 °C of day/night temperature, relative air humidity was 60/70% and 14/10 h light/dark regime. Four plant species were selected for this experiment: *Vigna radiata*, *Vigna unguiculata*, *Raphanus sativus* and *Phaseolus vulgaris*. For each species, seeds were divided into two experimental groups: a control group and a treatment group, each consisting of three replicates. The seeds were sown in Petri dishes lined with cotton and filter paper. Control seeds were sown in distilled water and for the treatment group, 3% (w/w) PET plastic particles of approximately 1 mm in size were uniformly spread above the paper layer prior to seed placement. The germination process was monitored over a five-day period and the number of germinated seeds was recorded daily; seed germination percentage was estimated from the data obtained. Data were statistically analysed using one-way Analysis of Variance (ANOVA). The results showed that among all tested species, only *Vigna unguiculata* demonstrated a statistically significant reduction in germination on Day 1 in the presence of 3% PET plastic ($p < 0.05$). No other species, including *Raphanus sativus*, *Vigna radiata*, or *Phaseolus vulgaris*, exhibited significant differences in germination between the control and treatment groups at any time point. These findings suggest that the inhibitory effect of plastic on seed germination may be species-specific and time-sensitive, with early-stage germination being more susceptible in certain plants. The lack of consistent significant differences across other species or days indicates that short-term exposure to plastic under controlled conditions may not universally impede seed germination. However, the observed sensitivity in *V. unguiculata* highlights the need for further investigation into species-specific interactions between plastic pollutants and seed physiology. This study contributes to the growing body of literature examining the ecological implications of plastic pollution in soil environments and underscores the importance of assessing plant responses at early developmental stages. This preliminary evidence raises concerns about the subtle but potentially harmful influence of microplastic contamination in agricultural soils. Understanding such early-stage disruptions is essential for predicting long-term impacts on crop productivity and food security.

Keywords: seed germination, PET microplastics, *Vigna radiata, Vigna unguiculata, Raphanus sativus, Phaseolus vulgaris*.

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Association Between *IL1RAP* Polymorphism and Pituitary Adenoma in the Lithuanian Population

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Abstract

Pituitary Adenoma (PA) is a commonly occurring benign tumor of the pituitary gland, often progressing slowly and initially without symptoms [1]. However, depending on the disease duration of the disease and the hormonal activity of the pituitary gland, various clinical signs may appear. Although the exact mechanisms of PA development are not fully understood, certain genetic mutations have been linked to tumor formation [2].

The *IL1RAP* gene encodes a protein found in the brain that acts as a co-receptor for IL-1 family cytokines, essential for regulating immune responses [3]. Altered cytokine levels can disrupt immune function and promote inflammation, with *IL1RAP* overexpression linked to tumor development and poorer survival outcomes [4].

The aim of this study was to investigate the association between the *IL1RAP* rs4624606 gene polymorphism and the occurrence of PA in the Lithuanian population.

This study included a total of 445 participants, divided into two groups: patients with PA (n =148) and a control group (n = 297). DNA was extracted from peripheral blood leukocytes using the DNA-salting out method. Genotyping was carried out using the real-time polymerase chain reaction (RT-PCR) method. Data were analyzed using IBM SPSS Statistics 29.0 software.

Our study demonstrated that the *IL1RAP* rs4624606 A allele was significantly less frequent among patients with non-functioning PA compared to the control group ($p = 0.031$). Furthermore, each A allele of *IL1RAP* rs4624606 reduces the odds of developing non-functioning PA form by 2.1-fold under the additive model (OR = 0.472; 95% CI: 0.360–0.957; $p = 0.033$).

In conclusion, the *IL1RAP* rs4624606 A alleles are associated with a reduced likelihood of developing non-functioning pituitary adenomas.

Keywords: Pituitary Adenoma, *IL1RAP*, rs4624606.

