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14th International Scientific Conference **THE VITAL NATURE SIGN**

October 15th-16th, 2020

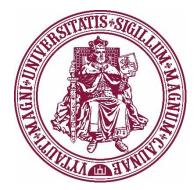
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ABSTRACT BOOK

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14th International Scientific Conference "The Vital Nature Sign" Program Thursday, 15th of October 2020

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10.30-10.45	Cellulose/Alginate Biocomposites and Their Properties A. Žiūkaitė, M. Strykaitė, J. Damašius
10.50-11.05	Quantitative Evaluation of <i>Irpex lacteus</i> and its Extracts Ability to Decolorize Textile Dyes M. Žagunis, N. Tiso, J. Mikašauskaitė-Tiso, A. Maruška
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12.05	Diversity and Plant Growth Promoting Traits of <i>Quercus robur</i> Cultivable Fungal Endophytes E. Beniušytė, D. Vaitiekūnaitė, S. Kuusienė
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- 9.40-9.55 Thermoluminescent Dosimetry of Low Energy Photons L. Lileikyte, B. G. Urbonavicius
- 10.00-10.15 Preliminary Analysis of Polymerization Dynamics in Dosimetric Gels Using Photospectrometric System M. Merkis, A. Jreije, B. G. Urbonavičius, D. Adlienė
- 10.20-10.35 Biofuel Cell Based on *Saccharomyces cerevisiae* Using Lipophilic and Hydrophilic Mediator Systems K. Blazevic, A. Zinovicius, J. Rozene, I. Morkvenaite-Vilkonciene, A. Ramanavicius
- 10.40-10.55 Smart Geiger-Muller Counter for the Internet of Things J. Beresnevičius, B. G. Urbonavičius
- 11.00-11.20 Coffee break

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- 11.30 Essential Oil Composition of Ground Ivy (*Glechoma hederacea* L) Growing in Northeast of Latvia

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11.35 Induced Decrease in Small Molecule Electrotransfer Efficiency by Extracellular Calcium

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- 11.40 Antioxidant and Anticancerous Properties of Eucalyptus Lignins *In Vitro* A. Oberemko, O. Oihana, G. Saulis, V. Baublys, J. Labidi
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- 11.50 Analysis of the Dynamics of Microplastics in the Urmia Lake Catchment R. Pashaei, R. Dzingelevičienė
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ORAL PRESENTATIONS

Serpin Expression in Chemoresistant Human Colorectal Carcinoma Cells HCT116

B. Aleksandravičiūtė, J. Navickaitė, V. Žitkutė

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Abstract

Colorectal cancer is one of the most commonly occurring cancers in men and women worldwide. Usually it is removed surgically, but if that fails it is often treated with a chemotherapy using a combination of 5-fluorouracil (5-FU) and oxaliplatin (OxaPt). These drugs cause DNA damage through different molecular mechanisms and it leads to a programmed cell death – apoptosis. However, the treatment often becomes complicated because of the intrinsic or acquired chemoresistance, caused by various molecular mechanisms: disrupted apoptosis, activated DNA reparation, altered drug metabolism, changes in cell signaling pathways. Furthermore, there is some evidence suggesting that serine protease inhibitors (serpins) might be one of the participants in the less examined chemoresistance mechanism. Serpins are a group of proteins, which are similar in their structure, but have a diverse profile of cellular interactions and roles. Depending on the type of tissue or serpin localization in a cell these proteins have a significant role in cell migration, proliferation, apoptosis or even extracellular microenvironment changes. Moreover, they function as biomarkers, tumour suppressors or oncogenes. However, there is a lack of significant evidence about their role in cancer or chemoresistance.

In this study we sought to reveal the patterns of the expression of four selected serpins (serpin E1, E2, B1 and B5) in the chemoresistant colorectal carcinoma cells (HCT116/FU and HCT116/Oxa) compared with chemotherapeutic drug sensitive cell line HCT116. We determined that serpin B5 transcript amount is higher in HCT116/FU cell subline compared to HCT116 cells. Based on this study, next we determined serpin B5 protein amount changes after 5-FU or OxaPt treatment in all cell lines. We found that serpin B5 protein amount increases after treatment with each drug in HCT116 and HCT116/FU cell lines, but only 5-FU treatment slightly increases serpin B5 protein amount in HCT116/Oxa cell line.

Keywords: colorectal cancer, serpins, serpin b5, chemoresitance, fluorouracil, oxaliplatin.

Smart Geiger-Muller Counter for the Internet of Things

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Abstract

Ongoing evolution of communication technologies such as LTE (4G) and upcoming 5G, as well as WiFi allows to transfer and receive data anywhere in the world. Such availability lets any device to connect to one network and act on incoming data or generate it from the environment (sensors). Such concept is also known as Internet of Things. In radiation protection field such technological invention helps to monitor radiation background over a large area and analyze such data in real time.

The aim of this work is to examine technological solutions for the development of a smart Geiger -Muller counter. The prototype is designed based on open source principles. To power Geiger tubes and receive its signal, high voltage source and processing circuit were designed. To ensure WiFi connection, ESP8266 module is selected as main microcontroller. Controller software was specially designed to have flexible functionality.

One of main principles of Internet of Things devices is low energy consumption. Developed system operating current is ~ 100mA. Such relatively low consumption allows to perform measurements using a lithium-ion battery.

Tests with the developed prototype have been made using different windowless and end-window Geiger-Muller tube types, which shown good reproducible results and let to confirm system reliability. High voltage source allows to change operating voltage and adjust it to the technical characteristics of the respective tube. The study of the influence of the operating voltage on the measurement results showed that the correct choice of the operating voltage significantly influences the operation of the end-window detector.

Keywords: Geiger-Muller counter, Internet of Things, ionizing radiation.

Biofuel Cell Based on Saccharomyces cerevisiae Using Lipophilic and Hydrophilic Mediator Systems

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Abstract

Saccharomyces cerevisiae, known as a baker's yeast, widely used in the food and beverage industries. Although, yeast cells are useful like a cell model system as it is a simple single-cell microorganism. Yeast metabolic mechanism is well known, inexpensive, could be cultivated in wastewater or in the media containing some unwanted industrial bio-products. Due to efficiency of redox reactions running during various metabolic processes [1] yeast can be used in the design of bio-electrochemical devices and systems [1-6]. However, the permeability of cell membrane and cell wall of yeast is very limited [2-5]. To improve electron transfer from cell to an electrode, double mediator system was used [7]. To determine electron transfer efficiency towards the electrode, cyclic voltammetry (CV) was applied. This electrochemical method illustrated that redox processes happening in yeast cells can be applied in the generation of electric current and energy of some chemicals suitable for metabolism of yeast can be converted into electrical one. During this research, concentration of yeast in the electrochemical cell was determined. Using this concentration of yeast, the influence of both lipophilic and hydrophilic mediators on the performance of the biofuel cell was assessed.

Keywords: *Saccharomyces cerevisiae*, biofuel cells, electrochemistry, redox mediator system, cyclic voltammetry.

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Determination of Glucose, Pyruvate, α-Ketoglutarate, Citrate, Succinate, Fumarate, Malate, Oxaloacetate and 2HG Levels *in vitro* by Using High Performance Liquid Chromatography Method

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Abstract

Metabolism is the biochemical reaction network where nutrients are converted into metabolites needed to maintain cell survival and proliferation [1]. Some basic metabolic pathways differ in cancer cells. Significant differences are observed in metabolites synthesized as a result of different phenotypes such as proliferation and invasion of cancer cells. This is observed in many energy metabolisms such as glycolysis, citric acid cycle, glutaminolysis, and oxidative phosphorylation [2]. In citric acid cycle, mutant isocitrate dehydrogenase 1/2 (IDH1/2) enzymes catalyze the conversion of 2-Hydroxyglutarate (2HG) from α -KG by gaining neomorphic enzymatic activity. While 2-HG is found in very small amounts in healthy cells, its excessive synthesis and accumulation in cancer cells directs the cell to metastasis [3]. In the present study, for the first time, the level of some intermediates such as pyruvate, α-ketoglutarate, citrate, succinate, fumarate, malate, oxaloacetate, 2HG, and glucose in citric acid cycle and glycolysis were investigated in primary (Caco-2) and metastatic character (SW620) colorectal cancer cell lines and the obtained data was compared with colon epithelial cell line (CCD18Co). Metabolites were extracted from the cells by the freezing-thawing method and their intracellular and extracellular levels were determined by an HPLC system. Significant differences were observed especially in the 2HG level between cancer and epithelial cell lines. The level of 2HG metabolite was 22.6 ± 1 and $152.6 \pm 1 \mu mol/10^6$ cell in Caco-2 and SW620 cells, respectively, whereas this metabolite could not be detected in CCD-18Co cell. Furthermore, as cells progressed to the metastasis stage, there was a significant increase in the 2HG level. According to the results, it can be concluded that 2HG metabolites may be a biomarker for early detection of colon cancer and these data are planned to be supported by in vivo and clinical studies in the future.

Keywords: colon cancer, glycolysis, metabolite, citric acid cycle, HPLC method.

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Development of a 2D Semiconductor Ionizing Radiation Detector

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Abstract

Various types of detectors (ex. gas ionisation, scintillation, thermoluminescence) as well as semiconductor detectors are used to detect ionizing radiation. Because these detectors are solid-state and have high speeds and, most importantly, are not exposed to external magnetic fields, their application in medical diagnostics is significant. However, the application of semiconductor detectors in radiometry has been little studied.

This research deals with the application of image analysis to radiometric measurements using a twodimensional semiconductor ionizing radiation detector. The CMOS sensor selected for the construction of the two-dimensional radiation detector is used in the Canon EOS 300D digital camera. Special hardware and software changes have been made to modify the camera to capture and record ionizing radiation. The individual mechanical components were specially designed and manufactured using the 3D printing technology.

Radiometric measurements using image analysis were performed using Fiji and MATLAB software environments, tested with several methodologies: video and continuous exposures. It has been observed that the video method does not allow to achieve unambiguous and detectable results. Using continuous recording radiation recording, a series of experiments were performed to determine the optimal values for the image analysis algorithms and finding the threshold effect value was particularly important.

For practical radiometric measurements, a check of the inverse distance square law was performed, the results of which coincided well with the results of the radiometer RKSB-104, using both Fiji and MATLAB algorithms. After confirming the reliability of the developed measurement system, an experiment was performed to determine the absorption coefficients of different materials, during which the absorption coefficients of the materials used in 3D printing for beta particles were evaluated for the first time.

Keywords: semiconductor detector, radiometric measurements, image processing, Otsu method.

Thermoluminescent Dosimetry of Low Energy Photons

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Abstract

Thermoluminescent dosimeters are one of the classical dosimetric methods used in the field of radiation safety and patient dosimetry. Patient dosimetry is particularly important for optimizing the radiotherapy and diagnostic procedures. Most challenging aspect of this optimization is performing in vivo dosimetry during diagnostic procedures. Most complex are low-energy and low-dose diagnostic procedures, such as mammography. In this work, dosimetry of mammography procedures using thermoluminescent dosimeters was investigated.

During the study, the radiation doses found in the mammography procedures were simulated using a Leybold Didactic 554 801 X-ray machine, selecting the X-ray energy in the range [10-35] keV. In order to evaluate the possible influence of the angle of incidence of radiation into the dosimeter on the measurement results, the range of angles of radiation incidence into the dosimeters $[0^{\circ}-30^{\circ}]$ was investigated.

