

Antioxidant activity and total polyphenol content of selected herbal medicinal products used in Poland

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Summary

The main aim of the study was to evaluate the antioxidant activity and total polyphenol content in fifteen popular herbal products often used in treatment of various diseases. The studied material were, among others, chamomile flower head, oak bark, St. John's-wort herb, hawthorn flower, dog rose and elder fruits, lingonberry leaf. Herbal extracts or infusions were prepared according to protocols provided by producer on the packaging. The highest antioxidant activity and polyphenols concentration were obtained for lingonberry leaves (725 mg Trolox/g d.w. and 109 mg catechin/g d.w., respectively). The oak bark, St. John's-wort herb, and lime flower were also a very rich source of antioxidant compounds, independent of their typical therapeutic action. Several herbs, such as dog rose fruit, lose their antioxidant activity when prepared at home conditions.

Key words: antioxidant activity, medicinal plants, traditional herbal specimens, polyphenols

INTRODUCTION

Many different herbaceous plants are used due to their therapeutic properties. There is a broad spectrum of diseases and disorders cured with herbs, from cardiovascular disease, rheumatic disorders, infections to AIDS and ma-

laria or even hypoxia [1-7]. Nowadays, about 35% of medicines are of plant origin and they are recognized as equal to chemical drug equivalents. Very convincing argument for natural preparations is their low price and a fact, that they can be used for a long time with quite good therapeutic efficiency and without any undesirable effects [8]. Moreover, they are easily available and often are applied without doctor consultation.

The chemical composition of particular herbs, or even particular parts of herbaceous plants, has been already defined. The main bioactive components are alkaloids, flavonoids, glycosides, vitamins, mineral salts, and active enzymes. Many of them have therapeutic effect on human body: they are able to heal, stimulate vital strength, prevent disease, or are neutral [9-11]. However, some herbs also contain poisonous substances and can be harmful, especially when taken in high amounts. Despite of therapeutic impact some herbaceous materials also exhibit antioxidant activity. Free radicals (FR) and reactive oxygen species (ROS) react with important biological macromolecules and therefore contribute to the pathogenesis of many diseases [12]. The supplementation of antioxidants can prevent destruction caused by those reactive particles. It was proved that some herbs contain antioxidants which are more active than those of fruits and vegetables. Among characterized herbs are well-known thyme, salvia, and garlic, as well as other medicinal plants known only to narrow local communities. Herbs typical to Polish cuisine and traditional medicine are less known and rarely examined with respect to their antioxidant activity.

The level of bioactive compounds and the final antioxidant activity of an extract depend on extraction method, especially on solvent and temperature [13-15]. Therefore, the majority of studies on herbs composition and their antioxidant activity were performed in model extracts. Those extracts were prepared with solvents that guarantee high efficiency of bioactive compounds recovery. The most often selected solvents are ethanol, methanol, ethyl acetate, acetone, and hot or boiling water [14, 16-18]. However, the consumer that buys the herbal remedy usually prepares it at home, in the mode given on the packaging by a producer. The home conditions during preparation of extracts, infusions and other herbal preparations significantly differ from those optimal and influence on antioxidant potential.

The aim of the study was to determine the antioxidant activity and total polyphenol content in selected herbs used for treatment, prepared accordingly with protocol suggested by a producer (statement on the herb packaging), widespread in Poland. All of the herbs selected are applied in practice for the traditional treatment of different disorders and are sold without medical prescription. Examined herbs are available both as single herbs and in herbal mixtures.

MATERIAL AND METHODS

Material

Dried, homogenous herbal products provided by local distributor (Herbapol S.A., Krakow, Poland) were used. The name of herbal material, common name of herb, its Latin name, and part of plant analyzed are presented in table 1.

Table 1.

