Czech Medicinal Plants as Possible Sources of Antioxidants

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Abstract

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The antioxidant activity of 17 Czech medicinal plants was studied and compared with the antioxidant activity of green tea. The antioxidant activities of water and ethanol extracts of the plants tested were determined by a spectrophotometric method using the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), and further the contents of the compounds with reducing properties in water extracts were determined by flow injection analysis with amperometric detection (using a detection potential +0.7 V). Considerable antioxidant activities were found in the extracts of plants from the *Rosaceae* family (rosehips and leaves of raspberry, blackberry, and strawberry), the *Lamiaceae* family (oregano, sweet balm, thyme, dead-nettle, and mint), and flowers of linden and elder.

Keywords: antioxidant activity; plant extracts; free radicals DPPH

Many human diseases are caused or negatively affected by free radicals. The natural defense of the human organism againts free radicals is not always sufficient mainly due to the significant exposition to free radicals from external sources in the modern world. The dietary intake of antioxidants plays an important role in the protection of the human organism againts free radicals. Many clinical and epidemiological studies show a connection between the antioxidant activity of the substances present in the diet and the prevention from such diseases as cardiovascular diseases or carcinogenesis (Hughes 2000; Kris-Etherton *et al*. 2002; Lindsay & Astley 2002).

Fruits, vegetables, grains, teas, wines, and some kinds of spices are natural sources of antioxidants (Rice-Evans *et al*. 1996). The intake of these food comodities is not always satisfactory. Therefore, the studies of possible new sources of antioxidants have become important in the last few years. The new sources of the antioxidants could be used for direct consumption or for the production of food supplements which could be used for enriching foods with the aim of increasing their nutritional value. Medicinal plants used in the traditional medicine and healing are one of these sources of antioxidants. In many countries, screening studies were carried out for the comparison of antioxidant activities of medicinal plants typical for the respective country (Chanwitheesuk *et al*. 2005; Ivanova *et al*. 2005; Katalinic *et al*. 2006; Proestos *et al*. 2006; Wong *et al*. 2006). There is no study of this kind in the available literature concerning the Czech medicinal plants.

The aim of this work was to compare the Czech medicinal plants in view of their antioxidant activ-

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ity. Seventeen plants resp. parts of plants were selected with respect to: popularity, the conventional use, and the recommendation for use (they can be taken for a long time) in the traditional medicine and healing, their use for beverage preparation, as well as legislation regulating the use of these plants in food industry. The antioxidant activity of water and ethanol extracts of the dry medicinal plants was estimated using the free radical DPPH, usually used in this type of screening study. To confirm the plants antioxidant activity, the contents of compounds with reducing properties were determined by flow injection analysis (FIA) using an amperometric detector. The antioxidant activities of the plants studied were compared with the antioxidant activity of green tea.

MATERIAL AND METHODS

Material. The studied medicinal plants and their parts were as follows: blueberry fruits (*Vaccinium myrtillus* L.), chicory roots and tops (mixture 1:1 m/m; *Cichorium intybus* L.), oregano tops (*Origanum vulgare* L.), fennel fruits (*Foeniculum vulgare* Mill.), camomile flowers (*Matricaria chamomilla* L.), nettle flowers (*Lamium album* L.), strawberry leaves (*Fragaria vesca* L.), rowan fruits (*Sorbus aucuparia* L.), linden flowers (*Tilia cordata* Mill. and/or *Tilia platyphyllos* Scopoli and/or *Tilia vulgaris* Hayne), blackberry leaves (*Rubus idaeus* L.), mint tops (*Mentha piperita* L.), thyme tops (*Thymus serpyllum* L.), sweet balm tops (*Melissa officinalis* L.), raspberry leaves (*Rubus fruticosus* L.), buckthorn fruits (*Hippophae rhamnoides* L.), rosehips (*Rosa canina* L.) and elder flowers (*Sambucus nigra* L.).

Practically all the plant materials studied (elder flowers excepted) can be used for the preparation of beverages resembling to tea without any limit in accordance with the Czech food regulations (Ministry of Agriculture 1997). Elder flowers can be used for this purpose only in mixtures with other plant materials up to 30% of weight. For that reason, the medicinal plants tested (elder flowers excepted) can be considered as safe, thus no disagreement will probably arise about using them in other ways than only for the preparation of beverages.

Of every medicinal plant studied from two to four dry samples were analysed. The samples were evaluated in this phase of research from the consumers' point of view. Therefore, the localities of the growth of these plants or the conditions of their drying were not found out. One series of dry samples (first series) was purchased from a well known Czech producer and the other from ordinary Czech shops. The antioxidant activity of water extracts was established in all samples. The antioxidant activity of ethanol extracts and the contents of compounds with reducing properties in water extracts were determinated in the first series.

