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Screening of radical scavenging activity of some medicinal and aromatic plant extracts

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Abstract

Extracts of 12 medicinal and aromatic plants were investigated for their radical scavenging activity using DPPH and ABTS assays: *Salvia sclarea*, *Salvia glutinosa*, *Salvia pratensis*, *Lavandula angustifolia*, *Calendula officinalis*, *Matricaria recutita*, *Echinacea purpurea*, *Rhaponticum carthamoides*, *Juglans regia*, *Melilotus officinalis*, *Geranium macrorrhizum* and *Potentilla fruticosa*. *Salvia officinalis* was used as a reference plant with well documented antioxidant activity. *G. macrorrhizum* and *P. fruticosa* extracts possessed very high radical scavenging activity (RSA) in both assays, higher than that of *S. officinalis* extract. High RSA was also characteristic to other *Salvia* species and *Rhaponticum carthamoides*. The content of total phenolic compounds, flavonoids and flavonols was measured in plant extracts. A correlation between radical scavenging capacities of extracts with total phenolic compound content was observed.

Keywords: Radical scavenging; DPPH; ABTS; Total phenolics; Flavonoids; Flavonols

1. Introduction

A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. Numerous studies were carried out on some of these plants, e.g. rosemary, sage, oregano, which resulted in a development of natural antioxidant formulations for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine, is still rather scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and neutraceuticals.

Chemical and biological diversity of aromatic and medicinal plants depending on such factors, as cultivation area, climatic conditions, vegetation phase, genetic modifications and others is an important impetus to study flora present in different growing sites, countries and geographical zones. This work is aimed at a preliminary screening of radical scavenging activities of the extracts isolated from some plants growing in Central and Eastern Europe. The following plants were selected for investigations: *Salvia sclarea*, *Salvia glutinosa*, *Salvia pratensis*, *Lavandula angustifolia* (all *Lamiaceae* family), *Calendula officinalis*, *Matricaria recutita*, *Echinacea purpurea*, *Rhaponticum carthamoides* (all *Asteraceae* family), *Juglans regia*, (*Juglandaceae* family), *Melilotus officinalis* (*Fabaceae* family), *Geranium macrorrhizum* (*Geraniaceae* family) and *Potentilla fruticosa* (*Rosaceae* family). To our knowledge there are few data on radical scavenging and/or antioxidant properties of these plants. Garden sage (*Salvia officinalis,* Lamiaceae) was used as a reference plant due to its well-known and widely documented antioxidant properties.

Salvia species are widespread plants in many countries. Clary sage (*S. sclarea*) is commercially cultivated for its aromatic and widely used essential oil (Lawrence, 1979); meadow sage (*S. pratensis*) is used in cosmetics and possesses some medicinal properties (Akbar, Tariq, & Nisa, 1984). *S. glutinosa* species were tested in

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enzyme-dependent and enzyme-independent systems of lipid peroxidation and found to be quite effective (Zupko et al., 2001). However, data about antioxidant properties of these plants are very scanty (Tang & Yuan, 1997).

Lavender (*L. angustifolia*) is an important source of a thoroughly studied essential oil, while antioxidant properties of this plant are much less documented. Some reports on the antioxidant properties of this plant are somewhat contradictory, most likely due to the differences in the assessment methodology. For instance, Dapkevicius, Venskutonis, Van Beek, and Linssen (1998) did not detect antioxidant activity of various plant extracts in the model linoleic acid-β-carotene system, while Hohmann et al. (1999) reported, that aqueous methanolic extracts of lavender were effective in lipid peroxidation media.

The use of German chamomile (*M. recutita*) teas and medicinal preparations has a long tradition in various countries. Although chamomile contains a great number of polyphenolic compounds (Hurrell, Manju, & Cook, 1999), it was reported that antioxidative properties of its extracts in rapeseed oil were not distinct (Lionis, Faresjo, Skoula, Kapsokefalou, & Faresjo, 1998).

