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ABSTRACT BOOK

May 12th-13th, 2022
Kaunas, Lithuania

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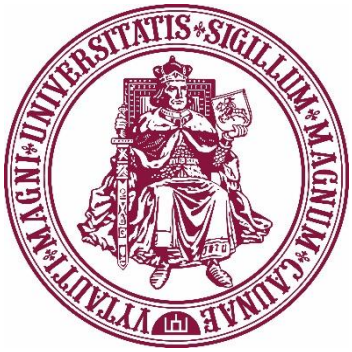
Organizing Institutions and Societies



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

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16th International Scientific Conference "The Vital Nature Sign"

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Program

Thursday, 12th of May 2022

9.00-9.15 (Time zone GMT+3) **Opening of the conference:** *Prof. Habil. Dr. Juozas Augutis (Rector of Vytautas Magnus university)*
Prof. Dr. Saulius Mickevičius (dean of Faculty of Natural Sciences)
Prof. Habil. Dr. Audrius Maruška (chairman of the conference)

Oral presentations. ***Chairpersons:*** *Prof. Ona Ragažinskienė; Prof. Audrius Maruška*

9.15-9.30 **Bread Sourdough Microorganisms - Possible Application and Challenges**
Elena Bartkiene, Modestas Ruzauskas, Egle Zokaityte, Vita Lele, Vytaute Starkute, Paulina Zavistanaviciute, Dovile Klupsaite, Laurynas Vadopalas, Sarunas Badaras, Darius Cernauskas, Vadims Bartkevics, Iveta Pugajeva, Zane Bērziņa, Romas Gruzauskas, Pranas Viskelis, Erika Mozuriene, and Grazina Juodeikiene

9.30-9.45 **Multifunctional Chewing Candy Formulations Based on Microbial and Plant Origin Ingredients**
Paulina Zavistanaviciute, Egle Zokaityte, Vytaute Starkute, Modestas Ruzauskas, Pranas Viskelis, Elena Bartkiene

9.45-10.00 **Application of Valorized Dairy and Cereal Industry By-Products for Nutraceutical Formulas Development**
Egle Zokaityte, Karolina Siriakovaite, Vytaute Starkute, Paulina Zavistanaviciute, Vita Lele, Erika Mozuriene, Dovile Klupsaite, Pranas Viskelis, Romas Ruibys, Raquel P. F. Guiné, Elena Bartkiene

10.00-10.15 **Safety Aspects of Fermented Traditional Lithuanian Edible Mushrooms**
Vytaute Starkute, Egle Zokaityte, Ernestas Mockus, Dovile Klupsaite, Justina Lukseviciute, Alina Bogomolova, Audrone Streimikyte, Fatih Ozogul, Elena Bartkiene

10.15-10.30 **The Impact of Lactic Acid Fermentation and Enzymatic Hydrolysis to Bee Pollen Antioxidant Properties**
Vaida Adaškevičiūtė, Vilma Kaškonienė, Audrius S. Maruška

10.30-11.00 ***Coffee break***

11.00-11.15 **Plant-Based Assays to Evaluate the Ecotoxicity and Genotoxicity of Bismuth in Different Environmental Matrices**
Massimo Zacchini, Fabrizio Pietrini, Laura Passatore, Serena Carloni, Lorenzo Massimi, Chiara Giusto, Valentina Iannilli

11.15-11.30 **The Mechanisms of Nickel Toxicity**
Sylwia Terpilowska

11.30-11.45 **Edible Wild Plants: A Sustainable and Healthy Food for the Future**
Enrica Donati, Isabella Nicoletti, Valentina Ramundi, Sara Cimini, Francesca Mariani

11.45-12.00 **The Contribution of Edible Cricket Flour to Quality Parameters and Sensory Characteristics of Wheat Bread, Including the Formation of Volatile Compounds and Their Relationship with Emotions Induced for Consumers**
Emilis Radvilavicius, Vytaute Starkute, Egle Zokaityte, Konstantinas Katuskevicius, Neringa Laukyte, Markas Fomkinas, Edikas Vysniauskas, Paulina Kasciukaityte, Skaiste Rokaite, Domantas Medonas, Emilija Valantinaviciute, Ernestas Mockus, Elena Bartkiene

12.00-12.15 (time zone GMT+3) **Novel Approach of The Wheat Bran Valorization to High Value Feed Stock for Broiler Chickens Nutrition**
Agila Dauksiene, Romas Gruzauskas, Grazina Juodeikiene, Dovile Klupsaite, Jolita Klementaviciute, Paulina Zavistanaviciute, Vytaute Starkute, Vita Lele, Egle Zokaityte, Erika Mozuriene, Elena Bartkiene

12.15-12.30 **Methodological Solutions in the Development of Methods Applied for the Determination of Neonicotinoids in Honey Samples Originated from Poland and Other Countries**
Magdalena Ligor, Małgorzata Bukowska, Renata M. Gadzała-Kopciuch, Bogusław Buszewski

12.30-12.45 **Application of Highly Ordered π-electron Materials and Contactless Conductivity Detector for Analysis of Bioactive Compounds from Plant Raw Material**
Audrius Maruška, Hirotaka Ihara, Makoto Takafuji, Elżbieta Skrzydlewska, Ona Ragažinskienė, Tomas Drevinskas, Mantas Stankevičius, Yutaka Kuwahara, Shoji Nagaoka, Nanami Hano, Rahman Mst Maria, Yongxing Hu, Domantas Armonavičius, Kristina Bimbiraitė-Survilienė, Vilma Kaškonienė, Loreta Kubilienė

12.45-13.15 Lunch break

Poster session I. ***Chairpersons:*** *Dr. Mantas Stankevičius, MSc. Kristina Bimbiraitė-Survilienė*

13.15 **The Influence of Bystander Effect on Viability of Untreated Cells After Combination of Calcium Electroporation, Irreversible Electroporation or Electrotransfer of Anticancer Drug Bleomycin**
Neringa Barauskaitė, Paulius Ruzgys

13.22 **The Connection Between Specific Glioma Stem Cells Genes and GBM Patients Outcomes**
Raminta Bagdanavičienė, Giedrius Steponaitis

13.28 **Allelopathic Effect on Germination of Invasive *Impatiens* spp.**
Ligita Baležentienė

13.35 **Photosynthetic Capacity of Some Invasive Species**
Ligita Baležentienė

13.42 **Impact of Soil Physical Parameters on Respiration Intensity**
Ligita Baležentienė

13.49 **Evaluation of Antioxidant and Antibacterial Activity of Bee Collected Pollen Fermented with Tibico and Kombucha Cultures**
Lukas Asanavičius, Vilma Kaškonienė, Nicola Tiso, Audrius S. Maruška

13.56 **Organic Farming Waste Material as a Driving Force for Development of Products for Use as Repellents**
Marta Berga, Laura Pastare, Liene Kienkas, Kristīne Berķe-Ļubinska, Ieva Mežaka, Arta Kronberga, Ilva Nakurte

14.03 **Extracellularly Triggered Cell Viability Decrease After Bleomycin Electrotransfer or Irreversible Electroporation**
Ugnė Borinskytė, Neringa Barauskaitė, Paulius Ruzgys, Saulius Šatkauskas

14.10 ***Bison Bonasus* Infection of *Babesia* Spp.**
Algimantas Paulauskas, Asta Aleksandravičienė, Dalia Černevičienė, Loreta Griciuvienė, Artūras Kibiša, Indrė Lipatova, Jana Radzijevskaja, Irma Ražanskė

14.17 **Diversity of Phenolic Compounds in Flowers of *A. kolomikta* (Rupr. & Maxim.) Maxim.**
Laima Česonienė, Paulina Štreimikytė, Mindaugas Liaudanskas, Vaidotas Žvikas, Pranas Viškelis, Jonas Viškelis, Remigijus Daubaras

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14.25-14.40 Coffee break

(time zone GMT+3)

Poster session II. *Chairpersons:* *Dr. Benas. G. Urbonavičius, Dr. Vilma Kaškonienė*

14.40 **Characterization and Liming Effect of Pelletized Lime Kiln Dust Combined with Biomass Combustion Ash on Soil Properties**
Donata Drapanauskaite, Thrinethra Shankar

14.47 **Chemical Diversity of Three Various Eleuthero Species Grown in Territory of Latvia**
Ilva Nakurte, Marta Berga, Arta Kronberga, Laura Kaļāne, Dainis Jakovels, Ieva Mežaka

14.54 **Repeated Heat Waves Negatively Affect Photosynthetic Energy Use Efficiency and Growth of Fescue**
Irena Januškaitienė

15.00 **Subclinical Mastitis detection in Dairy Cows Using Lactose as Biomarker**
Ramūnas Antanaitis, Vida Juozaitienė, Vesta Jonike, Walter Baumgartner, Algimantas Paulauskas

15.07 **Swallow-Wort (*Vincetoxicum lutea* L.) Leaves Extracts Aroma Profile and Antioxidant Properties**
Jovita Jovaišaitė, Laura Tamkutė, Renata Baranauskienė, Audrius Pukalskas, Petras Rimantas Venskutonis

15.14 **1*H*-Indol-3-ylcarbhydrazide and its Regioisomers: Synthesis, Chemical Modification and Investigation**
Gabrielė Juraitytė, Jiří Voller, Selvina Bezaraitė, Monika Iškauskienė, Alena Kadlecova, Vida Malinauskienė, Asta Žukauskaitė, Algirdas Šačkus

15.21 **Development of 3D Printable Radiation Shielding**
Kumar S. Mutyala, Benas G. Urbonavičius

15.28 **Seasonal Variation of Pollution with Organic and Biogenic Compounds in Jiesia River, Affected by Fish Farming**
Giedrė Kacienė, Monika Andriušytė

15.35 **Differences in Water Quality of Lithuanian Lakes and Reservoirs in 2005-2020**
Giedrė Kacienė, Mantas Jurkša

15.42 **Nonsteroidal Anti-Inflammatory Drug Bioremediation Using White-Rot Fungi**
Paulius Kačkauskas, Mantas Stankevičius, Rūta Mickienė, Audrius Maruška

15.49 **Changes of Minor and Major Cannabinoids in Hemp Methanolic Extracts During Storage**
Algimanta Kundrotaitė, Karolina Barčauskaitė

15.55 **Evaluation of the Dependence of Antioxidant Activity of Berries and Leaves of Medicinal Plants on Their Preparation**
Rugilė Karklytė, Vilma Kaškonienė, Audrius Maruška

16.02 **Effects of Chemoterapic FR901464 Compound on mRNA Expression in Hypoxic Cells**
Karina Šapovalovaitė, Laurynas Vilys, Arvydas Kanopka

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Friday, 13th of May 2022

Oral presentations. ***Chairpersons:*** *Dr. Paulius Ruzgys, Dr. Nicola Tiso*

9.00-9.15 (time zone GMT+3) **Ultrasound-Assisted Production of Resistant Starch in Rice Milling By-Products matrix: Evaluation of Physicochemical, Structural, and Technological properties**
Ruta Vaitkeviciene, Joana Bendoraitienė, Rimgailė Degutytė, Grazina Juodeikienė, Daiva Zadeike

9.15-9.30 **A Principal Component Analysis of Cold Plasma Treatment Effect on Morphometric and Biochemical Parameters of *Stevia rebaudiana***
Justinas Venckus, Rasa Žūkienė

9.30-9.45 **New Technologies for the Stimulation of Natural Sweeteners Biosynthesis in *Stevia Rebaudiana* Bertoni**
Erika Endriulaitytė, Augustė Judickaitė, Rasa Žūkienė

9.45-10.00 **Fluorescently Labelled Auxins and Elucidation of their Biological Activity**
Kristýna Bieleszová, Zuzana Gelová, Karel Doležal, Miroslav Strnad, Ondřej Novák, Asta Žukauskaitė, Jiří Friml

10.00-10.15 **TSP-1 in Glioma Patients Blood Serum and Tumor Tissue**
Egidija Satkevičiūtė, Daina Skiriutė, Kęstutis Skauminas, Rūta Urbanavičiūtė

10.15-10.30 **Environmental Fate of COVID-19 Antigen Test Kit**
Samanta Kaupaite, Ahmed Hosney, Karolina Barčauskaitė, Ahmed Azzam

10.30-10.45 ***Coffee break***

10.45-11.00 **Nano-Ag Particles Embedded in C-matrix Preparation, Properties and Application in Cell Metabolism**
Sylwia Terpiłowska, Stanisław Głuszek, Elżbieta Czerwosz, Halina Wronka, Piotr Firek, Jan Szmidt, Małgorzata Suchańska, Justyna Kęczkowska, Bożena Kaczmarska, Mirosław Kozłowski, Ryszard Diduszko

11.00-11.15 **Silver Nanostructures in LDI-MS of Low Molecular Weight Compounds**
Gulyaim Sagandykova, Piotr Piszczek, Aleksandra Radtke, Radik Mametov, Oleksandra Pryshchepa, Dorota Gabryś, Mateusz Kolankowski, Paweł Pomastowski

11.15-11.30 **The Study on the Formation of Supersaturated Complex of Iron with Bovine Lactoferrin**
Oleksandra Pryshchepa, Katarzyna Rafińska, Adrian Gołębiowski, Mateusz Sugajski, Agnieszka Rogowska, Gulyaim Sagandykova, Paweł Pomastowski, Bogusław Buszewski

11.30-11.45 **Lactoferrin As a Carrier of Iron Ions**
Katarzyna Rafińska, Oleksandra Pryshchepa, Adrian Gołębiowski, Paweł Pomastowski, Bogusław Buszewski

12.00-12.15 **New Methods of Preparing Plant Material for the Extraction of Bioactive Compounds**
Aneta Krakowska-Sieprawska, Anna Kiełbasa, Katarzyna Rafińska, Magdalena Ligor, Bogusław Buszewski

12.15-12.30 **New Approaches in Analytics of Plant Secondary Metabolites**
Magdalena Ligor, Bogusław Buszewski

12.30-13.00 ***Lunch break***

(time zone GMT+3)

Poster session I. ***Chairpersons:*** *Dr. Mantas Stankevičius, MSc. Kristina Bimbiraitė-Survilienė*

13.00 **Screening of Anticancer Compounds Accumulating Local Plants and Evaluation of Their Activities**
Domantas Armonavičius, Audrius Maruška

13.07 **Investigation of Biologically Active Compounds of *Chamerion angustifolium* L.**
Kotryna Kašėtaitė, Mantas Stankevičius, Rūta Mickienė, Audrius Maruška

13.14 **Soil Microbial Biomass Response to Application of Different Organic Fertilizers**
Rammukund Kishore Kumar, Modupe Doyeni, Vita Tilvikiene, Karolina Barcauskaite

13.21 **Influence of Different Treatments on Tannins in *Quercus robur* Acorns**
Aurelija Kondratavičiūtė, Vilma Kaškonienė, Audrius Maruška

13.28 **Essential Oil Composition and Antimicrobial Activity of Blackcurrant (*Ribes nigrum* L.) Buds**
Renata Baranauskienė, Renata Žvirdauskienė, Ugnė Navickaitė, Petras Rimantas Venskutonis

13.35 **Screening of Estonian Plants for Anti-*Borrelia* Phytochemicals**
Pille-Riin Laanet, Olga Bragina, Piret Saar-Reismaa, Merike Vaher

13.42 **Analysis of Secondary Metabolites and Antimicrobial Properties of *Levandula* L. (*Levandula angustifolia* L.) and *Helichrysum* L. (*Helichrysum arenarium* L., *Helichrysum italicum* L.)**
Karolina Lankaitė, Rūta Mickienė, Audrius Maruška

13.49 **Development of Gels as Stationary Phases for Chiral Capillary Electrochromatography**
Ernesta Lisauskaitė, Audrius Sigitas Maruška, Mantas Stankevičius, Tomas Drevinskas, Kristina Bimbiraitė-Survilienė

13.56 **Combined Addition of Biochar, Bioactivators and Plants as Synergic Strategy for the Treatment of PH-Contaminated Soil**
Valentina Mazzurco Miritana, Laura Passatore, Massimo Zacchini, Fabrizio Pietrini, Serena Carloni, Eleonora Peruzzi, Sara Marinari, Luisa Massaccesi, Anna Barra Caracciolo, Ludovica Rolando, Isabel Nogues

14.03 **An Impact of Volatile Disinfectants on Airborne Microorganisms**
Austėja Narauskaitė, Rūta Mickienė, Vilma Kaškonienė, Audrius Maruška

14.10-14.25 ***Coffee break***

Poster session II. ***Chairpersons:*** *Dr. Vilma Kaškonienė, Dr. Rūta Mickienė*

14.25 **The Research and Application of Medicinal (Aromatic) Plants for the Solution of Human Health Problems**
Nina Favergeat, Ona Ragažinskienė, Audrius Sigitas Maruška

14.32 **Evaluation of $CaCl_2$ influence on pDNA Electrotransfer Efficiency and Cell Viability Dynamics**
Rūta Palepšienė, Saulius Šatkauskas

14.39 **Bioremediation of Polycyclic Aromatic Hydrocarbons from Railway Wooden Sleepers Under Field Conditions**
Erika Raibužytė, Mantas Stankevičius, Audrius Maruška, Rūta Mickienė, Nicola Tiso, Kristina Bimbiraitė-Survilienė

14.56 (time zone GMT+3) **Physicochemical Properties of Chitosan Vary Depending on Living Source and This Change Has Effects on Blood Plasma Parameters**
Aurelija Ramanauskaitė, Bahar Akyüz-Yılmaz, Ivan Al-Jaf, Behlül Koç-Bilican, Vykintas Baublys, Murat Gül, Sinan İnci, Hediye Çiğdem Şimşek, Lalehan Akyüz, Murat Kaya

