Summary

According to the World Health Organization, diseases of affluence/noncommunicable diseases (NCDs) currently cause 71% of deaths globally, almost 40% of which are premature deaths. Due to the mass prevalence of NCDs and the associated massive demand for the healthcare systems, there is a need to pay more attention to prophylaxis, including the use of high-quality food and functional products, as well as supplementing conventional pharmacotherapy with rational phytotherapy, which might decrease the economic and social costs of NCDs. In this context, particularly interesting are plant products rich in polyphenols, widespread in food and medicinal plants, and showing a well-documented biological activity of a broad spectrum, corresponding to the complex pathophysiology of NCDs. It is acknowledged that the prophylactic and therapeutic influence of plant polyphenols on the progression of NCDs is associated with their normalizing and regulating effect on oxidative and inflammatory processes as well as metabolic disorders occurring in living cells, which are the elementary etiological factors of NCDs. Among the polyphenol-abundant plant materials, fruits are especially auspicious as they are willingly consumed fresh or processed. Some of them are also used in traditional medicine, which suggests their significant preventive and therapeutic potential. However, the available research demonstrates that the biological activity of plant phenolic fractions is conditioned by the presence of a specific range of compounds in appropriate proportions. Hence, the rational use of plant substances/preparations requires their complete characterization, including the establishment of their active components and mechanisms of action, phytochemical standardization, and activity parameters, as well as evaluation of toxicity and formulation/dosage to guarantee the effectiveness and safety of the therapy.

Considering the above premises, the subject of this dissertation is the fruit of *Prunus spinosa* L. (sloe, blackthorn) – a dietary product and a polyphenolic plant material, mentioned in traditional medicine as a substance with pleiotropic biological properties. For dietary purposes, fresh sloe fruits are consumed mainly as preserves (including juices and tinctures), while dried fruits are used to prepare herbal teas. Both fresh and dried fruits have the status of traditional herbal medicines. However, the ethnomedical sources suggest differences in the activity of both materials. For instance, dried fruit decoctions are mainly used to treat gastrointestinal tract inflammation; in contrast, fresh fruit preparations are recommended for systemic disorders, e.g., metabolic diseases, like diabetes. Despite the documented therapeutic application, until the publication of the manuscripts constituting this dissertation, the chemical composition of the fruit remained only fragmentarily recognized, and the impact of drying on its composition was not the subject of any research. However, it has been assumed that the primary pharmacologically active molecules of sloe fruits are polyphenols, most of which have been only partially identified. The data on the biological activity of sloe fruits was also incomplete. The previous studies have focused mainly on the antioxidant activity of fresh blackthorn fruits but they primarily used simple *in vitro* chemical tests, which are difficult to relate to *in vivo* conditions. On the other hand, the accumulated research suggested a high antioxidant potential of the fruit, encouraging further studies on the subject, especially because the only one available study of the antioxidant properties in a cellular model (U937 human promonocytes) showed significant and dose-dependent protective properties of sloe juice in the conditions of oxidative stress. Concerning the anti-inflammatory effect, the ethanol

extract of fresh sloes has only been demonstrated to reduce the expression of the pro-inflammatory cytokine IL-6 and some adhesion factors in stimulated human endothelial cells. Moreover, in the context of antidiabetic properties, the alcoholic extracts of fresh blackthorn fruits have been proven to inhibit α-glucosidase and α-amylase in vitro.

Considering the above, this dissertation aimed to identify biologically active components of extracts/fractions obtained from fresh and dried *P. spinosa* fruits as well as compare their phytochemical profile and their potential antioxidant, anti-inflammatory and antidiabetic effects in vitro in the context of the prevention and treatment of inflammatory bowel diseases, diabetes, and its vascular complications.

