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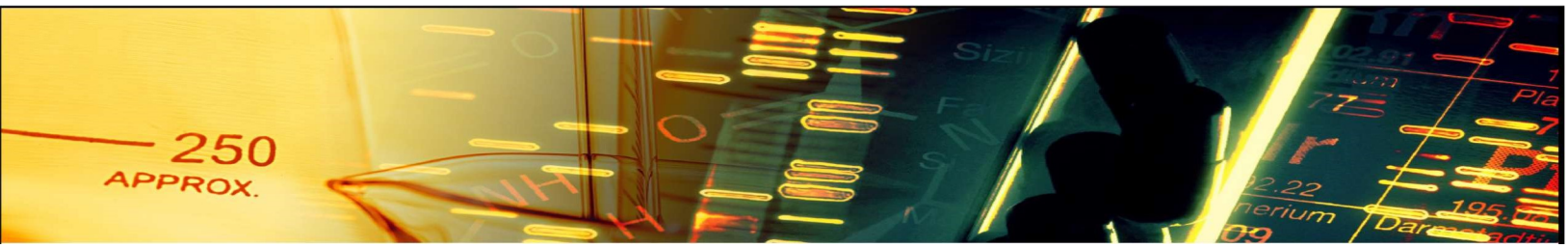

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ABSTRACT BOOK

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Capillary Electrophoresis-Contactless Conductivity Detection Compensation Techniques in Changing Environment

Tomas Drevinskas, Audrius Maruška

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High performance, precision, accuracy and robustness separations and detection in changing environment are hardly achieved. Usually, fluctuations of temperature, electromagnetic radiation, mechanical vibration and other factors negatively affect the quality of the data. Using common separation techniques not only the migration, or retention time, but also baseline drift and peak areas are shifted distorting the final results. In electromigration techniques, this is even more visible due to the fact that current density is proportional to the temperature. What is more important, conductivity readings also provide spoiled data during the change of the temperature.

Conventional way to deal with this problem is buffering and regulation of the temperature and other changing conditions. Usually, these devices are operated in the laboratory due to large dimension design and inoperable field-portability.

In this work an algorithm is proposed for effective real-time data treatment and improvement of quality of the results. Applied methodology improved the precision of migration time and reduced baseline shifting effectively.

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Oral presentation

Development and Application of Instrumental Analysis for Environmental, Biological and Synthetic Samples

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Trends in instrumental analysis area and ongoing projects in the research group will be reviewed. It will include bioremediation experiments with used wooden railway sleepers, the use of fermentation of medicinal plants as additives for preservation and increase of value of food products, searching for antiviral substances in the plants, searching and evaluation of natural biocides, analysis of chemical products and chemical evaluation of plants and soil coverage during regeneration of forests. The trends in miniaturization and integration of analytical methods and tools will be discussed.

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Oral presentation

Step-by-Step Isolation of Valuable Fractions from Berry Pomace by High Pressure and Enzyme-Assisted Extraction Methods

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Many berry species are known for their excellent flavor and abundance of healthy compounds possessing antioxidant and other beneficial properties. However, due to a rapid decay after harvesting, the majority of berry crops are processed into juices and other products. Pressing of juice results in large quantities of by-products, called pomace, press-cake or marc. These residues contain various valuable compounds such as polyphenolics, vitamins; however, currently they are used rather inefficiently and in many cases wasted, mainly due to a lack of scientific and technological valorization of their processing methods.

This study provides examples of biorefining of black currant (*Ribes nigrum*), chokeberry (*Aronia melanocarpa*), raspberry (*Rubus idaeus*) and European cranberry bush berry (*Viburnum opulus*) pomaces into high-value functional ingredients by using high pressure and enzyme-assisted extraction/fractionation methods. The residues are further extracted by using pressurized liquids, e.g. water or its mixtures with ethanol while remaining non-soluble substances are treated with various enzymes to obtain additional water soluble products. The composition and antioxidant properties of the fractions obtained were analysed by chromatography and mass spectrometry while antioxidant properties were evaluated by the batch *in vitro* assays (DPPH, ABTS, FRAP, ORAC, Folin-Ciocalteu values) and the on-line HPLC-UV-DPPH radical scavenging assay.

The results indicate that the fractions isolated from berry pomaces contain valuable bioactive compounds, which might find applications in functional foods, nutraceuticals, cosmetics, and other products. Firstly, lipophilic fractions, consisting mainly of triacylglycerols, were extracted with supercritical carbon dioxide. At optimal conditions, the yields of oily extracts from berries were from 3 to 15 %. These extracts were rich in polyunsaturated fatty acids and tocopherols. Higher polarity fractions were extracted from the residues and the total yield of extracts was up to 80 %. These fractions contained various phytochemicals; most of them were strong antioxidants.

Conclusion: the concept of biorefining, which is defined as ‚*a sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power, and/or heat)*‘ may be successfully applied to berry pomaces for the development of high added value functional ingredients; technological and economical aspects of upscaling the processes should be in the focus of future research.

Keywords: berry pomace, supercritical fluid extraction, pressurised liquid extraction, enzyme-assisted extraction, antioxidant capacity

Acknowledgements: this research was funded by the Research Council of Lithuania (Grant No. SVE-01/2014).

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Poster presentation

Development and Validation of HPLC Method for Bromazepam, Fluoxetine and Rispiridone Simultaneous Analysis

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There are many HPLC assays that can successfully detect the same psychotropic drug class, however, analysis to simultaneously detect different classes of psychotropic drugs are few. The aim of this research is to develop an HPLC method suitable for the qualitative and quantitative analysis of a mixture of bromazepam, risperidone and fluoxetine medicines.

Material and method: For HPLC method optimization bromazepam, risperidone, fluoxetine and their mixture stock solutions (0,1 mg/ml) in methanol were analysed. The analysis was carried out on Waters HPLC system equipped with PDA detector and HPLC BEH C18 analytical column. Gradient separations were carried out using aqueous 0.05% phosphoric acid and acetonitrile as the A and B solvents, respectively. Method validation was performed by evaluating specificity, linearity, LOD, LOQ and precision. Optimized method was applied for assay analysis of pharmaceutical products solutions.

Results: Method specificity was deducted according to retention time of the tested compound (2,49, 2,33 and 2,64 for bromazepam, risperidone and fluoxetine respectively) and PDA spectrum matching the standard. Absorption of UV light was similar as in scientific literature. An optimum condition was observed with the use of solvent A and B starting with the ratio 95:5 till 5:95 through 10 min chromatographic run. Calibration curve for the analyzed drugs with new method was investigated over a concentration range of 0,000781- 0,050000 mg/ml. LOD for bromazepam, risperidone and fluoxetine were 0,68 µg/ml, 0,586 µg/ml and 0,434 µg/ml and LOQ were 2,27 µg/ml, 1,95 µg/ml and 1, 45 µg/ml respectively. Method precision was measured as the average relative standard deviation (RSD). RSD for bromazepam, risperidone and fluoxetine were 0,1 and SSD were 0,002, 0,003, and 0,004 respectively.

Conclusion: This HPLC method is suitable for the qualitative and quantitative analysis of psychotropic drugs in the mixture and determination in pharmaceutical products.

Keywords: bromazepam, risperidone, fluoxetine, high-performance liquid chromatography, qualitative determination.

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Poster presentation

Optimization of Capillary Electrophoresis-Contactless Conductivity Detection Method for Analysis of Inorganic Cations and Amino Acids in Wheat, Rye and Oat Flour

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Abstract

According to the literature all flour types have different composition. This is important for people, who have allergy or disease caused by food, e.g. celiac disease. Usually flour quality is considered by determining gluten content, but it is also important to identify other constituents like minerals and amino acids.

Comparison of minerals and amino acids in different flours was performed between three types of flour. Wheat, rye and oat flour was chosen by its popularity in all world cuisines.

Capillary zone electrophoresis method with capacitance-to-digital based contactless conductivity detector was optimized and used for determination of inorganic cations and amino acids and comparison of different types of flours. Optimized conditions were following: 0.5 M acetic acid was used as a background electrolyte. 0.05 grams of flour were extracted by 50 % of propyl alcohol solution. Analysis in fused silica capillary were irreproducible, therefore it was decided to coat internal wall of the separation capillary. Mentioned procedure improved analytical data significantly.

Keywords: Capillary electrophoresis, Wheal flour, Rye flour, Oat flour, Contactless conductivity detection, Inorganic cations, Amino acids

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Poster presentation

Capillary Electrophoresis-Contactless Conductivity Detection – a Tool for Sample Suitability Testing in Liquid Chromatography-Mass Spectrometry Separations

Tomas Drevinskas[1], Mantas Stankevičius[1], Kristina Bimbiraitė – Survilienė[1], Vilma Kaškonienė[1], Olga Kornyšova[1], Violeta Bartkuvienė[1], Gintarė Naujokaitytė[1], Loreta Šernienė[2], Kristina Mulkytė[2], Neringa Kasnauskytė[2], Mindaugas Malakauskas[2], Audrius Maruška[1]

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Abstract

High performance liquid chromatography coupled with mass spectrometry is a useful analytical technique, however it faces difficulties when separating biological and high inorganic content containing samples. In this case, special sample preparation with effective desalting procedure is needed.

In this work capillary electrophoresis coupled with capacitance-to-digital based contactless conductivity detection was used for determination of inorganic cations in biological nisin containing samples. The procedure allows rapid separation and determination of inorganic cations in biological samples. Capillary electrophoresis separations before and after solid phase extraction – sample preparation of nisin containing samples indicate dramatic decrease of inorganic compounds, which allow further analysis using high performance liquid chromatography – mass spectrometry. Additionally, after sample processing, nisin is still present and can be quantified.

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Poster presentation

Optimization of Capillary Electrophoresis-UV Absorption Detection Method for Analysis of Thymol and Carvacrol in Essential Oil of *Thymus serpyllum* L.

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Abstract

Wild Thyme *(Thymus serpyllum L.)* is a medicinal plant, native to most of Europe. In Lithuania wild thyme usually grows in loam soil and in dry meadows. Wild thyme is often used in herbal medicine and as ingriedient in cooking. Biologically active compounds are most often found in essential oil. About forty-five different chemical compounds where found in essential oil Thymol and carvacrol are two main compounds in essential oil. Both thymol and carvacrol have strong antimicrobial and antifungal properties.

Plant material was collected in Utena county. Essential oil extractions were conducted as following: 0.5 grams of dried plant material was extracted for 24 hours in 75% and 100% methanol.

Capillary zone electrophoresis method with UV absorption detector was optimized and used for determination of thymol and carvacrol in the extract of *Thymus serpyllum L.* Optimized conditions were following: 25mM pH=9,35 sodium tetraborate soliution was used as a background electrolyte. 30mM sodium hydroxide solution was used washing the capillary. Applied voltage was 25kV, total capillary length was 60 cm, capillary length to detector was 50 cm, separation temperature was 23ºC

Keywords: Capillary electrophosesis, Wild Thyme, Tymol, Carvarcol, UV absorption detection, Esential oil

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Poster presentation

Optimization of Capillary Electrophoresis-Contactless Conductivity Detection Method for Analysis of Inorganic Cations in Chicken Egg Shell

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Abstract

Literature reports that structural composition of chicken egg shell is different and the difference is caused by inefficient feeding and lack of physical activity. It is assumed that quality of egg can be determined by quantity of Ca^{2+} and other inorganic elements and organic compounds, found in egg shell.

Comparison of minerals in different chicken egg shells was performed between two types of eggs, which were taken from egg industry farms, where chickens live in cages and another type was picked from ecological Lithuanian farms, where chickens' feeding and housing conditions are considered of being sufficient.

Capillary zone electrophoresis method with capacitance-to-digital based contactless conductivity detector was optimized and used for determination of mineral inorganic compounds and comparison of different egg shells. Optimized conditions were following: 0.5M acetic acid was used as a background electrolyte. 0.25 grams of chicken egg shell powder, from egg industry farms, dissolved in 16.65mM acetic acid solution and ecological egg shell powder dissolved in 66.65mM acetic acid solution. Total capillary length was 48 cm and capillary length to detector was 38 cm. Applied voltage was 14 kV and separation temperature was 30°C.

Keywords: Capillary electrophoresis, Chicken egg shell, Contactless conductivity detection, Inorganic cations, Acetic acid

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Poster presentation

Chemical Analysis of Biologicaly Active Compounds of *Rhaponticum carthamoides* DC. Iljin Using Gas Chromatography-Flame Ionization Detector Method in Different Vegetation Phases

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Abstract

Since ancient times people were using various herbs to heal illnesses, regain strength and improve health. One of many valuable herbs is Rhaponticum carthamoides (Rhaponticum carthamoides DC. Iljin) also known as Maral root, Leuzea carthamoides, Cnicus carthamoides. Rhaponticum is poorly examined, however researches which were made had shown that plant has lots of beneficial qualities. It is known that Rhaponticum has varios volatile compounds. Rhaponticum (rootstock, blossom and leafs) was collected and prepared in Vytautas Magnus University Botanic Garden. There were analysed 5 phases: intensive growth, budding, blossoming, massive blossoming, ending of blossoming and fructification. For extract preparation we used Supercritical fluid extraction method. The data were processed using Microsoft Excel.

The results showed that the in all vegetation phases there are 2 dominant compounds: β-Elemene(56.98-33.45%) and Salvial-4(14)-en-1-one(42.84-22.07%). The biggest amount of compounds was found in ending of blossoming and fructification phase (total 25) and the least in blossoming phase (total: 16).

Keywords: *Rhaponticum carthamoides*, Gas Chromatography-Flame Ionization Detector.

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Poster presentation

Introduction and Chemical Analysis of *Angelica archangelica* L. Grown at Kaunas Botanical Garden, Vytautas Magnus University

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Abstract

At present and in the future, it is necessary to enrich the assortment of the cultivated medicinal plant. Species of medicinal plants have been introduced *ex situ* in the medicinal plants collection at Kaunas Botanical Garden of Vytautas Magnus University since 1924 for this purpose. Biodiversity of plant resources, scarce information on bioactive compounds and their properties in many species, as well as increasing demand for naturalness of herbal medicines and food were the main motivation aspects of the present study. *Angelica archangelica* L., is a medicinal plant known for its cooking properties and its therapeutic uses. There is a link between medicinal properties and chemical compounds of the plant. That's why, the aim of this study was to carry out a comparative determination of total content of phenolic compounds, flavonoids and radical scanvenging activity between five different growth stages of this plant. *Angelica archangelica* L. was collected and air-dried at the Kaunas Botanical Garden in 2015. 0,5g of each stage were extracted with 20mL of methanol 75%, shaked during 24h, filtered and prepared for spectrophotometric analysis. Spectrophotometric methods were used to determine the total content of phenolic compounds (using Folin-Ciocalteu reagent), the total content of flavonoids (using a stock solution), and the total radical scavenging activity (using DPPH as a radical reagent). The results of this study suggest that the total radical scavenging activity is less important than the total content of phenolic compounds for each growth stages because all phenolic compounds can't reduce DPPH. However, the differences between both total are not significant. Moreover, this experiment demonstrates that the more important concentration of phenolic compounds and radical scavenging activity in *Angelica archangelica* L. are in the growth stage early bloom. Also, the total content of flavonoids is particularly lower than total content of phenolic compounds. In fact, there are several different phenolic compounds as tannins for example. Their presence in the plant is influenced by environmental conditions. In the medicinal plant *Angelica archangelica* L., total content of phenolic compounds, flavonoids and total radical scavenging activity are contingent on growth stage of the plant.

Keywords: *Angelica archangelica* L., phenolic compounds, flavonoids, radical scavenging activity, medicinal plant

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Poster presentation

The Impact of Different Fermentation Parameters on Antioxidant Activity of Bee Pollen

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Abstract

The aim of this study was to get product similar to the bee bread, which has higher biological value compared to the natural bee pollen. The influence of different parameters, such as temperature, pH, fermentation term, to fermentation process was determined. Free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, total amounts of phenolic compounds and flavonoids were evaluated by spectrophotometric methods. Fermentation of bee pollen was performed by lactic acid bacteria, namely *Lactobacillus rhamnosus.* Results showed that temperature range from 32°C to 37°C had no impact to fermentation and had no influence to total amount of phenolic compounds or radical scavenging activity. Changes of pH in the samples resulted the decrease of radical scavenging activity by an average of 0.8 times, but increased total amount of flavonoids by 1.7 times. Alkaline fermentation mixture resulted an increase of total phenolic compounds almost twice, but acidification had less impact and resulted an increase of total phenolic compounds by an average of 1.1 times. Control samples were not affected by changes of pH. Fermentation term had impact only on the total amount of flavonoids, which after fermentation increased significantly in all samples by 1.7 times, except samples, that were fermented only 3 days, where increase of total amount of flavonoids was only by 1.1 times.

Keywords: Bee pollen, fermentation, lactic acid bacteria, flavonoids, antiradical activity

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Poster presentation

Simultaneous Determination of Nifedipine, Propranolol, Verapamil and Diltiazem in Human Plasma Using Thin Layer and Ultra Performance Liquid Chromatography Method

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Drugs used in the treatment of cardiovascular disorders are among the most frequently used in Lithuania and in the world. However poisoning from cardiovascular drugs ranks among the leading agents in pharmaceuticals. The aim of this research is to develop a thin layer chromatographic (TLC) and ultra performance liquid chromatographic (UPLC) methods suitable for separation and identification of nifedipine, propranolol, verapamil and diltiazem from human plasma.

Material and method: A mixture of nifedipine, propranolol, verapamil and diltiazem solutions (0.1 mg/ml) from human plasma was extracted by solid phase and liquid-liquid extractions. Samples were analysed using TLC and UPLC. The UPLC analysis was carried out on Waters UPLC system equipped with PDA detector and UPLC BEH C18 analytical column. Mobile phase consisted of acetonitrile and 0.05% trifluoroacetic acid in water**.** Mobile phase elution was gradient under our set conditions. For TLC DC-Fertigfolien alugram chromatographic plates were used and two solvent systems were developed: MP-1 ethyl acetate : methanol : glacial acetic acid (16:24:2); MP-2 acetone : methanol: glacial acetic acid (16:30:1).

Results: UPLC method validation was performed by evaluating specificity, linearity, LOD, LOQ, intraday and inter-day repeatability. The validation results demonstrated the reliability of this method and antihypertensive drugs from human plasma were fully identified. The retention times (RT) of tested compounds were 4.105, 4.416, 4.770, 5.117 for propranolol, diltiazem, verapamil and nifedipine respectively. RT of compounds from human plasma were identical. For TLC good mixture separation and identification of its components from human plasma was produced. The R_f values (n=5) of MP-1 were 0.95, 0.62, 0.47 and 0.28 for nifedipine, propranolol, verapamil and diltiazem respectively. R_f of MP-2 were 0.93, 0.71, 0.60 and 0.43 for nifedipine, propranolol, verapamil and diltiazem respectively.

Conclusion: Our TLC and UPLC methods are suitable for the separation and identification of antihypertensive drugs from human plasma.

Keywords: nifedipine, propranolol, verapamil, diltiazem, thin layer chromatography, ultra performance liquid chromatography, solid phase extraction, liquid-liquid extraction.

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Oral presentation

Contributions to Macromycota of Turkey from Belgrad forest (Istanbul)

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Abstract

Belgrad Forest is situated in İstanbul province at the eastern part of the Thracian Peninsula. The forest covers 5441.71 hectares and it has characteristics of an Euro-Siberian as well as Mediterranean climate features. The forest vegetation is dominated by broadleaved trees such as oak, beech, hornbeam, chesnut, alder, poplar, lime trees, maple and elm. Planted coniferous trees such as fir, spruce, black pine, scots pine and pseudomacchie can also be seen [1-3].

This study is based on macrofungi samples collected in Belgrad Forest (İstanbul) between 2013-2015. As a result of the field and laboratory studies, totally 204 species belonging to 119 genera, 57 families, 16 order, 2 division have been identified. 15 species are belonging to *Ascomycota*, 204 taxa belonging to *Basidiomycota*. Taxa are given in alphabetical order and are listed together with locality, notes on habitat, geographical position, collection date, and accession numbers (A: Akata).

Keywords: Macrofungi, Biodiversity, Belgrad Forest, İstanbul

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Oral presentation

Contributions to the Macrofungal Diversity of Sinop Province

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Abstract

This investigation covers only Sinop province, which is located in the Western Black Sea region of Turkey, Although the study area is affected by the Black Sea climate predominantly, the influence of a Mediterranean climate is also seen in some parts of the study area [1].

The material of this study comprises macrofungi specimens collected from different localities in Sinop district (Turkey) in 2014 and 2015. As a result of field and laboratory studies, 81 taxa under 30 families belonging to the classes Ascomycetes and Basidiomycetes are described: 2 of them, namely *Mycena leaiana* (Berk.) Sacc., *Lepiota apatelia* Vellinga&Huijser and are new records for Turkey [2-5].

Keywords: Macrofungi, taxonomy, new records, Sinop, Turkey

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Oral presentation

Biocontrol of Mosquitoes *(Aedes, Culex)* in Pavlodar Region (Kazakhstan)

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Abstract.

Currently, there are several methods of regulation number of *Diptera* bloodsucking, but the most appropriate in terms of "efficiency and ecological safety" is a biological method using microorganisms pathogenic for bloodsuckers.

A classic example is the use of various biological preparations (drugs) based on *Bacillus thuringiensis* bacteria. The basic principle of action of drugs of this group is to identify bacteria endotoxin in cells of epithelial tissue of the intestine of the insect.

Typically, before this time the temporary and small reservoirs were conducted with the use of chemical insecticides. A feature of the Pavlodar region is the presence of a large river Irtysh, which has a great economic importance. Spring period characterized by extensive floods of the river and form flood inundated areas, is the optimal location for the development of mosquitoes.

Scientific support of disinfection measures carried out in the period from 15 April to 15 July 2015. Detected faunal composition and phenology of mosquito, places of their mass breeding were identified and there were given recommendations to the processing areas, specifying the date and dose of insecticides. Monitoring of the effectiveness of treatments was conducted regularly. The laboratory studies carried out to determine the effective doses of drugs conducted histopathological examination of mosquitoes larvae of the *Aedes* genus.

It revealed that the main (by mass) species in Pavlodar city and its surrounding areas are *Culex pipiens pipiens forma molestus, Aedes caspius caspius, Culex pipiens pipiens*, *Anopheles messeae Mg*. The first type of mosquito is developed only in flooded basements, and it is the earliest type, the development of the rest of the larvae takes place on open water.

During the spring-summer period, we made phenological observations over development of phases of blood-sucking mosquitoes

Processing's by a preparation were carried out with taking into account physical data of a reservoir (depth, impurity, quantity of vegetation, temperature)

Total processed 10 large and diverse areas, with a total area - 5 hectares. The average efficiency based on the results of this work to control the number of mosquito larvae using bio-insecticides is 93.15%. For comparison the effectiveness of treatment with a chemical drug, Averfos^{TM} was 90-97%

Thus, Baktitsid^{TM} drug treatment technique can be considered as sufficiently effective.

Keywords: Diptera, mosquito larvae, regulation number, bloodsuckers, Baktitsid

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Oral presentation

Analysis of Consumer Grade Medical Devices for Skin Care

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Abstract

Bioelectrical impedance analysis (BIA) is a widely used method for determining the total body water. Popularity of this measurement method is common because of the simplicity of the equipment and ease of use. Measurement techniques are established for total body water and body fat. However this method can be found to be used for skin analysis in various equipment. Research related to BIA application for skin analysis is not widespread, although the consumer grade devices are widely available.

Several common skin analyzers based on bioelectrical impedance analysis were picked for this research. The analysis of the electronic circuits built in for skin condition estimation allowed to evaluate the implementation of the bioelectrical impedance analysis in hardware level. The repeatability of the measurements were investigated under controlled conditions using human body and skin electronic emulator. Finally statistical analysis of real life measurements were performed in order to compare different devices regarding skin condition estimation variance and its dependence on various influence factors (pressure, measurement position, etc.).

Results revealed that some of the devices have a simplified bioelectrical impedance analysis technique implemented, which relies on measurements of the resistance part only of the impedance, omitting the reactance. However, there are no scientific papers proving the idea of such skin measurements. The experiment with human body/skin electronic emulator showed that the pressure applied during measurement on the device was one of the main factors influencing the increased scatter of the measurement results. This observation was confirmed by statistical analysis of the real-world measurements using the selected skin analyzers.

The research exposed that not all devices on the market have implemented bioelectrical impedance analysis technique to estimate skin condition, despite the fact manufacturers state so. Thus, further attention is required to justify the use of BIA for skin condition analysis.

Keywords: Bioelectrical impedance analysis, medical devices, metrology, skin analyzer

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Poster presentation

Superoxide Dismutases Activity in Mice Brain Under Selenium and/or Aliuminium Ions Treatment

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Abstract

Within a cell superoxide dismutases (SOD) are the first line of defence against reactive oxygen species. The present study was conducted to investigate the influence of Se and/or Al ions on the SOD activity in brain as well as distribution of these elements in the blood and the brain of laboratory mice.

Experiments were done on 4-6 weeks old outbreed mice. SOD activity was determined in brain after 24 h and 14 d. Al and/or Se solution i.p. injections. SOD activity was determined spectrofotometrically. The concentration of protein was measured by the Warburg-Christian method. Se and Al concentrations were determined by inductively coupled plasma mass spectrometer.

It was evaluated the effect of Al on SOD activity in mouse brain after a single i.p. Al injection. The results showed that SOD activity was the same value in control and experimental groups. After a single Se dose injection SOD activity decreased by 28.6% (compared to the control group). Subsequently, were evaluated changes in SOD activity following a single Se+Al mixture injection. It was observed a significant decrease in SOD activity (17.8%).

In further experiments, there was evaluated the effects of Al and/or Se on SOD activity after 14 d. i.p. injections. The results showed that injections of these elements alone did not cause changes of SOD activity. The data of the effect of both elements showed that SOD activity decreased by 41.5% (compared to the control group).

Estimation of the element distribution in brain homogenates showed that after 24 h and 14 d. concentrations did not differ from controls. While in blood: after 24 h exposure levels of Se increased in Se and Se+Al group (22% and 41% respectively) and after 14 d. increased Se and Al concentrations. It is also established in Se+Al group, but Al concentrations are significantly lower.

Our studies revealed that the Se and Al total effect in the mice brain reduce the enzymatic SOD activity after 24 h and 14 d. repeated exposure.

Keywords: Oxidative stress, superoxide dismutases, aliuminium, selenium

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Poster presentation

Larger Ascomycota of Turkey

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Abstract

Turkey is a large rectangular peninsula situated between south-eastern Europe and Asia and its land mass is about 780.000 km^2. The country is meeting ground of three phytogeographical regions: Euro-Siberian, Mediterranean, and Irano-Turanian. Turkey is among the richest floristic centres in the world and the wide range of temperature and precipitation conditions and the irregular topographic structure of Turkey have created a great diversity of ecological conditions [1].

The Kingdom Fungi is a large group of organisms that includes more than 100,000 species but the global biodiversity of the fungi has been estimated at about 1.5 million species. *Ascomycota* is a division of the kingdom Fungi and it includes organisms from unicellular yeasts to complex cup fungi. The division has approximately 40.000 species and its members produce asci that contain ascospores. Larger *Ascomycota* is specific part of this division and their sporocarps are visible without using a magnifying apparatus [2-3].

The aim of this study is to decument Larger *Ascomycota* taxa that have reported from Turkey. According to literature sources, the main lists of correct names of larger *Ascomycota* are presented. The checklist provides 258 species belonging to 36 families. Each taxon is presented in alphabetical order along with the distribution throughout to country.

Keywords: Larger *Ascomycota*, fungal diversity, Turkey

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Poster presentation

Ecological Features and Diversity of Turkish *Hymenoscyphus*

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Abstract

Hymenoscyphus is a large genus of family *Helotiaceae* within the order *Helotiales*. It contains approximately 160 species in the world [1]. The members of the genus are characterized by sessile to stipitate, cup to disc or cushion shaped apothecia, whitish to yellowish hymenium surface, cylindric to clavate, asci with amyloid pores, hyaline, smooth, cylindric paraphyses, ellipsoid, clavate, cylindric or fusoid spores [2].