15.03 **Study of Chitosan Antifungal Properties**
Aurelija Ramanauskaitė, Povilas Mulerčikas, Vykintas Baublys, Sonata Kazlauskaitė

15.10 **Can Deforestation Affect the Diversity of Testate Amoebae?**
Šatkauskienė Ingrida

15.17 **Study of Qualitative and Quantitative Composition of Phenolic Compounds in *Artemisia absinthium* L. Raw Material During Different Vegetation Stages**
Sandra Saunoriūtė, Ona Ragažinskienė, Lina Raudonė, Liudas Ivanauskas, Mindaugas Marksa

15.24 **The Role of Hypoxic Microenvironment in Cancer Related Genes Pre-mRNA Alternative Splicing**
Kostas Sivickis, Aistė Semionovaitė, Inga Pečiulienė

15.31 **Evaluation of Volatile Compounds in Organic and Conventional Production Vegetable Raw Materials (edible Carrots, *Daucus Sativus* Röhl.)**
Justina Zykevičiūtė-Laugks, Laima Česonienė, Audrius Maruška

15.38 **Closing of conference**

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ORAL PRESENTATIONS

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FLASH PRESENTATIONS

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ORAL PRESENTATIONS

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Bread Sourdough Microorganisms - Possible Application and Challenges

Elena Bartkiene[1]*, Modestas Ruzauskas[1], Egle Zokaityte[1], Vita Lele[1], Vytaute Starkute[1], Paulina Zavistanaviciute[1], Dovile Klupsaite[1], Laurynas Vadopalas[1], Sarunas Badaras[1], Darius Cernauskas[1], Vadims Bartkevics[2], Iveta Pugajeva[2], Zane Bērziņa[2], Romas Gruzauskas[3], Pranas Viskelis[4], Erika Mozuriene[1], Grazina Juodeikiene[3]

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Abstract

Most agro-industrial by-products contain highly valuable components that are not used efficiently. A potential source for efficient use by-products is the extraction of those beneficial compounds. However, in most cases, solvent extraction is not economical as it involves (I) the use of toxic extractants that hinder their management, (II) high temperatures, which lead to degradation of thermolabile compounds. In addition, toxic compounds can be concentrated in the end-product - extract during extraction. From this point of view, the use of technologies that enable valorization of the whole by-product is a promising way to increase the efficiency and sustainability of the process. Our research group presented the concept of berries and fruits (raspberries, blackcurrants, apples, rowanberries), dairy industry (sour whey, milk permeate), and cereal industry by-products valorization by using antimicrobial lactic acid bacteria (LAB) strains, previously isolated from spontaneous sourdough. Based on the research results, it can be stated that sourdough LAB can be applied in the food industry to the effective valorization of by-products and the preparation of safer (lower mycotoxin and biogenic amines concentration), higher-value food (with desirable antimicrobial, prebiotic and probiotic properties), feed (with probiotic, antimicrobial, microbiota modulation properties), and nutraceuticals (with immune system modulation characteristics).

Keywords: sourdough, lactic acid bacteria, valorization, sustainability.

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Multifunctional Chewing Candy Formulations Based on Microbial and Plant Origin Ingredients

Paulina Zavistanaviciute[1], Egle Zokaityte[1], Vytaute Starkute[1], Modestas Ruzauskas[1], Pranas Viskelis[2], Elena Bartkiene[1]

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Abstract

The aim of this study was to develop multifunctional chewing candy formulations based on microbial and plant origin ingredients. To increase sustainability of the formulations, berries by-products were used for product development. The optimal quantities of lyophilised raspberry and blackcurrant by-products were selected according to product overall acceptability, also, two texture forming ingredients for chewing candy preparation were tested (gelatin and agar). The highest overall acceptability showed chewing candies prepared with gelatin and 5 g of raspberry and 5 g blackcurrant by-products. In addition, product functionality was improved with the addition of pure *Lactiplantibacillus plantarum* LUHS135, *Liquorilactobacillus uvarum* LUHS245, *Lacticaseibacillus paracasei* LUHS244, and *Pediococcus acidilactici* LUHS29 (previously multiplied in milk permeate and lyophilised) strains. Prior to experiment, an antimicrobial activity of the berry by-products and lactic acid bacteria strains was evaluated. It was established, that the tested lactic acid bacteria strains inhibited 3 pathogens out of 11, and blackcurrant by-products inhibited all the tested pathogens. For the developed chewing candies further mentioned analyses were performed: texture hardness, colour coordinates, total phenolic compound content, antioxidant activity, viable lactic acid bacteria count, overall acceptability, and emotions induced for consumers. Chewing candies with raspberry by-products and *Lactiplantibacillus plantarum* LUHS135, showed the highest total phenolic compounds content (147.16 mg/100 g d.m.), antioxidant activity (88.2%), lactic acid bacteria count after 24 days of storage (6.79 $\log_{10}$ CFU/g), the highest overall acceptability (9.52 scores) and induced the highest intensity of the emotion 'happy' (0.231). However, the chewing candies with 5.0 g of blackcurrant by-products in combination with lactic acid bacteria showed slightly worser results for the tested parameters. Finally, it can be stated that gelatin, raspberry and blackcurrant by-products, and *Lactiplantibacillus plantarum* LUHS135 are promising ingredients for desirable antimicrobial properties chewing candies preparation in a sustainable manner.

Keywords: lactic acid bacteria, raspberry by-products, blackcurrant by-products, milk permeate, sustainability, chewing candies.

Acknowledgments: The authors gratefully acknowledge the COST Action 18101 SOURDOMICS-Sourdough biotechnology network towards novel, healthier and sustainable food and bioprocesses.

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Application of Valorized Dairy and Cereal Industry By-Products for Nutraceutical Formulas Development

Egle Zokaityte[1], Karolina Siriakovaite[1], Vytaute Starkute[1], Paulina Zavistanaviciute[1], Vita Lele[1], Erika Mozuriene[1], Dovile Klupsaite[1], Pranas Viskelis[2], Romas Ruibys[3], Raquel P. F. Guiné[4], Elena Bartkiene[1]

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Abstract

The zero-waste economy has many challenges, of which the most important is the need for attractive technologies for the effective recycling of by-products. One of challenges in dairy industry is to effectively use large quantities of dairy by-products, e.g., the milk permeate (MP). MP is a dairy industry by-product obtained during milk protein concentrate production. The aim of this study was to develop nutraceutical in chewing form (NC) preparations. The main ingredients used for the preparation of added-value nutraceuticals were MP (containing GOS), extruded and fermented wheat bran (containing $\geq 6.0_{\log 10}$ colony-forming unit CFU/g viable antimicrobial property showing the LAB strains), psyllium husk (PH) (source of desirable hydrocolloids), and apple by-products (AP) (source of phenolic compounds). Also, for the preparation of NC, gelatin and agar were tested. In the NC formulations, citric acid (CA) was replaced to ascorbic acid (AA) to obtain a desirable hard texture of samples with agar. The optimal quantities of bioactive ingredients were selected by performing an overall acceptability (OA) test and evaluating emotions induced by consumers. Moreover, viable LAB count during storage, colour, texture, and antioxidant characteristics were evaluated. The highest OA (score 8.5) was shown for samples consisting of MP, PH, AP, CA, and xylitol (XY), a very strong correlation was found between OA and the emotion "happy" ($r = 0.907^{**}$). After 14 days of storage, samples consisting of gelatin, MP, PH, AP, and CA showed a LAB count higher than $6.0_{\log 10}$ CFU/g, but higher antioxidant activity were found for the NC prepared with agar. Finally, it can be acknowledged that fermented MP, PH, and AP can be used for preparation of added-value NC in a sustainable manner.

Keywords: nutraceuticals, overall acceptability, emotions induced in consumers, milk permeate, psyllium husk, apple by-products.

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Safety Aspects of Fermented Traditional Lithuanian Edible Mushrooms

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Abstract

The aim of this study was to ferment edible mushrooms (*Suillus luteus* (SL), *Boletus edulis* (BE), *Cantharellus cibarius* (Ca), and *Rozites caperata* (RC)) with lactic acid bacteria (LAB) strains possessing antimicrobial properties (*L. uvarum* LUHS245 and *L. casei* LUHS210) and to evaluate the influence of the technology used on the colour characteristics, pH, sensory properties, volatile compound (VC) profile, and formation of biogenic amines (BA). In addition, pre-treatment with ultrasound before fermentation and the emotions induced for consumers by the treated mushrooms were evaluated. The LUHS245 strain showed better preservation properties in the case of fungal inhibition; however, prolonged thermal treatment and/or ultrasound pre-treatment ensure safer fermentation. Mushroom species, type of pre-treatment, and the interaction of these factors had a significant effect on the samples' colour coordinates and pH ($p \leq 0.0001$). A greater variety of VC was identified in pre-treated and fermented samples, with VC 1-octen-3-ol predominant. Significant differences were established between the emotions induced for consumers, the emotion 'happy' being most strongly expressed for ultrasonicated and fermented BE. Significant correlations were found between the emotion 'happy' and some of the mushroom VC. Ultrasonication before fermentation, in comparison with thermal treatment before fermentation, increased the sum of BA in BE and RC (on average, by 15.3 and 2.4 times, respectively) but reduced it in Ca (on average, by 2.4 times). Despite good overall acceptability, further studies are needed to select appropriate LAB strains for fermentation of edible mushrooms, to ensure their safety in the case of BA formation.

Keywords: edible mushrooms, fermentation, lactic acid bacteria, volatile compounds, biogenic amines.

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The Impact of Lactic Acid Fermentation and Enzymatic Hydrolysis to Bee Pollen Antioxidant Properties

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Abstract

Bee collected pollen is one of the most recognized well-balanced food additives. Varied biochemical composition of this natural product, have significant pharmaceutical effect including antibacterial, antiviral, anti-inflammatory, anti-cancerogenic, hepatoprotective, antioxidants properties [1]. In order to improve influence, it is necessary to find, evaluate and apply the most effective way to break double layer pollen cell wall. The aim of this study was to compare the impact of enzymatic hydrolysis and lactic acid fermentation to antioxidant properties of bee collected pollen.

Nine bee collected pollen samples from different regions of Europe (Lithuania, Poland, Spain, Malta, Italy, Netherlands, Sweden, Denmark and Slovakia) were fermented spontaneously and with *L. rhamnosus* lactic acid bacteria culture. Also, samples were hydrolysed using *Clara-diastase*, lipase, *Viscozyme* L, protease, amyloglucosidase and cellulase enzymes. Spectrophotometric methods were used to evaluate total phenolic content, total flavonoid content, and antioxidant activity [2]. Moreover, chemometric analysis of the results was also performed to statistically compare effects of both processes in the samples.

Positive effect of lactic acid fermentation to bee pollen antioxidant properties was determined. Total phenolic content increased by 1.4-2.3 times, total flavonoid content by 1.1-1.6 times, and radical scavenging activity by 1.4-2.3 times. Enzymatically hydrolysed samples results showed significantly ($p \leq 0.05$) higher content of total phenolic compounds (after hydrolysis increased by 1.3-2.5 times), total flavonoid content (increased by 1.2-1.9 times) and radical scavenging activity (increased by 1.2-2.1 times) of bee collected pollen. According to the determined results, these processes can be considered as the simplest possible way to improve the antioxidant properties of bee pollen and to increase the digestibility and release of biologically valuable pollen compounds to the human organism.

Keywords: bee pollen, enzymatic hydrolysis, lactic acid fermentation, antioxidant activity.

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Plant-based assays to evaluate the ecotoxicity and genotoxicity of bismuth in different environmental matrices

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Abstract

Bismuth (Bi) is a minor metal with a natural abundance on Earth estimated as 0.025 ppm, ten-fold less than antimony. It is considered a "green metal", being recognized less toxic than other metals for many applications. From ancient times, Bi is utilised for treating many health diseases and, at present, Bi-based formulations are used for curing malignant tumors, stomach ulcers and the severe acute respiratory syndrome coronavirus (SARS-CoV) [1]. Given the raising utilisation of Bi in many different industrial and commercial products, a rapid increase of its presence in the environment has predictable. In this regard, extremely high Bi concentrations were reported in different environmental matrices due to its release from civil and industrial discharge. Despite the growing interest towards Bi use, scarce information on the toxicity of Bi on biota is actually present, particularly on plants. Even if no biological processes are associated to the presence of Bi in living organisms, the exposure to this metal is reported to negatively affect seed germination and growth in different plant species [2, 3, 4]. To expand the knowledge on the matter, a multi-scale experimental approach was performed by targeting: 1) germination index, root elongation and DNA damages (Comet assay) in *Lepidium sativum* L. seeds exposed for 72-h to different Bi concentrations; 2) biometric, physiological and biochemical endpoints in *Lepidium sativum* L. plantlets grown for 21 d in Bi-supplied soils in growth chamber; 3) biometric and physiological proxies in fronds of *Lemna minor* L. exposed to different Bi levels in nutrient solution under a 7-d toxicity assay. The ecotoxicological and genotoxic effects of Bi on the model plant organisms assayed in this work could contribute to shed light on Bi toxicity mechanisms on biota and to set the toxicity thresholds for the different environmental compartments.

Keywords: aquatic plants, bioindicators, chlorophyll fluorescence, Comet assay, duckweed, heavy metals, *Lepidium sativum* L., photosynthesis.

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The Mechanisms of Nickel Toxicity

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Abstract

Nickel is distributed around the world. This element is used in industry processes, such as alloy, stainless steel manufacturing, electroplating and welding, manufacturing batteries, catalysts and nanomaterials. Nickel exists in five valences or oxidation states of -1, +1, +2, +3, and +4, +2 being the most common. Nickel compounds are taken up by mammalian cells, where nickel ions are released and then they can induce cytotoxicity, apoptosis, chromosomal aberrations and morphological transformation.

Nickel(II) in cells induces ROS production. Increasing concentration of intracellular ROS and MDA causes the decrease of SOD, CAT and GPx activities. It means that nickel(II) weakens cell defence mechanisms and plays the role of prooxidant. ROS react with cell biomolecules: DNA, proteins, and unsaturated fatty acids. ROS can lead to DNA destruction and induce mutations such as: base-pair, rearrangement, deletions, insertions, and sequence implication. Nickel(II) induces the increase of percentage of tail DNA in comet assay. What is more, nickel chloride evokes frameshift mutation more often than base–pair substitution mutation. In our experiments the incubation with nickel(II) caused the increase of the percentage of cells in G2/M phase, while the percentage of cells in the S and G0/G1 phases decreased. Moreover, aneuploidy peak (subG1) of DNA content was observed. It can be concluded that nickel(II) arrests cell cycle in G2/M phase. Nickel(II) induces G2/M cell accumulation by inhibiting the activity of cdk1 through directly stimulating the expression of p53. Microscopic analysis showed giant or multinuclear cells and cytoplasmic vacuolation. Additionally, the treated cells displayed characteristic apoptosis in comparison to control cells. Additionally, Ni^{2+} induces the increase of FasL level and caspase-8 activity and NF-κB activation after ROS production leading to apoptosis. The increase of caspase 3, 6 and 9 was observed after nickel(II) treatment. Additionally, Bax induction, while Bcl-2 inactivation were observed.

Nickel(II) interacts with other microelements. Chromium(III) and nickel(II) show antagonistic effect – chromium(III) protects from nickel(II) toxicity. Synergistic effect was observed after high doses of iron(III) and nickel(II). However, iron(III) at low concentrations plays a protective role against nickel(II) toxicity.

Keywords: nickel, cytotoxicity, genotoxicity, apoptosis.

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Edible Wild Plants: A Sustainable and Healthy Food for the Future

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Abstract

Mediterranean countries are characterized by an enormous biodiversity and a rich heritage of edible wild plants (EWPs), which grow spontaneously in nature without human intervention, particularly in agricultural lands, fallow lands, forests and near streams and roadside [1]. Since ancient times EWPs were employed by rural community as source of food and for medicinal purpose. In the last century, the industrialization of agriculture together with cultural transformations have led to changes in eating habits, causing the loss of the knowledge acquired over generations regarding the use of these plants. Despite that, the increasing demand for healthy foods and natural antioxidants, combined with the development of sustainable cuisine, has renewed interest in the use of wild flora [2]. In addition, besides having very positive health effects, eating wild plants also provides economic benefits for rural areas through the sale of high-quality local products and promotes a sustainable development concept ("zero kilometer"). The health-promoting effect is due to the presence in these matrices of biologically active secondary metabolites like phenolic compounds, which have been reported to exhibit multiple pharmacological activities such as anticancer, anti-inflammatory, antimicrobial and antioxidant activities [3]. The present study is aimed at showing useful data for furthering the knowledge of edible wild plants properties. In particular, the purpose of this research was to identify and quantify the main phenolic compounds that were found to be characteristic for the EWPs under investigation, notably *Glechoma hederacea* and *Sonchus oleraceus*.

A simple method coupling Ultra Performance Liquid Chromatography (UPLC) with Photodiode-Array detector (PDA) and Electrospray Ionization Mass Spectrometry (ESI-MS) was developed for the identification and quantitation of the main phenolic compounds in the plants extract. Polyphenols determination was then followed by the analysis of the EWPs antimicrobial properties by Minimal Inhibitory and Bactericidal Concentration assays (MIC/MBC) and by determining the Minimum Biofilm Inhibitory Concentration (MBIC) using a microtitre plate assay.

Keywords: edible wild plants, phenolic compounds, antimicrobial properties, sustainable food.

