

Antioxidant activity of selected herbal plants

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Summary

The main aim of the study was to evaluate the antioxidant activity and total polyphenols content in fifteen herbal plants, such as chamomile flower head, oak bark, St. John's-wort herb, hawthorn flower, dog rose and elder fruits, lingonberry leaf, and many more. From the herbs both the water and methanolic extracts were prepared. The highest antioxidant activity and the highest polyphenols level were obtained for lingonberry leaves (1752.9 mg Trolox/g d.w. and 199.6 mg catechin/g d.w., respectively). The oak bark, St. John's-wort herb, and flowers of hawthorn and elder were also a very valuable source of antioxidant compounds. It was demonstrated that, in contrary to water, methanol was more efficient solvent for extraction of antioxidant compounds from lingonberry leaf, lime flower, fruits of elder and doge rose, oak bark, flowers of hawthorn, St. John's-wort herb and heartsease herb. Therapeutic activity of some examined herbs did not depend on antioxidant activity.

Key words: herbaceous plants, medicinal plants, antioxidant activity, polyphenols, extraction, solvent choice

INTRODUCTION

Free radicals and reactive oxygen metabolites can react with proteins, nucleic acids and lipids, causing changes in genetic material and inactivation of enzymes [1, 2]. Therefore, the human health depends on the efficiency of antioxidant mechanisms. Because of detrimental influence of peroxides and oxygen radicals

on organism there is growing interest in natural antioxidants, especially in polyphenols [3]. Lower level of those compounds was observed in samples collected from smokers, elders, and patients with some disorders, as well as among people subjected to health-threatening agents [4]. Quite efficient scavengers of free radicals are also some vitamins, such as ascorbic acid, tocopherols, carotenoids and retinol [5]. Antioxidants are present in fruits, vegetables, cereals, leguminous plants, juices, wine, tea, and many herbs [6]. Herbaceous plants, besides improving taste of dishes, are the rich source of antioxidants, which are more active than those from fruits and vegetables. Recently, the correlation between antioxidant activity and level of polyphenols, betalains or carotenoids in plants was proved [7-9].

In past decades the growing consumption of synthetic medicines and therapeutic preparations, both prescribed by doctors and available without prescription, has been observed. Simultaneously to the increased amounts and frequency of their use, the number of documented adverse or even toxic effects raised and caused the consumer confidence fall. Despite the broad information campaigns, supported by results of pharmacologic researches as well as aggressive advertising, the interest in synthetic medicines offered by chemical industry is still decreasing. All abovementioned causes that natural therapeutics and plant preparations are gaining popularity. At the moment, about 35% of medicines are of plant origin and they are recognized as equal to their chemical equivalents. Very convincing argument for natural preparations is their low price and ability of long-term use with quite good therapeutic efficiency and with no side effects [10-12].

Particular herbs, or even particular parts of herbaceous plants, have defined chemical composition including different organic and non-organic substances such as mineral salts, active enzymes, alkaloids, flavonoids, glycosides, vitamins and provitamins. Bioactive compounds are usually accumulated in vacuoles in plant cells. Many of them exert a pharmaceutical effect on human organism. Some herbal compounds can heal, other stimulate organism or are neutral. Although, also harmful or poisonous substances can be found in herbs, especially when taken in high amounts. The influence of individual compound depends, among other things, on dose.

Many of herbs and spices are already well characterized. Among them are thyme, ginger, garlic, onion, marjoram, poppy, nutmeg, saffron, turmeric, fennel, and many others [11, 13-15]. However, typical for Central Europe, and especially for Polish culture and tradition herbs are less known and examined when antioxidant properties are taken into account.

The main aim of the study was to determine and compare the antioxidant activity and polyphenols content in selected herbal plants, especially typical for Polish culture, depending on solvent used for extraction.

MATERIALS AND METHODS

Material

Fifteen herbs were used in the investigation. Dried, homogenous samples of herbs were purchased in Herbapol S.A. in Kraków. The name of herbaceous material, common name of herb, its Latin name and part of plant analyzed are listed in table 1.

Table 1.