The essential oils of pot marigold (*C. officinalis*) are used as medicines soothing central nervous system and exhibiting other useful healing properties. The oil is also rich in carotenoids and used as a dye, as a lubricant and for other purposes (Marvin, Mastebroek, Becu, & Janssens, 2000). The information on antioxidant properties of this plant was not found in the literature.

R. carthamoides is a widespread and used medicinal plant in the Siberian part of Russia. It is included in formulae of various beverages, medicinal-hygienic preparations, lubricants, creams, shampoos, etc. It has been reported that certain fractions of the plant possess some pharmacological properties (Plotnikov et al., 1999 and Petkov, Roussinov, Todorov, Lazarova, Yonkov & Draganova, 1984).

Purple coneflower (*E. purpurea*) is a well-known medicinal plant possessing bactericidal, antiviral and antifungal effects against some microbial cultures. *E. purpurea* and *M. officinalis* were tested for antioxidant activity using $ABTS^{\bullet+}$ (generated by metmyoglobin and H_2O_2 in the presence of peroxidase) scavenging method and it was found to be rather ineffective (Pietta, Simonetti, & Mauri, 1998).

Sweet clover (*M. officinalis*) is applied in the production of some beverages and foods (Ehlers, Platte, Bork, Gerard, & Quirin, 1997). Honey of *M. officinalis* obtained during the plant flowering period was found to possess quite high antioxidant activity as it distinctly reduced polyphenol oxidase (Lei, Mehta, Berenbaum, Zangerl, & Engeseth, 2000).

Walnut tree (*J. regia*) has been thoroughly studied, however, the main attention was paid to its nuts, while the properties of other parts are less known. It was found that the leaves of *J. regia* have antimicrobial activity against some bacteria (Alkhawajah, 1997); they also possess some antioxidative properties, that have been assessed by the electron spin resonance (ESR) techniques (Ohsugi et al., 1999).

Although shrubby cinquefoil (*P. fruticosa*) is a native north American perennial plant, it is also widely cultivated in many European countries and has numerous medicinal virtues (Mitich, 1995). It has been reported that shoot extracts inhibited lipid peroxidation and had some other protective effects in vivo (Aryayeva et al., 1999). Quite high antioxidant activity of plant extracts was also reported using photochemiluminescence method (Bolshakova, Lozovskaya, & Sapezhinskii, 1998).

G. macrorrhizum is a grassy perennial plant with long roots; the centre of its distribution are Balkan countries (Leslie, 1993). The plant is mainly used for the production of essential oil. Extracts of *G. macrorrhizum* were reported to possess a broad spectrum of antimicrobial activities, it also has strong hypotensive, astringent, cardiotonic, capillary and sedative action (Ivancheva, Manolova, Serkedjeva, Dimov, & Ivanovska, 1992). Antioxidant properties of the plant have not been reported.

It can be concluded that the antioxidant activity of the selected plants was poorly investigated, therefore testing of their antiradical properties is of interest, primarily in order to find new promising sources for natural antioxidants, functional foods and neutraceuticals. Free radicals can be generated by metabolic pathways within body tissues; also they can be introduced by external sources, with food, drugs, can be caused by environmental pollution etc. Use of natural antioxidants, as food additives for inactivating free radicals receives a lot of attention nowadays, not only for their scavenging properties, but also because they are natural, non-synthetic products, and their appreciation by consumers is very favourable.

2. Materials and methods

2.1. Plant material

The following plants were harvested from the collection of medicinal plants at Kaunas Botanical Garden (Vytautas Magnus University, Lithuania) at different vegetation phases during May–August, 2000 [plant name (anatomical parts, harvesting stage)]:

Salvia officinalis (leaves and stems, initial blossom stage);
Salvia sclarea (leaves and stems, full bloom stage);
Salvia glutinosa (leaves, full bloom stage);

Salvia pratensis (leaves and stems, full bloom stage);
Lavandula angustifolia (leaves, stems and blossoms, full bloom stage);
Calendula officinalis (perianths, full bloom stage);
Matricaria recutita (blossoms, full bloom stage);
Echinacea purpurea (leaves, stems and blossoms, full bloom stage);
Rhaponticum carthamoides (leaves and stems, growing stage);
Juglans regia (leaves and stems, growing stage);
Melilotus officinalis (leaves, stems and blossoms, initial blossom stage);
Geranium macrorrhizum (leaves, growing stage); and
Potentilla fruticosa (blossoms, full bloom stage).