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The Contribution of Edible Cricket Flour to Quality Parameters and Sensory Characteristics of Wheat Bread, Including the Formation of Volatile Compounds and Their Relationship with Emotions Induced for Consumers

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Abstract

This study evaluated the influence of edible cricket flour (ECF) on the quality parameters and sensory characteristics of wheat bread (WB), including the formation of volatile compounds (VC) and their relationship with emotions (EM) induced for consumers. ECF reduced dough pH, redness, and yellowness. At 5%, ECF increased the porosity of WB (by 7.87%). The quantity of ECF significantly affected WB specific volume ($p = 0.030$), porosity ($p = 0.0001$), shape coefficient ($p = 0.0001$), and mass loss ($p = 0.023$). All WB with ECF had a more intense colour and additive odour. Breads with 10% and 15% ECF had more intense overall, additive, acidity, and bitterness flavours. However, all WB had similar overall acceptability (OA) and no correlations were found between OA and VC. The EM 'happy' and 'sad' were expressed more intensely for WB with 15% ECF and significant correlations were established between the EM 'happy' and separate VC. The main VC in WB were ethanol; 1-butanol, 3-methyl-; 1-hexanol; estragole; and hexanoic acid. Finally, 5% ECF could be incorporated in the main WB formula without having a negative impact on bread quality. Also, ECF influences VC formation, and separate VC could be related to emotions induced for consumers.

Keywords: sourdough, lactic acid bacteria, valorization, sustainability.

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Novel Approach of The Wheat Bran Valorization to High Value Feed Stock for Broiler Chickens Nutrition

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Abstract

Corn, wheat, and soybean meal are the primary ingredients of poultry diets [1]. However, its global price is increasing because of the limited world yield in covering the demands for both humans and livestock [2]. Minimizing the feed cost and improve in sustainability could be achieved through the use of wheat bran (WB), a by-product of the dry milling of wheat grains into flour [2]. Promising methods that can be applied to improve the nutritive quality of WB are extrusion and microbial fermentation [3]. The growth performance, weight of internal organs, caecal pH and short chain fatty acids (SCFAs) of broiler chickens (BC) fed diets containing WB replacing the wheat portion of the diets were investigated in this study. BC were assigned to 2 groups: control group (CON group) – was fed by basic compound feed. The 3% of basal were replaced by extruded (at 130 °C by two different speeds of the extruder screw (20 and 25 rpm)) and fermented by *Lactobacillus casei* and *Lactobacillus paracasei* WB in the diet of treatment group (WB group) and fed for 15 days of fattening. After 15 days, the broilers of WB group received basic compound feed. Results demonstrated that that the body weight (BW) and feed conversion ratio (FCR) of broilers was increased by 4% in supplementation of WB, compared with control group ($p>0.05$). The weight of heart, liver and intestinal tract were increased in WB group ($p<0.05$). Compared to the control group, the WB group, showed significantly increased caecal propanoic, butanoic and pentanoic acids levels ($p<0.05$). In conclusion, an extruded and fermented WB replacement in the diet could ameliorate digestive processes and productivity in BC.

Keywords: Wheat bran, extruded, fermented, digestive tract, broiler chickens.

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Methodological Solutions in the Development of Methods Applied for the Determination of Neonicotinoids in Honey Samples Originated from Poland and Other Countries

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Abstract

The chemical composition of honey confirms that it is very complex matrix. It can be evidenced by over 300 components determined in different type of honey belonging to various chemical groups of compounds. The composition of honey depends on many factors such as climatic (insolation, humidity) and environmental conditions, available plants, a botanical and geographical origin, welfare of bees and many others. Among compounds being labelled as harmful and even toxic to pollinators are as follows: imidacloprid, acetamiprid, clothianidin, thiacloprid, thiamethoxam. The main aim of the investigations was the method development for the separation and determination of neonicotinoid residues in honey samples. The proposed methodology consisted in a QuEChERS (quick, easy, cheap, effective, rugged, and safe) method used for sample preparation and UHPLC/UV (ultra-performance liquid chromatography with ultraviolet detection) utilized for chromatographic analysis. The method has been validated in detail. The linearity, regression coefficients, limit of detection (LOD), limit of quantification (LOQ) and recoveries for thiamethoxam, clothianidin, imidacloprid, acetamiprid and thiacloprid have been determined. The calculated LOD value was in the range from 60.80 to 80.98ng/g, hence LOQ value was in the range from 184.26 to 245.40 ng/g. The developed method has tested on Polish honey and applied to honey from various countries. Several honey samples mainly buckwheat, honeydew, goldenrod, phacelia, rapeseed, linden, raspberry, acacia, polyfloral, sunflower were purchased in Poland. Additional investigations have been carried out for samples from different countries in Europe (Bulgaria, Czech Republic, France, Greece, Italy, Portugal, Romania) and other countries of the world like Australia, Brazil, Cameroon, Russia, USA and Turkey. The qualitative and quantitative analysis have been performed to evaluate the presence of neonicotinoid residues in honey. There was a total lack of these compounds in sunflower and honeydew honey. Except of these two varieties, only two samples of rapeseed honey and two samples of acacia honey from Poland and Romania were neonicotinoids free. For all other investigated samples, the neonicotinoid were found at least at the LOD or LOQ level.

Keywords: neonicotinoids, honey, QuEChERS, UHPLC/UV.

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Application of Highly Ordered π-electron Materials and Contactless Conductivity Detector for Analysis of Bioactive Compounds from Plant Raw Material

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Abstract

Research in area of anticancer materials plays an important role in today's scientific world. Phytochemical compounds with antioxidant, anti-inflammatory, and anti-cancer properties are most researched using physicochemical methods. These are renewable sources of raw materials that are gaining increasing demand for sustainable, green biotechnologies. In some cases, these compounds can be hardly distinguishable using analytical methods due to the similarity of their physicochemical properties.

One of the promising strategies for the synthesis of new generation sorptive materials is organic compounds with a highly ordered structure. These kinds of materials have a high selectivity potential when interacting with low-hydrophobic amphiphilic compounds. Some challenges must be overcome before using these high-ordered materials for analysis of the biologically active compounds of raw plant materials. One of them is the lack of transferability of light, which prevents using conventional detection systems like UV/VIS light detectors in capillary column. A contactless conductivity detection system also can be applied for microanalysis of bioactive compounds in plant raw material.

The main purpose of this study is to develop high-selectivity separation microsystem using a highly ordered π-electron structures, which could be integrated into the capillary format and applied for the determination of antioxidant, anti-inflammatory, and anti-cancer compounds using the contactless conductivity detector.

Keywords: highly ordered π-electron materials, contactless conductivity detector, antioxidant, anti-inflammatory, anti-cancer.

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Ultrasound-Assisted Production of Resistant Starch in Rice Milling By-Products matrix: Evaluation of Physicochemical, Structural, and Technological properties

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Abstract

Resistant starch (RS) is one of the resistant carbohydrates that is resistant to the digestion in the human small intestine. On the basis of the structural and chemical characteristics, RS has been divided into five categories. RS1 is starch unavailable to be digested due to physical constraints, such as an intact hull or incomplete milling, leaving the grain or seed partly intact. RS2 refers to native starch granules (such as banana, potato, and high amylose maize starch) that resist digestion due to the conformation or structure of the starch granule. RS3 represents the most RS fraction and is mainly retrograded amylose formed during cooling of gelatinized starch. RS4 is chemically modified starch, and RS5 is a helical structure lipid-amylose complex. The increase in consumers demand for high-quality food products has led to the growth in the development of new technologies and functional ingredients, such as RS.

In this work, a dual ultrasonication-retrogradation process was provided for the enhanced production of resistant starch (RS) in rice bran (RB) and its effect on RS physicochemical, structural, and technological properties was analysed. A low-frequency (850 kHz, power output 120 W) ultrasound (US) was applied for the treatment of RB material to produce resistant starch. The samples of isolated RS were evaluated for the granule morphology, crystallinity pattern, FT-IR spectroscopy, pasting and hydration properties. The highest yield of RS of 12.58 g/100 g d.w. was achieved by applying 1.8 W/cm^2 US power, 40 ^{o}C temperature with 20 min of processing time. Finally, ultrasonication resulted in disintegrated RS particles with affected chemical structure as was confirmed by SEM and FT-IR, respectively. The US cavitation for 20 min at temperatures of 40–70 ^{o}C reduced crystallinity of RS, improved mechanical and thermal stability of the RS paste, increased oil and water absorption, solubility, and swelling power. The developed method and technology bring low-temperature US processing of the rice milling by-products to create a functionalised ingredient for food.

Keywords: rice bran, ultrasound cavitation, resistant starch production, structure, pasting properties, hydration properties.

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A Principal Component Analysis of Cold Plasma Treatment Effect on Morphometric and Biochemical Parameters of *Stevia rebaudiana*

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Abstract

Stevia rebaudiana contains natural low-calorie sweeteners steviol glycosides (SGs) and other secondary metabolites, mainly flavonoids and cathechins, that have various beneficial effects on health [1]. Stevia plant grown from seeds are heterogenous in the morphological traits, the concentration, and the composition of bioactive phytochemicals. Cold plasma (CP) is known to improve various plant properties on the basis of the hormesis effect on seeds [2]. So far, only one study has been published on the effects of CP on stevia seeds whose findings suggests that CP impact on heterogeneity of desirable morphometric parameters (plant height, number of leaves on each plant, leaf dry mass) and its biochemical properties (SGs, total phenolic (TPC) and total flavonoid content (TFC), antioxidant activity (AA) in different extracts of leaves) is uncertain [3]. Taking into account the assumption that these multivariate parameters interact with each other during vegetation, the aim of this study is to understand the differences and relationship between them by carrying out principal component analysis (PCA).

Seeds of *S. rebaudiana* were irradiated with CP for 2, 5, and 7 min by using an atmospheric pressure scalable dielectric barrier discharge plasma device. The control group did not receive the treatment. Stevia seeds were allowed to germinate in a climate-controlled growth chamber. Morphometric parameters of seedlings were assessed 12 weeks after the sowing. 12-week-old stevia leaves were dried and powdered using a batch mill with the disposable grinding chamber. The extraction was carried out in triplicate by ultrasonication in 70% (v/v) ethanol. The most abundant and commercially important SGs found in stevia, rebaudioside A (RebA) and stevioside (Stev), were separated and quantified using high-performance liquid chromatography. TPC in extracts was measured spectrophotometrically by Folin–Ciocalteu method, whereas TFC was evaluated using $AlCl_3$ colorimetric assay. AA was assessed using a 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method.

Despite the fact that CP had no effect on morphometric parameters, it increased RebA and Stev, whilst decreasing TPC, TFC, and AA in treatment groups. PCA revealed that dry leaf mass inversely correlated with AA, TPC and Stev, but along with plant height it showed a positive correlation with RebA, TFC, and RebA/Stev ratio. Plant height and the number of leaves negatively correlated with Stev and the total amount of SGs.

Keywords: cold plasma, *Stevia rebaudiana*, principal component analysis, steviol glycosides.

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New Technologies for the Stimulation of Natural Sweeteners Biosynthesis in *Stevia Rebaudiana* Bertoni

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Abstract

Plant secondary metabolites are widely used in the pharmaceutical, food, cosmetic industries, therefore ways to increase their concentrations in plants are intensively investigated. Seed treatment with cold plasma (CP) and electromagnetic field (EMF) are modern organic farming technology that has been proposed to promote plant growth. Our research group demonstrated for the first time that *Stevia rebaudiana* Bertoni seed treatment with various types of cold plasma and electromagnetic field increase the biosynthesis of valuable secondary metabolites steviol glycosides (SGs) that are responsible for the sweetness of stevia and are widely used as natural sweeteners. Stevioside (Stev) and rebaudioside A (RebA) are the most abundant SGs in stevia. RebA is preferred over Stev for better taste and lack of bitterness. We have demonstrated that seed treatment by CP can increase Stev and RebA concentrations several times.

The aim of this study was to overview our research group results on the effect of *Stevia rebaudiana* Bertoni seed treatment (2-7 min) with different types of CP (dielectric barrier discharge (DBD) and capacitively coupled (CC) CP) and EMF on the amount and ratio of main SGs, stevioside (Stev) and rebausioside A (RebA) in the leaves of stevia and the kinetics of stimulated biosynthesis. CC CP increased the RebA concentration 1.5-fold and the concentration of Stev 7-11-fold depending on treatment duration. The optimal 2-min pre-sowing seed treatment with DBD CP increased the RebA concentration 2-fold, Stev - 14%, RebA/Stev ratio - 1.7-fold. The treatment of longer duration (5-7 min) had lesser effect than 2 min. EMF stimulated SGs at treatment durations 5-15 min. Stimulating effect of CP treatment persisted at least 14 weeks. The concentrations of other bioactive compounds as phenolics and flavonoids were decreased or unchanged by both types of CP treatment resulting in lower or unchanged antioxidant activity of stevia leaf extracts rich in SGs.

It can be concluded that a short time pre-sowing treatment of seeds with CP and EMF can be a powerful tool for the enhancement of biosynthesis/accumulation of SGs in stevia plants.

Keywords: *Stevia rebaudiana* Bertoni, seed treatment, cold plasma, electromagnetic field.

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Fluorescently Labelled Auxins and Elucidation of their Biological Activity

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Abstract

Auxin is one of the most important phytohormones, which controls various aspects of plant growth and development [1]. Establishment of auxin concentration gradient, collectively regulated by auxin biosynthesis, metabolism and polar transport, determines plant organ positioning and growth responses to environmental stimuli [2, 3]. Fluorescently labelled auxins provide a great opportunity to study and visualize auxin transport and localization sites without the need of specific marker lines.

Here we report novel fluorescently labelled derivatives of the natural auxin, in the form of a conjugate with different fluorophores and elucidation of their biological activity. These compounds do not possess auxin activity but on the contrary, they inhibit auxin-induced effects in a dose-dependent manner. The most promising compounds are further investigated for their effect on auxin-mediated growth and development. Visualization of tissue-specific localization of the compounds by confocal microscopy is also performed.

Keywords: auxins, IAA, *Arabidopsis thaliana*, fluorescent labelling, biological activity.

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TSP-1 in Glioma Patients Blood Serum and Tumor Tissue

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Abstract

Glioma is a neuroepithelial tumor originated from the CNS glial cells [1]. As the degree of malignancy of gliomas increases [2], the tumors become more infiltrated and the probability of their relapse grows too [3]. In order to avoid the high mortality, these tumors should be diagnosed and treated as early as possible [2]. The TSP-1 protein is thought to be a potential marker for glioma. It is a multifunctional glycoprotein that acts as an endogenous inhibitor of angiogenesis [4]. TSP-1 has been shown to reduce tumor vascular density, thereby inhibiting tumor progression and increasing patient survival in several other tumors [5].

The aim of this study was to evaluate the potential of TSP-1 as a molecular marker of gliomas in patient blood serum and postoperative tumor tissue.

In this study 48 blood serum and 43 postoperative tumor tissue specimens were tested. RT-PCR analysis was performed to examine the expression of the *TSP-1* gene in tumor tissues, and Western blot analysis was performed to determine TSP-1 protein levels in patient blood serum before and after tumor resection. By using statistical analysis of the data, gene expression and protein distribution were analyzed according to patient clinicopathological characteristics (*IDH1* gene mut. status, *MGMT* promoter met. status, age, gender). In addition, correlation analysis and Kaplan-Meier survival analysis were performed in this study. The statistical significance level was $p<0.05$.

Results showed statistically significant difference ($p<0.05$) between low-grade and high-grade glioma based on *TSP-1* gene expression. While evaluating TSP-1 protein levels in blood serum no significant differences were found between different malignancy grades before ($p>0.05$) and after ($p>0.05$) tumor resection. We also found that *TSP-1* gene expression is related with patient survival time ($p<0.05$) but protein levels in blood serum are not. Patients with lower gene expression survived significantly longer. As mentioned, all values were compared with individual patient characteristics: significant gene expression connection with *IDH1* gene mutation status ($p<0.05$) was found. However, no other associations were detected. Weak ($r=0.24$) and insignificant ($p>0.05$) correlation was identified between protein levels in serum and gene expression. Finally, we showed, that TSP-1 protein levels in serum before and after tumor resection differed significantly ($p<0.05$).

In conclusion, results confirmed that *TSP-1* gene expression in tumor tissue could be more reliable molecular marker of glioma than TSP-1 protein levels in blood serum.

Keywords: glioma, thrombospondin-1.

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Environmental Fate of COVID-19 Antigen Test Kit

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Abstract

Nowadays, a COVID-19 antigen test kit can be easily conducted at home, workplace, universities, and airports as an initial indicator for the infection of coronavirus. Some workplaces perform the test on a mandatory or optional basis for their employees, travellers, or students according to each country's restrictions. However, each manufacturer of COVID-19 antigen tests has illustrated how to dispose of the components of the test, the environmental fate is still unclear regarding end-user behaviour in disposing of the waste of the test, or due to the deficiency of a specific biohazard container for the medical waste disposal or the waste may be collected from the location by the non-authorized hazardous waste hauler. Eu regulation specified a special treatment for medical and biohazard waste to avoid transmission of infectious diseases and to reduce the environmental impact resulting from the medical waste components. The main aim of this study is to determine the environmental fate of the COVID-19 antigen test kit. A survey has been conducted to determine how the people behave toward disposing of COVID-19 antigen test kit waste components showed that 47.4% of people who participated in this study did not read the manufacturer disposal instructions of the COVID-19 antigen test kit, 67.5% did not dispose of the waste ingredients of COVID-19 antigen test kit according to manufacturer disposal guidelines. In addition, 69.9% disposed of the waste of the test components in one regular garbage, while 30.1% of people who participated in this study sorted the waste components of the COVID-19 antigen test in specific containers. Also, about 50% of interviewers answered that they do not have containers for bio-hazardous waste in their workplaces, universities, or homes, while 13.9% said that they already have specific bio-hazardous waste containers for a COVID-19 antigen test kit at their workplaces, universities, and homes. Moreover, 43.4% answered that the waste of the COVID-19 antigen test kit is not collected by an authorized hazardous waste hauler but collected by the municipal hauler as regular garbage collection. More attention needs to be paid by workplace directors, municipalities, and decision-makers to issue internal restrictions and protocols for disposing of the large numbers of COVID-19 antigen waste components in a safe way that could reduce the spreading of infectious diseases and reduce the environmental degradation of biohazardous waste. More studies and investigations are needed to have a clear view and better disposal options of the COVID-19 antigen test kit.