According to literature [3-8], 7 *Hymenoscyphus* species (*H. calyculus* (Sowerby : Fr.) W. Phillips, *H. fructigenus* (Bull.: Fr.) Fr., *H. herbarum* (Pers.: Fr.) Dennis, *H. immutabilis* (Fuckel) Dennis, *H. lutescens* (Hedw.) W. Phillips, *H. robustior* (P. Karst.) Dennis, *H. scutula* (Pers.) W. Phillips) have previously been recorded from Turkey.

With this study, *Hymenoscyphus serotinus* (Pers.) W. Phillips is reported for the first time from Turkey and it will be 8th member of Turkish *Hymenoscyphus*

Notes are presented on the members of genus *Hymenoscyphus* and their distributions, morphological and ecological features along with the status in Turkey.

Keywords: *Hymenoscyphus*, biodiversity, new record, Turkey

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Poster presentation

Electroporation as a Tool for Studying the Role of Plasma Membrane in the Mechanism of Cytotoxicity of Bisphosphonates and Menadione

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Abstract

In this study, the role of the cell plasma membrane as a barrier in the mechanism of the cytotoxicity of nitrogen-containing bisphosphonates and menadione was studied and the possibility of increasing the efficiency of bisphosphonates and menadione (vitamin K_3) as chemotherapeutic agents by permeabilizing the cell plasma membrane has been investigated *in vitro*. The plasma membrane barrier was reduced by electropermeabilization with the pulse of strong electric field. Two membrane-impermeant bisphosphonates with different hydrophilicity were chosen as study objects: ibandonate and pamidronate. For the comparison, an amphiphilic vitamin K_3, which is able to cross the cell membrane, was studied as well.

The impact of nitrogen-containing bisphosphonates and vitamin K_3 on MH-22A cells viability was evaluated for the case of long (9 days) and short (20 min) exposure. When cells were cultured in the medium with vitamin K_3 for 9–10 days, it exhibited toxicity of 50 % over the control at 6.2 mM for mouse hepatoma MH-22A cells. Ibandronate and pamidronate were capable of reducing drastically the cell viability only in the case of long 9-days incubation and at high concentrations (~20 μM for pamidronate and over 100 mM for ibandronate) [1].

Single, square-wave electric pulse with the duration of 100 μs and the field strength of 2 kV/cm was used to electroporate mouse hepatoma MH-22A cells *in vitro*. The results obtained here showed that the combination of the exposure of cells to membrane-impermeable bisphosphonates pamidronate and ibandronate with electropermeabilization of the cell plasma membrane did not increase their cytotoxicity. In the case of membrane-permeable vitamin K_3, cell electropermeabilization did increase vitamin K_3 killing efficiency. However, this increase was not substantial; within the range of 20–30% depending on the duration of the exposure. Electropermeabilization improved cytotoxic efect of vitamin K_3 but not of pamidronate and ibandronate [1].

Keywords: Pamidronate, Ibandronate, Vitamin K_3, Elektrochemotheraphy, Electropermeabilization.

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Poster presentation

Morphological Comparison of Some Riverine *Batrachium* Species

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Abstract

Genus *Batrachium* (DC.) Gray is evolutionary young but very important group of aquatic plants. It is very essential component in the river for its ability to affect flow dynamics, silt deposition and provide refuge for ecologically important macroinvertebrate and fish populations and can be considered as the indicator of the sustainability of riverine ecosystem. Communities with *Batrachium* species are regarded as significant for European Union and listed in Apendix 1 of Habitat Directive (code 3260). Plants of this genus are important not only from the scientific point, but also and in terms of plant conservation. By reason of this, studies of morphological features of *Batrachium* species are very relevant. Our study is an introduction to further detail research of *Batrachium* species. It was performed in 16 river catchments. During this studies 185 *Batrachium* individuals were collected and measured. The morphological features such as the presence of floating leaves, number of capillary leaves furcations, length of terminal leaf segments, length of petiole, hairiness of stipules, length and number of petals, and hairiness of receptacle, were used in this analysis. The species level taxonomic identification of the individuals was performed using keys after Cook (1966), Webster (1988), Dahlgren, Jonsell (2001), Bobrov (2003), Webster, Rich (1998). Nonparametric Kruskal-Wallis test was used to compare morphological features. Mann-Whitney U test was used to identify differences between specific populations. The analysis showed that not all analysed features were uniformly important in *Batrachium* species identification. Acording to some morfological features all samples were grouped in two groups. Individuals from the first group distinguished glabrous or sparse hairy receptacle, had 5-8 petals (length ranged from 0.4 to 1.1 cm), had only capillary leaves, length of secondary leave ranged from 4 to 9 cm, and length of the sixth leave ranged from 6 to 12 cm. While specimens from another group had densely pubescent receptacle, five petals (length ranged from 0.2 to 0.5 cm), length of secondary capillary leaves ranged from 3 to 7 cm and length of the sixth leave ranged from 5 to 8 cm. Some individuals from Skroblus, Ūla and Merkys populations had intermediate leaves. We suppose, that individuals from first group can be described as *Batrachium fluitans*, whereas plants from second group – as *Batrachium penicillatum* s.l. or *Batrachium trichophyllum*.

Keywords: *Batrachium fluitans*, *Batrachium penicillatum* s.l., *Batrachium trichophyllum* morphopogical features

Acknowledgements

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Poster presentation

Macular Pigment Optical Density's Association with *CYP2C19* rs4244285 in Healthy Subjects

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Abstract

Macular pigments are mainly made up of two oxycarotenoids, lutein and zeaxanthin. The yellow macular pigment is mainly located in the ganglion cell layers and inner plexiform layers of the retina [1]. The CYP2C19 gene has nine exons and is highly polymorphic, with over 25 variant star (*) alleles currently defined by the Human Cytochrome P450 Allele Nomenclature Committee. rs4244285 (c.681G > A) is the defining polymorphism of the CYP2C19*2 allele (previously referred to as CYP2C19m1) and is a synonymous G > A transition in exon 5 that creates an aberrant splice site [2].

Purpose: to determine the effect of *CYP2C19* (G681A) Rs4244285 single nucleotide polymorphisms (SNPs) on macular pigment optical density (MPOD) in a healthy participants of Lithuanian origin with normal ocular health.

Methodology: 176 healthy subjects were included into our study. The optional macular pigment density module for the Visucam 200 used the reflectance of a single 460-nm wavelength based on a single blue-reflection fundus image to determine MPOD and its spatial distribution. The genotyping of Rs4244285 was carried out using the real-time polymerase chain reaction method.

Results: There were no significant differences between genotypes in the healthy participants group on MPOD (p=0.9240). GG median (min., max.): 0.111 (0.01185, 0.163) and GA median (min., max.):0.1155 (0.0905, 0.1595). There was no AA genotype in healthy subjects, so we can assume that it would correlate with unhealthy subjects.

Conclusion: Our results indicate that MPOD is not dependent on CYP2C19 (G681A) Rs424428 gene variants in healthy people.

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Poster presentation

The Association of Rs1551839 Gene Polymorphism and Macular Pigment Optical Density in Healthy Subjects

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Abstract

The carotenoid pigments of the macula, collectively known as macular pigment (MP), are constituted by the oxycarotenoids lutein (L) and zeaxanthin (Z) (1). MP is not synthesized by the human body and is entirely of dietary origin, and L and Z reach their peak tissue concentrations at the macular retina, far higher than in any other tissue compartment (2). Z predominates in the fovea, whereas L is predominant in the peripheral macula and other body tissues (2). Rs1551839 (CYP4F2) is a single nucleotide polymorphism (SNP) that is encoded by cytochrome 450 family which metabolizes arachidonic acid. This mechanism regulates blood flow, pressure and vascularization which are important for macular pigment clusters in the retina (3). There were no other researches done that inspect CYP4F2 association with macular pigment optical density (MPOD).

Purpose:To determine the association of rs1551839 gene polymorphism and macular pigment optical density in healthy participants.

Methodology:We examined 115 healthy people. The MPOD was determined by Visucam 200 using the reflectance of a single 460-nm wavelength based on a single blue-reflection fundus image.The genotyping of*CYP4F2* rs1551839 was carried out usingRT-PCRmethod.Comparisons between MPOD levels and genotypes were analysed using one-way ANOVA analysis by statistical analysis software "*Graph Pad Prism* 7.0" (*GraphPad* Software Inc., San Diego, CA).

Results:We found no statistical differences comparing MPOD levels between three genotype groups: GG (0,1129±0,026), GA (0,1162±0,017), AA (0,1149±0,017), p=0,7618.

Conclusion: There were no significant differences between genotypes and macular pigment optical density in healthy participants group.

Key words: gene polymorphism, macular pigment optical density.

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Poster presentation

The Dynamics of Biochemical Blood Parameters of Bulls with Spontaneous Sarcocystosis Infection

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Abstract

Some *Sarcocystis* species are important pathogens of humans, domestic and wild animals. In different countries some research results confirmed the high prevalence of Sarcocystis in cattle. The studies of sarcocystosis infection in domestic and wild animals are very important because of their potential threat to humans. In the absence of accurate (uniform) criteria for assessing cattle Sarcocystosis infection intensity it is not able to make an objective veterinary sanitation evaluation of slaughter products. The sarcocystosis monitoring , searching, collecting and systematizing new diagnostic methods information about Sarcocystis prevalence of food-producing animals, it is possible to create food products list that pose a risk to human health.

The aim of this study was to evaluate blood enzyme assay suitability for diagnose, monitoring and data collection of spontaneous cattle sarcocystosis.

Materials and Methods. Meat and blood samples were collected and examined in 2015 – 2016. Sarcosporidiosis infection was evaluated in 20–24 months of Lithuanian Black-and-White cattle bulls. Sarcocysts were diagnosed by means of the compressor-microscopic method. The samples were conditionally divided into 4 groups by the number of sarcocysts in 28 oat-size muscle sections, i.e. 0 cysts – no infection; 1-10 cysts – low infection; 11–40 cysts – moderate infection; > 40 cysts – intense infection. Biochemical blood tests were performed with automated computerized biochemistry analyser SELECTRA Junior (Netherlands, 2006). Biochemical tests were performed using Spinreact (Spain) reagents. Amounts of urea, GOT (AST – aspartate aminotransferase), GPT (ALT – alanine aminotransferase), alkaline phosphatase, iron, creatinine, triglycerides and total protein were determined in the bull's blood serum.

Results. GPT and ALP levels in all examined bull groups with different sarcocystosis infection were higher than the upper physiological limit and were statistically significant ($p<0,001$). Iron levels were lower than the lowest physiological limit and were statistically significant between no infection, moderate infection and intense infection groups ($p<0,001$). The amount of total proteins in blood serum were higher than normal in low infection and intense infection groups and were statistically significant between these groups ($p<0,05$).

Keywords: Spontaneous sarcocystosis, infection, bulls, enzyme assay, blood serum, slaughter.

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Poster presentation

Generation of Hydrogen Peroxide by High-Voltage Pulses in Cell-Free Media

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Abstract

Electroporation offers a number of applications in biology, oncology, immunology, and biotechnology. However, when a high-voltage is applied to the electrolyte solution, besides membrane permeabilization, various electrolysis reactions occur at the electrode-solution interfaces. One of the results of these electrochemical reactions is generation of reactive oxygen species (ROS). Although ROS formation following electroporation with long (μs–ms) electric pulses or nsPEF exposure has been reported earlier, no detailed analysis of this process has been carried out yet. In this study, generation of hydrogen peroxide as a result of the exposure of a cell-free media by high-voltage pulses has been studied.

Generation of hydrogen peroxide in highly buffered solution HB1, Dulbecco's Modified Eagle's medium (DMEM), Phosphate buffered saline (PBS), and distilled water was studied. The cuvette with stainless-steel electrodes was used. Hydrogen peroxide was detected with AmplexRed® (10-Acetyl-3,7-dihydroxyphenoxazine), which has a great specificity, stability, and selectivity. AmplexRed is nonfluorescent until, in the presence of horseradish peroxidase, it reacts with hydrogen peroxide to produce highly fluorescent resorufin. 50 μM of the AmplexRed dye were added to the medium and treated with high-voltage pulses. Fluorescence intensity in a solution was measured using TECA7 GeniosPro spectrophotometer (Tecan Group, Männedorf, Switzerland).

Treatment of HB1 medium by a single square-wave electric pulse with the duration of 0.5–2 ms and the amplitude of 100– 400 V (0.5-2.0 kV/cm) significantly increased dye emission. The AmplexRed fluorescence intensity was also dependent on the number of pulses, the conductivity, and composition of the medium (PBS, H2O, DMEM, HB1).

Conclusion: During high–voltage electric pulses, hydrogen peroxide is generated in cell–free media. Pulses of micro–millisecond duration increased fluorescence of hydrogen peroxide indicator AmplexRed depending on the duration, voltage, and/or number pulses. The conductivity and composition of the medium were also important.

Keywords: reactive oxygen species, AmplexRed, electrochemical reactions, stainless steel.

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Poster presentation

Physical and Mechanical Properties of Bacterial Cellulose

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Abstract

Cellulose is the main plant structural polysaccharide and is the most common organic compound on earth. While the most cellulose is being produced by plant cellulose synthase, bacterial cellulose (BC) is produced from certain types of bacteria.

Chemical structure of BC is the same as that of plant cellulose [1], but it has different physical and mechanical properties. BC have a three dimensional network structure of ultrafine fiber made from pure cellulose. The fibers of BC are 100 times thinner than that of plant cellulose [2], BC has no hemicellulose or lignin to be removed, and exhibits higher degree of polymerization and crystallinity. The biomaterial has high tensile strength and absorbent per unit volume [3 -5].

The biofilm of BC has potential as a natural easy produced biomaterial suitable for eco-friendly design projects, because it is renewable, non-toxic and biocompatible polymer. BC represents a promising alternative to plant-derived cellulose for specific applications in bio-medicine, cosmetics, high-end acoustic diaphragms, paper making, food industry, textiles and other applications.

In this study bacterial cellulose (BC) was produced using Kombucha strains primary referred to the gram-negative bacteria species *Acetobacter xylinum*. Four different carbon sources, namely D-sucrose (commercial named as white sugar), D-sucrose (commercial named as brown sugar), D-fructose and D-glucose were chosen as liquid medium for BC production. BC biofilms were characterized in terms of dry weight production, biofilm yield, moisture content, water sorption and mechanical properties such as tensile strength and strain. The influence of carbon source on physical and mechanical properties of BC has been investigated in this research.

Keywords: bacterial cellulose, biofilm, carbon source, yield, mechanical properties.

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Poster presentation

The Function of *Drosophila* Carboxypeptidase D (*Silver*) in Peptide Processing

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Abstract

Neuropeptides form the largest group of neuromodulators in the brain. They are processed from larger precursor proteins – the prepropeptides – by a concerted action of a set of dedicated enzymes. We could earlier on show that the first step of prepropeptide processing in catalyzed by prohormone convertase 2 (dPC2, *amon*). Typically, AMON C-terminally cleaves the prepropeptide at dibasic cleavage sites. Subsequently, the resulting C-terminal extension of the cleaved-out peptide needs to be removed by a carboxypeptidase. In mammals, the main carboxypeptidase involved in peptide processing in carboxypeptidase E (CPE), yet a gene for CPE is missing in the *Drosophila* genome. Carboxypeptidase D (CPD) encoded by *silver* appears thus as the most likely CP candidate involved in peptide processing in Drosophila. With the help of a newly generated rescue construct and peptidomics, we here provide first evidence that a loss of CPD function indeed leads to an accumulation of C-terminally extended peptides. Moreover, heatshock-induced expression of *silver* rescue the lethality in loss-of-function mutants.

Keywords: Carboxypeptidase D (CPD), *Drosophila* Carboxypeptidase D (CPD), Neuropeptide, MALDI-TOF MS

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Poster presentation

Overview of Amplified Fragment Length Polymorphism Markers Used for Investigation of Genetic Diversity of Invasive Plants

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Abstract

The objective of our study was to analyse what amplified fragment length polymorphism (AFLP) marker combinations are usually used for assessment of genetic diversity of invasive plants. Overview of thirty recently published papers concerning genetic structure of populations of alien species was performed with the purpose to find out what AFLP markers are the most frequently used to reveal molecular diversity of invasive plants such as *Senecio inaequidens*, *Erigeron annuus*, *Spartina alterniflora* and others. According to literature, for invasive plant analyses, seventy eight AFLP maker combinations were found. For one species populations the number of AFLP marker combinations tested ranged from 2 to 9. The most often examined AFLP marker pairs were as follows: EcoRI-AAC/MseI-CAC, EcoRI-ACG/MseI-CAA, EcoRI-ACG/MseI-CAC, EcoRI-ACC/MseI-CAT, EcoRI-AGC/MseI-CAT, EcoRI-ACT/MseI-CTA, each of these combinations had been applied at least in 4 independent surveys of invasive plant species. For evaluation of genetic diversity of *Impatiens parviflora* populations, we have screened thirty two AFLP marker combinations and chose eight of them (EcoRI-ACG/MseI-CAC, EcoRI-ACC/MseI-CAC, EcoRI-AGC/MseI-CAC, EcoRI-AGG/MseI-CAC, EcoRI-AAG/MseI-CAG, EcoRI-ACG/MseI-CAT, EcoRI-AAC/MseI-CTC, EcoRI-AAC/MseI-CTG). Our examinations revealed that these eight combinations are the most polymorphic and distinct ones for *Impatiens parviflora* studies of populations. Study was funded by Lithuania Science Council, Project No. LEK-07/2012, SIT-02/2015.

Key words: Small Balsam, *Balsaminaceae*, alien plants, molecular markers, population genetics

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Poster presentation

Evaluation of *Juniperus communis* Populations in Relation to Ellenberg Indicatory Values of Associated Species

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Abstract

Juniperus communis L. is one of the most cosmopolitan conifer species found in both the eastern and western hemispheres. Our present study was aimed at evaluation of *Juniperus communis* populations according to indicatory values of associated species diversity. Fourteen sites of *J. communis* L. located in such habitats as coastal brown dunes covered with natural Scots pine forests; *J. communis* shrubs; transition mires and quaking bogs; subcontinental moss Scots pine forests and xero-thermophile fringes were selected. Light, temperature, continentality, soil moisture / reaction / nitrogen of the sites were calculated by weighted average method [1] using species indicatory values [2]. Average indicatory values of herbaceous species in proportion to species percentage cover indicated that the most contrasting (having the highest indicatory values) habitats of *J. communis* according to the light gradient was xero-thermophile fringes also transition mires and quaking bogs; according to the temperature gradient – xero-thermophile fringes; according to the continentality – xero-thermophile fringes and coastal brown dunes covered with natural Scots pine forests; according to the moisture – transition mires and quaking bogs; according to the soil reaction and nitrogen – xero-thermophile fringes. In conclusion according to the Ellenberg indicatory values the most distinct habitat type of *J. communis* was xero-thermophile fringes.

Keywords: species diversity, *Cupressaceae*, Indicatory values, EIV, populations, juniper, conifers

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Poster presentation

Selection of Dominant Markers for Investigation of Genetic Diversity of *Cucurbitaceae* Family Species

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Abstract

In addition to cultivated for food and forage representatives of *Cucurbitaceae* family, some species belonging to *Echinocystis, Bryonia*, *Ecballium and Sicyos* are either cultivated for ornamental purposes or growing wild in Lithuania. The objective of present study was to select dominant Inter Simple Sequence Repeat (ISSR) markers for evaluation of genetic diversity of ornamental species *Echinocystis lobata* and *Sicyos angulata*. The total DNA was extracted by DNA Purification Kit (#KO512, Thermo Scientific, Lithuania). Three randomly chosen ISSR markers (UBC 810, UBC 881 and UBC 890) were selected and conditions suitable for DNA amplification were screened. Amplification reactions were carried out in volumes of 20 µl containing of 10 µl PCR Master mix (0.05 U/µl Taq DNA Polymerase; 4 mM $MgCl_2$; 0.4 mM each dNTP), 0.4 µl 20 mg/ml BSA (0.6 µl of BSA was also tested), 0.5 µl 10 µM primer and 80 ng of DNA (40 ng of DNA was also tested). Two different polymerase chain reaction (PCR) programs were tested. The first one consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 45 s and a final extension at 72 °C for 10 min. According to this program, none of the fragments of DNA were amplified. The second type PCR protocol included an initial denaturation at 94 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 45 s, annealing at 54–56 °C for 45 s, extension at 72 °C for 2 min and a final extension at 72 °C for 5 min, the following program allowed to amplify DNA fragments with all selected markers, although for separate primers annealing temperatures were different. To obtain polymorphic and reproducible fragments of DNA the most favorable annealing temperature for the marker UBC 810 was 54 °C and for the markers UBC 881, UBC 890 temperature was 56 °C. Obtained results revealed that chosen ISSR markers are valuable for analyses of genetic diversity of wild and one seed bur cucumbers. Study was funded by Lithuania Science Council, Project No. SIT-02/2015.

Keywords: ISSR, inter-simple sequence repeat markers, genetic diversity, molecular markers.

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Poster presentation

Ixodes ricinus Ticks and Gamasine Mites (Parasitiformes: Mesostigmata) Infestation of Rodents in Curonian Spit, Lithuania

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Abstract

Ectoparasites generally involved in spreading of various pathogens in the nature. Ticks are known to be participate in the transmission of babesiosis, some encephalitis viruses or arboviruses and also of Lyme disease. For human health perspective, the rodent-tick associations have a huge importance in most ecosystems. The aim of this study was to investigate ectoparasites (ticks and mites) parasitizing small rodents and estimate ectoparasite infestation patterns in different rodent species in particular habitats in Lithuania. Small mammals were captured in different locations in the Curonian Spit during 2013–2014. A total of 251 small rodents representing 8 species were trapped. Captured rodents were identified as *Apodemus flavicollis*, *A.agrarius*, *Myodes glareolus, Micromys minutus*, *Microtus oeconomus*, *M.arvalis*, *M.agrestis*, and *Ratus ratus.* Collected rodents harbored *Ixodes ricinus* ticks and parasitic mites from suborder Mesostigmata (Gamasida), family Laelapidae (genus *Laelaps, Hyperlaelaps, Eulaelaps, Haemogamassus, Myonyssus*). Mites from Laelapidae family dominated between other mesostigmatid members found on rodents. A total 1056 ticks and mites were removed from small rodents trapped with snap traps and live – traps. Although two stages of *I. ricinus* were found on trapped rodents: larvae and nymphs, considerably higher larval infestation were recorded. Generally, rodents were more infested with Laelapidae mites, than with *I. ricinus* ticks. The values of abundance and mean intensity of infestation with *I. ricinus* and mites varied between species of hosts.

Keywords: *Ixodes ricinus*, rodent, Mesostigmata, *Laelaps*, *Hyperlaelaps*, *Eulaelaps*, *Haemogamassus*, *Myonyssus,* Lithuania.

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Poster presentation

Strength Analysis of Soft Denture Relining Materials

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Abstract

The aim of our work was to determine whether the thickness of the soft prosthesis reliner has an impact to its adhesive strength. It is very important to know this property in order to use these materials for each clinical situation individually. The objective of our work was to assess and compare adhering strength of different soft relining materials to the base based on strength measurements.

Methods and materials. Investigation was done for 3 materials (VOCO UFI Gel SC, UFI Gel P and Zhermac Clinical Elite Soft Relining), which were tested under the same conditions. There were 3 groups for each material tested. Each group had 5 specimens: 1 mm, 2 mm or 3 mm thickness of the soft relining material applied in between the two 314mm^2 circles, made out of methyl-methacrylate (BMS014 Ordinary Cure). Each materials adhesive system has been used. The analysis was done by using a tension evaluating machine (Tinius Olsen H25KT) in Kaunas Technology University, which dragged and separated both circles into different directions, which made the soft relining material to tear apart. This gave us the results of the strength of the material. In statistical analysis the selected significance level was P=0.05. The calculations were carried out using IBM SPSS Statistics 23.0 application program.

Results. Zhermack Clinical "Elite Soft Relining", VOCO "UFI Gel SC" and VOCO "UFI Gel P" were assessed and compared when researching the strength of their adherence to the base, based on the strength measurements. The results obtained showed that the maximal strength was sustained by VOCO UFI Gel SC when it was used in the thickness of 3 mm; the average sustained strength: 551.0 N (SD=121.8; n=5, P<0,05). The other two materials were very similar. The average material by the strength sustained was Zhermack Clinical Elite Soft Relining; the average sustained strength: 498.0 N (SD=66.9; n=5, P<0,05). This result was obtained for the material thickness of 1 mm. And the lowest result of the research was for the hand-prepared VOCO UFI Gel P; the average sustained strength: 480.0 N (SD=133.9; n=5, P<0,05). This result was also obtained for the material thickness of l mm. The results obtained showed that the most rigid material is VOCO UFI Gel SC when it was used in the thickness of 3 mm.

Conclusions. If we need more strength and durability from the relined prosthesis, we should use the material with higheest strength - UFI Gel SC.

Keywords: Denture rebasing, Adhesion, Soft Relining.

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Poster presentation

Application of Codominant DNA Markers for Investigation of Molecular Diversity of Lithuanian Reed Canary Grass Populations

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Abstract

Reed canary grass (*Phalaris arundinacea* L.) is a perennial herbaceous plant widespread all over the world, belonging to *Poaceae* / *Gramineae* family, *Phalaris* genus, which includes 15 species. This species is common for the temperate climate zone and is naturally growing in Lithuania. Attempts to use reed canary grass as a forage or decoration increases need for molecular analysis [1]. The objective of this study was to select microsatellite DNA markers appropriate for evaluation of molecular diversity of Lithuania populations of *Phalaris arundinacea.* DNA was extracted using CTAB method. The quality of DNA was assessed spectrophotometrically and by gel electrophoresis. The quantity of DNA of each sample was standardised to approximately 20 ng/µl. Four SSR primer pairs (PHI071, UMC2185, UMC2272, and UMC2779) developed for *Zea mays* were tested for amplification of *Phalaris arundinacea* DNA. Polymerase chain reaction (PCR) was carried out in the volume of 8 µl, containing 20 ng of DNA of reed canary grass, 3.5 µl PCR Master mix (0.05 U/µl Taq DNA Polymerase; 4 mM $MgCl_2$; 0.4 mM each dNTP), 0.5 µl 10 µM of the forward primer, the same was true for the reverse primer, 0.31 µl 20 mg/ml BSA and 0.13 µl 5 M betaine. Amplification was performed following these conditions: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 54–56 °C for 45 s, extension at 72 °C for 2 min and a final extension at 72 °C for 20 min. The amplified DNA products were screened on an agarose (1.5 %) gel and visualized under UV light after staining by ethidium bromide. Three annealing temperatures were tested to get a clear band on an agarose gel. Annealing temperature at 54 °C was suitable for application of the primers PHI071, UMC2185 and UMC2779. For the primer UMC2272 annealing temperatures at 54 °C, 55 °C and 56 °C did not cause reaction of polimerisation. Concerning this primer further search of PCA conditions remains to be done. At present moment three out of four tested microsatellite primers designed for *Zea mays* appeared applicable for studies of molecular diversity of *P. arundinacea.* Study was funded by Lithuania Science Council, Project No. SIT-02/2015.

Key words: *Poaceae, Phalaris arundinacea*, *Zea mays*, SSR, microsatellite DNA, genetic diversity, molecular markers.

