

# ORIGINAL PAPERS

Adv Clin Exp Med 2005, 14, 3, 423–433  
ISSN 1230-025X

ZBIGNIEW SROKA, WOJCIECH CISOWSKI

## The anti-ROS Activity of Various Plant Extracts

### Aktywność anty-RFT różnych wyciągów roślinnych

Department of Pharmacognosy, Wrocław Medical University, Poland

#### Abstract

**Background.** Ethyl acetate and aqueous extracts were obtained from fourteen plant raw materials and investigated for the content of phenolic compounds, antiradical and anti-H<sub>2</sub>O<sub>2</sub> activity.

**Material and Methods.** The measured activities were expressed as absorbancy decrease of sample in the presence of extract and in the absence of extract (control). Then antiradical ( $TAU_{515}$ ) and anti-H<sub>2</sub>O<sub>2</sub> ( $TAU_{610}$ ) activities of extracts were calculated per 1 g of raw material. The activities of ethyl acetate and aqueous extracts of each raw material were summing up and presented as a total activity per 1 g of raw material and marked as  $RAU_{515}$  (antiradical activity) and  $RAU_{610}$  (anti-H<sub>2</sub>O<sub>2</sub> activity). The antiradical activity of extracts was measured by the colorimetric method with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH<sup>•</sup>) as a substrate. The anti-H<sub>2</sub>O<sub>2</sub> activity was assayed by the enzymatic method using reaction with peroxidase (EC 1.11.1.7) type II from horseradish. The content of phenols in the extracts was measured by colorimetric method with phosphotungstic acid reagent.

**Results and Conclusions.** The strongest antiradical as well as anti-H<sub>2</sub>O<sub>2</sub> activity was demonstrated for ethyl acetate extract 6 obtained from flowers of meadowsweet (*Ulmariae flos*). The weakest antiradical and anti-H<sub>2</sub>O<sub>2</sub> activity was observed for aqueous extracts from respectively rind of grapefruit 19 (*Paradisi pericarpium*) and Iceland moss 16 (*Lichen islandicus*). The greatest  $TAU_{515}$  was calculated for 6 and lowest for 3 (aqueous extract from rind of banana *Musa pardisiaca* L.). The greatest  $TAU_{610}$  was calculated for 20 (aqueous extract from flowers of meadowsweet) the lowest for 16. The highest  $RAU_{515}$  and  $RAU_{610}$  were calculated for flowers of meadowsweet and the lowest for Iceland moss. The high positive correlation was observed between the content of phenolic compounds in extracts and their antiradical and anti-H<sub>2</sub>O<sub>2</sub> activities (Adv Clin Exp Med 2005, 14, 3, 423–433).

**Key words:** antiradical activity, anti-ROS activity, plant extracts, phenolic compounds.

#### Streszczenie

**Wprowadzenie.** Z czternastu surowców roślinnych otrzymano wyciągi octanowe i wodne, które badano w kierunku zawartości związków fenolowych, aktywności przeciwrodnikowej i przeciwwodoronadtlenkowej.

**Materiał i metody.** Aktywności określono jako zmniejszenie absorbancji próbki w obecności wyciągu w porównaniu do próbki niezawierającej wyciągu (próbka kontrolna). Następnie obliczono aktywność przeciwrodnikową ( $TAU_{515}$ ) i przeciwwodoronadtlenkową ( $TAU_{610}$ ) wyciągów w przeliczeniu na 1 g surowca. Aktywności wyciągów octanowego i wodnego każdego z surowców sumowano i podano jako całkowitą aktywność wyrażoną na 1 g surowca jako  $RAU_{515}$  (aktywność przeciwrodnikowa) i  $RAU_{610}$  (aktywność przeciw H<sub>2</sub>O<sub>2</sub>). Aktywność przeciwrodnikową wyciągów mierzono metodą kolorymetryczną z zastosowaniem rodnika 1,1-difenylo-2-pikrylohydrazylowego (DPPH<sup>•</sup>) jako substratu, aktywność anty-H<sub>2</sub>O<sub>2</sub> – metodą enzymatyczną, stosując peroksydazę (EC 1.11.1.7) chrzanową typu II, a zawartość związków fenolowych – metodą kolorymetryczną, wykorzystując jako odczynnik kwas fosforowolframowy.

**Wyniki i wnioski.** Najwyższą aktywność przeciwrodnikową oraz przeciw-H<sub>2</sub>O<sub>2</sub> obserwowano dla wyciągu octanowego 6 otrzymanego z kwiatów wierzby błotnej (*Ulmariae flos*). Najniższą aktywność przeciwrodnikową i przeciwwodoronadtlenkową obserwowano dla wyciągu wodnego odpowiednio z naowocni grejfruta 19 (*Paradisi pericarpium*) i porostu islandzkiego 16 (*Lichen islandicus*). Najwyższą wartość  $TAU_{515}$  obliczono dla 6, a najniższą dla 3 (wyciąg wodny ze skórki banana, *Musa pardisiaca* L.). Najwyższą wartość  $TAU_{610}$  obliczono dla 20 (wyciąg wodny z kwiatów wierzby błotnej), najniższą dla 16. Najwyższą wartość  $RAU_{515}$  i  $RAU_{610}$  obliczono dla kwiatów wierzby błotnej, a najniższą dla porostu islandzkiego. Zaobserwowano dużą dodatnią korelację między ilością związków fenolowych w wyciągach i ich aktywnością przeciwrodnikową oraz przeciw-H<sub>2</sub>O<sub>2</sub> (Adv Clin Exp Med 2005, 14, 3, 423–433).

**Słowa kluczowe:** aktywność przeciwrodnikowa, aktywność anty-RFT, wyciągi roślinne, związki fenolowe.

The medical literature is full of claims that reactive oxygen species and free radicals are involved in various human and animal diseases [1, 2]. Such diseases as rheumatoid arthritis, haemorrhagic shock, cystic fibrosis and many others are often mentioned. Chronic inflammatory diseases lead to activation of macrophages what in turn is connected with increased concentration of reactive oxygen (ROS) and nitrogen (RNS) species. ROS and RNS play important role in normal physiology but in excess they enhance the oxidative stress and tissue and cells injury leading to many diseases such as cancer and atherosclerosis [3, 4].

Numerous current scientific investigations focus on looking for compounds which can be used as free radicals, ROS and RNS scavengers in such pathological conditions [5–7]. Plant raw material seems to be a rich source of polyphenolic compounds which in majority are very effective antiradical and antioxidant scavengers [8, 9].