The freshly cut plants were sorted out, dried in the drying room with active ventilation at ambient temperature, packed in paper bags and stored at ambient temperature for 4–5 months before use. These upper parts of following plants later were used for investigation.

2.2. Extraction

Dried plants were milled with sample mill (300 Waufn S2, Germany) and in turn extracted with three solvents: acetone (OBR PR, Plock, Poland), ethyl acetate (99.7%) and methanol (99.5%), (both from Lachema, Brno, Czech Republic). Two-step extraction was applied by shaking flasks with 6–10 g (±0.01 g) of plant and 100 ml (50 + 50) of solvent in a shaking machine (Sklo Union LT, Teplice, Czech Republic). Each extraction step was completed in 2 h. The extracts were filtered and concentrated in a rotavapor apparatus (Bûchi, Flawil, Switzerland) at approximately 40 °C.

2.3. Evaluation of antioxidant activity

2.3.1. DPPH radical scavenging assay

Radical scavenging activity of plant extracts against stable DPPH• (2,2-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH• reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep—violet to light—yellow) were measured at 515 nm on a UV/visible light spectrophotometer (Spectronic Genesys 8, Rochester, USA).

Radical scavenging activity of extracts was measured by slightly modified method of Brand-Williams, Cuvelier, and Berset (1995), as described below. Extract solutions were prepared by dissolving 0.025 g of dry extract in 10 ml of methanol. Acetone and ethyl acetate extracts were not fully soluble in methanol (even after treating solutions for 5 min in an ultrasonic bath), therefore they were filtered and only the soluble part was further analysed. The solution of DPPH• in methanol (6×10^{-5} M) was prepared daily, before UV measurements. Three ml of this solution were mixed with 77 (38 or 19 in additional assays) μl extract solution in 1 cm path length disposable microcuvettes (final mass ratio of extracts with DPPH• was approximately 3:1, 1.5:1, 0.75:1). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH• solution was prepared and measured daily. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$\%\ \text{Inhibition} = [(A_B - A_A)/A_B] \times 100, \quad (1)$$

where: A_B—absorption of blank sample ($t = 0$ min); A_A—absorption of tested extract solution ($t = 15$ min).

2.3.2. ABTS radical cation decolourisation assay

ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma-Aldrich Chemie, Steinheim, Germany) radical cation decolourisation test is also a spectrophotometric method widely used for the assessment of antioxidant activity of various substances. The experiments were carried out using an improved ABTS decolourisation assay (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999). It is applicable for both lipophilic and hydrophilic compounds. $ABTS^{+\bullet}$ was generated by oxidation of ABTS with potassium persulfate (BDH, Poole, UK). Three ml of ABTS cation solution were mixed with 30 μl methanol extract solution in 1 cm path length disposable microcuvette and the decrease of absorption was measured during 6 min. All determinations were carried out in triplicate.

2.4. The amount of phenolic compounds, flavonoids and flavonols

The following reagents were used: 2.0 M Folin-Ciocalteu phenol reagent, gallic acid, rutin (hydrate, min 95%), anhydrous sodium carbonate (all from Sigma-Aldrich Chemie, Steinheim, Germany), 98% acetic acid, (Lachema, Brno, Czech Republic), aluminium trichloride hydrate, sodium acetate (both from Reachim, Riga, Latvia).

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu method (1927). For the preparation of calibration curve 1 ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml ethanolic gallic acid solutions were mixed with 5 ml Folin–Ciocaleu reagent (diluted ten-fold) and 4 ml (75 g/l) sodium carbonate. The absorption was read after 30 min at 20 °C at 765 nm and the calibration curve was drawn. One ml methanolic plant extract (10 g/l) was mixed with the same reagents as described above, and after 1 h the absorption was measured for the

determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$C = c \cdot V/m' \quad (2)$$

where: C—total content of phenolic compounds, mg/g plant extract, in GAE; c—the concentration of gallic acid established from the calibration curve, mg/ml; V—the volume of extract, ml; m—the weight of pure plant methanolic extract, g.

