Antioxidant activity and total phenolic content of *Rheum palmatum* roots

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S u m m a r y

The aim of our work was to investigate the antioxidant activity of *Rheum palmatum* extracts. Antiradical activity against DPPH and ABTS radicals, reducing power FRAP and total phenolic contents, were investigated in one-, two- and three-year-old roots of rhubarb fertilized with nitrogen at the rates of 50, 100 and 200 kg N/ha. It was proved that nitrogen dose as well as the age of plantation did significantly influence antioxidant activity and total phenolic of root extracts. The highest values were determined in one-year-old plants, antioxidant activity ranged the level of 112–203 μM Trolox/g and total phenolic compounds average content was 22 mg GAE/g FW. Two-year-old roots were characterized by 3–11%, and three-year-old ones by 15–23% lower antioxidant activity and phenolic compounds content. When nitrogen dose increased, polyphenols content, DPPH and FRAP values increased as well, although, ABTS showed a different tendency.

Key words: *Rheum palmatum, age of plant, phenolics, antioxidant activity*

INTRODUCTION

Oxidative stress is a principal factor that shows health or disease. Increased antioxidative protection of human organism is one of the methods of treatment. Flavonoid-

rich herbal extracts are known for their chemoprotective, cardioprotective and antioxidant properties. *Rheum palmatum* roots and other *Rheum* species, like *R. tanguticum, R. officinale* are to be found among the oldest and best known herbal medicines. They are present in Chinese Pharmacopeia and used or recommended as laxative, antiphlogistic, in treatment of indigestion and other disorders. Applied in low doses (0.1–0.3 g), it shows contractive and anti-diarrhea activity, while in higher doses (1.5–5.0 g) it is used as a laxative. This herb is also applied to stimulate gastric juice and bile excretion. Rhubarb roots have an antibacterial and antioxidant activity. The most important components of roots are antrachinons (2–8%) such as chrysophanic acid, emodin, aloe-emodin, rhein and physcion, with their O-glycosides such asglucorhein, chrysophanein, glucoemodin; sennosides A-E, reidin C and others, their glycosides, bianthrones, hydrolysable and condensed tannins (5–10%), mainly catechin, stilbenes (resveratrol, rhapontigenin), volatile oil (containing paeonol, di-isobutyl phaltate, cinnamic aldehyde, methyl eugenol); rutin, fatty acids, calcium oxalate etc. [1-6]. Studies have shown that aloe-emodin holds several properties such as laxative, antiviral and hepatoprotective effects. Observations on neutroectodermal tumors found that aloe-emodin had an anticancer activity [7].

Food like fruits, vegetables and grains is reported to contain a wide variety of antioxidant components, including phenolic compounds. These compounds are found to be well correlated with antioxidant potential. Rheum roots have been studied for their antioxidant activity [8, 9]. Considering the phenolic constituent profile of *Rheum* species, especially their flavonoids, stilbenes and anthraquinones, they seem to be a potential source of antioxidants. Rhubarb is cultivated in Poland for processing industry. The harvest of raw material takes place in 3rd or 4th year of plant growing period, at the beginning of flowering or in late autumn [1].

The aim of this work was the comparative study of antioxidant activity of *R. palmatum* root extracts obtained from annual, biennial, three-year-old plants fertilized with different doses of nitrogen.

MATERIALS AND METHODS

Plant material and preparation of extracts

The Chinese rhubarb was cultivated in 2003–2005 in Experimental Station of Wrocław Environmental and Life Sciences University. The seeds were sown in the 3rd week of March to the 7 cm diameter pots filled with peat substrate. Seven-week-old transplants were planted in the middle of May on plots fertilized with ammonium nitrate at nitrogen rates: 50, 100 (50+50) and 200 (100+100) kg N/ha applied before transplants planting. The fertilizer was applied as a top dressing in the first year of cultivation, the second and the third year of cultivation in the first week of April and in the third week of May. In the third year of the experiment

roots of one-, two- and three-year-old rhubarb plants were harvested in October. The experiment was conducted as two-factorial design with plot area of 9 m^2.

The amount of about 2 g of roots was homogenized and extracted with 80% aqueous methanol at a room temperature, then filtered, and extracts were subjected to analyses.

Chemicals and spectral measurements

1,1-di-phenyl-2-picrylhydrazyl (DPPH) ferrous chloride, ferric tripyridyltriazine (TPTZ), kaliumperoxodisulfat, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), Folin-Ciocalteu's reagent were obtained from Sigma Chemical Co. (Sigma-Aldrich, Poland, Poznań). Methanol was obtained from POCh Poland. All chemicals and solvents were of analytical grade.

All UV–V measurements were recorded on a Shimadzu UV–2401PC (Kyoto, Japan).

Analytical methods

Ferric reducing antioxidant power (FRAP) was measured suing Benzie & Strain [11]. DPPH assay was carried out as described by Yen & Chen [12]. ABTS assay was done according to the method of Re et al. [13]. Results of antioxidant activity were expressed in μMol Trolox equivalents (TE)/g. Total phenolic contents were determined according to Folin-Ciocalteu's method [10]. The results were calculated as mg of gallic acid equivalent (GAE)/1 g.

Statistical analysis

Analysis of variance was performed by ANOVA procedures. Statistical analysis was performed using Statictica 8.0. Significant differences between means were determined by Duncan multiple range tests. Correlations among the data were obtained using a correlation coefficient (r).

RESULTS AND DISCUSSION

In this study three methods: DPPH and ABTS radical scavenging activities as ell as ferric reducing power FRAP were used. The concentrations of total phenolic were also calculated for the extracts. The results are shown in table 1.