Plant phenolic compounds are known to be strong free radical scavengers and antioxidants *in vitro* [8–10]. Phenolic compounds have also been evidenced to exhibit antioxidant effect *in vivo* [11–13]. For example, phenolic compounds in red wine were found to inhibit LDL oxidation *in vitro* and they might exert cardioprotective effect *in vivo*. This may explain the low incidence of heart attacks in France (French paradox) in these regions in which factors promoting cardiovascular disease (smoking and high fat intake) are common. Other studies showed inverse relation of the incidence of coronary heart disease in men with the dietary intake of phenolic compounds, first of all flavonoids such as quercetin which originated from fruits and vegetables [14, 15].

In this work, ethyl acetate and water extracts obtained from fourteen plant raw material were investigated for their activity as scavengers of free radicals and  $H_2O_2$  and the general phenolic compounds content was measured colorimetrically. Besides Spearman's rank correlation coefficient ( $r_s$ ) and type 1 error probability ( $p$ ) were calculated between phenol content and antiradical activity and anti- $H_2O_2$  activity of these extracts.

## Materials and Methods

### Chemicals and Biochemicals

Polish Chemical Reagents – methanol, ethyl acetate, Sigma – 1,1-diphenyl-2-picrylhydrazyl radical (DPPH $\cdot$ ), Peroxidase EC 1.11.1.7 (donor: hydrogen-peroxide oxidoreductase) type II from horseradish, Riedel-de-Haën – Phenol Red.

## Plant Material

“Herbapol” Herb Factory, Poland – Inflorescence of mountain arnica (*Arnicae inflorescentia*), Iceland moss (*Lichen islandicus*), bearberry leaves (*Uvae ursi folium*), mountain cranberry leaves (*Vitis idaeae folium*), birch leaves (*Betulae folium*), senna leaves (*Sennae folium*), comfrey root (*Taraxaci radix*), blackberry leaves (*Rubi fruticosi folium*).

Kawon, Herb Factory, Gostyn, Poland – meadowsweet flowers (*Ulmariae flos*), gentian root (*Gentianae radix*).

CNOS (seeds production), Wroclaw, Poland – celery seeds (*Petroselinum semen*).

Material obtained from fruits of edible quality – apple rind (*Malus domestica* Borkh.), banana rind (*Musa paradisiaca* L.), grapefruit rind (*Paradisi pericarpium*).

## Preparation of Extracts

The amount of raw material taken to the extraction is showed in Tables 1 and 2. Each raw material was extracted with 250 ml of 50% aqueous solution of methanol (70°C) for 2 days. Then methanol was removed under reduced pressure and remaining aqueous solution (125 ml) was left in refrigerator (4°C) for 3 days. The precipitate was filtered off (filter discs, grade 388, Filtrak) and discarded. Next, water extract was extracted with (5 × 50 ml) ethyl acetate. The ethyl acetate extract and remaining water solution were evaporated under reduced pressure to obtain dry extracts (the weight of extracts is given in Tables 1 and 2).

The yield of extraction expressed in percentages was calculated according to the equation below:

$$Y = \frac{W_E}{W_R} \times 100\%,$$

$Y$  – yield of extraction (%);  $W_E$  – weight of extract (g);  $W_R$  – weight of raw material taken to the extraction (g).

Ethyl acetate and aqueous extracts obtained from raw materials are marked in the text with the following numbers (the numbers of respective aqueous extracts are given in brackets): inflorescence of mountain arnica (*Arnica montana* L., *Asteraceae*) – 1 (aq. 15); Iceland moss (*Lichen islandicus*, *Parmeliaceae*) – 2 (aq. 16); rind of banana (*Musa paradisiaca* L., *Musaceae*) – 3 (aq. 17); rind of apple (*Malus domestica* Borkh., *Rosaceae*) – 4 (aq. 18); rind of grapefruit (*Citrus paradisi* Macfayden, *Rutaceae*) – 5 (aq. 19); flowers of meadowsweet (*Filipendula ulmaria* L. Maxim., *Rosaceae*) – 6 (aq. 20); leaves of bear-

berry (*Arctostaphylos uva-ursi* L., *Ericaceae*) – 7 (aq. 21); leaves of mountain cranberry (*Vaccinium vitis-idaea* L., *Ericaceae*) – 8 (aq. 22); root of gentian (*Gentiana lutea* L., *Gentianaceae*) – 9 (aq. 23); leaves of birch (*Betula pubescens* Ehrh., *Betulaceae*) – 10 (aq. 24); leaves of senne (*Cassia angustifolia* Vahl, *Caesalpiniaceae*) – 11 (aq. 25); seeds of celery (*Apium graveolens* L., *Umbelliferae*) – 12 (aq. 26); root of comfrey (*Symphytum officinale* L., *Boraginaceae*) – 13 (aq. 27); leaves of blackberry (*Rubus plicatus* Whe. et N. E., *Rosaceae*) – 14 (aq. 28).

## Measurement of Total Phenolic in Extracts

The measurement of general phenolic compounds was carried out according to the method Singleton et al. [16] with a modification described below.

Solution A – 5.77 g of sodium tungstate ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ) was dissolved in 75 ml of water and 8 ml of 85% phosphoric acid was added. The solution was heated in boiling water bath for 3 hours and after cooling adjusted with water to the final volume of 100 ml;

Solution B – 18% solution of  $\text{Na}_2\text{CO}_3$  in water.

Procedure – 0.5 ml of A solution was added to 1 ml of 50% methanol/water solution of extract (1 mg/ml) and 8.5 ml of B was added. The absorbancy at 750 nm was measured in a 1 cm glass cuvette 2 minutes after the addition of solution B. The results were calculated as gallic acid equivalents and expressed as general phenolic compounds amount (mg) per 1 mg of the extract. All measurements were made three times and standard deviation was calculated.

## Measurement of Antiradical Activity

The antiradical activity of the extracts was measured according to Brand-Williams et al. [17] Solution I was freshly prepared by dissolving 2 mg of DPPH' (1,1-diphenyl-2-picrylhydrazyl) in 54 ml of MeOH. Solution II was obtained by dissolving studied material in MeOH at 2.9 mg/ml concentration (1.45 mg/ml for 20 extract or 0.72 mg/ml for 6 and 7 extracts see legends of Tables 1 and 2). Then 40  $\mu\text{l}$  of II were added to 1460  $\mu\text{l}$  of I at room temperature. The absorbancy at 515 nm was measured in a 1 cm glass cuvette at 0 time and after 1 min of the reaction versus blank (40  $\mu\text{l}$  of II added to 1460  $\mu\text{l}$  of MeOH). The control samples were prepared in the following way: 40  $\mu\text{l}$  of

MeOH were added to 1460  $\mu\text{l}$  of I and the absorbancy was measured (515 nm) at 0 time and after 1 minute.