The obtained results allowed to evaluate the possibilities and dosimetric characteristics of the application of TLD-100 in the field of low doses and energies. It was found that the measuring limit (energy) of the used TL-100 dosimeters is at 20 keV, and the angular dependence of the dosimetric response is very strongly expressed in the whole studied angular range $[0^{\circ}-30^{\circ}]$.

Keywords: thermoluminescence, low energy photons, dosimetry, mammography, absorbed dose.

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Heavy Metals Contents in Polish Bee Pollen, Royal Jelly and Propolis

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Abstract

Bee pollen, royal jelly, and propolis are bee products, which provide nutrition and contain significant amounts of biologically active compounds: amino acids, proteins, vitamins, mineral salts, and phenolics [1]. They show health-promoting properties, appreciated in natural medicine since ancient times. To this day, bee products are recommended as dietary supplements for people whose lifestyle is active, and for a recovery. However, due to their natural origins, they could contain heavy metals contaminants [2].

The aim of this study was to analyze a broad spectrum of heavy metals in bee pollen, royal jelly and propolis, in order to evaluate bee exposure to various chemicals and to check the quality of their products. The studied samples were collected in Greater Poland Voivodeship in Poland. The qualitative analyses were performed with inductively coupled plasma - mass spectrometry (ICP-MS) technique.

The methodology proposed in this study allowed for the determination of 16 heavy metals in the samples. The results revealed notable differences in contaminants levels depending on the selected bee product. In general, propolis and bee pollen was found to contain higher levels of almost all examined metals in comparison to royal jelly. The most visible differences involved levels of manganese, cobalt, aluminium, nickel, barium, cadmium, and lead. The differences in metal contaminants of bee pollen, royal jelly, and propolis may result from the origins of bee products – royal jelly is a pure secretion of bees' glands, whereas bee pollen and propolis contain constituents derived directly from plants.

The contamination of bee products may arise both from environmental pollution and beekeeping practice. Since excessive heavy metals intake may lead to adverse effects, the acceptable levels of metals and other contaminants in bee products should be precisely defined. It is particularly important regarding the increasing interest of apitherapy and natural foods.

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Preliminary Analysis of Polymerization Dynamics in Dosimetric Gels Using Photospectrometric System

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Abstract

Polymer gel dosimetry is becoming increasingly important with the development of sophisticated radiation therapy techniques which require precise planned dose distribution verification. Control measures are necessary in order to avoid severe damage to the patient. Polymer gel dosimeters have unique capabilities when compared to conventional dosimetry techniques: these dosimeters allow to measure dose distribution in three dimensions with high spatial accuracy, moreover, they are tissue-equivalent in terms of absorption of ionizing radiation. Working principle of polymer gel dosimeters is based on radiation-induced polymerization process which is proportional to the absorbed dose. Due to polymerization, physical properties of the gel, for example optical density, are affected. These physical changes can be detected with various imaging techniques (spectroscopy, magnetic resonance imaging, computed tomography, etc.).

During evaluation of polymer gel dosimeters particular attention must be drawn to processes occurring after irradiation of the polymer gel because they can affect stability of the acquired dose distribution. Despite the popularity of polymer gel dosimeters, research regarding the polymerization dynamics is scarce, due to the complex processes and measurement techniques involved. In this work post-irradiation characteristics of nMAG polymer gel dosimeter were explored. A unique real-time photospectrometric measurement system was developed for this purpose. Light absorbance in the polymer gel sample was continuously monitored during the irradiation and up to 24 hours after. Data was acquired at particular wavelength (660 nm) where changes in absorbance were the greatest. From the acquired post-irradiation absorbance curves it was notified that polymerization processes continue throughout the entire observation period. However, the majority (80%) of post-irradiation polymerization ends in first 8-10 minutes. For more complete analysis of post-irradiation effects in polymer gel dosimeters longer investigation period should be selected that would enable to determine when the gel dosimeter becomes relatively stable.

Keywords: polymer gel dosimetry, polymerization dynamics, photospectroscopy.

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Enzyme-Assisted Extraction New Method of Biologically Active Compounds Isolation

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Abstract

Plant material is virtually an unlimited source of biologically active compounds used in the pharmaceutical, cosmetic and food industries, and its extraction is a key stage in obtaining metabolites. The synthesis and storage of bioactive compounds are multi-stage processes occurring both in the intracellular membrane system of plant cells and in the extracellular matrix (cell walls, intercellular spaces). Hence, some biologically active compounds are localized intracellularly, and some are bound by hydrophilic interactions or hydrogen bonds with components of cell walls, e.g. pectins, cellulose or hemicellulose. Accordingly, an effective enzyme-assisted extraction protocol has been developed that uses cell wall degrading enzymes such as pectinases, cellulases, glucanases and xylanases. The aim of the research was to develop techniques for extracting biologically active compounds from plant material with the use of enzymes that degrade cell walls. Obtained results indicate that the use of the commercially available kemzyme preparation, which includes, among others, cellulase, pectin and glucanase effectively increase the extraction efficiency. The extracts from enzymatically hydrolized plant material are characterized by a higher content of biologically active compounds and a higher antioxidant activity. The analysis of the material subjected to enzymatic hydrolysis at the level of the transmission electron microscope allowed to observe characteristic changes in the organization of cell walls, including the relaxation of interactions between individual cellulose fibrils. This process significantly improves the permeability of cell walls and facilitates the elution of bioactive compounds. Therefore, the enzymatically assisted extraction of plant material is an effective alternative to conventional extraction methods.

Keywords: enzymatic-assisted extraction, biologically active compounds, transmission electron microscopy, plant material.

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Evaluation of Glucose Oxidase Catalyzed Reaction Using Scanning Electrochemical Impedance Microscopy

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Abstract

Analysis and diagnostics play a pivotal role in modern medicine. During the COVID-19 worldwide pandemic diagnostic tests are getting more attention from the scientific community. More and more tests use biological compounds as a sensing element. Sensors that use such elements are called biosensors [1].

Electrochemical impedance spectroscopy (EIS) was successfully applied to evaluate biosensors with recognition element immobilized directly onto the electrode [2]. In this case, the diffusion of reaction products towards the electrode is blocked. To solve this problem, the recognition element is immobilized on the chosen surface, and detecting electrode is at some distance from modified surface. In this case, microelectrodes with the positioning possibilities are used. Such system is called scanning electrochemical microscopy (SECM). By combining SECM with EIS (SEIM) it is possible also to perform non-distractive experiments, since EIS has no influence on the system of interest, and measurements can be performed in biological-friendly medium to sustain biological activity during the experiments.

During research, the enzyme – glucose oxidase was immobilized on the dielectric surface. SEIM was applied for the evaluation of glucose oxidase catalyzed reaction. To ensure superior sensitivity, redox competition mode was used. Experimental data showed that charge transfer resistance seems to be the best parameter evaluating the glucose oxidase catalyzed reaction at different glucose concentrations. The glucose concentration can be determined up to 15 mM.

To conclude, scanning electrochemical impedance microscopy is a viable method for glucose biosensor evaluation *in situ*.

Keywords: glucose oxidase, redox-competition mode, scanning electrochemical impedance microscopy, biosensor.

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Cellulose/Alginate Biocomposites and Their Properties

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Abstract

Lately the interest in biodegradable and environmentally friendly packaging has grown rapidly. The main material used for bio-based packaging are biopolymers like proteins, lipids, polysaccharides, etc. [1, 2]. Thus, polysaccharides are the most promising components as bio-packaging material considering their great mechanical properties, availability and low cost. Therefore, there has been a wide interest in biopolymer-based edible or non-edible packaging for food products. However, most of food bio-packaging are sensitive to moisture, so the wider application of this type of packaging is limited [3]. Furthermore, there has not been enough work done in creating inedible biodegradable food packaging which would be suitable for raw meat, confectionery, and other products which have high moisture content.

The aim of the research was to produce cellulose/alginate biocomposites by laminating prepared cellulose (C) fiber plates with sodium (SA) and calcium alginates (CA) containing glycerol as a plasticizer and evaluate their properties.

Cellulose/alginate biocomposites were prepared by the solvent casting method using sodium alginate solutions of different concentrations. Half of the prepared cellulose/sodium alginate biocomposites were treated with calcium chloride solution to produce cellulose/calcium alginate biocomposites. The morphology, moisture content, solubility in water, water vapor permeability (WVP), and surface hydrophobicity of prepared cellulose/alginate biocomposites were evaluated and compared.

During this experiment, 61 different samples of cellulose/alginate biocomposites were prepared. The biocomposite surface morphology evaluation showed that all analyzed samples had smooth surfaces without cracks. Also, obtained results showed that the concentration of glycerol had a positive effect on gloss, flexibility, moisture content, solubility in water and negative effect on surface hydrophobicity of C/SA and C/CA biocomposites. It was observed that C/CA10-5 sample had a hydrophilic surface and its measured contact angle was 35.595° while all other samples had partly hydrophobic surfaces which contact angles varies from 37° to 63° . The highest WVP was determined in C/SA1-5 (18.568 g·mm·kPa⁻¹·h⁻¹·m⁻²) and C/CA1-5 (15.090 g·mm·kPa⁻¹·h⁻¹·m⁻²) biocomposites.

The results confirm that the addition of a plasticizer had a positive impact on biocomposite surface gloss appearance and a negative impact on the moisture content and solubility in water of cellulose/alginate biocomposites. Moreover, the results showed that all biocomposites have partly hydrophobic surfaces except for the C/CA10-5 biocomposite, which surface was hydrophilic.

Keywords: food packaging, biocomposite, cellulose, alginate, hydrophobicity

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Quantitative Evaluation of *Irpex lacteus* and its Extracts Ability to Decolorize Textile Dyes

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Abstract

Textile dyes are widely used in the textile industry due to their broad range of colors, high stability and simplicity of usage. Increased demand for these dyes creates ecological problems because considerable amounts are discharged into wastewater during manufacturing [1]. These dyes are difficult to remove due to their synthetic nature and complex molecular structures. Physical and chemical treatment methods have proven to be costly and harmful to the environment. An alternative, inexpensive and eco-friendly solution for this problem is the use of microbial bioremediation using microorganisms such as white rot fungi [2].

This study describes the application of *Irpex lacteus* and its extracts to decolorize a variety of textile dyes. The ability of *I. lacteus* to decolorize textile dyes was spectrophotometrically quantified by preparing malt extract liquid mediums supplemented with 0.02 % (w/v) textile dyes that were inoculated with isolates of *I. lacteus* and were observed during a 7-day period. Decolorization efficiency was evaluated by measuring and comparing the absorbance of the samples with a control at a predetermined wavelength for each textile dye using a UV-vis MILTON ROY spectronic 1201 spectrophotometer (USA). It was estimated that *I. lacteus* can degrade up to 99% of the investigated textile dyes contained in the liquid medium during observation period. Extracts of liquid and solid-state fermentations of *I. lacteus* were prepared from 7-day old inoculated malt extract liquid mediums without any supplementary dye and 28-day old solid-state fermentation medium using wood pellets. The extracts were supplemented with 0.02 % (w/v) of textile dye in the presence of 0.1 mM H₂O₂. Decolorization was evaluated during a 3-day period. An average of 30% of textile dye was decolorized by the liquid extract and an average of 39% by the solid-state fermentation extract.