In the phytochemical part of the research, a detailed analysis of the polyphenolic profile of dry extracts/fractions from fresh and dried blackthorn fruits was carried out. The qualitative profiling, performed by the LC-PDA-ESI-MS^3 method, allowed for the first time to identify the dried fruit constituents and significantly increased the knowledge about the active components of fresh sloes. In total, 57 and 45 polyphenols were identified in fresh and dried plant material, respectively, including 35 new polyphenolic compounds for *P. spinosa* fruits. A less diversified polyphenolic composition was observed for the dried compared to the fresh fruits. The dried material also contained lower total polyphenolic content (TPC) than the fresh one; however, the determinations confirmed sloes as a rich source of these phytochemicals. Apart from polyphenols, three compounds formed through the dehydration of sugars in the Maillard reaction (MPRs) were also confirmed for the first time in dried sloe fruits. Due to the potential high exposure to these compounds in a standard human diet and the alarming results of preliminary toxicological studies available in the literature, it is generally recommended to control the MPRs content in food. However, the present dissertation revealed that the content of MRPs in blackthorn fruits is at the lower range limit reported in the literature for numerous commonly consumed fruits, including prunes, and the fruits might be thus regarded safe.

Taking into account the critical role of immune cells, such as neutrophils and peripheral blood mononuclear cells (PBMCs), in the pathogenesis and progression of inflammatory diseases, including gastrointestinal tract inflammations, the influence of blackthorn extracts/fractions and model compounds on the secretion of key inflammatory mediators, i.e., ROS, ELA-2, TNF-α, IL-8, IL-6 and IL-10 by neutrophils and PBMCs was evaluated in the first step of biological activity studies. The tested extracts/fractions were characterized by a high modulation efficiency of pro-inflammatory and pro-oxidant functions of immune cells, both at concentrations of 1–5 μg/ml, which are nominally achievable for plasma polyphenols, and those found in the gastrointestinal tract (25–100 μg/ml) after oral administration of polyphenol-rich products. It might, therefore, be expected that they may be able to alleviate both systemic and local inflammation. Among the tested factors, the analytes most effectively inhibited the secretion of ROS, pro-inflammatory ELA-2 and TNF-α, and increased the secretion of anti-inflammatory IL-10.

The second step of biological activity studies included the verification of ethnomedical indications of sloe fruits in the treatment of diabetes. First, the potential of fruit extracts/fractions to inhibit glycolytic enzymes α-glucosidase and α-amylase was examined. All extracts/fractions from fresh and dried plant material presented a higher ability to inhibit α-glucosidase than acarbose, a reference antidiabetic drug. In turn, the fruit extracts/fractions

had only moderate activity in the α-amylase inhibition test. Moreover, because the overproduction of ROS modifies the main biochemical processes underlying the pathogenesis of diabetic vascular complications, including the glycation of various biomolecules and the formation of advanced glycation products (AGEs), a broad scavenging activity towards a range of *in vivo* generated free radical and non-radical oxidants, including $O_2^{\bullet-}$, $HO^{\bullet}$, H_2O_2, $NO^{\bullet}$ and HOCl, was documented for extracts/fractions from fresh and dried sloe fruits in the next step. Furthermore, the tested extracts/fractions were proven to inhibit the formation of AGEs with similar or higher effectiveness than aminoguanidine, a reference standard with anti-glycation properties. In the last step of activity studies, the analytes were tested in a human plasma model exposed to $ONOO^-$-induced oxidative stress. It was documented that the tested analytes effectively prevent the oxidative/nitrative damage to human plasma components (proteins and lipids) in vitro, and also have a high ability to increase the non-enzymatic antioxidant capacity of plasma.

In most of the performed biological activity tests, the extracts/fractions obtained from dried fruits presented slightly lower effectiveness than those from fresh fruits. However, the observed activity differences between dried and fresh fruit extracts were not critical, and the dried plant material was also characterized by a high antioxidant, anti-inflammatory and anti-diabetic potential. The activity parameters achieved for model polyphenols and the correlation studies showed that the presence of polyphenols determines the biological activity of sloe fruits. On the other hand, the activity tests of a model MRPs – HMF – suggested that these compounds exhibit partially opposite effects to polyphenols, especially towards immune cells (they stimulated the secretion of TNF-α and inhibited the release of IL-10); however, their presence did not influence the therapeutic value of the analyzed extracts/fractions negatively due to the low concentration. Eventually, the viability tests of neutrophils and PBMCs enabled the preliminarily confirmation of the cellular safety of the tested extracts/fractions and their model compounds.

The results of this dissertation prove the high potential of *P. spinosa* fruit extracts in the context of prophylaxis and complementary therapy of NCDs. Moreover, the obtained data may become the starting point for further, extended phytochemical, pharmacological and toxicological studies leading to the complete characterization of the plant material and, finally, to the development of valuable functional food products or therapeutic preparations.