Green tea samples were purchased from ordinary Czech shops. Their antioxidant activities were determined in water extracts.

The dry parts of plants and green teas were kept in closed containers, in the dark, at laboratory temperature, and they were used within the recommended period for their consumption.

Extraction. For the preparation of water extracts, 50 ml of deionised water (MILLI-Q-RG, ZFMQ 050 RG, Millipore, USA) were added to 1 g of dry ground plant material (or green tea). The water temperature was 98°C, infusion time was 20 minutes. For the preparation of ethanol extracts, 50 ml of ethanol (96%) were added to 1 g of dry ground plant material at laboratory temperature. The mixture was left in a dark place. Infusion time was 24 hours. All extracts prepared were analysed shortly after the preparation.

Scavenging DPPH radicals. The method used was almost the same as that used by other authors (Gadow *et al*. 1997; Ibañez *et al*. 2003; Dorman *et al*. 2004), but was modified in details. 2 ml of methanol solution of DPPH radical (2,2-diphenyl-1-picrylhydrazyl, Sigma Aldrich, St. Louis, USA) in the concentration of 0.05 mg/ml and 1 ml of plant extract or dilute plant extract were placed in cuvettes. The decrease in absorbance at 522 nm (experimentally established wavelength) was measured using a spectrophotometer Cary 100 Bio (Varian, Palo Alto, USA) until the difference between the absorbance of the sample and the control sample remained stable (24 h). As the control sample, DPPH radical solution with 1 ml of deionised water was used. Analytical grade methanol (Penta, Chrudim, Czech Republic) was used to zero the spectrophotometer.

The difference between the absorbance of the DPPH radical solution containing the plant extract and that of the control sample was expressed as mg of L-ascorbic acid (Sigma Chemical Co, St. Louis, USA) per 1 g of dry plant material. Calibration was used in such cases, where the plant extracts

were replaced with a freshly prepared solution of ascorbic acid in deionised water (concentration from 0 to 1.6 mg/100 ml).

All determinations were performed in duplicates and the particular assesment included also the preparation of extracts.

Reduction ability. FIA equipment consisted of a non-steel pump LCP 4020.31 (ECOM, Prague, Czech Republic) and an amperometric detector HP 1049A (Hewlett Packard, Avondale, USA) equipped with a glassy-carbon electrode (operating at a potential of +0.7 V), a reference Ag/AgCl electrode and a platinum counter electrode. Data were recorded using a 1.6 DataApex chromatography system (Prague, Czech Republic). A mixture of acetonitrile (Merck, Darmstadt, Germany) and 0.2% (m/m) *o*-phosphoric acid (Lachema Neratovice, Czech Republic) (4:1, v/v) containing sodium chloride (0.005 mol/l) (Lachema Neratovice, Czech Republic) was used as the electrolyte. The injection volume was 20 µl and the flow rate of 1 ml/min was applied.

The contents of oxidable substances were expressed as mg of ascorbic acid per 1 g of dry plant material. Calibration of freshly prepared solutions of ascorbic acid in deionised water was used for this purpose (concentration from 0 to 4 mg/100 ml).

All determinations were performed in duplicates and the particular assesment included also the preparation of extracts.

Statistical analysis. The results of the antioxidant activity determination using stable synthetic free radical DPPH were statistically analysed applying the Student *t*-test (on the level of probability 0.05). The correlation coefficients between the results of the antioxidant activity determination and the contents of compounds with reduction properties contained in water extracts of the respective medicinal plants were determined applying the Excel software (Microsoft).

RESULTS AND DISCUSSION

The antioxidant activity in water extracts of all samples of the medicinal plants studied is shown in Table 1. Antioxidant activities are expressed as milligrams of ascorbic acid per 1 g of dry plant material. These data are mismatched with the

Table 1. Antioxidant activity of water extracts determined using DPPH radical

Plant material	Number of samples	Antioxidant activity (mg/g)
Strawberry leaves[a]	4	123.0 ± 32.4
Oregano tops[a]	3	116.9 ± 4.7
Blackberry leaves[a]	3	111.5 ± 15.7
Raspberry leaves[a, b]	3	90.6 ± 10.6
Sweet balm tops[a, b]	4	87.5 ± 23.7
Thyme tops[a, b, c]	3	83.4 ± 22.8
Rosehip[b, c]	3	69.7 ±11.5
Netlle flowers[b, c]	3	64.9 ± 0.8
Mint tops[b, c]	4	61.7 ± 21.3
Elder flowers[c]	3	60.2 ± 3.3
Linden flowers[c]	3	58.8 ± 6.3
Chamomile flowers[d]	3	31.8 ± 4.0
Blueberry fruits[d]	3	31.8 ± 2.7
Buckthorn fruits[d, e]	2	21.4 ± 4.6
Rowan fruits[d, e]	3	15.6 ± 8.1
Chicory roots and tops[e]	3	13.2 ± 1.1
Fennel fruits[f]	2	3.2 ± 1.4