Keywords: Irpex lacteus, decolorization, textile dyes, bioremediation.

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POSTER PRESENTATIONS

Microbiological Quality of Biltong Marketed in Lubumbashi, Haut-Katanga Province, RD Congo

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Abstract

Biltong is a stable and ready-to-eat traditional meat product stored under ambient conditions. This work was carried out with the aim of characterizing several biltong samples marketed in Lubumbashi according to their microbiological characteristics.

75 samples of biltong were taken at random from five supermarkets in the city of Lubumbashi with 15 samples per supermarket. Thus, aerobic mesophilic flora, yeasts and moulds, *Staphylococcus aureus* and *Clostridiums perfringens* were counted on these samples after decimal dilutions and seeding in selective media.

The results of the analyses revealed that the aerobic mesophilic germs were the predominant flora of the biltong studied in five large supermarkets of the city of Lubumbashi. In fact, of the 75 samples collected, 45 (60%) were above the limit in aerobic mesophilic germs. Counts of *Staphylococcus aureus* in the samples we investigated showed higher maximum values in supermarkets 1 and 2 of 3.34×10^2 CFU/g and 7.22×10^2 CFU/g, respectively. *Clostridium perfringens* revealed lower average values, 2.90×10^1 CFU/g of biltong below the threshold set by the regulations. The mean contamination for yeasts and molds was $2.51 \times 10^2 \pm 0.17$ CFU/g of biltong examined.

The study shows that biltong produced and marketed in Lubumbashi is not of satisfactory hygienic quality, because the loads of aerobic mesophilic germs, yeasts and moulds are higher than the microbiological standards that raw and dried meat products must meet to be recognized as fit for consumption. Improved hygiene conditions during the meat processing process and preservation would significantly reduce the presence of these germs.

Keywords: Biltong, transformation, pathogenic germs, food poisoning.

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The Evidence of "Bystander" Effect after Bleomycin Electrotransfer

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Abstract

According to the World Health Organization, cancer is the cause of death of 9.6 million people in 2018. One of the options of cancer treatment is anticancer drug delivery or gene therapy. Therefore, intensive research is being done to obtain efficient and controlled transfer of drugs and genes to cells and tissues. Electrochemotherapy (ECT) is one of the possible options for novel cancer therapy that utilizes the method of electroporation. Electroporation occur once cells are stimulated with electric field at significant intensity, thus affected cell plasma membrane becomes temporarily permeable to various hydrophilic substances, such as the anticancer drug bleomycin (BLM). After BLM enters the cells its mechanism of action triggers the generation of, reactive oxygen species (ROS), resulting in multiple genomic DNA breaks hence leading to cell death.

Similar phenomenon of ROS generation is being observed when radiotherapy performed. Then ionizing radiation is the cause of ROS generation in the target cells, that cause cell death. It is known that radiation-exposed cells when killed in the environment, secrete signaling molecules that affect adjacent cells but are not directly affected by ionizing radiation. This phenomenon is called the Bystander effect. Although the effect of Bystander is known in radiotherapy, it is unexplored in electrochemotherapy - there is no evidence that this phenomenon is possible during electrochemotherapy. Therefore, the aim of this study was to evaluate the effect of the Bystander effect on cell viability after irreversible electroporation and in vitro electrotransfer of the anticancer drug bleomycin.

The study presented here is performed using CHO-K1 cells. The electrotransfer of the anticancer drug bleomycin is achieved using a single electrical pulse of 1400 V/cm amplitude and 100 μ s duration. Irreversible electroporation is achieved using a single electrical pulse with an amplitude of 2800 V/cm and a duration of 100 μ s. The Bystander effect is produced by incubating the cells after electroporation in a 24-well plate in a 0.2 ml DMEM growth medium for 24 hours. After incubation, the culture medium is collected and centrifuged twice. Finally, this growth medium is poured onto cells that have not been exposed to anything before. These cells are subjected to a colony formation test and the size (mm) and the number of colonies are assessed.

Based on the obtained results, we can state that after the electrotransfer of the anticancer drug bleomycin, the viability of surrounding cells, but not directly affected by electric fields, are affected (both phenomenon of mitotic arrest and cell viability change significantly). This effect is negligible after irreversible electroporation - although cell viability decreases, but the mitotic arrest remains unchanged.

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

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Effect of Different Types of Marinating Formulation and Grilling Methods on Chicken Quality and N-nitrosamine Formation

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Abstract

Meat products can be contaminated with carcinogenic *N*-nitrosamines, which is ascribed to the reaction between a nitrosating agent, originating from nitrite or smoke, and a secondary amine, derived from protein and lipid degradation. Although in model systems it is demonstrated that many amine containing compounds can be converted to *N*-nitrosamines, the yield is dependent of reaction conditions (e.g., low pH and high temperature).

The effects of five different marinates (e.g., pH, spices, starter culture *Lactobacillus plantarum*) and three different heat treatment (coal (220 °C temperature), firewood (240 °C temperature) and oven (300 °C temperature)) were investigated on *N*-nitrosamines, biogenic amines, and residual nitrites contents in heat-treated chicken. The various *N*-nitrosamines were isolated by a steam distillation method and analyzed by gas chromatography mass spectrometry (GC-MS). The biogenic amines were determined after extraction with perchloric acid as dansyl derivatives by high-performance liquid chromatography (HPLC) method. Descriptive statistics were used for data analysis.

The nitrite residues in inoculated samples with *L. plantarum* were significantly lower than in control samples (P<0.05). Also *L. plantarum* had a high inhibitory effect on N-nitrosodimethylamine (NDMA), N-nitrosopiperidine (NPIP), N-nitrosodiphenylamine (NdphA), N-nitrosodipropylamine (NDPA), N-nitrosodiethylamine (NDEA), N-nitrosodiisobutylamine (NDBA) and N-nitrosopyrrolidine (NPYR) after different heat treatments in chicken (P<0.05). Three different heat treatments gave different total *N*-nitrosamine concentrations (oven $- 6.46 \mu g/kg$, coal $- 7.70 \mu g/kg$, firewood $- 9.22 \mu g/kg$). From the above treatments we can conclude that the healthiest and best way is to use the oven. However, the amount of spermine in chicken samples was highest in heat treatment with wood than in control samples or other heat-treated sample (P<0.05).

These results demonstrate that *L. plantarum* could be used for marinating chicken to improve quality characteristics and reduce *N*-nitrosamine and nitrate formation during different types of grilling.

Keywords: L. plantarum, N-nitrosamine, marinated poultry, biopreservation, nitrite.

Diversity and Plant Growth Promoting Traits of *Quercus robur* Cultivable Fungal Endophytes

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Abstract

Endophytes are microorganisms, such as fungi, yeast, or bacteria that can be found in plant hosts for at least a part of their life cycle, but do not cause their host any harm or an evident infection. All plants tested so far have been shown to have them.

Fungi are common plant endophytes, and they are usually of the *Ascomycota* phylum. Endophytic fungi can exhibit PGPTs (plant growth promoting traits), which include phosphate mineralization and solubilization, and iron transport agent-siderophore production. These properties can be crucial for the host, especially in environments, where phosphorus and iron are not readily available.

Quercus robur or the common oak is a model tree for its genus. It is native in Europe, while *Quercus* genus grows widely throughout the Northern Hemisphere. Oaks have an extensive ecological and economical importance. However, their endophytic diversity is not thoroughly documented.

In this study endophytic fungi were isolated using a surface sterilization method from common oak trees. Pure strains were characterized using microscopy, colony morphology and DNA sequencing. The strains were screened for phosphate mineralization/solubilization and siderophore production using selective media.

Fourteen pure strains have been isolated, 5 of them were yeasts and 9 were molds. Based on sample viability, colony morphology and DNA sequencing results, 7 strains were screened for PGPTs. All tested strains were positive for phosphate mineralization/solubilization and siderophore production. DNA sequencing results revealed that the majority of isolates were from the *Ascomycota* phylum. In total strains from 8 different genera were isolated: *Aspergillus* sp., *Byssochlamys* sp., *Epichloë* sp., *Lecanicillium* sp., *Meyerozyma* sp., *Microstroma* sp., *Neocucurbitaria* sp. and *Talaromyces* sp.

Keywords: common oak, Quercus robur, plant growth promotion, fungal endophytes, endophytic yeast.

Analysis of Thuja (*Thuja spp.*) Biological Activity

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Abstract

The object of investigation of this research were leaves and bark of *Thuja standishii* (Gord.) Carr., *Thuja occidentalis* L. and *Thuja occidentalis 'Aurescens'*. The aim was to measure quantities of flavonoids and phenolic compounds, radical scavenging activities, compare the results to find out which parts of the plant and which species produce the most of the compounds, evaluate the effects of storage allelopathic and antimicrobial properties.

Gas chromatography-mass spectroscopy revealed that the main compounds in *T. occidentalis* leaves and bark are α -thujone, fenchone and β -thujone, in the wood – sabinene, α -thujone and terpin-1-en-4-yl acetate, in the cones – α -fenchene, α -thujone and myrcene. The main compounds in *T. occidentalis* '*Aurescens*' leaves are limonene, delta-3-carene and α -pinene, in the bark – caryophyllene and humulene, in the wood – sabinene, terpin-1-en-4-yl acetate and caryophyllene, in the cones – α -pinene and delta-3-carene. The main components in the extract of *T. standishii* leaves are sabinene and α -pinene, in the distillate of the leaves – terpinen-4-ol, sabinene and β -selinene, in the distillate of the leaves – terpinen-4-ol and α -terpinol, in the bark and cones – sabinene, α -pinene and myrcene.

All of the *T. occidentalis* extracts had some allelopathic properties against *Lepidium sativum* L. Vegetation period had little effect on the allelopathic properties. The extracts of *T. occidentalis* leaves inhibits the growth of *L. sativum* more than the extracts of cones and bark (including viability, the length of the stem and of the root). Nevertheless, in some cases the extracts made of cones have shown higher effect comparing to the extracts of leaves.

Antifungal activity was measured against *Penicillium candidum* and *Penicillium roqueforti*. None of the extracts had any inhibition in the growth of these fungi. However, the essential oils had some effect against *P. candidum*: *T. occidentalis* - 8 mm, *T. occidentalis Aurescens* - 9 mm, *T. standishii* - 7 mm. The extracts had no antibacterial activities against *Staphylococcus aureus* and *Proteus vulgaris*. Nevertheless, the essential oils had some antibacterial activities. Inhibition zones against *S. aureus*: *T. occidentalis* - 12 mm, *T. occidentalis 'Aurescens'* - 9 mm, *T. standishii* - 8 mm; against *P. vulgaris*: *T. occidentalis* - 10 mm, *T. occidentalis 'Aurescens'* - 10 mm, *T. standishii* - 9 mm.

Keywords: phenolic compounds, flavonoids, radical scavenging activity, storage effects, allelopathic properties, antifungal activity, antibacterial activity, *Thuja standishii, Thuja occidentalis, Thuja occidentalis 'Aurescens'*.

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Electrochemical Deposition of Polypyrrole Films Modified with Phenothiazine Derivatives

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Abstract

Electrochromism is the phenomenon, where the color of a material changes by applying a voltage [1]. This feature is very important for a wide range of materials such as smart windows, screens, thermal modulators and others [1]. Electrochromic properties are characteristic for some metal oxides and also some organic materials such as polymers too [2]. Polymers have a wide range of possibilities for both synthesis and application, and therefore can be used in the production of electrochromic materials [4]. Phenothiazine derivatives are biologically active compounds [3], which may be polymerized [4].

