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The screening analysis of antiradical activity of some plant extracts*

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Summary

Introduction:

There is a need for screening studies in order to select plant extracts or plant raw materials with strong antiradical activity which could be used as medicines or substances to protect food from oxidation. In this paper the antiradical activities of some plant raw materials were investigated.

Material/Methods:

The intensity of antiradical activity of extracts was investigated using DPPH[•] (1,1-diphenyl-2-picrylhydrazyl) radical as a substrate. The antiradical activity unit was defined and the number of antiradical activity units EAU_{515} per 1 mg of plant extract and TAU_{515} per 1 g of plant raw materials were calculated. Plant extracts were obtained with a methanol or methanol-water (1:1) solution.

Results and Discussion:

The highest numbers of antiradical activity units EAU_{515} were found for ethyl acetate extracts from the leaves of green and black tea. The lowest EAU_{515} value was demonstrated for garlic extracts. When the number of activity units TAU_{515} was calculated per 1 g of raw material, the highest value was found for the leaves of green tea, much lower for bee propolis and the leaves of black tea. On the basis of the presented results, green tea leaves, bee propolis, and the leaves of black tea could be considered as potential sources of extracts with strong antiradical activity.

Key words:

plant extracts • antiradical activity • antioxidants

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INTRODUCTION

Plant extracts rich in phenolic acids exhibit strong antioxidant and antiradical activity *in vitro* [7,13,18] and *in vivo* [4,22,30]. Among the compounds with strong antiradical features one could mention tannins, flavonoids, and phenolic acids. Tannins are high-molecular compounds with complicated and variable structure, exhibiting usually strong antiradical and antioxidant activity [14]. An interesting group of compounds are derivatives of catechins and gallic acid, the so-called gallo catechins, which are present in green tea [21]. Research has shown that these compounds have strong antioxidant activity as well as some anticancer features [24].

Flavonoids belong to the plant phenolic dyes with smaller molecules than tannins. Their antioxidant and antiradical activity varies from very weak, as in the case of naryngenin or hesperidin, to very strong, as in the case of compounds belonging to the flavonols, such as quercetin and miryctin [23]. Another group of plant compounds are red, blue, and violet anthocyanins, often with a glycosidic character. Among these, cyanidin and delphinidin exhibit strong antiradical and antioxidant activity [1,11].

The next group of plant phenolic compounds exhibiting antiradical and antioxidant activity are simple phenolic acids. We can divide them into derivatives of shikimic acid, such as caffeic and rosmarinic acids, or derivatives of benzoic acids, such as protocatechuic and gallic acids. Most of the phenolic acids mentioned above are very effective antioxidants and antiradicals [19,27].

In a number of chronic diseases, reactive oxygen species (ROS), such as singlet oxygen, superoxide radical, hydrogen peroxide, or hydroxyl radical, are formed as a consequence of the action of macrophages. The use of plant extracts with substantial antioxidant features could soften the negative effects of ROS action. The antiradical and antioxidant activity of plant extracts and phenolics was demonstrated many times *in vivo* [16]. In spite of the fact that the phenolic compounds described earlier are probably the main agents causing the antiradical or antioxidant activity of plant extracts, they are not the subject of this study. The main aim of this research was to obtain extracts from selected plant raw materials and to measure their antiradical activity. The antiradical effectiveness of extracts obtained from various raw materials were compared with the effectiveness of extracts with strong antioxidative activity obtained from the leaves of different kinds of teas.

The activities of the extracts and raw materials were described in defined antiradical activity units. The use of the antiradical activity unit allows one to compare the antiradical activities of different plant extracts and plant raw materials and to evaluate the yield of the isolation process of antiradical substances counted in activity units. The antiradical activity units were calculated per 1 mg of extract and per 1 g of raw material.

MATERIAL AND METHODS

Origin of raw material

The raw materials were chosen on the basis of literature information as materials containing high amounts of

phenolic compounds. The other criterion for choosing the type of raw material was the widespread usage of the raw material as a medicine and vegetable. Leaves of different kinds of teas, known as a source of strong antiradical extracts and compounds (gallo catechins, theaflavins), were used as a comparative raw material.

