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ANTIRADICAL PROPERTIES OF EXTRACTS FROM ROOTS, LEAVES AND FRUITS OF SIX *RUMEX* L. SPECIES

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We used the DPPH method to assess in vitro the antiradical activity of extracts from the roots, leaves and fruits of six *Rumex* L. (dock) species. Data from preliminary screening indicated that all the tested extracts showed antioxidant properties. The degree of antiradical activity depended upon the plant part. Fruit extracts from *R. hydrolapathum* Huds., *R. obtusifolius* L. and *R. confertus* Willd. showed stronger antiradical properties than the other tested material. We also determined tannin content levels in the extracts and their correlation with antioxidant activity.

Key words: *Rumex*, antioxidant activity, DPPH, tannins.

INTRODUCTION

The genus *Rumex* L. (dock) of the *Polygonaceae* family is represented by 25 plant species in Poland. Docks have been used in medical practice for many centuries for their antidiarrheal, spasmolytic or cholagogic action, and externally for different skin diseases (Wegiera and Smolarz, 2005). The varied biological activity of *Rumex* species is due to the presence of various groups of biologically active substances in them: flavonoids, tanning agents, phenolic acids, anthraquinones, polyunsaturated fatty acids and other substances (Hegnauer, 1973; Demirezer et al., 2001; Wegiera et al., 2007; Jimoh et al., 2008; Litvinenko and Muzychkina, 2008; Smolarz et al., 2008). Docks are still commonly gathered for consumption – raw leaves are eaten as salads or cooked as an ingredient of soup (Łuczaj and Szymański, 2007). Leafy vegetables in the *Polygonaceae* family, including docks, have been clearly identified as high-oxalate plants (Kasai et al., 1982; Guil et al., 1996; Torlá et al., 2005; Siener et al., 2006; Guerra et al., 2008), and for this reason they should not be eaten in large amounts or should be boiled to remove the oxalate content.

Oils, fats and other organic compounds easily lose their stability and produce free radicals during oxidative deterioration. These radicals can be scavenged by antioxidants; thus an antioxidant-rich diet is vital for health (Karpińska et al., 2001). In recent years particular attention has been paid to a specific class of antioxidant phytochemicals, the polyphenols, which are naturally present in essentially all plant material. They are ubiquitous in vegetables, cereals, fruits and nuts, but also in plant-derived products such as wine, cider, beer, tea and cocoa (Guendez et al., 2005). Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Pietta, 2000; Kaviarasan et al., 2007). Natural antioxidant polyphenol molecules, such as tannins, present in food as endogenous factors or added as preservatives, are believed to scavenge radical and oxygen species, thus modulating exogenous and endogenous oxidative stress; they are at the center of interest because of their beneficial physiological property as scavengers of reactive oxygen intermediates (ROI) (Labieniec et al., 2003). Gallic acid is a well known natural antioxidant found widely in plants, and its derivative, methyl gallate, has a protective effect against hydrogen peroxide-induced oxidative stress and DNA damage. Gallotannins showed stronger antioxidant activity than ascorbic acid did, and it was increased when

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there were more gallolyl groups in the molecule (Wang et al., 2007). There are also reports on the antiradical properties of oxalic acid, a typical organic acid in *Rumex* species (Kayashima and Katayama, 2002; Yoruk et al., 2004; Altunkaya and Gökmen, 2008).

Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases such as cancer, coronary heart disease, arteriosclerosis, inflammatory disorders and aging processes (Gülçin et al., 2003; Wang et al., 2007). Several studies have shown that phenolic compounds reduce in vitro oxidation of low-density lipoprotein, particularly phenolics with multiple hydroxyl groups, which are generally the most efficient in preventing lipid and low-density lipoprotein (LDL) oxidation and thereby, by inference, atherogenesis (Moure et al., 2001).

Antioxidant activity has been examined in a number of *Rumex* species: *R. acetosa* L. (Mantle et al., 2000; Alzoreky and Nakahara, 2003), *R. crispus* L. (Yildirim 2001; Coruh et al., 2008), *R. ecklonianus* Meiss. (Jimoh et al., 2008), *R. hymenosepalus* Torr. (Vanderjagt et al., 2002), *R. induratus* Boiss. & Reut. (Guerra et al., 2008), *R. japonicus* Houtt. (Nishina et al., 1991), *R. patientia* L. (Demirezer et al., 2001; Lone et al., 2007) and *R. thyrsiflorus* F. (Litvinenko and Muzychkina, 2008). Demirezer et al. (2001) reported that tannins isolated from the roots of *R. patientia* exhibited potent DPPH radical scavenging activity, while anthracene derivatives did not show any such activity.