The aim of this work was to synthesize polypyrrole films modified with three phenothiazine derivatives: methylene blue (MB), azure A (AA), thionine (TH) and to investigate the properties of the obtained materials.

Before electrochemical deposition of the films, the glass/ITO electrode was washed in the solution consisting of 27% NH₄OH and 30% H_2O_2 mixed at ratio 3:1 preheated up to 50°C for 3 min. Later the electrode was cleaned at room temperature in ultrasonic bath subsequently in water, acetone and water for 15 min in each liquid.

The films were electrochemically deposited by potential cycling in a range from -0.2 V until +1.0 V, at potential sweep rate of 50 mV/s, by 25 cycles with a step lift of 2.44 mV. A polymerization solution was prepared in water with 10 mM of phenothiazine derivative (MB, AA, or TH) and 50 mM of pyrrole.

Keywords: polypyrrole, phenothiazine derivatives, methylene blue, Azure A, thionine, electrochromism.

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Dependence of Bleomycin Cytotoxicity on Plasmid Size Using Concomitant Bleomycin and Plasmid DNA Electrotransfer

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Abstract

Electroporation is a process, when applied short and intense electric pulses that transiently permeabilize the cell plasma membrane, thus allowing transport of molecules otherwise non-permeant through the membrane. The method has been applied for anticancer treatment, as anticancer drugs, can be easier transported into electroporated tumour cells. Currently, the method is used in clinics and termed as electrochemotherapy (ECT). At this moment, various methods are being explored to increase the impact of ETC, such as gene transfer.

Preliminary studies have shown that plasmid DNA and bleomycin (BLM) in the medium allows simultaneous transport of both molecules. Also, we have shown that the presence of DNA in the medium can increase bleomycin transport and thereby cytotoxicity These effects can be used to increase the efficiency of ECT and to regulate the immune response, by delivering genes, encoding specific cytokines.

Chinese Hamster Ovary cells were used for bleomycin and plasmid DNA electrotransfer experiments. BLM was used at the concentrations ranging from 0.1 to 10 nM. pEGFP (4.7 kb) and piggyBac (7.1 kb) plasmids in concentrations of 200 μ g/ml were used. Cells were suspended in electroporation medium (conductivity 0.1 S/m, osmolarity 270 mOsm, pH 7.1). Cell electroporation was performed by using combination of 1 electric pulse of 1400 V/cm pulse strength and 100 μ s pulse duration.

Our study has shown that a combination of plasmid DNA, bleomycin, and electroporation increases the cytotoxic effect of the anticancer drug. Furthermore, the results have shown that the cytotoxic effects of anticancer drug (BLM) are dependent on plasmid DNA size.

Keywords: plasmid DNA, bleomycin, electrotransfection.

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Development of an Electrochemical Polypyrrole Sensor with L-Tryptophan Imprints

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Abstract

Molecular imprinting is a technique, in which functional monomers are polymerized in the presence of the target analyte, which acts as a template. Subsequent removal of template liberates binding sites complementary in shape and size to the template structure, allowing the polymer to re-bind the analyte with high specificity [1]. The resulting molecularly imprinted polymers can be used in various recognition-based applications.

The template molecule used in the experiment was L-tryptophan. L-tryptophan is an essential amino acid. It plays an important role in protein synthesis and it is also the precursor of a variety of biologically active compounds including serotonin, melatonin, NAD, and NADP [2].

The synthesis of molecularly imprinted polymers (MIPs) was performed electrochemically and the layer of polypyrrole imprinted with L-tryptophan molecules was formed onto the graphite electrode. The electrochemical synthesis was performed by potential cycling method. After the synthesis, template molecules were removed by washing MIP in water. The recognition ability of L-tryptophan was evaluated in a solution of Britton-Robinson buffer, pH 2.50. Differential pulse voltammetry method was carried out to determine the characteristics of developed L-tryptophan sensors, which were based on molecularly imprinted polymers. L-tryptophan electrochemical oxidation peaks were observed at potential around 0.9 V vs Ag/AgCl_(3 M KCl).

Keywords: polypyrrole, molecularly imprinted polymers (MIP), L-tryptophan.

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Determination of Total Phenolic Content and Antioxidant Activity of the Kombucha Beverage Derived from Lemon Balm, Linden Flowers, Oak Bark and Caraway Seeds

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Abstract

Kombucha is a fermented drink original made of *Camellia sinensis* L. tea sweetened with sugar, produced with symbiotic culture of bacteria and yeasts (SCOBY). Kombucha tea contains cultures of acetic acid bacteria, lactic acid bacteria and yeasts. Chemical assays of kombucha beverage have indicated various types of compounds, such as polyphenols, vitamins, organic acids, sugars, ethanol. The concentration of the compounds depends on fermentation conditions and raw material. Due to the wide range of bioactive compounds, kombucha might have a positive effect on the living organism [1]. The aim of this research was to evaluate total amount of phenolic compounds and antioxidant activity of kombucha prepared from dried lemon balm, linden flowers, oak bark and caraway seeds infusions. This work gains new knowledge on the use of different herbs, which have never been or insufficiently considered as a substrate for kombucha beverage. For this research fermentation was performed using kombucha drink as a starter culture, purchased from a local supermarket. All herbs infusions were sugared with sucrose and infused with broth containing kombucha culture. Black tea was used for comparison reason. Prepared infusions were left for 60 days in room temperature. Beverage pH were measured until the data stabilized.

The total amount of phenolic compounds and antiradical activity were evaluated using spectrophotometric methods [2]. The highest quantity of phenolic compounds was found in lemon balm tea before fermentation 983.7 \pm 52.3 mg/L (expressed in rutin equivalents), while after fermentation it reduced to 417.1 \pm 22.2 mg/L, respectively. The highest antiradical activity was observed in black tea infusion 733.3 \pm 9.7 mg/L, while after fermentation it reduced to 320.97 \pm 3.16 mg/L. The pH of tested fermented drinks settled to 3.43-3.34 after 12 days of fermentation.

The study found that total phenolic compounds content and antiradical activity reduced after 60 days of fermentation. We believe that this was due to the excessive duration of fermentation, which led to biodegradation by the action of some enzymes produced by various microorganisms in kombucha beverage.

Keywords: kombucha, Camelia sinensis, fermentation, SCOBY

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Variation of Biologically Active Compounds of *Geranium sanguineum* L. during Different Vegetation Stages

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Abstract

With the increase of different diseases, medicinal (aromatic) plants (MAPs) and their biologically active compounds have been used as the most successful mean for prevention of various diseases [3].

The aim of our study was to determinate total content of phenolic (flavonoids) and radical scavenging activity of *Geranium sanguineum* methanolic extracts in different vegetation stages.

The object of investigation was *Geranium sanguineum* L. – a herbaceous, medicinal plant of *Geraniaceae* (Juss.) family.

The investigation was conducted in Scientific Sector of Medicinal and Aromatic Plants, Scientific Department of Botanical Garden at Vytautas Magnus University (VMU) during vegetation period in 2017-2018. Phytochemical analysis was performed in Instrumental Analysis Open Access Center of Faculty of Natural Science at VMU.

Methanolic extracts was analysed in different vegetation stages: growth and leaf production, flower bud development, the beginning of flowering, massive flowering and the end of flowering. In this study determination of total content of phenolic compounds, flavonoids and radical scavenging activity were performed using spectrophotometric analysis methods. The radical scavenging activity as well as the total amount of phenolic compounds, flavonoids, expressed as rutin equivalent of RE mg/g of air-dried raw material [2, 3].

The chemical composition of biologically active compounds of *G. sanguineum* was investigated. in collection of Medicinal plants *ex situ* of Botanical Garden at Vytautas Magnus University, Lithuania was investigated As the results show, the highest content of phenolic compounds was determined in the end of flowering (54.43 RE mg/g), flavonoids in growth and leaf production (11.27 RE mg/g), radical scavenging activity in the beginning of flowering (47.31 RE mg/g) vegetation stage.

Keywords: Geranium sanguineum, biologically active compounds, vegetation period.

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Aggregation-Induced Emission Enhancement of Triphenylethylene-Functioned Salicylaldehyde-Based Schiff Base: Synthesis and DFT Studies

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Abstract

The concepts of aggregation-induced emission or aggregation-induced emission enhancement (AIEE) provide new possibilities in the design and synthesis of new practical luminescent materials [1]. Development of luminescent materials in with high photoluminescence quantum yields i the solid state is a hot research topic because of great potential of such materials for the applications in organic light-emitting diodes (OLEDs), organic solid-state lasers, chemical sensors etc. [2]. The Schiff bases (RHC[¬]NR), also known as azomethines, anils or imines, are widely used organic compounds which offer great flexibility in the design of useful and interesting organic electroactive compounds. Schiff bases were reported to display superior AIEE properties due to the limitation of intramolecular rotation around a single bond [3]. In this presentation we report on, the synthesis of triphenylethylene-functioned salicylaldehyde as a potential ligand for the preparation Schiff bases spectrometry, nuclear magnetic resonance spectroscopy, as well as computationally by the density functional theory method. The calculations have been performed in vacuum using the B3LYP method with the 6-31 G(d,p) basis sets.

Keywords: aggregation-induced emission, organic light-emitting diode, Schiff base.

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Cellular Response to Short-term Hypoxia by Changes in Alternative Pre-mRNA Splicing

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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. The ability to rapidly respond to changes in the cellular environment is essential for survival, and numerous strategies [1]. One of the ways cells adapt to altered environmental conditions is changes to alternative pre-mRNA splicing. The splicing process is carried out by the spliceosome, a complex macromolecular machinery, composed of five small nuclear ribonucleoprotein particles (U1, U2, U4, U5 and U6 snRNPs) and more than 200 auxiliary proteins [2]. Up to 95% of all human genes are alternatively spliced producing RNA isoforms that code for functionally distinct proteins [3, 4]. How short hypoxia, as cell stress, influences mRNA isoform formation only limited data are available. In this study we have analyzed the changes in splicing patterns of genes, previously reported as chronic hypoxia-dependant in order to investigate how they respond to short hypoxic stress.

Keywords: hypoxia, alternative splicing, cancer, cell stress.

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An Influence of Q65A Point Mutation on the Ligand Binding and Catalytic Activity of S-adenosyl-L-homocysteine Hydrolase from *Pseudomonas aeruginosa*

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Abstract

S-adenosyl-L-homocysteine hydrolase (SAHase) is a very highly conserved enzyme that controls a cellular concentration of *S*-adenosyl-L-homocysteine (SAH), a byproduct of methylation reactions that utilize *S*-adenosyl-L-methionine (SAM) as a methyl donor. SAH is a negative feedback inhibitor of SAM-dependent methyltransferases, therefore the enzyme serves as a key regulator of SAM-dependent biological methylation reactions. Selective inhibition of SAHase in targeted cells is an excellent possibility for a drug intervention at the molecular level of cell metabolism.

Our study has been focused on bacterial SAHase from *Pseudomonas aeruginosa* (PaSAHase) and the main goal was to elucidate a role the highly conserved glutamine residue (Q65) on an enzymatic activity of the enzyme, as well as on a ligand (substrate or product) binding. Within this project, we used various experimental techniques, including biocrystallography, enzyme kinetics study and isothermal titration calorimetry (ITC).