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Cell Electrofusion and Viability Assessment after Electroporation

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Abstract

Electroporation (EP) is a prevalent method based on the use of electric pulses to permeabilize cell membranes, allowing molecules to be transferred into the cells. Reversible EP disrupts membrane integrity just temporarily, while irreversible electroporation (IRE) damages cell membranes permanently by using more intense electric pulses than used for EP and causes instant cell death. However, intense permeabilization using IRE can promote fusion of adjacent cells, forming multinucleated cells which can have higher survivability. These cells were observed to form after tissue electroporation [1] or without using electric pulses [2]. The behaviour of these cells inside a human body is not fully explained, but known that multinucleated cells can cause negative effects on human health by becoming invisible to the immune system [3-5]. In summary, assessing the efficacy of chemotherapy in reducing post-IRE multinucleated cell formation is crucial, and this effect is not investigated yet. In this research, the potential of the anticancer drug bleomycin (BLM) to reduce the formation of hybrid cells after electroporation-induced electrofusion was evaluated.

Experiments were performed using Chinese hamster ovary (CHO) cells. Cell membranes were in contact during electroporation to replicate human tissue. Cells were treated with 8 HV electric pulses of 100 µs at the frequency of 1 Hz with different strengths: 1 kV/cm, 2 kV/cm and 3 kV/cm. To assess the relative cell viability in the areas affected by electrical pulses, custom trained AI cytoplasm and nuclei segmentation models were used.

The results demonstrated that cell viability decreased when joining IRE with BLM. Bleomycin also inhibited the formation of giant cells with no toxicity to non-electroporated cells.

In conclusion, combining bleomycin and IRE is an effective strategy for reducing dangerous multinucleated cell formation and cancerous cell survival, enhancing the safety and efficacy of electroporation-based therapies.

Keywords: electroporation, electrofusion, electrochemotherapy, cell fusion, multinucleated cell.

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The Content of Phenolic Compounds in Extracts from Different Color Carrot (*Daucus carota L.*) Root

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Abstract

Carrot (*Daucus carota* L.) is a nutrient-rich root vegetable that is widely cultivated worldwide and is valued for its high content of bioactive compounds with health benefits. Interest in functional foods and natural sources of antioxidants continues to grow, so the study of the phytochemical profiles and antioxidant activity of different carrot varieties is very relevant [1]. The resulting studies may significantly influence plans for growing certain carrot varieties, which in turn will affect dietary approaches, the food industry and pharmacological production.

The aim of this study was to determine and compare the total phenolic compounds content in the different varieties of edible carrot roots extracts.

The different colored roots of carrots were extracted with 70% of ethanolic solution with ultrasound assisted extraction. The total phenolic content (TPC) was determined quantitatively in extracts using the Folin-Ciocalteu methodology, with gallic acid as the standard [2].

The total phenolic content (TPC) in the tested extracts differed among carrot varieties. The significantly highest TPC was demonstrated in the extracts obtained from black carrots ($p < 0.05$), while the lowest content of phenolic compounds was detected in the orange carrot extracts ($p < 0.05$).

Keywords: total phenolic compounds, *Daucus carota L.*, the Folin-Ciocalteu methodology.

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A Shift in Lithuanian Rivers Water Quality during the Past Two Decades

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Abstract

The Water Framework Directive [1] aims to achieve good ecological status for all surface water bodies in the European Union until 2015. However, even by 2023, almost 60% of European rivers have not achieved this target according to the total nitrogen and total phosphorus indicators, which are key to controlling the eutrophication of lakes and seas [2]. The aim of this study was to evaluate the long-term changes of Lithuanian rivers, monitored by Lithuanian Environmental Protection Agency from 2005 till 2022. Our results have shown that average concentrations of biochemical oxygen demand (BOD7), ammonium-nitrogen (NH4-N) and phosphate-phosphorus (PO4-P) gradually reduced in Lithuanian rivers, their ecological status class (ESC) reached 'very good' in 2022, starting from 'average' in the beginning of monitoring period. However, the level of nitrates (NO3-N) in rivers was increasing, the ESC shifted from 'good' or even 'very good' during the previous years of monitoring till 'average' in the last 5 years of monitoring. Regression analysis confirmed significant reduction of NH4-N, BOD7, total suspended solids (TSS) and total P (R equals -0.065; -0.218; -0.06 and -0.032) as well as increase of NO3-N concentrations (R equals 0.277) ($p<0.05$). During the period 2016-2022 more than 80% of river monitoring sites could be attributed to 'good' or 'very good' ESC according to BOD7, NH4-N, PO4-P and total P; however, approximately 50% of monitoring sites are attributed to worse than 'good' ESC with respect to NO3-N and total N. Our study revealed the importance of river size for its ecological status and potential anthropogenic pollution. The worst ecological status is characteristic for small rivers (discharge <0.1 m^3/s). The percentage of 'good' and 'very good' ESC during the investigated period ranges from 77% (for NH4-N) till 44% (for total N). Whereas the biggest Lithuanian rivers (Nemunas and Neris) are attributed to 'good' or better ESC from 98% (for NH4-N) to 90 % (for total N) of all observations. Moreover, these rivers are never attributed to 'bad' ESC. To conclude, our study revealed that the ecological condition of Lithuanian rivers, especially those with the highest discharge, is good compared to other European rivers [3]; However, the increase in total N loads in small and medium-sized rivers, as well as the clearly poorer ecological status of the smallest rivers and streams, should be of concern.