Keywords: COVID-19 antigen test kit, environmental fate, biohazardous waste.

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Nano-Ag Particles Embedded in C -matrix Preparation, Properties and Application in Cell Metabolism

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Abstract

The aim of this investigations is to show the influence of the size of nano-Ag grains embedded in carbonaceous matrix on the cell viability, genotoxicity in L929 cells and mutagenicity.

The carbonaceous – silver nanoparticles NPs powders were obtained on Si wafer substrate using PVD technology. The prepared material in the form of a film was studied with SEM with energy-dispersive X-ray spectrometry (EDX), x-ray diffraction (XRD) and Raman spectroscopy. Material in the form of powder was applied for biological studies. The specimen P1 was obtained by removing of C-Pd film from Si substrate, the specimen P2 was obtained by removing the C-Pd film from distance. Samples of nano-Ag mixed with carbon powder were dissolved in a complete medium at following concentrations: 1.5 g/ml for P1 and 2 g/ml for P2. Biological investigations were performed on L929 fibroblasts. The MTT reduction, the LDH release, the Neutral Red Uptake, Comet and Micronucleus assays and AMES assay were performed.

This material consists of Ag nanograins embedded in a carbonaceous matrix which stabilizes the silver nanograins preventing their aggregation, which was shown by SEM studies. These studies also allow us for determination of maximal size of Ag nanograins. This size is between 10 and 30 nm for a film sample while the size of Ag nanograins is much lower in a powder. Raman spectroscopy results showed that the carbonaceous matrix is composed of graphene–like domains with the size of ~14 nm.

The studied properties provide a comprehensive understanding of the influence of size of silver nanograins on cell viability, genotoxicity and mutagenicity. It can be seen that mitochondria may be the first part of the cell to be affected by both P1 and P2 specimens. The disintegration of cell membrane and lysosomes follows mitochondria damage. Both of the specimens used do not induce genotoxicity and mutagenicity. Based on toxicity studies it can be concluded that P1 and P2 were non toxic in the L929 cell line.

Keywords: genotoxicity, mutagenicity, cytotoxicity, nanocomposite, nano-Ag.

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Silver Nanostructures in LDI-MS of Low Molecular Weight Compounds

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Abstract

Mass spectrometric techniques may provide wealth of information on molecular profiles of the studied sample. Low molecular weight compounds represent a diverse class of compounds that can be found in food, environmental samples as well as their analysis have implications in forensics science and pharmaceutical industry. Moreover, their identification and comparison of the molecular profiles in low mass region (< *m/z* 500) in biological samples may bring benefits for metabolomics and thus, studies of diseases pathogenesis. For this purpose, there is a need to develop efficient MS platforms. Laser desorption ionization (LDI) with nanomaterials assisting ionization bring advantages for analysis of small biomolecules such as low chemical background, chemical stability and enhanced light adsorption in the UV-region. However, existing LDI systems based on noble metals such as silver and gold have multiple drawbacks for applications for clinical samples. Thus, the goal of the research was to evaluate the synthesized LDI-MS systems for analysis of small biomolecules. In this work, we present the LDI-MS targets synthesized via chemical vapor deposition based on silver nanostructures. The effect of the mass of precursor on the LDI-MS response of low molecular weight compounds has been studied. The LDI-MS efficiency was studied for standards of low molecular weight compounds of biological relevance such as adonitol, glucose, fructose, shikimic acid, cholesterol, phosphatidylcholine, oleic and palmitic acids, amino acids namely alanine, serine, methionine and phenylalanine in both ion-positive and ion-negative modes. In addition, sensitivity of the plates towards triacylglycerols has been studied.

Keywords: laser desorption mass spectrometry, small biomolecules, silver nanostructures, chemical vapor deposition

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The Study on the Formation of Supersaturated Complex of Iron with Bovine Lactoferrin

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Abstract

The study aimed the investigation the formation of iron-bovine lactoferrin (bLTF) supersaturated complex. The existence of such a complex was previously predicted and described in the literature [1]. Among others, the bLTF supersaturation with iron may be a reason for the beneficial effect of lactoferrins on the non-heme iron bioavailability from food and dietary supplements. Thus, the studies can provide knowledge about the fate of iron in products fortified with microelements while respective complexes can be utilized as a dietary supplement. However, it also should be mentioned that lactoferrin (LTF) was shown to take part in the development of neurodegenerative diseases such as Alzheimer. There is no exact knowledge about the role of LTF in the process, but it was found in the senile plaques accompanying the degenerative neuronal elements. Senile plaques also can include iron-oxide magnetic nanoparticles in their core which can be accounted for the negative aspects of the formation of supersaturated Fe-protein complexes [3]. Thus, the study results can also be useful for the description of the processes and mechanisms of iron toxicity. Multiple analytical techniques were utilized during the studies, namely spectrometric (ICP-MS, MALDI-TOF MS), spectroscopic (UV-Vis, ATR-FTIR, Raman), and separation techniques (CE-ICP MS, SDS-PAGE) as well as electron microscopy (TEM-EDX). Finally, molecular docking was performed to predict possible Fe^{3+}-binding sites in the protein. The results indicate that the iron supersaturated complex (Fe-bLTF) can be synthesized where each molecule of the protein binds ≈ 50 atoms of Fe^{3+}. The respective complex is stable at a pH higher than 6.8 which was shown by desorption studies as well as with CE-ICP-MS studies.

Keywords: bovine lactoferrin, supersaturated iron-lactoferrin complexes, metal-protein interactions, biologically active substances.

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Lactoferrin as a Carrier of Iron Ions

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Abstract

Lactoferrin (LTF) is a multifunctional protein from the transferrin group that shows high affinity for iron ions. This glycoprotein is present in many body fluids and glandular secretions, such as milk, saliva, tears, and nasal gland secretions. Lactoferrin is believed to exert its main biological effects by interacting with receptors on target cells. The presence of a small lactoferrin receptor that play a key role in internalization of this protein was confirmed in many types of cells. The presence of lactoferrin receptors inter alia in intestine endothelium suggests that lactoferrin can be a good carrier for metal ions or therapeutic compounds. In our work we investigated the bioavailability of new iron-rich complexes, that bind 36 mg iron per g of protein. The aim of the study was to analyze absorption of iron from lactoferrin complexes by Caco-2 cells in order to verify the possibility of their use in supplementation of iron in anemia.

Caco-2 cell line that is human colon epithelial cancer cell line was used as a model of intestinal absorption of obtained complexes. These cells show many features typical for absorptive enterocytes with brush border layer as found in the small intestine and lactoferrin receptors.

First step was to determine the range of safe concentrations. For this purpose, L929 normal cell line as well Caco-2 cell line and two types of tests, i.e. MTT and LDH release test were used. To monitor the effect of iron-rich complexes interaction at the cellular level spectroscopic methods were applied as Attenuated Total Reflection Fourier Transform Infrared (ATR-FT-IR) and Raman.

In second part of experiment the kinetic of iron absorption by Caco-2 cells in time was studied. After an hour, the maximum concentration of iron was reached in the cells. To confirm obtained results, Single Particle ICP-MS (SP-ICP-MS) analysis that enables rapid measurement the metal content in individual cells was performed. To test cells treated with iron-rich complexes and for comparison for holo-LTF and known iron supplement iron citrate were used. The Caco-2 cells took up only slightly less iron from the lactoferrin complexes than from citrate. At the same time, the new form of iron ions administration will probably be devoid of many side effects, such as diarrhea, nausea and vomiting, and in others abdominal pain and constipation.

Keywords: lactoferrin, iron supplementation, Caco-2, cytotoxicity.

Acknowledgements: The research was financially supported in the frame of the project “Advanced Biocomposites for Tomorrow's Economy BIOG-NET”, FNP POIR.04.04.00-00-1792/18-00, B.B., K.R. O.P. and P.P. are members of Toruń Center of Excellence “Towards Personalized Medicine” operating under Excellence Initiative-Research University.

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New Methods of Preparing Plant Material for the Extraction of Bioactive Compounds

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Abstract

Bioactive compounds are desirable substances in many branches of industry. They are characterized by plenty of valuable properties, which improve the quality of products and enrich them with additional nutritional values. Considering that the cell wall is not an easy matrix to isolate bioactive compounds, it is necessary to use various methods that will facilitate this process. Currently, the methods of preparation are expected to modify the particles of the plant material in such a way that will contribute to the release of bioactive compounds loosely bonded to cell wall polymers. The method selection is very important for both analytical research at the laboratory level as well as for the industrial level where the main aim is to extract as many bioactive compounds as possible. In order to do this enzymatic treatments are often used. Enzymes speed up the reaction remaining unchanged. The factors that affect the speed of reaction are temperature, substrate and enzyme concentration, inhibitors, and pH. Enzymatic-assisted extraction is based on the use of a suitably matched enzyme to obtain the extract. Currently, experiments are being carried out with enzyme-assisted extraction in order to determine appropriate factors, which will allow it to isolate the expected component.

The research focused on the development of new methodologies for obtaining non-nutritive substances of plant origin with a high concentration of antioxidant compounds with the use of supercritical fluid extraction assisted with enzymes. To improve the extraction efficiency proposed the use of a mixture of enzymes that hydrolyze plant cell walls, and thus facilitate the release of bioactive compounds. In order to find the optimal hydrolysis conditions, a statistical response surface methodology was used. The obtained extracts were analyzed using HPLC-MS/MS analysis, to gain a deeper insight into the changes in the chemical composition.

The results of the research confirm that the methodology used allowed for the maximization of the recovery of biologically active compounds from plant material. The improvement of the efficiency of the extraction of polyphenols and flavonoids by the use of enzymes that degrade the plant cell wall was successful.

Keywords: sample preparation, plant material, enzymatic processes, fermentation.

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New Approaches in Analytics of Plant Secondary Metabolites

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Abstract

Generally, in most cases for the determination of secondary metabolites like polyphenols separated from green plants, the liquid – liquid and/or liquid – solid extraction and the high performance liquid chromatography (HPLC) are applied. New methods use modern equipment and various devices for isolating and determining the tested analytes, which increases the method sensitivity and shortens the analysis time. The main contributors are new extraction methods and liquid chromatography. Detectors for HPLC are characterized by high versatility as the ability of indication of a large number of substances, selectivity with respect to a certain number of substances, a wide linearity range, to work independent on the temperature and velocity of the mobile phase flow, ease of use. Many detectors as follows: UV/Vis, diode array detector (DAD), light scattering, corona discharge, fluorescence, conductivity, electrochemical, radioactivity, chemiluminescent, are available in various HPLC equipment and depending upon the application. Currently, in various methodologies applied for the determination of mentioned above compounds have been transformed by the use of automated liquid handling and HPLC tandem mass spectrometry detection techniques (LC-MS/MS). On the other hand, classical chromatographic techniques such as thin layer chromatography (TLC) still find various applications in the analysis of plant extracts. Interfacing TLC with mass spectrometry needs the analyte molecules remain adsorbed on the TLC gel bed after the separation process. Accordingly, the use of mass spectrometry to successfully characterization of phytochemicals on TLC plates requires that the analyte molecules first be separated or sampled from the surface of the adsorbent. The analyte molecules are subsequently entering the ion source of a mass spectrometer in gas or liquid phase. Several TLC–MS techniques have been developed that differ based on the methods used to separate or sample the analytes from the adsorbent and also based on the mass spectrometric ion sources used.

The main aim of our investigations was to prepare methodologies for the extraction and determination of biologically active compounds from green plants including families *Fabaceae* and *Asteraceae*. Various methods like supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and classical solvent extraction allow to obtain extracts rich in phenolic compounds and characterized by a high antioxidant activity. The method of rapid assessment of the qualitative analysis of extracts by means of chromatographic techniques LS-MS^n and TLC-MS was proposed.

Keywords: plant secondary metabolites, liquid chromatography, mass spectrometry.

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FLASH PRESENTATIONS

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Evaluation of Antioxidant and Antibacterial Activity of Bee Collected Pollen Fermented with Tibico and Kombucha Cultures

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Abstract

Bee pollen is a natural bee-made product, which the main component is various plant pollen. A plethora of phenolic compounds, vitamins, and minerals are present in pollen, which can improve the health, immune system, or well-being of the person [1]. Unfortunately, pollen has one major flaw, it is its specific wall called exine. The exine is made from sporopollenin, which makes the was sturdy, elastic, and resilient to chemicals, because of that people only digest 10-15% of pollen [2]. Fermentation is one of a few methods to disintegrate the exine wall and increase the bioavailability of pollen.

The aim of the study – determine and compare the antioxidant and antibacterial activity of bee collected pollen which were fermented with tibico and kombucha cultures. Research object: Bee pollen from five different regions of Lithuania. Research methodology: Microorganism count in tibico and kombucha drinks was determined using a selective medium. Bee pollen was fermented using tibico and kombucha drinks. Phenolic compounds, flavonoids, and radical scavenging activity from water fractions and methanol extracts were determined using spectrophotometric tests [3, 4]. Antibacterial activity against *Bacillus mojavensis* and *Azotobacter vinelandii* bacteria was determined using the well-diffusion into agar method [5].

It was determined that bacteria predominate in tibico culture, while yeasts predominate in kombucha culture. Phenolic compounds increased by 4.8-44.2% after fermentation with tibico, and by 14.5-57.0% after kombucha fermentation; flavonoid amount increased by 26.9-106.2% after fermentation with tibico, and by 20.5-115.1% using kombucha. The radical scavenging activity fermenting with tibico increased by 10.0-36.6%, while with kombucha – by 56.8-186.9%. Antibacterial activity against *Bacillus mojavensis* after fermentation with tibico and kombucha cultures increased from 0-14.6 µg/ml to 47.5 µg/ml, and against *Azotobacter vinelandii* increased from 0-19.3 µg/ml to 28.9 µg/ml ceftazidime equivalents.

Keywords: bee pollen, phenolic compounds, flavonoids, radical scavenging activity, antibacterial activity.

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The Connection Between Specific Glioma Stem Cells Genes and GBM Patients Outcomes

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Abstract

Glioblastoma is the most common and aggressive primary malignant brain tumor in adults. Despite current treatment encompassing surgery, radiotherapy, and chemotherapy with alkylating agents, the median survival of glioblastoma patients do not exceeds 15 months, and less than 10% of patients survive more than 5 years after surgery [1]. It is thought that the heterogeneity and glioma stem cells (GSC) might be responsible for therapy failure of glioblastomas, tumor regrowth, and high patients mortality rate [2]. It is now known that cancer stem cells that are resistant to chemo-radiotherapy are fully or partially responsible for tumor regrowth and progression after removal [3]. Nevertheless, it has not been researched whether all glioblastomas have GSC or only some of them, and whether the type and fraction of GSC in the tumor may affect patient outcomes.

The aim of this study was to evaluate the importance of glioma stem cell signature in glioblastomas in determining the association between changes in specific gene expression and clinical characteristics of patients.

Methods. Three gene expression datasets (GDS3885, GDS5671, and Laboratory of Molecular Neurooncology NCH421K and U87-MG sequencing data) comparing GSC and glioblastoma transcriptomes were used for GSC-specific genes selection. From each dataset, the top 100 most GSC-specific genes were selected, the lists were overlapped, and 13 overlapping genes were selected for the study. The mRNA was extracted from 32 human glioblastoma samples using Trizol method. The expression was performed using reverse transcriptase real-time PCR method with SYBR-Green I dye. Data analyzed and visualized using IBM SPSS Statistics.

Results. A statistically significant association between patient survival and gene expression was found for *S100B* ($p = 0.001$), *FREM2* ($p = 0.014$), *AGT* ($p = 0.024$), and *GAS7* ($p = 0.016$) genes. Patients with tumors showing increased mRNA expression of the *S100B*, *FREM2*, *AGT*, and *GAS7* genes were more likely to survive longer than patients with reduced expression of these genes.

In conclusion the molecular signature of glioma stem cells in glioblastoma tumors is related to patient outcomes and is likely to represent the fraction of GSC in the tumor mass. Further cytological studies are needed to confirm the dependence of the analyzed markers exclusively on the GSC fraction and thus to confirm their potential.

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Allelopathic Effect on Germination of Invasive *Impatiens* spp.

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Abstract

Invasive alien plant species induce a significant hazard to the biodiversity of native systems [1]. The changed biodiversity procures the alteration of their ecological services. Therefore, the understanding the dispersion of the alien species in invaded habitats outside their native range remains crucial finding the prevention means of their spread [2]. Biochemical interactions based on some hypothesis used to explain these phenomena.

This investigation has aimed for the evaluation of the phytotoxicity on germination using the photospectroscopy of total phenolics content *ex-situ* of Himalayan balsam (*Impatiens glandulifera)*, and *Impatiens parviflora* during growth period [3]. The tested acceptor species have chosen wheat (Monocot) and rapeseed (Dicot).

