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SUMMARY OF THE DOCTORAL THESIS

Preparation of the plant-derived polyphenolic-polysaccharide conjugates with the pro-health potential assisted by selected physical factors

The main subject of the doctoral thesis was to modify and optimize the existing isolation process of the macromolecular product from the plant *Erigeron canadensis*, which exhibit anticoagulant activity. The main goal of the accomplished dissertation was to reduce the duration time of the multistep bioproduct isolation procedure and possible increase of its biological activity and selectivity. It was possible to be achieved by the implementation of the physical factor at the very early stage of the isolation procedure, in a form of an ultrasound or microwave assisted extraction. The thesis focuses on determining the influence of the applied physical factors (assisting preliminary extraction) on the yield of the conducted process, on the possible change of a chemical character of the bioproducts obtained, as well as, on their biological activity, including the mechanisms of action.

The theoretical part of the doctoral thesis consist of a literature review, which presents actual state of knowledge within the scientific fields connected with the main aspects of the thesis. The first thing that was described is the division and characterisation of the plant-derived polysaccharides, which are the main building block of the plant cell walls. Moreover, the most important conjugates which plant polysaccharides can form with other compounds, especially focusing on the polyphenolic glycoconjugates and their pro-health properties, were described. Further part of the thesis introduces conventional and modern techniques used to acquire plant originated glycoconjugates, including novel methods that utilize physical factors. The last chapter of the theoretical part discusses the most important issues related to the design and optimization of the technological processes, that uses modern computational techniques from the field of the response surface methodologies (RSM).

Experimental part of the work represents the description of the own research, conducted by the PhD student in the field of the doctoral dissertation, together with its results and discussion. As first, the research material was describe – Canadian horseweed (*Erigeron canadensis*) – along with the basic characteristics and presentation of the results of a conventional two-phase extraction process in an alkaline environment. Those results were treated as a starting point for the further research, i.e. introduction of the physical factors assisting the extraction process and its optimization. The aim was to propose such extraction conditions, that would allow to significantly shorten or simplify the overall process of obtaining bioproducts from horseweed and make it economical and environmentally friendly, while still offering attractive biological properties of the product. The following chapters describe the results of the research related to the conduction of the series of ultrasound and microwave assisted extractions with variable power, time and temperature parameters of the process. The obtained range of crude *E. canadensis* extracts was subjected to preliminary

analyses – extraction yield from the dry plant mass was determined, the pH vale of the aqueous extracts was measured and their anticoagulant activity against blood plasma was determined *in vitro*. Obtained results allowed in the further part of the doctoral thesis to propose a computational model for the optimization of the physical factor assisted extraction process. As an effect, it was possible to determine, using a response surface methodology, the optimum conditions for the ultrasound assisted and microwave assisted extraction process. This allowed to select the potentially the best horseweed extracts in terms of their biological activity (not worse than the extracts obtained in a conventional process) and the efficiency of the process itself. In the next part of the PhD student's own research the selected raw extracts were subjected to purification by a multi-step process, mainly using a series of the two-phase extractions in different organic solvents. In the chapter connected with the purification of the selected plant extracts, the results of determination of the basic chemical characteristics of purified bioproducts have been described. What is more, their anticoagulant activity has been also verified. Based on that, two, potentially the most attractive, plant products obtained through the modified isolation process utilizing ultrasound and microwave assisted extraction were selected. The last chapter describing the own research, presents works connected with the separation of the best obtained products into homogenous fractions. These products and their separated fractions were re-evaluated for *in vitro* anticoagulant activity. Furthermore, their mechanisms of action have been determined on the selected enzymes of the coagulation cascade. Finally, for both the best products and their fractions, a thorough chemical analysis was performed, using range of colorimetric (UV-Vis), chromatographic (GC-MS, molecular weight estimation by GPC) and spectroscopic techniques, such as FT-IR, ^{1}H NMR oraz ^{1}H – ^{13}C HSQC NMR.

All of the results obtained during the studies conducted as a part of the doctoral thesis have been summarized. Particular emphasis has been put on the possibility of obtaining, in an economical and more environmentally-friendly way, a bioproduct from *E. canadensis* that is attractive in the terms of biological activity and selectivity in comparison to a product obtained in a conventional process.