The antiradical activity of extracts ( $AU_{515}$ ) is expressed as absorbancy decrease after 1 minute of the reaction and calculated according to the equation:

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

where  $AU_{515}$  – radical scavenging activity,  $A_0$  – absorbancy of sample at 0 min of the reaction,  $A_1$  – absorbancy of sample after 1 min of the reaction,  $A_{0K}$  – absorbance of control sample at 0 min of the reaction,  $A_{1K}$  – absorbance of control sample after 1 min of the reaction. Because the result of  $(A_{0K} - A_{1K})$  was equal to 0 during the test the above equation was simplified to  $AU_{515} = (A_0 - A_1)$ .

The measurements were made in three times and standard deviations were calculated.

The anti-DPPH' activities ( $TAU_{515}$ ) for ethyl acetate and aqueous extracts were calculated per 1 g of raw material according to the following equation:

$$TAU_{515} = \frac{At}{0.1102 \text{ WR}} \times AU_{515},$$

$At$  – total weight of extract (mg) (see Tables 1, 2), 0.1102 – the amount of extract in test sample (mg), WR – weight of raw material taken to the extraction (g) (see Tab. 1, 2).

Total anti-DPPH' activity per 1 g of raw material ( $RAU_{515}$ ) was calculated according to the following equation:

$$RAU_{515} = TAU_{a515} + TAU_{e515},$$

$TAU_{a515}$  – anti-DPPH' activity of aqueous extract calculated per 1 g of raw material,  $TAU_{e515}$  – anti-DPPH' activity of ethyl acetate extract calculated per 1 g of raw material.

## Measurement of anti-H<sub>2</sub>O<sub>2</sub> Activity

Hydrogen peroxide scavenging activity was determined using a Pick and Keisari [18] method, modified by Bahorun et al.[19].

100  $\mu\text{l}$  of water solution of extracts at 0.71 mg/ml (or 0.42 mg/ml for 6, 7, 8, 10, 14, 20, 21, 22 see legend of Tab. 1 and 2) or 0 mg/ml (sample without extract) were added to 100  $\mu\text{l}$  of 0.002%  $\text{H}_2\text{O}_2$ . Then 0.8 ml of 0.1M phosphate buffer ( $\text{Na}_2\text{HPO}_4$  :  $\text{KH}_2\text{PO}_2$  + 100 mM NaCl) were added. The reaction mixture was preincubated for 10 minutes at 37°C. Then 1 ml of 0.2 mg/ml phenol red dye with 0.1 mg/ml horseradish peroxidase in 0.1M phosphate buffer was added. After

**Table 1.** The weight of extracts, content of general phenolics, scavenging of DPPH<sup>•</sup> radical and scavenging of hydrogen peroxide by ethyl acetate extracts isolated from some plant raw materials. The content of phenolic compounds is expressed in mg of phenolics per 1 mg of the extract. The antiradical and anti-H<sub>2</sub>O<sub>2</sub> activity has been defined in the Material and Methods section

**Tabela 1.** Waga wyciągów, ogólna zawartość fenoli, zmniejszenie rodnika DPPH<sup>•</sup> i zmniejszenie nadlenku wodoru przez wyciągi octanetylu uzyskane z wybranych surowców roślinnych. Zawartość związków fenolowych wyrażono w mg na 1 mg wyciągów. Aktywność przeciwrodnikowa i przeciw-H<sub>2</sub>O<sub>2</sub> została zdefiniowana w części Materiał i metody

Number of extract (Surowiec)	Weight of raw material (Waga surowca) g	Weight of extract (Waga wyciągu) g	Yield of extraction Y%	Contents of phenolics – mg/mg extracts (Zawartość fenoli – mg/mg wyciągu)	Scavenging of DPPH <sup>•</sup> radical $AU_{515}$ – absorbancy at 515 nm (Zmniejszenie rodnika DPPH <sup>•</sup> $AU_{515}$ absorbancją 515 nm)	Scavenging of H <sub>2</sub> O <sub>2</sub> $AU_{610}$ – absorbancy at 610 nm (Zmniejszenie H <sub>2</sub> O <sub>2</sub> $AU_{610}$ absorbancją 610 nm)	Anti-DPPH <sup>•</sup> activity of extracts calculated per 1 g of raw material – $TAU_{515}$ (Aktywność przeciw- DPPH <sup>•</sup> wyciągów przeliczona na 1 g su- rowca – $TAU_{515}$ )	Anti-H <sub>2</sub> O <sub>2</sub> activity of extracts calculated per 1 g of raw material – $TAU_{610}$ (Aktywność przeciw- H <sub>2</sub> O <sub>2</sub> wyciągów przeliczona na 1 g surowca – $TAU_{610}$ )
1. Inflorescence of mountain arnica (Kwiatostan arniki)	10	0.2524	2.5	0.1212 ± 0.0110	0.5067 ± 0.0230	0.1067 ± 0.0058	116	38
2. Iceland moss (Porost islandzki)	10	0.1785	1.8	0.0232 ± 0.0010	0.1033 ± 0.0058	0.1067 ± 0.0058	17	27
3. Rind of banana (Skórka z banana)	12	0.0468	0.39	0.0963 ± 0.0106	0.1667 ± 0.0115	0.4500 ± 0.0173	6	25
4. Rind of apple (Skórka z jabłka)	10	0.1068	1.1	0.1044 ± 0.0060	0.2167 ± 0.0058	0.2400 ± 0.0000	21	36
5. Rind of grapefruit (Skórka z grejfruta)	16	0.5242	3.3	0.0215 ± 0.0010	0.0900 ± 0.0173	0.1100 ± 0.0100	27	49
6. Flowers of meadowsweet (Kwiaty wiązówki)	12.5	1.4659	11.7	0.2564 ± 0.004	3.5800* ± 0.0200	0.9599 <sup>§</sup> ± 0.0085	3810	1583