The content of flavonoids was determined by a pharmacopeia method (1989) using rutin as a reference compound. One ml of plant extract in methanol (10 g/l) was mixed with 1 ml aluminium trichloride in ethanol (20 g/l) and diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min at 20 °C. Blank samples were prepared from 1 ml plant extract and 1 drop acetic acid, and diluted to 25 ml. The absorption of rutin solutions was measured under the same conditions. Standard rutin solutions were prepared from 0.05 g rutin. All determinations were carried out in duplicate. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula:

$$X = (A \cdot m_0 \cdot 10)/(A_0 \cdot m), \quad (3)$$

where: X—flavonoid content, mg/g plant extract in RE; A—the absorption of plant extract solution; A_0—the absorption of standard rutin solution; m—the weight of plant extract, g; m_0—the weight of rutin in the solution, g.

The content of flavonols was determined by Yermakov method (1987). The rutin calibration curve was prepared by mixing 2 ml of 0.5, 0.4, 0.3, 0.2, 0.166, 0.1, 0.05, 0.025, and 0.0166 mg/ml rutin ethanolic solutions with 2 ml (20 g/l) aluminum trichloride and 6 ml (50 g/l) sodium acetate. The absorption at 440 nm was read after 2.5 h at 20 °C. The same procedure was carried out with 2 ml of plant extract (10 g/l) instead of rutin solution. All determinations were carried out in duplicates. The content of flavonols, in rutin equivalents (RE) was calculated by the following formula:

$$X = C \cdot V/m, \quad (4)$$

where: X—flavonol content, mg/g plant extract in RE; C—the concentration of rutin solution, established from the calibration curve, mg/ml; V, m—the volume and the weight of plant extract, ml, g.

2.5. Statistical analysis

Correlation coefficients (R) to determine the relationship between two variables (between different RSA tests; RSA tests and content of total phenolic compounds, flavonoids and flavonols) were calculated using MS Excel software (CORREL statistical function).

3. Results and discussion

The results of DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ inhibition by different plant extracts are summarized in Table 1 and Fig. 1 respectively.

Methanol extracts were the most effective DPPH radical scavengers. Seven of the investigated methanol extracts almost completely inhibited DPPH absorption (*S. sclarea*—92.9%, *S. glutinosa*—91.5%, *S. pratensis*—93.0%, *R. carthamoides*—87.6%, *G. macrorrhizum*—91.7% and *P. fruticosa*—93.9%). These percentages can be considered as a full absorption inhibition of DPPH, because after completing the reaction the final solution always possesses some yellowish colour and therefore its absorption inhibition compared to colourless methanol solution can't reach 100%. Permanent residual absorption results in up to 7% of total absorption inhibition. The extracts of *J. regia* and *M. officinalis* were also good radical scavengers with the inhibition of 67.8% and 75.9% respectively. The extracts of *E. purpurea* and *C. officinalis* contained remarkably lower amounts of radical scavenging compounds.

Ethyl acetate and acetone extracts were considerably less effective radical scavengers compared to methanolic extracts. However, RSA of ethyl acetate and acetone extracts of *S. officinalis* and acetone extract of *P. fruticosa* was similar to the RSA of methanol extracts of these plants. The acetone extract of *E. purpurea* was more effective than its methanolic extract, however, all the extracts from this plant were very weak radical scavengers. It should be pointed out that ethyl acetate and acetone extracts during test were dissolved in methanol, and this procedure could have some effect on the measurements of RSA so far as the extracts were not fully soluble in methanol. Acetone extracts were more active

Table 1
DPPH absorption inhibition (%) of plant extracts isolated with methanol, ethyl acetate and acetone