The herbaceous material used in study

raw material	common name of herb	latin name of herb	part of plant
<i>Fructus Rosae</i>	dog rose	<i>Rosa canina</i> L.	fruit
<i>Fructus Myrtilli</i>	lingonberry	<i>Vaccinium vitis-idaea</i> L.	fruit
<i>Cortex Quercus</i>	english oak	<i>Quercus robur</i> L.	bark
<i>Folium Vitis idaeae</i>	lingonberry	<i>Vaccinium vitis-idaea</i> L.	leaf
<i>Folium Urticae</i>	nettle	<i>Urtica dioica</i> L.	leaf
<i>Folium Plantaginis</i>	ribwort plantain	<i>Plantago lanceolata</i> L.	leaf
<i>Flos Tilia</i>	small-leaved lime	<i>Tilia cordata</i> Mill.	flower
<i>Flos Sambuci</i>	elder	<i>Sambucus nigra</i> L.	flower
<i>Flos Crataegi</i>	hawthorn	<i>Crataegus laevigata</i> (Poir.) DC	flower
<i>Chamomillae Anthodium</i>	chamomile	<i>Matricaria recutita</i> L.	flower head
<i>Herba Hyperici</i>	St. John's-wort	<i>Hypericum perforatum</i> L.	herb
<i>Chelidoni Herba</i>	celandine	<i>Chelidonium majus</i> L.	herb
<i>Herba Violae tricoloris</i>	heartsease	<i>Viola tricolor</i> L.	herb
<i>Herba Equiseti</i>	horsetail	<i>Equisetum arvense</i> L.	herb
<i>Herba Visci</i>	mistletoe	<i>Viscum album</i> L.	herb

Chemicals

Diammonium salt of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS diammonium salt); 2,2'-diphenyl-1-picrylhydrazyl (DPPH); (\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 the SIGMA-Aldrich Company. The chemicals: potassium persulfate ($K_2S_2O_8$) and methanol (analytically pure) were obtained from the POCh Company, and a 96% ethanol from the Chem-Pur Company.

Extracts preparation

Methanolic extracts

A portion of the herbs was placed in a container of the laboratory mill and grounded (2×12 seconds). An amount of 40 mL of 80% (v/v) methanol was poured over a 0.500 g of ground sample and mixed for 2 h by a magnetic stirrer (500 rpm). The whole mixture was filtered and centrifuged for 10 min ($1467 \times g$, 20°C), and the supernatants obtained were collected into twisted test-probes. Those methanolic extracts were then stored in a freezer (-20°C).

Aqueous extracts

A portion of the herbs was placed in a container of the laboratory mill and grounded (2×12 seconds). An amount of 40 mL of boiling redistilled water was poured over a 0.500 g of ground sample and herbs were brewed for 20 min.

Assessment of the antioxidant activity

The antioxidant activity was assayed on the basis of a protocol represented by Re et al. [15] with some modifications incorporated. The ABTS radical was generated during a chemical reaction between the 7 mM aqueous solution of diammonium salt of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid 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) in such a way that allowed for obtaining the final absorbance of $A=0.70 \pm 0.02$ ($\text{ABTS}_{0.7}$) measured at 734 nm (spectrophotometer BECKMAN DU 650). 100- μL aliquots of the properly diluted extract or of Trolox solutions (their concentration ranging from 0 to 100 mg/L) were added to 1 mL of $\text{ABTS}_{0.7}$, and the absorbance was measured 6 min after mixing. The antioxidant capacity of extracts under study was calculated using a standard curve drawn up for solutions of the synthetic vitamin E (Trolox) and expressed in mg of Trolox/g of dried weight. All determinations were performed in 5 replications.

Total polyphenol content assay

Total polyphenols content was assayed by Folin-Ciocalteu method on the basis of a protocol represented by Swain and Hillis [16]. An amount of 45 mL of redistilled water, 0.25 mL of Folin-Ciocalteu reagent (water dissolved 1:1 v/v) and 0.5 mL of 7% Na_2CO_3 were added to the 5 mL of herb extract. The mixture was left out for 30 minutes in dark. Then the absorbance was measured on a spectrophotometer (BECKMAN DU 650) at 760 nm. The obtained results of total polyphenols content were expressed as mg of catechin/g of dry weight based on the standard

curve drawn up for catechin methanol solutions (their concentration ranging from 0.1 to 15 mg/L). All determinations were performed in 5 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 a statistical analysis. A Kolmogorov-Smirnov test was applied to examine the normality of distribution.

RESULTS

Analysis of antioxidant activity

Among all the methanol solutions examined, the lingonberry extracts were characterized by the highest antioxidant activity (1752.9 mg of Trolox/g d.w.). The oak bark, St John's-wort herb and flower of hawthorn showed twice lower antioxidant activity (fig. 1). The lowest ability to ABTS radical scavenging had extracts from nettle leaves and from herbs of mistletoe, heartsease and horsetail.