Structural studies showed that for SAHase in the open form, the Q65 residue is disordered and localized quite far from the ligand binding site. Upon ligand binding (substrate or product), Q65 side chain is ordered and moves toward a cation coordination loop involved in the ligand binding, as the result of the movement of two consecutive domains of the enzyme. The side chain of Q65 residue is directly involved in coordination an alkali metal cation, preferably potassium. The role of cation is an ordering and accurately positioning of the amide group of Q65 residue to allow its interaction with the ligand.

An importance of Q65 in ligand binding and enzymatic activity of PaSAHase is reflected in our mutagenesis study. In the presence of preferred K⁺ ions, the Q65A mutant is over four times less active and the K_M value increases over three times compared to the activity of the wild type enzyme, measured also in presence of K⁺. The catalytic proficiency index k_{cat}/K_m is over one order of magnitude lower. ITC titrations also confirmed the importance of the Q65 residue for the catalytic cycle.

Keywords: S-adenosyl-L-homocysteine hydrolase, point mutation, Pseudomonas aeruginosa.

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Synthesis and Evaluation of Antibacterial and Antioxidative Activities of Carbazole Compounds

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Abstract

Excessive and improper uses of antibiotics led microorganisms to develop resistance to various antibacterial agents. Fighting against such drug-resistant bacteria has become one of the most important and challenging health problems worldwide. Therefore, there is an urgent need to develop new compounds with potential antibacterial activities to help overcome drug resistance [1]

Carbazole and its derivatives are important type of nitrogen containing aromatic heterocyclic compounds [2] that are extensively used in various chemistry fields such as photoelectrical materials, dyes, supramolecular recognition and medical applications [3,4,5,6,7]. Therefore, carbazole based compounds show a lot of potential to develop and design new antibacterial agents, that could be used to challenge drug resistance problem.

In the work 7 compounds were synthesized by known methods and were screened for their in vitro antibacterial activity against Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* according to the disc diffusion method. Antioxidative activities were evaluated using free DPPH radical scavenging assay and ferric reducing antioxidant power (FRAP) methods. 6 out of 7 compounds exhibited antibacterial activities at MIC values ranging from to 31.25 to 1000 μ g/mL. 3-Cyano-9H-carbazole, 3-iodo-9H-carbazole and 3,6-diiodo-9H-carbazole displayed lower MIC values against *Bacillus subtilis* than that of reference drug amoxicillin. Against *Escherichia coli* stronger activity was shown by the 1,3,6-tribromo-9H-carbazole. According to DPPH radical scavenging assay and FRAP assay methods all tested compounds revealed weak antioxidative activities.

Keywords: antibacterial agents, antioxidative activity, carbazole, disk diffusion method, antioxidative activities.

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Synthesis and Properties of Novel Dimethoxycarbazole and 1,4-Dicyanobenzene-Based (D-A-D) Moieties

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Abstract

Organic light-emitting diodes (OLEDs) have been broadly produced and designed over more than twenty years. The rapid OLED technology development and pervasion in the market occurred because of its unique advantages, such as simple design, flexibility, thinness, lightness, fast respond time, brightness, etc. Nowadays OLED-based matrixes are widely applied in mobile phones, TV screens and interior and exterior lighting devices etc. In spite of mentioned benefits, OLEDs have some drawbacks that still needs to be improved: morphological stability, short lifetimes and low efficiencies. [1,2] Considering these issues, three new (D-A-D) organic compounds possessing dicyanobenzene (as acceptor) and 3,6-dimethoxy- or 2,7-dimethoxy-9H-carbazole (as donor) moieties were synthesized (Fig. 1).

The main aspects of the synthesis, characterization (photophysical, optical, thermal, electrochemical, charge transporting properties) and theoretical calculations will be reported in the presentation.

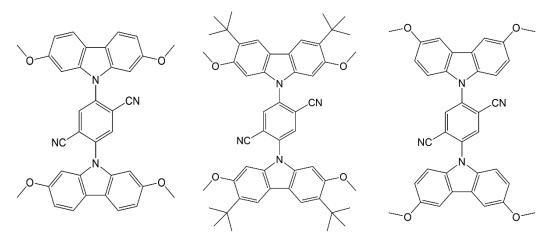


Figure 1. The series of new organic compounds having dicyanobenzene and dimethoxycarbazole moieties.

Keywords: semiconductors, bipolar, methoxycarbazole, OLED.

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Hypoxia's Influence on MAPT Alternative Pre-mRNA Splicing and its Regulation by U2AF

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Abstract

Lack of oxygen in the brain cells (hypoxia) is involved in the development of neurodegenerative diseases [1, 2]. One of the adaptations to hypoxic conditions are changes in alternative pre-mRNA splicing that occur, when mRNA isoforms are formed and proteins translated from them promote cell survival under unfavorable conditions [3, 4]. Splicing is catalyzed by a multiprotein complex called the spliceosome. A heterodimeric factor U2AF plays an important role in formation of the early splicesome complex. This splicing factor is composed of two subunits: 65 kDa (U2AF65) and 35 kDa(U2AF35) [5]. Data from the literature states that *MAPT* gene which are involved in Alzheimer's and Parkinson's disease pathology can be alternatively spliced [6]. In this study, we have investigated the influence of hypoxia on neurodegenerative disease associated *MAPT* gene alternative pre-mRNA splicing and if/how U2AF65 and U2AF35 individual subunits are involved in splicing regulation under unfavorable cellular conditions.

Keywords: hypoxia, alternative splicing, neurodegenerative disease, splicing factor U2AF, MAPT.

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Design and Synthesis of Dibenzothiophene-Based Compounds for TADF-based OLEDs

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Abstract

Materials exhibiting thermally activated delayed fluorescence (TADF) have emerged as the most promising candidates for constructing high-performance organic light emitting diodes (OLEDs), due to their efficient harvesting of both singlet and triplet excitons through the reverse intersystem crossing [1,2]. In this work emitters were designed and prepared by linking the donor and acceptor moieties around the central phenyl core. The key intermediates were synthesized from the corresponding chloro-substituted benzoic acids through acyl chlorination and Friedel-Crafts reactions. Then, the corresponding intermediates reacted with (4-(diphenylamino)phenyl)boronic acid to yield the target products by means of the Suzuki reaction. The TADF emitters were purified through column chromatography before the measurements and device fabrication. The molecular structures were identified by nuclear magnetic resonance spectroscopy and mass spectrometry.

Keywords: TADF, emitter, OLED.

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Potential Usage of Acorns for Human Food and Health: A Review

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Abstract

Currently, due to the growing population, new materials are being sought that would fulfill the needs of people in a variety of ways. Oaks (Quercus) are trees common in the Northern Hemisphere that have nutritious and valuable fruits called acorns, which were used as a food source thousands of years ago. Presently, acorns are being used by making such products as flour, coffee, starch, and oil. Moreover, there are some traditional foods and beverages like Turkish drink *Raccahout*, Portuguese alcoholic drink Licor de Bolota, acorn coffee Eichel Kaffee, Sardinian bread Pan'Ispeli which includes acorns as required ingredient. It is known that acorns feature beneficial composition (such as vitamin A and C, fibers), a high content of phytochemical compounds and biological activity [1]. One of the most advantageous acorns peculiarity is bioactive compounds such as phenolic compounds, which have antioxidant properties. Besides acorns, also oak leaves are suitable for human health. Both contain compounds useful for alleviating symptoms connected with Alzheimer disease, other neurodegenerative diseases, and diabetes [2]. In addition, Quercus species have antibacterial effect against many important pathogens, which can be a solution to the problem of unlimited use of antibiotics [3]. The properties of Quercus spp. acorns show the potential benefits for human health, so it is necessary to study the various species of *Quercus* and embrace their properties in daily life which can be done by increasing further applications of acorns in the food and pharmaceutical industries. So, considering the composition of oak acorns they could be used as a functional food and as an alternative to other food sources while simultaneously improving people health.

Keywords: acorns, Quercus, antioxidant, antibacterial, food, health, pharmaceutical.

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A New Approach to Flavonoids Isolation from *Medicago sativa* Leaves – Optimization the Parameters of Extraction Process and Enzymatic Hydrolysis

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Abstract

In contemporary analytical chemistry, more and more interest is put to biologically active substances, especially their isolation from plants increases. Most of these are secondary metabolites and play important functions in the interaction of plants with the external environment. Many of them exhibit biological activity, therefore they can have a positive effect on human health. However, due to the fact that the concentration of biologically active substances in plant raw materials is small, many studies have been carried out to develop more effective and selective methods of extracting these compounds. Special attention was put on the development of chemical process that relay on use environmentally-friendly solvents and hydrolyzation of plant material.

The aim of this study was to optimize together the parameters of extraction process and enzymatic treatment of plant material to develop a practical and comprehensive extraction protocol for the enhanced release of biological active compounds from plant cells. Extraction of plant material was carried out by SFE with 96% ethanol as a co-solvent. The RSM based on Box-Behnken design was used to optimize the extraction conditions (temperature, pressure and percentage of co-solvent) and the enzymatic treatment parameters (pH, enzyme concentration, time and temperature), as well as assess the impact of these conditions on response variable (total flavonoids content). HPLC-MS/MS analysis allowed to evaluate the content of individual biological active compounds in obtained extracts. The total content of phenolic acids and flavonoids in the extract obtained from non-hydrolyzed material (274.68 \pm 22.50 μ g/g) and in the extract obtained by maceration (161.86 \pm 19.80 μ g/g). Moreover, it was evidenced that the extract is characterized by the highest content of flavonoids which can support cellular antioxidant system by directly neutralizing free radicals or by activating enzymatic antioxidative mechanisms.

The high efficiency of the enzyme-assisted supercritical fluid extraction (EA-SFE) established in our research might result from strong surface damage of *M. sativa* plant material by using the enzymatic formulation, so that the phenolic compounds are liberated more easily. The developed methodology is economically efficient and represents an advance in modern technological processes, as well as it can be successfully implemented for any type of plant material.

Keywords: EA-SFE, enzymatic hydrolysis, flavonoids, HPLC-MS/MS

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Through-Space Charge Transfer in Bipolar Hosts of High-Efficiency Blue OLEDs

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Abstract

Organic light-emitting diodes (OLEDs) nowadays represent one of the most promising and dominant technology in the market of displays and illumination equipment. OLEDs are superior to competitive technologies with respect of mechanical flexibility, low response time, quality of image, transparency, low mass and wide range of working temperatures [1,2]. In this work blue phosphorescent organic light-emitting diodes (PhOLEDs) with maximum external quantum efficiency from 22.7 to 28.4% were fabricated using new bipolar derivatives of benzimidazole linked through phenyl spacer with the different number of *tert*-butyl substituted carbazolyl groups, as hosts. Applicability of the newly synthesized compounds as hosts for blue PhOLEDs was justified by their high triplet levels (2.94-2.98 eV), bipolar charge transporting properties with charge mobilities up to 2.34×10^{-3} cm²/V·s at electric field of 2.7×10^5 V/cm, and high ionization potentials (5.63-5.81 eV).

Keywords: OLED, host, benzimidazole, bipolar.