7. Leaves of bearberry (Liście mącznicy)	14	1.2334	8.8	0.2588 ± 0.0080	3.6467* ± 0.0115	0.9288 <sup>β</sup> ± 0.0100	2915	1152
8. Leaves of mountain cranberry (Liście borówki brusznicy)	9	0.9591	10.6	0.1549 ± 0.0017	0.5383 ± 0.0076	0.7419 <sup>β</sup> ± 0.0049	520	1113
9. Root of gentian (Korzeń goryczki)	13	0.1926	1.5	0.0980 ± 0.0010	0.1333 ± 0.0058	0.2183 ± 0.0029	18	45
10. Leaves of birch (Liście brzozy)	10	0.3648	3.6	0.1764 ± 0.0020	0.5533 ± 0.0058	0.6626 <sup>β</sup> ± 0.0000	183	340
11. Leaves of senne (Liście senesu)	10	0.1400	1.4	0.0545 ± 0.0010	0.1533 ± 0.0115	0.4183 ± 0.0029	19	82
12. Seeds of celery (Nasionie seleru)	10	0.1293	1.3	0.0754 ± 0.0010	0.2467 ± 0.0058	0.4583 ± 0.0029	29	83
13. Root of comfrey (Korzeń żywokostu)	11	0.1245	1.1	0.2112 ± 0.0040	0.3833 ± 0.0058	0.5200 ± 0.0000	39	83
14. Leaves of blackberry (Liście jeżyny)	8	0.2431	3.0	0.2147 ± 0.0020	0.7950 ± 0.0087	0.9254 <sup>β</sup> ± 0.0085	219	396

Extracts 6, 7 exhibited very strong antiradical activity and extracts 6, 7, 8 exhibited very strong anti-H<sub>2</sub>O<sub>2</sub> activity what forced us to use these extracts in lower concentrations in tests. In order to compare the activities of these extracts to the activities of other extracts the measured activity was multiplied by 4 and 1.69 and marked in Table with respectively \* and <sup>β</sup>. (see also section Materials and Methods).

Wyciągi 6, 7 wykazywały bardzo silną aktywność przeciw wolnorodnikową i wyciągi 6, 7, 8 wykazywały bardzo silną aktywność przeciw-H<sub>2</sub>O<sub>2</sub>, co spowodowało konieczność badania tych wyciągów w mniejszych stężeniach. W celu porównania aktywności tych wyciągów do aktywności innych wyciągów zmierzona została pomnożona przez 4 i 1.69 i zaznaczona w tabeli odpowiednio przez \* i <sup>β</sup> (patrz Materiał i metody).



**Table 2.** The weight of extracts, content of general phenolics, scavenging of DPPH<sup>•</sup> radical and scavenging of hydrogen peroxide by water extracts isolated from some plant raw materials. The content of phenolic compounds is expressed as mg of phenolics per 1 mg of extract. The antiradical and anti-H<sub>2</sub>O<sub>2</sub> activity has been defined in the Material and Methods section

**Tabela 2.** Waga wyciągów, ogólna zawartość fenoli, zmiatanie rodnika DPPH<sup>•</sup> i zmiatanie nadtlenu wodoru przez wyciągi wodne uzyskane z wybranych surowców roślinnych. Zawartość związków fenolowych wyrażono w mg na 1 mg wyciągów. Aktywność przeciwrodnikowa i przeciw-H<sub>2</sub>O<sub>2</sub> została zdefiniowana w części Materiał i metody

Number of extract (Surowiec)	Weight of raw material (Waga surowca) g	Weight of extract (Waga wyciągu) g	Yield of extraction Y%	Contents of general phenolics – mg/mg extracts (Zawartość fenoli – mg/mg wyciągu)	Scavenging of DPPH <sup>•</sup> radical $AU_{515}$ – absorbancy at 515 nm (Zmiananie rodnika DPPH <sup>•</sup> $AU_{515}$ absorbancją 515 nm)	Scavenging of H <sub>2</sub> O <sub>2</sub> $AU_{610}$ – absorbancy at 610 nm (Zmiananie H <sub>2</sub> O <sub>2</sub> $AU_{610}$ absorbancją 610 nm)	Anti-DPPH <sup>•</sup> activity of extracts calculated per 1 g of raw material – $TAU_{515}$ (Aktywność przeciw- DPPH <sup>•</sup> wyciągów przeliczona na 1 g su- rowca – $TAU_{515}$ )	Anti-H <sub>2</sub> O <sub>2</sub> activity of extracts calculated per 1 g of raw material – $TAU_{610}$ (Aktywność przeciw- H <sub>2</sub> O <sub>2</sub> wyciągów przeliczona na 1 g surowca – $TAU_{610}$ )
15. Inflorescence of mountain arnica (Kwiatostan arniki)	10	0.9336	9.3	0.0690 ± 0.0044	0.3533 ± 0.0058	0.3933 ± 0.0058	299	517
16. Iceland moss (Porost islandzki)	10	0.8123	8.1	0.0058 ± 0.0010	0.0567 ± 0.0058	0.0167 ± 0.0058	42	19
17. Rind of banana (Skórka z banana)	12	3.9982	33.3	0.0325 ± 0.0010	0.1367 ± 0.0058	0.0833 ± 0.0058	413	391
18. Rind of apple (Skórka z jabłka)	10	2.8363	28.4	0.0186 ± 0.0010	0.0633 ± 0.0058	0.0333 ± 0.0058	163	133
19. Rind of grapefruit (Skórka z grejfruta)	16	1.5000	9.4	0.0162 ± 0.0010	0.0467 ± 0.0058	0.0700 ± 0.0100	40	92
20. Flowers of meadowsweet (Kwiaty wiązówki)	12.5	2.5000	20	0.1538 ± 0.0010	1.5733 <sup>α</sup> ± 0.0115	0.9401 <sup>β</sup> ± 0.0259	2855	2648

21. Leaves of bearberry (Liście mącznicy)	14	3.023	21.6	0.1050 ± 0.0010	0.7667 ± 0.0058	0.7957 <sup>β</sup> ± 0.0130	1502	2420
22. Leaves of mountain cranberry (Liście borówki brusznicy)	9	2.2593	25.1	0.0789 ± 0.0010	0.3233 ± 0.0058	0.6484 <sup>β</sup> ± 0.0130	736	2292
23. Root of gentian (Korzeń goryczki)	13	3.0324	23.3	0.0093 ± 0.0010	0.0633 ± 0.0058	0.0250 ± 0.0087	134	82
24. Leaves of birch (Liście brzozy)	10	1.6388	16.4	0.0655 ± 0.0040	0.2533 ± 0.0058	0.3267 ± 0.0153	377	754
25. Leaves of senne (Liście senesu)	10	2.4455	24.4	0.0186 ± 0.0010	0.0600 ± 0.0000	0.1100 ± 0.0100	133	379
26. Seeds of celery (Nasionie seleru)	10	0.6887	6.9	0.0255 ± 0.0010	0.0767 ± 0.0058	0.1600 ± 0.0000	48	155
27. Root of comfrey (Korzeń żywokostu)	11	2.4343	22.1	0.0336 ± 0.0027	0.0933 ± 0.0115	0.1050 ± 0.0050	187	327
28. Leaves of blackberry (Liście jeżyny)	8	1.7690	22.1	0.0893 ± 0.0050	0.7567 ± 0.0058	0.5583 ± 0.0029	1518	1739

