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ScienceDirect
Food Chemistry 105 (2007) 940–949
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity and phenolic compounds in 32 selected herbs

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Received 3 November 2006; received in revised form 7 February 2007; accepted 18 April 2007

Abstract

Total equivalent antioxidant capacities (TEAC) and phenolic contents of 32 spices extracts from 21 botanical families grown in Poland were investigated. The total antioxidant capacity was estimated by the following methods: $ABTS^{\cdot+}$ (2,2′azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)), $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl radical) and ferric reducing/antioxidant power (FRAP) expressed as TEAC. The total phenolics were measured using a Folin–Ciocalteu assay. Qualitative and quantitative analyses of major phenolics by reverse-phase high-performance liquid chromatography (RP-HPLC) were also used. Major phenolic acids identified in analyzed species were caffeic, *p*-coumaric, ferulic and neochlorogenic, while predominant flavonoids were quercetin, luteolin, apigenin, kaempferol and isorhamnetin. Myricetin was detected only in *Epilobium hirsutum*. Many investigated spices had high levels of phenolics and exhibited high antioxidant capacity. The TEAC values of the spices ranged from 1.76 to 346 μM trolox/100 g dw, from 7.34 to 2021 μM trolox/100 g dw, and 13.8 to 2133 μM trolox/100 g dw for $ABTS^{\cdot+}$, $DPPH^{\cdot}$ and FRAP, respectively. The total phenolic content, measured using a Folin–Ciocalteu assay, ranged from 0.07 to 15.2 mg of gallic acid equivalents (GAE)/100 g dw. The herbs with the highest TEAC values were *Syzygium aromaticum*, *E. hirsutum* and the species belonging to the Labiatae and Compositae family. A positive relationship between TEAC ($ABTS^{\cdot+}$ and FRAP) values and total phenolic content, measured by HPLC, was found only in family groups with many representative herbs within Labiatae and Compositae.

Keywords: Herbs; Antioxidant activity; Phenolic compounds; Radical-scavenging activity; $ABTS^{\cdot+}$; $DPPH^{\cdot}$; FRAP

1. Introduction

Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kähkönen et al., 1999). Herbs are used in many domains, including medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances, cosmetics (Djeridane et al., 2006). Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anticarcinogenic potential (Aaby, Hvattum, & Skrede, 2004; Luo, Cai, Sun, & Corke, 2004). Crude extracts of herbs and spices, and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.

The basic flavonoids structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C6–C3–C6), labelled A, B, and C (Fig. 1). Various classes of flavonoid differ in the level of oxidation and saturation of ring C, while individual compounds within a class differ in the substitution pattern of rings A and B. The differences in the structure and substitution will influence the phenoxyl radical stability and thereby the antioxidant properties of the flavonoids.

Plant species belong to several botanical families, such as Labiatae, Compositae, Umbelliferae, Asteracae, Polygonacae and Myrtacae (Table 1). Many spices have been investigated for their antioxidant properties for at least 50 years.

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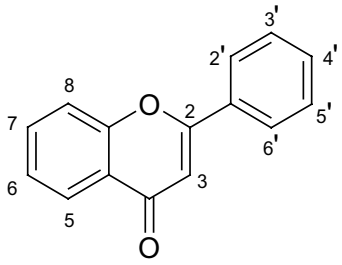
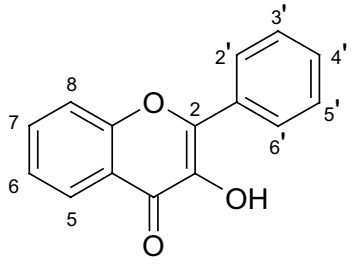
doi:10.1016/j.foodchem.2007.04.038



Apigenin (4', 5, 7 - OH)

Luteolin (3', 4', 5, 7 - OH)

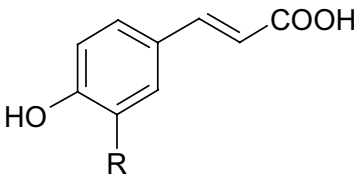


Quercetin (3', 4', 5, 7 -OH)

Myricetin (3', 4', 5', 5, 7 - OH)

Kaempferol (4', 5, 7 - OH)

Isorhamnetin (4', 5, 7 – OH; 3' – OCH_3)



R- H: *p*-coumaric acid
R- OH: caffeic acid
R- OCH_3: ferulic acid

Fig. 1. Structures of major phenolic compounds identified in the species.

