Poster session

Plant in vitro culture as source of biologically active secondary metabolites

Chairs:
Professor Irena Matławska, PhD habilitated in pharmaceutical sciences
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16. The potential of plant stem cells as a source of bioactive compounds with anti-aging effect

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17. Extracts from *Schisandra henryi* suspension cultures rich in polyphenolic compounds with potential application in the treatment of neurodegenerative diseases

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18. Cytotoxic activity of hydromethanolic extract from hairy roots of *Salvia bulleyana* Diels grown in optimized conditions

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19. Antioxidant, antiproliferative and antibacterial activities of biomass extracts from various types of undifferentiating *in vitro* cultures and from the herb of *Verbena officinalis* L.

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20. **Antioxidant and antibacterial activity of *Verbena officinalis* L. extracts from various types of microshoot cultures and extracts from plants grown *in vivo***

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21. Bioaccumulation of health-promoting elements in chia (*Salvia hispanica* L.) microshoots as a new model for fortified food

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16.The potential of plant stem cells as a source of bioactive compounds with anti-aging effect

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Introduction. The cosmetics industry has been promoting the innovative concept of "plant stem cells" (RKM) and their use in modern anti-aging cosmetics aimed at protecting and extending the life of human epidermal stem cells (LKM) for over a decade. Cosmetic formulations intended for aging skin contain the content of cells from callus and suspension released as a result of cell wall digestion, rich in specific epigenetic factors and bioactive compounds. Plant biotechnology is an alternative to the production of active cosmetic substances on an industrial scale.

Material and methods. *In vitro* cultures (induction and proliferation of callus cells / establishment and stabilization of cell suspension) of the following species: *Chaenomeles japonica* (Japanese quince), *Eryngium planum* (Flat sea holly), *E. campestre* (Field eryngo), *E. maritimum* (Sea holly), *Lychnis flos-cucul*i (Ragged robin), *Linnaea borealis* (Twinflower), and *Plantago ovata* (Plantain).
Qualitative and quantitative phytochemical analyzes conducted by chromatographic methods (TLC, HPLC).

Results. The stabilized callus cultures (and cell suspensions) were obtained, characterized by good biomass growth parameters and the accumulation of bioactive compounds (mainly antioxidants) from the above-mentioned species.

Conclusions. Plant stem cells of *Ch. japonica*, *E. planum*, *E. campestre*, *E. maritimum*, *L. flos-cuculi*, *L. borealis*, and *P. ovata* may be potential sources of bioactive anti-aging compounds.

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17. Extracts from *Schisandra henryi* suspension cultures rich in polyphenolic compounds with potential application in the treatment of neurodegenerative diseases

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Introduction. Neurodegenerative diseases are a group of diseases of an acquired or innate nature of the nervous system in which nerve cells are lost. The main etiological factor contributing to the development of this type of disease is chronic oxidative stress resulting from disturbances in homeostasis between the production of free radicals and their inactivation by cellular protective mechanisms [1]. Plants are the main source of secondary metabolites, especially polyphenolic compounds with valuable activities, like: antioxidant, anti-inflammatory and anti-cancer [2]. *Schisandra henryi* C.B. Clarke is an endemic, East Asian plant belonging to the Schisandraceae family. The carried out by us former phytochemical studies on *S. henryi*, were confirmed the presence of polyphenols, such as phenolic acids and flavonoids [2].

Materials and methods. The studies were performed on *S. henryi* suspension cultures, which were initiated and cultivated on Murashige and Skoog [3] liquid medium containing plant growth regulators: 2 mg/l indolyl-3-butyric acid and 0.5 mg/l 6-benzyladenine. The experiment was carried out in flasks on an Ohaus shaker (model SHEX1619DG, 120 rpm). The 10, 20 and 30 day breeding cycles (3 series) were tested. Methanol extracts were prepared from the obtained biomass, in which polyphenolic compounds were determined by the DAD-HPLC method [4].

Results. The chromatographic qualitative and quantitative analyzes confirmed the presence of 8 phenolic acids: gallic, caftaric, neochlorogenic, 3,4-dihydroxyphenylacetic, chlorogenic, vanillic, caffeic and syringic acids, as well as 6 flavonoids: hyperoside, rutoside, quercitrin, trifolin, quercetin and kaempferol.

The contents of individual compounds depended on the duration of the growth cycle. The maximum total phenolic acid content was 584.79 mg/100 g d.m. (day 20 of growth cycle). The contents of individual compounds ranged from 0.80 to 178.08 mg/100 g d.m. (3,4-dihydroxyphenylacetic acid, day 20 of growth cycle).

The maximum total content of flavonoids was 144.79 mg/100 g d.m. (day 10 of the breeding cycle). The contents of individual compounds ranged from 0.12 to 82.32 mg/100 g d.m. (kaempferol, day 10 of growth cycle).