Keywords: surface water monitoring, Water Framework Directive, eutrophication, ecological status class, rivers.

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Effect of DNA Methyltransferase 1 Inhibitors on Protein Expression in Cells

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DNA methylation in eukaryotes is a key epigenetic process, which is involved various processes within the cell, such as regulating gene expression, cell differentiation, embryonic development, adaptation to changes in environmental conditions and maintaining the stability of the genome via mobile genetic element silencing. In animals, this process is performed and maintained by cytosine DNA methyltransferase (Dnmt) enzymes, which recognise cytosine-phosphate-guanine (CpG) dinucleotides and catalyse the methyl group transfer reaction from an S-adenosyl-L-methionine cofactor (SAM or AdoMet) onto the fifth position of cytosine's pyrimidine ring, resulting in 5-methylcytosine (5mC). Dnmt1 is responsible for the maintenance of DNA methylation patterns during DNA replication and ensures the fidelity of epigenetic inheritance [1].

Aberrant DNA methylation is common in many cancers. Excessive methylation of tumour suppressor genes results in faster, more aggressive progression of disease and Dnmt1 overexpression is correlated with a worse prognosis for patients in several cancers [2]. For this reason, Dnmt1 is being further investigated as a potential target for chemotherapy. Previously discovered Dnmt1 inhibitors are nucleoside analogues, which have flaws like high cytotoxicity, mutagenic effects and non-specificity. A novel class of Dnmt1 inhibitors are dicyanopyridine derivatives, which show a stronger, less toxic, more specific and reversible inhibition of Dnmt1 [3]. In this study, we investigated the effect of two dicyanopyridine-based DNA methyltransferase 1 inhibitors - GSK1 and GSK2 - on relevant protein expression in two mouse cell lines (MEF and LLC1). Cells in culture were treated with inhibitors, then the protein expression of inhibitor target Dnmt1, target's partner Uhrf1, and target's paralog Dnmt3a were evaluated utilising western blotting.

Keywords: Dnmt1 inhibitors, Dnmt1 protein expression.

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Determination of Qualitative and Quantitative Composition of Phenolic Compounds in Chaga (*Inonotus obliquus* (Ach. ex Pers.) Pilát) Samples

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Abstract

Chaga is an edible mushroom that primarily grows on birch trees in northers countries. Due to the phytochemical composition chaga has been used for centuries in medical field [1]. Some of the most important biologically active compounds are polyphenols. They have a wide range of pharmacological effects for humans' health: anti-aging, anti-inflammatory, antioxidant and antiproliferative agents [2]. The aim of this research was to compare phytochemical composition of polyphenols of different chaga samples.