[a, b, c, d, e, f]statistically significant differences were not found in groups of plants marked, with the same letters data was expressed as mean ± SD as milligrams of ascorbic acid per 1 g of dry plant material

contents of antioxidants, because one molecule of ascorbic acid is able to inactivate two free DPPH radicals while phenolic acids, e.g., are able to inactivate 4 to 6 free DPPH radicals per molecule (Brand-Williams *et al*. 1995). Therefore, the antioxidant contents are probably lower.

Antioxidants are secondary metabolites and their contents in plants depend on varied stress conditions of vegetation (Verpoorte *et al*. 1999). However, considerable differences were not found between the antioxidant activities of the samples of the same plant. Water extracts of strawberry leaves, oregano tops, raspberry leaves, blackberry leaves, sweet balm tops, and thyme tops had generally a high antioxidant activity. Rosehips, dead-nettle flowers, mint tops, elder flowers, and linden flowers had a medium antioxidant activity, and camomile flowers, blueberry fruits, buckthorn fruits, rowan fruits, chicory roots and tops and fruits of fennel had a low antioxidant activity.

Green tea is known as one of the richest sources of natural antioxidants (Kris-Etherton *et al*. 2002). Therefore, the antioxidant activities of the plants tested were compared with the antioxidant activity of green tea. All investigated plants had a lower antioxidant activity than the water extracts of green tea (a typical result with three samples was 313.3 ± 15.2 mg of ascorbic acid per 1 g of dry plant material) prepared in the same way as water extracts of medicinal plants. However, the plants studied contain no caffeine, which is why the plants with a lower antioxidant activity than that of green tea could be an interesting source of natural antioxidants.

Antioxidant activity depends on the manner in which the extracts are prepared (and in which antioxidant activity is determined, see next). Therefore, antioxidant activity was established in the first series, including the samples of all the plants studied, in both their water and ethanol extracts (and, further, the contents of the compounds with reducing properties in water extracts of these samples were determined, see next). The extraction of substances with the antioxidant activity

Table 2. Antioxidant activity of water and ethanol plant extracts (first series of samples) determined using DPPH radical

Plant material	Antioxidant activity (mg/g)	
	water extract	ethanol extract
Strawberry leaves	121.6 ± 14.2	33.6 ± 3.9
Oregano tops	118.2 ± 7.0	11.8 ± 0.6
Blackberry leaves	114.8 ± 2.9	30.1 ± 1.3
Thyme tops	109.4 ± 0.7	21.4 ± 0.3
Raspberry leaves	102.7 ± 1.2	17.1 ± 0.7
Sweet balm tops	100.0 ± 11.2	6.5 ± 0.5
Mint tops	72.2 ± 2.4	21.3 ± 2.3
Netlle flowers	65.8 ± 3.6	14.3 ± 0.4
Linden flowers [a]	63.0 ± 3.8	36.7 ± 1.8
Rosehip	62.7 ± 1.1	6.3 ± 1.1
Elder flowers	60.8 ± 0.1	13.5 ± 0.5
Chamomile flowers	34.8 ± 0.2	5.5 ± 0.2
Blueberry fruits	34.2 ± 0.0	14.9 ± 0.1
Rowan fruits	25.0 ± 2.5	9.4 ± 0.1
Buckthorn fruits	24.6 ± 0.5	0.9 ± 0.1
Chicory roots and tops	12.4 ± 0.4	4.0 ± 0.5
Fennel fruits	4.2 ± 0.2	1.3 ± 0.1

[a]value is the mean of four determinations, the other values are the mean of two determinations; data was expressed as mean ± SD as milligrams of ascorbic acid per 1 g of dry plant material

was markedly more efficient using hot water than using ethanol at laboratory temperature with all plants studied (Table 2).