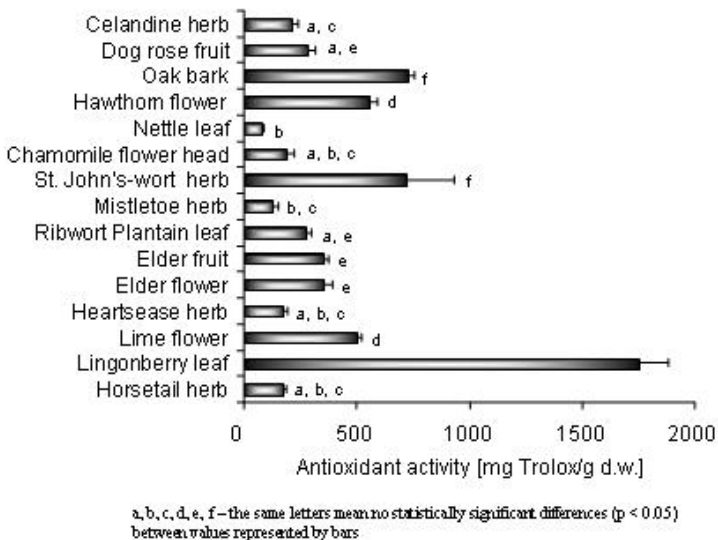


Figure 1. Antioxidant activity of herbal plants extracts prepared with methanol and expressed as mg of Trolox/g of dry weight (mean \pm SD, n = 5)

When the aqueous extracts were analyzed, lower values had been obtained. The lingonberry leaf had the highest antioxidant activity, reaching the value of 1136.2 mg of Trolox/g d.w. Relatively high antioxidant properties had also lime flower (812.5), oak bark (447.1), St. John's-wort herb (367.4) and elder flower (350.9) (fig. 2). The extracts prepared from fruits of dog rose and elder, as well as from horsetail herb were characterized by the lowest activity (24.3, 65.4, and 64.3, respectively).

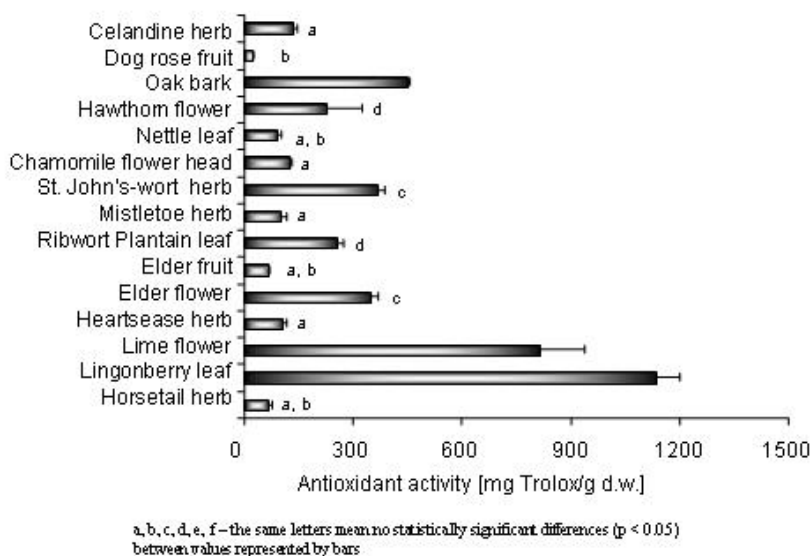


Figure 2. Antioxidant activity of aqueous extracts of herbal plants expressed as mg of Trolox/g of dry weight (mean \pm SD, n = 5)

Determination of total polyphenols content

The results of analysis showed that the highest concentration of polyphenol compounds had methanolic extracts of lingonberry leaves (199.6 mg of catechin/g d.w.) (fig. 3). Similarly, like in antioxidant activity evaluation, twice lower values were obtained for lime flower (105.8), St John's-wort herb (90.9), hawthorn flower (90.9) and oak bark (83.5 mg catechin/g d.w.). The lowest polyphenol content had extracts from nettle leaves (5.3) and from herbs of horsetail (10.6), mistletoe (11.2) and heartsease (12.0). The values obtained for aqueous extracts were lower than for methanolic ones and a very high differentiation among the results was observed, ranging from 0.5 (for dog rose fruit) to 80.7 mg of catechin/g d.w. for lingonberry leaves (fig. 4).