I. obliquus powder was bought from two different producers. The extracts were prepared using 1 g of raw chaga powder and 70% ethanol, followed by ultrasonication for 20 min. The resulting extracts were filtered through cotton wool with a help of a vacuum pump into 10 ml volumetric flasks. If necessary, the volume was adjusted to the mark with 70% ethanol. Prior to UHPLC-MS/MS analysis, the extracts were further filtered through a 0.22 µm membrane filter. Statistical analysis was performed using "MS Excel 2023" ("Microsoft", Redmond, USA). Both samples contained phenolic compounds: caffeic acid, chlorogenic acid, protocatechuic acid (3,4-dihydroxybenzoic acid), isoquercitrin and ferulic acid. Higher caffeic acid amount (13.37±0.001 µg/g) was found is a sample Number 1 and the lower caffeic acid content (8.46±0.43 µg/g) was found in sample Number 2. Higher content (36.07±0.31 µg/g) of protocatechuic acid was found also in sample Number 1. Sample Number 2 showed lower amount (17.49±0.29 µg/g) of protocatechuic acid. Higher content (2.91±0.61 µg/g) of chlorogenic acid was ascertained in a sample Number 2. Sample Number 1 had lower chlorogenic acid amount (0.92±0.05 µg/g). Also sample Number 2 showed higher content of isoquercitrin (0.63±0.14 µg/g). Lower amount (0.40±0.002 µg/g) of isoquercitrin was ascertained in a sample Number 1. Sample Number 2 showed higher ferulic acid content (0.14±0.081 µg/g) than sample Number 1 (0.07±0.03 µg/g).

In conclusion, our results indicate that the content of phenolic compounds, including caffeic acid, protocatechuic acid, chlorogenic acid, isoquercitrin and ferulic acid, varies significantly between two different producers.

Keywords: chaga, flavonoids, *Inonotus obliquus*, phenolic acids, phenolic compounds.

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Development of an Inflammatory Organoid Model of the Large Intestinal and Assessment of Its Response to *Janus* Kinase Inhibitors

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Abstract

Ulcerative colitis (UC) is a chronic inflammatory bowel disease associated with immune dysregulation and compromised intestinal barrier function. Despite advances in treatment, a significant proportion of patients become resistant to conventional and biologic therapies, underlining the need for reliable *in vitro* models to study disease mechanisms and test new targeted therapies [1].

In this study, we aimed to develop and validate a 3D colonic organoid model for mimicking human colonic epithelium. Organoids were shown to contain key epithelial cell types and preserved apical-basolateral polarity, confirming their structural and functional relevance by immunofluorescence analysis. Inflammation was induced by a TNF-α and IFN-γ cytokine mix, and organoids were treated with Janus kinase (JAK) inhibitors – upadacitinib (UPA) and tofacitinib (TOFA) – at two concentrations (UPA at 0,1 µM and 1 µM; TOFA at 1 µM and 10 µM). Gene expression was analyzed using RT-qPCR. A time-dependent increase in *TNF*, *CXCL9*, and *CCL5* gene expression confirmed inflammation induction, with 24 h being the peak. UPA and TOFA both reduced inflammatory gene expression, particularly *CXCL9*, which was significantly suppressed at 24 h across all treatment conditions. UPA 1 µM also significantly reduced *CCL5*, while TOFA 10 µM lowered *TNF* expression. Both inhibitors restored *TJP1* and *OCLN* expression, indicating improved epithelial barrier integrity, and TOFA 10 µM also increased *MKI67* expression, suggesting enhanced regeneration [2].

In conclusion, this organoid-based UC model effectively mimics intestinal inflammation and demonstrates responsiveness to JAK inhibitors, proving to be a promising platform for drug screening and applications in personalized medicine.

Keywords: Ulcerative colitis, organoids, JAK inhibitors, upadacitinib, tofacitinib, *CXCL9*, intestinal barrier, inflammation, precision medicine.

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Waste food polymers and microbial biocontrol agents as promising enhancers of growth, development and quality of oregano (*Origanum vulgare* L.)

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Abstract

The study was aligned with existing research on how environmental factors affect the growth and chemical composition of cultivated plants, as well as on cultivation techniques aimed at improving the quality and efficiency of bioactive compound production.

The study evaluated the influence of variable soil amendment strategies using food-grade polymers in the form of sodium alginate, guar gum and xanthan gum, both independently and in combination with *Trichoderma virens* TRS 106 and *Trichoderma atoviride* TRS 25 spores (P, TRS, TRS+P) and different light regimes, on the morphophysiological responses of *Origanum vulgare* L. plants. The study assessed the effects of the above factors on (i) fresh and dry weight biomass and shoot length of plants, (ii) moisture content, (iii) average leaf number and leaf blade area, and (iv) content of plant pigments, free sugars and terpenoids.