Raw materials extracted by method **I** are marked with **I–VI** and those extracted by method **II** are marked with **1–12**. These materials were:

Kawon, Gostyń: herb of goldenrod (*Virgaureae herba*) – **II**, herb of knotgrass (*Polygoni avicularis herba*) – **IV**, herb of marjoram (*Majoranae herba*) – **1**, herb of common wormwood (*Absinthii herba*) – **7**, herb of greater celandine (*Chelidonii herba*) – **8**, flower of linden (*Tiliae inflorescentia*) – **10**, herb of southernwood (*Abrotani herba*) – **11**;

Flos, Mokrsko: leaf of red pu-erh tea (*Theae folium*) – **6**, seed of black mustard (*Sinapis nigrae semen*) – **9**, herb of echinacea (*Echinaceae herba*) – **12**;

Gospodarstwo Pasieczne, Wambierzyce: bee propolis (*Propolis*) – **I**;

Kamis-Przyprawy S.A, Stefanowo, Wólka Kosowska: seed of pepper (*Piperis nigri semen*) – **V**;

Multeafil, Dobrzyca: leaf of black tea (*Theae folium*) – **2**;

Bio-Active Sp. z o.o., Warszawa: leaf of green tea (*Theae folium*) – **3**;

Yunnan Heichao Teablocks Co Ltd.: leaf of red pu-erh tea in briquettes (*Theae folium*) – **4**;

Origin of other raw material: spruce shavings from a forest in the Sudety Mountains (*Picea abies*) – **III**, bulb of garlic (*Allium sativum bulbosum*) – **VI**, fruit of red pepper (*Capsici fructus*) – **5**.

Extracts preparation

Two methods of extraction were used **I** and **II**. Method **I** was used to extract raw materials **I–VI**. In this method, methanol was used as the first solvent. In raw materials **I–VI**, flavonoids and phenolic acids, which are soluble in methanol, were recognized as the main phenolic fraction with antiradical activity.

Method **II** was applied to extract raw materials **1–12** with 50% methanol in water. In these raw materials, tannins were considered as the important fraction with antiradical activity. Tannins are insoluble in methanol, but well soluble in a mixture of methanol: water (1:1).

Method I of extraction

In method **I**, methanol was chosen as the first solvent in order to extract a wide range of phenolic compounds with a limited amount of tannins. Seven to nineteen grams of raw material was extracted with 250 ml of methanol at 50°C for three days. After separation of the raw material by filtration, the methanol was evaporated under reduced pressure and the dry residue was dissolved in hot water. After three days of storage at 4°C, the solution was filtered (filter discs, grade 388, Filtrak). The precipitate was collected and marked as **Wa**, with the number of the appropriate raw material (see above). The remaining solution was extracted with ethyl acetate (5×50 ml). The ethyl acetate extract was evaporated under reduced pressure to obtain an

extract marked as **Wb** with the number of the raw material. The remaining aqueous solution was concentrated to dryness under reduced pressure to obtain extract **Wc** with the appropriate number.

Yield of extraction

(Y%) was calculated for two methods **I** and **II** according:

$$Y\% = \frac{Cle}{w_R} \cdot 100\%$$

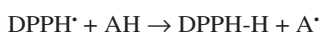
where w_R is the weight of raw material taken to extraction [g], Cle is total amount of extracts [g].

Method II of extraction

In method **II**, 50% methanol in water was used to obtain an extract with a large quantity of tannins. Five to fifty grams of raw material was extracted with 250–900 ml of a solution of methanol-water (1:1 v/v) for two days at 50°C. Twenty percent of the total volume of the methanol-water extract was evaporated to dryness under reduced pressure to obtain extract **WA**. The methanol was evaporated (reduced pressure) from the remaining extract and the aqueous remainder was stored at 4°C for two days. Then the aqueous solution was filtered and the precipitate discarded. The solution was extracted with ethyl acetate (200–600 ml). The ethyl acetate extract and aqueous remainder were concentrated to dryness to obtain extracts **WB** and **WC**.

Measurement of antiradical activity

DPPH[•] (1,1-diphenyl-2-picrylhydrazyl) radical in its radical form has a characteristic absorbance at 515 nm which disappears after its reduction by an antiradical compound (AH). The reduction of DPPH[•] can thus be monitored by measuring of the decrease in its absorbance at 515 nm during the reaction.



The method is simple, precise, and inexpensive, which is important in a screening investigation. All details related to the method are described by Brand-Williams et al. [5].