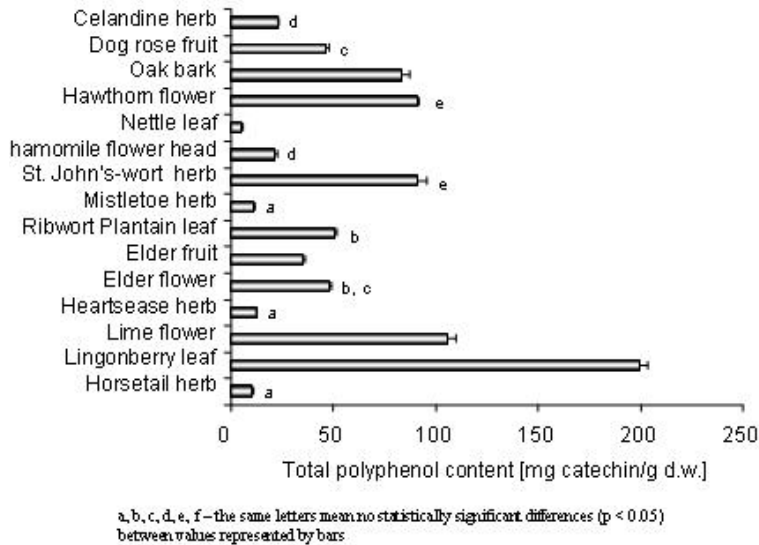


Figure 3. Total polyphenols content of methanolic extracts of herbal plants expressed as mg of catechin/g of dry weight (mean \pm SD, n = 5)

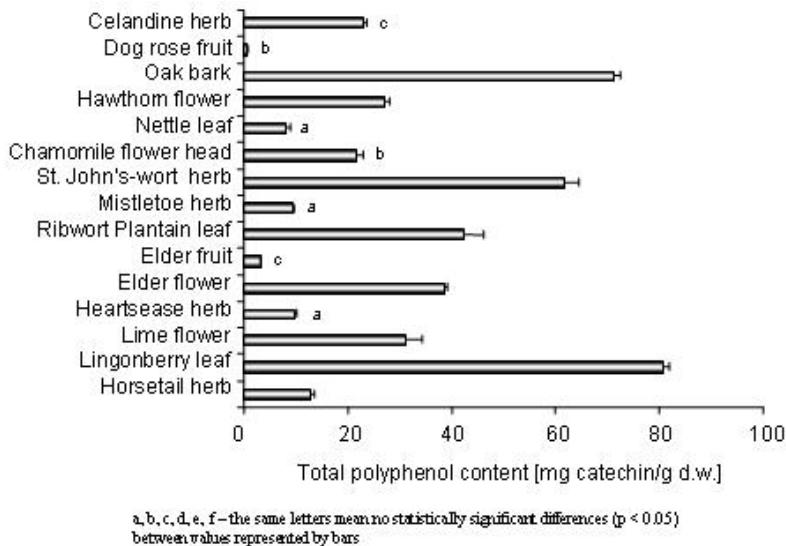


Figure 4. Total polyphenols content of aqueous extracts of herbal plants expressed as mg of catechin/g of dry weight (mean \pm SD, n = 5)

DISCUSSION

Among the examined herbs, leaves of lingonberry appeared to be the richest source of antioxidants compounds of a very high activity. In culinary tradition the fruits of lingonberry are quite popular, but its leaves are herbaceous material of high interest. Lingonberries contain plentiful organic acids, vitamin C, β -carotene, vitamins B₁, B₂, B₃, and the mineral elements (K, Ca, Mg, P). Its leaves are also rich in glycosides (5–7%), with arbutin and methylarbutin being of the most importance; they contain tannins (~12%), flavonoids (hyperoside, miricetin, quercetin glycosides), and benzoic acid (allowing to prepare lingonberry preservatives without boiling) [17-19]. For the sake of healthful nutrients contents, lingonberries preservatives and herbal preparations are used to counteract colds and urinary-tract infections. In our work it has been shown that they can also be used as a valuable source of antioxidants. We also proved that methanol was better solvent for extraction of those compounds, allowing to obtain almost twice higher values both of polyphenol content and antioxidant activity.