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1,8-Naphthalimide-Based Derivatives Exhibiting Orange-Red Thermally Activated Delayed Fluorescence and Their Application in OLED

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Abstract

Over the past few years, development of orange-red thermally-activated-delayed-fluorescence (TADF) emitters for organic light emitting diodes (OLEDs) have been lagging when compared with the blue and green fluorophores [1]. The record-high external quantum efficiencies (EQEs) of 39% and 38% have been reported for blue and green TADF-OLEDs respectively [2]. On the other hand, the EQEs of orange red to red TADF-OLEDs with emission maxima beyond 580 nm rarely exceed 25% [3]. Many efforts have been devoted towards development of highly efficient orange-red/red TADF compounds by increasing the twist angle between the donor and acceptor, by introducing multiple donors or acceptors, adjusting donor and acceptor strength of the molecule, or enlarging the molecular rigidity [4]. In this work, four 1,8-naphthalimide derivatives were synthesized and investigated. The synthesis of the compounds was carried out in three steps. It included bromination, imidization and Buchwald-Hartwig cross-coupling reactions. The structures of the synthesized compounds were confirmed by nuclear magnetic resonance spectroscopy and mass spectrometry. Photophysical, electrochemical, thermal properties and performance in OLEDs were studied.

Keywords: OLED technology, orange-red/red TADF, EQE.

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Targeted Metabolomic Study of Prolidase-treated Human Dermal Keratinocytes under the Condition of IL-1β-induced Inflammation.

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Abstract

Introduction. Prolidase, apart from its catalytic activity, is an EGF receptor (EGFR) ligand. Stimulation of this receptor in damaged cells intensifies pro-proliferative pathways that are the basis for tissue regeneration [1]. Among 4 phases of wound healing processes, inflammation is an important step reflecting the outcome of skin regeneration. Prolonged inflammation may result in a delay in wound healing. The unique feature of prolidase is supplying proline for biological processes through hydrolysis of proline-containing peptides originating from collagen degradation [2]. It is known that proline mediates the tricarboxylic acid (TCA), urea, and proline cycles [3]. Thus, we have presumed that prolidase regulates all aforementioned metabolic pathways playing an important role in wound healing. Aim. We aimed to evaluate the effect of extracellular prolidase and IL-1 β on the concentration of selected metabolites in TCA, urea, and proline cycles of the human keratinocytes.

Methodology. Before LC-MS-based analysis, the human dermal keratinocytes (HaCaT cells) were incubated with prolidase (1-100 nM) and IL-1 β (1 ng/ml) for 24h. High-performance liquid chromatography coupled with mass spectrometry (HPLC-QTOF-MS, Agilent) was applied for the quantitative analysis of citric acid, α -ketoglutarate, and succinate (TCA cycle), arginine (urea cycle) and glutamine, glutamate, and proline (proline cycle).

Results. We observed significant changes in the levels of the metabolites involved in the proline, TCA, and urea cycles under conditions of IL-1 β -induced inflammation in the keratinocytes. The addition of prolidase and IL-1 β to cell culture medium induced an increase of intracellular proline and citric acid concentrations while glutamine and arginine levels drop in a dose-dependent manner. Glutamate, α -ketoglutarate, and succinate levels were not affected upon prolidase and IL-1 β treatment of the cells.

Conclusion. Prolidase under conditions of IL-1 β -induced inflammation modulates the concentration of selected metabolites of TCA, urea, and proline cycles in human dermal keratinocytes. The phenomenon could affect the process of wound healing.

Keywords: prolidase, EGFR, targeted metabolomics, IL-1β, inflammation.

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Essential Oil Composition of Ground Ivy (*Glechoma hederacea L*) Growing in Northeast of Latvia

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Abstract

Interest in bioactive compounds obtainable from natural sources has increased considerably in recent years mainly from the industry of food, cosmetics and medicine. The attention paid to bioactive compounds is confirmed by the large amount of literature published in this field. In Europe there are more than 30 000 wild plant species, 400 are considered medicinal. 60-90% of the medicinal and aromatic plants in Europe believed to be wild collected. *Glechoma hederacea* var. longituba (Labiatae) is a perennial vine plant. It is distributed widely in Asia, Europe and America. *G. hederacea* is commonly known as 'ground ivy' and it is one of the first spring plants and the first of the labiates to come into flower. After flowering it busies itself in spreading widely through its underground runners. A recent phototherapeutic monograph [1] suggests that the chemistry of ground ivy is well studied and that documented pharmacological activities for its constituents support some herbal uses. Within this research the amount and composition of essential oils obtained from both *G. hederacea* samples collected in wild and harvested in organic production system of Northeast Latvia was to determine. Whole plants of *G.hederacea* were harvested at different their vegetation periods within two seasons in 2019 and 2020. Essential oil of the air-dried plant was extracted by hydro-distillation using the Clevenger-type apparatus for 3h. Hydro-distillation of the *G. hederacea* yielded pale yellow-green,

pleasant smelling essential oil with concentration varying in range from 0.32 to 2.98 mL kg⁻¹. Separation of the volatile constituents was determined by gas chromatography-mass spectrometry. The chemical compositions of the essential oil were determined according to their retention time and spectrometric electronic library (NIST). The identity of the constituents of the oils was established using GC retention indices (RI). The major compounds identified in the oil were Germacrene D, Germacrene B, Eucalyptol, β -Ocimene and 1-Octen-3-ol.

Keywords: Glechoma hederacea L., genetic resources, essential oils, GC-MS.

Acknowledgments: The work has been supported by ERAF project Nr.1.1.1.1/18/A/043 "Innovative solutions for growing technologies and applications of spring medicinal and aromatic plants".

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Induced Decrease in Small Molecule Electrotransfer Efficiency by Extracellular Calcium

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Abstract

Electroporation is a method, developed in order to deliver exogenous biocompounds (anticancer drugs, nucleic acids, proteins, etc.) to cells and tissues, which are mostly designed for cancer treatment purposes. An alternative to chemotherapeutic drugs can be Ca²⁺ ions which are universal signal messengers that regulate a multitude of cellular functions. The application of calcium electroporation is a novel anticancer treatment that selectively kills cancer cells by necrosis, a cell death pathway that can induce the immune system due to the high release of antigens. However, the mechanism behind the calcium electroporation remains unclear to this day. Calcium ions are also known to play a key role in membrane resealing, potentially altering the pore dynamics and molecular delivery during electroporation. To elucidate the role of calcium ions in the process of electroporation, we used different extracellular calcium concentrations with different fluorescent dyes (Propidium iodide (PI), YO-PRO-1 and ethidium bromide (EtBr) in electroporation media. Experiments were performed using flow cytometry and fluorescent microscopy methods. In conclusion, we report that extracellular calcium induces a negative effect to the small molecule (Propidium iodide (PI), YO-PRO-1 and ethidium bromide (EtBr), transfer into the cells after electroporation.

Keywords: calcium electroporation, microsecond electroporation, calcium, PI electrotransfer, pore resealing, membrane repair.

Antioxidant and Anticancerous Properties of Eucalyptus Lignins in Vitro

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Abstract

The specific characteristics of kraft lignin, its high industrial availability together with previous reported biological activities of the lignin molecule were the main reasons that motivated this research work. Thus, the objective of the present study was the assessment of in vitro cytotoxic activity of industrial kraft lignins precipitated at two different pH on mouse hepatoma MH-22A, melanoma B16 and Chinese hamster ovary (CHO, non-cancerous) cells.

This work reports the study of in vitro antioxidant and cytotoxic activity of two isolated industrial kraft lignins as well as the analysis of their composition and main structural characteristics. Aside from presenting low molecular weights, Eucalyptus kraft lignins showed high-condensed structure with a high content of phenolic hydroxyl groups. These structural features together with their chemical composition, rich in phenolic compounds, contribute to their potent antioxidant activity. Furthermore, the cytotoxic test revealed that kraft lignins induced apoptosis- and necrosis like processes acting cytotoxically on both tumor and normal cells. However, it was observed that mouse hepatoma cells (MH-22A) presented the highest sensitivity to kraft lignin samples, while melanoma B16 and non-cancerous CHO cells had similar behavior showing more tolerant to kraft lignins treatment even at high concentrations.

Keywords: lignins, cytotoxicity, antioxidant.

The Role of SRSF7 in Hypoxia Induced Alternative Splicing

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Abstract

Alternative pre-mRNA splicing regulation is an important cellular process used to adapt to changes in the microenvironment. Changes in splicing have been identified as the cause of many disorders including cancer, neurodegenerative and other diseases. Up to 95% of all human genes are alternatively spliced producing RNA isoforms that code for functionally distinct proteins [1, 2]. The splicing process is carried out by the spliceosome, a complex macromolecular machinery, composed of five small nuclear ribonucleoprotein particles (U1, U2, U4, U5 and U6 snRNPs) and more than 200 auxiliary proteins [3]. SR family proteins have been identified as one of the regulators of alternative splicing [4]. In this study we aim to investigate the individual role of SRSF7 protein in hypoxia dependant alternative splicing in brain cells.

Keywords: hypoxia, alternative splicing, neurodegenerative disease, SR proteins, SRSF7.

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Analysis of the Dynamics of Microplastics in the Urmia Lake Catchment

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Abstract

The current case study is focused on Urmia Lake located in the North West of Iran between Tabriz and Urmia cities. The lake is the second largest salty lake worldwide and the largest permanent hypersaline aquatic inland ecosystem in the Middle East [1]. With the area of 5700 km² [2] being the largest lake in Iran. Urmia Lake is an important water source for agriculture in Iran, Turkey, Azerbaijan Republic, Iraq, and Armenia. Microplastics (MPs) and the associated toxins, persistent organic pollutants, and potentially toxic elements are the main threat in the aquatic environment [3-7]. There is a lack of study of MPs pollution in Urmia Lake. In the present study, we investigated MPs pollution amount during 2016 and 2019 in Urmia Lake located in the North West of Iran. Due to the limitations in the sample matrix (fragile filters), Thermogravimetric analysis (TGA), and optical microscopy methods were used. Also, we performed field observations to investigate abundance, and characteristics (size, color, shape). Additionally, nano plastics were isolated and characterized by the use of flow field-flow fractionation (F4) technology of MPs in Urmia Lake. Surface MPs sampling were collected at 24 stations across the Urmia Lake in August, September, and October 2016 and October 2019. Our field survey results indicate the ubiquity of MPs in 2019 is higher than in 2016. Due to our knowledge, this is the first report on MPs pollution in Urmia Lake.

Keywords: microplastic, Urmia lake, thermogravimetric analysis, optical microscopy, flow field-flow fractionation.

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Synthesis and Properties of Bicarbazolyl-Based Hole-Transporting Materials

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Abstract

Organic hole-transporting materials (HTM) are widely used in perovskite solar cells (PSCs).

Although it is widely known that HTM, derived from conductive organic molecules, are renowned for their uses in PSCs, the threshold of certified power conversion efficiency of ~25.2% has yet to be breached [1].

A HTM layer which is between the perovskite film and the anode is to reduce the energy barrier and suppress the potential interfacial charge recombination [2]. Among the viable organic HTM, aromatic compounds with methoxy substituents are beneficial for lowering the highest occupied molecular orbit (HOMO) energy level, which is related with a higher open circuit voltage [3]. The increased number of phenyl rings and methoxy groups allows to achieve increased hole mobility, stronger intermolecular interactions and better positioned orbital energies.

In this work, we report on the synthesis and properties of methoxyphenyl and dimethoxycarbazolyl substituted 3,3'-bicarbazolyl-based hole transporting materials. The 3,3'-bicarbazolyl moieties were chosen because it offers excellent charge transporting properties, low ionization potentials and good thermal, morphological and chemical stability [4].