The characteristics of herbaceous material used in the study

raw material	common name of herb	Latin name of herb	part of plant
<i>Quercus cortex</i>	English oak	<i>Quercus robur</i> L.	bark
<i>Crataegi folium cum flore</i>	Hawthorn	<i>Crataegus laevigata</i> (Poir.) DC	flower
<i>Sambuci flos</i>	Elder	<i>Sambucus nigra</i> L.	flower
<i>Tiliae flos</i>	Small-leaved Lime	<i>Tilia cordata</i> Mill.	flower
<i>Matricariae flos</i>	Chamomile	<i>Matricaria recutita</i> L.	flower head
<i>Sambuci fructus</i>	Elder	<i>Sambucus nigra</i> L.	fruit
<i>Rosae pseudo-fructus</i>	Dog rose	<i>Rosa canina</i> L.	fruit
<i>Hyperici herba</i>	St. John's-wort	<i>Hypericum perforatum</i> L.	herb
<i>Chelidonii Herba</i>	Celandine	<i>Chelidonium majus</i> L.	herb
<i>Violae herba cum flore</i>	Heartsease	<i>Viola tricolor</i> L.	herb
<i>Equiseti herba</i>	Horsetail	<i>Equisetum arvense</i> L.	herb
<i>Visci herba</i>	Mistletoe	<i>Viscum album</i> L.	herb
<i>Vitis idaeae folium</i>	Lingonberry	<i>Vaccinium vitis-idaea</i> L.	leaf
<i>Urticae folium</i>	Nettle	<i>Urtica dioica</i> L.	leaf
<i>Plantaginis lanceolatae folium</i>	Ribwort Plantain	<i>Plantago lanceolata</i> L.	leaf

Chemicals

Diammonium salt of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS diammonium salt); (\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox); catechin hydrate; Folin-Ciocalteu phenol reagent; and a phosphate buffer saline (PBS): 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride; pH 7.4 at a temperature of 25°C. All the chemicals listed were purchased from SIGMA-Aldrich Company (Germany). The following chemicals: potassium persulfate ($K_2S_2O_8$) and methanol (analytically pure) were obtained from POCh Company (Poland), and a 96% ethanol from ChemPur Company (Poland).

Producer's protocols of herbs preparation

All analyzed herbs were prepared according to the procedure suggested by producer on the herb packaging. The producers' protocols for extracts or infusions and their medical use are listed in table 2.

Table 2.

Protocols of herbs preparation according to the producers' suggestions and their medical use

raw material	producer protocol	medical use/disease treated [3, 4, 7, 19-22]
English oak bark	Pour 1 cup of water over 1 spoon (3 g) of bark. Boil for 5 min. Leave for 15 min. Strain.	antidiarrhetic, anti-inflammatory
Hawthorn flower	Pour 1 cup of boiling water over 1 teaspoon (1 g) of flowers.	myocardium and coronary arteries diseases
Elder flower	Pour 1 cup of boiling water over 2 teaspoons (1 g) of flowers.	diaphoretic, strengthening capillaries
Lime flower	Pour 1 cup of boiling water over 1 teaspoon (1.5 g) of flowers. Leave for 15 min under the cover.	expectorant, anti-inflammatory agent in respiratory tract diseases, antipyretic, anxiolytic
Chamomile flower head	Pour 1 cup of boiling water over 1 spoon (3 g) of flower heads. Leave covered for 10 min. Strain.	anti-inflammatory in gastrointestinal tract, antiseptic for the mouth and throat
Elder fruit	Pour 1 cup of boiling water over 1 teaspoon (3.5 g) of fruits.	detoxificant and analgesic activity
Dog rose fruit	Pour 1 cup of boiling water over 1 teaspoon (4 g) of fruit and boil for 3 min.	anti-inflammatory and supporting physical activity
St. John's-wort herb	Pour 2 cups of boiling water over 2 spoons (5 g) of herb in thermos. Cover and leave for 1 hour. Strain.	liver disorders, gastric and duodenal ulcer, regenerative, anti-inflammatory agent in digestive tract diseases, choleric
<i>Celandine herb</i>	Pour 1 cup of boiling water over 1 spoon (2.5 g) of herb. Infuse covered for 10 min.	spasmolytic and anti-inflammatory agent in bile ducts diseases, skin inflammations
Heartsease herb	Pour 1 cup of cold water over 1 spoon (2.5 g) of herb. Heat to boiling and boil covered for 5 min. Strain.	diuretic agent, detoxificant in acne
Horsetail herb	Pour 1 and a half cup of warm water over 2 spoons (4 g) of herb. Boil slowly for 10 min. Leave for 15 min and strain into thermos.	diuretic and haemostatic agent, skin diseases
Mistletoe herb	Pour 1 l of hot water over 3 spoons (6 g) of mistletoe herb. Leave for 5 to 10 min covered. Strain.	chronic hypertension, slight bleedings
Lingonberry leaves	Pour 1 L of water over 3 spoons (6 g) of lingonberries leaves, heat to boiling and keep boiling under cover for 5 min. Strain.	antiseptic in urinary tract inflammation
Nettle leaves	Pour 1 cup of boiling water over 1 spoon (3 g) of leaves. Infuse covered for 10 min.	diuretic agent, astringent in diarrhea
Ribwort Plantain leaves	Pour 1 l of water over 2 spoons (6 g) of leaves. Heat to boiling and keep boiling covered for 15 min. Strain.	gastroenteritidis, damages of stomach and intestinal mucosa, ulcer disease, dermatosis