In the case of fruit of elder and dog rose both antioxidant activity and polyphenol content were several times higher in methanolic extracts than in aqueous ones. Among the antioxidants of elder phenolic compounds constitute the majority, reaching about 20 mg/g d.w. There are mainly cyanidin and pelargonin glycosides, and to less extend quercetin glycosides and chlorogenic acid [20, 21]. In fruits of dog rose carotenoids (about 0.7 mg/g d.w.), like lycopene and carotene are also present [22]. Such polyphenols profile contributes to higher efficiency of methanolic extraction, as affinity of those compounds to organic solvents is much higher than to water [23-25]. Both fruits contain high levels of vitamin C, assessed for 29 mg/g d.w. of dog rose fruit and about 18 mg/100 g of dry weight of elder [3, 26]. This is another cause of lower antioxidant activity of aqueous extracts; ascorbic acid lost its properties in high temperature.

The different situation is when the flower of elder was used as herbaceous material. The use of methanol as a solvent allowed increasing the efficiency of polyphenols extraction by about 25%. However, the antioxidant activity of methanolic extracts did not vary significantly from those obtained by aqueous extraction. Similar results were obtained for leaf of ribwort plantain and mistletoe herb; the polyphenol extraction yield was near 20% higher when methanol was used. The flowers of elder are rich in vitamin C which is antioxidant and protects polyphenols present in extract. The other important compounds are: rutin, isoquercitrin, kaempferol, hyperoside, chlorogenic acid, and tannins [18]. During long exposition to boiling water the vitamin C can be lost, but higher amounts of other antioxidant components can be extracted from the plant material and the final antioxidant potential of aqueous extract can achieve the level of methanolic extract. In the study of Stoilova et al. [27] the antiradical activity of elder flower were shown. Elder flower extract effectively inhibited conjugated dienes formation at the concentration lower than that used of the standard BHT. It had also significantly gre-

ater antiradical activity against $\cdot\text{OH}$ in comparison to DPPH, neutralizing it in a more effective manner than BHT and BHA. It suggests that natural herbal extracts can be used in cosmetics, medicine or food industry with the same or even more efficiency like synthetic antioxidants.

The ribwort plantain leaves contain iridoid glycosides (1%), such as aucubin and catalpol. They exert strong effect on human organisms, are implicated in wound healing and stimulating immunological system, act as sedative, anaesthetic, alterative, antiseptic, antiviral, antitoxic, antihistaminic, antiinflammatory, antirheumatic and anticancer agents. Moreover, plantain contains vitamins A, C, and K and high levels of calcium, magnesium, sodium, phosphorous, zinc, copper and cobalt. Among phenolic acids the most important are fumaric, benzoic, cinnamic, and vanilic acid, and among flavonoids baicalin and apigenin are the most familiar [17, 28]. Also sorbitol (1.5%), tannins (~6%), pectins, alkaloids, and mucilage are responsible for properties of plantain [18].

The level of polyphenols in mistletoe extracts is rather low, however, it contains many bioactive compounds (lectins like viscumin and polypeptides like viscotoxin, omelotoxin) characterized by anticancer properties. The mechanism of their activity is not based on the antioxidant activity, it is rather cytotoxic and immunostimulatory influence. Mistletoe extracts are marketed under several trade names, such as Iscador, Helixor, Eurixor, and Isorel, most of which are available in Europe, and are used for cancer treatment and for complementary therapy [29].

The hawthorn flower, oak bark, and St. John's-wort appeared to be a quite valuable source of antioxidant compounds. In all those cases, methanol used for extraction allowed to obtain higher both polyphenols level and antioxidant activity. It was expected as the flower of hawthorn contains mainly flavonoids (~1%), such as vitexin, isovitexin, rutoside, hyperoside, kaempferol, catechin, epicatechin and their derivatives, procyanidins, triterpens (about 0.3%), phenolic acids (caffeic, chlorogenic), coumarins and others [18]. The majority of those compounds is better extracted by alcohols than by water. In oak bark the main compounds are tannins and proanthocyanins (about 4%), then catechin mono-, di- and trimers, flavonoids and free phenolic acids [30], while in St. John's-wort herb, antioxidant activity depends mainly on the high concentration of carotene (13%), tannins (up to 12%), anthocyanins (6%), polyphenols (hyperoside, rutin, quercetin, quercitrin), and vitamin C [18].

Recently, many studies were done with water extracts of nettle herb. They confirm nettle's ability to reduce blood pressure, and its antioxidant, antimicrobial, antiulcer, immunostimulatory, and analgesic effect [31-33]. In our research the extracts of nettle leaves appeared to be rather poor antioxidants when compared with other examined herbs. Although water was a better solvent and allowed to extract more polyphenols, the antioxidant activity in methanolic and aqueous extracts was almost the same.

In the case of lime flower, the methanolic extract was characterized by 3 times higher polyphenols level, but its antioxidant activity was lower by almost 40%.