Solution **A** was prepared by dissolving 2 mg of DPPH[•] radical in 54 ml of methanol. Solution **B** was prepared by dissolving the investigated extract in methanol at concentrations dependent on the activities of the extracts. Then 40 µl of solution **B** was added to 1460 µl of solution **A** at room temperature. A control sample was prepared by adding 40 µl of methanol to 1460 µl of solution **A**.

Absorbance was measured at 515 nm in a 1-cm glass cuvette at time 0 and after 1 min of reaction against a blank (40 µl of solution **B** was added to 1460 µl of methanol).

The antiradical activity (AU_{515}) was calculated according to the equation:

$$AU_{515} = (A_0 - A_1) - (A_{0K} - A_{1K})$$

where AU_{515} is the antiradical activity of the extract, A_0 the absorbance of the sample at the beginning of the reaction

(0 min), A_1 the absorbance of the sample after 1 min of the reaction, A_{0K} the absorbance of the control sample at the beginning of the reaction, and A_{1K} the absorbance of the control sample after 1 min of the reaction. Because $A_{0K} - A_{1K}$ was always equal to 0, the above equation was simplified to:

$$AU_{515} = A_0 - A_1$$

The absorbance of the samples was measured three times and the standard deviation was calculated.

The antiradical activity unit was defined as the activity decreasing the absorbance of a sample at 515 nm of 1 after 1 minute of reaction at 20°C under the defined test conditions (described in this study).

The number of antiradical activity units (EAU_{515}) was calculated per 1 mg of each extract according to the following equation:

$$EAU_{515} = \frac{AU_{515}}{Ie}$$

where Ie is the amount of extract in the sample [mg] and AU_{515} the antiradical activity of the extract.

Then the total number of antiradical activity units extracted from each raw material was calculated per 1 g of raw material (TAU_{515}) as described below.

For raw materials extracted according to method **I**, PAU_{515} (total number of antiradical activity units in the extract) was calculated separately for each extract **Wa**, **Wb**, and **Wc** according to the equation:

$$PAU_{515} = \frac{Cle}{Ie} \cdot AU_{515} \quad (1)$$

where Cle is the total amount of extract [mg] and Ie the amount of extract in the measured sample [mg].

Then the number of antiradical activity units (TAU_{515}) isolated from 1 g of raw materials **I–VI** was calculated as:

$$TAU_{515} = \frac{PAU_{515}(a) + PAU_{515}(b) + PAU_{515}(c)}{w_R}$$

where $PAU_{515}(a)$ is PAU_{515} calculated for extracts **Wa**, $PAU_{515}(b)$ is PAU_{515} calculated for extracts **Wb**, $PAU_{515}(c)$ is PAU_{515} calculated for extracts **Wc**, and w_R is the weight of raw material taken for extraction [g].

For raw materials extracted with method **II**, PAU_{515} was calculated separately for extracts **WA**, **WB**, and **WC** according to equation (1). Then TAU_{515} isolated from 1 g of raw materials **1–12** was calculated as:

$$TAU_{515} = \frac{PAU_{515}(A) + PAU_{515}(B) + PAU_{515}(C)}{w_R}$$

where $PAU_{515}(A)$ is PAU_{515} calculated for extracts **WA**, $PAU_{515}(B)$ is PAU_{515} calculated for extracts **WB**, $PAU_{515}(C)$ is PAU_{515} calculated for extracts **WC** and w_R is the weight of raw material taken for extraction [g].

Table 1. Antiradical activity of extracts **Wa**, **Wb** and **Wc** (EAU_{515}) obtained with extraction method *I*. The number of antiradical activity units in 1 mg of extract (EAU_{515}) and 1 g of raw material (TAU_{515}). Bee propolis – **I**, herb of goldenrod – **II**, spruce shavings – **III**, herb of knotgrass – **IV**, seed of black pepper – **V**, bulb of garlic – **VI**