In this study we investigated antioxidant activity and tannin content in the roots, leaves and fruits of six *Rumex* species growing in Poland, and assessed whether tannin content was related to antiradical properties, with a view to improving the therapeutic use of these raw materials.

MATERIALS AND METHODS

PLANT MATERIAL

We collected roots, leaves and fruits from six *Rumex* L. species from the vicinity of Lublin, Poland: *R. acetosa* L., *R. acetosella* L., *R. confertus* Willd., *R. crispus* L., *R. hydrolapathum* Huds. and *R. obtusifolius* L. Voucher specimens are deposited in the Herbarium of the Chair and Department of Pharmaceutical Botany, Medical University, Lublin, Poland.

PREPARATION OF METHANOL EXTRACTS

Dried and ground plant material was extracted four times with 80% aq. methanol in an ultrasonic water bath for 15 min. The extracts were mixed and concentrated in a vacuum to obtain dry residue. Then the crude extracts were dissolved in 4 ml methanol and refrigerated for use in the analyses.

DPPH FREE RADICAL SCAVENGING ASSAY

We determined the radical scavenging activity of *Rumex* extracts against stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals according to the method of Brand-Williams (1995), which has been widely used to evaluate the activity of natural antioxidants.

Changes in color from deep violet to light yellow were measured at 515 nm with a UV-VIS light spectrophotometer (Lambda 40; Perkin-Elmer). Solution of DPPH in methanol (6×10^{-5} M/dm^3) was prepared, and 1.96 ml of it was mixed with 40 µl methanol extract. The decrease in absorbance at 515 nm was determined continuously, with data capturing at 1 and 8 min intervals. The blank sample contained 40 µl methanol instead of extract. All determinations were made in duplicate. Radical scavenging activity was calculated by the following formula:

$$\% \text{ inhibition (IP)} = [(A_B - A_A) / A_B] \times 100,$$

where AA is the absorption of the tested solution and AB the absorption of the blank sample.

The basal methanol extracts (1 g/ml) were diluted with methanol to obtain concentrations of 1, 2, 3.33, 5, 20 and 40 mg/ml of the investigated samples.

DETERMINATION OF TANNIN CONTENT

Content of tannins was determined by the titrimetric method described in *Polish Pharmacopoeia V* (2000). The tannins were precipitated from water extract and examined as insoluble copper salts. All analyses were made three times.

Extract preparation

Two grams of dry powdered plant material was extracted with 500 ml distilled water. The mixture was heated to boiling and left to cool, stirring from time to time. The cold aqueous extract was filtered through a wad of cotton wool into a 1000 ml volumetric flask. The plant sample was extracted again with 300 and 200 ml distilled water (heated for 10 min after boiling). The obtained extracts were filtered through filter paper, rejecting the first 20 ml of filtrate.

Determination of tannins

In the final determination, 50 ml of the tested extract and 25 ml of 0.1 mol/dm^3 cupric acetate solution were measured and mixed with a glass rod. After 12 h the precipitated copper salts were

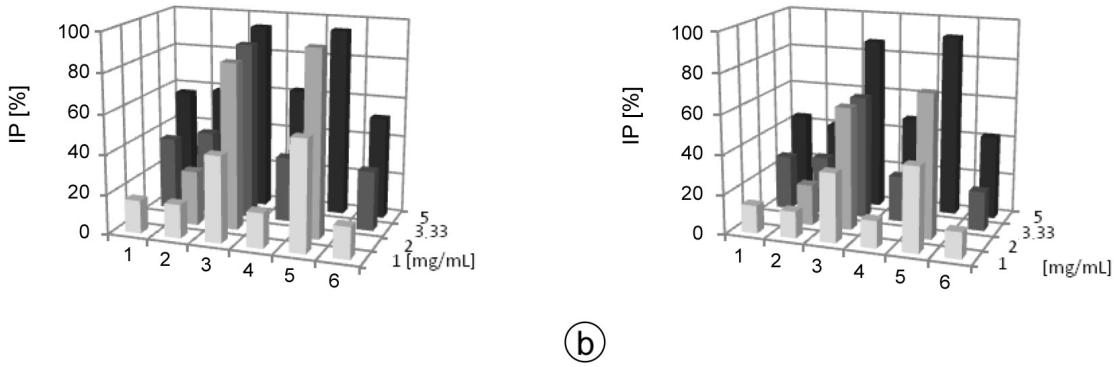

Fig. 1. Radical scavenging activity (as inhibition percentage) of different concentrations (1, 2, 3.33 and 5 mg/ml) of extracts from roots of selected *Rumex* species at (**a**) 1 min, (**b**) 8 min. 1 – *R. acetosa*; 2 – *R. acetosella*; 3 – *R. confertus*; 4 – *R. crispus*; 5 – *R. hydrolapathum*; 6 – *R. obtusifolius*.