The total phenolics content alternated between 0.001 and 0.008 mg/ml in aqueous extracts of investigated *Impatiens* spp. The germination and seedlings growth of tested acceptor species belonged to different systematic classes of Gymnosperm, i.e. monocot and dicot, have been supressed in the aqueous extracts of invasive *Impatiens* spp. Therefore, the acceptors' germination and seedlings data confirmed the phytotoxity of tested *Impatiens* spp. However, the acceptors' germination exhibited different response to the extracts due to permeability of their seed coats. Depending on *Impatiens'* growth stage and extract concentration, the germination of thick-walled wheat recorded by 8-54% higher than that of rapeseed. The maximal phytotoxicity and germination suppression was determined in 0.2% extracts of flower and seed of tested *Impatiens* spp.

The data justify that phytotoxicity of tested invasive *Impatiens* species might depress the germination and thus regeneration and spread of native species in invaded ecosystems.

Keywords: allelopathy, germination, phenolics, *Impatiens* spp.

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Photosynthetic Capacity of Some Invasive Species

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Abstract

Solar radiation is mainly assimilated in the leaf to photosynthesis' energy and transpiration rate, which constitute of the essential integrated functional system in plants [1, 2]. The estimations of these components are essential for the understanding of plant physiological conditions. From the ecological perspective, attention has mainly focused on the yield of chemical energy assimilated from light energy and indicating the photosynthetic efficiency. However, photosynthesis constitutes a complex, and responds to many environmental factors (light intensity, vapour pressure deficit, CO_2 concentration, etc.) that force invasive plant acclimation success [3]. The main objective of present research was to evaluate the ration of photosynthesis as indices of acclimation of invasive species to environmental conditions in invaded habitat. The invasive *Fallopia japonica*, *Heracleum sosnowskyi* and *Rumex confertus* were assessed. *Taraxacum officinale* presented as a control species. LCpro+Photosynthesis System (ADC BioScientific, UK) used for the measurement photosynthesis (μmol/m^2 s)) and photosynthetically active radiation (PAR, μmol/(m^2 s)) parameters *in situ* of invasive plant species enlisted in National List of invasive species (2016). Measurements implemented every month along the grassland or bushland ecotones in Kaunas distr. during plant vegetation period.

Photosynthesis (r=0.8) responded to PAR intensity which related on habitat type and meteorological conditions during measurements. Nonetheless, differences between PAR values recorded at particular dates varied insignificantly in invaded habitats. PAR values of separate species determined their photosynthesis rates (r=0.8) during growth period. Though invasive species exhibited different capacity of photosynthesis in invaded habitats however the light-access was sufficient for carbon assimilation maintaining plant' growth, and thus guarantee their acclimation strategy in temperate climate of Lithuania making them resistant to the competition of native species. Acceptable gain of photosynthesis during growth period contributed to invasive plant success, consequently it might attest as useful indicator for predicting invasiveness within particular environments.

The photosynthesis data confirmed that assessed invasive species exhibited intensive photosynthesis in invaded habitats as compared to control species. Nonetheless, photosynthesis can be limited by canopy or water availability. The data revealed that photosynthesis quantifying might support to identify the acclimation potential of invasive species.

Keywords: phorosynthesis, invasive species, habitat.

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Impact of Soil Physical Parameters on Respiration Intensity

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Abstract

The ecosystems absorb atmospheric CO_2 and assimilate ca. one quarter of anthropogenic emissions per year. However, content of CO_2 increased from 277 ppm in 1750 to 410 ppm during Anthropocene due to intensive human activity. The challenges to control and decrease the anthropogenic emissions are of great importance in agrosector as well.

The investigation of respiratory emissions has carried out in order to evaluate and compare the impact of physical conditions in different agroecosystems of organic farming. Intensity of soil respiration carried out in agroecosystems of the barey+ley undercrop, ley, wheat, vetch + oat mixture at the Training Farm of Agricultural Academy.

Soil thermal mode offerings important driver for different parameters in agroecosystems that increase numerous vital processes. Soil temperature exhibited strong positive stimulation on soil respiration (r=0.7-0.9; p=0.01). However, soil humidity determined anaerobic conditions that depended on precipitation' amount and significantly negatively correlated with soil respiration (r=-(0.3-0.7); p=0.04). Undesirable surplus of moisture appeared in autumn and early spring determining the oxygen deficiency and thus unfavourable environment for soil biota and plant root' respiration. Otherwise, the soil electrical conductivity depends on dissolved ions that are important for functioning of plant and soil biota. El. conductivity determined different in agroecosystem' soils and insignificantly correlated with soil humidity (r=0.3), temperature (r=0.2), and soil respiration (r=0.1-0.6).

Keywords: soil respiration, emissions, environment, agroecosystem.

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The Study of Bystander Effect After Combining Calcium Electroporation, Electrotransfer of Anticancer Drug Bleomycin or Irreversible Electroporation

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Abstract

The technique of electroporation (EP) is used to improve exogenous molecule transfer thru the cell membrane. When cells are exposed to a high-intensity electric field, EP occurs and this process causes the creation of hydrophilic pores, as a result of increased transmembrane potential. Reversible EP is used in anticancer therapies, such as calcium electroporation or bleomycin electrotransfer. If the transmembrane potential is significantly higher than the EP threshold, irreversible electroporation occurs, and this process could be used as a method for cancer tissue ablation.

Both therapies share similarities with another anticancer therapy – radiotherapy. It is known that at lethal doses irradiated cells secrete signaling molecules that affect adjacent unirradiated cells. The "Bystander effect" is the term given to this phenomenon. "Bystander effect" is well-known in radiotherapy, but it has not been explored in the process of electrochemotherapy. According to our recent publication, this effect could occur during Bleomycin electrotransfer and irreversible electroporation [1]. Further research into the Bystander Effect led to the use of the combination of different anticancer therapies. The aim of this research is to assess the "Bystander effect" after combination of $CaCl_2$ electrotransfer. electrotransfer of anticancer drug bleomycin or irreversible electroporation.

In this study, we used 4T1 (mouse breast cancer) cells. The BTX electroporator was used to create electric fields. One 100 ms long and 1400 V/cm amplitude pulse was used to electrotransfer $CaCl_2$ and bleomycin molecules and for irreversible electroporation, we used the amplitude of 2800 V/cm. After electroporation, the cells were incubated for 10 minutes on a 24-well plate, then 0.2 ml RPMI growth medium was added and incubated for 48 hours. After that time, the media was withdrawn and put on untreated cells and after 6 days the viability of cells was measured by colony formation assay.

We obtain, that the Bystander effect occurs after a different combination of calcium electroporation, irreversible electroporation, and bleomycin electrotransfer. Then the media after treatment is diluted twice the Bystander effect did not occur. However, combining two different bystander effects by adding half of the media from cells after bleomycin electrotransfer and half of media from cells after irreversible electroporation resulted in a significant reduction of cell viability. Also, we can obtain similar results if we combine bleomycin electrotransfer and calcium electroporation.

Keywords: electroporation, Bystander effect, bleomycin electrotransfer, irreversible electroporation, calcium electroporation.

Acknowledgments: This work was supported by grant Nr. S-MIP-19-13 from the Research Council of Lithuania.

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Organic Farming Waste Material as a Driving Force for Development of Products for Use as Repellents

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Abstract

In the last decade trade of agricultural products has increased three times. To feed the human population of 2050, outputs of agricultural sector must rise by 60% [1]. Increasing threats due to climate change, abiotic stresses and weeds, diseases and pests put immense pressure on crop production [2]. Plant pests globally can reduce yields of food crops by 40% [1]. The conventional approach for pest control is spraying of pesticides. Resistance to pesticides as a problem is rising. Multi-resistance to pesticides may lead to situation where insect invasions become uncontrollable therefore novel formulations for pest management are needed. The European Commission has set an aim to decrease the use of chemical pesticides by 50 % and achieve 25% organically managed agricultural land. Since 2009 EU Directive 2009/128/EC establishes a framework to achieve a sustainable use of pesticides by reducing the risks and impacts of pesticide use and promoting the use of alternative approaches or techniques such as non-chemical alternatives to pesticides. Plant-based pesticides are the first choice for pest management in integrated pest management systems [3]. Production of essential oils from organic medicinal and aromatic plants such as chamomile, pine, caraway in SIA Field and Forest (Latvia) results in high value-added products, as well as herbal waste as a by-product. The market potential of the essential oils is high enough, while the potential of the remaining waste biomass, which is a rich source of bioactive compounds, has not been sufficiently explored. Aqueous extraction of biomass right after distillation process is very effective and requires few technological resources. This is a great method to extract non-volatile compounds for the potential use in pest repellent formulations.

The aim of this research is to characterize obtained aqueous extracts and to compare results with commercial repellent extracts available in the market to develop agricultural plant-based pest repellents that are compatible with organic farming practices. Furthermore, allelopathic effects of chemically characterized extracts on host plants (cabbage and cucumber), as well as impact on common agricultural insects are also tested.

Keywords: plant-based repellents, herbal waste.

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Extracellularly Triggered Cell Viability Decrease After Bleomycin Electrotransfer and Irreversible Electroporation

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Abstract

Cancer is a frequent causes of death in the world, hence is a burden of the society. Amongst novel anticancer therapies are electrochemotherapy and electroablation [1]. Both treatments are based on electric field application, that trigger transmembrane potential in the affected cell membranes [2]. When transmembrane potential does not greatly surpass this threshold the transient electropores are created, hence reversible electroporation occurs. However, if the transmembrane potential greatly exceeds this threshold the electropores does not close, hence irreversible electroporation is triggered.

Reversible electroporation is mostly utilized in clinical approach in combination with anticancer drug bleomycin - termed electrochemotherapy. Irreversible electroporation also is applied to another anticancer treatment termed as electroablation.

Both methods are locally based, hance can be compared to radiotherapy – also local cancer therapy. Also, cells that are affected with ionizing radiation can induce supplementary effect on adjacent cells called, the bystander effect. Lately published publications showed bystander effect after electrochemotherapy as well as electroablation [3]. However, too low number of publications indicate a lack of research that could show the bystander effect after both electroporation triggered cancer treatment methods. One of the unexplored parts of this phenomena is the effect difference in between cell lines.

Human adenocarcinoma cells (A549) and Chinese hamster ovary cells (CHO-K1) were utilized in the experiments. Electroporation was triggered with one electrical pulse at (1400 V/cm) for the duration of 100 µs for BLM (20 nM) electrotransfer. Irreversible electroporation triggered after a single electrical pulse at (2800 V/cm) for the duration of 100 µs. Then after 10 min a 1 ml growth media (RPMI) was put on top and incubated for 72 hours. Afterwards the medium is collected and put on 400 cells in the 4 cm radius petri dish and clonogenic assay was done.

The results show that utilized A-549 and CHO cell lines make similar bystander effect after bleomycin electrotransfer even if the affected media was switched in between cell lines. However, A-549 cells showed higher sensitivity tendency to electroporation-based treatments than CHO treated cells.

Keywords: electroporation, Bystander effect, bleomycin electrotransfer, irreversible electroporation**.**

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Bison Bonasus Infection of *Babesia* spp.

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Abstract

Canopy animals are the main carriers of mite distributed pathogens and tanks in Europe. *Bison bonasus* is the largest wild animal of Europe, which is recorded not only to the Lithuanian Red Book, but also to the International Red Book. For this reason, studies related to tick pathogens in the ticks gathered from animals and from spleen samples are complicated. The aim of this study is to investigate the pathogens of ticks in spleen samples and ticks collected from *Bison bonasus* in Lithuania.

The material was collected in the territory of Lithuania in the period from 2014 year up to 2022 year from the dead (car or train accidents, dead for unknown reasons) or removed from nature by protection plan because of illness or genetic disorders.

Following the analysis of the data and the analysis of the sequences found that *Babesia divergens* and *Babesia capreoli* were found in spleen samples and in the ticks collected from *Bison bonasus* were found *Babesia divergens* and *Babesia divergens, Babesia mircoti* and *Babesia venatorum.* Also, in the bison samples and the ticks were found *Anaplasma phagocytophilum* and *Borrelia burgdorferi* pathogens.

Keywords: *Bison bonasus*, *Babesia* spp., tick, spleen.

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Diversity of Phenolic Compounds in Flowers of *A. kolomikta* (Rupr. & Maxim.) Maxim.

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Abstract

The plants of *Actinidia kolomikta* are functionally dioecious so this species is characterized by both male and female flowers [1]. *A. kolomikta* flowers do not contain nectar and berry yields can be greatly reduced if pollinators do not visit the flowers. On the other hand, various chemical compounds in the flowers can attract pollinators. The aim of this work was to assess the diversity of phenolic compounds in male and female flowers of *A. kolomikta*. Separation of phenolic compounds was performed with the Acquity H-class UHPLC system (Waters, Santa Clara, CA, USA) equipped with a triple quadrupole tandem mass spectrometer (Xevo, Waters, USA) with an electrospray ionization source (ESI) to obtain MS/MS data using a previously described and validated UHPLC-ESI-MS/MS technique [2]. A total of 14 phenolic compounds were identified both in male and female flowers. Flavonols were the primary class found in flowers, other identified phenolic compounds were flavan-3-ols, phenolic acids and flavones. The major flavonols identified were kaempherol-3-*O*-glucoside, rutin, kaempherol, isorhamnetin, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, quercetin, and quercitrin. Flavon-3-ols (+)-catechin, (-)-epicatechin, and procyanidin C1 represented the second most abundant class of phenolic compounds. For flavone class, small amounts of apigenin were found. Two compounds belonging to the hydroxycinnamic acids class, i.e., chlorogenic and neochlorogenic acids were detected in both male and female flowers.

Keywords: flowers, phenolic compounds, pollinators.

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Characterization and Liming Effect of Pelletized Lime Kiln Dust Combined with Biomass Combustion Ash on Soil Properties

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Abstract

Extensive application of mineral fertilizers resulted in high soil acidity, which is one of the major problems for crop production and soil degradation. In acid soils the improvement of the productivity depends on soil relevant management practices. Liming is the most economical method of ameliorating soil acidity [1]. The cost and the efficiency of liming depends on the chosen liming material. The use of natural recourses is limited, not all countries have large quantities of them. One way is to use solid alkali waste, which is kept in landfills. Industrial solid waste, such as lime kiln dust and wood ash, can be used as alternative liming materials to benefit sustainable agricultural development [2-4]. The purpose of this study was to investigate the effects of recovered waste from lime processing plants as soil liming materials on soil properties. Pelletized lime kiln dust with and without biomass ash were utilized and their liming properties were compared to those of natural minerals, such as ground chalk and crushed dolomite. A field experiment was performed to evaluate the performance of liming materials under natural conditions. For the investigation we used: ground chalk, crushed dolomite, pelletized lime kiln dust (two different fractions) and pelletized lime kiln dust with biomass combustion ash. The application rate 5 t/ha is calculated by the amount of active substance – $CaCO_3$ in liming materials. The research was conducted for the three years in crop rotation – spring barley, spring wheat and pea. After the field experiments the results showed that the application of 5 t/ha of pelletized lime kiln dust and pelletized lime kiln dust with biomass combustion ash increased soil pH_{KCl} 0.58 and 0.50 pH units after three years of liming. Moreover, increased soil mobile Ca, Mg, P_2O_5 and K_2O contents and reduced mobile Al concentration in soil.

Keywords: liming, pelletized biomass combustion ash, soil neutralization.

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Chemical Diversity of Three Various Eleuthero Species Grown in Territory of Latvia

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Abstract

Eleutherococcus (Rupr. & Maxim.) is a shrub which is collected in the wild in Russia and north-east China. In addition to local use, it has also gained popularity in Eurasia and North America for treatment of fatigue and weakness as well as anti-stress and adaptogen action. The medicinal properties of Eleutherococcus are due to presence of eleutherosides which are mainly present in the roots and rhizomes. According to European Pharmacopoeia (01/2008:1419) Eleutherococci radix are standardized based on the sum of eleutherosides B and E (min. 0.08%). However, the leaves, bark and flowers are also used. Eleutherococcus leaves have been reported for glycosidase inhibition as well as having antibacterial properties. For this reason, leaves are commonly consumed as tea, soup, and wine. For example, *E. senticosus* leaves have been developed as a functional beverage called *ci-wu-jia* tea in China and Siberian ginseng tea in the United States and Europe [1]. Siberian ginseng has the potential to grow in Latvian agricultural and climatic conditions as well, as some plants have been introduced in Latvia and grow well, for instance, in the collection of the National Botanical Garden. The aim of the current study was to evaluate eleutheroside content in leaves of different eleutherococcus plants obtained from nurseries and gardens across territory of Latvia, as a potential for their further commercial cultivation. Chemical diversity of different eleutherococcus plant leaves was evaluated by detection of total phenolic compound and total triterpenoid saponin content, DPPH free-radical scavenging activity, as well as detection of the sum of eleutherosides B and E. Phytochemical capabilities were measured using both spectrophotometric analysis methods and liquid chromatography mass spectrometry. Total phenol content varied from 2.54+0.09 to 22.09+1.18 g of gallic acid equivalents on kg DW, while total triterpenoid saponin content varied from 53.20+2.43 to 88.95+2.76 g of Escin equivalents on kg DW. Eleutheroside B content varied from 0.9mg to 33.5mg on kg dried leaves, while eleutheroside E content varied from 22.5 mg to 52.0 mg on kg dried leaves. The obtained results confirm that the leaves collected from *Eleutherococcus* plants growing in the territory of Latvia have future potential to be used as herbal medicines or dietary supplements.

Keywords: *Eleutherococcus,* eleutherosides B and E, liquid chromatography mass spectrometry.