Raw material	Yield of extraction (Y%)	Antiradical activity (AU_{515})	The number of antiradical activity unites in 1 mg of extract (EAU_{515})	The number of antiradical activity unites in 1 g of raw material (TAU_{515})
Wa I	42.6	0.603±0.0115	10.9±0.21	4830±93.9
Wb I	1.24	0.652±0.0144	11.8±0.26	
Wc I	1.24	0.140±0.0115	1.3±0.10	
Wa II	3.65	0.623±0.0210	5.6±0.19	1081±34.4
Wb II	4.18	0.440±0.0115	16.0±0.42	
Wc II	6.33	0.360±0.0173	3.3±0.16	
Wa III	0.97	0.280±0.0115	2.5±0.10	40.3±1.8
Wb III	0.64	0.283±0.0115	2.6±0.10	
Wc III	0.14	0.020±0.0115	0.2±0.10	
Wa IV	2.92	0.383±0.0115	13.9±0.42	738±29.4
Wb IV	1.27	0.492±0.0144	8.9±0.26	
Wc IV	9.70	0.247±0.0158	2.2±0.14	
Wa V	4.62	0.153±0.0115	1.4±0.10	114±19.2
Wb V	0.60	0.180±0.0115	1.6±0.10	
Wc V	1.97	0.223±0.0115	2.0±0.70	
Wa VI	2.18	0.067±0.0158	0.6±0.14	35±10.53
Wb VI	0.12	0.077±0.0115	0.7±0.10	
Wc VI	6.96	0.033±0.0115	0.3±0.10	

RESULTS AND DISCUSSION

The number of antiradical units (EAU_{515}) calculated per 1 mg of extract are shown in Tables 1 and 2 and Figures 1 and 2.

Of the **Wa** extracts, the strongest EAU_{515} were calculated for the extracts obtained from the herb of knotgrass **Wa IV** (Table 1, Figure 1), bee propolis **Wa I**, and the herb of goldenrod **Wa II**, respectively 13.9, 10.9, and 5.6. The weakest EAU_{515} were demonstrated for extracts from garlic **Wa VI** and seed of black pepper **Wa V**, with values of 0.6 and 1.4, respectively. The highest EAU_{515} values for **Wb** (Table 1, Figure 1) were calculated for the extracts obtained from the herb of goldenrod **Wb II**, bee propolis **Wb I**, and the herb of knotgrass **Wb IV**, respectively 16.0, 11.8, and 8.9. The lowest EAU_{515} were calculated for the extracts from bulb of garlic **Wb VI** and black pepper **Wb V**, respectively 0.7 and 1.6. Extracts **Wc** had the lowest number of antiradical activity units (EAU_{515}) calculated per 1 mg of extract (Table 1, Figure 1). Of these, the highest EAU_{515} values were calculated for the extracts from the herb of goldenrod **Wc II** and the herb of knotgrass **Wc IV**, with values of 3.3 and 2.2, respectively. The lowest EAU_{515} were calculated

for the extracts from spruce shavings **Wc III** and garlic **Wc VI** with 0.2 and 0.3.

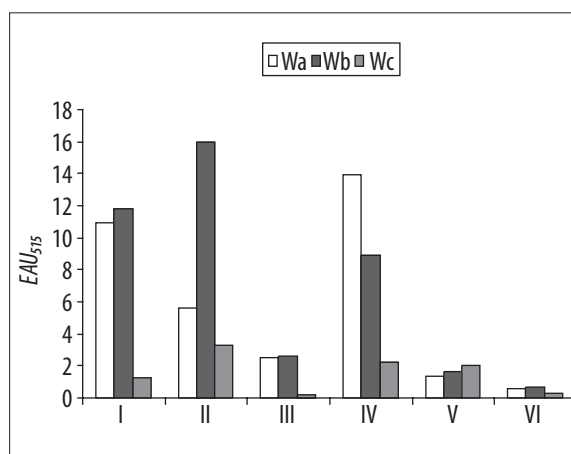


Figure 1. The number of antiradical activity units (EAU_{515}) calculated for 1 mg of extracts **Wa**, **Wb**, and **Wc** obtained by extraction method *I* from the following raw materials: bee propolis – **I**, herb of goldenrod – **II**, spruce shavings – **III**, herb of knotgrass – **IV**, seed of black pepper – **V**, bulb of garlic – **VI**

Table 2. Antiradical activity of extracts **WA**, **WB** and **WC** (AU_{515}) obtained with extraction method **II**. The number of antiradical activity units in 1 mg of extract (EAU_{515}) and 1 g of raw material (TAU_{515}). Herb of marjoram – **1**, leaf of black tea – **2**, leaf of green tea – **3**, red tea in briquets – **4**, fruit of red pepper – **5**, leaf of red tea – **6**, herb of common wormwood – **7**, herb of greater celandine – **8**, seed of black mustard – **9**, flower of linden – **10**, herb of southernwood – **11**, herb of echinacea – **12**