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Poster presentation

Verticillium Wilt (*Verticillium* spp.) on Oilseed Rape (*Brassica napus*) in Lithuania

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Abstract

The objective of this work was to overview the long-term research on Verticillium wilt in oilseed rape conducted at the Department of Plant Pathology and Protection, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Since 1991, field experiments and observations have been carried out to monitor and investigate various crop-damaging fungal diseases. For years, Verticillium wilt has been considered as an insignificant and economically unimportant oilseed rape disease. Generally, the disease incidence (DI) did not exceed 1 % during the 1991 – 2007 period, only in 1998 the DI was recorded higher – 5 %. The first outbreak of Verticillium wilt was noted in 2008 when the DI was as high as 39.1 %. The area under oilseed rape cultivation in Lithuania is steadily increasing. In addition, a high concentration in the crop rotation as well as frequent return to the same field has resulted in Verticillium wilt becoming a major threat to oilseed rape production in Lithuania. One of the risk-factors for the occurrence of Verticillium wilt is crop rotation. Research on different cultivation technologies suggested that a long crop rotation cycle (crops return to the same field every 4 years) was superior to continuous cropping (growing of a crop in the same field year after year). The lowest incidence and severity were observed in the fields grown with oilseed rape every 4 years 13 – 27 % and 0.6 – 7.8 %, respectively. Compared to the continuous cropping for two years in succession, the disease incidence was 78 % and severity 33 %. Another important factor in the control of Verticillium wilt is the choice of a cultivar. The data on the different susceptibility to the disease, obtained over the two growing seasons (2013/2014 and 2014/2015) were rather controversial. Among winter oilseer rape cultivars, 'Nelson' showed the highest susceptibility to Verticillium wilt. Among spring oilseed rape cultivars, 'Kaliber' was less susceptible in the first growing season, while 'Tamarin' and 'Mojang' proved to be more susceptible in the second season. These results indicate that completely resistant oilseed rape cultivars are currently not available on the Lithuanian market.

Keywords: *V. longisporum*, fungal diseases, crop rotation, cultivars

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Poster presentation

Investigation of *Babesia* spp. Infecting *Dermacentor reticulatus* and *Ixodes ricinus* Ticks in Lithuania

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Abstract

Babesia species, the causative agents of babesiosis, are protozoan blood parasites that are transmitted by ticks to their vertebrate hosts. *Ixodes ricinus* ticks are considered the main vectors of *Babesia* species causing diseases in human, domestic and wild animals, while other tick of Ixodidae family *Dermacentor reticulatus* is recognized as the most important vector of canine babesiosis. In Lithuania up to date, no human cases due to *Babesia* spp. have been reported, however in the beginning of 21th century were registered many cases of canine babesiosis in dogs. The aim of this study was investigate the presence of *Babesia* spp. in *D. reticulatus* and *I. ricinus* ticks in various regions in Lithuania, and to assess the risk of *Babesia* infection in the human population and to better understand epidemiology of canine babesiosis. A total of 2259 *D. reticulatus* and 370 *I. ricinus* were collected from 40 locations in Lithuania.This study represents the first investigation and characterization of *Babesia* spp. infecting *D. reticulatus* and *I. ricinus* in Lithuania. Different regions of the 18S rRNA gene of the genus *Babesia* were amplified by using nested PCR. The prevalence of pathogens in *D. reticulatus* (1,2%) and *I. ricinus* (9.5%) ranged in different locations from 0% to 6.7% and 0% to 18.5%, respectively. Sequence analyses of DNA from these positive samples indicate the presence of *B. canis* and *B. venatorum* in *D. reticulatus* ticks, *B. microti* and *B. venatorum* in *I. ricinus* ticks. This study represents the first investigation of zoonotic *Babesia* parasites in Lithuania.

Keywords: *Babesia*, *Dermacentor reticulatus*, *Ixodes ricinus*, Lithuania

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Poster presentation

Prevalance of Tick-Borne Rickettsiae Pathogen in *I. lividus* Ticks from Sand Martin (*Riparia riparia*) Nest in Lithuania

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Abstract

The aim of the present study was to investigate the presence of *Rickettsia sp*., as well as to determine their prevalance in *I. lividus* ticks from Sand martin (*R. riparia*) nests. In 2013 and 2015 years fourthy Sand martin nest from 2 different locations were collected and examined for ticks. Microscopic and morphometric analyses were used for the indentification of ticks to the species level. All 2676 (3 females, 7 nymphs, 1 male and 2665 larvae) captured ticks was identified as *I. lividus*. For *Rickettsia sp*. detection *gltA* and *17kDa* genes were used and DNA was sequenced for species identification. Phylogenetic analysis showed that samples fitted neatly into a group containing strains *Rickettsia japonica*, *Rickettsia raoultii* and *Rickettsia heilongjiangensis* wich couse dangerous ricketsioses in humans. The prevalance of pathogens in *I. lividus* ranged in different location and years from 75 % to 100 % respectively. Following on from this small study, futher research is required to examined *Rickettsia* transmision potential of *I. lividus* and other tick species associated with migratory hosts.

Keywords: *Rickettsia sp*., *I. lividus*, *gltA* gene, *17kDa* gene, prevalence

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Poster presentation

Evaluation of Synergy of Antifungal Activity of the Secondary Metabolites Extracted from *Eryngium* L. Species

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Abstract

The antifungal activity of methanolic extracts of three *Eryngium* L. genera (*E. planum*, *E. campestre*, *E. maritimum*) originated from the sector of medicinal plants, botanical garden of Vytautas Magnus University, Lithuania - were tested by the method of series dilutions, against different fungi species. Investigated microorganisms were *Aspergillus flavus, Penicillium chrysogenum, Cladosporium cladosporioides, Alternaria alternata, Rhizopus microsporus, Trichoderma viride.* The antifungal activities of extracts were described by determination of the Minimal Inhibitory Concentration (MIC). Preliminary results show that the MIC range between 5 %v/v and 20 %v/v of the extracts for the different *Eryngium* L. extracts and fungi species. The total amounts of phenolic compounds, total amounts of flavonoids and essential oils were tested in the methanolic extracts of the plants. The extracts chemical composition were analyzed by chromatography methods. Synergy of phenolic fraction and essential oils of the plants was determined against the tested fungi.

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Keywords*: Eryngium L.,* antifungal activity, plant extract

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Poster presentation

Preliminary Characterization and Antiviral Properties of Some Secondary Metabolites in Selected Medicinal Plants

Rūta Mickienė[1], Tomas Drevinskas[1], Audrius Maruška[1], Nicola Tiso[1], Algirdas Šalomskas[2], Raimundas Lelešius[2], Agneta Karpovaitė[2], Ona Ragažinskienė[3], Loreta Kubilienė[4]

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Abstract

Medicinal plants have been used in traditional health care since prehistoric times and are still the most important health improvement resource for the vast majority of the population all over the world. World Health Organization emphasized importance of scientific research into herbal medicine. The use of antiviral synthetic drugs is often unsatisfactory and limited. Mutant viruses resistant to the existing antiviral agents arise upon treatment or these agents may cause side or toxic effects besides their high costs.

In our research different medicinal plants, their parts and two types of propolis were selected for screening. Total phenolic content, total flavonoid content and radical scavenging activity was determined. The results indicated that optimal extrahent is 40%, vol. of ethanol – water mixture. Determined total phenolic content, total flavonoid content and radical scavenging activity indicated that extracts of *Origanum Vulgare* L., *Mentha piperita* L., *Geranium macrorrhizum* L., *Melissa officinalis* L. and *Desmodium canadence* L. contains highest amount of extractable phenolic compounds (7.31-7.16 rutin equivalents (mg/ ml) respectively), flavonoid content (2.14 - 1.51 rutin equivalents (mg/ml) respectively) and radical scavenging activity (11.98 - 12.22 rutin equivalents (mg/ml) respectively). Composition of the extracts is analyzed using HPLC. Evaluation of cytotoxicity effect (CE) was tested using Vero cells. Extract of *Agastache foeniculum* (Pursh) Kuntze showed the highest CE 6.32 ± 0.13 $\log_2 CE_{50}$.

The research showed that all extracts have various not high CE and they can be used for testing of antiviral properties. The study of antiviral activity of extracts is in progress.

Acknowledgements. The research was granted by Research Council of Lithuania, project No. MIP-065/2015.

Keywords: antiviral, medicinal plants, viruses

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Poster presentation

Matrix Metalloproteinase Gene Polymorphism in Multiple Sclerosis and Optic Neuritis

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Abstract

Introduction. Multiple sclerosis (MS) is the most common immune-mediated inflammatory demyelinating disease of the central nervous system. MS is characterized pathologically by multifocal areas of demyelination with loss of oligodendrocytes and astroglial scarring. It is more common among young and middle aged individuals. For 20 percent of people with MS, the first signs of disease are optic neuritis (ON) symptoms, as painful, usually monocular, visual loss with decreased visual acuity, defects of the visual field and colour vision, and it may occur in up to two-thirds of individuals. The most important factor is probably the loss of signal transmission in some axons due to conduction block or ganglion cell death

Endopeptidase matrix metalloproteinase-2 is increased in neuroinflammation and implicated in myelin basic protein break down and oligodendrocytes death, and could be important in demyelination process and autoimmune inflammatory disease like MS and ON.

Aim. To determine the influence of *MMP-2 Rs243865* genotype on the development of multiple sclerosis with optic neuritis.

Material and methods. We selected with 21 patients with ON and MS, 19 patients only with ON and 318 control subjects (reference group) to perform a case – control study. The genotyping test of *MMP-2 (-1306 C/T)* was carried out using the real-time polymerase chain reaction method. The genotype distribution was compared between the patients and the reference group, and analyzed.

Results. In our study *MMP-2 (-1306) C/T* gene polymorphism has not revealed any differences in the genotypes distribution between ON without MS and ON with MS. But research revealed differences between the ON without MS group and control group subjects: C/C genotype 84.21% vs. 59.75%, p=0.0499. Patients with optic neuritis were the carriers of the C/C genotype significantly more frequently than their control counterparts.

Conclusion: *MMP-2 (–1306) C/C* genotype may have an influence only on ON development. It is known, that the *C/C* genotype is increasing gene expression but further research with larger patients group is required.

Keywords: gene polymorphism, optic neuritis, matrix metalloproteinase-2.

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Poster presentation

Selection of Co-dominant Markers for Estimation of Genetic Diversity of *Juniperus communis*

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Abstract

Codominant markers took a priority for low cost and accuracy evaluation of genetic diversity. There are two different categories of codominant genetic markers, one originating from coding sequences (allozymes) and the other from sequences of the nuclear genome that, as is common for microsatellites. Big sensitivity to environment of allozymes caused generation of the new genome sequence-related evaluations of populations. The development of electrophoretic techniques provided more exact methods for estimation of genetic diversity. High levels of polymorphism and codominance found in microsatellites (simple sequence repeats, SSR) presently outstand these markers as more informative and the most valuable ones. Microsatellites are more suitable, compared to the other for population analyzes within last decades often used methods such as Randomply Amplified Polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP). Following co-dominant nuclear microsatellites were developed and characterized for the genera of *Juniperus*: Jc031, Jc037, Jc166, for *Juniperus excelsa*, Jc031, Jc032, Jc035, Jc166, for *Juniperus brevifolia*, even thirteen SSR - Jce01-Jc13, *Juniperus cedrus*. For

Juniperus communis microsatellites Jc 016 Jc 031 Jc 032 Jc 035 Jc 037 were created. They were all applicable for comparisons of populations of Lithuanian *J. communis*.

Keywords: genetic diversity, molecular markers, populations, *Cupressaceae*, juniper

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Poster presentation

Roughness of Different Surface Treatments on Lithium Disilicate Ceramics

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Relevance of the problem: Ceramic restorations require efficient surface treatment after hot pressing technique and final occlusal reduction. After these procedures surfaces become rough. Roughness leads to surface damage which causes increased bacteria adhesion and wear of antagonist teeth.

Aim of the work: To determine which lithium disilicate ceramic treatment develops smoother surface and to ascertain if it is possible to polish the surface sufficiently after surface reduction.

Material and methods: Twenty 10 mm x 4 mm disc-shaped wax specimens were modeled using CAD/CAM system (ZIRKONZAHN M5, South Tyrol, Italy) and then milled. Wax was changed to lithium disilicate ceramic (IPS e.max Press, Ivoclar Vivadent AG, Schaan, Liechtenstein). Specimens were divided into two groups by surface treatment method. P group specimens (n=10) were polished and G group specimens (n=10) were glazed. Then surface reduction was made using 40µm diamond bur and the intraoral polishing imitation was performed using polishing system (NTI CeraGlaze P336, P3036, P30036, NTI-Kahla GmbH, Germany). After every procedure mean surface roughness (Ra) was estimated with profilometer (Ambios Technology Inc., XP-Plus 200 Stylus, USA) and analyzed with scanning electron (Hitachi S-3400N VP-SEM, Singapore) and optical (Nikon Eclipse LV150, USA) microscopes. Statistical analysis was performed using SPSS 20.0. Roughness compared using Student *t,* Mann-Whitney and Wilcoxon tests.

Results: P group Ra after polishing was 1,532±0,729µµm, G group after glazing - 2,519±1,513µm. G group Ra after surface reduction was 2,585±0,529µm, P group - 2,685±0,538µm. G group Ra after intraoral polishing imitation was 1,983±1,220µm, P group - 1,611±0,685µm. There is statistically insignificant ($p>0,05$) difference between the average surface roughness of P and G groups after all the procedures. Regardless of the initial sample preparation method, statistically greater surface roughness after reduction (2,635 ± 0,521µm) was found in comparison with the roughness after the initial preparation (2,025 ± 1,261µm) and intraoral polishing imitation (1,797 ± 0,981µm).

Conclusions:

Using both lithium disilicate ceramic final surface preparation methods similar ceramic surface roughness is achieved. Regardless of the ceramic samples distribution of the initial surface preparation method, the surface is rougher after the reduction. Lithium disilicate ceramic surface can be polished to initial roughness values using intraoral polishing system.

Keywords: Lithium disilicate, roughness, polishing, glazing, reduction.

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Poster presentation

Pathogenic Fungus *Dothistroma Septosporum* Distribution in West and Central Parts of Lithuania

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Abstract

Dothistroma septosporum is a pathogen of *Pinus spp*. and some species in the genera *Larix*, *Pseudotsuga* and *Picea*. This pathogen causing red band needle blights of pine also known as Dothistroma needle blight (DNB), is a serious needle disease across the globe. Disease causes needle necrosis and premature needle loss, resulting substantial growth loss. At present *Dothistroma septosporum* has a worldwide distribution. In Lithuania the first report appeared in 2005. In late autumn of 2015 infected needles of *Pinus sylvestris*, *Pinus mugo*, *Pinus nigra* were collected in west and central parts of Lithuania. Needle samples were collected from each *Pinus spp*: 8 trees, from each tree 8 needle pairs collected from a single tree. Our main idea of this work was to compare infection frequecy in *Pinus spp*. in the different parts of Lithuania. Research was made in Vytautas Magnus University plants and animals laboratory and Nature Research center, Botanical institution. Molecular analysis was performed with classical PCR and real-time PCR. Samples were analysed and results show that in Lithuania western part most infected was *Pinus sylvestris,* than *Pinus mugo* and least *Pinus nigra.* In Lithuania central part most infected was *Pinus nigra,* than *Pinus mugo* and least *Pinus sylvestris.*

Keywords: Dothistroma septosporum, Red band needle blight, Dothistroma needle blight, pathogn

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Poster presentation

Some Morphophysiological Parameters of *Impatiens glandulifera* in Relation to Climate Fluctuations

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Abstract

In the temperate climate zone plants alternate due to seasonal variation of the environmental factors. The development of specific plant organs and essential functional and biochemical changes occur during certain periods of plant vegetation. However, climate change interferes with ordinary plant life. Nowadays it becomes more and more important to evaluate patterns of the plant dynamics and thus detect aberrations. According to the World Meteorological Organization, the years 2011-2015 have been the warmest five-year period on record. Global climate warming enhances the invasion of non-native species. As a result alien species invade natural habitats, threatening the biodiversity of numerous ecosystems worldwide. So the seasonal variation of invasive species has become a major research object of modern phenology. In Lithuania Ministry of Environment confirmed an expanded list of non-native species in 2015 years. Himalayan balsam (*Impatiens glandulifera* Royle) continues to be enlisted among the most invasive plant species. It has spread widely in Europe, Asian countries, North America, New Zealand. Himalayan balsam is the large annual plant that tends to form dense monotypic growth areas gradually resulting in degradation of unique environment and valuable habitats.

In this work morphology changes of Himalayan balsam (*Impatiens glandulifera* Royle) during vegetation period were investigated and it was aimed to determine how the growth of this invasive plant was effected by warming of the climate. This kind of research can help determine plant characteristics that predispose the success of invasion. For this purpose some locations of Lithuania with different populations of Himalayan balsam were chosen. It was observed morphological indicators change of plant and gravimetric analysis was carried out. Selected populations were compared with each other and the influence of Lithuania climate on phenology of Himalayan balsam was estimated. The last year's drought was disastrous for some Himalayan balsam populations.

Keywords: Himalayan balsam, invasive species, climate change, morphophysiological parameters.

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Poster presentation

First Records of Freshwater Gastrotrichs (*Gastrotricha*) in Lithuania

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Abstract

Gastrotrichs (phylum *Gastrotricha*) are worm-like microinvertebrates that represent an important component of the miobenthic fauna of marine, brakish-water and freshwater habitats. This phylum includes about 780 free living species of which 450 species can be found in freshwater habitat. As there are no researches about gastrotrichs species composition and ecology in Lithuania the objective of this study was to explore the knowledge of freshwater gastrotrichs in Lithuania.

During the study gastrotrichs specimens were collected by sampling freshwater bodies in different regions of Lithuania. There were selected 8 still water bodies in Kaunas district for seasonal sampling (4 times in 2016 year vegetation period - April, June, August, October). Other 23 selected still water bodies in different regions of Lithuania were examined one time in vegetation period. In order to increase species diversity water bodies were selected according to the different ecological characteristics and spatial distribution. After every collection water samples were stored in laboratory (4 °C, no specialised aeration, no lightning) and observed with a light microscope in 14 days after collection. The specimens that were found in water samples have been captured in photos which were used for following species identification.

This research resulted in the collection of photographs of 98 gastrotrich specimens. Preliminary were identified 6 species belonging to *Chaetonotidae* family. As observation of gastrotrichs with light microscope may not provide all morphological data needed for species identification, some specimens were identificated only to genus. This is a completely new data on Lithuanian gastrotrichs, no researches have been carried out before in Lithuania.

Key words: gastrotricha, freshwater, species composition, Lithuania.

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Poster presentation

Comparison of Three European *Impatiens* Species by Nuclear DNA Markers

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Abstract

Invasion of *Impatiens parviflora* and *I. glandulifera* is very important problem nowadays, solving it, valuable messages might be obtained comparing invasive range *Impatiens* of Europe with native species of the same genera. Comparison of genetic information of three *Impatiens* species is still at initial stage. The aim of this study is to determine genetic variability of *Impatiens* species growing in Lithuania and Czech Republic by RAPD (Randomly Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeat) markers. Lithuania and Czech Republic were selected as different in climate, edaphic conditions also pathways of invasion, with significant amount of *I. parviflora* and *I. glandulifera* populations present. In total, 24 populations of *I.* noli-tangere. *I. parviflora* and *I. glandulifera* (8 populations of each species) were examined employing 8 RAPD and 5 ISSR markers. Study was funded by Lithuania Science Council, Project No. LEK-07/2012; SIT-02/2015.

According to the mean values of polymorphism at RAPD loci, the closest were *I. noli-tangere* and *I. parviflora* (respectively mean *P*% = 13.9% and *P*% = 17.3%), while at ISSR loci, the most similar were *I. parviflora* and *I. glandulifera* (respectively mean *P*% = 26.5% and *P*% = 22.0%). The highest genetic differentiation at RAPD loci (G_{ST} = 0.81) was characteristic for *I. parviflora* and the highest genetic differentiation at ISSR loci (G_{ST} = 0.73) was documented for *I. glandulifera*. According to genetic differentiation, the most similar species were *I. noli-tangere* and *I. parviflora* at RAPD loci, while at ISSR loci – the most comparable were both invasive species of balsams. According to Nei's genetic distances between populations, significant correlations were documented for *I. noli-tangere* and *I. parviflora* ($r = 0.79$; $p < 0.05$), also for *I. parviflora* and *I. glandulifera* ($r = 0.76$; $p < 0.05$) based on RAPD loci and for *I. parviflora* and *I. glandulifera* ($r = 0.89$; $p < 0.05$) based on ISSR loci. UPGMA dendrograms revealed that the closest species were *I. noli-tangere* and *I. parviflora* by both RAPD and ISSR data. *I. noli-tangere* and *I. glandulifera* were the most different species by almost all RAPD and ISSR markers related parametres. Present study did not separated species unambiguously; further research selecting other molecular or biochemical markers is required.

Keywords: *Balsaminaceae*, balsams, invasive species, alien species, dominant markers

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Poster presentation

Genetic Diversity of Invasive in Lithuania Populations of *Bidens frondosa* along Nemunas and Neris Riversides

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Abstract

Riparian and river ecosystems are permanently deteriorated by anthropogenic factors, related to industry, transport, intensive agriculture or tourism. As a consequence of chemical and physical intervention, favorite conditions are created for invasive species introduction, long-term settlement and spread. On the banks of the ponds and the rivers two species of the *Bidens* genera – *B. tripartita* and *B. cernua* are widely spread in Lithuania. Since 1982, in addition to these native ones, *B. frondosa* as alien species, started be recorded for the same habitats. Presently this species is abundant along banks of the Nemunas and its tributaries, also around Curonian Lagoon. Sharing the same habitat, native and invasive *Bidens* representatives are of similar flowering time. In the areas where individuals of two different species are overlapping there is big probability for hybridization events. It is realistic, that part of the hybrids might contain broader adaptations and in longer term could create elimination threat for individuals of local *B. tripartita*. Our study aimed at evaluation of anthropogenic pressure on the spread, structure and genetic diversity of *B. frondosa* populations, also interactions between native and invasive species. Populations growing along Nemunas and its tributary Neris were sampled. Genetic diversity was studied using four markers at Inter-Simple Sequence Repeat loci. Our assessment of molecular variation demonstrated significant genetic differentiation between populations of *B. frondosa* (Φ_{PT} = 0.335; $p < 0.001$). In addition, interaction of different *Bidens* species with special stress on the genetic impact of *B. frondosa* on adjacent native species is discussed.

Keywords: *B. frondosa*, genetic diversity, ISSR markers.

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Poster presentation

Rs 2108622 Gene Polymorphism Association with Dry Age-related Macular Degeneration by Gender

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Abstract

Introduction: Age-related macular degeneration (AMD) is a progressive neurodegenerative disease and the leading cause of irreversible blindness among individuals aged 65 and older, particularly in western countries [1]. AMD, which affects the macula , and is a leading cause of significant and irreversible loss of central vision [2]. The aetiology and pathophysiology of age-related macular degeneration is not absolutely clear. Macular degenerative lesions are manifest by drusen formation, retinal pigment epithelium changes, retinal pigment epithelium and choroid capillary layer, Bruch's membrane lesion, geographic atrophy of the central fovea, exudative AMD with choroidal neovascularization, retinal pigmentary epithelium detachment or submacular disciform scarring changes. The pathological hallmark of the disease is amorphous deposits of protein and lipid, termed drusen [3]. Knowing that the main pathological changes of age-related macular degeneration are drusen formation, which include about 30% lipids [2], there have been attempts to find relation with age-related macular degeneration and gene controling lipid metabolism.

Aim: To determine the frequency of the genotype of Rs 2108622 in patients with dry age-related macular degeneration by gender.

Material and methods: The study enrolled n = 190 patients with dry age related macular degeneration and a random sample of the population n = 210 (reference group).

The genotyping of Rs2108622 was carried out using the real-time polymerase chain reaction method. Exudative age-related macular degeneration was confirmed after optical coherent tomography examination.

The DNA extraction was carried out from samples of whole blood using a *Thermo Scientific GeneJET Genomic DNA Purification Kit* and *Thermo Scientific GenJET Genomic DNA Purtification Kit*, according to the manufacturer's recommendations. Genotyping was performed using *TaqMan® SNP Genotyping Assay* for *Rs2108622* and real-time polymerase chain reaction method.

Results: A total 190 patients with dry AMD were enrolled into analysis according to the subject inclusion and exclusion criteria. The control group comprised of 210 persons. There were 70% (n=161) women in control group and 67.4% (n=122) women in exudative AMD group.

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Poster presentation

The Estimation of Cobalt Effects on Barley Homeotic Mutants Using Callus Cultures as a Model System

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Abstract

Due to the increasing rates of industry, more heavy metals are accumulated in the soil. It is showed, that in small amounts cobalt (Co) plays a role in the development of ectopic reproduction organs [1] through the ethylene biosynthesis pathway and *barley knox3* (*bkn3*) gene, mutations in which leads to the formation of barley *Hooded* phenotype [2]. Therefore, considering growing population, it is important to analyse the influence of cobalt on important edible plants, including barley, which is among the most consumed crops in the world.

It is known that increased concentrations of heavy metals have negative effect on plants However, our earlier experiments using intact barley plants demonstrated that even extremely high Co concentrations induce only very mild effects on vegetative and generative organs of barley, suggesting barley to be highly resistant to Co exposure. In order to escape the influence of plant protective tissues for Co absorption and penetration into the meristematic zones, callus cultures that were induced from mature barley embryos were used as a model system that allows to observe Co effects directly on meristematic cells. In the present study, two *Hooded*-type mutants (*Lemma hooded* and *Hooded (H)*), mutant *tweaky spike 2* (*tw*$_2$) and their hybrids, that have ectopic transformations of different flower organs, were used for callus induction. Mature embryos were exposed to different concentrations of $CoCl_2$ in MS medium and callus growth intensity was determined and Co-induced oxidative stress was evaluated by measuring the lipid peroxidation level[3]. The strongest negative effect of cobalt on callus growth was determined on *Lemma hooded* (*Lh*) mutant – its growth rate decreased almost three times in comparison to control group, while Co effect on *H* hybrid was signifficantly less expressed. The utmost lipid peroxidation level after Co-exposure was observed in wild type plants (cv. 'Auksiniai II') and *H* hybrid, while the *Lh* showed the lowest level of lipid peroxidation that allow to consider them as the most resistant to Co-induced oxidative stress.

Keywords: Barley mutant, Callus culture, Cobalt, *Hooded,* Lipid peroxidation, *tweaky spike*

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Poster presentation

Frequency of Rs762551 Genotype in Males And Females In Patients With Early Age-Related Macular Degeneration

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Abstract

Introduction: Age-related macular degeneration (AMD) is the leading cause of blindness in high-income countries and the third highest cause of blindness in the world. AMD is a complex disease to which many factors contribute. Age and genetic factors are the most important risk factors for AMD development.

Purpose: To determine if the frequency of the genotype *CYP1A2* (g.-163C>A) Rs762551 have an influence on the development of early age-related macular degeneration in males and females.

Methodology: The study enrolled 150 patients with early age-related macular degeneration (42 men and 108 women), and a random sample of the population 296 (76 men and 220 women) (reference group). The genotyping test of *CYP1A2* were carried out using the rPCR. Statistical analysis was performed using the SPSS / W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

Results: The comparison of the Rs762551 genotype in males and females between patients with AMD and the control group showed statistically significant differences. The C/A genotype was more frequent in healthy the control group males and females compare to AMD patients males and females (52.6% vs. 28.6%, p=0.0128 and 44.1% vs. 31.5%, p=0.0312)

Conclusion: The study showed a significantly greater prevalence of C/A genotype in healthy males and females.

Keywords: Age-related macular degeneration, CYP1A2, gene polymorphism.

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Poster presentation

Species Composition of Freshwater Bryozoan (Bryozoa) in Some Regions of Lithuania

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Abstract

Freshwater bryozoans are among the most fascinating invertebrate animals, although many aspects of their ecology, physiology and development are still poorly understood. Their common name "moss animals" refers to the mossy appearance of encrusting species.

Bryozoans perform an important role in water ecosystems. They are widespread, can be found in inland waters as well as in seas and oceans. These animals likewise corals are sensitive to environmental changes and are affected of global warming. Thus bryozoans can be used as bioindicators. Also freshwater bryozoans are one of the most important water biofilters.