DPPH and ABTS radical were used as a stable free radicals to determine antioxidant activity of extracts. The methods are based on the reduction of DPPH or ABTS solutions in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical compounds.

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Table 1.

Total phenolic content and antioxidant activity of *Rheum* roots extracts (average±SD)

analysis	fertilization [kg N/ha]	year of cultivation 1	2	3	*average*
DPPH [μM TE/g]	50	108.8 ± 1.9	94.6 ± 1.1	92.8 ± 0.1	***98.7***^{c}
	100	113.9 ± 0.7	114.9 ± 0.2	76.9 ± 0.7	***101.9***^{b}
	200	115.7 ± 0.3	118.6 ± 0.7	110.7 ± 0.7	***115.2***^{a}
	average	***112.8***^{a}	***109.3***^{b}	***93.5***^{c}	***105.2***
ABTS [μM TE/g]	50	187.8 ± 1.8	162.1 ± 1.2	162.2 ± 5.4	***170.7***^{c}
	100	207.9 ± 2.9	211.2 ± 3.5	147.4 ± 3.2	***195.5***^{b}
	200	214.0 ± 1.6	217.3 ± 2.7	205.1 ± 1.4	***212.1***^{a}
	average	***203.3***^{a}	***196.8***^{b}	***171.5***^{c}	***192.7***
FRAP [μM TE/g]	50	118.2 ± 0.5	97.0 ± 1.0	95.9 ± 1.0	***103.7***^{c}
	100	132.9 ± 1.3	134.5 ± 0.4	82.9 ± 0.4	***116.8***^{b}
	200	142.4 ± 1.1	139.8 ± 0.6	124.4 ± 1.0	***135.5***^{a}
	average	***131.1***^{a}	***123.7***^{b}	***101.1***^{c}	***118.6***
tTotal phenolics [mg GAE/g]	50	21.0 ± 0.2	16.5 ± 0.8	17.1 ± 0.0	***18.2***^{c}
	100	22.0 ± 0.1	22.1 ± 0.1	14.8 ± 0.8	***20.0***^{b}
	200	23.1 ± 0.4	23.4 ± 0.3	19.5 ± 0.6	***22.0***^{a}
Average		***22.0***^{a}	***20.6***^{b}	17.1^{c}	19.9

Values expressed are means ±SD of three parallel measurements ($p<0.05$).
Statistically homogeneous (p value ≤0.05) groups are designated with the same letters.

The best DPPH and ABTS radical scavenging activity (112.3 and 203.3 μM TE/g FW, respectively) was obtained for extracts from one-year-old cultivated plants, as well as the highest dose of fertilization. Antiradical activity decreases according to the decrease in the amount of nitrogenous fertilizer and in the second (at about 3–7%) and the third (on average 16–17%) year of cultivation. Reducing power exhibited a similar tendency.

A considerable variability of antiradical activity among *Rheum* species was observed. Antioxidant activity against ABTS radical of other *Rheum* species was from about 90 μM TE/g FW in *R. cordifolia* to 320 in *R. officinale* μM TE/g FW [14]. *R. palmatum* has similar activity like (+)catechin [8]

According to Cai et al. [14], total phenolic compounds content in *R. heum* in comparison with other herbs is relatively high and in *R. officinale* amounts about 30 mg GAE/g FW. In our experiments, total phenolic content was about 20 mg

GAE/g FW. The highest phenolic compound amounts were discovered for one-year root extracts. The concentration of polyphenols in two- and three-year roots extracts was lower (at about 10% and 22%, respectively).

The linear correlation between phenolics concentration and antioxidant activity (R value 0.92–0.96) in the examined plan material was recorded (tab. 2).

Table 2.

Correlation coefficients between total phenolic content and antioxidant activity of *Rheum palmatum* root extract

	DPPH	*ABTS*	*FRAP*	*phenolics*
DPPH	1			
ABTS	0.93	1.00		
FRAP	0.96	0.95	1.00	
phenolics	0.93	0.92	0.95	1

CONCLUSIONS

The results presented in this study have been, so far, the first information on the antioxidant activity of one-, two- and three-year-old roots extracts of *R. palmatum*.

1. The highest antioxidant activity and phenolic compounds were found in roots from one-year cultivation.
2. Intensive nitrogen fertilization had a positive effect on phenolics content and antioxidant activity, estimated as FRAP, ABTS and DPPH tests.

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AKTYWNOŚĆ ANTYOKSYDACYJNA KORZENI RZEWIENIA DŁONIASTEGO (*RHEUM PALMATUM* L.)

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S t r e s z c z e n i e

Celem badań była ocena aktywności antyoksydacyjnej oraz zawartości polifenoli w ekstraktach z korzeni rzewienia dłoniastego. Aktywność antyoksydacyjna mierzona testami ABTS, DPPH i FRAP i zawartość polifenoli ogółem, była oceniana w roślinach jedno-, dwu-, i trzyletnich nawożonych azotem w dawce 50, 100, 200 kg N/ha. Stwierdzono, że zarówno dawka azotu jak i wiek rośliny miały istotny wpływ na aktywność antyoksydacyjną i poziom polifenoli w surowcu. Największą aktywność antyoksydacyjną 112–203 μM Trolox/g stwierdzono u roślin jednorocznych. Również u tych roślin odnotowano największą zawartość polifenoli (22 mg GAE/g św. m.). U roślin dwuletnich wartości te były o mniejsze o 3 i 11%, zaś u trzyletnich o 15 i 23%. Wraz ze wzrostem dawki azotu wzrastała aktywność antyoksydacyjna (testy DPPH i FRAP) i zawartość polifenoli.

Słowa kluczowe: *Rheum palmatum, wiek plantacji, aktywność antyoksydacyjna, polifenole*