In *O. vulgare* plants TRS+P grown under 174 µmol/(m^2·s) light conditions, an increase in fresh biomass and moisture content values was observed compared to the control. With regard to dry biomass weight and shoot length, no significant effect of *Trichoderma* spores and food biopolymers was observed in plants grown under 174 and 600 µmol/(m^2·s) light conditions. At the same time, an increase in leaf blade area was observed in TRS and TRS+P plants grown under 174 and 600 µmol/(m^2·s) light conditions. The application of polymeric components and *Trichoderma* resulted in an increase in chlorophyll a and b, anthocyanins and carotenoids mainly in TRS, P, and/or TRS+P plants grown under 174 µmol/(m^2·s) light conditions. In plants grown under 174 µmol/(m^2·s), a significant effect of biopolymers on the increase in glucose and terpenoid content was shown in the P plants and in glucose and fructose in the TRS+P plants. An increase in sucrose content was only observed in the P plants, grown under 600 µmol/(m^2·s) light conditions. In the same light conditions, an increase in terpenoid content compared to the control was observed mainly in the TRS, P, and TRS+P plants. The results obtained indicate a positive effect of the inoculated polymers and Trichoderma fungi on the growth and water content of oregano plants and on the content of photosynthetic pigments, particularly in plants grown under 174 µmol/(m^2·s) light conditions. However, it was under light conditions of 600 µmol/(m^2·s) that the plants produced more terpenoids, with potentially beneficial health effects.

Keywords: *Origanum vulgare*, *Trichoderma*, food polymers.

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Spectrophotometric Analysis of *Canabis sativa* L. Extracts and Evaluation of Their Anticancer Activity *in vitro*

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Abstract

Cannabis sativa L. is one of the oldest plant species cultivated by humans and has increasingly gained attention in scientific research due to its wide-ranging pharmacological properties, particularly those associated with methanolic extracts. These extracts are rich in bioactive compounds - such as flavonoids, terpenoids, and other phytochemicals - which, as shown in recent studies, significantly contribute to their antioxidant activity. The presence of these compounds suggests that *Cannabis sativa* L. may serve as a valuable natural source of health-promoting substances, especially antioxidants [1].

The aim of this research is to evaluate the total phenolic content (TPC), total flavonoid content (TFC), and radical scavenging activity (RSA) of *Cannabis sativa* L. using spectrophotometric analysis, as well as to assess the anticancer activity of the extracts against the renal carcinoma cell line (Caki-1). Extracts were prepared from dried plant material, specifically from separate plant parts - leaves, blossoms, and seeds. The extraction was carried out using the maceration method with 50% and 75% methanol. TPC, TFC, and RSA were quantified using spectrophotometric methods. The antioxidant properties of the bioactive compounds were assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay [2].

The results, expressed in rutin equivalents (mg RE/g), indicated that the total phenolic content across the blossoms, leaves, and seed methanolic extracts of *Cannabis sativa* L. ranged from 72.35 mg RE/g to 106.71 mg RE/g. The total flavonoid content ranged from 35.14 mg RE/g to 75.87 mg RE/g. The radical scavenging activity ranged from 45.38 mg RE/g to 87.35 mg RE/g, depending on the plant part analyzed and the extraction solvent used. The anticancer activity of the extracts was evaluated in vitro using the MTS assay on Caki-1 cell lines. The assay was conducted after 24, 48, and 72 hours of treatment with various concentrations of bioactive compounds. The extracts obtained from dried blossoms, leaves, and seeds of *Cannabis sativa* L. inhibited Caki-1 cell proliferation in a concentration - and time-dependent manner.

Keywords: anticancer activity, bioactive compounds, spectrophotometric analysis.