The results show that rosemary, oregano, sage and others belonging to the Labiatae family, exhibit antioxidant properties. Some researchers report that other species, e.g. clove, cinnamon and coriander also exhibit antioxidant properties (Dragland, Senoo, Wake, Holte, & Blomhoff, 2003; Wang, 2003; Wu et al., 2004). The rich world of herbs, with thousands of species and varieties, demands study. Especially, phenolic composition and antioxidant activity of wild and cultivated plants need investigation. However, there are few reports about these proprieties in other plants. Antioxidant activity of herbs is generally studied with regard to total phenolic content, using traditional methods and only one test is used for radical-scavenging activities. Although extensive studies of bioactive compounds and their total content in many species have been carried out, the phenolic identification data are still insufficient and incomplete, in particular, quantitative data on phenolics in the species are still missing. Also, there are few comparisons of phenolic constituents identified in various species of different spice families. The structure–activity relationships of phenolic compounds present in spices require further investigation (Czapecka, Mareczek, & Leja, 2005; Ivanova, Gerova, Chervenkov, & Yankova, 2005).

The most commonly used antioxidant methods are $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. Both of them are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their response to antioxidants. The DPPH free radical $(DPPH)^{\bullet}$ does not require any special preparation, while the ABTS radical cation ($ABTS^{\bullet+}$) must be generated by enzymes or chemical reactions (Arnao, 2000). Another important difference is that $ABTS^{\bullet+}$ can be dissolved in aqueous and organic media, in which the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples. In contrast, DPPH can only be dissolved in organic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidants. Both radicals show similar bi-phase kinetic reactions with many antioxidants. However, the ferric reducing antioxidant power (FRAP) method is based on the reduction of a ferroin analogue, the Fe^{3+} complex of tripyridyltriazine $Fe(TPTZ)^{3+}$ to the intensely blue-coloured Fe^{2+} complex $Fe(TPTZ)^{2+}$ by antioxidants in acidic medium. However, the reducing capacity does not necessarily reflect antioxidant activity, as has been suggested by Wong, Li, Cheng, and Chen (2006) and Katalinic, Milos, and Jukic (2006).

The objectives of this study were to: (1) evaluate and compare total antioxidant capacity by three common antioxidant activity methods, presented as total equivalent antioxidant capacity (TEAC) and phenolic content of 32 common Polish species extracts; (2) identify and quantify major phenolic compounds present in the tested species by RP-HPLC; (3) determine the relationship between antioxidant activity and phenolic compounds of 32 species extracts to confirm that phenolic constituents are responsible for antioxidant activity of the plants.

2. Materials and methods

2.1. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl radical, 2,2′azinobis-(3-ethylbenzthiazoline-6-sulfonic acid), potassium persulfate, methanol, acetonitrile, β-glucosidase, β-xylosidase, β-galactosidase and β-hesperidinase, Sulfatase type H-2 were purchased from Sigma–Aldrich (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium bisulfite and formic acid were from Fluka Chemie AG (Buchs, Switzerland). Chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, quercetin, kaempferol, apigenin, luteolin, izorhamnetin and myricetin were purchased from Extrasynthese (Lyon Nord, France).

Table 1
Antioxidant capacity and total phenolic content in 32 selected herbs[a]