filtered on filter paper pre-dried to constant weight. The precipitate was washed with distilled water until a negative reaction with the copper ion (1 ml filtrate should not turn brown after the addition of 1 ml 5% solution of potassium ferrocyanide). Filter paper and precipitate were dried at 100°C to constant weight, then 25 ml filtrate containing unbound copper acetate was measured into a flask, and 10 ml 10% sulfuric acid and 2 g potassium iodide dissolved in 5 ml distilled water was added. After shaking, 2 ml starch solution was added to the flask and titrated with 0.1 N sodium thiosulfate until the color changed from brown-blue to white. A blank control was also made. All analyses were done in triplicate.

Tannin content was calculated as:

$$X = C - (A - 3B) \times 6.354 \times 1.252$$

where X is the amount of tannins (mg) in 50 ml aqueous extract, A is the number of ml 0.1 mol/l copper acetate (II), B is the number of ml 0.1 mol/l sodium thiosulfate (VI) used for titration of copper ions not bound by the tannins, C is the weight of copper tannins (mg), 6.364 is the quantity of Cu corresponding to 1 ml 0.1 mol/l copper acetate (II), and 1.252 is the molecular weight ratio of copper oxide (II) to Cu.

STATISTICAL ANALYSIS

All results are expressed as means of three replicates. Statistica 7 (StatSoft®, Cracow) was used for the calculations. We evaluated relationship between antioxidant activity and total content of tannins with Pearson rank correlations (r). Values of $p < 0.05$ were considered statistically significant and $p < 0.01$ highly significant.

RESULTS AND DISCUSSION

We tested the antioxidant activity of extracts of roots from six *Rumex* species at dilutions of 1, 2, 3.33 and 5 mg/ml. At 5 mg/ml more than half of the investigated extracts indicated very high antioxidant activity after 1 min (Fig. 1a). At the lowest concentration (1 mg/ml) the extract of *R. hydrolapathum* showed 42.63% inhibition of DPPH, and *R. confertus* extract gave 35.03% inhibition.

After 8 min all the root extracts diluted to 5 mg/ml achieved 50% inhibition (Fig. 1b). The IP value was highest (more than 94%) for *R. hydrolapathum* and *R. confertus*. The extracts from *R. obtusifolius* and *R. acetosa* showed the weakest antioxidant activity: at 1 mg/ml concentration, only 16% inhibition of free radicals. Of all the root extracts, *R. hydrolapathum* revealed the strongest activity in reducing DPPH. Extracts of dock roots from *R. obtusifolius*, *R. acetosa*, *R. acetosella* and *R. crispus* showed the weakest and very similar antiradical activity.

We also tested the antioxidant activity of leaf extracts at 2, 5, 20 and 40 mg/ml. At the 20 mg/ml concentration only the leaf extract of *R. acetosa* gave an IP value of more than 50% after 1 min (Fig. 2a). At 5 mg/ml it also gave the highest IP value (33.23%); the lowest (4.07%) was for *R. hydrolapathum*. There were large increases of antioxidant activity after 8 min; at the 40 mg/ml concentration the majority of IP values were close to or above 50% (Fig. 2b).

The antioxidant activity of fruit extracts at 2, 3.33, 5 and 20 mg/ml concentrations was stronger than that of leaf extracts leaves and close to the activity of root extracts. After 1 min most of the investigated extracts and concentrations achieved IP values of 50% (Fig. 3a). This indicates that the dock fruit extracts possess the strongest antioxidant