The synthesized compounds were amorphous materials with glass transition temperature higher than 160 °C. Their films were characterized by UV photoelectron spectroscopy in air and by time of flight method. Ionization potentials values of the compounds depended on their chemical structure and were in the range of 5.23 - 5.42 eV. The layers of the compounds show hole mobility of 1.6×10^{-5} cm²/Vs at high electric fields.

Keywords: ionization potential, hole transporting, 3,3'-bicarbazolyl.

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The Usefulness of MALDI-MSI in Diagnosing Ovarian Tumours – a Pilot Study

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Abstract

Ovarian tumours often present a diagnostic challenge for both clinicians and histopathologists. The correct diagnosis of benign ovarian tumours requires histopathological examination complemented by immunohistochemistry – both methods are time consuming but essential for a proper diagnosis. In this study two ovarian neoplasms were analysed - low-grade serous borderline ovarian tumour and ovarian fibrothecoma. Both ovarian tumours were selected as examples of ovarian lesions whose correct diagnosis is challenging and they are often misdiagnosed as ovarian cancer. Fresh frozen tissues sections were washed with ethanol and transferred into a vacuum desiccator to dry for 30 minutes. The α-CHCA matrix was applied onto the prepared tissue sections using ImagePrep station. The analyses were carried out using MALDI-TOF mass spectrometer. The selection of differentiating peaks was carried out in flexImaging software. The analysis of the ovarian tumour tissue using MALDI-MSI showed a close correlation of the molecular map with its morphological and histopathological features and allowed to identify different tissue types within the ovarian borderline tumour tissue section. Moreover, molecular maps have shown the differentiating distribution of regiospecific peaks within the examined samples. The obtained results confirm the complementary nature of the MALDI-MSI method in the diagnosis of ovarian tumours. This pilot study highlights the potential significance of MSI in enabling a detailed morphological characterization of ovarian tumours and thus leading to a more accurate differential diagnosis of ovarian tumours, especially in the most challenging cases.

Keywords: proteomics, MALDI-MSI, ovarian tumours, mass spectrometry, tissue imaging.

Preliminary Study on the Fraction Collection of Royal Jelly Using High Performance Liquid Chromatography

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Abstract

The royal jelly (RJ) is a natural-origin secretion produced by the honey bee (*Apis mellifera*). This complex matrix of a wide spectrum of different chemical compounds shows various health-enhancing properties. These properties make RJ useful in traditional medicine, apitherapy. To better understand the biochemistry, biological, and pharmaceutical properties and due to origin-related composition differences of RJ, extensive studies on that natural product are needed.

The aim of the study was to develop the analytical method of separation of the water extract of RJ with a collection of the obtained fraction for further mass spectrometry-detection.

Chromatography separation and fraction collection were carried out using an Ultraperformance Liquid Chromatograph Ultimate 3000 (Thermo Scientific) connected with UV-Vis detector. The Kinetex F5 (100mm x 4.6mm; 2.6 μ m, Phenomenex) column maintained at room temperature enabled chromatographic separation. The mobile phase comprised 0.1% formic acid in methanol and 0.1% formic acid in water. Gradient flow was used with the constant flow rate of 500 μ L/min. Injection volume was set to 50 μ l. Total runtime was 45 minutes. Detection was performed at the wavelength of 205 nm. Successive eluate fractions were collected by the time of analysis.

The presented preliminary studies focus on attempting to investigate RJ as a complex biological matrix, taking into account the possibility of further analyzes of individual fractions of this bee-product. This newly developed method allows for the effective separation of RJ components and automated collection of the obtained fractions using the built-in fraction collector. The obtained fractions can be further analyzed to investigate the composition of each single fraction.

The obtained results may contribute to broadening the knowledge about the composition of such a complex matrix as RJ, thus leading to an in-depth understanding of the mechanisms responsible for its therapeutic properties, which still stay not fully investigate.

Keywords: bee products, high-performance liquid chromatography, HPLC, fraction collection, apitherapy.

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Antioxidant Activity and 10-HDA Content in Lithuanian Royal Jelly

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Abstract

Royal jelly is a natural bee product with great potential for use in medicine with many protein, free amino acids, carbohydrates, lipids, vitamins, minerals and phenolic compounds. Its wide chemical composition indicates that there are various bioactive substances such as 10-hydroxy-2-decenoic acid (10-HDA) [1]. This acid, also called queen bees acid, is only natural origin and is considered as one of the most important components from which the royal jelly biological activity derives [2]. Also the detection and quantification of 10-HDA is considered an indicator of the identity and quality of royal jelly [3]. Phenolic compounds and flavonoids are other important components which have influence to the antioxidative properties of royal jelly [4]. Due to its antioxidant activity and other properties royal jelly can be widely used in cosmetics, medicine and pharmaceutical industries. However, in Lithuania it is not as popular as in Asian countries. According to our data, there are not much scientific researches with Lithuanian royal jelly. The main task of this study was to evaluate the changes of antioxidant activity 10-HDA content in Lithuanian royal jelly, collected at different periods of the summer and from different apiaries. Different spectrophotometric tests were employed for evaluation of antioxidant activity of tested samples. The results showed that the total content of phenolic compounds, flavonoids, antiradical activity and 10-HDA content differed depending on the time of collection and apiary. The samples collected in the middle of July and the beginning of August characterized by the highest quantities of phenolic compounds and flavonoid, 10-HDA and antiradical activity. The phenolic compounds content ranged from 8.10±0.06 mg/g to 9.23±0.03 mg/g, the flavonoid content varied from 2.13±0.03 mg/g to 2.76±0.02 mg/g and antiradical activity varied from 2.44±0.05 mg/g to 3.04±0.02 mg/g. 10-HDA content ranged from 15.2 ± 0.18 mg/g to 37.3 ± 0.15 mg/g.

Keywords: royal jelly, antioxidant activity, 10-HDA.

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Symbiotic Relationships Between *Populus* Explants and *Pseudomonas* sp. Bacterium *in vitro*

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Abstract

Although plant-bacteria symbiosis is usually investigated in natural systems, increasing evidence also points to its possible continuance in vitro. The present study reports a symbiotic bacterium from the in vitro cultures of *Populus* trees. Three *Populus tremula* \times *P. tremuloides* genotypes (L191, Wa13, and 174/10) and one P. tremula \times P. alba genotype (IBL 91/78) were cultured in vitro by using apical shoot segments as explants. The presence of a bacterium, whose colonies stretched from the explants onto the nutrient medium, was observed in all three *P. tremula* \times *P. tremuloides* genotypes, but not in IBL 91/78. The bacterial DNA was extracted, and 16S rRNA gene analysis classified the bacterium as Pseudomonas sp. The rooting-related differences between the Pseudomonas-infected and uninfected Populus genotypes were assessed both in the dark and under a 16-hour white-light photoperiod. The uninfected genotype IBL 91/78 showed a much higher adventitious rooting rate in the light than in the dark (62.1% vs. 25.8%). This could be explained by light-dependent polar auxin transport from a viable shoot apex, which is a known hormonal mechanism in plants. In contrast, the *Pseudomonas*-infected *P. tremula* \times P. tremuloides genotypes Wa13 and 174/10 had lower rooting rates in the light than in the dark: 21.3% vs. 68.1% and 13.3% vs. 49.5%, respectively. The genotype L191 had similar rooting rates both in the light and dark (40.0% vs. 45.6%) but, interestingly, only 30.4% of L191 explants had non-browning shoot apices in the dark, in contrast to 95.0% of viable apices in the light. Thus, the adventitious rooting in the Pseudomonas-infected genotypes seemed to be independent from the viability of shoot apex and did not require light, indicating a possible role of the symbiotic bacteria rather than plant internal mechanisms only.

Keywords: aspen, white poplar, hybrid tree, *Pseudomonas*, symbionts, rooting.

The Influence of Biochar Fertilization on Spring Wheat Biomass Formation under Different Watering Condition

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Abstract

The importance of soil is obvious for all of us, but its degradation faced many problems and people looking for different solutions to improve soil quality. One of the solutions for the improvement of soil and increase of carbon content in it could be the use of biochar as soil amendment or fertilizer, because it is environmentally friendly and could enrich the soil. The biochar is a carbon-rich material which can be made from biomass (wood, waste products from agriculture, even from pig manure) by the pyrolysis under anaerobic conditions [1]. It can be use as alternative fuel, sorbent, but the most popular is using it as a soil amendment [2]. This study was aimed to identify the influence of biochar on spring wheat biomass formation under different watering conditions: optimal (soil moisture content about 14%), dry (soil moisture content about 7%), flooded (soil moisture content about 35%). Biomass yields of plants, chemical composition, chlorophyll fluorescence, moisture content in the soil, soil and biochar chemical composition, nitrogen and carbon content were measured. 10 seeds of spring wheat in pots of 10 kg of the soil and soil-biochar mixture were sown. The content of biochar in soil-biochar mixture was 247 g. Each treatment had four replicates. After 66 days of sowing the plants were harvested and weighted for the determination of biomass yield. The highest biomass was 14.288 g in the soil mixed with biochar grown under flooded condition. In comparison on the same condition but grown without biochar, biomass was 6.370 g, it is more than half lower. The least biomass was 5.115 g grown in soil under dry conditions (in comparison the biomass yield of wheat grown in the soil mixed with biochar was 7.783 g). In conclusion the weight of plants biomass after experiment was higher in the soil mixed with biochar in the dry and flooded condition. Only in the optimal watering condition the weight was highest in the normal soil 13.508 then soil mixed with biochar 10.930 g.

Keywords: biochar, soil, plants, biomass, dry, flooded, optimal, pots.

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Marine Compounds as Proteasome Activators

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Abstract

Proteasomes are constituents of the cellular proteolytic network that maintain protein homeostasis through regulated proteolysis of normal and abnormal (in any way) proteins. Proteasome activation in human primary fibroblasts has been shown to result in cellular lifespan extension. Using Caenorhabditis elegans as a model, we were also able to promote proteasome activation at the organismal level. More specifically, proteasome activation in C. elegans either through genetic means or through natural compounds resulted in enhanced levels of proteasome activities that led to a SKN-1-dependent lifespan extension. The elevated proteasome function conferred lower paralysis rates in various Alzheimer's disease (AD) nematode models accompanied by decreased AB deposits, thus ultimately decelerating the progression of the AD phenotype. Similar positive results were also produced in human neuroblastoma cells and in murine cortical neurons. Based on these results, we have searched for natural compounds that may act as "structural" proteasome activators (compounds that activate the multienzyme complex through direct stereochemical interaction with the 20S proteasome). In the context of the present study, marine compounds derived from the Mediterranean Sea were examined for their proteasome activating properties in the test tube through direct activation of highly purified 20S proteasome. We identified a diterpene, namely MAR174 that activated the proteasome in the test tube as well as in human primary fibroblasts in cellulo. We further tested the anti-aggregation properties of the compound. Administration of MAR174 on the CL4176 C. elegans strain (transgenic nematodes expressing the A β_{1-42} peptide in their muscle cells) delayed the paralysis onset of the animals. In total, our results suggest that proteasome activation may exert downstream positive outcomes on AD and they unveil the need for identification of compounds with proteasome activating properties that may act as anti-amyloidogenic agents.