According to Fauna European (2013) there are 21 freshwater bryozoan's species in Europe, with a number of these species being recorded in neighboring countries (Latvia, Estonia, Poland, and Belarus). Little is known about bryozoans in Lithuania, other than one thesis conducted during 1931-1933 by B. Pajiedaitė . She examined water samples from rivers, lakes and ponds in Kaunas, Šiauliai, Utena and Vilnius districts and described 7 freshwater bryozoan's species (*Paludicella articulata, Cristatella mucedo, Plumatella fungosa, P. repens, P. emarginata, P. fruticosa, P. punctata*).

Research on bryozoan diversity in Lithuania was renewing in 2015. 19 water bodies were examined during bryozoan vegetation period. By examination of the collected statoblasts there were identified 9 bryozoan species. Two species: *Pl. casmiana, Pl. geimermassardi* were found for the first time in Lithuania.

Keywords: bryozoan, statoblasts, diversity, Lithuania

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Poster presentation

Deciduous Tree Species Chilling Requirements and Its Importance to Spring Phenology

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Abstract

Timing of bud burst and leaf unfolding is widely used indicator of climate change and it is also a very important measure of trees chilling requirements detection by setting certain experiments in the laboratory. It is well known that different tree species has different chilling requirements during the period of dormancy, but the transition from true-dormancy to post-dormancy is not well understood. Therefore I investigated two various tree species: one climax – *Sorbus americana*, the other pioneer - *Betula papyrifera,* trying to detect the date when chilling requirements for these species are fulfilled. This investigation was done in Schoodic Penninsula at Acadia National Park (Maine USA) in 2015/2016 cold period of the year. First cutting date was shortly after end of leaf fall (middle of November) and last cutting date was before leaf unfolding in spring (end of March). Twigs of the trees were collected weekly and kept in a cups filled with a tap water in the laboratory with a certain air temperature and humidity (T-18ºC, humidity - 60%). The photoperiod was regulated automatically by full spectrum lamps and plants were exposed to 14 h of daylight. The observations of bud burst in the laboratory were done once (until March) and twice (since March) per week.

The results showed that successional strategy and temperature are linked and that pioneer species (*Betula papyrifera)* has lower chilling and forcing requirements as compared with climax species (*Sorbus americana).* After collection in November the twigs of paper birch reacted to forcing temperatures in the laboratory in the beginning of January and bud burst was detected after 48 - 71 days being in the lab. Twigs of mountain ash collected in November and December did not show any signs and first bud burst appeared in the middle of March after 57 - 69 days spent in laboratory (collection date – beginning of January). The sooner chilling requirements are reached the faster treles starts to react to the warming temperatures in the end of winter and spring. These results indicates that species with lower chilling requirements might benefit from climate change (warming winters) and that increased temperature might become an issue for late successional species such as mountain ash when chilling requirements are not fulfilled and trees development delays.

Keywords: Chilling requirements, dormancy, climate change

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Poster presentation

Use of *Tradescantia* Clone 4430 to Evaluate the Genotoxicity of Soil Collected from Vilnius City Closed Landfills

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Abstract

Landfills are the most common way for disposing of municipal waste. Many genotoxic substances including heavy metals and polycyclic aromatic hydrocarbons can be found in landfill leachates and it may be harmful for terrestrial plants, animals and humans. Genotoxicity of soil from Vilnius city closed landfills of Mickūnai and Polockas was evaluated. *Tradescantia* plants were planted into 5 soil samples from Mickūnai landfill and 6 soil samples from Polockas landfill. DNA was extracted from plant leaves after half, 1 and 2 years of growing in tested soils. RAPD-PCR was performed in order to find polymorphisms in DNA after direct long time exposure to contaminated soil. *Tradescantia* clone #4430 is very suitable test organism for RAPD assay, because any changes in banding patterns should only represent DNA damage after exposure to genotoxins. Additionally, one soil sample from Mickūnai landfill (as most contaminated according to chemical soil analysis) was selected for *Tradescantia* stamen hair mutation (Trad-SHM) test. Out of 48 primers tested only 2 (OBP 07 and 270-5) revealed reproducible polymorphic DNA changes in DNA banding patterns that can be considered as mutations caused by genotoxic compounds. Band appearance and changes in band intensity were observed in DNA samples from plants after 1 year exposure to three soil samples from Polockas landfill. There were statistically significantly higher colorless cell mutation counts in soil extract treated inflorescences than solvent controls in Trad-SHM test. Genotoxic effects were not detected by pink cell mutation test. After treatment with soil extracts branched hair counts were higher than solvent control but statistically significant differences were observed only in DMSO extract treatment.

Keywords: Soil Genotoxicity, landfills, RAPD test, Trad-SHM.

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Poster presentation

Role of Carbon Source in Bacterial Films Growth

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Abstract

Bacterial cellulose produced by aerobic bacteria received great attention due to its unique physiochemical properties compared to plant cellulose. It has a wide variety of current and potential future applications in the food industry, the medical field, commercial and industrial products, and other technical areas [1]. However, bacterial cellulose production depends heavily on several factors such as the growth medium, environmental conditions, and the formation of byproducts. Bacteria are most efficient when supplied with an abundant carbon source and minimal nitrogen source. On the other hand glucose and sucrose are the most commonly used carbon sources for cellulose production, while fructose, maltose, xylose, starch, and glycerol also have been tried. *Acetobacter*, *Rhizobium*, *Agrobacterium*, and *Sarcin* produce bacterial cellulose in synthetic and non-synthetic medium through oxidative fermentation [2]. The systematic study of physical and chemical properties of mono and polysacharides solutions is presented when physical and chemical properties were investigated by densitometry, UV-VIS spectrometry and polarimetry and refractometer methods. The influence of different carbohydrate sources on the properties of bacterial cellulose was investigated. The dependences between the parameters of primary growth solution and the optical and mechanical properties of biofilm was discussed.

Keywords: bacterial cellulose, bacterial films, carbon source.

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Poster presentation

Overview of Uroflowmetry Techniques

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Abstract

Lower urinary tract symptoms (LUTS) affect about 60% of men over age of 40. One of the most common diagnostic procedure for patients with these symptoms is urine flow measurements (uroflowmetry), which are taken using special equipment called uroflowmeters. Not only are these measurements performed for adults, but for children with LUTS as well.

Prostate cancer is one the most common cancer form amongst men in the EU. Various urodynamic testing and diagnostic procedures are performed as the primary condition evaluation step, which also could be used to monitor oncological patients after prostate cancer treatment periodically (e.g. prostate cancer brachytherapy).

Uroflowmeters are measuring systems providing information about urine flow like the flow rate and the total voided volume. The measurement results are presented in so-called nomogram (diagram showing the changes of the flow rate over time) form. The obtained nomogram allows the physician to diagnose the patient quickly and to make decision about performing other necessary diagnostic procedures.

The systems used for urine flow measurements in clinical practice provide high accuracy but are not suitable for patients monitoring at home. The possibility to take measurements of the urine flow at home gives an option to obtain many natural flow traces, which would provide a more complete picture about patient's urinary functions. Constant improvement of the uroflowmeters and their use procedures are also important, especially for clinical applications.

The goal of our investigation is to review and classify various developed uroflowmetry techniques employed to measure characteristics of the urine flow in clinical environment and at home particularly and to compare them with regard to various aspects.

Keywords: uroflowmetry, urodynamics, flow rate measurements.

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Poster presentation

Carriage of ESBL-Producing *E. coli* in European Herring Gulls (*Larus argentatus*)

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Abstract

Wild birds are normally not exposed to antimicrobial agents but can acquire antimicrobial resistant bacteria through contact with domesticated animals and environment. Residues of antibiotics disposed inappropriately can affect soil microbiota particularly in urban dumps where gulls are predominant fauna in recent years.

The aim of this study was to determine the carriage of extended spectrum beta-lactamases (ESBL) producing *Escherichia coli* in the population of European herring gulls (*Larus argentatus*) and to characterize the isolates regarding antimicrobial resistance.

Material/methods: One hundred samples of European herring gull faeces were collected on Kaunas city dump as well as on the pier of Ventes Ragas using sterile cotton swabs with transport medium (Transwab® Amies, UK). Material was inoculated onto Brilliance ESBL Agar (Thermo Scientific, UK) for screening of *E. coli*. Pink and blue colonies were selected and identified using GN-ID A biochemical identification system (Microgen, UK). ESBL-production was confirmed with the cefpodoxime/cefpodoxime + clavulanic acid double-disc test (Thermo Fisher, UK). Samples were regarded as ESBL producing and further analysed when zone diameter around cefpodoxime was ≥5 mm than the zone diameter around cefpodoxime + clavulanic acid, according to manufacturer's instructions. Susceptibility testing was performed using Sensititre® ARIS incubating and reading system with "Sensititre" plates (Thermo Scientific, UK). Interpretation of results was based on EUCAST clinical breakpoints.

Results: Seventeen samples of out of one hundred tested were positive for ESBL-producing *E. coli* (17.0%; CI 95 % 9.6-24.4). All of the isolates demonstrated resistance to monobactams. MIC of cefpodoxime toward 13 isolates was >8 mg/L and 2-4 mg/L toward 4 isolates. One isolate was resistant to imipenem. Thirteen isolates (76.5%) were resistant to ciprofloxacin. ESBL-producing isolates also demonstrated resistance to gentamicin (23.5%) and chloramphenicol (35.3%). Two isolates were intermediate susceptible to amikacin while all of the isolates were susceptible to nitrofurantoin.

Conclusions: ESBL-producing *E. coli* are highly prevalent in European herring gulls – migratory species that frequently changes residence location. Native marine birds now are often distributed in urban areas were they feed at garbage dumps and sleep onshore. Such habit may lead to the fast and wide dissemination of resistant bacteria through soil and water.

The study was funded by a grant (SIT-6/2015) from the Research Council of Lithuania.

Keywords: antimicrobial resistance, beta-lactamases, gulls, *Larus argentatus*, dumps

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Poster presentation

Characterization of Some Metabolites in the Needles of Common Juniper (*Juniperus communis* L.) from Lithuania

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Abstract

Common juniper (*Juniperus communis* L.) is representative of *Cupressaceae*, existing in the forms of the shrub or tree within very broad ecological scale. Juniper is known as a plant that is rich in secondary substances, particularly terpenoids and phenolic compounds. It's essential oils are widely used in pharmaceutical and technical preparations, cosmetic products and as a food additive. Our study aimed at evaluation of genetic diversity of juniper, relating metabolites with molecular parameters and habitat type. In September, 2013, 140 female individuals were sampled from 14 Lithuanian populations of common juniper. Current-year needles were taken from the middle part of the crown and dry material was prepared for biochemical analysis. Samples were analysed according to the protocol, described earlier [1]. Both terpenoids and soluble phenolic compounds were analysed. Terpenoids were isolated and characterized using gas chromatography (GC) with an HP-5 column 30 m x 320 μm x 0.25 μm (Agilent Technologies, Santa Clara, CA). Soluble phenolics were analysed using high performance liquid chromatography (Nexera2, LC-DAD-MS, Shimadzu) and column ODS2, 250 x 4.6 mm. Quantification of the major terpenoids and flavonoids showed very high variability of concentration in respect to different populations. In the current-year needles absolute values of ranged for terpenoids from 2.50 to 5.02 (mg/g d. m.) and for flavonoids from 3.05 to 8.18 (mg/g d.m.), respectively. These results allowed to compare secondary metabolites of the needles of populations belonging to different habitat type. Our data suggest that composition and quantity of secondary metabolites of common juniper are related to the habitat type.

Keywords: *Juniperus communis*, *Cupressaceae*, populations, terpenoids, flavonoids.

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Poster presentation

The Influence of Medium on *Amplex Red* as Indicator for Fluorescent Hydrogen Peroxide during High-Voltage Electric Impulses

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Abstract

Fluorescent dyes often use to study cell membrane electropermeabilization. When a high-voltage pulse is applied to the electrolyte solution, a variety of electrolysis reactions occur at the metal-electrolyte interfaces. Metal ions that released from the electrodes can react with the fluorescent dyes and quench their fluorescence. This may have an impact when estimating the efficiency of electropermeabilization. In this study, the influence of the medium treated by high-voltage pulse and Al^{3+}, Fe^{2+}, Fe^{3+}, Cr^{6+}, Ni^{2+} and Mn^{7+} ions on the fluorescence of fluorescent tracer molecules, including fluorescent indicator for hydrogen peroxide, in various solutions was studied.

Cell culture medium consisted of Dulbecco‘s modified Eagle‘s medium supplemented with 9 % fetal bovine serum and 1 % L-glutamine solution (all Sigma–Aldrich Chemie, Steinheim, Germany). 50 µl of the culture medium or a solution of a fluorescent dye was treated with a square-wave electric pulse with the duration of 0,1–2 ms and the amplitude 0,2–2,4 kV/cm. The fluorescence of calcein, meso-tetrakis (4-sulfonatophenyl) porphyrin (TPPS4), doxorubicin (Adriamycin) and H_2O_2 indicator *Amplex Red* was studied. The fluorescence was measured at room temperature using Tecan spectrofluorimeter (Tecan Group, Männedorf, Switzerland).

The medium which was treated by the electric pulse with the amplitude of 1,2 kV/cm and the duration of 500 and 2000 µs can almost completely quench calcein fluorescence. When the concentration of the metal ions increase in solution, the intensity of the fluorescence of calcein, TPPS4, Adriamycin and *Amplex Red* decrease. For example, 1 mM of Fe^{3+} or Ni^{2+} ions suppressed fluorescence of calcein by 15 and 79 % respectively. Fe^{3+} at the concentration of 1 mM totally suppressed the fluorescence of porphyrin-sulphonate, by 30 % – the fluorescence of Adriamycin, and by 30–50 % the fluorescence of *Amplex Red*.

It can be summarized that the cell culture medium pretreated by high-voltage pulse quenches the fluorescence of various fluorescent molecules, mainly due to the metal ions released from the stainless-steel and aluminum electrodes.

The results of the current study can be useful for optimizing the electroporation method as well as for introducing it to medicine or industry.

Keywords: Amplex Red, calcein, metal ions, fluorescence.

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Poster presentation

The Role of Rs1799750 Gene Polymorphism in Pituitary Adenoma

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Abstract

Pituitary adenoma (PA) is typically a benign monoclonal neoplasm with an overall prevalence of 16.7% (14.4% in autopsy studies and 22.5% in imaging studies) in the general population [1]. The majority of PAs, however, are small and nonfunctional tumours, and only 0.16–0.2% of them are macroadenomas ≥ 10 mm in diameter [1, 2]. With modern imaging methods and hormone assays, the diagnosis of pituitary adenomas has increased. The matrix metalloproteinase-1 enzyme (MMP-1, also called collagenase 1) plays a key role in the alteration of collagen fibers in the intercellular matrix. The insertion of a guanine residue has been found within the promoter region of the MMP-1 gene [3]. There is no literature where MMP-1 rs1799750 was compared to pituitary adenoma's recurrence.

Purpose**:** To determine if the frequency of the genotype of MMP-1 have an influence on the recurrence of pituitary adenoma (PA).

Methodology**:** Permission (Number P2-9/2003) to undertake the study was obtained from the Kaunas Regional Biomedical Research Ethics Committee. The study was conducted in the Departments of Ophthalmology and Neurosurgery, Lithuanian Health Sciences University Hospital. Study participants comprised of 100 subjects with a diagnosis of pituitary adenoma (35 men and 65 women (age 51,28)), and the control group involved 200 subjects (49 men and 151 women (age 49,57)). The genotyping tests of MMP-1 were carried out using the real-time polymerase chain reaction method. Statistical analysis was performed using the SPSS / W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

Results**:** The 1G/2G genotype was more frequent in females of control group compared to PA group females: 50.3% vs. 30.8%, p=0.011. The 1G/1G genotype was more frequent in the PA without recurrence group than in the control group: 27.7% vs. 16.5%, p=0.034.

Conclusion**:** The MMP-1 gene 1G/1G may play role in pituitary adenoma without recurrence.

Keywords: pituitary adenoma, matrix metalloproteinase-1, gene polymorphism, recurrence.

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Poster presentation

Genotoxicity Assessment of Soil from Vilnius Industrial Areas Using *Tradescantia* Clone #4430

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Abstract

Nowadays environmental contamination is one of the most important problems in the world. Tons of toxic and genotoxic pollutants that are dangerous to ecosystems and human health are emitted every day. Genetic toxicology is the scientific discipline dealing with the effects of exposure to genotoxins. The genotoxicity of soil from the territories of closed industrial enterprises in Vilnius was examined in the present work. A sensitive bioindicator – *Tradescantia* clone #4430 – was used as a test-system. Two genotoxicity tests – *Tradescantia* stamen hair mutation (*Trad*-SHM) and *Tradescantia* micronucleus (*Trad*-MCN) assays – were applied to investigate the effect of short-term exposure to aqueous and DMSO extracts of contaminated soil. Four types of biomarkers were scored – pink cells, colourless cells and branched hairs in stamen hairs, and micronuclei in tetrads of pollen mother cells. Additionally, the RAPD-PCR method was applied to investigate the effect of long-term (0.5, 1, 1.5 and 2 years) exposure to contaminated soil. 61 RAPD primers were tested, 29 of them were informative and could potentially show polymorphism. Reproducible changes were detected using 4 primers. Out of three soil samples from the territory of closed Electrography institute "Elmatronas" studied with *Trad*-tests, sample EL3 was found to be the most genotoxic according to the results of pink cell and micronucleus formation analyses. This would not correlate with the results of soil chemical analysis, which showed that sample EL3 was less contaminated than sample EL1. Sample EL2 was found to be the least genotoxic according to the results of pink cell and micronucleus formation analyses, and chemical analysis. Moreover, an extra RAPD band was detected in *Tradescantia* DNA extracted after 0.5 year of direct exposure to soil sample EL3. The same extra band was gained after 1 year of exposure to soil sample KA5 from the territory of closed "Kuro aparatūra" factory. Sample KA5 was found to be the most contaminated out of all of the soil samples examined in the chemical analysis.

Keywords: soil contamination, Tradescantia, Trad-SHM, Trad-MCN, RAPD, genotoxicity, micronucleus.

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Oral presentation

Genetics and Biotechnology of Orchard Plants in Institute of Horticulture, LRCAF

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Abstract

Orchard plant breeding programs of the Institute of Horticulture of the Lithuanian Research Centre for Agriculture and Forestry are focused on improving cold hardiness, resistance to diseases and pests, high yield. Research include the collection and preservation of genetic resources, study of the genetics and biology of plant disease resistance and adaptation to low temperature, and the development of new genetic lines for breeding. Over 790 apple, 300 pear, 250 cherry (sweet and sour), 160 plum, 180 currant and gooseberry, 100 strawberry, 50 raspberry and other orchard plant cultivars and species are being preserved in institute collections. Morphological, agronomical and genetic studies of these accessions take place at the laboratories of IH. Origin and polymorphism of Lithuanian cherries was evaluated using molecular markers. An investigation of inheritance of disease resistance in interspecific hybrids in *Ribes* genus resulted in a large number of the hybrids displaying resistance to powdery mildew, a small percentage showing resistance to Septoria leaf spot, and no plants demonstrating any resistance to anthracnose. AFLP marker related to gall mite resistance gene *P* in a *Ribes* germplasm with different genetic background was identified and it may be used for early diagnosis of resistant to gall mite hybrids. Apple disease resistance research at the IH, LRCAF is aimed at the identification and pyramidisation of resistance genes, mainly for polygenic resistance to fungal diseases and hypersensitive response based resistance to apple scab, in addition to expediting the resistance breeding process.

Biotechnology applications, such as *in vitro* methods are being developed and employed to guarantee high quality reproductive material of fruit plants for production practices and preservation. Methods for micropropagation of strawberry, apple, pear, cherry, currants, lingonberry and other orchard and ornamental plants were established. *In vitro* test method for selection of resistant to apple scab progeny at embryonic stage was developed; application of the method allows a 92-100% recovery of progeny and provides a valuable tool for efficient assessment of the disease resistance. Methods of *in vitro* embryo rescue were developed for sweet cherry, sour cherry and black currant, this technique was used to obtain interspecific hybrids of *R. nigrum* and *R. americanum*. As an alternative to the embryo rescue technique, polyploidisation of *R. nigrum* was used to overcome the reduced fertility during interspecific hybridization. Polyploid lines of japanese quince were developed using *in vitro* culture technique.

Keywords: orchard plant breeding, plant biotechnology, plant genetics, resistance to biotic and abiotic factors.

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Oral presentation

Hypoxia and Pre-mRNA Splicing

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Abstract

RNA splicing takes place in the nucleus and occurs either co- or post-transcriptionally. Noncoding sequences (introns) in nuclear mRNA precursors (pre-mRNA) are removed by dedicated splicing machinery. The coding sequences (exons) are joined to generate the mature mRNA that is exported to the cytoplasm and translated into protein. Splicing events are tissue-specific. This process plays an important role in cellular differentiation and organism development. The splicing machinery heavily contributes to biological complexity and especially to the ability of cells to adapt to different developmental stages and altered cellular conditions. A striking change has been observed in alternative splicing pattern of genes and alterations in splicing factor expression under pathologic conditions especially in human cancers. Hypoxic regions have been identified within all solid tumors and their presence has been linked to malignant progression, metastasis, resistance to therapy, and poor clinical outcomes following treatment. Cellular responses to hypoxia are mediated by hypoxia inducible transcription factors (HIFs).

The talk will focuses on hypoxia, hypoxia dependent changes in pre-mRNA splicing and available data on how regulation of alternative splicing under hypoxic conditions is achieved.

Keywords: hypoxia, pre-mRNA, splicing, splicing factors, cancer.

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Oral presentation

Surface Characteristics of Chitin and Chitosan

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Abstract

Chitin and chitosan (deacetylated form of chitin) are nontoxic, biocompatible and biodegradable biopolymers. Because of these useful properties chitin and chitosan have great application areas such as medical, pharmacy, food, agriculture, textile etc. Surface morphology of chitin and chitosan is one of the most important characteristics for determining their application areas. Especially surface characteristics have an important role in adsorption and absorption studies. Here, I tried to summarize which kinds of surface morphologies have been described for chitin and chitosan up to now. After checking the literature, mainly six different types of surface morphologies have been recorded and these are: 1) smoot surface without fibers or pores, 2) only fibrous morphology, 3) fibers and nanopores together, 4) fibers and two types of pores (nano and micro porous morphology), 5) only nanopores and 6) papillar surface structure with fibers. In the present study, I will discuss my original scanning electron microscopy (SEM) pictures of chitin samples obtained from various organisms. All the chitin samples were obtained in Aksaray University Scientific laboratories and characterized via a Quanta 200 FEG.

Keywords: chitin, chitosan, surface morphology, fibers, pores

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Oral presentation

Mutation Identification in *ZCCT2* and *WRKY71* Genes in Winter Wheat

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Abstract

Bread wheat (*Triticum aestivum*) is one of the main crop grown around the world due to is wide application in food industry. Climate change and unpredictable temperature fluctuations cause a considerable damage in winter wheat. Many genes responsible for cold-regulation are being activated during cold acclimation which subsequently increases plant's tolerance to freezing. Therefore it is important to identify genes which differentially regulated during cold acclimation, however genetic basis of these mechanisms is still poorly understood. The aim of this study was to identify mutations in cold-acclimation genes.

Targeting Induced Local Lesions in Genomes (TILLING) population of the two winter wheat cultivars ('Kena DS' and 'Gaja DS') were developed using ethyl methane sulfonate (EMS) mutagen. TILLING method was used in order to create point mutations in target genes. Vernalization gene *ZCCT2* and transcription factor gene *WRKY71* were chosen for mutation detection by High Resolution Melting (HRM) analysis with gene-specific primers in winter wheat TILLING population of generation M_2. A total of 229.8 kb and 235.5 of genomic DNA were screened for *ZCCT2* and *WRKY71* gene, respectively. Six novel alleles of *ZCCT2* gene in exon 1 were identified, of which four were missense and two silent mutations. Further 7 novel alleles were detected in exon 1 for *WRKY71*, of which two were missense and five silent mutations.

Mutant plants were propagated to generation M_4 in order to validate mutation phenotypes. We found 3 missense mutations of *ZCCT2* gene (G46D; A49T; G82E) and all four mutations of *WRKY71* gene were present in generation M_4, two of them being missense (G165D; H146Y) and two having a silent mutations (R127R; Q141Q). Missense mutants only will be chosen for further study to verify the effect of these mutations on freezing tolerance in winter wheat.

Keywords: TILLING, HRM, *ZCCT2*, *WRKY71*, *Triticum aestivum*

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Poster presentation

Extraction and Characterization of Chitin from *Gonepteryx rhamni* (*Lepidoptera*)

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Abstract

Because of its nontoxicity, biodegradability, biocompatibility also antitumor, antioxidant, antimicrobial properties chitin is unique natural polymer. It has broad application in biotechnology, medicine, food technologies and other fields. In this study chitin was isolated from male and female of *Gonepteryx rhamni* (Lepidoptera). Physicochemical properties of chitin were evaluated by SEM, TGA, XRD and elemental analysis. Chitin content of male (10.5%) was recorded slightly higher than female. Degree of acetylation (DA) of female chitin was observed closer to 100% than male chitin, so it can be concluded that purity of female chitin was determined higher than male chitin. Thermal stability of female chitin was observed slightly higher than male chitin. SEM showed that both male and female chitin isolates have chitin nanofiber on the surface. Crystallinities of the chitin isolates from male and female were measured very close to each other. During this study, chitin extraction from male and female of *G. rhamni* was carried out for the first time. Determined properties of chitin samples could be used for further applications.

Keywords: biopolymer, chitin, butterfly, characterization.

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Poster presentation

Mutations Identification in *WCOR14a* Gene in Winter Wheat TILLING Populations

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Abstract

Freezing temperatures is one of the main factor causing winterkill in winter cereals. Freezing tolerance of winter wheat develops during cold acclimation which is triggered by induction of cold responsive genes after exposure of plants to low non-freezing temperature for certain periods of time. Consequently, identification of mutations in candidate genes expressed during cold acclimation was applied.

Targeting Induced Local Lesions in Genomes (TILLING) populations of the two winter wheat varieties ('Kena DS', 'Gaja DS') were developed in order to create new allele forms of the candidate genes to verify their role in freezing-tolerance formation. *Wheat cold-responsive protein* gene (*WCOR14a*) was chosen for mutation detection by High Resolution Melting (HRM) analysis in wheat TILLING M_2 populations. A total of 185 kb and 283 kb of genomic DNA was screened for exons I and II of *WCOR14a* gene, respectively. Two heterozygous point mutations (C127C/T, C106C/T) in exon I and one (G395A/G) in exon II were identified. All identified mutations were missense type (R43C, P36C and G120D, respectively). An overall mutation density was one mutation per 156 kb in the populations.

In order to carry out further research the presence of identified mutations in the M_3 generation has been verified. It was found that 30 % of M_3 mutant plants preserved heterozygous mutation (G395A/G) in exon II of *WCOR14a* gene. Further work will estimate the effect of the mutation on the freezing tolerance in winter wheat.

Keywords: High-resolution melting analysis, *Triticum aestivum,* TILLING, *WCOR14a,*

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Poster presentation

Detection of Volatile Compounds in *Actinidia* L. Seeds by GC-MS Method

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Abstract

Berries of *Actinidia* L. genus plants are an excellent source of biologically active compounds. While the roles of sugars and acids in Actinidia fruit flavor are well known and described, the roles of volatile compounds that contribute to flavour and odour are more difficult to define [1]. Aroma, sweetness and acidity is one of the crucial factors that contribute to the berry flavour, which is the result of a subtle mixture of volatile compounds [2,3]. The aim of this study was to investigate volatile compounds in seeds of *A.deliciosa*, *A.kolomikta*, *A.melanandra*, and *A.arguta*.

Fresh Actinidia berries were collected in August and September in 2015. Berries were stored at -80 ºC until analysis. We applied gas chromatography-mass spectrometry (GC-MS) method by Mota et al. (2011) with modifications. For analysis seeds were picked from berries and washed with distilled water.