Conclusions. The obtained results are conducive to further research and give hope for the possibility of using the obtained extracts from the biomass of *S. henryi in vitro* cultures in the treatment of neurodegenerative diseases.

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18. Cytotoxic activity of hydromethanolic extract from hairy roots of *Salvia bulleyana* Diels grown in optimized conditions

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Introduction. Nowadays, formulations and compounds derived from plants become popular alternatives to conventional therapies. This is largely due to their effectiveness confirmed by many centuries of use and low toxicity. For many years, compounds obtained from plants have been used in the prevention and treatment of civilization diseases, including cancer therapy. *Salvia bulleyana* is native to the Chinese province of Yunnan. In traditional medicine, it was used as a substitute of *Danshen* (roots of *Salvia miltiorrhiza*) which was commonly used in cardiovascular diseases and as an anti-inflammatory agent.
Plant biotechnology can be used to increase the production of bioactive compounds, with transformed roots being a particularly promising material. In our research, we managed to obtain *S. bulleyana* hairy roots, which, due to the optimization of the cultivation conditions, produced much higher amount of phenolic acids (124.4 mg/g dry weight), and in particular rosmarinic acid (110.2 mg/g dry weight), than the roots of the field growing plant (23.09 mg/g dry weight).
Materials and methods. The aim of the study was to determine the cytotoxic activity of a methanol-water extract (4:1 v/v) from *S. bulleyana* hairy roots. The transformed roots were cultivated for 40 days in ½SH medium with 3% sucrose and subjected to a 3-day elicitation with 100 µM methyl jasmonate. The *in vitro* cytotoxic study was performed using human cervix (HeLa) and gastric (AGS) adenocarcinoma epithelial cells and also human colon epithelial (LoVo). Moreover, normal mouse fibroblasts (L929) were used as an extract safety indicator. The cell metabolism was assessed by the MTT test.
Results. In the concentration range 0.25-5 mg/ml, an increase in the activity of the extract against each of the examined tumor lines depending on its concentration was noted. It turned out that the cells of AGS line were the most sensitive to the extract. The cell viability decreased significantly after applying the 0.5 mg/ml concentration and reached only 30% of the initial value at the highest concentration of the extract used. In the case of the LoVo and HeLa lines, a statistically significant decline in cell viability was observed in the concentration ranges: 1-5 mg/ml and 1.25-5 mg/ml respectively. These treatments resulted in 22–46.5% and 21–39% of dead cells. *S. bulleyana* hairy root extract turned out to be safe for the L929 line in the concentration range of 0.25-2.5 mg/ml.
Conclusion. The obtained results indicate the anticancer potential of the extract from *S. bulleyana* hairy roots. The extract demonstrated significant cytotoxic activity against gastric cancer cell line, while being safe for mice fibroblasts.

19. Antioxidant, antiproliferative and antibacterial activities of biomass extracts from various types of undifferentiating *in vitro* cultures and from the herb of *Verbena officinalis* L.

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Introduction. Vervain herb contains various groups of bioactive compounds - mainly iridoids, phenylpropanoid glycosides, flavonoids and phenolic acids, which determine the valuable medicinal properties of this plant raw material [1]. Since 2008, the herb has the status of a pharmacopeial raw material in the European Pharmacopoeia [2]. The metabolism of cells from established undifferentiated *in vitro* cultures of this species is selectively focused on the production of verbascoside [3]. The aim of the presented research was to documented the biological activity of biomass extracts from various types of undifferentiating *in vitro* cultures and from the herb of *V. officinalis*.

Materials and methods. Agar callus cultures, suspension cultures and bioreactor cultures of vervain (in a commercially available stirred tank bioreactor produced in USA) were maintained. Extracts from *in vitro* biomass and herb of plants grown *in vivo* were tested for antioxidant (DPPH test, reduction of Fe^{+3} ions, chelating activity of Fe^{+2} ions), antiproliferative (human neuroblastoma cell line SH-SYSV) and antibacterial (4 Gram-positive and 8 Gram-negative bacteria strains) activity.

Results. All biomass extracts from *in vitro* cultures and plant material showed strong antioxidant activity in all three tests. In the DPPH and chelating capacity tests, the activity of biomass extracts was stronger than herb extract. The strongest antiproliferative and antibacterial properties were observed in biomass extracts from bioreactor cultures and herb. All tested extracts showed stronger activity against Gram-positive bacteria, especially against *Y. enterocolitica, K. pneumoniae* and *S. epidermidis*.

Conclusion. Proven directions of biological activity are extremely important in treatment and prevention of geriatric ailments. It can be assumed that the activity of biomass extracts is mainly associated with the high content of verbascoside (> 9g% in bioreactor cultures), while the activity of herb extracts is associated with the significant content of iridoids, mainly verbenalin. Biomass extracts from bioreactor cultures can be proposed as a biotechnological raw material for the prevention and treatment of geriatric diseases.