Plant	DPPH inhibition (%)		
	Ethyl acetate extract	Acetone extract	MeOH extract
	$[M_{extract}]:[M_{DPPH}] = 3:1$		
Salvia officinalis	91.7±0.5	92.6±0.6	92.3±0.5
Salvia sclarea	21.5±1.8	17.8±3.4	92.9±0.4
Salvia glutinosa	16.2±1.5	41.0±0.9	91.5±0.5
Salvia pratensis	17.2±1.4	26.0±0.3	93.0±0.5
Lavandula angustifolia	2.5±1.4	7.4±1.3	35.4±1.7
Calendula officinalis	1.6±1.4	2.6±1.0	12.9±0.8
Matricaria recutita	6.4±1.7	8.2±1.1	44.7±2.6
Echinacea purpurea	3.5±0.1	14.2±1.8	6.8±1.5
Rhaponticum carthamoides	11.4±1.8	30.0±0.3	87.6±1.1
Juglans regia	33.9±2.3	25.3±1.6	67.8±0.2
Melilotus officinalis	8.1±1.7	7.6±5.2	75.9±1.8
Geranium macrorrhizum	26.9±1.4	44.6±1.2	91.7±0.6
Potentilla fruticosa	46.3±0.5	93.0±0.2	93.9±0.7

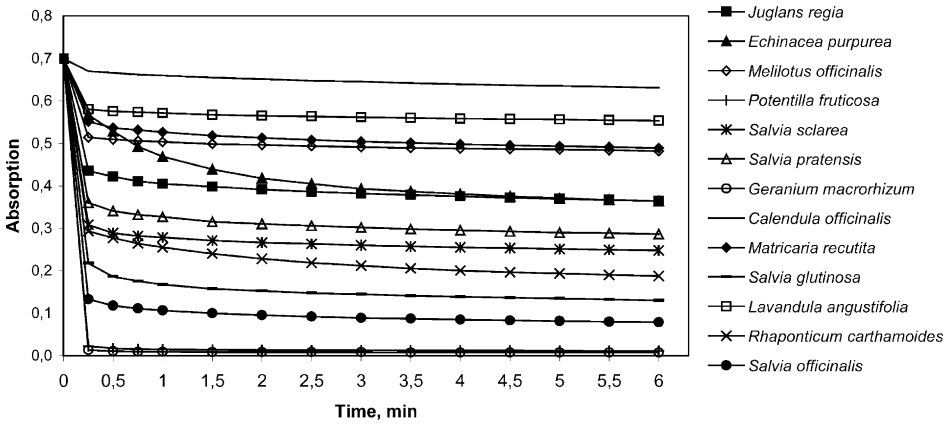

Fig. 1. $ABTS^{+\bullet}$ absorption inhibition assay of investigated plant methanolic extracts.

against the DPPH radical than ethyl acetate extracts except for *J. regia* and *S. sclarea*. As the polarity of these two solvents is quite similar (ethyl acetate is slightly more polar than acetone) it can be concluded that the use of ethyl acetate for extraction of radical scavenging property compounds from the selected herbs was not effective.

For further DPPH scavenging assessment the most effective plant extracts were diluted 2- or 4-fold. The final mass ratio of extracts with $DPPH^{\bullet}$ was 1.5:1 and 0.75:1 (Table 2).

The results provided in Table 2 demonstrate that the most active radical scavengers were the methanolic extract of *G. macrorrhizum* and the acetone extract of *P. fruticosa*. RSA of these extracts after their four-fold dilution remained also the same as that of the initial extracts, while RSA of *S. officinalis* at the same conditions reduced approximately twice. The methanolic extract from *P. fruticosa* was also a strong radical scavenger indicating that active compounds of different polarity could be present in this plant.

Another antioxidant activity screening method, applicable for both lipophilic and hydrophilic antioxidants—ABTS radical cation decolourisation assay, showed quite similar results compared to those obtained in DPPH reaction. The extracts of *G. macrorrhizum* and *P. fruticosa* were the most active: they nearly fully scavenged $ABTS^{+\bullet}$ (the absorption after 6 min was close to 0). In the ABTS assay the activity of *S. officinalis* extracts was slightly lower; the absorption after 6 min decreased to 0.08, i.e. by 89% (Fig. 1). The extracts of *S. glutinosa*, *R. carthamoides* and *S. sclarea* also possessed strong activity: absorption with these extracts decreased by 81, 72 and 64% respectively.