Headspace Solid-Phase Microextraction. One commercial fibre was used to extract volatiles. According to the recommendations of the supplier (Supelco, USA), the fibre coated with PDMS/DVB as stationary phases and 65-μm film thickness is the most adaptable to determine the compounds in kiwi matrix. Approximately 0,5 g of seeds sample was homogenized and was loaded in 10 ml vial and then sealed with metal cap and PTFE/silicone septa (ROTH, Germany). Then the fibre was exposed to the headspace for 20 min in 50 ºC. Afterwards, the fibre was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption at 260 °C for 1 minute. All samples were analyzed in triplicate.

Gas Chromatography/Mass Spectrometry Analyses. HS-SPME analysis was performed using a Shimadzu GC-2010 gas chromatograph and mass spectrophotometer GC-MS-QP2010, and workstation software GC-MS solution version 2.71 (Shimadzu Corporation, Japan). The column used for samples analysis was a RTX-5MS (30 m×0.25 mm× 0.25 μm) from Restek (USA). The injector port was heated to 260 °C. The carrier gas was helium 5.0 (Aga, Latvia), at a constant flow of 1,5 mL/min. The oven temperature was set at 50 °C for 2 min, and then increasing at 8 °C/min to 280 °C and held for 2 min. Ionization was maintained off during the fifth minute. Electron ionization detector was at 70 eV. A scan was used from 40 to 400 m/z.

The comparison of MS fragmentation pattern with those of pure compounds and mass spectrum database search was performed using National Institute of Standarts and Technology (NIST) MS 08 spectral database.

Seeds of eight accessions were analysed. Following volatile and semi-volatile compounds were identified after GC-MS analysis: limonene, α-pinene, myrcene, phenylacetaldehyde, terpinolene, oxalic acid, furazan-3-ol, thiophene, methoxyacetic acid, nonane, benzoic acid, butane and phthalic acid.

Keywords: Actinidia, kiwi fruit, volatile compounds, GC-MS method, seeds.

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Poster presentation

Chemical Analysis of Polycyclic Aromatic Hydrocarbons and Investigation of Bioremediation of Used Railway Sleepers

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Abstract

Thousands of wooden sleepers have to be change to the new ones every year worldwide. Wooden sleepers impregnated with creosote can be used for about 15 years before they lose their functionality due to strong abiotic factors and microbial activity. According to the regulations of the European Union, any wastes containing polycyclic aromatic hydrocarbons (PAH) can not be used for compost or fuel before treatment and degradation of polycyclic aromatic hydrocarbons in their composition. PAH found in creosote have strong cancerogenic properties. Sixteen of them have also mutagenic properties and are reglamented in EU as the most hazardous and dangerous compounds for human health.

One of the ways to degrade PAH is to treat them in high temperature but it is too expensive for such a small country as Lithuania where about 30,000 sleepers are changed annually. Also transportation of ashes to special burning facility in Norway increases expenses. Bioremediation, as a part of the ecological biotechnology, could be successfully applied for hazardous wastes neutralization. Moreover, it is cost effective and environmentally friendly. Bioremediation is based on microorganisms that have ability to degrade selected compounds using their enzymatic properties. The strongest enzymatic properties to degrade PAH containing 3-6 aromatic rings were determined in white-rot fungi species. Downscaled in vitro test was applied for bioremediation screening test. The advantages of this type of experiment: small volume of media needed, lower risk of contamination and lower amount of hazardous consumables, higher precision and repeatability.

In this work we successfully analysed chemical composition of used wooden sleepers in Lithuania and Sweden. Bioremediation procedure of PAH was performed *in vitro* using white-rot fungi *I.lacteus*.

Keywords: polycyclic aromatic hydrocarbons, bioremediation, wooden sleepers, creosote, *Irpex lacteus.*

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Poster presentation

Highly Porous and Three Dimensional Hoop Shaped Chitin Extraction and Characterization from *Ommatoiulus sabulosus* (Diplopoda)

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Abstract

A new hoop shaped three dimensional chitin was obtained successfully from the body segment of a diplopod species (*Ommatoiulus sabulosus*) by following the procedure decolorization, demineralization and deproteinization. Purity of the hoop shaped three-dimensional chitin was proved by FT-IR analysis and chitinase digestive test. The important bands for α-chitin such as 1654.2, 1619.7 and 1552 cm^{-1} were found after FTIR analysis. And the chitinase digestive test revealed the purity of chitin (with digestion rate of 94.7%). SEM analysis showed that the chitin surface consisted of highly porous structure (the size between 400-600nm) with nanofibers. Thermal stability of the hoop shaped chitin was recorded quite high (DTG_{max}= 383 ºC).The nitrogen, carbon and hydrogen contents of the hoop shaped chitin were determined as 6.81, 46.23 and 6.43% respectively. And also degree of acetylation (DA) of the chitin indicated the purity with 95.85%. This new type of three-dimensional chitin obtained from the diplopod body segments can find more effective applications in further studies comparing to the conventional dust forms.

Keywords: 3D, chitin, diplopod, pores, chitinase test

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Poster presentation

The Comparison of Antioxidant Activity of Fermented and Unfermented Bee Pollen

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Abstract

Bee collected pollen is a very valuable natural product. It contains a large amount of polyphenolic compounds. Pollen consists mainly of flavonoids, which may act as potential antioxidants. According to the published data, the bee bread possesses higher antioxidant activity than bee pollen. However, the production of bee bread by the bees is a long process. Bee pollen is fermented by the bees by addition bee saliva, honey or nectar in the comb. There are no data about artificially fermented bee pollen. The main task of this analysis was to perform the fermentation of five different bee pollen samples using lactic acid bacteria and compare its antioxidant activity with natural bee pollen and bee bread. Four pollen samples were collected in Lithuania and one sample was collected in Latvia. Bee pollen fermentation process was performed by *Lactobacillus rhamnosus GG (*ATCC 53103*)* for 12 days, at 32 °C temperature. Phenolic compounds were analysed using spectrophotometric methods with Folin-Ciocalteau reagent; flavonoids analysis was carried out by colorimetric reaction with aluminium trichloride. Radical scavenging activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical spectrophotometric assay. All results were expressed in mg/g rutin equivalents. A qualitative characterization of antioxidant activity from fermented and unfermented bee pollen extracts was carried out by high performance liquid chromatography with DPPH reaction detector.

Keywords: Bee pollen, fermentation, antioxidant activity, lactic acid bacteria, online HPLC-DPPH.

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Poster presentation

Evaluation of Phenolic Compounds in Herbal Teas

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Abstract
The aim at this work was to assess and compare the radical binding activity and to identify the biologically active compounds of ETNO line of teas and the same composition tea blends made of medicinal plant raw materials derived from VMU Kaunas Botanical garden.
In order to achieve the aim it was divided into several tasks: to determine the total content of phenolic compounds and flavonoids, total antioxidant activity of medicinal plant materials by means of spectrophotometry; make blends of teas adequate to commercial ones and determine the total content of phenolic compounds and flavonoids, total antioxidant activity of the blends and commercial teas; to compare the results obtaint for medicinal plant raw materials at different stages of vegetation teas and tea blends. The total content of phenolic compounds has been evaluated by Folin–Ciocalteu method. The total flavonoid content has been measured using the spectrophotometric method after the reaction with aluminum choride. The antioxidant activity has been measured using the spectrophotometric method with DPPH.
Several commercially available teas: „Velvety Evening“, „Elfin“ and medicinal plant raw materials from VMU Kaunas Botanical Garden: camomile (*Matricaria recutita* L.), thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita* L.), raspberry leaves (*Rubus idaeus* L.), caraway seeds (*Carum carvi* L.), rosehip fruit (*Rosa canina* L.) and balm leaves (*Melissa officinalis* L.) were investigated. The total content of phenolic compounds was highest in *Melissa officinalis* raw material 79.04 mgRE/g, and „Velvety Evening“ tea mixture 76,48 mgRE/g. Minimum amounts were determined in *Carum carvi* L. 5.9 mgRE/g and „Elfin“ mixture 44.1 mgRE/g. Flavonoids highest amounts were in *Mentha piperita* L. raw material 40,33 mgRE/g and „Velvety Evening“ mixture 48.65 mgRE/g. The minimum total content of phenolic compounds was in *Carum carvi* L. raw material 4.57 mgRE/g and „Elfin" tea - 7,56 mgRE/g. The highest radical binding activity was determined for *Rosa canina* L. raw material 12,46 mgRE/g and „Velvety Evening“ tea blend 16.56 mgRE/g. Minimum radical binding activity was determined for *Carum carvi* L. 6.83 mgRE/g and „Elfin“ tea 7,82 mgRE/g. The minimum total phenolics and flavonoids quantities as well as radical binding activity was determined for *Carum carvi* L. Phenolic compounds content was highest in *Melissa officinalis* L., content of flavonoids in *Mentha piperita* L. and the radical binding activity in *Rosa canina* L. Comparing commercial teas and blends the radical binding activity as well as content of phenolic compounds and flavonoids were slightly higher in the blends than commercial teas.
Keywords: Phenolic compounds, flavonoids, antiradical activity

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Poster presentation

Isolation of Proteins Which Interact with Phospholipase A2 (IIA) from Human Serum after MyocardialInfarction. Flavonoids from *Bidens tripartita* as Phospholipase A2 Inhibitors.

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Abstract

Secreted phospholipases A_2 ($sPLA_2$, E.C. 3.1.1.4) are enzymes, which catalyzes the hydrolisis of the ester bond at the sn-2 position of glycerophospholipids, forming free fatty acids and lysophospholipids. These 13–18 kDa enzymes are structurally very similar [1], but display an impressive variety of different physiological and pathological activities [2]. Most of $sPLA_2$ have been identified in mammals - they are found in components of pancreatic juices, liver cells, synovial fluids and in many different mammalian tissues. In normal condition, $sPLA_2$ regulate the turnover of free fatty acids in membrane phospholipids, affecting membrane stability and fluidity. Phospholipase activity is also responsible for the generation of intracellular messengers, including arachidonic acid metabolites [3]. Interestingly, *Viperidae* snake venome contains $sPLA_2$ enzymes which induce effects like neurotoxicity and myotoxicity [4]. What is more, certain snake venom phospholipase A_2 have been identified as specific, non-competitive blood coagulation inhibitors that bind to human activated blood coagulation factor X (FXa) [4]. Secreted PLA_2s can also interact with others proteins, for example calmodulin, antibodies anti-PLA_2 and protein kinase C (PKC) [5,6]. In the other hand , those enzymes can interact with non-protein targets - natural phospholipase inhibitors (PLI) and flavonoids and polyphenols (eg. rutin and quercetin) [7,8].

The aim of work was to isolate proteins which interact with phospholipase A_2 (IIA) from human serum after myocardial infarction. What is more, during this study the effect of flavonoids inhibitors from the extract of *Bidens Tripartita* were examined. At the poster are shown the results of the phytochemical characterisation of compounds found in *Bidens tripartita* plant extract (metods as HPLC and GC) and results of pull-down methods.

Keywords: phospholipase A2, protein-protein interactions, pull-down methods, flavonoids, Bidens tripartita, HPLC, GC-MS

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Poster presentation

The Influence of Antimicrobial Microorganisms for Cattle Feeding

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An alternative and effective approach to antibiotic administration to livestock is the use of probiotics, which can help to improve gut microbial balance and therefore the natural defence of the animal against pathogenic bacteria [1]. Lactic acid bacteria (LAB) antimicrobial effect and their producing bacteriocin-like inhibitory substances (BLIS) they have a potential to be used as modifiers of the ecosystem in the gastrointestinal tract [2] and stimulators of animal production [3].

The aim of this study was to investigate the influence of *Lactobacillus sakei* KTU05-6 and *Pediococcus pentosaceus* BaltBio02 on dairy cattle milk production and rumen fluid parameters. Also, determination of antimicrobial activities of tested LAB against variety of pathogenic and opportunistic bacterial strains previously isolated from diseased cattle was performed. By using produced supplement, feeding experiment was performed in the winter at the farm of Black & White dairy cattle. Control (A) and trial (B and C) groups received identical diet; however, during 65 days, the trial groups received additionally 100 g of the supplement per cow daily (10^{11} colony-forming units (CFU) of LAB/head/day, B group – *L. sakei*, C group – *P. pentosaceus*).

It was found that at the end of the experiment supplement fermented with *L. sakei* do not have significant influence on dairy cattle milk production and rumen fluid parameters. Therefore, the interaction between analysed factors (type of microorganisms and supplements feeding duration) has been determined as statistically significant on milk yield ($F(8.212) = 54.609$, $p < 0.001$) and total count of aerobic and facultative anaerobic microorganisms (TCM) ($F(9.000) = 56.109$, $p < 0.0001$). Type of used LAB have a significant influence on microbiological parameters of rumen on glucose fermentation reaction (GF) ($F(4.061) = 0.677$, $p < 0.045$), (TCM) ($F(146.203) = 7.188$, $p < 0.0001$), total count of lactobacilli (TCL) ($F(157.042) = 7.219$, $p < 0.0001$), total count of enterobacteria (TCE) ($F(599.210) = 21.851$, $p < 0.0001$). According to results could be stated, that milk yield could be increased ($p \leq 0.05$) by the using *P. pentosaceus* BaltBio02 supplement.

We conclude that *Lactobacillus sakei* KTU05-6 supplementation by 10^{11} CFU/head/day may not be beneficial as probiotic bacteria for dairy cattle, as positive effects on the milk production and rumen fluid parameters was not observed in the animals tested. However, milk yield could be increased ($p \leq 0.05$) by the using *P. pentosaceus* BaltBio02 supplement, and it can be recommended for the cattle feeding.

Keywords: Lactic acid bacteria (LAB), dairy cow, ruminal fermentation, rumen microorganisms, milk.

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Poster presentation

Studies of *Lactococcus lactis* Infection by Phage sk1

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Abstract

Bacteria *Lactococcus lactis* is one of the most commonly used in the dairy industry lactic acid bacteria [1]. The infection of these bacteria inhibits lactose conversion to lactic acid. Therefore, quality of the product changes, in some cases the production is stopped. *L. lactis* is usually infected by bacteriophages belonging to the *Siphoviridae* family. The aim of this study was to determine the influence of incubation conditions on *L. lactis* viral infection.

It is usual in the dairy industry to inactivate bacteriophages by heat [2], but higher the temperature is, more taste and presentation of the final product is affected. In order to explore the bacteriophage inactivation by heat, not the infected bacterial culture but the bacteriophage suspension was heated. Bacteriophage sk1 suspension was heated at 63°C and 90°C for various time periods. It was observed that heating affects the course of infection: the phage remained infective at 63°C but after 30 min. from the start of the incubation at 90°C, phage was completely inactivated.

Multiplicity of infection (MOI) has an influence on the course of infection: the increase of MOI 8 times (from 2 to 16) leaves less unlysed virus-resistant cells. It is known [3] that the supplement of a medium with divalent cations, such as calcium, magnesium, or manganese, is required for the productive infection. Our results also indicated that the reproduction of bacteriopages does not occur if the growth medium is not supplemented with these ions, and Mn^{2+} ions are the most efficient.

L. lactis cells are unable to synthesise heme. The bacterial culture grows longer and produces more biomass after the addition of hemin to the medium. Studies of the infection showed that bacterial lysis progress faster in the medium with hemin. However, after examination of the medium aeration effects on infection, it was discovered that the infection progresses worse at high aeration conditions. In the case of strong aeration bacterial lysis started very quickly but ineffeciantly and the growth of bacteriophage-resistant cells immediately started. These results show that strong aeration prevents virus infection on *L. lactis* cells.

Keywords: Lactococcus lactis, lactic acid bacteria, bacteriophages.

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Poster presentation

Gel Based Proteome Analysis of Oxidative Stress Response in *Malus* sp.

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Abstract

The domesticated apple (*Malus* × *domestica* Borkh.) is one of the major economically important fruit crops of temperate regions and has been designated as one of three model species of the *Rosaceae* family. For northern regions, plants of *Rosaceae* family, such as apple, cherry and strawberry are often damaged low temperatures in winter. Plant responses to abiotic stress conditions, such as cold, drought and salinity, share common cellular processes associated with osmotic stress and involves increased generation of reactive oxygen species (ROS). The ROS production elicits cellular signaling networks and may cause physiological damage to plant cells, therefore the production of ROS must be strictly controlled in order to avoid oxidative stress. A manifestation of plant tolerance to osmotic stress phenotype is a consequence of integration of multiple environmental and biotic interaction signals. The non-pathogenic interaction with endophytic microorganisms modulates plant physiological responses such as growth, stress and disease response. Aim of this study was to investigate the effect of the ROS production regulating activity of the endophytes on osmotic stress signaling and changes of protein expression profile during osmotic stress response in apple cells. An osmotic stress was induced in apple cell suspension *in vitro* by incubation of the cells with PEG-8000. The cell suspension was inoculated with the endophytic bacteria strain that was previously shown to have ROS production reducing activity in apple. Intracellular ROS production was assessed and analysis of lipid peroxidation was used to estimate level of oxidative stress injury. Differential protein expression was assessed using 2D DIGE analysis. Statistical analysis and protein identification using LC-MS/MS approach revealed proteins involved in stress response, plant defense, gene expression and metabolic processes.

Keywords: osmotic stress tolerance, reactive oxygen species, in vitro cell suspension, endophytic bacteria, proteomics

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Poster presentation

Chitin Extraction from Spider Waste Cuticle

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Abstract

After the molting process, the exoskeletons of spiders remain as a waste material. Here in this study it was tried to obtain chitin from these kinds of waste materials. Chitin is known as a biopolymer with its nontoxic, biodegradable and biocompatible nature. Thanks to these useful properties, it has large set of applications in medical, pharmacy, agriculture, textile, food and feed industries. In the present study, chitin was isolated from spider (*Poecilotheria regalis*) waste cuticle through chemical method (demineralization, deproteinization and decolorization). This isolated chitin was analyzed by FTIR and it was determined that the chitin is in alpha form. SEM analysis revealed that the obtained chitin was consisted of both fibers and rare pores. The chitin content was recorded as around 12% on dry basis.

Keywords: *Poecilotheria regalis*, chitin, SEM, FTIR, exoskeleton

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Poster presentation

Analysis of Factors Influencing Selected Whey Proteins Concentration in Cow's Milk

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Abstract

Milk contains many peptides and proteins, which exhibit bacteriostatic and bactericidal properties in their intact form [1]. Especially the whey proteins contain peptides that improve the immunomodulatory activity (stimulating defence mechanisms of the body) and antibacterial activity [2]. The concentration of the whey proteins varies not only depending on the degree of infection of the udder but it is also affected by the cow age, lactation period, keeping conditions [3].

The aim of the study was to evaluate the amount of chosen whey proteins, i.e. lactoferrin (Lf), immunoglobulin G (IgG), alpha-lactalbumin (alpha-LA), beta-lactoglobulin (beta-LG) and bovine serum albumin (BSA) in cow milk in relation to somatic cell count (SCC), stage and number of lactations and different seasons.

Milk samples from individual quarters were collected once during the spring, summer and autum (10 cows/40 quarter samples/each season). All cows were clinically healthy without any signs of udder infection at the sampling time. Milk samples were obtained from quarters of the 1st, 2nd and 3rd lactations at early (< 120 days), middle (121–200) and late (201–305) stages of lactation. For determination of SCC milk samples were analysed with the flow cytometric analysis method using a somascope cell counter. Lf and IgG concentrations were measured by the enzymatic method ELISA based on the competition between marked antigen-antibody. Each sample was examined for alpha-LA, beta-LG and BSA determination using reversed-phase high-performance liquid chromatogaphy (RP-HPLC) with UV-VIS detector.

Criterion SCC in milk was used to assess the health status of the quarters. Grouping the data according to SCC revealed increased Lf (0.07±0.01 vs 0.06±0.01 mg/ml), IgG (0.27±0.05 vs 0.23±0.02 mg/ml), beta-LG (3.53±0.14 vs 3.28±0.09 mg/ml) and BSA values (0.34±0.03 vs 0.33±0.02 mg/ml) in diseased quarters (SCC from 201,000 ≥ 401,000 cells/ml) compared to healthy quarters (SCC up to 200,000 cells/ml). In contrast, decreased values were found for alfa-LA (0.83±0.04 vs 0.93±0.02 mg/ml) in diseased quarters compared to healthy quarters and indicate significant influence ($p<0.05$) of SCC on alfa-LA content in milk. The milk Lf, IgG and alpha-LA levels were effected by stage of lactation ($p<0.01$, $p<0.05$ and $p<0.01$ respectively). The results showed that selected whey proteins concentrations are associated with seasons of the year ($p<0.001$). Nevertheless, SCC and subsequent lactation ($p>0.05$) had no effect on these whey proteins, exept beta-LG ($p>0.01$).

Keywords: whey proteins, somatic cell count, lactation, stage of lactation, season, cow milk

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Poster presentation

Naphthoquinones Exert Cytotoxic and Antiproliferative Effects on *Glioblastoma multiforme* in a Cell Culture Model

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Abstract

Glioblastoma multiforme (GBM) is the most common and most aggressive primary central nervous system malignancy in adults.[1] Despite combined surgery, radiotherapy and chemotherapy median survival rate is extremely low - 14.6 months.[2] The anticancer activities of bioactive compounds such as 1, 4-naphthoquinones have been the focus of much research to discover novel anticancer agents.Studies have shown that plumbagin, menadione and lawsone have anticancer effects on GMB.[3–5]

The aim of this study is to investigate and compare the results between different concentrations of three different 1,4-naphthquinones: plumbagin, menadione and lawsone effectiveness on rat glioblastoma cell culture viability, and assess their impact to the GMB cell proliferation.

Methods: Cell viability was evaluated using propidium iodide and Hoechst 33258 assay, additionally cell viability was assessed by measuring the ability of cells to metabolize MTT dye. Cell staining with propidium iodide and Hoechst 33258 also was used to investigate anti-proliferative effect.

Results: Our results have shown that investigated 1,4-naphtoquinones induced dose-depend reduction in viability and proliferation of C6 cells. The EC_{50} values after 24 h were approximately 7.7 ± 0.28μM of plumbagin, 8.6 ± 0.75 μM of menadione and 1368 ± 280 μM of lawsone. Furthermore, propidium iodide/Hoechst assay showed that higher concentrations of tested compounds initiate cell death by necrosis.

Incubation of cells with 1 - 5 μM of menadione and 1 - 4 μM of plumbagin after 24 hours reduced cell proliferation by 16-27 %, lawsone reduced cell proliferation by 48% after incubation of cells with 500–1000 μM.

Conclusion: Plumbagin and menadione in a concentration-depend manner showed similar anti-proliferative and citotoxic effect to rat glioblastoma cells, however the effect of lawsone is approximately 150-fold lesser compared with other investigated naphthoquinones.

Keywords: glioblastoma, naphthoquinones, plumbagin, menadione, lawsone.

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Poster presentation

Promoter of *MMP-14* Gene Methylation in Pituitary Adenoma

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Abstract

Background: Pituitary adenoma (PA) is one of the most common abnormalities in the *sellar region.* Even knowing that PA is a benign monoclonal neoplasm, it can cause serious complications such as ophthalmological, neurological and endocrinological abnormalities. Until now, all the causes that increase the progression of tumours are unknown. Epigenetic silencing of the *MMP-14* gene may be related to the development of PA, because this gene plays an important role in the processes of tumour metastasis and angiogenesis.

Methods: The methylation status of *MMP-14* promoter was investigated by methylation specific PCR reaction (MS-PGR). For statistical analyses the IBM program "SPSS Statistics 20" was used. Statistical analysis was conducted to investigate the associations between the methylation status, age and gender of 120 PA patients.

Results: *MMP-14* was methylated in 30% patients of pituitary adenoma. It was also discovered that promoter methylation of *MMP-14* correlate with the male gender (58.8 % vs. 35.7 %, p=0.022) and unmethylated *MMP-14* with the female gender (64.3 % vs. 41.7 %, p=0.027).

Conclusion: However *MMP-14* and *TGFB-1* promoter methylation cannot be considered as a prognostic marker in pituitary adenomas.

Keywords: pituitary adenoma, MMP-14, promoter, methylation.

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Poster presentation

3D Chitin Isolation from *Blaberus giganteus*

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Abstract

Chitin is second most abundant polysaccharide, synthesized by an enormous numbers of living organisms. This natural polymer has very good properties, such as biodegradability, biocompatibility and non – toxicity. Chitin could be found in many applications: biomedical, pharmaceutical, food and environmental industries. This polymer could be separated in three – dimensional form. Aim of our study was to separate and characterize 3D chitin from Giant Cave Cockroaches (*Blaberus giganteus*) wings. This species were selected because they are one of the biggest cockroaches in the world and has two wing span of proximately 15 cm length. For 3D chitin isolation we used chemical method. Sodium hypochlorite for bleaching the samples, hydrogen chloride was used for demineralization and sodium hydroxide for deproteinization. Dried samples were characterized using FT-IR analysis. During our study *B. giganteus* chitin was first time successfully isolated. We detected, that 3D chitin had alpha form. Comparison among different cockroaches' chitin was made. Because of size, easy breeding, very high survival level and tough wings structure, we state that *B. giganteus* is one of the most attractive source of all cockroaches.

Keywords: cockroach, chitin, isolation.

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Poster presentation

Free Amino Acid Profile and Biogenic Amines in Fermented *L. angustifolius* and *L. luteus* Lupin Seeds

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Abstract

Lupins are native European legumes that can become true alternatives to soya bean, given their elevated content of high-quality proteins, potential health benefits, suitability for sustainable production, and acceptability to consumers (Lucas et al., 2015). Lupin may be a better alternative to soybeans, as it is not commonly genetically modified, has lower levels of phytoestrogens, and is lower in cost (Villarino et al., 2015).

The aim of the study was to investigate the ability of *Pediococcus pentosaceus* strains KTU05-8, KTU05-9, and KTU05-10 producing bacteriocin-like inhibitory substances (BLIS) to degrade biogenic amines (BAs) during submerged (SMF) and solid-state fermentation (SSF) of *Lupinus luteus* (variety Vilčiai) and *Lupinus angustifolius* (variety Vilniai, and newly bred hybrid lines No.1700 and No.1701). In addition, the influence of fermentation on the free amino acid (FAA) profile was assessed.

Lupin is a suitable substrate for the *pediococci*, and a significant correlation exists between the number of LAB cells and pH ($R=0.1731$, $P=0.0432$) in fermented lupin seeds. The selection of lupin varieties, fermentation methods, and the type of *pediococci* significantly affected the FAA profile and BAs concentration in lupin seeds and the interaction of these factors was ($F(5.989)=1755.321$, $P<0.0001$) and ($F(5.660)=130.736$, $P<0.0001$), respectively. By optimizing the technology of lupin seed fermentation, it is possible to produce bioactive peptides, which are of great interest for the design of higher nutritional value.

Keywords: Lupin, fermentation, pediococci, amino acids, biogenic amines

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Poster presentation

Efficiency Evaluation of Phenothiazine-Based Inhibitors of MDR Efflux Pumps in Gram-Negative Bacteria Cells

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Abstract

Antibiotics are considered the keystone of modern medicine, but their excessive use gives the consequences. Frequent usage of it for human therapy, as well as for farm animals resulted pathogenic bacteria resistance to multiple drugs. Multidrug resistance MDR efflux pumps are the main reason of bacterial resistance to antibiotics. Inhibitors of drug efflux pumps have great potential as pharmacological agents that restore the drug susceptibility of multidrug resistant bacterial pathogens. The idea to investigate phenothiazine derivatives as efflux pump inhibitors derived from the previous studies of methylene blue and chlorpromazine antimicrobial activity against infectious bacteria. It is very important to discover molecules which inhibit efflux pumps and to investigate the mechanism of efflux pump inhibition.

The aim of our work was to evaluate the inhibition efficiency of various phenothiazine derivatives of MDR efflux pumps in bacteria cells.