Extract 20 exhibited very strong antiradical and anti-H<sub>2</sub>O<sub>2</sub> activity and extracts 21 and 22 exhibited very strong anti-H<sub>2</sub>O<sub>2</sub> activity what forced us to use these extracts in lower concentrations in the tests. In order to compare the activities of these extracts to the activities of other extracts the measured activities were multiplied by 2 and 1.69 and marked in Table respectively by <sup>α</sup> and <sup>β</sup> (see also section Material and Methods).

Wyciąg 20 wykazywał bardzo silną aktywność przeciwwolnorodnikową i przeciw-H<sub>2</sub>O<sub>2</sub> i wyciągi 21, 22 wykazywały bardzo silną aktywność przeciw-H<sub>2</sub>O<sub>2</sub>, co spowodowało konieczność badania tych wyciągów w mniejszych stężeniach. W celu porównania aktywności tych wyciągów do aktywności innych wyciągów zmierzona aktywność została pomnożona przez 2 i 1.69 i zaznaczona w tabeli odpowiednio przez <sup>α</sup> i <sup>β</sup> (patrz Materiał i metody).

15 minutes 50 µl of 1M NaOH were added and absorbancy was measured immediately at 610 nm.

The H<sub>2</sub>O<sub>2</sub> scavenging activity ( $AU_{610}$ ) of extracts was calculated according to the following equation:

$$AU_{610} = B_0 - B_R.$$

$AU_{610}$  – scavenging of hydrogen peroxide activity;  
 $B_0$  – absorbancy of sample without extract,  $B_R$  – absorbancy of sample with extract.

The measures were made three times and standard deviations were calculated.

The anti-H<sub>2</sub>O<sub>2</sub> activity ( $TAU_{610}$ ) for each extract was calculated per 1 g of raw material according to the following equation:

$$TAU_{610} = \frac{At}{0.071W_R} \times AU_{610},$$

$At$  – total weight of extract (mg) (see Tab. 1, 2),  
 0.071 – the amount of extract in sample (mg),  $W_R$  – weight of raw material taken to the extraction (g) (see Tab. 1, 2).

Total anti-H<sub>2</sub>O<sub>2</sub> activity per 1 g of raw material ( $RAU_{610}$ ) was calculated according to the following equation:

$$RAU_{610} = TAU_{a610} + TAU_{e610},$$

$TAU_{a610}$  – anti-H<sub>2</sub>O<sub>2</sub> activity for aqueous extract calculated per 1 g of each raw material,  $TAU_{e610}$  – anti-H<sub>2</sub>O<sub>2</sub> activity for ethyl acetate extract calculated per 1 g raw material.

## Statistical Analysis

Statistical analysis was performed by calculation of Spearman's rank correlation coefficient ( $r_s$ ) between phenol content and antiradical or anti-H<sub>2</sub>O<sub>2</sub> activities of extracts.

The probability of type 1 error (p) was also calculated.

## Results and Discussion

Anti-DPPH• and anti-H<sub>2</sub>O<sub>2</sub> activity of ethyl acetate and aqueous extracts is demonstrated in Tables 1 and 2.

The strongest anti-DPPH• activity among ethyl acetate extracts was measured for the extracts 7 and 6 respectively 3.647 and 3.580  $AU_{515}$ . Lower antiradical activity was observed for 14, 10, 8 and 1 respectively: 0.795, 0.553, 0.538 and 0.507  $AU_{515}$ . Other ethyl acetate extracts exhibited antiradical features in the following decreasing order 13 > 12 > 4 > 3 > 11 > 9 > 2 > 5 with activities respectively: 0.383, 0.247, 0.217, 0.167, 0.153, 0.133, 0.103

and 0.090  $AU_{515}$ . Among aqueous extracts the strongest anti-DPPH• activity was measured for 20 (1.573  $AU_{515}$ ). Lower activity was showed for 21, 28 respectively: 0.767, 0.757  $AU_{515}$ . Antiradical activity other extracts was in decreasing order 15 > 22 > 24 > 17 > 27 > 26 > 18 = 23 > 25 > 19 with activities respectively: 0.353, 0.323, 0.253, 0.137, 0.093, 0.077, 0.063, 0.063, 0.060, 0.047  $AU_{515}$ .

The strongest anti-H<sub>2</sub>O<sub>2</sub> activity of ethyl acetate extracts was measured for 6, 7 and 14 with activity equal to 0.960, 0.929 and 0.925  $AU_{610}$  respectively. A lower anti-H<sub>2</sub>O<sub>2</sub> properties was observed for 8, 10, 13, 12, 3 and 11 extracts with activities 0.742, 0.663, 0.520, 0.458, 0.450 and 0.418  $AU_{610}$  respectively. Activities of other extracts are in the following decreasing order 4 > 9 > 5 > 1 = 2 with 0.240, 0.218, 0.110, 0.107, 0.107  $AU_{610}$ .

The strongest anti-H<sub>2</sub>O<sub>2</sub> activity of aqueous extracts was measured for 20, 21, 22 and 28 with activities 0.940, 0.796, 0.648 and 0.558  $AU_{610}$  respectively. Activities of 15 and 24 was 0.393 and 0.327  $AU_{610}$ . Activity of other extracts was as follows 0.160, 0.110, 0.105, 0.083, 0.070, 0.033, 0.025, 0.017  $AU_{610}$  respectively for 26 > 25 > 27 > 17 > 19 > 18 > 23 > 16 extracts.