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Phytochemical Analysis and Evaluation of Antimicrobial Activity of *Aegopodium podagraria* L. Aerial Parts

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Abstract

Aegopodium podagraria L. is a common plant in the *Apiaceae* family. It is widely distributed throughout the country and is commonly found in forests. It spreads extensively across the ground by underground rhizomes and outcompetes other plant species, so it is often considered disadvantageous. However, it has been used since ancient times to treat gout, inflammatory states in kidneys and bladder or to facilitate wound healing which is linked to its antimicrobial activity [1]. The chemical composition of *Aegopodium podagraria* has not been fully identified, but it is known that its primary active compounds are polyacetylenes, such as falcarinol and falcarindiol [2]. However, to date, very little information is available on other compounds of the plant, and it is not yet known which compounds contribute to its antimicrobial properties. Thus, the aim of the study was to identify volatile compounds of *A. podagraria* leaves, flowers and stems by collecting them by headspace solid phase microextraction and analysing by gas chromatography with mass spectrometry detector (HS-SPME-GC-MS) and to evaluate antimicrobial activity against different bacteria. Antimicrobial activity was evaluated by well diffusion method with extract of leaves, stems and flowers, using 75% methanol as an extraction solvent.

In total, 26 compounds were identified in aerial parts of *A. podagraria*. Main components, found in all parts of the plant was caryophyllene, α-pinene, β-pinene, cymene and spathulenol. β-farnesene and germacrene were identified as the major constituents in stems and flowers, with relative percentages of 8.84% and 45.01% for β-farnesene, and 28.00% and 14.97% for germacrene, respectively. Among the analysed plant parts, the stems exhibited the highest diversity of volatile compounds, while leaves were found to have the least diverse volatile profile.

As for antimicrobial activity determination, extracts of aerial parts of *A. podagraria* demonstrated antimicrobial activity against *Escherichia coli* and *Micrococcus luteus*. The most effective extract for microorganism growth inhibition was obtained from the flowers, as it also contained the highest concentration of some volatile compounds, such as β-farnesene. This study provided insights about the compounds found in aerial parts of *A. podagraria* and their correlation with the plant's antimicrobial properties.

Keywords: *Aegopodium podagraria*, volatile compounds, antimicrobial activity.

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An Overview of Plant-Derived Bioactive Compounds Inhibiting Oral Pathogens: Potential for Natural Oral Care Solutions

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Abstract

The aim of this review is to explore and summarize how plant-derived bioactive compounds can inhibit oral pathogens, with an emphasis on their potential role in natural oral care products. Bioactive compounds derived from plants have shoved potential as natural alternatives for conventional antimicrobials to target oral pathogens. They include antibiofilm, antimicrobial and anti-inflammatory properties [1]. *In vitro* and *in silico* studies demonstrate the ability of polyphenolic compounds like quercetin, catechin, and cinnamic acid to inhibit biofilm formation by 65–87% in clinical isolates (*Pseudomonas aeruginosa*, *Bacillus* spp.) at low concentrations (MIC: 0.0625–0.5 mg/ml). This primarily happens through membrane disruption and interference with energy metabolism rather than quorum sensing [2]. Mediterranean herbs such as *Rosmarinus officinalis* (rosemary) and *Origanum vulgare* (oregano) exhibit potential activity against *Streptococcus mutans* biofilms (MIC: 0.01–1.25 mg/ml), competing with chlorhexidine in efficacy [3]. Improvements in formulation, such as oral sprays and nanoparticle delivery methods, enhance absorption and clinical application. Studies using products infused with plant extracts have shown lower levels of *S. mutans* in high-risk populations [4]. For standardizing those extracts and validating their long-term safety further *in vivo* studies are necessary, despite all promising results. These findings underscore the viability of plant-based strategies to address antibiotic resistance and advance sustainable oral healthcare solutions.

Keywords: oral pathogens, antibiofilm activity, polyphenols, mediterranean herbs.

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Comparison of the Phytochemical Composition and Biological Activity of Bee Pollen and Flower Pollen

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Abstract

As interest in natural health supplements continues to grow, understanding the differences between flower pollen and bee pollen becomes increasingly important. Flower pollen serves as the raw material that bees collect and enzymatically modify to produce bee pollen – a process that can alter its chemical and biological properties. It is suggested that pollen-bearing blooms evolved more than 140 million years ago. The earliest evidence of human consumption of bee products can be traced back to a painting from between 8000-5000 B.C. in Spain; pollen was considered to be the food of kings, due to its therapeutic potential [1]. Now using the latest analytical technologies, we can confirm these hypotheses.