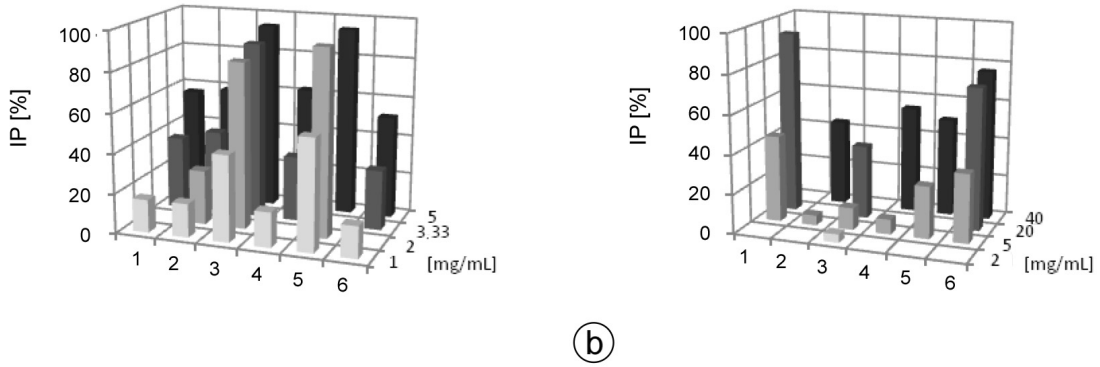

Fig. 2. Radical scavenging activity (as inhibition percentage) of different concentrations (2, 5, 20 and 40 mg/ml) of extracts from leaves of selected *Rumex* species at (**a**) 1 min, (**b**) 8 min. 1 – *R. acetosa*; 2 – *R. acetosella*; 3 – *R. confertus*; 4 – *R. crispus*; 5 – *R. hydrolapathum*; 6 – *R. obtusifolius*.

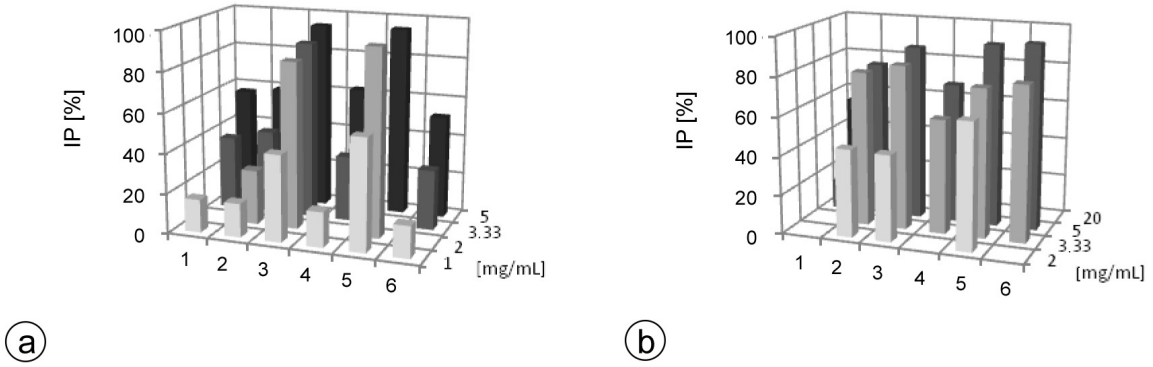

Fig. 3. Radical scavenging activity (as inhibition percentage) of different concentrations (2, 3.33, 5 and 20 mg/ml) of extracts from fruits of selected *Rumex* species at (**a**) 1 min, (**b**) 8 min. 1 – *R. acetosa*; 2 – *R. acetosella*; 3 – *R. confertus*; 4 – *R. crispus*; 5 – *R. hydrolapathum*; 6 – *R. obtusifolius*.

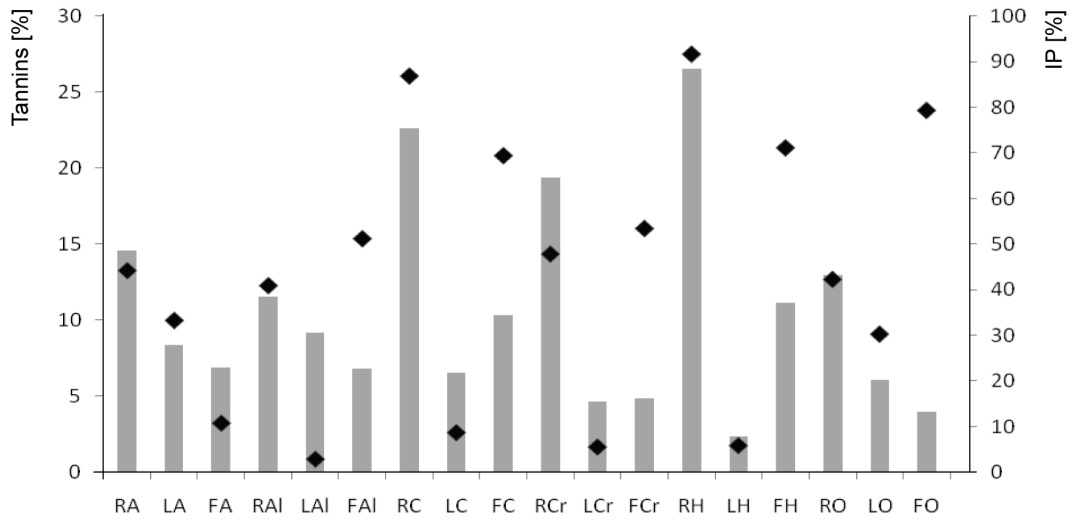

Fig. 4. Relationship between tannin content (% d.w. – columns) and antioxidant activity (% inhibition after 1 min – diamonds). Samples as described in Table 1.