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Repeated Heat Waves Negatively Affect Photosynthetic Energy Use Efficiency and Growth of Fescue

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Abstract

Extreme weather events such as heat waves and severe droughts are projected to become more common and intense as a result of climate change. Heat waves have a variety of effects on plants, including physiological changes, restriction of developmental processes, and, ultimately, yields. Heat has a species-dependent effect, and with ideal water availability, it can be negligible or even positive; nevertheless, a series of droughts exacerbates the negative effects on carbon uptake and growth. The goal of this study was to see how chlorophyll *a* fluorescence parameters of hybrid fescue (*Festulolium loliaceum* (Huds.) P. Fourn.) responded in a controlled setting and under the stress of recurrent heat waves. Plants were grown in pots filled with a mixture of field soil, perlite and fine sand (5:3:2). Two 4-day heat waves (35/28 ^{o}C day/night temperature) were combined with dryness to evaluate the effect of heat waves on plants (10% soil moisture). A 5-day regeneration period followed the 4-day heat wave. Plants were re-exposed to the second heat wave (4-day) and re-evaluated for their recovery potential after 5 days in an analogous heat wave effect and recovery experiment. On the last (4th) day of each heat wave exposure and after each recovery period, measurements of chlorophyll *a* fluorescence parameters were taken with the Plant Efficiency Analyzer, PEA (Hansatech Instruments, Ltd., England) with randomly selected youngest fully expanded leaves.

When compared to control plants, the first heat wave reduced the photosynthetic performance (PIabs) index by 61% ($p<0.05$). The statistically significant loss continued at 30% ($p<0.05$) after the first recovery phase. The second heat wave reduced the photosynthetic performance (PIabs) index by 51% ($p<0.05$), which was slightly less than the first heat wave. The detrimental effects of the second heat wave persisted after the second recovery phase, when the PIabs showed statistically significant lower changes (46 percent, $p<0.05$) than the control plants. Other examined parameters, such as the quantum yield of electron transport (phi(Eo)) and the quantum yield for the reduction of end acceptors of PSI per photon received (phi(Ro)), revealed similar regularities. The rise in the levels of dissociated energy DIo/CSo in plants during the first and second heat waves may have caused these alterations in photosystem viability. During the first heat wave, for example, fescue DIo/CSo increased by 41% compared to control plants ($p<0.05$). After the second wave and both recovery periods, the pattern remained the same. Meanwhile, during and after the recovery periods of both heat waves, the share of energy transported in the form of electron transport (ETo/CSo) or used for the regeneration of reaction centers (REo/CSo) has dropped. During the first and second heat waves, the above-mentioned variations in energy demand resulted in dry biomass losses of 21% and 33% ($p<0.05$), respectively. According to this study, plants do not recover even after regeneration periods, and repetitive heat waves have a negative influence on energy usage efficiency and development of fescue.

Keywords: heat wave, hybrid fescue, chlorophyll fluorescence, forage crops.

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Subclinical Mastitis detection in Dairy Cows Using Lactose as Biomarker

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Abstract

High negative impact on milk production and quality in dairy herds are caused by subclinical mastitis. To maximize the management of subclinical mastitis distribution and assure the high quality of milk as an industrial product, better diagnostic technologies are required. For this reason, seventy-two dairy farms were evaluated for bovine subclinical mastitis causative agents between 2015 and 2020 to determine the link between lactose levels, seasons, and subclinical mastitis infection. The dominant species found in tested milk samples were mixed microbiota, coagulase-negative *Staphylococcus,* and *Staphylococcus aureus.* After a comparison of microorganism diversity between seasons, it was noticed that *Enterococcus* spp. and *E. coli* were only found in the summer and autumn, while yeast was identified only in autumn. Milk lactose and the number of somatic cells in milk showed a negative correlation (-0.471; $p < 0.001$). A negative correlation was detected between the number of somatic cells in milk and lactose levels (-0.471; $p < 0.001$). Significant changes in lactose levels in milk samples were associated with the prevalence (%) of subclinical mastitis pathogens, such as *Streptococcus agalactiae* ($y = -1.8011x + 10.867$, $R^2 = 0.9298$), *Staph. aureus* ($y = -3.5216x + 25.957$, $R^2 = 0.8604$) and other *Streptococci* ($y = -0.5956x + 7.6179$, $R^2 = 0.6656$). These data suggest that milk lactose could be used as a biomarker for suspected udder inflammation in modern cow health prevention programs to lower the prevalence of subclinical mastitis infections in dairy herds.

Keywords: cow mastitis, mastitis pathogens season dynamics, milk lactose, Lithuania.

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Swallow-Wort (*Vincetoxicum lutea* L.) Leaves Extracts Aroma Profile and Antioxidant Properties

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Abstract

Swallow-wort (*Vincetoxicum lutea L.*) grows in Europe, Asia and North America. It is an herbaceous perennial plant belonging to the family *Apocynaceae*, subfamily *Asclepiadoideae*. Historically, the rhizomes, leaves and dry seeds of swallow-wort have been used in folk medicine for various medicinal purposes. A decoction of the herb and roots has been used in folk medicine to treat neurosis and malaria. In Chinese medicine, it has been used to treat internal fever and scaling [1]. In addition, swallow-wort has been claimed to have diuretic and laxative properties and a powder made from the roots has been shown to accelerate wound healing. It should also be stressed that swallow-wort is a poisonous plant, and in large doses has a severe effect on the nervous system and the heart. Glycosides, saponins and small amounts of volatile oils were found in the leaves and other parts of the plant [2]. However, there is still a lack of information on the composition of these plants, especially if modern extraction methods are used. The aim of this work was to evaluate the advantages of supercritical fluid extraction with carbon dioxide (SFE-CO_2) for the extraction of phytochemicals from *V. lutea*. The profile of aromatic compounds in the lipophilic extract of *V. lutea* was investigated using GC-TOF/MS. The chemical composition of the *V. lutea* extract obtained by three solvent extraction and post-hydrolysis extraction methods was studied by UPLC-QTOF. The properties of the extract were evaluated by in vitro assays, namely DPPH° absorbance and oxygen radical absorbance capacity (ORAC), and the total phenolic content was determined by Folin-Ciocalteu method. The antioxidant capacity of the solids (dried leaves and UAE residues) was assessed using the Quencher procedure for the above spectrophotometric tests. The SFE-CO_2 method yielded a lipophilic fraction of 1.52%. More than 50 volatile compounds consisting of saturated n-alkanes, acids, esters, amides, terpenes, aromatics, diterpenoids, triterpenoids, tocopherols, etc. were detected in the *V. lutea* extract by GC-TOF/MS. The main compounds were oleic acid, phytol, hexacosane, triacontane, α-tocopherol, octacosane, tetracosane, henicosanol, squalene, 2-pyrrolidinone and ethyl hexadecanoate. The UPLC chromatograms obtained from the three solvent extractions and the post-hydrolysis extracts revealed several phenolic compounds: chlorogenic acid, caffeic acid, isoquercitrin, kaempferol and various flavonoid glycosides. The results of the UPLC-QTOF analysis also suggest that hydrolysis results in the disappearance of the hydrolysable compounds, but the appearance of a relatively higher number of non-hydrolysable compounds such as organic acids and other compounds that are difficult to hydrolyse. The antioxidant capacity of *V. lutea* leaves and residues after extraction, measured by different methods, decreased with increasing polarity of the solvent used for extraction. The raw material and the resulting extract contain different classes of bioactive compounds with strong antioxidant properties. It can be concluded that a well-designed extraction process of *V. lutea* leaves allows the extraction of valuable bioactive compounds with high antioxidant capacity.

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1*H*-Indol-3-ylcarbhydrazide and its Regioisomers: Synthesis, Chemical Modification and Investigation

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Abstract

Friedreich's ataxia is a rare condition that has been documented as an inherited neurodegenerative disease. It is transmitted through an autosomal recessive model that causes gradual degeneration of the cerebral and spinal ganglia in patients. This disease is classified as a rare disease because its incidence is 2-3 cases per 100,000 population, and treatment is currently only available symptomatically. In previous our research three of the prepared compounds, protected FA-fibroblasts against glutathione depletion induced by γ-glutamylcysteine synthetase inhibitor BSO [1]. The structure-activity analysis showed that 5-(butylthio)-1,3,4-oxadiazole structural unit is potentially important molecule site responsible for interaction with active center leading to desired antioxidant properties.

Inspired by the obtained results, it was decided to further elucidate protective potential of 1,3,4-oxadiazole-*N*-heterocycles hybrids. In a three-step synthesis, a small library of 5-(butylthio)-1,3,4-oxadiazole-indole regioisomers was prepared starting from alkoxycarbamoylindoles. The first step was reaction of 2-, 3-, 4-, 5- or 6-alkoxycarbamoylindoles with $NH_2NH_2{\times}H_2O$ providing intermediate hydrazides. The next synthesis step was formation of 1,3,4-oxadiazole-2(3*H*)-thione structural unit by base-mediated crude hydrazide treatment with CS_2, followed by *in situ* acidification. In the final step *S*-alkylation with 1-iodobutane was carried out in presence of K_2CO_3 as a base catalyst.

Upon proper purification of target products their structure was confirmed by detailed ^{1}H and ^{13}C NMR, IR and MS spectrum data analysis.

Keywords: 1*H*-indole, 1,3,4-oxadiazole, oxidative stress.

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Seasonal Variation of Pollution with Organic and Biogenic Compounds in Jiesia River, Affected by Fish Farming

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Abstract

The rapid development of the aquaculture industry causes many environmental problems, such as water pollution [1] and ecosystem degradation [2]. Fishpond wastewater is usually discharged in autumn and contains huge amounts of organic residues and biogenic compounds. Rivers in urban or agricultural landscapes are also polluted by diffused and point pollution sources whole year. Therefore, the aim of this study was to evaluate the effect of two fishing farms AB 'Išlaužo žuvis' and Šilavotas Subdivision of the Fisheries Service under the Ministry of Agriculture on Jiesia river water pollution with organic and biogenic (N and P) compounds in different seasons. Water sampling was performed in 2021-2022 at the end of each month at ten places below and above the fishponds. Concentrations of NH_4^--N, NO_3^--N, PO_4^{3-}-P, was evaluated by spectrophotometry, total suspended solids (TSS), pH, redox potential and BOD7 was evaluated by Multi 9630 IDS analyzer (WTW, Germany). Significant seasonal variation was detected for physico-chemical parameters, with the lowest pH and redox potential in winter and highest in warm season. Pollution with biogenic compounds were highly dependent on the season as well: the highest concentration of NH_4^--N was detected in autumn (1.74 mg/l), significantly exceeding the limit value (1 mg/l); the level of NO_3^--N in December-March (4.14 mg/l) was also exceeding the limit value (2.3 mg/l) and especially the lowest level in summer and autumn (0.40 mg/l). The lowest concentration of PO_4^{3-}-P was detected in summer (0.10 mg/l), and the highest – in February (0.40 mg/l), exceeding the limit value (0.065 mg/l) whole year. Strong seasonal variation (up to 10-fold) in N and P compounds is also detected by other authors and are mostly determined by autotrophs consumption in the warm season [3,4]. Although significant pollution was found in the investigated section of the river, we did not detect long-term reduction of water quality bellow the fishponds. It indicates that anthropogenic pollution from point sources (sewages from urban areas) and diffused pollution from agricultural fields, both of which are widespread in the territory, can be stronger agents than effluents of aquaculture facilities.

Keywords: aquaculture pollution, ammonium, nitrates, phosphates, seasonal effects.

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Differences in Water Quality of Lithuanian Lakes and Reservoirs in 2005-2020

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Abstract

Monitoring of surface water bodies of the Lithuanian Environmental Protection Agency is carried out in several dozen lakes and ponds. According to summarized reports, the quality of these water bodies is good or very good with respect to most chemical and physico-chemical parameters. The aim of this study was to evaluate the shift of the water quality during the last 15 years, comparing natural Lithuanian lakes and reservoirs as well as differentiating it by river basin districts (RBDs). We found that water quality of reservoirs is significantly lower than lakes with respect to almost all investigated parameters (except pH). Reservoirs are characterized by lower Secchi depth, higher electrical conductivity, alkalinity, and total dissolved solids (from 1.5 to 2-fold), less dissolved oxygen, higher concentrations of chlorophyl a (chl a), N and P compounds, as well as BOD7. The most important reason of stronger eutrophication of reservoirs is the reduction of stream velocity above the dam and sedimentation of particulate phosphorus, originating from the upstream catchment [1]. Low self-purification capacity and nutrients originating from soil also play a role [2]. Little significant correlation was detected between reservoirs water quality parameters and time flow, with exception of pH (Pearson $R = 0.263$, $p<0.0001$), dissolved O_2 (Pearson $R = -0.153$, $p<0.05$), and suspended matter (SM) (Pearson $R = -0.182$, $p<0.05$). Only reduction in SM indicates a slight increase in water quality, whereas significant increase in pH and reduction in O_2 content indicate intensification of eutrophication. The lowest water quality was found in reservoirs in Northern and Western Lithuania, belonging to RBDs of Venta, seaside rivers and especially Lielupė RBD. Reservoirs of Lielupė RBD had the worse water quality with respect to nitrates, mineral N, total N and P, chl a and BOD7; whereas reservoirs of Venta were characterized by the lowest O_2 and highest concentrations of phosphates. Poor water quality of Northern Lithuanian rivers is also detected by other authors and are closely related to intensive agriculture and urbanization in this region [3]. A slight and insignificant improvement of water quality of reservoirs of these RBD can be seen with respect of SM, chl a, ammonium and/or P compounds. However, water quality is obviously deteriorating in terms of nitrites, nitrates, mineral and total N. To conclude, reservoirs of Lithuania are characterized by much worse water quality than lakes; increasing pollution with N compounds, especially in the most polluted RBDs should gain more scientific attention.

Keywords: water reservoirs, surface water monitoring, river basin districts.

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Nonsteroidal Anti-Inflammatory Drug Bioremediation Using White-Rot Fungi

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Abstract

Nonsteroidal anti-inflammatory drugs are easily available as over the counter medicine, widely used for pain or fever relief and as such, the usage of these drugs is rising each year. In Lithuania there was a 6,5% increase in drugs used per capita between 2016 and 2018 [1]. It is estimated that due to unpredictable differences between patients, up to 90% of the drug used may end up in wastewater [2]. Research shows that at the moment, such contamination is not cleared reliably by activated sludge within wastewater treatment plants and more specialized treatment may be needed [3].

As a result, the aim of this research was to explore and evaluate white-rot fungi application possibilities for nonsteroidal anti-inflammatory drug bioremediation. Three study objects, ibuprofen, diclofenac and naproxen, were chosen, as they are the most widespread. Fungi cultures chosen for this work were *Pleurotus ostreatus*, *Pleurotus eryngii* and *Irpex lacteus*. A miniaturized bioremediation experiment was carried out according to the procedure described by Drevinskas et al. [4]. Samples from the bioremediation experiment were processed using an optimized solid-phase extraction procedure, after which the obtained solutions were analyzed using high performance liquid chromatography. Results from a preliminary analysis show an up to 99% decrease in drug concentration over the course of two weeks with *P. eryngii* and *I. lacteus* fungi cultures, beyond the limits of quantification. *P. ostreatus* showed lower resistance to contamination, therefore its bioremediation capabilities remain unknown. While this study suggests that *P. eryngii* and *I. lacteus* are capable of eliminating selected nonsteroidal anti-inflammatory drugs, more research is required, especially in terms of resulting metabolite identification and toxicity evaluation.

Keywords: nonsteroidal anti-inflammatory drugs (NSAIDs), bioremediation, white-rot fungi, solid phase extraction (SPE), high performance liquid chromatography (HPLC).

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Evaluation of the Dependence of Antioxidant Activity of Berries and Leaves of Medicinal Plants on Their Preparation

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Abstract

Drying is one of the most popular methods of processing plant raw materials, allowing them to be used all year round. Drying has been found to reduce the biological activity of medicinal plants [1-4], so other possible treatments that preserve biological activity more effectively should be considered. The aim of this study was to evaluate the variation of antioxidant activity in a medicinal plant material depending on the type of preparation. Leaves and berries of *Hippophae rhamnoides*, *Rubus idaeus*, *Aronia melanocarpa*, *Crataegus monogyna* were selected for the study. Two treatment methods were chosen: freezing at -20 °C and convection drying at +35 °C temperature. Fresh sample was used as a control one. Methanolic extracts were prepared from treated and not-treated material. The DPPH radical scavenging method was used to evaluate antiradical activity. The study showed a decrease of the antiradical activity in material of frozen and dried leaf, except for *C. monogyna*. The increase in antiradical activity was observed in the berry raw material, except for dried and frozen *H. rhamnoides* berries.

Keywords: drying, freezing, antioxidant activity, sea buckthorn, raspberry, chokeberry, hawthorn.

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Screening of Anticancer Compounds Accumulating Local Plants and Evaluation of Their Activities

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Abstract

According to statistics from World Health Organization in 2020, cancer is one of the leading causes of death worldwide. It is closely related to age, general trends in people's lifestyles and environmental pollution. Therefore, research into the search for natural antioxidants in plants is highly relevant globally. Polyphenols, including ellagitannins, make up a large proportion of such compounds.

Vytautas Magnus University scientist has been conducting research on the isolation of phyto-compounds from herbal medicinal raw materials and their antioxidant properties for many years [1-3]. As Lithuania's vegetation is diverse, it is essential to research the plant's accumulation of anticancer ellagitannins and accumulation of other biologically active compounds. For this task, the total content of phenolic compounds, flavonoids and radical scavenging activity is determined in 11 plants [4-6]. It was estimated that *Epilobium angustifolium*, *Solidago virgaurea*, *Quercus robur*, *Fragaria vesca* and *Fagopyrum esculentum* have the most significant biological activity.

Therefore, future studies will include the extraction and fractionation of anticancer ellagitannin-accumulating plants using different strategies. The research will also develop and adapt solid-phase anticancer plant separation equipment and raw materials for quality assessment. Anticancer studies of the extract fractions will also be performed using *in vitro* cancer cell lines.