It also should be noted that the reaction with $ABTS^{+\bullet}$ was quite fast and almost in all cases was completed in 0.25–0.5 min. During the remainder of the reaction time the changes in absorption were negligible, except for *E. purpurea*, when the absorption decrease continued during 3 min.

Comparing results of two radical scavenging tests good correlation between them can be observed (correlation coefficients between DPPH and ABTS assays were 0.64, 0.83 and 0.76 for ethyl acetate, acetone and methanol extracts respectively). *G. macrorrhizum*, *P. fruticosa* and *S. officinalis* possessed the highest activity in both tests. Quite similar results were obtained also for other plants, except for *E. purpurea*; the activity of its extracts in ABTS test was higher than in the DPPH test. One the other hand slow reaction time was observed in this case. It suggests that the kinetics of radical scavenging reactions in these two systems differ.

Table 2
DPPH absorption inhibition (%) of methanolic and acetone extracts

Plant	DPPH inhibition (%)			
	MeOH extr.	Acetone extr.	MeOH extr.	Acetone extr.
	$[M_{extract}]:[M_{DPPH}]$ = 1.5:1		$[M_{extract}]:[M_{DPPH}]$ = 0.75:1	
Salvia officinalis	91.1	87.8	57.5	45.9
Salvia sclarea	74.5	n.a.[a]	n.a.	n.a.
Salvia pratensis	80.3	n.a.	n.a.	n.a.
Salvia glutinosa	77.6	n.a.	n.a.	n.a.
Potentilla fruticosa	92.5	92.4	79.6	91.4
Geranium macrorrhizum	92.3	n.a.	89.0	n.a.
Rhaponticum carthamoides	66.9	n.a.	n.a.	n.a.

[a] n.a.—values were not estimated.

3.1. Content of phenolic compounds

So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics (Agrawal, 1989). These compounds possess a broad spectrum of chemical and biological

activities including radical scavenging properties. Such properties are especially distinct for flavonols. Therefore, the content of both groups of phenolics was also determined in the extracts (Table 3).

The content of phenolic compounds (mg/g) in methanolic extracts, determined from regression equation of calibration curve ($y = 10.738x + 0.061$, $R^2 = 0.98$) and expressed in gallic acid equivalents (GAE), varied between 4.1 and 37.9. The highest amounts were found in the extracts of *P. fruticosa* and *G. macrorrhizum*. High content of phenolic compounds also contained all *Salvia* species and *R. carthamoides*. It can be observed that the content of phenolics in the extracts correlates with their antiradical activity (e.g. correlation coefficient between data of ABTS assay and total phenolic compounds is 0.84), confirming that phenolic compounds are likely to contribute to the radical scavenging activity of these plant extracts.

The content of flavonoids (mg/g), in rutin equivalents varied from 0.3 to 13.8. The highest amounts of flavonoids were found in extracts of *G. macrorrhizum* and *J. regia*, while *S. officinalis* and other *Salvia* species contained remarkably lower amounts of these compounds. Relatively low amounts of flavonoids were also determined in *P. fruticosa* which contained the highest amount of phenolics. It can be observed that the amount of flavonoids in the analysed plant extracts showed only low correlation with the total amount of phenolics ($R = 0.43$), also with RSA (e.g. correlation coefficient between data of flavonoid content and DPPH assay with methanolic extracts was only 0.32). It is known that only flavonoids of a certain structure and particularly hydroxyl position in the molecule determine antioxidant properties; in general these properties depend on the ability to donate hydrogen or electron to a free radical.