This review focuses on comparison of the phytochemical composition and biological activity of flower and bee pollens. Both bee pollen and floral pollen contain a unique content of phytochemicals that vary depending on the plant origins from which they are gathered. The protein content varies among different flowers between 2-60% depending on the plant species [2]. The amino acid profile of bee pollen is typically richer and more diverse than that of flower pollen, probably due to the nectar or saliva addition to bee pollen during gathering [3].

There is a lack of scientific data comparing biological activity of both type of pollen collected from one botanical source; however, studies show bee pollen and flower pollen possess antioxidant and antimicrobial activity against Gram-positive and Gram-negative bacteria [4, 5].

Overall, bee pollen is generally superior to flower pollen for human consumption. This superiority is attributed to its rich composite of nutrients, enhanced digestibility, and established health benefits, making it more suitable for dietary inclusion [6,7]. This diverse nutritional profile, coupled with its functional attributes, positions bee pollen as a more advantageous choice over flower pollen [8].

Keywords: biological activity, flavonoids, phytochemical composition.

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Prevalence of Micromycetes in Strawberries in Relation to Cultivar and Ripening Stage

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Abstract

Micromycetes are a diverse group of microscopic fungi, many of which are important plant pathogens that cause fruit and berry deterioration both before and after harvest [1]. Strawberries, with their high water and sugar content and soft skin, are particularly vulnerable to mould infections [2,3]. The most identified micromycetes in strawberries are *Botrytis cinerea*, *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., and *Alternaria* spp. [4,5]. The spread of such pathogens depends on several factors, including the genetic characteristics of the cultivar, environmental conditions, growth technology and fruit maturity [6]. Studies have shown that *Botrytis cinerea* is the main causal agent of strawberry blight, especially in humid weather and dense vegetation [7]. Cultivars '*Elsanta*', '*Albion*' and '*Honeoye*' have a higher susceptibility to this pathogen, while '*Sonata*' is considered to be more resistant to *Cladosporium herbarum* and *Alternaria alternata* [6,8]. The stage of ripeness is a key risk factor, with a significantly higher prevalence of micromycetes in fully ripe berries, due to a reduction in the concentration of acidic and phenolic compounds and an increase in the sugar content, which favours fungal growth [9,10]. In addition to genetic characteristics, the method of cultivation is also important: some authors have suggested that strawberries grown in organic systems are often less infected by micromycetes than those grown intensively, possibly due to the higher diversity of the microbiota and the lower chemical stress [11]. Integrated measures, including varietal selection, optimised harvest timing, and maintenance of natural antifungal properties, could help to reduce the impact of pathogens and increase the shelf life of berries [12].

Keywords: micromycetes, strawberries, cultivar, ripening stage.

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Sporulation and Production of Secondary Metabolites in *T. harzianum*

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Abstract
Fungi of genus *Trichoderma* are widely used in agriculture as a biocontrol agent; however, the medicinal use of this microorganism was not explored until recent years [1]. It is accepted that the success of *Trichoderma* in the field of agriculture is due to its ability to produce a vast variety of secondary metabolites [2]. This study explores what effect different nutritional mediums have on *T. harzianum* spore and secondary metabolite production. Sporulation of each sample was first evaluated spectroscopically and later quantitively estimated using a serial dilution method. For the detection of secondary metabolites, various colorimetric tests were performed, notably – modified Arnow's test for catecholate type siderophore detection. Early results suggest that samples grown on malt extract medium supplemented with $CaCl_2$, as well as on 10% malt extract medium, shown an increase in number of spores produced, meanwhile, malt extract medium with $CaCl_2$ added at the beginning of sporulation, showed a significant decrease in spore production. Experiments with catecholate type siderophores revealed that malt extract medium with $CaCl_2$ showed a slight increase in production compared to control medium, while the rest showed a decrease. These preliminary results demonstrate that by modifying various growth factors such as media composition and stress, we can stimulate *T. harzianum* to produce bigger amounts of beneficial metabolites that could be used in the pharmaceutical industry.

Keywords: *T. harzianum*, sporulation, siderophores, catecholate type siderophores, calcium chloride.