Raw material	Yield of extraction (Y%)	Antiradical activity (AU_{515})	The number of antiradical activity unites in 1 mg of extract (EAU_{515})	The number of antiradical activity unites in 1 g of raw material (TAU_{515})
WA 1	23.1	0.307±0.0115	2.8±0.10	1341±50.5
WB 1	4.0	0.854±0.0346	31.0±1.26	
WC 1	14.1	0.227±0.0115	2.0±0.10	
WA 2	28.0	0.463±0.0158	16.8±0.57	3003±102.2
WB 2	6.1	0.493±0.0158	35.9±1.14	
WC 2	12.0	0.442±0.0173	4.0±0.16	
WA 3	40.8	0.393±0.0158	28.6±1.15	8646±346.7
WB 3	11.6	0.437±0.0115	63.5±1.68	
WC 3	12.3	0.470±0.0115	4.3±0.10	
WA 4	32.5	0.570±0.0115	5.1±0.10	992±20.1
WB 4	6.7	0.603±0.0115	10.9±0.21	
WC 4	19.5	0.063±0.0115	0.5±0.10	
WA 5	17.8	0.060±0.0115	0.5±0.10	313±60.3
WB 5	0.81	0.137±0.0115	1.2±0.10	
WC 5	44.9	0.087±0.0115	0.8±0.10	
WA 6	31.1	0.490±0.0115	8.9±0.21	1705±40.2
WB 6	5.2	0.575±0.009	20.9±0.31	
WC 6	20.0	0.197±0.0115	1.8±0.10	
WA 7	26.0	0.210±0.0115	1.9±0.10	340±18.7
WB 7	1.4	0.313±0.0115	2.8±0.10	
WC 7	16.4	0.180±0.0115	1.6±0.10	
WA 8	28.0	0.153±0.0115	1.4±0.10	287±18.7
WB 8	0.62	0.313±0.0210	2.8±0.19	
WC 8	16.2	0.163±0.0210	1.5±0.19	
WA 9	15.0	0.120±0.0115	1.1±0.10	112±10.8
WB 9	0.53	0.500±0.0115	4.5±0.10	
WC 9	8.3	0.100±0.0115	0.9±0.10	
WA 10	24.3	0.147±0.0158	1.3±0.14	800±85.9
WB 10	2.9	0.453±0.0115	16.4±0.41	
WC 10	17.7	0.273±0.0231	2.5±0.21	
WA 11	19.8	0.510±0.0115	4.6±0.10	1041±23.5
WB 11	3.9	0.540±0.0210	9.8±0.38	
WC 11	19.6	0.390±0.0115	3.5±0.10	
WA 12	9.2	0.330±0.0115	3.0±0.10	420±14.6
WB 12	0.71	0.270±0.0115	2.4±0.10	
WC 12	19.9	0.243±0.0173	2.2±0.10	

