## Poster session

## Scientific studies on the biological activity of plant originated products

Chairs:

Professor Irena Matławska, PhD habilitated in pharmaceutical sciences

Andrzej Ostrowicz, PhD in pharmaceutical sciences

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   Department of Biology and Pharmaceutical Botany, Medical University of Gdansk

2. **Study of the neuroprotective activity of betulin in Alzheimer's disease**
   Agnieszka Zakrzeska[1], Natalia Szymańska[2] Ph.D., Paweł Kitlas[2] MSc, Mikołaj Tomulewicz[2] Ph.D.
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   Agnieszka Zielińska, Ph.D., Mariusz Bochnia, MSc
   Department of Organic and Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw

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5. **Synthesis of curcumin co-crystals with gallic acid and analysis of their antioxidant activity**
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6. **"Pepper" as a source of compounds with anti-cancer potential - application of in silico methods in the search for Aurora A kinase inhibitors**
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## 1. Yamogenin – a steroidal saponin of plant origin with anticancer activity

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**Introduction**. Steroid saponins are a group of high-molecular-weight compounds with the spirostane or furostane structure [1]. One of the best known steroidal saponins is diosgenin with specific biological and pharmacological properties [2, 3]. Much less is known about neodiosgenin, called yamogenin ((25S)-spirost-5-en-3beta-ol) which occurs among others in *Trigonella foenum-graecum*, *Dioscorea collettii* and *Asparagus officinalis* [4-6]. Yamogenin has cytotoxic properties against cancer cells, but its mechanism of action is not yet known. The aim of the study was to determine the cytotoxic effect of yamogenin and selected cellular factors involved in the death of human ovarian cancer SKOV-3 cells *in vitro*.

**Material and methods.** Yamogenin was dissolved in absolute ethanol using an ultrasonic bath, then the solution was added to SKOV-3 cells (in the concentration range of 10-70 µg/mL). The cells were incubated for 24 h. Cytotoxicity, including determination of $IC_{50}$ values (inhibitory concentration) were performed using the MTT test. Determination of the level of oxidative stress in the cells, change of mitochondrial potential, inhibition of the cell cycle and caspase-8/-9 activity were performed with flow cytometry and a luminometer.

**Results.** The results obtained with the MTT assay indicate that yamogenin has cytotoxic effect in SKOV-3 cells ($IC_{50}$ value was 16.70 ± 0.10 µg/mL). Yamogenin caused a strong inhibition of the cell cycle in the sub-G1 phase, which may indicate the induction of the apoptotic process in the tested cells. Moreover, yamogenin caused an increase in the level of oxidative stress, a decrease in the mitochondrial potential and a strong activation of caspase-8/-9 in SKOV-3 cells incubated with the compound.

**Conclusions.** Yamogenin strongly inhibits the proliferation of human ovarian cancer SKOV-3 cells and causes cell death by apoptosis.

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## 1. Study of the neuroprotective activity of betulin in Alzheimer's disease

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**Introduction.** Betulin *(łac: betula)* is a natural chemical compound extracted from birch bark by solvent extraction. As a substance, it occurs as an almost white crystalline powder. The first isolation of betulin from birch bark was made by the chemist Tobias Lowitz in 1788 [1]. The birch bark extract was mainly used to treat tuberculosis and diseases of the lymphatic system. Nowadays, betulin has also been shown to support the treatment of neurodegenerative diseases [2]. Alzheimer's disease (AD) is a chronic condition characterised by progressive loss of memory and cognitive abilities. β-amyloid deposition and neurofibrillary tangles are the main neuropathological features of AD, occurring in the hippocampus and frontal cortex.
The aim of this study is to evaluate the protective effect of betulin in AD pathology induced in an animal model and to elucidate its mechanism of action.
**Materials and methods.** 40 adult male Wistar rats (200-220 g). Alzheimer's pathology was modelled with ICV-administered streptozotocin (1.5 mg/kg) and the neuroprotective effects of betulin were assessed using behavioural tests: beam walking test and Morris water maze test) and biochemical parameters of brain tissue.
**Results.** The administration of betulin to the animals definitely improved cognitive memory in a statistically significant manner (the time to reach the platform in the Morris maze was comparable to the control group - healthy animals). The situation is similar for the beam-walking test. The administration of betulin does not improve memory and does not eliminate gait disturbances 100%, whereas the administration of betulin in complex with cyclodextrin allows 100% improvement. TNF-alpha levels, on the other hand, decreased to the value observed in the control group after betulin in complex with cyclodextrin (control 7.29±0.51 vs ICV-STZ +C 6.88±0.54; $p<0.05$).
**Conclusions.** The results show that betulin can be used in the prevention and possible treatment of AD. It is necessary to establish the cellular mechanism of action of betulin on neuronal cells and possible clinical trials before a new drug can be introduced into clinical practice. Nevertheless, ongoing research is very promising.
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3. **Qualitative and quantitative analysis of dietary supplements containing monacolin K**