ABTS radical cation decolorization assay

The antioxidant activity was measured with use of ABTS radical decolorization, described previously [16]. The ABTS radical was generated during a chemical reaction between the 7 mM aqueous solution of ABTS diammonium salt and the 2.45 mM potassium persulfate. The solution was kept at a room temperature in darkness throughout the night, in order to complete the reaction and to stabilize the ABTS cation-radical. Prior to analysis, the radical solution was diluted with PBS (pH 7.4) to obtain final absorbance value of $A = 0.70 \pm 0.02$ (ABTS_{0.7}) measured at 734 nm (spectrophotometer BECKMAN DU 650). 100- μ L aliquots of the properly diluted extract or of Trolox solutions (with concentration ranging from 0 to 100 mg/L) were added to 1 mL of ABTS_{0.7}, and absorbance value was measured 6 min after mixing. The antioxidant capacity of the studied extracts was calculated using a standard curve drawn up for solutions of the synthetic vitamin E (Trolox) and expressed in mg of Trolox equivalent/g of dried weight of plant material. All determinations were performed in five replications.

Total polyphenol content assay

Total polyphenol content was assayed by Folin-Ciocalteu method on the basis of a protocol represented by Swain and Hillis [23] with slight modifications. 5 mL of herb extract was diluted with 45 mL of redistilled water. Then 5 mL of this solution was mixed with 0.25 mL of Folin-Ciocalteu reagent (dissolved with water 1:1 v/v) and 0.5 mL of 7% Na₂CO₃. The mixture was left for 30 minutes in dark and the absorbance value was measured at 760 nm. The obtained results of total polyphenol content were expressed in mg of catechin/g of dry weight of plant material based on the standard curve drawn up for catechin methanolic solutions (their concentration ranged from 0.1 to 15 mg/L). All determinations were performed in five replications.

Statistical analysis

The results were shown as an arithmetic mean \pm standard deviation. A single-factor Analysis of Variance test (ANOVA) with a *post hoc* Tukey test was applied to perform the statistical analysis. A Kolmogorov-Smirnov test was applied to examine the normality of distribution. Pearson product-moment correlation coefficient (r) was calculated to measure the strength of linear relationship between the antioxidant activity and total polyphenol content.

RESULTS

The antioxidant activity

Among all examined herbs, the extract prepared from lingonberry leaves exhibited the highest antioxidant activity (725.2 mg Trolox/g d.w.). The oak bark (495.2) and St John's-wort herb (432.5) showed almost twice-lower antioxidant activity than lingonberry leaves (fig. 1). The lowest ability to scavenge the ABTS radical was observed for extracts from dog rose fruit and horsetail herb (20.6 and 25.3 mg Trolox/g d.w., respectively). Hawthorn, plantain and celandine extract also presented low antioxidant activity.