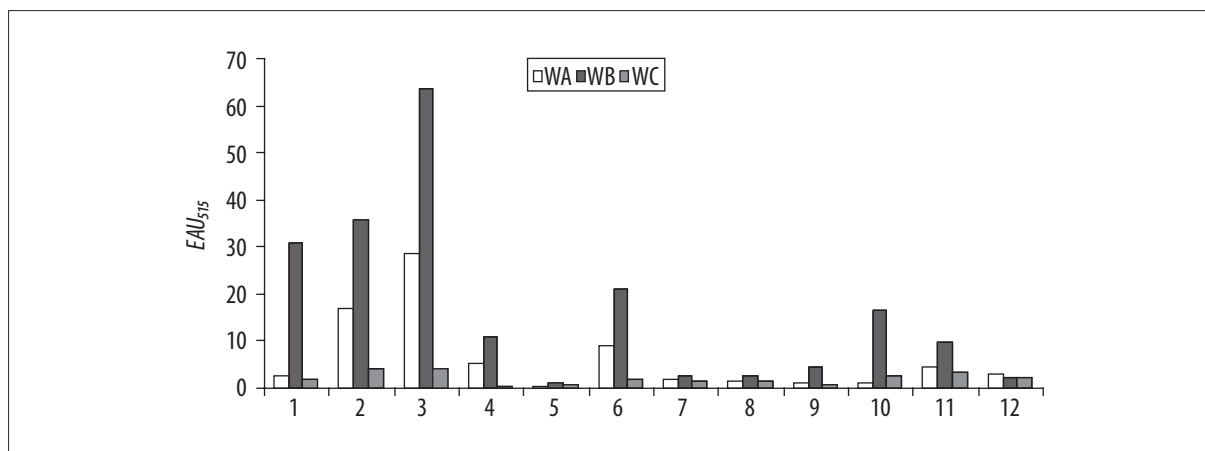


Figure 2. The number of antiradical activity units (EAU_{515}) calculated for 1 mg of extracts **WA**, **WB**, and **WC** obtained by extraction method **II** from the following raw materials: herb of marjoram – **1**, leaf of black tea – **2**, leaf of green tea – **3**, red tea in briquettes – **4**, fruit of red pepper – **5**, leaf of red tea – **6**, herb of common wormwood – **7**, herb of greater celandine – **8**, seed of black mustard – **9**, flower of linden – **10**, herb of southernwood – **11**, herb of echinacea – **12**

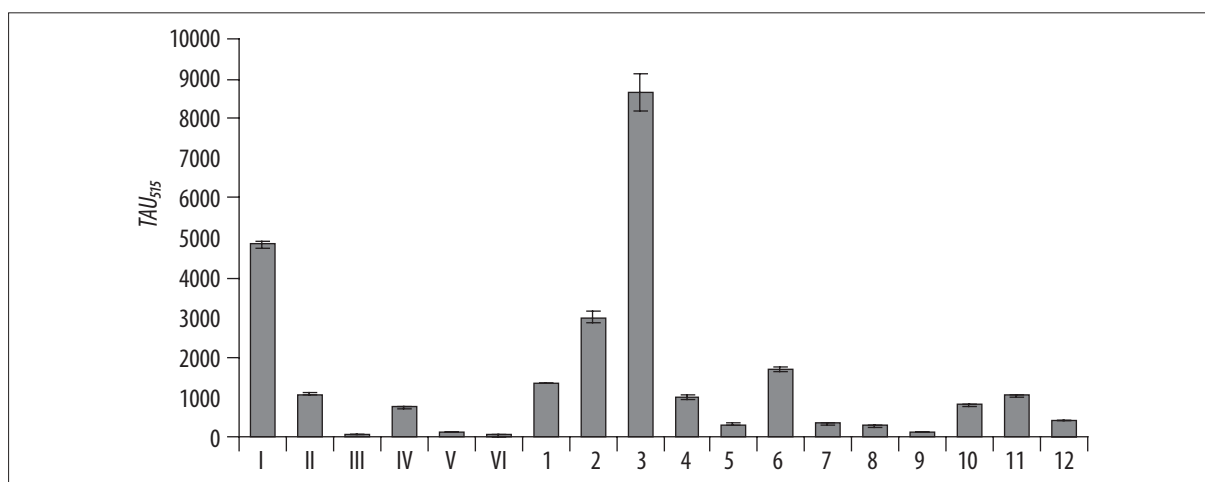


Figure 3. The number of antiradical activity units (TAU_{515}) calculated for 1 g of raw material. Raw materials **I–VI** were extracted with methanol, while extracts **1–12** were obtained with 50% (v/v) methanol in water. Raw materials: bee propolis – **I**, herb of goldenrod – **II**, spruce shavings – **III**, herb of knotgrass – **IV**, seed of black pepper – **V**, bulb of garlic – **VI**, herb of marjoram – **1**, leaf of black tea – **2**, leaf of green tea – **3**, red tea in briquettes – **4**, fruit of red pepper – **5**, leaf of red tea – **6**, herb of common wormwood – **7**, herb of greater celandine – **8**, seed of black mustard – **9**, flower of linden – **10**, herb of southernwood – **11**, herb of echinacea – **12**

