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The analysis of the polyphenolic profile and biological activity of leaves of *Sorbus domestica* L.

PhD dissertation based on a series of scientific publications

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Summary

High levels of reactive oxygen/nitrogen species (ROS/RNS), generated as a consequence of disturbed redox balance, are the cause of oxidative stress, which together with chronic inflammation is considered to be among the major causes of cardiovascular disorders, insulin resistance, and other chronic non-communicable diseases (NCDs). Healthy lifestyle and, in particular, proper diet are one of the suggested strategies for limiting NCDs progression. Positive results are obtained by the increased consumption of plant products rich in structurally diverse polyphenols, which exert multidirectional effects on human body by the regulation of redox homeostasis and inflammation processes in cells and tissues. The proven efficacy of polyphenolic antioxidants in prevention and therapy of NCDs has generated interest in plant products, their sources, and the rules of potential supplementation. As a result, a large amount of research has focused on the analysis of plants raw materials that, after proper phytochemical, pharmacological and toxicological evaluation, could be used as herbal medicines, dietary supplements or functional food ingredients. In that context, the attention has been paid to one of the species from the Mediterranean region, i.e. service tree (*Sorbus domestica*). Despite the documented traditional usage of *S. domestica* leaves, the lack of research limits their boarder medical application. Previous studies have focused on fruits of service tree. However, despite being rich source of bioactive polyphenols, the fruits are available only regionally due to the weakened flowering and fruiting of *S. domestica* in the temperate climate. Moreover, in case of many fruit plants, the leaves are actually a better source of active phenolics than fruits, which was confirmed by numerous studies and is reflected in the increasing number of leaf-based products placed on the market (e.g. grapevine, or blackcurrant).

Taking into account the above mentioned premises, the evaluation of the leaves of *Sorbus domestica* cultivated in Poland as the source of natural, bioactive polyphenols become the objective of the presented dissertation. The studies consisted of two interdependent parts, i.e. phytochemical analysis and bioactivity assessment. The phytochemical part aimed for the detailed characterization of polyphenolic profile of dry extracts/fractions from *S. domestica* leaves and included qualitative UHPLC-PDA-ESI-MS^3 assays, optimization and validation of the quantitative HPLC-PDA method, as well as isolation and identification of active markers for quality control of the plant material and extracts. In the bioactivity studies, the extracts and model polyphenols (active markers) have been subjected to *in vitro* tests of antioxidant and anti-inflammatory activity in complementary chemical and biological models, including evaluation of their cellular safety. Furthermore, taking into account the impact of the climatic conditions on the quality of plant products, the comparative analysis of the composition and antioxidant activity of the plant material from Poland (cultivation) and Croatia (natural habitat) was carried out.

The total phenolic content in the methanol-water extract from *S. domestica* leaves (283 mg GAE/g dw) and its polyphenol-richest fractions (464-700 mg GAE/g s.m.) was comparable to or higher than that found in the extracts recognized in prevention of oxidative stress-related ailments, such as extracts from grape seeds (630-790 mg GAE/g dw), grape leaves (60-260 mg GAE/g dw), green tea (447 mg GAE/g dw) or blackcurrant leaves (249 mg GAE/g dw). In the course of detailed qualitative and quantitative chromatographic analysis of the extracts/fractions (UHPLC-PDA-ESI-MS^3; HPLC-PDA-fingerprint) over 40 polyphenolic compounds were detected, and the contents of the dominant groups of polyphenols (flavan-3-ols and proanthocyanidins, flavonoids and phenolic acids) and individual

analytes were determined. Moreover, two flavonoid diglycosides, i.e. quercetin 3-*O*-(2''-*O*-β-D-glucopyranosyl)-α-L-rhamnopyranoside (HRQ) and quercetin 3-*O*-(2''-*O*-β-D-xylopyranosyl)-α-L-rhamnopyranoside (PRQ) were isolated for the first time from genus *Sorbus*. The final step of the phytochemical studies was the development and validation of the HPLC-PDA method for quantitative analysis of polyphenols in the tested extracts, as well as the selection of nine active markers of *S. domestica* leaves, i.e. (–)-epicatechin (ECA), procyanidin B2 (PB2), procyanidin C1 (PC1), chlorogenic acid (CHA), quercetin (QU), quercitrin (QCT), rutin (RT), HRQ and PRQ.

During the biological activity studies, the extracts and their active markers were proven to protect the components of human plasma (proteins and lipids) from the oxidative/nitrative damage induced by $ONOO^-$ as well as to increase the non-enzymatic antioxidant capacity of blood plasma. The ROS/RNS scavenging potential of the extracts and markers was suggested as one of the possible mechanisms of their activity in the applied model, after they were demonstrated *in vitro* as wide-spectrum antioxidants, effective against *in vivo*-operating reactive species, i.e. $O_2^{\bullet-}$, H_2O_2, $NO^{\bullet}$, HClO, $HO^{\bullet}$, $ONOO^-$. Moreover, the study of the interactions between the representatives of the main group of polyphenols responsible for the extracts activity (ECA, QCT and CHA) implied some synergistic effects, which might explain the similar or even higher activity of the extracts/fractions in comparison to pure compounds, both in the biological model and towards particular ROS/RNS. Furthermore, the safety of the extracts was preliminarily confirmed after demonstrating, that they have no impact on the viability of peripheral blood mononuclear cells (PBMCs). The anti-inflammatory potential of the extracts and their activity markers was established in the lipoxygenase and hyaluronidase inhibition assays.

Additionally, comparative analysis of the composition and antioxidant activity of the leaves from Polish cultivation and Balkans area showed, that despite the differences in phytochemical profile, caused by climatic factors, samples from both sources may be valuable materials for production of bioactive extracts. The tested samples differed mainly in terms of the proportion between flavonoids and proanthocyanidins. Even though the latest were the dominant group in all samples, in leaves from Poland they accounted for 59-64% of total polyphenols, while in the samples from Croatia their percentage share was lower (45-52% of total polyphenols) with accordingly higher share of flavonoids.

The results obtained in the present dissertation imply that structurally diverse polyphenols are the active compounds of *S. domestica* leaves and emphasize the importance of the antioxidant and anti-inflammatory mechanisms for their biological effects. The observed multidirectional activity of the investigated *S. domestica* leaf extracts indicates their high value as potential components of herbal medicines for the prevention or adjunctive therapy of oxidative stress/inflammation-related diseases. Leaves of service tree, used so far only in the traditional medicine, seem to be a promising material for further phytochemical, pharmacological and toxicological studies, that would evaluate their properties more thoroughly and, possibly, enable their introduction to the official medicine.