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**Introduction.** According to statistics, hypercholesterolaemia affects 60 percent of the adult Polish population, i.e. about 18 million people. The most popular drugs used in the treatment of this disease are statins. Compounds from this group of drugs can also be found in dietary supplements with red yeast rice extract, containing monacolin K, structurally and functionally identical to lovastatin (a statin medication). When using the extract, an effect similar to the results obtained in pravastatin and lovastatin therapies can be observed. Studies show identical side effects to taking supplements containing red yeast rice extracts compared to using statin therapy. Therefore, the simultaneous intake of lovastatin in the form of a drug and supplementation with products containing the extract can take place, that causes the accumulation of statin dosages and increasing the risk of side effects hazardous to health.

The aim of the research was the quantitative and qualitative analysis of selected dietary supplements with monacolin K available on the Polish market, and in particular to compare the content of this substance with the product labeling. Any discrepancies may be a field for discussion on the safety of using dietary supplements containing statins.

**Material and methods**. Seven supplements from different manufacturers with similar labelled amounts of the extract were selected for the study. The optimal conditions for the extraction of active substances were developed. The measurements were performed using the methods of UV/VIS spectrophotometry and HPLC-DAD chromatography.

**Results.** Data obtained from HPLC measurements allowed to confirm the presence of monacolin K in products, calculate its content and confront the obtained results with manufacturers' declarations placed on the packaging. Percentage differences between the actual content and the labelled content ranged from -77% to + 45%.

**Conclusions.** The occurrence of discrepancies between the measured and labelled values may contribute to an increased risk of side effects for supplements that exceed the declared content of monacolin K or to a reduction of the therapeutic effect when the substance is too low or not released from the product form.

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**4. *In silico*, *in vitro*, and *in vivo* studies of curcumin**

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**Introduction.** Curcuminoids are a class of organic compounds that are primarily responsible for the yellow colour of turmeric, the spice commonly used in Indian cuisine. Curcumin is the most studied and most potent compound among curcuminoids. It is a polyphenol that is derived from the rhizomes and plant stems of the herb, turmeric [1]. Curcumin has antioxidant, anti-inflammatory, and anticancer activities [2]. The biological action of curcumin can be due to its binding to various molecular targets.
**Materials and methods.** In this work molecular docking calculations were carried out using Surflex software. Capsaicin, dihydrocapsaicin, nonivamide (chili pepper), curcumin (turmeric), and piperine (black pepper) were selected as a set of ligands for molecular docking to human and zebrafish γ-estrogen receptor (to compare curcumin results to constituents of other peppers spices). Then the Zebrafish toxicity test was performed in the first 48h of zebrafish embryo development. Compounds were tested at the concentrations 5 and 10 mg/l. Antioxidant studies were determined by FRAP test and determination of total polyphenols content in 11 samples of beverages/drinks with curcumin available on Polish market.
**Results.** Molecular docking studies showed a high affinity of dihydrocapsaicin and capsaicin to Zebrafish γ -estrogen-related receptor. The high value of the $R^2$ factor (0.823) for the correlation of total score functions between human and zebrafish receptors was observed. Zebrafish studies revealed that the least toxic compounds at the concentration of 5 mg/l were piperine and curcumin. Only at the concentration of 10mg/l, they lead to high mortality (around 50-75%). As a second step, we tested. The antioxidant activity determined by FRAP test ranges from 255 to 6314 µM $Fe^{2+}$/ml. The polyphenol content ranges from 127 to 1315 mg GAE/l.
**Conclusions.** Curcumin is a potent antioxidant and consumed in beverages may support our health. Our studies show also a high safety of using curcumin not only to humans, but also to the aquatic environment.
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**5. Synthesis of curcumin co-crystals with gallic acid and analysis of their antioxidant activity**