In this work we studied effects of the phenothiazine based MDR efflux pump inhibitors on the accumulation of TPP^+ ions in different bacteria. Data on the effective concentrations of the phenothiazine inhibitors and the efficiency of inhibition of MDR efflux pump activity will be presented.

Keywords: antibiotics, multidrug resistance, efflux pump inhibitors, phenothiazines.

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Poster presentation

A Research of Impact of PAHs Alone and in Interaction with *Saccharomyces* Family Yeast on *Populus tremula L.*

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Abstract

Heterocyclic derivatives of polycyclic aromatic hydrocarbons (PAHs) are of great concern to microbiologists, because they are hard to be degraded in the environment. Data from various studies indicate that organic aromatic compounds as pollutants are formed during industrial or human activities and are found in air, drinking water and all surface soils [1,2]. Part of PAHs are adsorbed from air on needles and leaves. It is known that plants can help clean up many types of contaminants but high concentrations may limit plant growth and take too long to clean up. The major source of human PAH exposure are considered to be vegetables, grains and fruits due to deposition of PAHs on plants [3]. Thus it is important to minimize the uptake of PAHs into the plants. The goal for these studies was to explore the effects of two heterocyclic PAHs, *9H*-carbazole (CBZ) and *10H*-phenothiazine (PTZ) on grow and quality of *Populus tremula L.* plants in vitro and ex vitro. The vitality of plants under the influence of different concentration of organic compounds solutions have been investigated using usual methods. It was found that vitality of plants in *9H*-carbazole solution differs from that one in *10H*-phenothiazine solution. Microorganisms play a key role in the removal of many types of chemical pollutants from the environment, including PAHs. The plant growth kinetics in microorganism solutions with organic pollutants was analyzed as well.

Keywords: PAHs, *10H*-phenothiazine, *9H*-carbazole, organic pollutants, yeast.

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Poster presentation

The Antioxidant Properties of *Brassica* Microgreens Grown in Different Substrates

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Abstract

Microgreens is a type of young vegetables with two fully developed cotyledon leaves and mostly one pair of small true leaves. Due to the young age, microgreens are known as an excellent source of vitamins, minerals and antioxidants. The selection of a suitable substrate is very important factor for accumulation of nutrients in short time as microgreens grow. The objective of our study was to evaluate the antioxidant properties of *Brassica* microgreens grown in different types of organic substrates. Red pak choi (*Brassica rapa* var. *chinensis* cv. 'Rubi F1') and tatsoi (*Brassica rapa* var. *narinosa*) microgreens were grown in greenhouse (9 days, 22/18 ± 2 °C day/night temperature, 60 ± 5 % a relative air humidity) during winter season. Three different substrates were chosen for experiments – peat (PROFI 1), coconut fibre's (ORGANIX COCOSUBSTRATAS) and compost (Organic compost Universal). The obtained data showed that antioxidant properties of microgreens depended on substrate and varied among species. The total phenols and anthocyanin contents significantly ($P \leq 0.05$) increased in both *Brassica* species grown in coconut fibres substrate. Meanwhile, significantly reduced content of phenols was determined in tatsoi and red pak choi microgreens grown in compost. Tatsoi grown in peat substrate accumulated more phenolic compound, however, significantly lower content of phenols in red pak choi was determined. Contrary, compost and peat substrate had no influence for accumulation of anthocyanins in red pak choi, and significantly reduced in tatsoi. The similar tendency was observed on flavonols in both microgreens species when significantly lower index was determined. Leaf flavonol index significantly increased in tatsoi, which were grown in compost, and red pak choi grown in coconut fibre's substrate. The highest DPPH radical scavenging activity was in both microgreens species grown in coconut substrate, and tatsoi in peat. However, the compost influenced reduced DPPH radical scavenging activity in both microgreens species. In summary, coconut fibre was the most appropriate substrate for enhanced antioxidant properties of microgreens, and less positive effects of compost and peat substrates were determined.

Keywords: anthocyanins, DPPH, flavonols, microgreens, phenols, substrate

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Poster presentation

Effect of Photoperiod and Temperature on *Brassicaceae* Family Plant Metabolism and Growth

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Abstract

The experiments were carried out in the phytotron complex of Plant Physiology Laboratory of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. The objective of our studies was to investigate the influence of photoperiod and temperature on radish (*Raphanus sativus* L.) and rocket (*Eruca sativa* L.) growth parameters, photosynthetic pigments and primary photosynthesis metabolites.

Radish 'Faraon' and rocket 'Rucola' were grown in boxes in a peat substrate (pH 5–6) mixed with sand in the ratio of 3:1. The plants were grown in a greenhouse at 21-25 / 15-18° C (day / night) temperature. Sprouted plants were transferred to four phytotron chambers, with controlled temperature and photoperiod conditions: 1) + 10/14° C (night / day) and 8 hours (photoperiod); 2) + 10/14° C and 16 h.; 3) + 17/21° C and 8 h.; 4) + 17/21° C and 16 h. Plants were irradiated with high-pressure sodium lamps (HPS). Total photosynthetically active radiation photon flux density of analyzed light combinations were ~160 μmol cm^2 s^{-1}. Plants from planting to technical mature stage were grown for 30 days.

Our results showed that the *Brassicaceae* family plants, radish and rocket formed four or more leaves, were elongated and accumulated more dry and green mass and radish formed the biggest roots at 17/21° C (night / day) and 16 h photoperiod. Lower, 10/14° C (day / night), temperature regime suppressed the growth of tested plants. Radish and rocket were more compact and had less leaves but the highest content of chlorophyll *a* and *b* and the ratio of sugars (fructose, glucose and sucrose) was determined in the leaves at 10/14° C and 16 h photoperiod. Direct correlations between the biochemical and biometric indices were established.

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Oral presentation

Possibilities and Challenges in Transdermal Drug Delivery

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Abstract

Human skin is considered as potentially valuable site for local and systemic drug delivery. At the same time the skin is a multifunctional organ, and one of the most important is a protective barrier function against external physical, chemical and microbiological factors. The barrier function of the skin is resulting from its multilayer structure. External skin layers exhibit different extent of cellular differentiation. The skin's barrier function is accomplished mainly by the outermost layer of the skin – the stratum corneum, which is comprised of dead cells skin cells located within a lipid rich matrix. Thus only relatively lipophilic compounds can diffuse into the deeper layers of the skin.

The development of transdermal drug delivery systems is stimulated by numerous benefits offered by this route: ease of use and dose termination in the event of any adverse reactions, patient acceptance and compliance, convenient and painless administration, avoidance of GI tract pH variations, gastric emptying time, first pass metabolism, sustained and controlled delivery, reduction in side effects, direct access to target or diseased site, and possible alternative in circumstances where oral dosing is not possible. Some challenges and limitations of using transdermal route should always be considered: active substance 500 Da molecular weight limit concept, sufficient aqueous and lipid solubility, variability of intact and diseased human skin permeability, presystemic metabolism, skin irritation and sensitization.

The conventional dosage forms for delivering drugs to the skin include gels, ointments, creams, which apply passive diffusion technology. Nowadays similar dosage forms have been developed by enhancing the driving force of drug diffusion and modifying skin permeability. These approaches include the use of penetration enhancers, supersaturated systems, prodrugs, nanostructured carriers, and other vesicles. However, the dose of drug that can be delivered using these methods is limited as the barrier properties of the skin are not sufficiently modified. Active delivery methods include iontophoresis, electroporation, microneedle based devices, mechanical removal of skin barrier layer or skin perforation, needles injecting, pressure application, sonication, laser radiation, radiofrequency ablation, thermophoresis etc. The costs and complexity of the systems are becoming important challenges in development of new transdermal drug delivery technologies. The scientific, technological, regulatory, and consumer needs must be considered in development of new transdermal drug delivery systems.

Keywords: Transdermal, Dermal, Drug Delivery, Skin, Pharmaceutical formulation.

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Oral presentation

Pharmaceutical Improvement of Pharmaceutical Characteristics of Anthocyanins Using the Incorporation into the Iota or Kappa Carrageenans

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Abstract

Anthocyanins (ATCs) are the most important water-soluble natural pigments of vascular plants). ATCs have a high potential for use as phytopharmaceutical active compounds due to their antioxidant and phytopharmacological activity which plays the vital role in the prevention of various diseases. The European bilberries are characterized by 15 ATCs. Up to now the extraction methods proposed are not selective for ATCs since they are able to co-extract a great number of other compounds present in fruits which accelerate the degradation of ATCs. Degradation of ATCs is the main problem for introduction of new innovative technological forms and phytopreparations in to pharmaceutical or nutraceutical markets.

Stabilization and prolongation of dissolution/relaxation time of ATCs was experientially confirmed when innovative copolymer forms of ATSs and carrageenans (CARGs) were introduced technologically. CARGs are natural water-soluble hydrocolloids composed of a linear chain of sulfated galactans and extracted from certain species of red seaweed. For the pharmaceutical improvement, the ATCs from the bilberries water extracts have been incorporated into the iota or kappa carrageenans during complexation in dilute solution or adsorption onto microgranules of CARGs.

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Poster presentation

The Roles of FYCO1 and Midbody Degradation in Regulating Cancer and Stem Cell Maintenance and Differentiation

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Abstract

At late mitosis, the mother cell divides, leaving two daughter cells connected by a thin intercellular bridge (ICB). During abscission of the ICB, the ingression of the cleavage furrow is formed, and the central spindle microtubules are compacted into the structure known as midbody (MB). The MB is situated within the ICB, with the abscission usually occurring at one side of the MB. As a result, only one daughter cell inherits the post-mitotic MB [1]. These MBs can then either accumulate in the cytoplasm or be degraded. Recent studies have identified MBs as novel signaling platforms regulating stem cell fate and proliferation. Indeed, stem cells as well as cancer cells were shown to accumulate post-mitotic MBs, resulting in reprogramming of cell fate and conversion to highly-proliferative, stem cell-like phenotypes.

It has been proposed that regulated macroautophagy may be playing a key role in mediating pots-mitotic MB degradation. In this study we identified FYCO1 as a protein, which associates with post-mitotic MBs and may regulate their degradation. Interestingly, FYCO1 is also known to be present on autophagosomes, and overexpression of FYCO1 can induce the formation of enlarged LC3-containing autophagocytic structures. Here we demonstrate that FYCO1 knock-down leads to defects in autophagic MB degradation, and that FYCO1 functions by targeting endocytic membranes to the autophagic phagophore during early stages of MB degradation. Additionally, we showed that FYCO1 depletion leads to increased proliferation and cell growth in soft agar. Based on all these data, we hypothesize that FYCO1 mediates selective MBs degradation via endosome-dependent extension of the phagophore around the post-mitotic MBs, and that MBs may be the regulators of cancer proliferation and progression.

Keywords: cytokinesis, midbody, degradation, autophagy, cancer

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Poster presentation

Formulation and Characterization of Lyophilizated Aqueous Extracts of Propolis

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Introduction. Freeze – drying, also known as lyophilization, is a method, in which water from frozen materials is removing by sublimation of ice crystals (primary drying) and desorbtion (secondary drying) [1,2]. Advantages of lyophilization: rapid reconstitution time, long preservation period owing to 95%-99.5% water removal, minimal changes in the properties because microbe growth and enzyme effect can not be exerted under low temperature, etc [2]. Freeze drying can be used in the formulation of aqueous extracts of poorly soluble in water substances. For this property, propolis was choosen to make its aqueous extract, instead usual ethanolic.
The aim of the study. To formulate lyophilizated aqueous extracts of propolis powder and characterize formulation quality: powder loss of drying, angle of repose, flow, tap density.
Materials and methods. Four formulations of 10% aqueous extracts of propolis with 10% bulking agents (D-sorbitol, D-mannitol, sucrose and D(+)-glucose anhydrous) were developed. The extracts were frozen at -80°C in freezer and lyophilized in a lyophilizator under 0,5-0,1 mm-Hg pressure at -50 °C. The quantitative composition of lyophilized powder was investigated with HPLC. Loss of drying was measured by electronic moisture analyzer KERN MLS, angle of repose and flow was measured by flow tester ERWEKA AG, tap density – by tap density tester TD1. A ratio of released and adsorbed heat of developed formulations was determined by microcalorimetry. Mean values and standard deviations of the results were calculated using Microsoft Office Excel 2007 software.
Results. The highest amount of fenolic acids and the most appropriate loss of drying was detected in lyophilizated powders with mannitol – respectively 15,28 μg/ml and 0,81%. Excellent flow – 12,5 g/s, angle of repose – 26,5° and tap density – 524,5 kg/m^3 was observed of powder with sorbitol. Formulation with mannitol as bulking agent demonstrated absence of change in adsorbed thermal energy when compared to propolis – free formulation.

Conclusions. The obtained results comply with the standarts for this type of dosage forms with loss of drying, flow, angle of repose, tap density. It was determinated that mannitol is the most suitable bulking agent for lyophilizated aqueous extracts of propolis.

Keywords: lyophilization, propolis, mannitol, sorbitol.

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Poster presentation

Synthesis of New 4-thiazolidinone Compounds and Their Evaluation of Metabolic Activity against *S. aureus*

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Introduction

Rhodanines exhibiting broad range of significant biological and pharmacological activities such as: antidiabetic, anti-inflamatory, antiapoptotic, antiviral, antifungal, antibacterial. Recent data shows that rhodanine compounds bearing 5-arylidene moieties are active against both Staphylococcus aureus and MRSA (1). There are few methods to evaluate metabolic processes of pathogens: NMR, LC-MS, LC-MS Q-TOF, MALDI TOF, DESI, SIMS, NIMS.

Aim. To synthesize new rhodanine derivatives with substituents in 5 position with possible antimicrobial activity against *S.aureus* by evaluating metabolomics profile.

Methods. Modifications of a rhodanine ring were performed in a 5 position by the Knoevenagel condensation reaction using various aromatic aldehydes. The rate of reaction was monitorised using FT-IR spectroscopy and TLC methods. Moreover, metabolic profiling of S.aureus metabolites to precisely determine enzymatic targets was performed using LC-MS (Q–TOF) technique (2). The whole metabolome profile evaluated of a microbial pathogen in the stationary phase. This procedure disposed after rhodanine derivatives influence 10 µM vs 10^9 CFU *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™) (2).

Results. (5Z)-5-[(4-nitrophenyl)methylidene]-2-sulfanylidene-1,3-thiazolidin-4-one synthesized by Knoevenagel condensation reaction. Yield 67.2%, orange crystals (pur: flash column chromatography $CHCl_3$:C_6H_6 ratio 9:1). The structure of Rhodanine derivative determined by the means of FT–IR and MS spectral data respectively: $\upsilon_{max/cm}$ 1702,28 (C=O), 1603,90 (C=C), 1503,19 (N=O); MS (ESI): calcd mass 266.9830 Da for $C_{10}H_6N_2O_3S_2$ $[M+H]^+$; found 266.9813 Da. The treated and untreated samples of *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™) were prepared by lysation and injected in LC-MS (Q–TOF). Preliminary screening results showed, that the synthetic rhodanine derivative acts upon the DNA synthesis pathway and adenosyl tetraphosphate hydrolase inhibition. The 2.2-fold and 2.3-fold up-expression changes ($\log_2(A_1/A_2)$ = 1,13; 1,18) respectively of *S. aureus* deoxyuridine triphosphate (dUTP) and adenosine tetraphosphate metabolites were observed suggesting that the rhodanine derivative could potentially target DNA polymerase. This research is currently continued in order to evaluate further S. aureus intracellular targets.

Conclusions. The metabolomics profiling of the synthesized rhodanine compound against *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™), revealed 2.2-fold increased changes ($\log_2$(A1/A2) = 1,13) of *S. aureus* dUTP and adenosine tetraphosphate and 2.3-fold increased changes ($\log_2$(A1/A2) = 1,18 adenosine tetraphosphate, suggesting that synthetic rhodanine derivative is interfering with the DNA polymerase biosynthesis pathway.

Keywords: rhodanine derivatives, five–membered heterocycles, synthesis, *S. aureus*, metabolome.

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Poster presentation

Synthesis of Tetrazole Compounds

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Introduction

Derivatives bearing tetrazole scaffold posses broad pharmacological activity. Due to the unique polyazo structure, mimicking many functional groups, tetrazole has specific bioisosteric properties, which are a useful tool in the drug R&D. Furthermore, tetrazole derivatives, particularly active against *S. aureus,* could help to identify intracellular targets by tracing metabolites with the LC-MS (QTOF) technique. [1]

Aim

The main aim of this work is to evaluate the metabolomic profile *vs. S. aureus* searching for potential intracellular targets by treating them with synthesized tetrazole derivatives.

Methods

In order to obtain derivatives with the tetrazole ring, cyclization or diazotization reactions were performed. The structure of synthetic compounds was determined by the means of FTIR and MS spectral data. Likewise, the metabolites of the *S. aureus* determinated in the late stationary phases after treatment with 10 μM of the synthetic tetrazole compound *vs* 10^9 CFU of the bacterium. [2]

Results

5-Aminotetrazole synthesized by diazotization reaction yields 40%. The structure of tetrazole derivative determined by the means of FTIR and MS spectral data respectively: IR $\upsilon_{max/cm^{-1}}$: 3331 (NH), 1637(C=N), 1045(C-N), 997(NH_2); HRMS (ESI^+): calcd mass 86,0461 for CH_3N_5 $[M+H]^+$; found 86,0454. The treated and untreated samples of *S. aureus* were prepared by lysation and injected in LC-MS (QTOF). Primary screening results showed, that the synthetic tetrazole derivative acts upon the RNA and the DNA synthesis pathway. The 3,5-fold change ($\log_2$(A1/A2) = 1,79) of *S. aureus* deoxycytidine diphosphate (dCDP) metabolite was observed suggesting that the tetrazole derivative could potentially target DNA polymerase, ribonucleotide reductase and nucleoside-diphosphate kinase (NDPK). This research is currently continued in order to evaluate more potential S. aureus intracellular targets.

Conclusions

The metabolomic profiling of the synthesized tetrazole compound against *S. aureus*, revealed 3,5-fold change ($\log_2$(A1/A2) = 1,79) of *S. aureus* deoxycytidine diphosphate (dCDP) metabolite, suggesting that synthetic tetrazole derivative is interfering with the RNA and the DNA synthesis pathway.

Keywords: tetrazole, synthesis, metabolites, antibacterial properties, dCDP.

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Poster presentation

Synthesis of Various Five-Membered Heterocyclic Compounds and Their Evaluation of Metabolic Activity against *S. aureus*

Monika Remezaitė[1], Liudas Šlepikas[1], Giedrė Tautkevičienė[1], Eligijus Kupriūnas[1], Rytis Mačionis[1], Hiliaras Rodovičius[1]

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Introduction. Compounds containing 1,2,4-triazole and 1,2,3,4-thiatriazole moieties are an important class of heterocycle which possess diverse biological and pharmacological activities, including, anticancer, antibacterial, antitubercular and antifungal. Furthermore, broad activity of 1,2,4-triazole and 1,2,3,4-thiatriazole derivatives particularly against *S. aureus* could help to confirm and validate intracellular targets by tracing metabolites with LC-MS Q– TOF technique [1].
Aim. The main aim of this work is to synthesize various five membered heterocyclic compounds, which have antistaphylococcal properties and to evaluate the metabolomic profile of *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™).
Methods. In order to obtain products with the triazole and 1,2,3,4-thiatriazole scaffolds the following reactions were performed: the cyclization of aminoguanidine oxalate and sodium hydrocarbonate in water; the recyclization of rhodanine with hydrazine in water; the diazotization reaction of thiosemicarbazide with sodium nitrite and hydrochloric acid in water. Thin layer chromatography (TLC) was used to monitor the rate of reaction. These compounds were determined by the means of melting point, FT–IR spectroscopy. Furthermore, for clarifying the chemical structures of derivatives used LC-MS (Q–TOF) technique [2]. Likewise, the whole metabolome profile evaluated of a microbial pathogen in the late growing phase. This procedure disposed after five membered heterocyclic compounds influence 10 μM vs 10^9 CFU *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™) [3].
Results. 3-amino-1H-1,2,4-triazole-5-carboxylic acid synthesized by cyclization reaction. Yield 77.3%, orange crystals (recryst. from a water), mp 182–183 ºC. The structure of 1,2,4-triazole derivative determined by the means of FT–IR and MS spectral data respectively: υ_{max}/cm^{-1} 3231 (N–H), 2987 (C–H), 2893 (OH), 1677 (C=O), 1637 (N–H), 1447 (COOH);

MS (ESI): calcd mass 129.0407 Da for $C_3H_4N_4O_2$ $[M+H]^+$; found 129.0401 Da. The treated and untreated samples of *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™) were prepared by lysation and injected in LC-MS (Q–TOF). Preliminary screening results showed, that the synthetic 1,2,4-triazole derivative acts upon the folate, and the DNA synthesis pathway. The 5.5-fold, 4.1-fold and 5.5-fold down-expression changes ($\log_2(A_1/A_2)$ = 2,45; 2.05; 2.45) respectively of *S. aureus* deoxyuridine (dU), deoxycytidine and 10-formyl-dihydrofolate (10-formyl-DHF) metabolites were observed suggesting that the 1,2,4-triazole derivative could potentially target folate biosynthesis pathway, DNA polymerase, ribonucleotide reductase and nucleoside-diphosphate kinase (NDPK). This research is currently continued in order to evaluate further S. aureus intracellular targets.
Conclusions. The metabolomics profiling of the synthesized 1,2,4-triazole compound against *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™), revealed 5.5-fold decreased changes ($\log_2$(A1/A2) = 2,45) of *S. aureus* dU and 10-formyl-DHF metabolites, suggesting that synthetic 1,2,4-triazole derivative is interfering with the folate biosynthesis pathway. Also 4.1-fold down-expression change of dC metabolite, implying that synthesized 1,2,4-triazole molecule is affecting with DNA biological synthesis pathway.

Keywords: five–membered heterocycles, synthesis, metabolome, S. aureus, antistaphylococcal properties.

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Poster presentation

Essential Oils as Potential Penetration Enhancers for Cutaneous Administration of Taxifolin into Human Skin *Ex Vivo*

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Abstract

Taxifolin (TXF) is a flavonoid with reported high antioxidant properties and it could be considered as a natural agent for UV induced skin damage prevention [2]. However, the data about possible TXF application into skin is limited.

Stratum corneum (SC) is the main barrier for cutaneous drug delivery. Overcoming *SC* barrier is challenged by its structural organization. Chemical penetration enhancers (CPEs) are one of the possible strategies to increase drug permeation through *SC*. Recent studies have focused on essential oils (EOs) as natural penetration enhancers with relatively low skin irritancy [1].

The aim of this study was to evaluate essential oils as CPEs for cutaneous delivery of TXF. Spruce needle oil (SO) and pine needle oil (PO) were selected as CPEs. 1% carbopol 980 gels, containing 1 % TXF and 3% CPE were formulated. 1% TXF gel without CPE was used as a control. *Ex vivo* skin penetration experiments were carried out using full-thickness human skin in modified Bronaugh-type flow-through diffusion cells. Infinite dose of TXF gel formulation was applied on *SC* side for 6 h. TXF was determined in epidermis and dermis by UPLC analysis. *Stratum corneum* barrier properties were evaluated by using tape stripping method.

After 6 hours application of gel formulations, TXF was detected both in epidermis and dermis. TXF amounts penetrated in epidermis and dermis from control formulation were 1.1986 ± 0.1377 µg/mg and 0.0030 ± 0.0010 µg/mg. TXF amounts detected in epidermis after application of gels with SO and PO were 3.2074 ± 0.2392 µg/mg and 2.2325 ± 0.3751 µg/mg, dermis – 0.0225 ± 0.0017 µg/mg and 0.0091 ± 0.0014 µg/mg. Enhancing ratios for SO and PO were calculated: epidermis – 2.68 and 1.86, dermis – 7.49 and 3.05, respectively. Both SO and PO increased TXF penetration into human skin layers at statistically significant level ($p<0.05$). Application of SO containing gel after *stratum corneum* removal showed 1.6 – 1.7 fold higher amounts of TXF in skin layers ($p<0.05$).

Both selected essential oils significantly increased TXF amounts in skin layers. Spruce needle oil was more effective as CPE than pine needle oil for TXF. The barrier properties of *stratum corneum* for TXF were demonstrated. Results suggest possible application of selected EOs as chemical penetration enhancers for TXF.

Keywords: taxifolin, essential oils, chemical penetration enhancers

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Poster presentation

In vitro Characterization and Optimization of Naftifine Hydrochloride 1% Medical Nail Lacquers

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Introduction. Topical delivery systems offer significant benefits in dermatology, but those formulations are not adapted for the transungual delivery since they are easily removed by washing or rubbing. Medical nail lacquers offer good attachment to the nail surface thus promising reliable delivery and reservoir of an active substance in the infected nail plate.

The aim of the study. Optimization of medical nail lacquer containing 1% naftifine hydrochloride.

Materials and methods. Twenty experimental formulations of nail lacquer containing 1% (w/v) naftifine hydrochloride, film-former Eudragit RL 100 and plasticizer triacetin were developed. 96% ethanol, butyl acetate and ethyl acetate were used as formulations solvent systems. Water resistance of formed film had been evaluated every 24h for 7 days; glass slides with nail lacquer films were soaked in a water and visual film changes were evaluated every 24h. Structural changes of films were visualized by means of inverted microscope Olympus IX71 combined with LCAchN40xPH lens. Microcalorimetric analysis of lacquer films was performed using microSC microcalorimeter. Release of naftifine hydrochloride was evaluated by applying modified Franz cells. Pseudoternary phase diagrams were constructed and optimal composition of nail lacquer was obtained using Design Expert software. Mean values and standard deviations of the results were calculated using Microsoft Office Excel 2007 software.

Results. All developed formulations produced homogenous smooth and transparent films. It was determined that film water resistance depends on amount of film-former, plasticizer and their both ratio. The most stable and resistant to water was formulation containing 13.6% Eudragit RL 100 and 2% triacetin. Film containing 15.6-17.8 % Eudragit RL 100 and 2.1 % triacetin washed out after 1st day of an experiment, this result was apparent in structural changes – visible non-homogeneity was visualized. Formulation containing 15.3% Eudragit RL 100 and 0.5% triacetin was the most stable to microcalorimetric heat changes. It was determined, that amount of film-former has significant influence on the release of active substance. Nail lacquer with 15% Eudragit RL 100 showed the highest release of active substance – 98.5% after 6 hours. Optimized composition of medical nail lacquer containing 1% naftifine hydrochloride was obtained by *in vitro* release results.

Conclusions. The obtained results comply with the standarts for this type of dosage forms with regard to the drying time, water resistance, as well as film appearance. A ratio of film former impacts drying time and *in vitro* release of active substance. Critical parameters for composition of nail lacquers were determined by optimization experiments.

Keywords: medical nail lacquer, naftifine hydrochloride, *in vitro* release, optimization.

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Poster presentation

Synthesis of Isatin Compounds and Their Evaluation of Metabolic Activity against *S. aureus*

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Introduction

Isatin, is a precursor for a large number of pharmacologically active compounds. The substitution of 3_{rd} and 5_{th} position of an isatin aromatic ring has been reported to be associated with antimicrobial properties (1). Although isatin derivatives mechanism against *Staphylococcus aureus* Rosenbach subsp. *aureus* (ATCC® 25923™) is not clear, but using LC-MS (QTOF) technique we could identify intracellular targets by tracking metabolites, such as folate, DNA biological pathways.

Aim

The aim of this work is to synthesize 3_{rd} and 5_{th} substituted isatin compounds, which have antibacterial properties and to identify it metabolomic profile of *S. aureus*.

Methods. In order to obtain the isatin derivatives the following reactions were performed: the chlorsulphonation reaction of isatin (2); the recyclization from boiling water. These compounds were determined by the means of melting point, FT–IR spectroscopy. Additionally for identifying the chemical structures of compounds LC-MS (Q–TOF) technique was used (3). Also, the whole metabolome profile of a microbial pathogen in the stationary phase was evaluated. This procedure was disposed after isatin derivatives influence 10 μM vs 10^9 CFU *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™) (3).