Table 3
Total amount of plant phenolic compounds, flavonoids and flavonols

Plant extracts	Total phenolic compounds, mg/g plant extract (in GAE)	Total flavonoids, mg/g plant extract (in RE)	Total flavonols, mg/g plant extract (inRE)
Salvia officinalis	22.6±0.9	3.5±1.6	0.6±0.0
Salvia sclarea	24.0±1.1	4.8±0.5	0.7±0.1
Salvia glutinosa	17.1±0.6	5.7±0.3	0.9±0.0
Salvia pratensis	9.7±0.4	1.4±0.1	0.5±0.0
Lavandula angustifolia	5.4±0.2	0.3±0.0	0.1±0.0
Calendula officinalis	6.6±0.3	3.4±0.1	0.3±0.0
Matricaria recutita	7.5±0.1	7.1±0.4	0.5±0.1
Echinacea purpurea	4.1±1.2	2.5±0.1	0.2±0.0
Rhaponticum carthamoides	13.3±0.3	8.4±0.6	1.1±0.1
Juglans regia	11.5±1.0	11.3±0.4	1.7±0.0
Melilotus officinalis	4.3±0.6	1.0±0.1	0.1±0.0
Geranium macrorrhizum	25.9±0.2	13.8±0.2	0.9±0.0
Potentilla fruticosa	37.9±2.1	6.1±1.1	0.7±0.0

The concentration of flavonols, expressed in rutin equivalents (regression equation of calibration curve $y = 5.242x + 0.222$, $R^2 = 0.93$) in mg/g of plant extract, varied in a less wide range as compared with total phenolics and flavonoids, i.e. from 0.1 to 1.7. In general, some correlation between total phenolics and flavonols can be observed, although the highest amounts were found in *J. regia* and *R. carthamoides*, the plants with medium concentration of phenolics. Flavonols are known as important compounds in terms of radical scavenging properties. In our study their content had higher correlation with antiradical activity of plant extracts as compared to flavonoids (R between flavonol content and ABTS assay was 0.46). However this correlation was even less remarkable in case of *J. regia* and *M. recutita* extracts, which possessed medium or even low DPPH and ABTS scavenging properties comparing to high amounts of flavonols. Detailed examination of phenolic composition in plant extracts is required for the comprehensive assessment of individual compounds exhibiting antioxidant activity.

4. Conclusions

Methanol extracts of *G. macrorrhizum* and *P. fruticosa* were the strongest radical scavengers in both DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ assays among the plants screened. They are promising plants for more detailed investigation of their antioxidant properties and application possibilities. Their antiradical activity was higher than that of *S. officinalis* which is one of the most effective plant in terms of antioxidant activity. The extracts of all tested *Salvia* species and *R. carthamoides* also possessed high radical scavenging abilities, while those of *C. officinalis*, *L. angustifolia and M. recutita* were relatively weak antioxidants. Good correlation was obtained between DPPH and ABTS radical scavenging assays. The amount of total phenolic compounds in investigated plant extracts in most cases correlated with their antiradical activity.

References

Agrawal, P. K. (1989). *Carbon-13 NMR of flavonoids*. New York: Elsevier.

Akbar, S., Tariq, M., & Nisa, M. (1984). A study on CNS depressant activity of *Salvia haematodes* Wall. *International Journal of Crude Drug Research*, *22*, 41–44.

Alkhawajah, A. M. (1997). Studies on the antimicrobial activity of *Juglans regia*. *American Journal of Chinese Medicine*, *25*, 175–180.

Aryayeva, M. M., Azhunova, T. A., Nikolaev, S. M., Aseeva, T. A., Lesiovskaya, E. E., & Nikolaeva, I. G. (1999). Effect of *Pentaphylloides fruticosa* (L.) O. Schwarz shoot extract on the course of experimental diabetes. *Rastitelnye Resursy*, *35*, 91–97.

Bolshakova, I. V., Lozovskaya, E. L., & Sapezhinskii, I. I. (1998). Antioxidant properties of a number of plant extracts. *Biofizika*, *43*, 186–188.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie*, *28*, 25–30.

Dapkevicius, A., Venskutonis, R., Van Beek, T. A., & Linssen, J. P. H. (1998). Different Isolation Procedures from some aromatic herbs grown in Lithuania. *Journal of the Science of Food and Agriculture*, *77*, 140–146.

Ehlers, D., Platte, S., Bork, W. R., Gerard, D., & Quirin, K. W. (1997). HPLC analysis of sweet clover extracts. *Deutsche Lebensmittel Rundschau*, *93*, 77–79.

