

Aspects of biopharmaceuticals extraction from *Rosa canina* and *Crataegus monogyna* micromycetes

The increased demand for biopharmaceuticals issues new challenges for pharmaceutical industry due to the exigency for search of promising producers of bioactive metabolites and effective methods of their isolation. The data on a technique applied to extraction of bioactive compounds from plants of the family *Rosaceae* and their associated fungi are analyzed.

Method of extraction highly affect the quality and purity level of targeted compounds. The plants of *Rosa* genus are known as a rich source of vitamin C, carotenoids, phenolic compounds, etc. The presence of flavonoids (quercetin, hyperoside, vitexin), tannins, pectins, organic acids, vitamins and minerals is characteristic for the representatives of *Crataegus* genus. The plant material of *Crataegus* and *Rosa* species is applied in the composition of numerous medicinal preparations for improving the function of cardiovascular and digestive systems, as well as in tinctures, phytocompositions and food supplements in Ukraine [1]. Fungi associated with those plants gain more attention as they may serve as a source of new important biopharmaceuticals. For example, presence of *Mycosphaerellaceae* fungi has been registered on representatives of 72 families of medicinal plants [2]. During long time of plants and micromycetes coevolution, fungi gain capability to produce some similar substances to their hosts as to influence host's metabolism. Thus, when dealing with plant associated fungi the preliminary analysis of plant metabolites is recommended.

The typical methods of biopharmaceuticals extractions involve maceration, remaceration, percolation, that are widely applied for plant material [3]. However, for fungal metabolites extraction foregoing methods cannot be directly used due to peculiarities of fungal cell wall structure. Chitin presence provides the strength and specificity to fungal cell wall, thus preliminary treatment and its demolishing are necessary. The optimization of extraction method requires stage of disintegration that should be followed by filtration. Fungal cells disintegration techniques during extraction process involve mechanical or enzymatic treatment: those include application of lytic enzymes, ultrasound or via grinding with quartz sand. Under the submerged fungi cultivation, cultural liquid or the broth could directly serve for extraction of bioactive compounds. In such case, the most appropriate methods involve evaporation with subsequent solvent extraction. Selection of solvent and isolation technique are rather essential as target compounds could be produced by fungi in low amounts and their full recovery is crucial. The most common solvents used in extraction of fungal metabolites include ethanol, methanol, ethyl acetate, dichloromethane,

hexan; and the selection of applicable one is dependent on the nature of targeted compound [4]. New techniques of biopharmaceuticals extraction are developing nowadays, amongst are microwave and ultrasound assisted extractions, as well as supercritical fluids or switchable solvents application [3].

Preliminary phytochemical analysis of *Rosa canina* and *Crataegus monogyna* plants was carried out for getting the information on bioactive compounds present in sampled material for subsequent comparison with available fungal metabolites. Water and ethanol extracts of *C. monogyna* and *R. canina* fruits were prepared from dried plant material via maceration. Obtained extracts were tested on the presence of bioactive substances and preliminary results demonstrated the presence of phenolic compounds, as well as bioflavonoids, 2-methyl-1,4-naphthoquinone (3-) derivatives and ascorbic acid in all of them. The assay of ascorbic acid content was carried out by the Tillman's method with 2,6-dichlorophenol indophenol sodium salt. The resulting values were 393,09 mg/g and 70,41 mg/g for extracts from *R. canina* and *C. monogyna* fruits, respectively, that correlated well with known data. The next stage included isolation of fungi associated with studied plant species by standard mycological protocols. Species of *Microsphaeropsis olivacea* and *Alternaria alternata* were characteristic for leaves and fruits of the common hawthorn, and *Aspergillus terreus* and *Diplocarpon rosae* were isolated from the dog rose. Further cultivation and testing of isolates on the presence of biologically active compounds and comparison of their content with results of phytochemical analysis are under the consideration.

Thus, preliminary study supports the view that some plants of the family *Rosaceae* and their associated fungi could be a potent and still do not well studied source of biologically active substances, and serve for further biotechnological and pharmaceutical application. Special attention should be paid on selection of appropriate methods of compounds extraction and assay as to propose ways to achieve the maximum product yield. Besides, dealing with plant associated fungi is quite complex and requires the optimization of existing extraction and compounds study methods.

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