TABLE 1. Tannin content obtained from the roots, leaves and fruits of selected *Rumex* species (% of dry weight)

		Extract	Tannins [% of dry weight]
R. acetosa	roots	RA	14.56 ± 0.33
	leaves	LA	8.39 ± 0.48
	fruits	FA	6.88 ± 0.45
R. acetosella	roots	RAl	11.51 ± 0.44
	leaves	LAl	9.20 ± 0.71
	fruits	FAl	6.80 ± 0.57
R. confertus	roots	RC	22.65 ± 0.22
	leaves	LC	6.55 ± 0.49
	fruits	FC	10.36 ± 0.29
R. crispus	roots	RCr	19.36 ± 0.41
	leaves	LCr	4.63 ± 0.37
	fruits	FCr	4.88 ± 0.56
R. hydrolapathum	roots	RH	26.55 ± 0.87
	leaves	LH	2.33 ± 0.25
	fruits	FH	11.16 ± 0.17
R. obtusifolius	roots	RO	12.98 ± 0.35
	leaves	LO	6.05 ± 0.63
	fruits	FO	4.00 ± 0.35

properties. After 8 min the IP values for the 5 mg/ml concentration exceeded 90% for two extracts and 70% for three of them (Fig. 3b). Antiradical activity was strongest for the extracts from fruits of *R. hydrolapathum*, *R. obtusifolius* and *R. confertus*.

The largest amount of tannins was found in roots of *R. hydrolapathum*, in accord with literature data (tannin content 30%) (Jędrzejko, 2002). Roots of *R. crispus*, *R. obtusifolius* and *R. acetosella* showed a higher level of tannins than given in the literature (5–8% in Mowszowicz, 1986; 4–7% in Hegnauer 1973; 2% in Schöttelndreier et al., 2001). The level of tannins in the roots of *R. confertus* is given here for the first time. The tannin content we found in root extract of *R. acetosa* is comparable with data from other investigators (7–15%) (Torlá et al., 2005).

This is the first time the tannin concentration was measured in fruits of *R. acetosella* and *R. obtusifolius*. Our results were comparable to literature reports for *R. crispus* (4.88%, versus 5% given by Hoppe, 1975) and *R. hydrolapathum* (11.16%, versus 14% given by Fairbairn and Muhtadi, 1972). The tannin concentrations in our work were higher than reported elsewhere for *R. acetosa* (6.88%, versus 5% given by Hoppe, 1975) and *R. confertus* (10.36%, versus 7.4% given by Bargman, 1972).

For most of the dock species in this study this is the first time that leaf extract has been assessed for tannin content. Among the investigated extracts, the tannin concentration was lowest in leaves of *R. hydrolapathum* (2.33%); this result is similar to data from other work (1%) (Hoppe, 1975).

The level of tannins and the antiradical activity were highest in roots of *R. hydrolapathum* (Fig. 4). There was a very significant relationship between antioxidant activity and total content of tannins in the methanol extracts of the investigated *Rumex* species ($p<0.01$; $R^2 = 0.598$). These results are similar to those for extracts of leaves and seeds of *R. crispus* (Yildirim et al., 2001). There was a statistically significant correlation between the amount of phenolic compounds and DDPH scavenging activity ($p<0.05$; $R^2 = 0.892$). Total phenolics in some edible Yemeni plants, including leaves of *R. acetosa*, were highly correlated ($R^2 = 0.937$) with Trolox equivalent antioxidant capacity (Alzoreky and Nakahara, 2001). Other work also confirmed a positive correlation between total phenolic content and antioxidant activity (Nazaruk, 2008; Wijngaard et al., 2009). In malts, however, no correlation between antioxidant activity and phenolic content was found, since other compounds were responsible for the antioxidant activity, nor was a relationship between antioxidant activity and phenolic composition found in citrus residues, fruit berry or fruit wines (Moure et al., 2001).

CONCLUSIONS

1. All the tested extracts of *Rumex* species showed certain levels of antioxidant activity.
2. The compounds in the extracts neutralized radicals quickly.
3. Within species, fruit and root extracts showed higher antioxidant activity.
4. Fruit extracts from *R. hydrolapathum*, *R. obtusifolius* and *R. confertus* showed the strongest antiradical action.
5. *R. hydrolapathum* roots are plant material with strong antioxidant properties and higher tannin content, and as such deserve special attention.

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