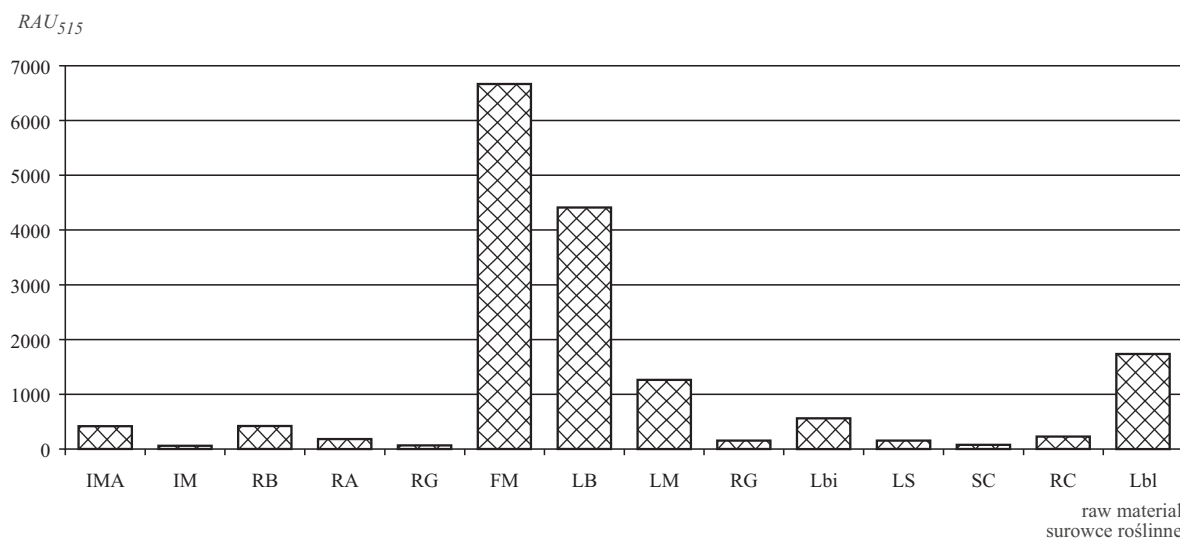
Average anti-DPPH• activity calculated for fourteen ethyl acetate and aqueous extracts was equal to 0.794  $AU_{515}$  and 0.330  $AU_{515}$  respectively. Average anti-H<sub>2</sub>O<sub>2</sub> activity calculated for fourteen ethyl acetate and aqueous extracts was respectively 0.489  $AU_{610}$  and 0.304  $AU_{610}$ . One can say that ethyl acetate extracts exhibit stronger anti-DPPH• as well as anti-H<sub>2</sub>O<sub>2</sub> activities than aqueous extracts.

The antiradical and anti-H<sub>2</sub>O<sub>2</sub> activity of ethyl acetate extracts well correlated with the amount of phenol contents in the extracts. The correlation coefficient ( $r_s$ ) between phenol content and antiradical activity and probability (p) were equal to 0.93 and 0.0004 but between phenol content and anti-H<sub>2</sub>O<sub>2</sub> activity respectively 0.81 and 0.0018.

The antiradical and anti-H<sub>2</sub>O<sub>2</sub> activity of aqueous extracts well correlated with phenolic compounds amount in these extracts. The correlation coefficient ( $r_s$ ) between phenolic amount in aqueous extracts and their antiradical or anti-H<sub>2</sub>O<sub>2</sub> activities and probability (p) were the same 0.95 and 0.0003.

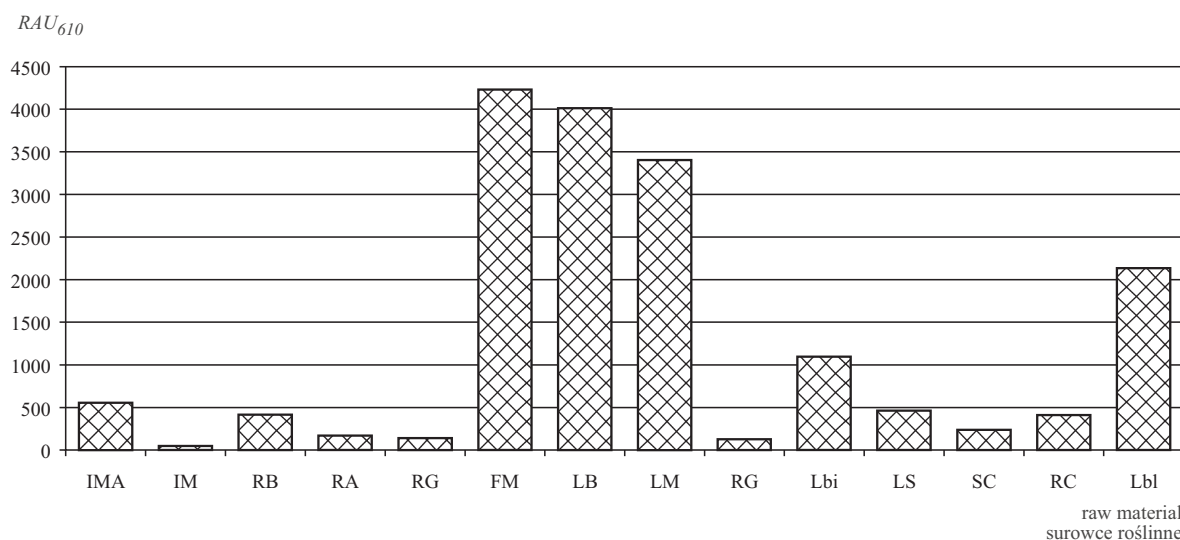
The total anti-DPPH• ( $TAU_{515}$ ) and anti-H<sub>2</sub>O<sub>2</sub> ( $TAU_{610}$ ) (Tab. 1 and 2) activity was calculated for ethyl acetate and aqueous extracts and additionally calculated per 1 g of raw material in order to compare the yield of activity extraction from different raw materials for ethyl acetate





**Fig. 1.** Total anti-DPPH• activity (RAU<sub>515</sub>) of each raw material calculated as a sum of activities of ethyl acetate and aqueous extracts and expressed per 1 g of raw material (see Material and Methods): IMA – inflorescence of mountain arnica, IM – iceland moss, RB – rind of banana, RA – rind of apple, RG – rind of grapefruit, FM – flowers of meadowsweet, LB – leaves of bearberry, LM – leaves of mountain cranberry, RG – root of gentian, Lbi – leaves of birch, LS – leaves of senne, SC – seeds of celery, RC – root of comfrey, Lbl – leaves of blackberry