Results. 2,3-dioxo-2,3-dihydro-1*H*-indole-5-sulfonylchloride synthesized by nucleophilic substitution reaction. Yield 95 %, yellow powder (recryst. from a water), mp 150-152ºC. The structure of 5-chlorsulfonisatin determined by the means of FT–IR and MS spectral data respectively: $\upsilon_{max/cm^{-1}}$ 1669,72; 1731,6 (C=O), 1616,92 (C=C), 1376,08 (S=O); 552 (S-Cl); MS (ESI): calcd mass 243,0311 Da for $C_8H_4ClNO_4S$ $[M+Na]^+$; found 243,0304 Da. The treated and untreated samples of *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™) were prepared by lysation and injected in LC-MS (Q–TOF). Preliminary screening results showed, that the synthetic 5-chlorsulphonylisatin derivative inhibit the DNA synthesis pathway.The1,2-fold up-expression changes ($\log_2(A_1/A_2)$ = 0,25) of *S. aureus* deoxycytidinediphosphate (dCDP) metabolites were observed suggesting that the 5-chlorsulphonylisatin derivative could potentially target DNA synthesis. Also the thymidine diphosphate linked sugar metabolites was identified. It's possible that synthesized compound act other metabolomics pathway. This research is currently continued in order to evaluate further S. aureus intracellular targets.

Conclusions. The metabolomics profiling of the synthesized 2,3-dioxo-2,3-dihydro-1*H*-indole-5-sulfonyl chloride compound against *S. aureus* Rosenbach subsp. *aureus* (ATCC® 25923™), revealed 1,2-fold increased changes ($\log_2(A1/A2)$ = 0.25) of *S. aureus* dCDP metabolites, suggesting that synthetic 5-chlorsulphonyl derivative is interfering with the DNA biosynthesis pathway.

Keywords: isatin derivatives, synthesis, metabolome, *S. aureus*, antibacterial properties.

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Poster presentation

Release of Propolis Phenolic Acids and Vanillin from Cellulose-Based Hydrogels *In Vitro*

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Introduction. Hydrogels are hydrophilic polymer networks which may absorb up to thousands of times their dry weight in water [1]. Cellulose-based hydrogels have a lot of suitable properties such as hydrophilicity, biocompatibility, transparency, low cost, non-toxicity and can be used as controllable delivery system [2].

The aim of the study: to investigate the propolis phenolic acids and vanillin release from cellulose-based hydrogels *in vitro*.

Materials and methods. Four formulations of cellulose-based hydrogels with aqueous extract of propolis were developed. Each hydrogel was made with one type of cellulose: hydroxyethyl cellulose, hydroxypropylmethyl cellulose, methyl cellulose and sodium carboxymethyl cellulose (medium viscosity). The pH value and the dynamic viscosity of developed hydrogels were estimated. *In vitro* release experiments were performed using the modified Franz type diffusion cells. The samples from the receptor solution were removed at 0.25, 0.5, 1, 2, 4 hour and 37°C temperature was maintained. All samples were analyzed by HPLC. Mean values and standard deviations of the results were calculated using Microsoft Office Excel 2007 software.

Results. All developed formulations were homogenous, transparent, light yellow in colour. The dynamic viscosity of prepared hydrogels ranged from 2.34±0.01 to more than 11 Pa·s. The pH values were estimated from 4.70±0.01 to 6.00±0.02. Release data demonstrated that after 4 hours hydroxyethyl cellulose-based hydrogel showed the highest stream value of propolis phenolic acids and vanillin – 56.43±1.84 μg/cm^2. Sodium carboxymethyl cellulose-based hydrogel showed 48.81±3.09 μg/cm^2 of phenolic acids and vanillin, hydroxypropylmethyl cellulose-based hydrogel – 44.59±6.94 μg/cm^2, methyl cellulose-based hydrogel – 39.27±2.72 μg/cm^2.

Conclusions. All developed formulations were suitable appearance, the estimated pH values were close to the skin pH. The obtained results comply with the standarts for this type of dosage forms with release data results.

Keywords. Hydrogels, propolis, *in vitro* release.

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Poster presentation

Air Quality Evaluation of the Factory AB "Achema" Environment Using Two Methods: Passive Lichenoindication and Lichens Bioaccumulation

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Abstract

Air pollution remains one of the most important environmental problems inherent not only for urban but also for surrounding areas of industrial facilities. Recently the use of bioindicative methods is increasing worldwide and allows monitoring and evaluating complex negative environmental impact of known and unknown factors. The aim of this study was to evaluate air quality in the area surrounding the biggest producer of industrial chemical products in the Baltic States – AB "Achema" by means of passive lichenoindication and lichens bioaccumulation methods. The abundance of epiphytic lichen species, factor characterizing the condition of lichen community (index of poleotolerance PI) and nitrogen concentration in lichens were investigated at six study sites: control zone, factory premises (0-5 km), and study sites at 11-17 km, 17-23 km, 23-30 km away from the factory. The study site at 11-17 km away from the factory is characterized by the greatest diversity of species of lichen (n=5.1), the lowest diversity was established at control point (n=3.6). The highest projection coverage is in the premises of AB "Achema" (64%), the lowest coverage was established in the zone of 23-30 km away from the factory (21%). The premises of the factory have the highest PI (7.1) and the lowest PI was in the control zone (5.8). The correlation analysis revealed that the abundance of species, lichen coverage and poleotolerance index were related with the concentrations of accumulated nitrogen.

Keywords: Air quality; Lichens; Bioindication; Bioaccumulation; Nitrogen; Fertiliser production

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Poster presentation

Short-term Effects of Elevated Temperature and CO_2 on Carbon Sequestration in Winter Wheat and Summer Rape

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Abstract

Climate change seems to be one of the biggest ecological problem at this time. Terrestrial ecosystems may provide a positive feedback in a climate change therefore it is important to investigate all its' possibilities. Agriculture lands ability to sequester carbon is one of the opportunities to mitigate climate change which depends on such factors as soil type, type of crop or management practices. In order to study carbon sequestration in different plant parts of different crop species, closed growth chamber experiment was performed with winter wheat and summer rape in a controlled environment at ambient [21 ºC/400 ppm] and elevated [25 ºC/800 ppm] temperature and CO_2 conditions. Measurements of carbon sequestration were carried out at 28-day period after the treatment. Carbon content was measured with Shimadzu TOC solid sample module SSM-5000A.

The results showed that after 4 weeks of treatment under elevated temperature and CO_2 conditions both species of crop have sequestrated the biggest amount of carbon in stems (575 mg g^{-1} in wheat and 545 mg g^{-1} in rape). Whereas under ambient temperature and CO_2 conditions wheat has sequestrated the biggest amount of carbon in leaves (566 mg g^{-1}), while rape again in stems (523 mg g^{-1}). Under conditions of [25 ºC/800 ppm] the amount of organic carbon in leaves of rape increased by 6.8% ($p<0.05$), as compared to conditions of [21 ºC/400 ppm]. However, there were no significant differences ($p>0.05$) between [21 ºC/400 ppm] and [25 ºC/800 ppm] in the amount of sequestrated carbon in stems and roots of rape. Contrary, roots of wheat under conditions of [25 ºC/800 ppm] after 4 weeks of treatment sequestrated significantly bigger (6.0%, $p<0.05$) amount of carbon, as compared to conditions of [21 ºC/400 ppm]. In the above-ground parts of wheat there were no significant differences ($p>0.05$) between ambient and elevated temperature and CO_2 conditions.

Our results suggest that under future elevated temperature and CO_2 conditions both investigated crop species will sequester more carbon in their biomass but in different manner.

Keywords: Carbon sequestration, closed chamber method, summer rape, winter wheat

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Poster presentation

Effect of Different Treatment with Antioxidants on Aging Stability of Paper Containing Iron-gall Ink

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Abstract

Degradation of cellulose in historic manuscripts with iron-gall ink leads our heritage to deterioration. The main causes of paper decay depend on Iron-gall ink constituents. Acids and salts containing transition metal ions can accelerate hydrolysis and oxidation of cellulose. The most prevalent technique to prevent acidic hydrolysis and oxidation is treatment with solutions of calcium phytate and calcium hydrocarbonate. However, such treatment must be applied aqueously, which is often found unacceptable because of drastic modification of the paper/ink composition, mechanical stress of damaged papers, substantial changes in colour and document appearance [1].

The aim of this study was to apply non-aqueous alkalization and treatment with different antioxidants to paper samples containing iron-gall ink by the intention to stabilize oxidative degradation and acidic hydrolysis of paper and to compare the efficiency of aqueous and non-aqueous treatments. Firstly, this research examined the effect of non-aqueous treatment with tetrabuthylammonium bromide (TBABr) and 1-ethyl-3-methylimidazolium bromide (IEMIMBr) without and with a combination of alkalization with calcium hydrocarbonate ($(Ca(HCO_3)_2$). Secondly, this work investigated the effect of alternative treatment of paper stabilization by compressing the samples between two interleaves that are charged with calcium carbonate and different active compounds, such as: TBABr and IEMIMBr, potassium iodide (KI) and potassium bromide (KBr).

The effect of stabilization treatment on paper samples during artificial thermal ageing was evaluated by determination of the molecular weight distribution by two methods: size exclusion chromatography (SEC) and viscosimetry. Visual and elemental analysis was carried out using scanning electron microscopy (SEM) coupled with energy dispersive x-ray spectroscopy (EDS). Color change of paper samples was determined by reflection measurement using integrating sphere with halogen light source. Determination of oxidized groups of cellulose was estimated by Fourier transform infrared spectroscopy (FTIR).

The results demonstrate that effective stabilization of paper containing iron-gall ink during ageing may be successfully achieved by interleaving with alkaline paper impregnated with KBr.

Keywords: Paper, iron-gall ink, stabilization, antioxidants, degradation, deacidification.

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Poster presentation

Changes of *Triticum Aestivum*, *Hordeum Vulgare* and *Vicia Faba* Chlorophyll Content and Fluorescence Parameters under Impact of Sapropel

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Abstract

Fertilizer usage is increasing worldwide and with that, a lot of environmental problems are being created. So it is becoming really important to avoid such problems, one of these alternatives could be sapropel. The aim of this work is to investigate effects of sapropel on wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and beans (*Vicia faba* L.) photosynthesis pigment content and fluorescence parameters. Plants were sown in 1 l pots with prepared substrate, 10 seeds each. There were investigated the effects of unfertilized soil (control) and different concentrations 2.2; 3.1 and 4 t/ha of sapropel on plants. Plants were grown in controlled environment conditions. Experiment lasted one month, at the end of the experiment the content of pigments was measured spectrophotometrically. Handy PEA chlorophyll fluorimeter was used for determination of chlorophyll fluorescence parameters. In wheat leaves the content of chlorophylls a+b increased more ($r=0.94$, $p<0.05$) than in barley ($r=0.81$, $p<0.05$), and in beans they even got lower, but insignificantly ($r=-0.18$, $p>0.05$). Content of carotenoids differed similarly to chlorophylls, i.e. by increasing sapropel concentration in growth substrate the content of carotenoids increased in wheat ($r=0.87$, $p<0.05$) and barley ($r=0.87$, $p<0.05$), meanwhile in beans – lowered ($r=-0.5$ $p>0.05$). Parameter of II photosystem efficiency – Fv/Fm ratio increased significantly together with sapropel concentration increase in wheat $r=0.97$ ($p<0.05$) and in barley $r=0.98$ ($p<0.05$). Fv/Fm ratio of beans increased too, but insignificantly ($r=0.94$; $p>0.05$). With increasing sapropel concentration photosynthesis index (PI) for wheat had low rise ($r=0.4$; $p>0.05$). But opposite results were detected for barley and bean, when correlation between PI and sapropel concentration increase were $r=0.82$ ($p<0.05$) and $r=0.91$($p<0.05$) respectively.

Keywords: Sapropel, fertilizer, wheat, barley, beans, growth, chlorophyll, fluorescence.

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Poster presentation

Influence of Chemical Modification of Cellulose Microfiber Surface on Their Structure and Properties

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Abstract

Cellulose microfibers (CMF) are being used as potential reinforcing materials because of so many advantages, such as highly expanded surface area, low density, their ubiquitous availability and low cost, their bio-renewable, recyclability character and very high aspect ratios of the fibres [1]. The properties of CMF are strongly influenced by many factors: chemical composition, internal fibre structure and microfibril angle [2]. However, CMF also display some limitations such as, highly polar and hydrophilic charecter, wich make them poorly compatible with used as filler in non-polar polymer matrix. Beside this, the hydrophilic nature of nature cellulose fibers adversely affects adhesion to a hydrophobic polymer matrix and as a result, it may cause a loos of strength [3]. To prevent this, cellulose surface has to be hydrophobize in order to promote compatibility. This provides an opportunity to make a modification the surface of cellulose microfibers by chemical modifications. Chemical modifications are considered to improve compatibly between fibre and polymer matrix [4]. In this work, we have present hydrophobicity to the hydrophilic CMF by treatment of silanes. Two different modification method were used in this study. The effect of chemical modification on surface properties was evaluated by water contact angle meaurement. The Fourier transform infrared (FTIR) was used to analyze the chemical structure of the cellulose microfibers before and after hydrophobization. The scanning electron microscopy (SEM) was used to observe the surface morfology of the cellulose.

It is known that CMF is hydrophilic and contact angle is $\theta = \sim 15°$, but after modification the water drop displayed a comparatively large contact angle were values reach ($\theta = 125°$). FTIR analysis of the modified CMF confirmed that hydrophobic dense top layer was formation. It was obtained from SEM imagines that a fairly rough surface is characteristic for modified and unmodified CMF.

Keywords: cellulose microfiber, chemical modification, hydrophobization.

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Poster presentation

Surface Active Agents and Microbiological Cultures Treatment of Oil Hydrocarbons Contaminated Soil

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Abstract

Contamination of soil with crude oil, diesel, gasoline, motor-oil hydrocarbons etc is one of the most important ecological problems in terrestrial ecosystems. This paper provides information about the ability of a biological (P. Aeruginosa DS-10-126 produced) surfactant and a synthetic (anionic sodium dodecylsulphate SDS and non-ionic polyoxyethylene(20) oleyl ether "Brij 98") surfactants to remove diesel and motor-oil mixture from contaminated soil under varying washing conditions. Soil was prepared in the laboratory by mixing black soil, clay and gravel at equal parts and then contaminated by mixing diesel, motor-oil and soil. Soil physical properties (density, humidity, granuliometric composition) was determined for both contaminated and clean soils. The washing parameters for syntethic surfactants usage were as follows: temperature 21 ◦C, time 20 min, shaking speed 200 rpm, surfactant concentration (below the CMC and CMC). Parameters for bacterial washing were the same as synthetic surfactant, except temperature (37 ◦C) and time (till the stationary optical density and 1 hour after reaching stationary optical density). The concentration of remaining hydrocarbons in soil was determined using GC/MS. The surfactants were found to have considerable potential in removing crude oil from contaminated soil. The removal of oil products contamination by biological surfactants was about 50% more effective than synthetic surfactants. Synthetic surfactants cleaned about 30% of oil hydrocarbons from contaminated soil, when biological surfactants cleaned more than 70% of them. The most influential parameters on oil removal were surfactant type and concentration.

Keywords: soil, hydrocarbons soil washing, surfactants.

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Poster presentation

Significance of Medicinal (Aromatic) Plants for Eco-Urbanism

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Abstract

The research work presents information on the most important issues in modern society's life – eco matters, in particular, eco – urbanism. Nowdays as ' green and eco ' issues are of great importance the European Commission promotes *Green Week* events that aspires citizens across Europe to engage and express their views or show examples of how today's investments can help the idea of a 'greener future' become a reality for the generations to come.

According to the global plant protection strategy and adopted Convention on Biological Diversity the determination of medicinal aromatic plants role in eco- urbanism is being considered. (Zajonc, 1980; Ayad, 1994; Global Strategy,2002).

The development of medicinal (aromatic) plants is based on long-term research data patterns and biological methods.

Landscaping functions in a number of high importance issues such as environmental aesthetic improvement, protection from air pollution and plant species diversity enrichment. Scientists and professionals of various fields deal with the problems of landscaping and design, development and maintenance, also security issues in a comprehensive manner. The medicinal (aromatic) plants grown for landscaping purposes are the property of state as well as the society and they must be not only cherished and protected but their diversity of species must also be enriched. The plants serve both as educational and at the same time as recreational and human healthy lifestyle optimization means in the processes of investigating, teaching/learning.

Scientists say that in those cities and towns where there are high quality green areas and flowerbeds significantly less crime occurs (Bratman et al., 2012). Research has shown that the medicinal (aromatic) plant view and smell affects the human brain chemical processes and cause positive impulses. (Zajonc, 1980*)*

Medicinal, spice (aromatic) plants develop people's skills and dispositions to knowledge and natural methods of analysis responding to emerging issues, finding solutions to human activities caused by natural changes in the preservation of the environment, respecting their own and other people's health and the sustainable development of society - it develops natural science excellence.

Medicinal (aromatic) plants are a recreational and educational tool for understanding the diversity of plants healthy lifestyle optimization and a component of eco -urbanism.

Key words: medicinal (aromatic) plants, eco – urbanism, landscaping, recreational.

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Poster presentation

Analysis of Tree Bark and Lichens for Detecting Contamination and Physiological Effects in the Urban Environment of Kaunas (Lithuania)

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Abstract

Biomonitoring of air pollution in urban area was studied analyzing samples of *Tilia* sp. tree bark and lichens. The samples of bark and naturally growing *Xanthoria parietina* were collected removing a surface layer of the bark and lichen thallus. Samples of the lichen *Evernia prunastri* were transplanted for 5 weeks at the study sites and changes in ecophysiological parameters (photosynthetic pigments, membrane lipid peroxidation, cell membrane damage) were assessed. The concentration of heavy metals (Pb, Cd, Cu) in the samples were used as an indicator of the level of air pollution in the three sites of different activities. The bark tissue analysis revealed that the highest metal concentrations were obtained in intense traffic zone. The conductivity of the bark samples was found to be a sensitive indicator of pollution. Lichen revealed the increased amount of heavy metals and changes in some physiological parameters in urban sites. Although the tree bark was not as sensitive to pollution as lichens, but it can be used as an indicator for various pollutants in areas, where other bioindicators are unavailable.

Keywords: air pollution, bioindicator, heavy metals, tree bark, lichen.

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Poster presentation

LED Lighting Device for Plant Experimentations: from Researches to Technology and Back

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Abstract

Plant LED experimentations started in ~1991. In Lithuania LED in plant photophysiological researches were first included in 2003, when LAMMC Institute of Horticulture started the research project with Vilnius University, Institute of Applied Research. The custom-designed lighting units, used for research provided novel and actual possibilities and also encouraged to make timely decisions: requirements and amenities for the lighting unit, arising from years of experimentations in the field of plant photophysiology, were transferred for designers and engineers to construct ergonomic device with controllable light parameters. The smart light emitting diode (LED) based lighting solution for diverse plant research environments (growth chambers, phytotrons, laboratories and greenhouses) was developed. The HLRD-series lighting units consists of numerous single LED lamps fitted on the panel. This construction allows to use any producer's LEDs; to compose various light spectra (red, blue, green, orange, yellow, UV-A) and to update the spectra with new wavelengths easily during operation. Light parameter control includes possibilities to regulate lighting photoperiod, spectra, intensity of each spectral component and blinking frequency via user-friendly software in mobile devices or computer. The lighting unit was designed seeking for versatility, ergonomics, flexibility and functionality, as well as precise control of lighting parameters to realize the researcher's ideas.

Keywords: plant lighting, light spectra, intensity, multiparametric lighting control

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Poster presentation

Toxicological Assessment of Closed Municipal Solid-Waste Landfill Impact to the Environment

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Abstract

The large number of municipal solid waste landfills in Lithuania poses a serious environmental threat to the quality of soil, surface and ground water. The physicochemical characteristics and toxicity of closed Panevėžys municipal solid-waste landfill leachate and its impact to soil and surface water were assessed. Landfill leachate is complex mixture of various inorganic and organic compounds. The toxicity of municipal solid waste landfill leachate and surface water was evaluated using bioassays with aquatic plants (*Lemna minor* L.) and microinvertebrates (*Daphnia magna*). The leachate was shown to be toxic to *D. magna*, reduced the growth and biomass of *L. minor*. The toxicity has been linked with ammonium and heavy metals content as the prime contributors to the toxicity.

Keywords: landfill leachate, *Lemna minor*, *Daphnia magna*, toxicity.

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Natural Products

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Oral presentation

The Use of Enzymatic Treatment and Fermentation with *P. acidilactici* BaltBio01 MSCL P1480 for Industrial Cereal By-products on Purpose to Produce Food/Feed Stock with high Amount of Lactic Acid Bacteria

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Abstract

A higher economical advantage could be achieved with the use of by-products from agro-food industries, such as from flour milling industry, as potential resources to produce a higher nutritional and biological value food/feed stock. The aim of this study was to apply the enzymatic treatment and fermentation with *P. acidilactici* BaltBio01 MSCL P1480 for industrial cereal by-products on purpose to produce food/feed stock with high amount of lactic acid bacteria (LAB). Also, the possibility to use the potato juice for *P. acidilactici* multiplication was analyzed. Potato juice was found suitable substrate for *P. acidilactici* cultivation (cells count 9.6 log CFU/mL).Two techniques (freeze- and spray-drying) were used for stabilization of *P. acidilactici* in potato juice. Stabilized by spray-drying LAB powder remained stable (7.0 log CFU/g of viable cells) during 12 months of storage and was used as bacterial starter for cereal by-products fermentation. The changes of microbial profile, BAs, mycotoxins, lactic acid (L+/D-), lignans and alkylresorcinols (ARs) contents of fermented cereal by-product were analysed. Cereal by-products enzymatic hydrolysis before fermentation allow to obtain a higher content of LAB during fermentation. Fermentation with *P. acidilactici* reduce mycotoxins content in fermented cereal by-products, and there was a significant effect of treatment with enzymes before fermentation on AFL, ZEA and DON concentration in cereal by-products. The BAs concentrations in fermented cereal by-products were found far below those levels associated with a health risk. The use of *P. acidilactici* for cereal by-product fermentation increased lignans content, however ARs contents were reduced. According to our results multiplied in potato juice *P. acidilactici* could be used for cereal by-products treatment, as a potential resource to produce safer food/feed stock with high amount of LAB for industry.

Keywords: Cereal By-products, Enzymatic treatment, Lactic Acid Bacteria, Fermentation, Food, Feed

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Oral presentation

Interference with DNA Damage Repair and Translesion Synthesis: a Possible Mechanism for Natural Products Chemoprevention and Indirect Genotoxicity

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Objectives of the study: Balance between DNA damage and repair appears vital to the cellular machinery, to health and longevity. Given the complexity of DNA lesion tolerance and repair mechanisms and the high number of proteins and cofactors involved, we hypothesize that natural products from food and medicinal plants may interfere in these processes, notably by activating or inhibiting repair.

Methods: Original methods were developed to probe the modulation of major mechanisms by natural products: *(i)* fidelity of translesion synthesis (TLS), a DNA damage tolerance mechanism that relies on specialized DNA polymerases (pols) able to insert a nucleotide opposite a lesion on the template strand (capillary electrophoresis); *(ii)* kinetics of rejoining strand breaks arising from damage and excision repair (comet assay); *(iii)* capacity of double-strand breaks repair by non-homologous end-joining (NHEJ), a preponderant mechanism in eukaryotes (on-chips microelectrophoresis); *(iv)* capacity of base excision repair, the major repair pathway responsible for removal of small DNA lesions (oligonucleotide repair chips). Nuclear extracts were obtained from either plant extracts-pretreated FHs 74 Int cells (treatment at non-lethal concentrations, determined by a MTT cytotoxicity test) or control cells. Common flavonoids were tested for their capacity to interact in these mechanisms. Food plants were treated following their usual processing for human consumption and extracted with ethanol/water 50/50, then evaporated to dryness.

Results: All methods were validated and could be applied to the study of modulation by natural products. None of tested flavonoids inhibits repair; quercetin increases non-specific endonuclease activity, apigenin and epicatechin increase the excision of damages; sakuranetin increases non-specific enzymatic activities and decreases or increases specific activities. Plant extracts variously modulate activities.

Conclusion: Although some of these protocols represent a simplification of the complexity of the *in vivo* organization of DNA into chromatin, data obtained so far show that plant extracts are likely to interfere in TLS and repair capacity.

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Oral presentation

Safety and Quality Improvement of Plants, Relevant for Healthy Food Production

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Abstract

Buckwheat, oats and spelt wheat are recognized as healthy foods in many countries. The current study was aimed to determine the concentrations of mycotoxins in buckwheat, oats and spelt wheat grains and hulls, establish the change of phenolic compound concentrations and evaluate the relationship between phenolic compounds and mycotoxins.

In recent years, aflatoxin B1 (AFLB1) contamination of buckwheat grains and their products has been increasing. The concentration of AFLB1 in buckwheat grains was found to vary from 47 $\mu g \cdot kg^{-1}$ at ripening stage to 5 $\mu g \cdot kg^{-1}$ after harvesting (allowable limit 2 $\mu g \cdot kg^{-1}$). Nearly 90% of the buckwheat, spelt and oats grains were contaminated with deoxynivalenol (DON); however, the concentrations did not exceed the allowable limits Regulation (EC) No.1881/2006. A trend was revealed that in most cases cereal hulls were several times more contaminated with AFLB1, DON and T2 toxin. There is evidence that hulls play an important role in protecting the grain kernel from mycotoxin contamination.

Analysis of phenolic compounds showed that the significantly highest total phenolic content (10.3±1.2 $mg \cdot g^{-1}$ dry weight (d.w.), antioxidant activity (2–4 times), rutin concentration (358.4±45 $\mu g \cdot g^{-1}$ d.w.) and hydroxybenzoic acids (*p*- hydroxybenzoic 15.4±3.0 $\mu g \cdot g^{-1}$ d.w. and 3,4-dihydroxybenzoic acid 34.8±15.0 $\mu g \cdot g^{-1}$ d.w.) concentrations of all groups of cereal samples were established for buckwheat grain. Oat grains were distinguished by the highest quercetin content (12.2 – 29.8 $\mu g \cdot g^{-1}$ d.w). Total phenolic acids content significantly ($P<0.01$) predominated in oats and spelt wheat samples, noting that ferulic and *p*-coumaric acids together accounted for about 90% of the total phenolic acids content.

The regression-correlation analysis between mycotoxin and phenolic compounds suggested that the content of DON in buckwheat grains significantly ($P<0.01$) decreased with increasing content of total phenolics and 3,4-dihydroxybenzoic acid (correlation coefficients r = -0.867 and r = -0,765, respectively). However, significant impact of phenolic compounds on mycotoxins in most cases was detected when grain contamination level was low. In order to use cereal grains for food, or hulls for human needs, the grains need to be thoroughly checked for mycotoxin presence.

Keywords: buckwheat, spelt, oat, grain, hulls, mycotoxin, phenolic compounds.

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Poster presentation

Fractionation of *Echinacea purpurea* L. (Moench) Proteins and Assessment of Glycosylated Proteins Distribution in Fractions

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Abstract

Plant lectins, non-immune origin glycoproteins, got their attention for possible beneficial effects to health just 3 decades ago and are quiet new group of compounds with pharmacological interest.

Echinacea purpurea L. (Moench) is a well-known medical plant. However, pharmacologically interesting proteins in this plant aren't well characterized.

In this study we extracted and fractionated proteins of *Echinacea purpurea* L. (Moench) roots and checked estimation of glycosylated proteins in all fractions.

Materials and Methods

30 g of fresh roots were homogenized in mortar with liquid nitrogen and extracted in phosphate buffer saline pH 7.4 in a ratio 1:5 for 2 hours at 4 °C. Proteins were precipitated from crude extract by adding 4 volumes of cold (-20 °C) 10 % Trichloracetic acid/acetone solution, containing 0.2 % β - mercaptoethanol. Protein pellets were washed with acetone solution, containing 0.2 % β – mercaptoethanol for 3 times and resuspended in phosphate buffer 7.4.