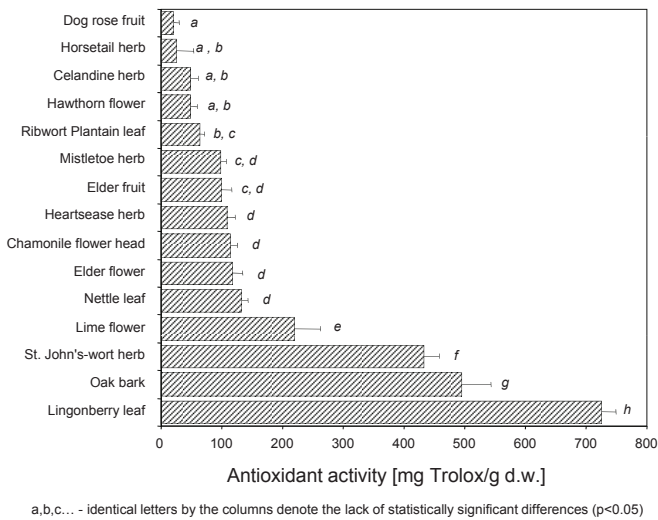
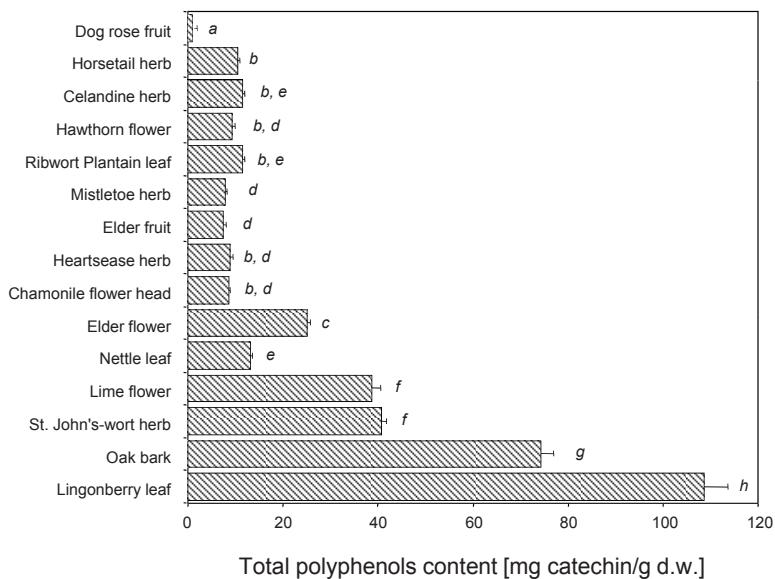


Figure 1. Antioxidant activity (mg Trolox/g d.w.) evaluated in herbal products prepared according to producer protocols (arithmetic mean \pm SD, $n = 5$)

Total polyphenol content

The analysis of total polyphenol content showed that lingonberry leaves extract is the richest source of those compounds among analyzed herbs (108.8 mg catechin/g d.w., see fig. 2). The lowest concentration of polyphenols was observed in dog rose fruit (1.1 mg catechin/g d.w.). Relatively high amounts of polyphenols were demonstrated also in oak bark (74.2), St John's-wort herb (40.7) and lime flower (38.6). The antioxidant activity and polyphenols content showed high and significant correlation within the results ($r = 0.964$, $p < 0.05$, see fig. 3)



a,b,c... - identical letters by the columns denote the lack of statistically significant differences ($p < 0.05$)

Figure 2. The total polyphenols content (mg catechin/g d.w.) in analyzed herbal products evaluated by Folin-Ciocalteu method (arithmetic mean \pm SD, $n = 5$)

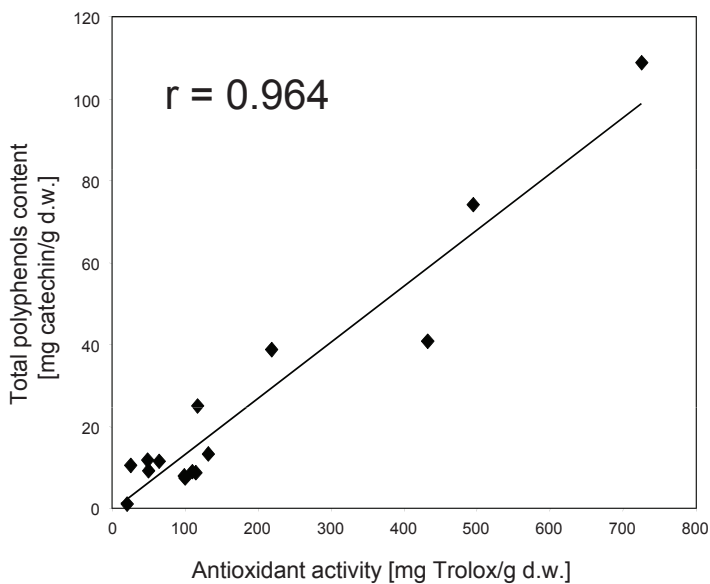


Figure 3. The relationship between the antioxidant activity and total polyphenols content, r - Pearson correlation coefficient