Acknowledge. The research was carried out in cooperation with the units of University of Messina and the Warsaw University of Life Sciences.

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20. Antioxidant and antibacterial activity of *Verbena officinalis* L. extracts from various types of microshoot cultures and extracts from plants grown *in vivo*

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Introduction. Vervain is a plant species present in the European Pharmacopoeia since the 6th edition (2008) [1]. Medicinal plant raw material - Verbena herb contains mainly iridoids, phenylpropanoid glycosides, flavonoids and phenolic acids [2]. In the established microshoot cultures of this species, the main metabolites are phenylpropanoid glycosides - verbascoside and isoverbascoside [3]. The aim of the presented research was to documented the biological activity of extracts from various types of microshoot cultures and from plants grown *in vivo.*

Material and methods. Agar cultures, stationary liquid and agitated cultures and bioreactor cultures of vervain microshoots (in commercially available temporary-immersion system – RITA® bioreactors produced in France) were maintained. Extracts of microshoot cultures and herb of plants grown *in vivo* were tested for antioxidant (using the DPPH test, Fe^{+3} ion reducing test and Fe^{+2} ion chelating test) and antibacterial activity (4 Gram-positive and 8 Gram-negative bacteria strains).

Results. All the investigated microshoot extracts showed a significant ability to remove free radicals (DPPH test), good Fe^{+3} to Fe^{+2} reducing activity and interfered with the formation of the Fe^{+2}-ferrozine complex. The antioxidant activity of *in vitro* cultures extracts was higher than of the herb extract. Bioreactor microshoot extracts showed the highest chelating activity, while agar cultures extracts showed the highest activity in the other two tests. All microshoot extracts showed antibacterial activity against tested Gram-positive and Gram-negative bacteria strains. This activity was stronger against Gram-positive strains, especially against *S. epidermidis*. The most active were extracts from stationary liquid cultures, the least active were herb extracts.

Conclusion. It can be assumed that the high content of phenylpropanoid glycosides in *in vitro* cultures is responsible for the strong antioxidant and antibacterial activity of microshoot extracts. These are known compounds with strong antioxidant activity. The proven directions of *in vitro* microshoot cultures extracts activity indicate the possibility of their use in the prevention and treatment of geriatric diseases.

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21. Bioaccumulation of health-promoting elements in chia (*Salvia hispanica* L.) microshoots as a new model for fortified food

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Introduction. *Salvia hispanica* L. (chia) is the source of chia seeds (*Salviae hispanicae semen*) - one of the most recognized food raw materials in the world [1,2]. Thanks to these bioactive components, seeds are increasingly used in the food and pharmaceutical industries to meet the nutritional needs of the elderly [3].
Plant *in vitro* cultures provide a valuable model for the development of new techniques into the use of plant biomass in medicine [4]. Fortification with bioelements is one of the modern techniques for enriching foods with essential macro- and micro-nutrients [5].
The aim of the present study was to investigate the bioaccumulation capacity of health-promoting metal ions: Mg, Ca, Zn, Fe, Cr, Se and Li, using a model of *S. hispanica* agitated microshoot cultures and to propose them as a potential source of therapeutic raw material used in phytotherapy of diseases of the elderly through supplementation with fortified food.
Materials and methods. *S. hispanica* microshoot cultures were grown on medium according to Murashige-Skoog [6], without the use of plant growth regulators. Salts of macroelements - calcium ($CaCl_2$ x $6H_2O$) and magnesium ($MgSO_4$ x $7H_2O$), and microelements - (FeNaEDTA x $2H_2O$), zinc ($ZnSO_4$ x $7H_2O$), selenium (Na_2O_3Se), chromium ($K_2Cr_2O_7$), and lithium (Li_2SO_4 x H_2O) were added to the media at concentrations of: 1, 5, 10, 25, 50 (mg per bioelement per liter of medium). The results obtained were compared with the control samples (cultures without the addition of bioelements). The culture growth cycles lasted 14 days (3 repetitions). Cultures were cultivated under continuous white light (LED). Analysis of the content of bioelements were performed by inductively coupled plasma mass spectrometry (ICP-MS).
Results. The conducted experiments proved the high ability of bioaccumulation of element ions in the biomass of *S. hispanica in vitro* cultures. The highest bioaccumulation (mg/100 g dry mass) was found for Cr (9.69), Zn (5.81), Se (2.24) and Li (0.49) ions supplemented at a concentration of 50 mg/L. A high bioaccumulation capacity of the tested bioelements was found in relation to the control cultures.
Conclusions. *In vitro* cultures of *S. hispanica* can be proposed as a "innovative food" that can be used to enrich a wide range of food products with health-value bio-elements, especially important in the diet of the elderly.
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