Keywords: anticancer compounds, ellagitannins, sorption-phase separation.

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Investigation of Biologically Active Compounds of *Chamerion angustifolium* L.

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Abstract

Nowadays, scientists are paying more attention to the quality of food and their health benefits, ability to treat some health disorders. Growing public interest in herbal medicine and the benefits of their biologically active substances are creating a worldwide need for research of medical herbs raw material. The aim of this study was to determine the total amount of biologically active compounds spectrophotometrically in a medicinal plant raw material of *Chamerion angustifolium* L. by comparing two extraction methods namely maceration and ultrasound and to evaluate the antimicrobial effect of the given extracts.

Determination of polyphenolic compounds by spectrophotometric method; Determination of antimicrobial activity by the disk diffusion method and the agar diffusion method were used.

Results of the study: The amount of biologically active compounds in the raw material of *Chamerion angustifolium* L. depends on different time of maceration or ultrasound extraction. The content of phenolic compounds, flavonoids and antioxidant activity when extracting the extracts in an orbital shaker was determined at 12, 24, 48, 72 h. and extracting the extracts in an ultrasonic bath for 10, 20, 30 min. increased. Narrow-leaved gourmet extracts had antimicrobial activity against both bacteria: *Bacillus subtilis, Azotobacter vinelandii*, and the fungus *Cryptococcus neoform*ans. It had no antimicrobial activity against *Ulocladium chartarum* fungi.

Keywords:biologically active compounds, maceration, ultrasound, antimicrobial, *Chamerion angustifolium* L.

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Soil Microbial Biomass Response to Application of Different Organic Fertilizers

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Abstract

Wastes derived from animal sources have been recognized as alternative sources of plant nutrients and hence have a great potential to be used in sustainable agriculture and circumvent issues posed by the usage of synthetic fertilizers. The aim of the study was to assess the influence of different variations of livestock (pig) waste sources and other amendments on soil microbial biomass.

A strictly controlled laboratory experiment was set up with six treatments involving synthetic mineral nitrogen, pig manure, pig manure digestate, pig manure digestate and biochar, pig manure digestate and ash and the unamended. Soil microbial biomass which is used as an early indicator for changes in soil was determined before and after the respective treatments using the fumigation extraction method.

The results showed that pig manure digestate amended with ash had the highest percentage increase of 32.16% in soil microbial biomass carbon (SMBC) with control having the least SMBC value of 170.58 μg/g after fertilization. SMBC due to synthetic nitrogen fertilizer application was significantly different ($p < 0.05$) compared to other treatments. However, pig manure treatment and other co-amendments had significantly higher SMBC after fertilization. Hence, it can be concluded that pig manure digestates could be effectively used, alone or in combination with biochar or ash to increase the soil microbial biomass leading to enhanced nutrient cycling and an overall soil fertility.

Keywords: pig manure, digestate, soil, biochar, ash.

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Influence of Different Treatments on Tannins in *Quercus robur* Acorns

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Abstract

Oaks (*Quercus* spp.) are wildly known perennial, widespread throughout the Northern Hemisphere trees. One of the naturally growing oaks in Lithuania is the common oak (*Quercus rubra L.*), which has acorns, also known as oak nuts. Acorns are attracting increasing interest from researchers and healthy lifestyle enthusiasts due to their potential in the field of pharmacy, as many studies demonstrate antioxidant and antibacterial activities of various *Quercus* species, as well as antifungal, anti-inflammatory, or antiviral activities [1]. However, there is a problem with acorns toxicity, mainly the amount of tannins in the acorns. It is known that tannic acid is moderately toxic by the inhalation and ingestion exposure pathways for humans and for animals after daily dosage of 50 g for 16 days can be lethal [2]. However, there is no deaths have been reported from acorns poisoning [3]. Even so, tannins are known and valued for their own antimicrobial and antioxidant activities, suggesting a possible application of using it as sources of natural antimicrobials and antioxidants [4]. A study was performed with the 5 different Lithuanian regions (Kaunas, Šilutė, Jonava, Kretinga, Kėdainiai) acorns of the *Q. robur*. The acorns were treated in different ways to determine their role in tannin reduction: (1) heat treatment at 200 °C for 15 minutes; (2) heat treatment at 37 °C for 3 days; (3) soaking in distilled water for 18 hours at 45 °C; (4) fermentation of lactic acid bacteria - 24 hours using a culture of *Lactobacillus rhamnosus*. The amounts of phytochemical compounds in *Quercus robur* acorns were found to vary depending on their treatment method and habitat. The highest amounts of tannins were found in the extracts at 37 ºC compared to other treatments, and the lowest in acorns soaked at 45 °C. Tannins ranged from 22.0 to 50.1 mg/g. Tannin levels increased both after spontaneous and after fermentation with *L. rhamnosus* bacteria.

Keywords: tannins, toxicity, *L. rhamnosus, Q. rubra,* acorns.

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Changes of Minor and Major Cannabinoids in Hemp Methanolic Extracts During Storage

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Abstract

Cannabis Sativa L. is an important species of herbaceous plant which contains wide variety of chemical compounds, but one of the most important phytochemicals are phytocannabinoids. Cannabis contains more than 113 different cannabinoids, which are divided into different types: cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), tetrahydrocannabinol (THC), cannabicyclol (CBL), cannabinol (CBN) and others [1]. Cannabinoids have a positive therapeutic effect on people suffering from pain, anxiety, nausea, muscle spasms or exhaustion, so cannabis products may be useful for people with multiple sclerosis or Tourette syndrome [2].

Previous studies have shown that not all cannabinoids respond equally to different environmental factors and that unsuitable storage conditions for extracts may adversely affect the amount of beneficial compounds so it is important to determine the optimal storage conditions for extracts.

In this study minor and major cannabinoids were examined in *Cannabis sativa* L. leaves and flowers methanolic extracts during storage. Extracts were prepared and divided into 3 equal parts. One part was stored at room temperature, the other part was refrigerated at 3-4 °C and the third part was stored in a freezer at -18 °C. Quantitative analysis of phytocannabinoids before storage, after 2 and after 4 weeks of storage in different temperatures was performed using GCMS QP2010 gas chromatography - mass spectrometry system (Shimadzu, Japan).

Results are presented as means of concentrations and demonstrate that the amount of minor and major cannabinoids decreases after 2 and after 4 weeks of storage in all temperatures.

Keywords: *Cannabis sativa* L., cannabinoids, methanolic extracts.

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Screening of Estonian Plants for Anti-*Borrelia* Phytochemicals

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Abstract

Lyme disease, caused by the spirochaete *Borrelia burgdorferi*, is the most common vector-borne disease in Europe [1]. Antibiotics are an effective cure during the early stages of the disease. However, at an advanced stage, the chronic infection can affect joints and muscles, nervous and cardiovascular systems, and withstand several rounds of antibiotic treatment [2]. The recalcitrance of the illness is tied to the latent bacterial forms [3]. Therefore, novel therapeutic approaches are required to combat the *B. burgdorferi* persisters. Research suggests that antibacterial phytochemicals could aid in the treatment of chronic Lyme disease, either acting synergistically with antibiotics or when administered separately. Antioxidative and antibacterial properties of several plants found in Estonia have been demonstrated. Our research team investigates these plants with the aim of discovering new lead compounds with distinct anti-*Borrelia* activity. In this work, ten different plant extracts were studied, namely those of *Galium verum*, *Galium aparine*, *Thymus vulgaris*, *Origanum vulgare*, *Taraxacum officinale*, *Allium ursinum*, *Lonicera caerulea* var. *edulis*, *Leonurus cardiaca*, *Rhodiola rosea*, and *Rheum rhaponticum*. We tested the anti-*Borrelia* activity of the plant extracts on the latent bacterial forms using the SYBR Green I/PI assay [4]. To identify the antibacterial phytochemicals, the active extracts were fractionated and tested on *B. burgdorferi*, and the active extracts and fractions were chemically characterized by HPLC-DAD-MS/MS. The cytotoxicity of the active extracts and fractions was evaluated on mammalian cells by both 48h end-point measurements using WST1 assay [5], and real-time measurements during 48h using RealTime-Glo™ MT Cell Viability Assay [6].

Keywords: Lyme disease, *Borrelia burgdorferi*, phytochemicals.

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Analysis of Secondary Metabolites and Antimicrobial Properties of *Levandula* L. (*Levandula angustifolia* L.) and *Helichrysum* L. (*Helichrysum arenarium* L., *Helichrysum italicum* L.)

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Abstract

The demand for essential oils and plant extracts is growing due to their various medicinal properties, including antimicrobial effects, due to the presence of secondary metabolites that are important for the plant protection mechanism [1].

The aim of the study was to evaluate radical scavenging activity, phenolic compounds, and flavonoids of plant extracts of *L. angustifolia* L. and *H. arenarium* L. and to compare the antimicrobial properties of extracts and essential oils of *L. angustifolia* L., *H. arenarium* L. and *H. italicum* L.

The total content of phenolic compounds and flavonoids and radical scavenging activity in plant extracts were determined spectrophotometrically. Antimicrobial activity was determined using filter paper discs and wells, inoculation of bacteria and fungi on an agar plate using Drigalski spatula and inoculation with liquid media.

The results showed that highest phenolic compounds amount in *H. arenarium* L. extracts were found using 75% methanol and in the 25% extract of *L. angustifolia* L. Highest amount of flavonoids were found in 75% of *H. arenarium* L. extract and 50% extract of true lavender. *H. arenarium* L. extracts had the highest radical scavenging activity using 50% methanol for extracts and *L. angustifolia* L. had the highest radical scavenging activity using 50% methanol. Essential oil of *H. italicum* L. inhibited the growth of *Azotobacter vinelandii, Bacillus subtilis, Ulocladium chartarum* and *Cryptococcus neoformans (Sanfelice*). *H. arenarium* L. extracts had a small inhibitory effect on *A. vinelandii*. Purchased true *L. angustifolia* L. essential oil inhibited the growth of all bacteria and fungi tested. *L. angustifolia* L. essential oil grown in Lithuania had lower antimicrobial activity against the tested bacterial and fungal strains. Extracts of true lavender showed inhibitory effects on *A. vinelandii* and *U. chartarum*.

Keywords: *L. angustofolia* L., *H. italicum* L., *H. arenarium* L.

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Development of Gels as Stationary Phases for Chiral Capillary Electrochromatography

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Abstract

The living nature around us has chiral origin – the majority of important biomolecules, such as amino acids, carbohydrates, polysaccharides and proteins are enantiomers or compounds made up of enantiomers. Therefore, such receptors may give different results when interacting with chiral drugs. One of the enantiomers may be neutral or even have adverse effects when the other exhibits the desired pharmaceutical activity. It is therefore particularly important that the medicinal products should be produced enantiomerically pure or have defined enantiomeric composition, where appropriate. This has been regulated since 1992 by the FDA (US Food and Drug Administration) and national drug agencies [1]. Previous results obtained in our research group were the first in the world to use molecularly homogeneous agarose gels for reverse phase capillary gel electrochromatography [2]. Capillary electrochromatography is a separation technique with high efficiency and high selectivity for many compounds. These two properties are the result of a combination of high-performance liquid chromatography and capillary electrophoresis. The mobile phase and the analytes in the capillary column are driven by a strong electroosmotic flow (EOF). Separation is usually performed in fused silica gel capillaries [3]. Gels can be used as the entirely homogeneous stationary phase. The method of capillary electrochromatography is widely used in various fields: pharmaceutical analysis, biotechnological research, food, agricultural, environmental analysis [4]. The aim of this study was to develop a gel composite by fusing agarose and gelatin gels for capillary columns suitable for capillary chiral electrochromatography, capable of generating electroosmotic flow and having chiral selectivity. The 2% gel composite was prepared by fusing gelatin and agarose gels, capillary column length 30 cm, effective length 20 cm, 75 μm I.D. fused silica capillary was filled with a molten gel composite. A mobile phase of 15 mM pH 3 phosphate was prepared. The quality of the filled capillary and the voltage dependence on the current was evaluated. As this is a new way to apply the method to the separation of chiral compounds, the results of the optimization of the conditions will be presented in the presentation.

Keywords: chiral capillary electrochromatography, electroosmosis, gel composite.

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Combined Addition of Biochar, Bioactivators and Plants as Synergic Strategy for the Treatment of PH-Contaminated Soil

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Abstract

Due to its potential applications in carbon sequestration and in soil remediation, biochar, a product of biomass pyrolysis, is receiving increasing attention [1]. Among its physicochemical characteristics, the surface area and the adsorption capacity of the pore structure have been highlighted as particularly suitable to immobilize toxic compounds [2]. Moreover, the pores and particles of biochar may promote the growth and reproduction of microorganisms, possibly playing a valuable ecological role.
Within this framework, a mesocosm experiment has been set up under greenhouse conditions to evaluate the remediation potential towards petroleum hydrocarbons (PHs) in different soil treatments (biochar and biochar plus bioactivators, in presence or not of plants) by analysing: i) PH content and ii) the microbial community structure in terms of the total microbial abundance and cell viability. Notwithstanding the initial sampling revealed a soil PH concentration of about 10.000 mg/kg, value significantly higher than the limit established for agricultural areas by Italian legislation (C10-C40 = 50 mg/kg; DM 46/2019), the structure of microbial community presented remarkable values of microbial abundance (1.95E+08 - 2.44E+08 N. total cells/g soil) and of cell viability (around 40%) for all the experimental mesocosms. The bioactivators (produced by Eurovix S.p.A) added to the soil showed a total microbial abundance estimated by DAPI staining of 4.38E+10 - 8.02E+08 N. total cells/g soil of which fungal population was represented by 1.36E+10 - 7.61E+08 N. fungi/g soil. Preliminary results evidenced that, despite the high PHs contamination, the agricultural soil maintained an abundant and vital microbial community. Biochar, bioactivator and plant addition to soil are expected to improve the microorganism activity in order to increase the remediation.

Keywords: bioremediation, microorganisms, microbial community, petroleum hydrocarbons.

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An Impact of Volatile Disinfectants on Airborne Microorganisms

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Abstract

Volatile disinfectants have an impact on the quality of air. There is a diverse community of microorganisms from animals, humans, plants, soil, and water sources in the environment. Air disinfection can be applied to prevent and control various diseases. The essential oils can be used as volatile disinfectants. Essential oils can effectively kill bacteria, micromycetes, and viruses, for example, *Enterococcus spp., Staphylococcus spp., Enterobacter spp., Bacillus spp., Mycobacterium spp., Staphylococcus spp., Aspergillus spp., Penicillium spp. Alternaria spp.,* and other.

Different methods can be used for microbial aerosol detection, such as microbial culture counting, gene chip technology, biosensor technology, and direct microscopic detection. We can use methods to characterize microbial systems via 16S rRNA sequence analysis – this technology can detect microorganisms that are difficult to obtain by culturing methods and has been used in many fields. As an example, using the dispersion of *Citrus limon* and *Abies alba* essential oils helped to reduce the contamination of microorganisms [1].

Keywords: essential oils, microorganisms, disinfectants, environments.

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The Research and Application of Medicinal (Aromatic) Plants for the Solution of Human Health Problems

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Abstract

According to the WHO Traditional Medicine Strategy 2014–2023, Global Strategy for Plant Conservation 2011 – 2020 and adopted Convention on Biological Diversity (1993) the issue of enrichment of medicinal (aromatic) plants (MAP's) and their biological active substances diversity research and their rational consumption for healthy lifestyle optimization is being debated.

In recent years a lot of attention has been paid towards research and application of medicinal (aromatic) plants and their raw material rational and professional usage [2, 3].

Scientific Sector of Medicinal and Aromatic Plants, Scientific Department, Botanical Garden of Vytautas Magnus University, with the living MAP's collections, as centre of introduction, research, and protection of Medicinal plants, are one of the important links in the conservation of the MAP's diversity, including rare and endangered species [4, 5].

Object of the research and application: diversity of perspective Medicinal (Aromatic) Plants (MAP's).

The data of the research were obtained by analyzing the data of Directives, scientific articles, and literature sources, applying the theoretical methods of systematic analysis [1, 4, 5, 6].

This research work presents details of introduction perspective medicinal (aromatic) plants and restocking diversity, the creation of green spaces and revealing MAP's diversity collections cognitive importance of public education activities, personal natural sciences competences, respect to human health, operational safety, and environmental interaction problem.

Keywords: medicinal (aromatic) plants, MAP's, human health.

Acknowledgments

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Evaluation of $CaCl_2$ Influence on pDNA Electrotransfer Efficiency and Cell Viability Dynamics

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Abstract

Electroporation is a physical delivery method that increases the permeability of cell membranes and then large molecules as DNA can be introduced into the cell. Although gene electrotransfer has been researched extensively in both *in vitro* and *in vivo* systems, the methods by which DNA enters and navigates through cells are still unknown. It's been proposed that the negative charge of DNA might induce its electrophoretic movement near to the cell membrane and thus facilitate nucleic acid transfer. What is more, divalent ions like Ca^{2+} which abolish electrostatic repulsion between DNA and the cell membrane, might enhance DNA electrotransfer efficacy [1]. Thereby, the aim of this study was to evaluate the effect of different extracellular $CaCl_2$ concentrations (0-1 mM) on the pDNA transfection efficiency and cell viability tendencies. Experiments were performed using two sets of electroporation parameters: 1400V/cm, 100 μs and 1200V/cm, 250 μs. pDNA transfection efficiency and fluorescence intensity were evaluated using flow cytometry and fluorescence microscopy. For the evaluation of cell viability tendencies, FCA and MTT methods were used. Results revealed that the higher Ca^{2+} (> 0.25 mM) concentrations induce decrease in plasmid electrotransfer efficiency and results in significant decrease in cell viability.