Folin, O., & Ciocalteu, V. (1927). On tyrosine and tryptophane determination in proteins. *Journal of Biological Chemistry*, *27*, 627–650.

Hohmann, J., Zupko, I., Redei, D., Csanyi, M., Falkay, G., Mathe, I., & Janicsak, G. (1999). Protective effects of the aerial parts of Salvia officinalis, Melissa officinalis and Lavandula angustifolia and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation. *Planta Medica*, *65*, 576–578.

Hurrell, R. F., Manju, R., & Cook, J. D. (1999). Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition*, *81*, 289–295.

Ivancheva, S., Manolova, N., Serkedjeva, J., Dimov, V., & Ivanovska, N. (1992). Polyphenols from Bulgarian medicinal plants with anti-infectious activity. *Plant Polyphenols*, *59*, 717–728.

Lawrence, B. M. (1979). Commercial production of non-citrus essential oils in North America. *Perfumer & Flavorist*, *3*, 21–33.

Lei, C., Mehta, A., Berenbaum, M., Zangerl, A. R., & Engeseth, N. (2000). Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. *Journal of Agricultural and Food Chemistry*, *48*, 4997–5000.

Leslie, A. C. (1993). Geranium macrorrhizum. *Garden*, *118*, 340–342.

Lionis, C., Faresjo, A., Skoula, M., Kapsokefalou, M., & Faresjo, T. (1998). Antioxidant effects of herbs in Crete. *Lancet*, *352*, 1987–1988.

Marvin, H. J. P., Mastebroek, H. D., Becu, D. M. S., & Janssens, R. J. J. (2000). Investigation into the prospects of five novel oilseed crops within Europe. *Outlook on Agriculture*, *29*, 47–53.

Mitich, L. W. (1995). Cinquefoils (Potentilla spp.). The five finger weeds. *Weed-Technology*, *9*, 857–861.

Ohsugi, M., Fan, W. Z., Hase, K., Xiong, Q., Tezuka, Y., Komatsu, K., Namba, T., Saitoh, T., Tazawa, K., & Kadota, S. (1999). Active-oxygen scavenging activity of traditional nourishing-tonic herbal medicines and active constituents of *Rhodiola sacra*. *Journal of Ethnopharmacology*, *67*, 111–119.

Petkov, V., Roussinov, K., Todorov, S., Lazarova, M., Yonkov, D., & Draganova, S. (1984). Pharmacological investigations on *Rhaponticum carthamoides*. *Planta Medica*, *50*, 205–209.

Pietta, P., Simonetti, P., & Mauri, P. (1998). Antioxidant activity of selected medicinal plants. *Journal of Agricultural and Food Chemistry*, *46*, 4487–4490.

Plotnikov, M. B., Aliev, O. I., Vasiljev, A. S., Maslov, M. Y., Chernyshova, G. A., Krasnov, E. A., & Zibareva, L. N. (1999). Haemorheological activity of extracts of the above-ground parts of *Lychnis chalcedonica* L. and *Rhaponticum carthamoides* (Willd.) Iljin under experimental myocardial infarction. *Rastitelnye Resursy*, *35*, 103–107.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology*, *26*, 1231–1237.

State Pharmacopeia of USSR (1989). *Moscow, Medicina*, *2*, 324–334 (in Russian).

Tang, X., & Yuan, H. M. (1997). Extraction of *Salvia sclarea* L. oil and its antioxidative activity in foods. *Science and Technology of Food Industry*, *3*, 10–13.

Yermakov, A. I., Arasimov, V. V., & Yarosh, N. P. (1987). *Methods of biochemical analysis of plants*. Leningrad: Agropromizdat (in Russian).

Zupko, I., Hohmann, J., Redei, D., Falkay, G., Janicsak, G., & Mathe, I. (2001). Antioxidant activity of leaves of *Salvia* species in enzyme-dependent and enzyme-independent systems of lipid peroxidation and their phenolic constituents. *Planta Medica*, *67*, 366–368.