Resuspended protein sample was loaded on HiTrap Q FF 1 mL column with gradient elution from 0 M to 1 M NaCl. 5 mL fractions were collected, dialyzed in phosphate buffer 7.4 and immunoblotted with anti-xylose antibody.

Results

After anion exchange fractionation we've collected 18 protein fractions, 11 of them contained glycosylated proteins. Glycoproteins started eluting from the column when sodium chloride concentration in eluent reached 0.4 M. Elution of glycosylated proteins decreased when sodium chloride concentration reached 0.7 M.

Conclusions

Protein fractions extracted from *Echinacea purpurea* L. (Moench) roots contained glycosylated proteins. Glycosylated protein patterns between fractions were different from each other.

Keywords: *Echinacea purpurea* L. (Moench), glycoproteins, ion exchange chromatography

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Poster presentation

Fatty Acid Composition and Radical Scavenging Properties of Raspberry (*Rubus idaeus* L.) Seed Extracts

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Abstract

A valuable by-product of berry juice production is pressing residue known as pomace. Berry pomace is a rich source of biologically valuable substances such as unsaturated fatty acids and natural antioxidants (phenolic compounds, tocopherols, carotenoids). The value-added use of pomace which mostly consists of seeds could expand the market for raspberries and the profit margins for growers and processors. Therefore, the aim of the work was to measure the content and the fatty acid composition of oil extracted from the seeds of eight raspberry cultivars as well as the radical scavenging activity of the seed flours (the solid residues left after oil extraction).

The oil content extracted from the dried raspberry seeds varied between 12% ('Nagrada') and 20% ('Ariadne'). The GS-MS analysis revealed that the predominating fatty acid in the raspberry seed oils was linoleic acid (51-57%), followed by α-linolenic acid (27-31%) and oleic acid (2-3%). The content of saturated fatty acids in raspberry seed oils amounted for less than 5% of the total fatty acids methyl esters (FAME) detected. The content of monounsaturated fatty acids (MUFA) varied between 11% ('Sputnica') and 14% ('Nagrada'), while the polyunsaturated fatty acids (PUFA) content varied between 82% ('Zorinka') and 85% ('Sputnica') of total FAME. The oils had favourable n-6/n-3 ratio (the average 1.9:1). In addition, the seed residues left after oil extraction exhibited strong free radical scavenging activities against DPPH and ABTS radicals. The highest radical scavenging activity exhibited methanolic seed flour extracts of 'Novokitaevskaja' and 'Toma'.

The results of this investigation indicate the possible food application of raspberry pomace and its fractions in improving human nutrition and potential value-adding opportunities in raspberry production and processing.

Keywords: Raspberry, by-product, fatty acids, seed flour, antiradical activity

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Poster presentation

Essential Oils and Antioxidant Activities of Phenolic Compounds Present in Mediterranean Spice Plants

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Abstract

Mediterranean diet is considered one of the healthiest diets, because the high consumption of fresh vegetables, olive oil as a principal source of fat and the presence of culinary spices with beneficial health effects.

In order to determine the activity of these phenolic compounds and essential oils present in this Mediterranean spices, we have analyzed three of them, namely basil (*Ocimum basilicum*), oregano (*Origanum vulgare*) and rosemary (*Rosamarinus officinalis*).

The analysis was carried out by means of spectrophotometric, chromatographic methods and by mass spectrometry. Determining the antioxidant activities of phenlic compounds we found that the compound with most presence is oregano, followed by basil and rosemary. Furthermore, the analysis carried out by gas cromatography followed by mass spectrometry (GC-MS) determined the presence of 24 volatile compounds.
Keywords: Phenolic compounds, chromatography, mass spectrometry.

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Poster presentation

Co-occurrence of Type-B Trichothecenes in Spring Wheat Grain from Different Production Systems

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Abstract

Deoxynivalenol (DON) together with two acetylated derivatives, 3-ADON and 15-ADON is present in cereal grains and their products. 15-ADON is more toxic than DON and 3-ADON. Co-occurrence of DON and acetylated derivatives in cereal grain is found worldwide. Until now, DON and its derivatives have been considered equally toxic by health authorities. In this survey, 48 spring wheat samples were analysed for co-occurrence of type-B trichothecenes (DON, 3-ADON, 15-ADON) from different agricultural production systems in Lithuanian. Samples were classified according to the production systems – organic, conventional and intensive. Mycotoxin levels in the spring wheat grain samples were determined by the HPLC method with UV detection. Results showed that a type-B trichothecenes tended to be present at higher concentrations in the grain from the intensive production system. Forty seven percent of the spring wheat grain samples from the intensive production system were co-contaminated with a combination of DON+3-ADON+15-ADON, 12% with DON+3-ADON and DON+15-ADON. 3-ADON and DON were found in 6% and 17 % of the tested samples respectively. Six percent of the samples were free from mycotoxins. In the grain samples from the conventional production system, DON and a combination of DON+3-ADON showed a higher incidence - 47% and 23%, respectively. The samples with a combination of DON+3-ADON+15-ADON accounted for 18%. Completely different results were obtained from the analyses of organic grain samples. A great number of organic spring wheat grain samples were contaminated with DON+3-ADON (55%), DON (36%). The combinations of DON+3-ADON+15-ADON and DON+15-ADON were present at the lowest concentrations - 0% and 9%, respectively.

The production systems did not lead to significant differences in mycotoxin levels, although a trend toward higher incidence and higher contamination was observed in the samples from intensive and conventional production systems. Given that grains are contaminated with DON and acetylated-DON, the higher toxicity of 15-ADON should be taken into account. In our study, 15-ADON was prevalent in the spring wheat grain samples from all production systems - intensive (59%), conventional (39%) and organic (9%).

Keywords: Trichothecenes, spring wheat, co-occurrence, grain production systems.

Acknowledgements: This abstract presents findings from the research programme ‘Harmful Organisms in Agro and Forest Ecosystems’ implemented by Lithuanian Research Centre for Agriculture and Forestry.

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Poster presentation

Analysis of Antioxidant Properties of Extracts of Medicinal Plants and Their Mixtures

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Abstract

Many science publications about various medicinal plants usually characterize one or a few plants separately, but none of them evaluate how these popular and widely used plants affect each other. The purpose of this research was to determine the total amounts of secondary metabolites in separate medicinal plants and their mixtures by spectrophotometric methods. In addition, elemental analysis of these separate plants was carried out. Seven medicinal plants were analysed: leaves of peppermint (*Mentha piperita* L.), lemon balm (*Melissa officinalis* L.) and raspberry (*Rubus idaeus* L.), thyme (*Thymus vulgaris* L.) herb, blossoms of chamomile (*Matricaria recutita* L.), seeds of caraway (*Carum carvi* L.) and rose hips (*Rosa* L.). All these plants were grown in the Kaunas Botanical Garden of Vytautas Magnus University. The three mixtures of plants were made parallel to the three Lithuanian commercial ETNO teas (JSC Švenčionių Vaistažolės). Spectrophotometric assays (evaluating total phenolic compounds content, total flavonoid content and antiradical activity) with separate medicinal plants showed that the highest total amount of phenolic compounds was found in peppermint leaves (242.36 mg rutin equivalents (RE)/g of dry plant material), the lowest – in the seeds of caraway (22.85 mg RE/g of dry plant material). The highest and lowest total flavonoid contents were detected in peppermint and in rose hips, i.e. 97.49 and 0.78 mg RE/g of dry plant material, respectively. The highest radical scavenging activity was in the leaves of peppermint and lemon balm (291.30 and 289.71 mg RE/g of dry plant material, respectively), while caraway seeds showed the lowest activity (18.54 mg RE/g of dry plant material). All of the herbal mixtures showed the synergistic effect of phenolic compounds, while the interaction of flavonoids was rather more antagonistic.
The highest amount of magnesium was determined in lemon balm (5.15 mg/g), whereas rose hips had the smallest amount (1.26 mg/g). The highest quantity of iron was found in thyme (0.42 mg/g), the smallest – in rose hips (0.02 mg/g).

Keywords: Antioxidant activity, phenolic compounds, flavonoids, radical scavenging activity, synergism, antagonism, elemental analysis

Acknowledgements

Authors want to thank the scientists Dr. Angelo Massacci, Dr. Daniela Di Baccio, Dr. Massimo Zacchini, technician Ermenegildo Magnani and others from IBAF, CNR (Italy) for the opportunity to perform the elemental analysis and for all of the assistance.

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Poster presentation

Evaluation of Extraction Factors Influence on Total Phenolic Content and Antioxidant Activity of *Melissa officinalis* L. Leaves Extracts

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Abstract

Extraction factors influence the quantity and quality of extracts. It is important to choose the suitable conditions for valuable product. The aim of this study was evaluate the extraction factors influence on quality of lemon balm extracts. Dry leaves of *Melissa officinalis* L. were extracting by dynamic maceration using ultrasound. Extraction factors were solvent – water and 50 % ethanol; extraction time - 5, 10, 15 and 30 min, raw material and solvent ratio was 1:10, 1:20 and 1:30, and particle size of dry leaves. Qualitative parameters - amount of total phenolic content and antiradical activity by 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical inactivation were determined spectrophotometrically. Total quantity of phenolic compounds of all determinations ranged between 13.6±0.99 – 78.49±8.06 mg/mL RAE. The higher amount of phenolics was extracted with 50% ethanol than with water. 10 – 15 min was optimal extraction time for the phenolics, longer time shows lower results. 1:10 ratio of leaves and solvent is most effective with all analyzed conditions. Reduction of particle size increased the yield of phenolics over 20%. Antioxidant activity of all measurements ranged between 37.60±5.83 % – 89.48±0.62 % inactivated DPPH radicals. The highest antiradical activity was achieved at 1:10 ratio after 15 minutes of ethanolic extracts using reduced particle size of leaves. Relationship between total content of phenolics and antiradical activity was evaluated and showed moderate correlation. Quantitative results were compared to the standardized lemon balm dry extract contained 7 % of RA. Total phenolic content was 0.941±0.1 mg/ml RAE and antioxidant activity 42.89±1.5 % inactivated DPPH radicals. Substantial results approve beneficial properties of liquid water extract and dry extract of *Melissa officinalis* L as valuable active substances.

Keywords: Melissa officinalis, phenolics, antioxidant activity, extracts.

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Poster presentation

Quantitative Composition of Phenolic Compounds in Apple Leaves during Vegetation Period

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The aim of the research – investigation of quantitative composition variation of phenolic compounds during vegetation period in apple leaf samples of cultivar *'Ligol'* grown in Lithuania and evaluation of the antioxidant activity of these extracts.

The objectives of the research:

1. Evaluation of quantitative composition variation of phenolic compounds during vegetation period in extracts of apple leaf samples of cultivar *'Ligol'*.
2. Evaluation of quantitative composition variation of flavonoids during vegetation period in extracts of apple leaf samples of cultivar *'Ligol'*.
3. Evaluation of quantitative composition variation of proanthocyanidins during vegetation period in extracts of apple leaf samples of cultivar *'Ligol'*.
4. Evaluation of the antioxidant activity in extracts of apple leaf samples of cultivar *'Ligol'* using ABTS, DPPH, FRAP and TFPH UV-VIS spectrophometric methods *in vitro*.

Research object and methodology: *'Ligol'* apple leaves were harvested at different phenology phases between June and November 2013. The total phenolic compound, flavonoid and proanthocyanidin content in the apple leaf extracts were determinated with UV-VIS spectrophotometry. Antioxidant activity in apple leaf extracts determinated by DPPH, ABTS, FRAP, TFPH, and UV-VIS spectrophotometric methods *in vitro.*

Results: the maximum total amount of phenolic compounds (235.15±10.34 mg/g) in apple leaf samples was established at the beginning of leaf growth (May 20), the smallest (145.81±7.29 mg/g) at the end of phenology period of leaf maturation (October 7). At the end of vegetation (November 4) the amount of phenolic compounds increased to 167.91±6.23 mg/g.

The maximum total amount of flavonoids (27.37±0.86 mg/g) in apple leaf samples was established at the end of the vegetation period (November 4), the minimum (4.4±0.12 mg/g) at the phenology period of leaf development (June 3).

The maximum total amount of proanthocyanidins (9.18±0.36 mg/g) in apple leaf samples was established at the stage of leaf aging (November 4), the smallest amount (1.41±0.03 mg/g) at the beginning of leaf growth (May 20).

The strongest antiradical activity in apple leaf samples was established during leaf maturation phase (August 8) and at the end of maturarion period (November 4) (134.93±5.62 and 133.93±8.5 μmol TE/g accordingly). The weakest antiradical activity in apple leaf samples was established in June 17 (43.16± 2.03 μmol TE/g).

Conclusions: Apple leaves contain large amounts of phenolic compounds therefore they are a promising material for food supplements enriched with apple leaf extracts and for production of individual compounds with specific biological activity.

Keywords: *'Ligol'*, apple leaves, phenolic compunds, vegetation period, antioxidant activity, UV-VIS spectrophometry.

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Poster presentation

Introduction and Chemical Analysis of *Grindelia Squarrosa (Purch) Dunal* in Vytautas Magnus Univesity

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Abstract

At present and in the future it is necessary to enrich the assortment of the cultivated medicinal plants. Species of medicinal (aromatic) plants have been introduced ex~*situ*~in the medicinal plants collection~*of Sector of Medicinal Plants*~at Kaunas Botanical Garden of Vytautas Magnus University~since 1924 for this purpose. Biodiversity of plant resources, scarce information on bioactive compounds and their properties in many species, including those growing in Lithuania, as well as increasing demand for naturalness of~herbal medicines and food were the main motivation aspects of the present study. Medicinal (aromatic) plants, their raw materials and biologically active compounds were investigated.

Grindelia is a famous genus of medicinal plant; the species *robusta* and *squarrosa* are, in particular, the most used in medicinal industry. The phenolic compounds, flavonoïds in particular, have a function in the medicinal property of *Grindelia.* But the concentrations of these products are changing between the different periods of development of the plant. The target of this experiment is to analyze the difference of the concentration of phenolic compounds, flavonoïds and radical scavenging activity between growing periods, budding period, start blooming, full blooming and end of blooming period. *Grindelia squarrosa (Purch) Dunal* was collected and air-dried at Kaunas Botanical Garden of Vytautas Magnus University. The plant was crush and 0,5g were extracted with 20 ml of 75% methanol and shacked during 24h. Spectrophotometry methods were used to find total phenolic compounds (using Folin-Ciocalteu Reagent), the content of flavonoïds and radical scavenging activity (using DPPH as a radical reagent). The results are showing that the radical scavenging activity is less important than the total phenolic compounds; we can explain this result because all the phenolic compounds don't have abilities to reduce DDPH. We can already say that the flavonoids are always lower than the total phenolic compounds, it's normal because the flavonoïd are already phenolic compounds but there are other types of phenolic compounds like tannins in the plant. The results suggest that the maximal amount of phenolic compounds is during the intensive growth period. So the scavenging activity is bigger during the intensive growth period as the content of total phenolic compounds already is higher in the plant during this period too. It also suggest that the maximal amount of flavonoids is find in the full blooming period, we can explain this results because the flavonoids are an element of the colorization of the flower.

Keywords: *Grindelia Squarroza (Purch) Dunal*, phenolic compounds, flavonoids, radical scavenging activity.

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Poster presentation

Seasonal Variation of Phenolic Compounds Composition and Antiradical Activity in *Sorbus aucuparia* L. Bark

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Abstract

Sorbus L. genus is one of the most numerous genera of the Rosaceae family. Various rowan species are used for food and as a source of medicinal raw plant material. Our research aim – to investigate qualitative and quantitative composition of phenolic compounds in bark samples of rowan (*Sorbus aucuparia* L.) plants during the growth season and to evaluate antiradical activity of ethanol extracts of rowan bark samples. Total content of phenolic compounds varied from 6.40 to 10.51 % during the growth season. In bark samples quantitative estimation of neochlorogenic acid (0.004-0.193 %), chlorogenic acid (0.008-0.026 %) and isoquercitrin (0.001-0.003 mg/g) was performed. Bark samples collected during budding stage were characterized by higher contents of neochlorogenic acid. Bark samples collected during budding stage and early flowering stages were characterized by higher contents of chlorogenic acid while bark samples collected during early flowering stage were characterized by higher contents of isoquercitrin. Studies of antiradical activity show that rowan bark samples are characterized by strong antiradical activity. The strongest antiradical activity was determined in rowan bark samples collected during budding stage, early flowering and fruit ripening stages. In conclusion, analysis of the composition of phenolic compounds in bark samples of rowan revealed a close relationship between the content of phenolic compounds in rowan raw plant material and different vegetation stages. Data on differences in phytochemical composition and antiradical activity of rowan bark samples in the course of various vegetation stages can be used for rational planning of the collection of raw plant material rich in phenolic compounds with specific biological effects.

Keywords: *Sorbus aucuparia*, bark, phenolic compounds, antiradical activity.

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Poster presentation

Variations of Probiotic Characteristics among Nisin Producing *L. lactis* Strains Derived from Milk and Fermented Grain

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Abstract

The aim of the study was to select nisin producing *Lactococcus lactis* strains isolated from milk and fermented grain with probiotic characteristics and suitable for industrial application. In total 12 nisin producing *Lactococcus lactis* strains isolated from raw cow, goat milk and fermented grain samples were evaluated for their probiotic potential. Properties such as bile tolerance, tolerance to low pH, antagonistic activity, demonstration of safety have been evaluated. Although acidity is reported to have the most negative effect on survival of probiotics, all tested strains were able to tolerate acid with a survival rate being over 84% after 3 h incubation with PBS (pH 2.5) solution. Two strains showed 98% survival rate after 3h incubation with the number of viable bacteria count being 7.8±0.13 and 7.5±0.01 log CFU/ml respectively. Not all strains were resistant to 0.3% ox bile after 24 h incubation. Only seven strains showed resistance whereas one strain showed 74% survival rate after 24 h incubation with the number of viable bacteria count being 5.6±0.03 log CFU/ml. All strains were evaluated for hemolytic activity on sheep blood agar. α-hemolytic activity was determined for three strains, none of the strains showed β-hemolytic activity and φ-hemolytic activity as determined for nine strains. Antibacterial activity against eight pathogenic and food spoilage bacteria including *Bacillus cereus*, *Salmonella typhimurium* and others were also assessed. All tested strains showed clear inhibition against tested bacteria. Against *Salmonella typhimurium* and *Eschericia coli* a better antagonistic activity was expressed by isolates from fermented grain samples and against *Listeria monocytogenes* a better antagonistic activity was expressed by isolates from raw milk samples. Six strains had properties that are characteristic for probiotics with potential for application in functional foods (1 isolated from raw cow milk and 5 from fermented grain samples). These strains after further testing could be applied for probiotic functional foods development.

Keywords: Probiotics, *Lactococcus lactis,* antagonistic activity, bile tolerance.

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Poster presentation

Analysis of Volatile and Non-volatile Biologically Active Compounds Found in the Ground Segment of *Silybum marianum* L. During Different Stages of Vegetation

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Abstract

Analysis of volatile and non-volatile biologically active compounds found in the ground segment of *Silybum marianum* L. by using GC-MS system and spectrophotometric methods was completed to determine the most appropriate medicinal raw materials of the ground segment collection time and analysis of meteorological conditions for accumulation of biologically active compounds in the ground segment of *Silybum marianum* L. was completed to determine the optimal conditions for the accumulation of these compounds. 21 volatile compounds have been identified. The amount of total phenolic compounds of the year 2014 plant raw materials ranged from 17.80 mg / g RE (rutin equivalents) to 26.58 mg / g RE, of the year 2015 plant raw materials ranged from 23.25 mg / g RE up to 60.45 mg / g RE. Total flavonoid content of the year 2014 plant raw materials ranged from 10.86 mg / g RE up to 17.81 mg / g RE. The year 2015 plant flavonoids of the raw material content ranged from 8.02 mg / g RE up to 42.45 mg / g RE. Radical scavenging activity varied from 5.56-5.63 mg/g RE of the year 2014 and from 10.27 -11.63 mg/g RE of the year 2015. A good time to plant medicinal raw material collection is full flowering vegetation stage. The most favorable meteorological conditions during vegetation period for accumulation of biologically active compounds of milk thistle is aired conditions. Hydrothermal coefficient HTC = 0.61 and lower duration of solar lighting.

Keywords: Milk thistle, volatile compounds, total phenolic compounds, radical scavenging activity, meteorological conditions, vegetation periods

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Poster presentation

Effect of Commercial Starter Cultures with Dihydroquercetin on Microbiological, Physical-Chemical Indexes and Acceptability of Cold-Smoking Pork Sausage

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Abstract

The need for high-quality safe products has resulted in the use of natural antioxidants with starter cultures (SC) for the production of cold-smoking pork sausages. The aim of this study was to investigate the effects of DHQ and mixture of DHQ with different SC: *Pediococcus pentosaceus, Staphylococcus xylosus*, *Leuconostoc carnosum* on the quality and safety parameters of the sausages.

During the storage time samples were kept at 4±0.5 °C temperature. The number of mesophilic aerobic bacteria counts were determined on PCA, *Enterobacteriaceae* on VRBGA, *Escherichia coli* on TBX, *Pseudomonas* spp. on PAB, yeast and mold on YMA and lactic acid bacteria on MRS, number of biogenic amines were identified too. We also measured pH, colour parameters (CIE L* a* b* system) and lipid oxidation (peroxide and acids values) in the cold-smoking sausages after 0, 19, 52, 147 and 200 days of storage.

Initial microbial counts revealed that, *E. coli, Pseudomonas* spp., yeast and mold counts (respectively, 0.99±0.13; 3.66±0.33 and 4.10±0.20 log CFU/g) were similar in all batches of sausages. Mixture of DHQ with SC had better antagonistic effect against these microbial groups compared to other cases of sausages. During the fermentation process in samples with mixture of DHQ and SC the count of *E. coli* decreased by 0.31±0.09 log CFU/g after 19 days of ripening, *Pseudomonas* spp. by 0.39±0.05 log CFU/g and *Enterobacteriaceae* by 0.70±0.17 log CFU/g after 52 days of ripening, reducing effect was higher compared to control sample with all cases of microorganisms, when $p \leq 0.05$. During the ripening period, samples with mixture of DHQ and *P. pentosaceus, S. xylosus* had the best reducing effect on yeast and mold count, which was decreased by 81.19±0.22 log CFU/g, compared to control sample ($p \leq 0.05$). Besides mixture of DHQ and *P. pentosaceus, S. xylosus* had the highest acceptability compared to other batches of sausages.

Statistical analysis displayed that instrumental colour and pH values were significantly affected by the ripening time and mixture of DHQ with SC (respectively, $p \leq 0.05$; $p \leq 0.05$). During ripening, DHQ without SC decreased the levels of peroxides and acids most considerably (respectively, by 4.00±0.50 meq/kg and 5.68±0.24 mg KOH/g). The amount of biogenic amines was far below the levels causing any health risk.

Mixture of DHQ, *P. pentosaceus* and *S. xylosus* is a natural and safe way to increase the acceptibility and the safety parameters of the cold-smoking pork sausages.

Keywords: Cold-smoking sausages, starter cultures, dihydroquercetin.

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Poster presentation

Comparative Study of Recovery and Selectivity of Extraction Methods of Winter Savory (*Satureja montana* L.) Essential Oil

Eventas Šaučiūnas[1], Audrius Sigitas Maruška[1], Ona Ragažinskienė[2], Tomas Drevinskas[1], Mantas Stankevičius[1]

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Abstract

Medicinal and aromatic plants are widely used for prevention and treatment of diseases There is increasing interest in medicinal plants which accumulate not only biologically active phenolic compounds or vitamins, but also essential oil [3]. Winter savory (*Satureja montana* L.) like other *Lamiaceae* family plants is rich in essential oil [2] and non-volatile phenolic compounds [1]. Therefore this plant was chosen as a model to investigate different extraction methods of essential oil such as aqueous 75 % methanol extraction, supercritical CO_2 fluid extraction and hydro distillation. The aim of this study was to compare recovery and selectivity of different extraction methods of *Satureja montana* L. essential oil. Results of the investigation have shown that the highest extraction recovery of *S. montana* L. essential oil is obtained by 75 % methanol extraction – 51.2 ± 2.2 %. Extraction methods differed by selectivity for different compound classes of the essential oil: hydro distillation method demonstrated the highest selectivity for monoterpenes while selectivity for oxygenated monoterpenes was relatively similar of all investigated extraction methods. It was shown that 75 % methanol extraction method is of maximum selectivity for sesquiterpenes which is comparable to selectivity determined by supercritical CO_2 fluid extraction method. Moreover, investigation revealed that supercritical fluid extraction method at the conditions investigated and hydro distillation selectively extract only volatile essential oil components.

Keywords: Essential oil, extraction methods, recovery, selectivity, carvacrol, *Satureja montana* L.

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Poster presentation

Agronomic and Biochemical Implements for Reduced Nitrate Contents in Green Vegetables

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Abstract

Green leafy vegetables occupy a very important place in human diet, but unfortunately, constitute a group of foods, which contributes maximally to nitrate consumption by living beings. This is of high importance in intense greenhouse horticulture systems, where the applied technologies tend more to the high vegetable productivity, external quality and post-harvest shelf-life, than to nutritional value of produce. There are multiple opinions about the harm of nitrates on adult organisms, however, the Regulations of European Commission restricts nitrate concentrations in green vegetables. The aim of this study was to review the works performed at Lithuanian Research Center for Agriculture and Forestry, institute of Horticulture, seeking to control nitrate contents in green vegetables and explain the metabolic traits between nitrate assimilation and photosynthesis processes. The effects of light parameters (spectra, intensity), as well as other environmental factors (substrate type, mineral nutrition, temperature, seasonality, etc.) have remarkable effects on nitrate assimilation processes, thus combining these implements with photophysiological knowledge it is possible to achieve the balance between agronomic objectives and improved nutritional value of green vegetables.

Keywords: nitrates, nitrites, nitrate reductase, photosynthesis intensity, proteins, sacharides.

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Poster presentation

Chemical Composition of Birch Sap and Its Quality Changes during Storage

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Abstract

The results of investigations, which were carried out by scientists of North countries, about birch sap chemical composition, mechanism of its secretion and medical effect show nutritional and healing value of this product [1, 2]. Birch sap was being used in Lithuania from old times in folk medicine. There are no results of sap quality investigations in Lithuania. This study evaluated biochemical, chemical and physical parameters of the collected fresh birch sap. There was found in birch sap on the average 1.1 % of dry soluble solids, 1.07 % of sugars, 7.2 mg 100 g^{-1} of ascorbic acid, small amount of phenolic compounds (1.45 mg 100 g^{-1}) and organic acids. Nitrates in birch sap samples weren't detected. In fresh birch sap there found nine macro- and microelements. The biggest amount comprised potassium (K) – 115 mg kg^{-1}. In order to create and produce good quality birch sap product, it is necessary to investigate the properties of this raw matter, their changes during storage and processing and to choose the parameters of production process. We didn't find the results of investigation of this product storage. Therefore, the aim of our study was to evaluate the changes of birch sap during storage and fermentation and to optimize storage parameters. It was established that birch sap for fresh usage can be stored up to two months. The most suitable storage temperature for this product is 0±0.5 °C, +2±0.5 °C. Titratable and active (pH) sap acidity during storage changes most of all, the amount of dry soluble solids – least of all. The indices of birch sap distinctive electrical conductivity changes lest of all when storage temperature is low (0±0.5 °C and +2±0.5 °C). When distinctive electrical conductivity coefficient reaches the limit of 1.4 mS/m, sap begins to opalesce. Nevertheless, storing at different temperature, the changes of sap colour quality aren't very significant and the dynamic of their change depends on storage temperature and duration. The depression of freezing temperature suggests that it is possible to store fresh sap also in low temperature up to -1.4 °C. This would help to prolong the duration of its storage.

Keywords: birch sap, biochemical composition, storage.