DISCUSSION

In present study, 15 different herbs popular in Poland and of widespread use in traditional treatment of different disorders were investigated as sources of plant antioxidants. They were provided in dry form, and the herbaceous materials were the whole aerial parts, flowers, fruits, or leaves.

Fruits of lingonberry are commonly used in many national cuisines, but their leaves are used rather as herbal remedy. We demonstrated that lingonberry leaves are very promising source of polyphenols (109 mg catechin/g d.w.) and characterized by extremely high antioxidant activity (725 mg Trolox/g d.w.). However, the amounts of polyphenols detected in extract prepared in the way suggested by producer are much lower than those demonstrated by other authors in methanol or hot water extracts. The methanolic and aqueous extracts were analyzed in our previous study [16] and in both cases the antioxidant activity obtained was much higher than in the present paper (1753 and 1136 mg Trolox/g d.w., respectively, in comparison with 725). However, the content of total polyphenols was higher only in methanolic extract (2-fold). According to references, lingonberry leaves are rich in tannins (up to 12%), arbutin and methylarbutin (5–7%), hyperoside, myricetin, and glycosides of quercetin. Benzoic acid, also present in the lingonberry, allows to prepare preservatives without boiling [22, 24]. All above suggests that different solvents used for herbal extract preparation allow to extract different compounds which obviously vary with the antioxidant capacity. Nevertheless, all those components can positively influence the human body. The lingonberry preservatives along with herbal preparations are utilized to counteract colds and urinary tract infections. They are also a rich source of antioxidant compounds and can contribute to prevent many disorders induced by reactive oxygen species.

Among other analyzed herbs, a relatively high antioxidant potential and polyphenols concentration was observed in oak bark, St. John's-wort herb and lime flower. In oak bark the main compounds are tannins and proanthocyanins, then catechin mono-, di- and trimers, flavonoids, and free phenolic acids [25]. It is an herbal product of external use (astringent, anti-inflammatory, wound healing, burnings), however it can be also used internally (as antidiarrheal or antibacterial remedy) [20, 26]. We proved that despite its astringent and antibacterial properties mentioned above, oak bark is also a rich source of antioxidants. St. John's-wort herb has been used in traditional medicine for years, and *Hyperici herba* is mentioned in many national pharmacopoeias. The main constituents are: phloroglucinol derivates (0.2–4%, mainly hyperforin and adhyperforin), naphthodianthrones (0.06–0.4%, such as pseudohypericin, hypericin, protohypericin, protopseudohypericin etc), flavonoids (2–4%, mainly quercetin glycosides like hyperoside, rutin, isoquercitrin, quercitrin), procyanidines, tannins (6–15%), essential oil (0.1–0.25%, 2-methyloctane, α -pinene) and small amounts of chlorogenic acid and other caffeoylquinic and p-coumaroylquinic acids, and also free amino acids [19]. Infusions prepared with water are widely used in traditional medicine at least in Central Europe, mainly as antidepres-