Of the **WA** extracts, the highest EAU_{515} were calculated for extracts obtained from the leaves of green tea **WA 3** (Table 2, Figure 2), leaves of black tea **WA 2**, and leaves of red tea **WA 6**, with values of 28.6, 16.8, and 8.9. The lowest values of antiradical activity units were calculated for the extracts from the fruit of red pepper **WA 5**, seeds of black mustard **WA 9**, and the flowers of linden **WA 10**, with values of 0.5, 1.1, and 1.3. Of the **WB** extracts (Table 2, Figure 2), the greatest numbers of antiradical activity units EAU_{515} were calculated for the extracts from the leaves of green tea **WB 3**, black tea **WB 2**, and the herb of marjoram **WB 1**, with values of 63.5, 35.9, and 31.0, respectively. The lowest EAU_{515} were calculated for the extracts from fruit of red pepper **WB 5** and herb of echinacea **WB 12**, with 1.2 and 2.4. Among the **WC** extracts (Table 2, Figure 2), the highest values of EAU_{515} were demonstrated for **WC 3** and **WC 2**, with 4.3 and 4.0, respectively. The lowest EAU_{515} were calculated for the extracts

from the leaves of red tea in briquets **WC 4** and the fruit of red pepper **WC 5**, with 0.5 and 0.8, respectively.

The number of antiradical activity units calculated per 1 g of raw material TAU_{515} are demonstrated in Tables 1 and 2 and Figure 3.

The highest TAU_{515} values were calculated for the leaves of green tea (8646), lower for bee propolis (4830). The rest of the raw materials in decreasing order with respect to TAU_{515} values were: leaves of black tea (3003), leaves of red tea (1705), herb of majoram (1341), herb of goldenrod (1081), herb of southernwood (1041), red tea in briquets (992), flower of linden (800), herb of knotgrass (738), herb of echinacea (420), herb of common wormwood (340), fruit of red pepper (313), herb of greater celandine (287), seed of black pepper (114), seed of black mustard (112), spruce shavings (40.3), and bulb of garlic (35).

Not only separated or synthesized substances, but also plant extracts could be very attractive additions to food and medicines as agents with antioxidative and antiradical features [26].

In this study, the highest numbers of antiradical activity units per 1 mg of extract (EAU_{515}) were calculated for **WB** extracts from the leaves of green, black, and red teas, the herb of marjoram, as well as for the **WA** extract from the leaves of green tea, and these extracts could be considered as effective antiradical agents. The high antioxidative features of the extracts obtained from different kinds of tea and marjoram are described in the literature [8,9,12,15,17,20,28,29]. Average EAU_{515} values were exhibited by extracts from bee propolis, herb of goldenrod, herb of knotgrass, flower of linden [3,6], and herb of southernwood. Other extracts were shown to have low antiradical features.

The highest number of activity units per 1 g of raw material (TAU_{515}) was calculated for the leaves of green tea. Bee propolis also exhibited a high number of antiradical units. The antiradical activity of bee propolis was concentrated mainly in the water-insoluble precipitate. The compound responsible for the antiradical activity of this raw material could be caffeic acid phenethyl ester [2,10,25]. Other raw materials exhibited much smaller antiradical features and were not interesting as sources of antiradical extracts.

The average number of activity units TAU_{515} calculated for raw materials **I–VI** was 1140, whereas the average TAU_{515}

value calculated for raw materials **1–12** was 1583. When the calculation was done after excluding the maximal and minimal results (**I**, **VI**, **3**, **9**), the average activities were 493 and 1024 for raw materials **I–VI** and **1–12**, respectively. Method **II** is more effective than method **I** for the isolation compounds with antiradical activity. The higher effectiveness of method **II** could be explained by the better extraction of tannins (strong antiradical compounds) with the methanol: water (1:1) mixture.

IN CONCLUSION ONE CAN STATE THAT:

- The highest numbers of activity units per 1 mg of extract were calculated for ethyl acetate extracts from the leaves of green **WB 3** and black tea **WB 2** (Table 2, Figure 2). The lowest EAU_{515} values were noted for extracts from spruce shavings **We III** and bulb of garlic **We VI** (Table 1, Figure 1).
- The highest numbers of antiradical activity units TAU_{515} per 1 g of raw material were calculated for the leaves of green tea, bee propolis, and the leaves of black tea (Tables 1 and 2 and Figure 3).

On the basis of these results, one can say that green and black tea and bee propolis could be recommended as a source of extracts with strong antiradical activity. The leaves of red tea and red tea in briquets exhibited average activity. Other raw materials appeared to be poor sources of compounds with antiradical activity

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