PhD Dissertation

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In vitro and in vivo cultures of Iris pseudacorus plants as a source of biologically active compounds.

Abstract

Plants are representatives of eukaryotic organisms that cannot actively move. However, in the course of evolution, they developed mechanisms of chemical interaction with the environment – secondary metabolites. They enable them to survive in difficult, changing environmental conditions and to interact with other organisms in their surroundings. Secondary metabolites produced in plant tissues exhibit a wide range of biological activities. They can be used i.e.: to attract insects, discourage herbivores or defend plants against pathogenic microorganisms. These compounds also affect numerous molecular targets in the human body, which is the reason why they have become an area of extensive research.

This doctoral dissertation presents a wide range of analyses regarding the possibility of using plants of the species *Iris pseudacorus* (yellow flag iris) as a source of biologically active compounds. The activity of extracts obtained from the tissues of these plants grown in soil and in *in vitro* cultures were analysed. It has been shown that extracts obtained from rhizomes of *I. pseudacorus* have an antibacterial activity ranging from 1.25 to 50 mg of dry tissue weight/mL against bacteria from the species: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, both for reference strains as well as sensitive and resistant clinical isolates. It has been shown that the extract from *I. pseudacorus* rhizomes, in a concentration approx. 2.5-fold higher than in the case of bacteria grown in planktonic culture, is effective in combating *S. aureus* biofilm.

The analyses of the antibacterial activity of yellow flag iris extracts were extended to the study of their interaction with antimicrobial silver nanoparticles (AgNPs). These studies were performed to verify if combination of extract and AgNPs can give synergistic interaction (to reduce the doses of both antibacterial agents while increasing their biological activity). Combining different antibacterial substances can increase efficiency against microorganisms, limiting the possibility of gaining resistance. Studies have shown an activity close to synergy between extract form iris rhizomes and AgNPs against *P. aeruginosa* and antagonism in the activity against *S. aureus*.

The studies also showed the cytotoxic activity of methanolic extracts from *I. pseudacorus* rhizomes against the human cancer cell lines: HeLa (cervical), HCT-116 (colon), MCF-7 (breast) and non-cancerous MCF-10A (mammary gland epithelial cells). The highest cytotoxic efficacy was obtained against cells from MCF-7 line (IC_{50} = 11.75 ± 0.7 µg of dry extract/mL). The obtained result indicated about 3.7-fold higher cytotoxic activity in relation to neoplastic cells as compared to cells from the non-neoplastic MCF-10A control line (IC_{50} = 44 ± 1 µg dry extract/mL).

As a part of the doctoral thesis, a method of micropropagation of *I. pseudacorus* by stimulation of hypocotyls and a model of tissue culture of anatomical roots were developed.

Anatomical root culture of yellow flag iris have been shown to synthesise phenolic compounds from isoflavone group, including the antibacterial iristectorigenin B and unidentified dimethoxy-dihydroxy-isoflavone. Moreover, thanks to the use of thin layer chromatography (TLC) and bioautography (soft top agar technique), the presence of active compounds against *S. aureus* in the post-culture medium was demonstrated.

To obtain hairy roots of *I. pseudacorus, Rhizobium rhizogenes* bacteria were used. Instead of hairy roots, a transformed shoot (teratoma) was obtained.

Detailed analyses of the metabolic composition of extracts using TLC and High Performance Liquid Chromatography with diode detection and particle mass determination with electrospray ionization (HPLC-DAD-ESI-MS) revealed the presence of numerous phenolic compounds, including tannins and isoflavones. Among them, genistein, daidzein and iristectorigenin B were identified. An antibacterial compound from rhizome extract was identified as galocatechin.

The obtained results clearly indicate the potential of the *I. pseudacorus* species as a valuable source of biologically active compounds.