sant, although can be useful in other nervous system and psychiatric disorders. The traditional use of liquid preparations (water or ethanol as extraction solvent) of St. John's-wort herb for wound healing is supported by pharmacological data. Anti-inflammatory, analgesic, astringent and antibacterial activities are also documented. According to different authors, it can be also useful in treatment of hepatobiliary, gastrointestinal, renal, urinary, respiratory, thoracic, endocrine, metabolism, and nutrition disorders [19, 21, 27]. Kaltschmidt et al. [28] demonstrated that St. John's-wort herb was able to induce short-time activation of NF- κ B which declined to basal levels after 24 h. Extracts of *Hypericum perforatum* exhibited significant protective effects on the trauma of PC12 cells induced by 200 μ M H₂O₂ in a dose-dependent manner within 24-hour treatment [29]. Intra- and extra-cellular ROS levels decreased significantly to 71.9% and 50.0% of control, suggesting that *Hypericum* extract could easily enter the cells and play important role in reducing ROS levels. Moreover, *Hypericum* extract blocked DNA fragmentation and prevented H₂O₂-induced apoptosis. The authors conclude that St. John's-wort extracts may be a candidate for application in ROS-dependent neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease. Those theses have already find confirmation in studies of Silva et al. [30] and Dinamarca et al. [31]. In the study of Jeziorek and Wasek [32], among 30 analyzed herbs, *Hyperici herba* was on the 2nd place when flavonoids content was evaluated and on the 4th place when the antioxidant activity was concerned. *Hypericum* extracts showed relevant antioxidant activity both *in vitro* and in a cell system by means of inhibiting free radical generation and lipid peroxidation [33]. Extracts from St. John's-wort herb are effective inhibitors of lipid oxidation which positively correlated with high concentration of polyphenols. The qualitative chromatographic analysis of the *hyperici* extract demonstrated that it contained caffeic, ferulic and chlorogenic acids, and quercetin derivatives (rutin, quercetin-7-glucoside, quercetin-3,7-dirhamnoside, quercetin-3-glucoside-7-rhamnoside) [19, 34]. However, the solvent chosen for extraction influences the composition of extract [19] and has impact on its antioxidant properties.

In lime flower, the methanolic extract was characterized by 3 times higher polyphenols level and 2 times higher antioxidant activity [16] than extract prepared in water according to producer protocol. According to the references [22], the flower of *Tilia cordata* contains mainly antioxidant compounds, such as flavonoids (derivatives of quercetin and kaempferol) and volatile oils (farnesol, eugenol, geraniol). They are also rich in mucilage and organic acids. It seems that methanol is a better solvent for extraction of those components. However, extracts prepared in this study had contained relatively high polyphenols level.

Many studies show that fruits of wild growing plants can be a rich source of antioxidants. Increasing interest in traditional and forgotten plants can be observed recently [35]. In the present study, the fruits of elder and dog rose were analyzed, as an example of traditional Polish herbal material used in treatment of many diseases. The studies concerning chemical composition demonstrated that those fruits contain significant amounts in polyphenol compounds (mainly cyanidin and pelargonin

glycosides, and, to less extent, quercetin glycosides and chlorogenic acid [36, 37]. In fruits of dog rose carotenoids (about 0.7 mg/g d.w.), like lycopene and carotene, are also present. Both fruits contain also high levels of vitamin C, assessed for 250-2900 mg/100 g d.w. of dog rose fruit and about 18 mg/100 g in dry weight of elder [38, 39]. Surprisingly, the antioxidant capacity and total polyphenols content of dog rose fruit was the lowest among herbs analyzed in our study. The main cause of differences is that we had prepared extracts by method suggested by producer. When the plant raw material is analyzed, usually the optimal conditions are set and the most efficient solvent is used for extracting examined compounds. For example, the antioxidant activity and polyphenols level were 10 and 4-times higher in dog rose fruit extracted with methanol, respectively [16], than in extracts prepared according to producer's protocol. It is known that many bioactive compounds have higher affinity to organic solvents than to water, so their recovery with methanol is better [40, 41]. Moreover, during extraction made in the present study, the fruits had been boiled for 3 minutes, which probably caused the lost of activity of many thermolabile substances such as ascorbic acid. Also difference in the mode of herb pretreatment is of great importance. In our previous experiment, fruits were grounded before extraction so the solvent had better access to compounds present in fruit tissue. In the present study, whole, dry fruits were poured with water without any crumbling. Hence, although dog rose fruit is of a very big potential, it can provide only a small part of it when prepared in home conditions.