**Ryc. 1.** Całkowita aktywność przeciw-DPPH• (RAU<sub>515</sub>) badanych surowców roślinnych obliczona jako suma aktywności wyciągów octanowych i wodnych oraz przeliczona na 1 g surowca (patrz Materiał i metody): IMA – kwiatostan arniki, IM – porost islandzki, RB – skórka z banana, RA – skórka z jabłka, RG – skórka z grejpfruta, FM – kwiaty wiązówki, LB – liście mącznicy, LM – liście borówki brusznicy, RG – korzeń goryczki, Lbi – liście brzozy, LS – liście senesu, SC – nasienie seleru, RC – korzeń żywokostu, Lbl – liście jeżyny



**Fig. 2.** Total anti-H<sub>2</sub>O<sub>2</sub> activity (RAU<sub>610</sub>) of each raw material calculated as a sum of activities of ethyl acetate and aqueous extracts and expressed per 1 g of raw material (see Material and Methods): IMA – inflorescence of mountain arnica, IM – iceland moss, RB – rind of banana, RA – rind of apple, RG – rind of grapefruit, FM – flowers of meadowsweet, LB – leaves of bearberry, LM – leaves of mountain cranberry, RG – root of gentian, Lbi – leaves of birch, LS – leaves of senne, SC – seeds of celery, RC – root of comfrey, Lbl – leaves of blackberry

**Ryc. 2.** Całkowita aktywność przeciw-H<sub>2</sub>O<sub>2</sub> (RAU<sub>610</sub>) każdego z surowców obliczona jako suma aktywności wyciągów octanowych i wodnych oraz przeliczona na 1 g surowca (patrz Materiał i metody): IMA – kwiatostan arniki, IM – porost islandzki, RB – skórka z banana, RA – skórka z jabłka, RG – skórka z grejpfruta, FM – kwiaty wiązówki, LB – liście mącznicy, LM – liście borówki brusznicy, RG – korzeń goryczki, Lbi – liście brzozy, LS – liście senesu, SC – nasienie seleru, RC – korzeń żywokostu, Lbl – liście jeżyny

and aqueous extracts (see section Material and Methods).

The greatest anti-DPPH<sup>•</sup> activity ( $TAU_{515}$ ) for ethyl acetate extracts (Tab. 1) was calculated for extracts 6 (3810  $TAU_{515}$ ) and 7 (2915  $TAU_{515}$ ) but the weakest anti-DPPH<sup>•</sup> activity was calculated for 3 (6  $TAU_{515}$ ) and 2 (17  $TAU_{515}$ ). The strongest anti-DPPH<sup>•</sup> activity 2855 and 1502  $TAU_{515}$  of aqueous extracts (Tab. 2) was calculated for extracts respectively 20, 21 but the weakest was calculated for 16 and 26 respectively 42 and 48  $TAU_{515}$ . The greatest anti-H<sub>2</sub>O<sub>2</sub> activity ( $TAU_{610}$ ) in ethyl acetate extracts (Tab. 1) was equal to 1583 and 1152  $TAU_{610}$  for respectively 6 and 7 extracts but the least for 2 and 3 with respectively 27 and 25  $TAU_{610}$ . The strongest anti-H<sub>2</sub>O<sub>2</sub> activity of aqueous extracts was calculated for 20, 21 and 22 with respectively 2648, 2420 and 2292  $TAU_{610}$ . The weakest anti-H<sub>2</sub>O<sub>2</sub> activity was 19  $TAU_{610}$  for extract 16.

The total anti-DPPH<sup>•</sup> ( $RAU_{515}$ ) and anti-H<sub>2</sub>O<sub>2</sub> ( $RAU_{610}$ ) activity was calculated per 1 g of raw material as a sum of total activities of ethyl acetate and aqueous extracts of each raw material (see section Material and Methods). The results are demonstrated in Table 3 and Figure 1, 2. The higher total anti-DPPH<sup>•</sup> ( $RAU_{515}$ ) (respectively 6665 and 4417) and anti-H<sub>2</sub>O<sub>2</sub> ( $RAU_{610}$ ) (respectively 4231 and 4012) activity was calculated for flowers of meadowsweet and leaves of bearberry. The lowest  $RAU_{515}$  value was calculated for Iceland moss and rind of grapefruit respectively 59 and 67 and the lowest  $RAU_{610}$  value was calculated for Iceland moss (46) and root of gentian (127).

In conclusion one can say that:

a) extracts 6 and 7 and extracts 20 and 21 exhibited the strongest anti-DPPH<sup>•</sup>  $AU_{515}$  and anti-H<sub>2</sub>O<sub>2</sub>  $AU_{610}$  activities;

b) extracts 2 and 5 and extracts 16 and 19 exhibited the lowest anti-DPPH<sup>•</sup>  $AU_{515}$  and anti-H<sub>2</sub>O<sub>2</sub>  $AU_{610}$  activities;

c) the greatest anti-DPPH<sup>•</sup> ( $TAU_{515}$ ) and anti-H<sub>2</sub>O<sub>2</sub> ( $TAU_{610}$ ) activity was calculated for extracts 6, 7. The lowest anti-DPPH<sup>•</sup> activity was calculated for 2 and 3, and the lowest anti-H<sub>2</sub>O<sub>2</sub> activity was calculated for 3 and 16 extracts.

d) the higher total anti-DPPH<sup>•</sup> ( $RAU_{515}$ ) and anti-H<sub>2</sub>O<sub>2</sub> ( $RAU_{610}$ ) was calculated for flowers of meadowsweet but the lowest for Iceland moss.

Our results showed that extracts obtained from leaves (leaves of bearberry, leaves of mountain cranberry, leaves of blackberry) show strong antiradical and anti-H<sub>2</sub>O<sub>2</sub> activities. An exception are leaves of senne which exhibited weak activity. Strong antiradical properties of extracts isolated from leaves was demonstrated also by Bahorun et al. [19].