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Total Peroxidase Enzymatic Activity of *L. edodes, I. lacteus* and *P. eryngii* after Heat Shock

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Abstract

When exposed to sudden increase in temperature, most organisms respond by transiently inducing the synthesis of a variety of metabolites, predominantly proteins.

Fungi, like many other living organisms, are very sensitive to temperature fluctuations, especially heat shock, which can significantly affect their cellular and physiological functions, including enzyme activity and protein conformation. This response to thermal stress can be used to induce and/or increase the production of a variety of molecules useful in medicine or industry. Therefore, the aim of this study was to evaluate the enzymatic activity of *L. edodes, I. lacteus, P. eryngii* after heat shock.

The experimental results shown that the enzymatic activity of all the investigated fungal strains, especially *P. eryngii*, increased when subject to heat shock. Among the procedures tested, the most evident increments were obtained by applying 40 °C for 40 min every 24 h for three times.

Keywords: white-rot fungi, ligninolytic enzymes, heat shock, peroxidases.

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Isolation and Purification of Enzyme Lysozyme from Chicken Egg White, Evaluation of Antibacterial and Antioxidant Effects

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Abstract

The present study focused on the isolation and purification of lysozyme from chicken egg white and the subsequent evaluation of its antibacterial and antioxidant activities. The experimental work employed two Gram-positive bacterial strains, *Azotobacter vinelandii* and *Lactiplantibacillus plantarum*, as test organisms, while enzymatic hydrolysis was facilitated using enzymes protease and amyloglucosidase. The experimental procedures included two successive purification and hydrolysis processes: the initial trial aimed to validate methodological reliability and serve as a control, whereas the subsequent application was conducted under optimized conditions to evaluate bioactivity of lysozyme. The results demonstrated that lysozyme purified using ammonium sulphate and cold acetone exhibited significant antibacterial and antioxidant properties, particularly against *Lactiplantibacillus plantarum*. Further analysis revealed that enzymatically hydrolysed proteins from chicken egg white produced bioactive peptides with enhanced efficiency. Notably, proteins treated with amyloglucosidase showed superior antioxidant capacity, while samples hydrolysed with protease presented stronger antibacterial effects, again with increased inhibition observed against *Lactiplantibacillus plantarum*. These findings highlight the potential of purified and enzymatically modified lysozyme as a functional biomolecule with both antimicrobial and free radical scavenging properties.

Keywords: chicken egg white, lysozyme, hydrolysis, isolation, purification, antibacterial activity, radical scavenging activity.

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Production of Ligninolytic Enzymes by Basidiomycetes Exposed to Thermal Stress

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Abstract

Basidiomycetes species are a particularly interesting species of fungi due to their remarkable adjustment abilities to adapt to harsh environmental conditions where they act as natural lignocellulose decomposers. Being exposed to different kinds of stress can confer detoxification abilities in white rot fungi by increasing extracellular discharge of ligninolytic enzymes. The main enzymes responsible for this process are lignin peroxidase, manganese peroxidase and laccase.

The applications of these enzymes are based on their capacity to degrade various compounds, and it's applicable to many industrial processes. Some of them are the food, paper and textile industries, bioremediation and organic, medical, pharmaceutical, cosmetic and nanotechnology fields.

For this reason, *B. adusta, H. erinaceus, P. eryngii* and *P. ostreatus* were subjected to 50 ºC and 10 ºC, temperatures above and below its optimal growth, for 20, 40 or 60 minutes, to compare the effects on the production of ligninolytic enzymes.

For a qualitative analysis, three solid mediums were used: MEA (1.5% agar + 3% malt extract), MEA + 0.015% Remazol Brilliant Blue R (RBBR) and MEA + 1g/L gallic acid for the detection of laccase and peroxidases. Liquid medium of 3% malt extract was employed when determining the concentration of enzymes.

Each strain had a different growth rate; however, all of them decreased as the duration of exposure to 50 ºC increased. Same justification was applied to their production of ligninolytic enzymes. In contrast, no significant alteration was observed when the same tests were performed at 10 ºC.

High temperatures impact negatively on the growth and production of these enzymes. However, lower temperatures do not appear to have an effect on either of these two matters.

Keywords: Basidiomycetes, thermal stress, ligninolytic enzymes, *P. ostreatus*, *H. erinaceus*.