Relatively high antioxidant capacity was demonstrated in flower of elder (fig. 1 and 2), although, when compared with our previous research [16], the results obtained, both antioxidant activity and polyphenol level, were lower. The most important components of elder flowers are vitamin C, rutin, astragaline, quercetin, isoquercitrin, kaempferol, hyperoside, chlorogenic acid, triterpenes, sterols and tannins [21]. In the present study, the dry herbaceous material was only poured with boiling water, while previously the aqueous extract had been boiled for 20 minutes. When prolonged exposition for boiling water was applied, the inactivation of vitamin C probably took place. However, the higher amounts of other antioxidant components could be extracted from the plant material and the final antioxidant potential of aqueous extract can achieve the level of methanolic extract (~350 mg Trolox/g d.w.). In the study of Stoilova et al. [42], the antiradical activity of elder flowers was shown. The extract effectively inhibited conjugated dienes formation at the concentration lower than that used for the standard BHT. It had also significantly greater antiradical activity against $\cdot\text{OH}$ in comparison to DPPH radical, neutralizing it in a more effective manner than BHT and BHA. It suggests that extracts from elder flowers can be used in cosmetics, medicine or food industry with the same or even higher efficiency than those of synthetic antioxidants.

Herbs selected to the study were chosen because they are popular, easy available and have widespread use in traditional medicine. Although many of them are quite effective in treatment of particular diseases and disorders, only four of the analyzed herbs (mentioned above lingonberry leaf, oak bark, St. John's-wort herb, lime

flower) could be considered also as a valuable source of antioxidants. Other examined herbs contain many different bioactive compounds, that can influence human organism and help during healing and treatment process, although, they are weak antioxidants. It can be concluded that the mechanism of their activity is not based on the antioxidant activity. Obviously, the protocols for herbal remedies preparation must be designed in such way that bioactive components, the most important in the treatment of particular disease, could pass from the herbaceous material into infusion or extract. In some cases, those conditions are not optimal for recovery of other components, such as antioxidants. However, many traditional herbal remedies, used typically for treatment of various diseases, can play additional role as rich source of antioxidant compounds providing adequate extraction conditions.

CONCLUSIONS

1. The way of herbs preparation is crucial for their final properties. Protocols of herbal remedies preparation suggested by producer were designed in such way that bioactive components, the most important in the treatment of particular disorder, could pass from the herbaceous material into infusion or extract. In many cases, those conditions are not optimal for recovery of antioxidant compounds.

2. The extracts of lingonberry leaf, oak bark, St. John's-wort herb, and lime flower are rich sources of polyphenols (39–109 mg catechin/g d.w.) and exhibit high antioxidant activity (219–725 mg Trolox/g d.w.). Regardless from their therapeutic action, they supply human organisms with bioactive compounds of antioxidant activity and can protect our tissues against free radicals.

3. The information on the most efficient way of antioxidant compounds extraction and possible losses at improper conditions, should be applied during production of herbal preparation as well as for supplementation of foodstuff in natural antioxidants (functional food).

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WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCE I ZAWARTOŚĆ CAŁKOWITA POLIFENOLI W WYBRANYCH PREPARATACH ZIOŁOWYCH STOSOWANYCH W POLSCE

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Streszczenie

Celem badań było określenie aktywności antyoksydacyjnej oraz zawartości polifenoli ogółem w 15 popularnych ziołach często wykorzystywanych do leczenia różnych schorzeń. Materiał do badań stanowiły między innymi koszyczek rumianku, kora dębu, ziele dziurawca, kwiat głogu, owoce dzikiej róży i bzu czarnego, liść brusznicy. Napary lub ekstrakty ziołowe zostały przygotowane zgodnie z przepisem zamieszczonym przez producenta na opakowaniu danego zioła. Wysoką aktywność przeciwutleniającą oraz stężenie polifenoli ogółem otrzymano dla ekstraktów z liści borówki brusznicy (odpowiednio 725 mg Troloxu/g s.m. i 109 mg katechiny/g s.m.). Niezależnie od właściwości terapeutycznych, liście borówki brusznicy, kora dębu, ziele dziurawca oraz kwiaty lipy stanowią także bogate źródło związków antyoksydacyjnych. Niektóre z ziół, jak na przykład owoce dzikiej róży, tracą właściwości antyoksydacyjne podczas przygotowywania preparatu leczniczego w warunkach domowych.

Słowa kluczowe: *aktywność antyoksydacyjna, rośliny lecznicze, tradycyjne preparaty ziołowe, polifenole*