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**Introduction.** Curcumin is one of the compounds of the complex of curcuminoids found naturally in the turmeric rhizome called turmeric. Turmeric is used as a spice, dietary ingredient, and part of traditional East Asian medicine for its immunomodulatory, anti-inflammatory and antioxidant properties. The biggest problem with curcumin's biological activity is that it is difficult to dissolve in water and has low bioavailability. The aim of the work is to obtain and physicochemical characterization of pharmaceutical curcumin cocrystals and to investigate their antioxidant properties.
**Material and methods.** In order to optimize the process of obtaining curcumin co-crystals with gallic acid, the method of dynamic grinding of substances with the addition of a solvent was used, and both substances were melted and cooled slowly. Then, X-ray diffraction measurements on the powder material (PXRD) were carried out. The antioxidant properties of curcumin co-crystals were also compared with gallic acid in relation to the properties of curcumin itself (FRAP and DPPH tests with spectrophotometric measurement were used).
**Results.** The method of grinding equimolar amounts of curcumin and gallic acid with the addition of 96% ethanol made it possible to obtain curcumin co-crystals, which was confirmed by PXRD analysis. The obtained co-crystals showed better antioxidant properties than pure curcumin in in vitro tests.
**Conclusions.** Later, studies of the dissolution profile of the obtained cocrystals are planned. However, preliminary results indicate that the obtained new form of curcumin may be an interesting alternative to pure curcumin used in popular dietary supplements, although this requires further, in-depth analyzes.
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**6. "Pepper" as a source of compounds with anti-cancer potential - application of in silico methods in the search for Aurora A kinase inhibitors**

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**Introduction.** The Aurora kinase family, including aurora kinases A, B, and C is a group of highly conserved serine/threonine kinases that are important for the correct transition through mitosis [1]. Although the faithful process of mitosis is connected with the activity of Aurora kinase's, its overexpression may be a connected with development and course of some cancers, such as breast and gastric cancer [2]. It is associated with the partial degradation of the p53 protein, the physiological function of which is leading damaged to the apoptotic pathway [3]. Moreover, that overexpression of Aurora kinases induces chemoresistance in breast cancer cells. Since therapeutic process demands the inhibition of overexpressed Aurora kinase, the use of several small molecule aurora kinase inhibitors as potential anticancer therapeutic is being investigated. Although, there are many studies of the synthetic molecules, still natural products or directly derived compounds play crucial role in the discovery of new drugs.

In this study we asked the question: whether the compounds contained in the *Piper*, *Capsicum*, and *Pimenta* (the main genera of peppers consumed worldwide) and their analogues may act as Aurora A (Aurka) inhibitors? Since capsaicin is one of the best known activator of ion-channel type TRPV1 receptor, we looked for connection between TRPV1- and Aurora inhibiting activity.

**Material and methods.** The study was performed using molecular docking. The set of ligands was composed of 16 compounds occurring in *Piper*, *Capsicum* and *Pimenta* genera or their derivatives. Molecular docking calculation were carried out to Aurka and TRPV1. To observe the relationship between measured variables, PCA (Principal Component Analysis) was applied. We compared our results with anticancer activity studied earlier of $IC_{50}$ on MCF-7 cell lines (breast cancer cells).

**Results.** The correlation between Aurka Total Score and $IC_{50}$ was statistically significant. It is possible, that inhibition of overexpression of Aurka is one of the mechanisms of anticancer activity observed in MCF-7 cell lines studies.

**Conclusions.** Our research indicates that the compounds contained in peppers may inhibit Aurora A. Further research *in vitro* is planned to confirm these reports.

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