The authors observed good positive correlation

**Table 3.** Total anti-DPPH<sup>•</sup> and anti-H<sub>2</sub>O<sub>2</sub> activity of each raw material calculated as a sum of activities of ethyl acetate and aqueous extracts and expressed per 1 g of raw material (see section Material and Methods)

**Tabela 3.** Całkowita aktywność przeciw-DPPH<sup>•</sup> i przeciw-H<sub>2</sub>O<sub>2</sub> badanych surowców roślinnych obliczona jako suma aktywności wyciągów octanowych i wodnych oraz przeliczona na 1 g surowca (patrz Materiał i metody)

Raw material (Surowiec)	$RAU_{515}$	$RAU_{610}$
Inflorescence of mountain arnica (Kwiat arniki)	415	555
Iceland moss (Porost islandzki)	59	46
Rind of banana (Skórka z banana)	419	416
Rind of apple (Skórka z jabłka)	184	169
Rind of grapefruit (Skórka z grejpfruta)	67	141
Flowers of meadowsweet (Kwiat wiązówki)	6665	4231
Leaves of bearberry (Liść mącznicy)	4417	4012
Leaves of mountain cranberry (Liść borówki brusznicy)	1256	3405
Root of gentian (Korzeń goryczki)	152	127
Leaves of birch (Liść brzozy)	560	1094
Leaves of senne (Liść senesu)	152	461
Seeds of celery (Nasienie seleru)	77	238
Root of comfrey (Korzeń żywokostu)	226	410
Leaves of blackberry (Liść jeżyny)	1737	2135

between the content of phenolic compounds in extracts and their antiradical and anti-H<sub>2</sub>O<sub>2</sub> activities what is discordant with the result obtained by Kahkonen et al. [20]. Strong positive correlation between the content of phenolic compounds in plant extracts and antioxidant or antiradical activity of these extracts was also demonstrated by Chinnici et al. [21], Kuti and Konuru [22] and Chun et al. [23].

The calculations demonstrated in the paper could be used to screening measurement and comparing of anti-ROS activity of different plant raw material.

Flowers of meadowsweet, leaves of bearberry, leaves of mountain cranberry appeared to have strong anti-ROS features. These raw materials could be used in the future as a source of powerful antiradical and anti-H<sub>2</sub>O<sub>2</sub> extracts or compounds.

## References

- [1] **Halliwell B, Gutteridge JMC:** Free radicals, other reactive species and disease. In: Free radicals in biology and medicine. Eds.: Halliwell B, Gutteridge JMC, Oxford University Press, Oxford 1999 3<sup>rd</sup> ed., 617–783.
- [2] **Noguchi N, Niki E:** Chemistry of active oxygen species and antioxidants. In: Antioxidant status, diet, nutrition and health. Ed.: Papas AM, CRC Press, London, New York 1999, 1<sup>st</sup> ed., 3–20.
- [3] **Abdi S, Ali A:** Role of ROS modified human DNA in the pathogenesis and aetiology of cancer. *Cancer Lett* 1999, 142, 1–9.
- [4] **Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H:** Role of oxidative stress in atherosclerosis. *Am J Cardiol* 2003, 91, 7A–11A.
- [5] **Burdette JE, Chen SN, Lu ZZ, Xu H, White BE, Fabricant DS, Liu J, Fong HH, Farnsworth NR, Constantinou AI, Van Breemen RB, Pezzuto JM, Bolton JL:** Black cohosh (*Cimicifuga racemosa* L.) protects against menadione-induced DNA damage through scavenging of reactive oxygen species: bioassay-directed isolation and characterization of active principles. *J Agr Food Chem* 2002, 50, 7022–7028.
- [6] **Cordova CA, Siqueira IR, Netto CA, Yunes RA, Volpato AM, Cechinel FV, Curi-Pedrosa R, Creczynski-Pasa TB:** Protective properties of butanolic extract of the *Calendula officinalis* L. (marigold) against lipid peroxidation of rat liver microsomes and action as free radical scavenger. *Redox Rep* 2002, 7, 95–102.
- [7] **Halder J, Bhaduri AN:** Protective role of black tea against oxidative damage of human red blood cells. *Biochem Bioph Res Comm* 1998, 244, 903–907.
- [8] **Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K:** Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. *J Nutr Sci Vitaminol* 2001, 47, 357–362.
- [9] **Okawa M, Kinjo J, Nohara T, Ono M:** DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. *Biol Pharm Bull* 2001, 24, 1202–1205.
- [10] **Robak J, Gryglewski RJ:** Bioactivity of flavonoids. *Pol J Pharmacol* 1996, 48, 555–564.
- [11] **MCS, KS, Kuttan R:** Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *J Ethnopharmacol* 2002, 83, 109–116.
- [12] **Zang LY, Cosma G, Gardner H, Shi X., Castranova V, Vallyathan V:** Effect of antioxidant protection by p-coumaric acid on low-density lipoprotein cholesterol oxidation. *Am. J. Physiol – Cell Ph* 2000, 279, 954–960.
- [13] **Zhong Z, Froh M, Connor HD, Li X, Conzelmann LO, Mason RP, Lemasters JJ, Thurman R.G:** Prevention of hepatic ischemia-reperfusion injury by green tea extract. *Am. J Physiol Gastro* 2002, 283, 957–964.
- [14] **Folts JD:** Potential health benefits from the flavonoids in grape products on vascular disease. *Adv Exp Med Biol* 2002, 505, 95–111.
- [15] **Mojzisova G, Kuchta M:** Dietary flavonoids and risk of coronary heart disease. *Physiol Res* 2001, 50, 529–535.
- [16] **Singleton VL, Rossi JA Jr:** Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965, 16, 144–153.
- [17] **Brand-Williams W, Cuvelier ME, Berset C:** Use of free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol* 1995, 28, 25–30.
- [18] **Pick E, Keisari Y:** A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. *J Immunol Methods* 1980, 38, 161–170.
- [19] **Bahorun T, Gressier B, Trotin F, Brunet C, Dine T, Luyckx M, Vasseur J, Cazin M, Cazin JC, Pinkas M:** Oxygen species scavenging activity of phenolic extracts from Hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimittel-Forsch* 1996, 46, 1086–1089.
- [20] **Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M:** Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* 1999, 47, 3954–3962.
- [21] **Chinnici F, Bendini A, Gaiani A, Riponi C:** Radical scavenging activities of peels and pulps from cv. Golden Delicious apples as related to their phenolic composition. *J Agric Food Chem* 2004, 52, 4684–4689.
- [22] **Kuti JO, Konuru HB:** Antioxidant capacity and phenolic content in leaf extracts of tree spinach (*Cnidoscolus* spp.). *J Agric Food Chem* 2004, 52, 117–221.
- [23] **Chun OK, Kim DO, Moon HY, Kang HG, Lee CY:** Contribution of individual polyphenolics to total antioxidant capacity of plums. *J Agric Food Chem* 2003, 51, 7240–7245, 2003.

## Address for correspondence:

Zbigniew Sroka  
Department of Pharmacognosy, Wrocław Medical University  
pl. Nankiera 1  
50-140 Wrocław  
Poland

Received: 1.09.2004

Revised: 27.09.2004

Accepted: 13.12.2004

Praca wpłynęła do Redakcji: 1.09.2004 r.

Po recenzji: 27.09.2004 r.

Zaakceptowano do druku: 13.12.2004 r.