On the basis of the contents of the compounds possessing reducing properties, the medicinal plants studied were lined up in the following order: sweet balm tops > thyme tops > oregano tops > strawberry leaves > mint tops > elder flowers > raspberry leaves > nettle flowers > blackberry leaves > camomile flowers > linden flowers > blueberry fruits > rosehips > rowan fruits > buckthorn fruits > chicory roots and tops > fennel fruits. The comparison between the antioxidant activities in water extracts and the contents of compounds with reducing properties contained in these extracts is shown in Figure 1. There is a significant linear correlation ($P < 0.01$; correlation coefficient $r = 0.602$ (resp. $r = 0.745$) where the trendline goes from the origin of coordinates (resp. does not go from the origin of coordinates) between these two parameters. Although all oxidable substances do not have antioxidant activity (the opposite, some compounds without reduction abilities can act as antioxidants, e.g. some substances that are able to bind metal ions), a close relation between the antioxidant activity and the content of compounds with reducing properties was described by other authors (Buratti *et al.* 2001; Cosio *et al.* 2006).

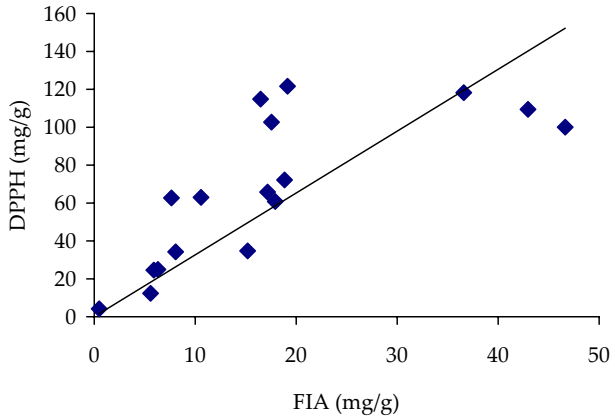

Figure 1. Relationship between antioxidant activities (determined using DPPH radicals) and the contents of compounds with reduction ability (determined by a flow injection analysis with amperometric detection) in medicinal plants water extracts ($r = 0.602$). Data was expressed in both methods as milligrams of ascorbic acid per 1 g of dry plant material

On the basis of the presented results, we can consider the studied plants of the *Lamiaceae* family (oregano, sweet balm, thyme, dead-nettle, mint) and the *Rosaceae* family (leaves of raspberry, blackberry, and strawberry) to be good sources of antioxidants. These results agree with the results published formerly. A good antioxidant activity of the *Lamiaceae* family was found by many authors (Zandi & Ahmadi 2000; Dorman *et al.* 2004; Capecka *et al.* 2005). The plants of the *Rosaceae* family were not investigated in detail from the antioxidant point of view unlike those of the *Lamiaceae* family. Katalinic *et al.* (2006) determined the antioxidant activities and phenolic contents of 70 medicinal plants. The leaves of raspberry, blackberry, and strawberry were among eleven of the most effective plants. In another study (Wang & Lin 2000), the antioxidant activities of leaves and berries of these plants were compared. The leaves were a richer source of antioxidants and had a greater content of phenolic substances than berries.

A medium antioxidant activity was found in the extracts of rosehips, elder flowers, and linden flowers in the present study. The majority of these results also agree with the results published formerly. For instance, the study by Su *et al.* (2007) established rosehips as a potential source of natural antioxidants. A significant antioxidant activity of linden flowers was established in a study by Yildirim *et al.* (2000). In this study, the authors compared the antioxidant activities of linden flowers, black tea, and sage. Water extract of linden flowers had a higher antioxidant activity than water extract of sage. But the study of Italian authors (Giamperi *et al.* 2003) detected poor antioxidant activity in the essential oil of elder flowers while their ethanol extract was inactive.

A low antioxidant activity was found in the extracts of camomile flowers, blueberry fruits, buckthorn fruits, rowan fruits, and chicory roots and tops (althought the extract of camomile flowers showed medium activity in the determination of compounds with reducing properties). The fruits tested, particulary buckthorn (Eccleston *et al.* 2002), are rich in the content of vitamin C. During drying, the content of vitamin C probably decreases as described with rosehips by Erenturk *et al.* (2005). Contrary to this, dry rosehips were classified as a plant material with medium antioxidant activity in this study. It may be due to the presence of other antioxidants in rosehips apart from sole vitamin C (Xianggun *et al.* 2000).

Conclusion

On the basis of our results, Czech medicinal plants from the *Rosaceae* family (rosehips and leaves of raspberry, blackberry and strawberry), the *Lamiaceae* family (oregano, sweet balm, thyme, dead-nettle and mint), and flowers of linden (*Tiliaceae* family) and elder (*Loniceraceae* family) appear to be good and safe sources of antioxidants. These plants could be used for direct consumption as various kinds of beverages or as extracts to increase the nutritional value of different foods and diets.

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