Keywords: electroporation, DNA electrotransfer, $CaCl_2$, cell viability.

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Bioremediation of Polycyclic Aromatic Hydrocarbons from Railway Wooden Sleepers Under Field Conditions

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Abstract

Polycyclic aromatic hydrocarbons are a hazardous class of chemicals found in coal, crude oil, and gasoline. Also are found when gasoline, oil, coal, wood, tobacco, and garbage is burned. Polycyclic aromatic hydrocarbons are among the compounds of concern for human health due to their toxic, carcinogenic, mutagenic, and bio-accumulative properties [1]. To prevent the contribution of wooden railway sleepers, which are no longer used, to the pollution, a lot of research is carried out into their biological treatment.

The aim of this study was to evaluate the possibility to use white rot fungi to bioremediate wooden sleepers from polycyclic aromatic hydrocarbons safely. Also, it is important to test which species of white rot fungi could be the candidates for the most successful bioremediation and what quantity of toxic wood they could effectively remediate.

In this experiment railway wooden sleepers were shredded into pieces of 0.5-50 mm. Three groups of those sleepers were investigated: first group had the least amount of polycyclic aromatic hydrocarbons and was the lightest colour, second group had average amount of hydrocarbons and was grey, the last group had the highest amount of hydrocarbons and the colour was the darkest brown. The pieces of wood were weighed by colour and placed in bags. The bags were placed in an experiment container (2.0 m × 1.0 m × 0.35 m), filled with layer of 125 kilograms of soil and layer of 375 kilograms of shredded wooden sleepers, as well as 3 equal layers of inoculants (*P. ostreatus* and *I. lacteus*). Experiment was conducted outside in weather conditions for two months, only humidity was controlled.

For extraction each bag was weighed and placed in a 100 ml bottle and filled with acetone and ethyl acetate mixture (1:1). A gas chromatography with ionic flame detection method was used to evaluate the polycyclic aromatic hydrocarbons concentration.

The results showed that the polycyclic aromatic hydrocarbons bioremediation degree with white rot fungi under field conditions occurred with an accuracy of about 60% for two months. Overall, even more efficient bioremediation could be performed using better environmental conditions. In conclusion, we can state that extraction with organic solvents can be safely used to remove polycyclic aromatic hydrocarbons from railway wooden sleepers.

Keywords: polycyclic aromatic hydrocarbons, white rot fungi, bioremediation.

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Physicochemical Properties of Chitosan Vary Depending on Living Source and This Change Has Effects on Blood Plasma Parameters

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Abstract

Chitosan is a versatile biopolymer obtained from arthropod cuticles and fungal cell walls, and used in medicine, pharmacy, food/feed, agriculture, textile, and water purifications. In earlier studies, it was well determined that the properties of chitosan related with the isolated source organism. Herein, we produced chitosan from adult and larvae of *Pyrrhocoris apterus* to compare the physicochemical properties. We proved the purity of the obtained chitosan samples by using Fourier-transform infrared spectroscopy (FT-IR). Thermogravimetric analysis (TGA) and Scanning electron microscope (SEM) were used to determine the properties of the chitosan products. Thermal stability of the larval chitosan was found slightly higher than the adult chitosan. Interaction of the produced chitosans with the blood plasma were analysed. Therefore, it has been revealed that plasma parameters vary considerably according to the chitosan source. Considering all the results, it has been noted that the physicochemical properties of chitosan vary according to the living source and this change has different effects on blood plasma parameters.

Keywords: *Pyrrhocoris apterus*, chitin, chitosan, blood plasma.

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Study of Chitosan Antifungal Properties

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Abstract

Micromycetes cause disease and crop loss in fields worked and harvested by farmers. In order to find an environmentally friendly natural and non-polluting fungicide, attention paid to chitosan. Our aim is to elucidate the influence of chitosan molecular weight on its antifungal properties. The antifungal activity of chitosan different molecular weights (high, medium and low) observed in this study. The gels used in the study for each molecular weight were made from preparations of 0.5 g/l, 1 g/l and 2 g/l chitosan. In the study, we use non-sterile wheat and barley seeds coated with chitosan gel of different molecular weights and concentrations. Micromycetes identified in a study with spring cereals 'Koksa': *Bipolaris* spp. *Alternaria* spp., *Penicilium* spp., *Ulocladium* spp., *Mycelia sterilia*, *Saccharomyces* spp., *Mucor* spp. In a test with seeds of spring barley 'Podarek', the following were identified: *Bipolaris* spp., *Aspergillus* spp., *Alternaria* spp., *Penicilium* spp., *Ulocladium* spp., *Mycelia sterilia*, *Saccharomyces* spp., *Mucor* spp. After evaluating the ability of chitosan to inhibit the growth of pathogenic fungi, the fungicidal effect of low molecular weight chitosan on *Bipolaris* spp. *Alternaria* spp. *Saccharomyces* spp.; effects of high molecular weight chitosan *Bipolaris* spp. *Penicilium* spp. and *Mycelia sterilia* micromycetes. Evaluation of the ability of chitosan to inhibit the growth of pathogenic fungi revealed no interaction between the molecular weight and concentration of chitosan.

Keywords: chitosan, antifungal activity, micromycetes, *in vitro*.

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Effects of Chemoterapic FR901464 Compound on mRNA Expression in Hypoxic Cells

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Abstract

All living organisms respond to, and defend against, environmental stresses. These include temperature shock, oxygen shock (hypoxia), nutrient deprivation and DNA damage. It is very important to mention that hypoxia is a pathological condition of the body that develops due to insufficient supply of oxygen to the cells of the body's tissue and causes disorders of homeostasis [1]. In hypoxia, not only changes in gene expression are important but also another post-transcriptional mechanism - alternative splicing is important for cell adaptation. Hypoxia – induced splicing changes are associated with a number of cellular processes such as maintenance of proliferation potential, activation of angiogenesis and metastasis, regulation of energy metabolism, and avoidance of components of apoptosis inducers and the immune system [2]. So one of the ways cells adapt to changed environmental conditions is pre-mRNA splicing. Splicing is a form of pre-mRNA rearrangement in which introns are removed from an original transcript and exons are linked [3]. RNA splicing mechanism increases the diversity of proteins in the cell, which gives the cell the ability to adapt to altered environmental conditions [4]. Changes in splicing are found in almost all types of cancer. Tumors show up to 30% more changes in alternative splicing compared to healthy tissues [5]. Also, organisms respond to the effects of chemical compounds. Chemotherapy is a treatment that uses highly active chemicals to kill fast – growing cells in the body. FR901464 has anti-tumor activity and acts as an inhibitor of SF3B1 and histone deacetylase. The compound effects cancer cells, including cell cycle arrest, differentiation, apoptosis and transcriptional activity [6]. The aim of this study was to investigate the effect of chemotherapeutic compound FR901464 on the viability of HCT116 cells cultured under hypoxic conditions (1% O_2), splicing factor U2AF35, U2AF65, SRSF1, SRSF2 and SRSF5 expression level changes at RNA and protein levels and the expression of cancer-related Fas, RacI, FHIT gene mRNA formation.

Keywords: hypoxia, pre-mRNA splicing, chemotherapy.

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Can Deforestation Affect the Diversity of Testate Amoebae?

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Abstract

The clearing of forests has an enormous impact on invertebrates, vertebrates, and climate [1, 2]. Little is known about how the loss of trees affect the protists, which inhabit the floor of forests [3]. This study analyzed the effect of clearing forests on testates amoebae (*Testacea*), which are common in soil, moss, and freshwater. Testates amoeba feeds on decaying organic material, bacteria, and algae, thus including them in the nutritional networks in an ecosystem and contributing to the decomposition and nutrient cycling of soil and litter in forests [6]. The goal of this study was 1) to evaluate the diversity of testates amoeba in the moss of pine forest and 2) to evaluate does deforestation can affect the testates amoeba? During this study, testates amoebae were collected in three pine forests and three logging sites and extracted from the moss *Pleurozium schreberri*. Dominate taxa in the moss were assessed counting no less than 150 specimens per sample [4]. The diversity and distribution of testate amoeba were evaluated by ecological diversity indexes [5]. Totally 23 species of testate amoeba were found in the studied forests. Obtained results revealed the slightly lower diversity and domination of certain functional groups of testates amoebas in logging sites compared with studied forests.

Keywords: testate amoebae, deforestation, diversity, moss.

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Study of Qualitative and Quantitative Composition of Phenolic Compounds in *Artemisia Absinthium* L. Raw Material During Different Vegetation Stages

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Abstract

In recent years special attention has been pointed to *Artemisia* (L.) genus plants and their biologically active compounds in various fields of treatment [1,2]. Previous studies have shown that *Absinthii herba* accumulates high levels of phenolic acids, flavonoids, tannins, coumarins, sterols, essential oils, organic and fatty acids, but there isn't enough scientific knowledge about biologically active compounds accumulation during vegetation [3;4]. The object of investigation was *Artemisia absinthium* (L.) − a perennial, medicinal (aromatic) plant of *Asteraceae* (Bercht. & J. Presl) family. The aim of this study was to investigate non-volatile compounds composition of *Artemisia absinthium* (L.) raw material during vegetation period.

The raw material of *Artemisia absinthium* (L.) were collected during vegetation period in collection of Spice-Milliferous plants of Scientific department, Scientific sector of Medicinal and Aromatic Plants at Botanical Garden of Vytautas Magnus University in 2018−2020. The qualitative and quantitative composition of phenolic compounds was determinated by high-performance liquid chromatography method.

Phenolic compounds identified in *Absinthii herba* were luteolin-7-rutinoside, luteolin-7-glicoside, isorhamnetin-3-rutinoside, rutin, chlorogenic, caffeic, neochlorogenic, 4-O-caffeoylquinic, 3,4-di-caffeoylquinic, 3,5-di-caffeoylquinic acids. Chlorogenic acid was a predominant component among all evaluated phenolic compounds in vegetation period, variation coefficient (CV = 0.76%). The highest content of chlorogenic acid (118.66 ± 0.52 mg/g) was estimated in butonization vegetation stage and the lowest content (11.26 ± 0.01 mg/g) in the flower bud development stage.

Keywords: *Absinthii herba*, phenolic compounds, vegetation period.

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The Role of Hypoxic Microenvironment in Cancer Related Genes Pre-mRNA Alternative Splicing

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Abstract

Oncological diseases remain one of the unsolved problems of the entire society as the oncology-related morbidity and mortality has been systematically increasing both in Lithuania and around the world. Tumor cell genomes analysis has led to significant advances in the development of cancer therapies. Nonetheless, treatment-resistant tumor cells undergo apoptosis and are responsible for the recurrence of the disease [1]. Important factor that impacts pathological processes in tumor cells is the cell microenvironment. One of the main characteristics of the cell microenvironment is hypoxia, which also can change pre-mRNA alternative splicing of enormous variety of genes. Certain formation of various mRNA isoforms from various oncogenes provide angiogenic, anti-apoptotic, invasive and survival properties of cells [2].

Leukemia is a group of blood cancers that usually begin in the bone marrow and characterized by an increase in the number of immature and functionally impaired types of their precursor cells in the blood. Several studies have shown a global aberrant RNA-splicing in patients with leukemia compared to healthy individuals [3]. Altered expression of splicing regulatory proteins is one of the factors that alters mRNA formation related with oncogenity [4]. Nevertheless, the altered expression of splicing factors and mRNA isoforms that is associated with blood oncological diseases in a hypoxic conditions is yet to be investigated. Our research describes how leukemia associated genes mRNA formation in K562 cells is dependent on reduced oxygen concentration in the environment. The obtained results show that hypoxic microenvironment affects the expression of MAX, GFI1B and PUF60 leukemia associated genes mRNA isoforms. It also reveals how hypoxia impacts SRSF1, SRSF2 and U2AF splicing factors expression in the same cell line. These results provide some knowledge how hypoxic microenvironment of cells alters expression of tumor-associated mRNA formation.

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Essential Oil Composition and Antimicrobial Activity of Blackcurrant (*Ribes nigrum* L.) Buds

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Abstract

Blackcurrant (*Ribes nigrum* L.) is a widely cultivated shrub all over the world, especially for its high value berries containing various bioactive constituents, such as ascorbic acid, anthocyanins and polyphenols, and possessing unique aroma [1]. Leaves and buds due to their characteristic colour and excellent flavour have also found applications as a raw material for food and cosmetic industries [2, 3]. Essential oil (EO) is other valuable blackcurrant fraction obtained by hydrodistillation. Due to high cost EO is mainly used as a fragrance ingredient in perfumery, however EO and buds can also be used as natural food grade flavourings. The aim of this study was to analyse chemical composition of EO and evaluate its antimicrobial activity against food spoilage bacteria and yeast, including pathogens. The buds were donated by *Juodoji uoga*. EO was hydrodistilled in a *Clevenger* apparatus and analysed by gas chromatography – time-of-flight mass spectrometry (GC-TOF/MS). The antimicrobial properties against *Bacillus subtilis, Escherichia coli, Listeria monocytogenes, Salmonella enteritidis, Micrococcus luteus* and *Candida albicans* were evaluated by the agar well diffusion method by measuring inhibition zone (mm) after incubations for 24 h at 37 °C in plate count agar. The yield of EO was 0.86-0.92% (v/w). In total, 55 compounds were identified, which constituted 96.8% of the total integrated peak area. The content of a major compound *δ*-3-carene, possessing "citrus, terpenic, herbal, pine, solvent, resinous, phenolic, cypress, medicinal, woody" odour was 48.5-49.10% followed by *p*-cymene (5.88-6.01%), *p*-cymen-9-ol (5.49-6.54%), α-pinene (4.65-5.31%), sabinene (3.48-3.72%), caryophyllene oxide (3.14-3.72%). The EO was effective antimicrobial agents against all tested cultures at 1, 3, 5 and 10% concentrations. *M. luteus* was the most sensitive bacteria to buds EO: at 1% concentration the inhibition zone was 5.33 ± 0.20 mm, while 5 and 10% fully inhibited bacteria growth. The most stable against EO was *E. coli,* the inhibition zone were from 1.42 ± 0.20 mm (1% EO) to 2.83 ± 0.21 mm (10% EO). The results show that buds of blackcurrant are valuable source of natural phytochemicals, and its fractionation to valuable flavourings (EO) and possible antioxidant fractions might be applied in the development of effective bioactive ingredients for functional foods, medicinal, nutraceuticals, flavours and cosmetics.

Keywords: blackcurrant buds, *Ribes nigrum* L., essential oil, antimicrobial activity.

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Development of 3D Printable Radiation Shielding

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Abstract

3D printing has now become a standard manufacturing technique in various field of engineering and science. In many cases printable filament properties are paramount for the desired result. Manufacturing 3D printable filament from various polymers is a well known process, which allows for the modification of mostly mechanical properties of the resultant filament. Not much is done in the field of 3D printable radiation shielding material research. Such 3D printable filaments would allow for a more flexible, in terms of manufacturing, shielding components for the aerospace and medical fields.

In this study two subsets of 3D printable filaments were experimented with, that were based on ABS and PLA polymers. Different metallic additives in the form of powder were added into the polymers prior to the extrusion into filament. These additives were selected based on their atoms Z number, which is directly related to mass attenuation properties of the said material. Radiation attenuation properties in the MeV energy range was evaluated, together with the mechanical/tensile properties of the ready to print filament.

Experiments showed that by adding metallic materials into the polymer matrix, attenuation properties of the resultant polymer measurably increases.

Keywords: radiation protection, thermoplastics, 3D printing

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Evaluation of Volatile Compounds in Organic and Conventional Production Vegetable Raw Materials (Edible Carrots, *Daucus sativus* Röhl.)

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Abstract

The main aim of investigations was to identify determine differences between some carrot cultivars and hybrids, produced organically and non-organically. Fresh, cultivated at the same conditions carrots were provided by Lithuania Horticulture institute, Babtai. One of objectives was evaluate volatiles content by determine essential oils of carrots.

Carrots (*Daucus sativa* cultivars: *'Garduolės'(* GA*), Sylva',* (SY)*, 'Karlena',* (KA), *'Karotan'* KAR, 6 hibrids: *'Ieva' H,* (IE)*, 'Jola' H,* (JO)*, 'Soprano' H,* (SO)*, 'Maestro' H* (MA), *'Senior' H,* (SE), *'Romance' H* (RO) stored for up to 12 months at -40 °C (frozen storage), followed by processing into shreds. Volatiles from the carrot shreds were determined using gas chromatography coupled with mass spectrometry (GC-MS), to determine the volatile composition and aroma active components of carrots stored under -40 degrees temperature conditions. Total 25 compounds were quantified, of which mono- and sesquiterpenes accounted for approximately 98% of the total volatile mass. Among the main components of carrot essential oils and, major volatile compounds were (-)-alpha-pinene, monoterpenes: beta-myrcene (β-myrcene), (-)-limonene, (+)-limonene, (+)-sabinene, gamma-terpinene, p-cymene, terpinolene, sesquiterpenes: α-humulene and β-caryophyllene, and (E)- and (Z)-gamma-bisabolene. Samples were prepared by applying solid phase microextraction (SPME). The concentration of Terpenes is much higher than other volatile organic compounds (17-32% in total contribution).

Essential oil produced of carrot contributes directly to its aroma and flavour. The oil consists predominantly of blends of volatile 10-carbon monoterpenes and 15-carbon sesquiterpenes that reside in highly interconnected phloem oil ducts in the above- and belowground tissues [1, 2]. Specific sensory attributes have been associated with different terpenes [3].

Keywords: volatile compounds, *Daucus sativus*, organic and conventional production methods.

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