According to the references [18] flower of *Tilia cordata* contain mainly antioxidant compounds, such as flavonoids (derivatives of quercetin and kaempferol) and volatile oils (farnesol, eugenol, geraniol). They are also rich in mucilages and organic acids. It seems that methanol is a better solvent for extraction of those components. However, antioxidant activity of those components is low. The properties of the aqueous extract were much higher and probably in only little extent dependent on polyphenols.

In case of chamomile, celandine and horsetail the amounts of polyphenols extracted by methanol were only slightly lower (not statistically significant) than those obtained with use of water. However, the activity of methanolic extracts was higher by 30–60%. Despite of that, all mentioned herbs were characterized by low antioxidant properties. It seems that the antioxidant activity is not the most important way of their therapeutic action. The studies of Then et al. [34] shown that celandine can act as antioxidant, and its activity is independent on alkaloid content.

The bioactive compounds present in selected herbs can influence human organism and help during healing and treatment process. Recently, many researches start to analyze antioxidant properties of traditional herbaceous plant. Pourmorad et al. [35] investigated, among others, methanolic extracts prepared from dried horsetail, nettle and plantain. His results varied from ours and indicated that horsetail had higher polyphenols level than other herbs. It can be caused by differences in soil and climate conditions, herb origin or way of cultivation and treatment. Ninety two medicinal plants were investigated by researchers from the University in Helsinki, Finland [36]. In the study, lingonberries were shown as a rich source of polyphenols (24.9 mg gallic acid /g d.w.). Lowest values were obtained for dog rose fruit (12.5) and flower head of chamomile (9.1). Although the values differ from ours (gallic acid was used instead of catechin as a standard), the relationship are similar. Kim et al. [37] analyzed the ability of 180 methanolic extracts of oriental herbal plants to oxidize linolic acid. Among selected herbs were celandine, horsetail and hawthorn. Also in this case, our results were in accordance with obtained in this study.

CONCLUSIONS

The main aim of the study was to compare antioxidant properties of several herbaceous plants depending of the solvent used for extraction. The herbs selected for analysis are traditional plants with well known therapeutic activity. However, they were poorly characterized with respect of antioxidant properties or polyphenols content. The results of our study can contribute to bigger interest in herbaceous material and its better utilization in food and cosmetic industry. Information on the most efficient way of antioxidant compounds extraction can be applied during production of herbal preparation as well as for supplementation of foodstuff in natural antioxidants (functional food).

Our results show that:

- 1) lingonberry leaf, oak bark, St. John's-wort herb, and flower of hawthorn and elder are a very valuable raw material rich in antioxidant compounds;
- 2) methanol is better and more efficient solvent in contrary to water for extraction of antioxidants from lingonberry leaf, flower of lime, fruits of elder and doge rose, oak bark, flowers of hawthorn, St. John's-wort herb and heartsease herb;
- 3) therapeutic activity of nettle, heartsease, mistletoe, chamomile, celandine and horsetail probably does not depend on antioxidant activity.

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WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCE WYBRANYCH ROŚLIN ZIELARSKICH

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Streszczenie

Celem badań było określenie aktywności antyoksydacyjnej oraz zawartości polifenoli ogółem w 15 wybranych surowcach zielarskich takich jak koszyczek rumianku, kora dębu, ziele dziurawca, kwiat głogu, owoce dzikiej róży i bzu czarnego, liść brusznicy i in. Z wybranych ziół zostały przyrządzone ekstrakty wodne oraz metanolowe. Najwyższą aktywność przeciwutleniającą oraz najwyższe stężenie polifenoli ogółem otrzymano dla ekstraktów z liści borówki brusznicy (odpowiednio 1752,9 mg Troloxu/g s.s i 199,6 mg katechiny/g s.s). Równie cennym źródłem przeciwutleniaczy okazały się kora dębu, ziele dziurawca, kwiaty głogu i bzu czarnego. Wykazano, że metanol jest wydajniejszym od wody ekstrahentem do wyodrębniania przeciwutleniaczy z liści brusznicy, kwiatów lipy, owoców bzu i dzikiej róży, kory dębu, kwiatów głogu, oraz ziela dziurawca i fiołka. Właściwości lecznicze niektórych z badanych ziół nie są zależne od potencjału antyoksydacyjnego.

Słowa kluczowe: *rośliny zielne, rośliny lecznicze, aktywność antyoksydacyjna, polifenole, ekstrakcja, dobór rozpuszczalnika*