Keywords: ubiquitin-proteasome system, proteasome activation, marine compounds, anti-aggregation.

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Determination of the Phenolic Profile of Vaccinium vitis-idaea L. Flowers

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Abstract

Lingonberry (*Vaccinium vitis-idaea* L.) leaves and fruits are widely used in the human diet and traditional medicine due to nutritional and health improvement benefits. This is because of their richness in bioactive phenolic compounds. High levels of phenolic compounds in lingonberry raw materials led us to explore lingonberry flowers, that had not been studied before.

The lingonberry flowers were collected at the second flowering cycle in late summer from Apuniškis forest (56°00'40.6"N, 25°31'29.4"E (WGS)). The phenolic composition of lingonberry flower extracts (sample/solvent ratio 1:10, solvent 70% ethanol) was determined by the HPLC-PDA method, using the ACE C18 reversed-phase column in the solvent system with 0.1% trifluoroacetic acid and acetonitrile under gradient elution. The analyte and reference compound retention time and UV absorption spectra were used for peak identification. All measurements were made in triplicate, and results were expressed as mean \pm standard deviation for dry weight (DW) raw material.

Results showed that 2 simple phenols, 6 phenolic acids, 13 flavonols, 2 flavanols, and 5 proanthocyanidins were detected in lingonberry flowers. The sum of identified phenolic compounds was 47.95 mg/g DW. The most (p<0.05) abundant phenolic compounds were catechin (15.19 ± 0.25 mg/g DW), following by 2-caffeoylarbutin, arbutin and procyanidin A1 (9.35 ± 0.13, 4.80 ± 0.03, and 3.73 ± 0.04 mg/g DW, respectively). Our results suggest that lingonberry flowers are promising health therapeutic candidates in the pharmaceutical industries because of phenolics, and further research of lingonberry flowers composition and activity is required.

Keywords: Vaccinium vitis-idaea, HPLC-PDA, phenolic compounds.

Evaluation of Binders for Lyophilized Raw Materials

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Abstract

Recently, the interest in lyophilized foods have increased in the way that lyophilized products differs from other food products by their biological and nutritional value, outside appearance and aroma properties [1]. Moreover, the lyophilization is a promising process as it allows to avoid wasting food by lyophilizing substandard, unripe and damaged fruits, berries, vegetables and meat [2]. Due to high biological and nutritional value lyophilized raw materials have high potential in developing new food products. Thus, to make a homogenic mass from dry lyophilized powders, it is necessary to find the most suitable material for bonding these raw materials.

Binders used in food products are divided into polysaccharides, proteins and fats. Polysaccharides and proteins are also known as hydrocolloids. Hydrocolloids have many functional properties applicable in the food industry, such as thickening, gelling, emulsifying, stabilizing, film-forming, and other properties [3]. Plant-based fats are attractive for their low moisture content and solid form. However, lipid oxidation has to be taken into consideration while using fats [4].

The aim of this research was to evaluate the most suitable binder for lyophilized raw materials. During this research, 7 different binders such as cacao butter (CB), coconut oil (CO), palm fat (PF), pectin (PC), gelatin (GL), agar (AG) and sodium alginate (SA) were selected. A total of 14 different samples were prepared, of which 7 were with lyophilized beef powder and 7 with lyophilized chicken powder. The outside appearance, structure and lipid oxidation of plant-based fats by the *Oxipress* method were evaluated.

Summarizing the results of the evaluation of binders for lyophilized raw materials obtained results of outside appearance and structure showed that the samples with a binder of plant-based fat (CB, CO and PF) had the smoothest surface and the strongest structures compared to other binders. Thus, the results of oxidative stability of CB, CO and PF showed that the most stable plant-based fat is CB, and its induction period was 3.8 hours at temperature of 130 °C.

Keywords: lyophilized, cacao butter, lipid oxidation.

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Synthesis and Properties of Triphenylamine and Methoxycarbazole-Based Hole Transporting Materials

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Abstract

During the last two decades the need of cheaper, more efficient and eco-friendly energy and effective light sources has increased drastically. Thus the focus of scientists and engineers was shifted from the incandescent and halogen light sources to more advanced technology - improved light emitting diodes (LEDs). Main issues using LED devices is related not only with their efficiency, colour purity, but their fragility and toxicity (toxic cadmium, gallium, indium are used), as well. Therefore, nowadays the interest of main LED alternatives, such as organic light emitting diodes (OLEDs) is growing in scientific community and world market. The structure of a typical OLED device consists of multiple organic layers deposited between two electrodes. Each electroactive layer (hole/electron transporting or emissive) has its own specific purpose. Our scientific interest was focused on hole transporting layer, which is responsible for efficient transportation of positive charges (holes) into the emissive layer, and blocking of electrons, that are passing emissive layer from the opposite electrode. In our current work, we synthesized a series of TCTA (popular commercially available hole transporting material) - tris(4carbazoyl-9-ylphenyl)amine [1] - alternatives possessing triphenylamine and various methoxycarbazole derivatives, such as, 2,7-dimethoxycarbazole, 3,6-dimethoxycarbazole and 2,7-dimethoxy-3-tertbutyl-9H-carbazole. By introducing tert-butyl- and methoxy- groups onto carbazole fragment we obtained better morphological characteristics and higher electrochemical stability of the target compounds. Additionally, the comparative study of physical, optical, thermal, electrochemical properties of new triphenyl and methoxycarbazole derivatives will be presented.

Keywords: TCTA, carbazole, OLED.

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Fabrication of Rachets for Macromolecular Separation and Single-Chip Contactless Conductivity Multi-Channel Detection System

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Abstract

Research on biomolecules and bio-particles is the most important part of life sciences. Currently, the methods used to extract biomolecules are expensive, requiring a lot of maintenance, their productivity is low or moderate. Modern 3D manufacturing technologies already allow the creation of hundred-nanometer-sized structures with controlled geometry and spatial layout. Also, such derivatives may be the chosen orientation, and most importantly, the structures created by 3D printing technologies can be particularly homogeneous. Structures used for homogeneous purification of biomolecules are significantly superior to non-homogeneous derivatives created by classical methods. The technology provided by the project would allow the separation of not only μ m-sized bioparticles, such as living, dead cells, healthy, cancer cells, but also different biomolecules and macromolecules.

In this phase of the project, multi-channel capillary format detection systems for coupling to microparticle filters and direct-simultaneous monitoring of separation results were developed and evaluated. Optimized microfiltration production technologies by forming the micropoles field for the output channels of particles of different sizes and forming the coating of this distribution field. In the further research work it is planned to perform separation of particles of various sizes and compositions, optimization of the distribution process and applications in continuous separation processes of biological objects. The stability, operational efficiency and possible service life of such installations and other qualitative indicators of the separation system will be assessed.

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Compound Produced by Bacteria and Their Antifungal Evaluation in a Model Study with Aspergillus spp.

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Abstract

Nowadays, a wide range of antifungal agents is used in combating biodeterioration and in preventing or treating fungal diseases of plants and for treating diseases in animals and humans. Antifungal compounds such as natamycin, nystatin, amphotericin increasing the permeability of the fungal cell membrane, after binding to sterols (e.g. ergosterol) causing the death of the cell. Iturins A contains antifungal compounds produced by *Bacillus subtilis* which are cyclic lipopeptides characterized by the presence of seven α -amino acids. The supernatant of *B. subtilis* was tested for activity against *Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus, Aspergillus fumigatus, Aspergillus versicolor*. Iturin A is efficacious as a seed treatment agent for many seed-borne fungi and stable over time on treated seeds, but it is not able to inhibit aflatoxin production by *Aspergillus flavus* [1, 2]. For analysis was used to differences in the sizes of the inhibition zones on media. Range of sensitivity of fungi to iturin A – 5 µl/disk to 40 µl/disk. Diameter of inhibition zone at various concentrations of iturin A on agar media are 3 mm – 25 mm. All of the fungi tested were sensitive to iturin A at the highest concentration.

Keywords: bacteria, fungi, antifungal, inhibition.

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Interaction Between Irpex lacteus, Pleurotus ostreatus, Pleurotus eryngii and Several Bacteria

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Abstract

Interactions between bacteria and fungi occur in many different situations and can be considered from different viewpoints. One of the most common bacterial - fungal interactions is antibiosis, characterized by the diffusion of detrimental and often chemically complex molecules from one partner to the other. A variety of different results for each partner may occur due to physical and molecular interactions between bacteria and fungi. The biotic and abiotic effects on the bacterial-fungal complex may be influenced by these changes [1]. In our research bacteria Bacillus thuringiensis, Bacillus mycoides, Klebsiella variicola, Bacillus licheniformis, Bacillus subtilis, Pseudomonas fluorescens are generally isolated from ground and purified in order to be tested for their interactions properties using fungi Irpex lacteus, Pleurotus ostreatus, Pleurotus eryngii. Interactions susceptibility between bacteria and fungi were tested using agar plug method modified according to the corrections published by the Clinical and Laboratory Standards Institute. When applying this method microbial cells secrete molecules which diffuse in the agar medium. Then the interaction activity of the microbial secreted molecules is detected by the appearance of the inhibition zone around the agar plug with fungi. *Irpex lacteus* showed inhibitory effect 3-10 mm and a strong inhibitory activity against the bacteria Bacillus subtilis. Pleurotus ostreatus observed zone of inhibition was comprised between 0 and 10 mm, while Pleurotus eryngii observed zone of inhibition was 2-7 mm using bacteria Bacillus thuringiensis, Bacillus mycoides, Klebsiella variicola, Bacillus licheniformis, Bacillus subtilis, Pseudomonas fluorescens.

Keywords: bacteria, mushrooms, interaction, inhibition.

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An Impact of Enzymatic Hydrolysis and Bacterial Fermentation to the Biological Properties of Some Natural Objects

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Natural plant raw materials, such as bee pollen, dill seeds, chia seeds and flaxseeds have gained huge attention worldwide for their positive effects on human health. Due to the high nutritional values they are being used as functional food. Chia seeds contain important fatty acids, dietary fibre, proteins, vitamins, and some minerals, also are a great source of polyphenols and antioxidants, such as caffeic acid, rosmarinic acid, myricetin, quercetin, and others [1]. The presence of some compounds, exhibiting health-promoting effect, such as n-3 fatty acids, soluble fibre, tocopherols, lignans, phenolic and peptide composites, promotes the usage of flaxseeds in food and medicine [2]. Essential oil, fatty oil, proteins, carbohydrates, fibre, mineral elements (potassium, calcium, magnesium, phosphorous, sodium), vitamin A and niacin are bioactive components of dill [3]. On the other hand, bee pollen gives to organism nutrients such as B group vitamins, minerals and unsaturated fatty acids. Moreover, it helps to overcome metabolic problems and reduces the activity of harmful bacteria [4].

However, the cell walls of bee pollen, dill seeds, chia seeds and flaxseeds are resistant to human digestive system, therefore restricted bioavailability is established. There are few methods and techniques in food biotechnology that might help to increase the biological properties of some natural objects, such as enzymatic and bacterial hydrolysis. The aim of this study was to compare an effect of enzymatic hydrolysis and bacterial fermentation on changes of the biological activity of bee pollen, dill seeds, chia seeds and flaxseeds. The results will be presented during the conference.

Keywords: bee pollen, chia seeds, dill seeds, flaxseeds, enzymatic hydrolysis, bacterial hydrolysis.

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