Family and scientific name		Parts of the herbs	Total phenolic content[b]	TEAC (μM trolox/100 g dw)		
				ABTS	DPPH	FRAP
Salvia officinalis	Labiatae	Herbal	8.25 ± 0.09	17.0 ± 0.23	41.2 ± 1.11	167 ± 1.01
Origanum vulgare	Labiatae	Herbal	0.15 ± 0.01	19.9 ± 1.00	79.6 ± 2.04	405 ± 2.22
Marrubium vulgare	Labiatae	Herbal	3.86 ± 0.05	11.8 ± 0.43	22.5 ± 2.04	138 ± 3.01
Rosmarinus officinalis	Labiatae	Herbal	1.71 ± 0.02	38.7 ± 0.11	513 ± 5.99	662 ± 4.66
Melisa officinalis	Labiatae	Herbal	13.2 ± 0.13	10.6 ± 0.09	36.1 ± 1.03	61.8 ± 0.91
Artemisia vulgaris	Compositae	Herbal	3.83 ± 0.43	7.42 ± 0.14	74.7 ± 2.01	51.7 ± 2.01
Inula helenium	Compositae	Root	3.65 ± 0.12	8.75 ± 0.56	144 ± 1.04	60.1 ± 1.11
Silybum marianum	Compositae	Seed	4.77 ± 0.09	12.3 ± 0.01	34.3 ± 2.01	65.7 ± 0.02
Taraxacum officinale	Compositae	Root	12.6 ± 0.34	1.76 ± 00.23	213 ± 4.76	15.9 ± 3.10
Tanacetum vulgare	Compositae	Leaf	1.68 ± 0.02	37.3 ± 2.09	469 ± 9.00	455 ± 5.66
Petroselinum sativum	Umbelliferae	Root	2.02 ± 0.09	11.8 ± 0.11	39.9 ± 1.34	40.9 ± 1.23
Carum carvi	Umbelliferae	Fruit	0.07 ± 0.00	13.1 ± 0.05	153 ± 2.34	75.6 ± 0.43
Levisticum officinale	Umbelliferae	Herbal	0.72 ± 0.02	18.9 ± 0.43	232 ± 5.03	123 ± 2.39
Archangelica officinalis	Umbelliferae	Leaf	0.29 ± 0.01	0.45 ± 0.01	7.34 ± 1.41	13.8 ± 1.10
Achillea millefolium	Asteraceae	Herbal	9.55 ± 0.11	11.2 ± 0.77	200 ± 3.33	191 ± 4.51
Echinacea purpurea	Asteraceae	Leaf	15.15 ± 0.13	12.3 ± 0.49	75.0 ± 3.04	94.6 ± 1.01
Acorus calamus	Araceae	Rhizome	12.45 ± 0.04	8.66 ± 0.23	79.9 ± 1.23	78.9 ± 1.56
Humulus lupulus	Cannabaceae	Cone	7.14 ± 0.16	10.8 ± 0.11	83.2 ± 2.00	50.3 ± 2.34
Herniara glebra	Caryophyllaceae	Herbal	0.00 ± 0.00	48.1 ± 0.78	50.5 ± 1.11	66.4 ± 5.91
Glycyrrhiza glabra	Fabaceae	Herbal	1.15 ± 0.03	30.8 ± 0.12	177 ± 3.06	67.3 ± 2.34
Hypericum perforatum	Hypericaceae	Herbal	0.55 ± 0.01	57.8 ± 1.23	82.3 ± 0.56	420 ± 5.89
Juglans regia	Juglandaceae	Leaf	0.24 ± 0.03	27.3 ± 0.34	119 ± 3.07	128 ± 1.32
Thymus vulgaris	Lamiaceae	Herbal	0.58 ± 0.02	35.4 ± 0.12	295 ± 5.83	693 ± 5.87
Cynamonum zeylanicum	Lauraceae	Seed	0.13 ± 0.01	140 ± 3.01	253 ± 3.56	233 ± 2.10
Trigonella foenum-graecum L	Leguminosae	Seed	7.60 ± 0.11	6.74 ± 1.01	364 ± 7.02	21.6 ± 1.00
Myristica fragrans	Myristicaceae	Fruit	8.95 ± 0.45	33.3 ± 3.04	182 ± 1.11	218 ± 3.21
Syzygium aromaticum	Myrtaceae	Fruit	8.96 ± 0.34	346 ± 5.34	884 ± 9.04	2133 ± 6.87
Epilobium hirsutum	Onagraceae	Herbal	4.03 ± 0.12	69.5 ± 1.22	2021 ± 22.1	275 ± 1.11
Polygonum aviculare	Polygonaceae	Herbal	11.2 ± 0.23	19.2 ± 0.99	141 ± 2.01	161 ± 4.99
Valeriana officinalis	Valerianaceae	Herbal	11.1 ± 0.13	10.7 ± 1.03	25.8 ± 0.11	59.3 ± 2.13
Chelidonium majus	Papaveraceae	Herbal	2.09 ± 0.02	9.56 ± 1.05	300 ± 3.34	62.2 ± 4.33
Curcuma longa	Zingiberaceae	Rhizome	1.72 ± 0.12	19.5 ± 0.45	100 ± 2.56	62.6 ± 1.01

[a] All values are the means of three measurements.
[b] Total phenolic content expressed as mg of GAE/100 g of dry weight (dw).

2.2. Plant materials

All plant materials (0.5 kg each sample) were collected in 2005 by Polish Pharmaceutical Enterprises "JURBO-AGRO" from Grabowo Wielkie. Fresh plant samples were cleaned, freeze-dried and ground into a fine powder by laboratory mill.

2.3. Phenolics analysis

Dried plants (50 g) were crushed using a laboratory mill. Ground dry plant material (500 mg) was weighed into a test tube and 2 ml of a mixture of enzymes (5 mg of each enzyme: β-glucosidase, β-xylosidase, β-galactosidase, and β-hesperidinase) and 0.5 ml Sulfatase type H-2 diluted in citrate buffer at pH 5.5 were added. Moreover, SO_2 (from $NaHSO_3$), in order to prevent oxidative losses of phenolics, was added. The tested sample with enzyme were hydrolyzed in a water bath for 1 h at 37 °C. Then, samples were chilled to 20 °C and kept in this condition for 24 h. Then 2 ml of methanol were added to each vial and sonificated for 10 min by shaking occasionally (BAS-10, Poland). Then, samples were centrifuged (5 min, 19000*g*; MPW-250, Poland) and the clear supernatant was injected into the HPLC equipment.

2.4. Identification and quantification of phenolic compounds

Twenty-microliter samples of each supernatant of spices were analyzed using an HPLC system equipped with an L-7100 pump (Merck Hitachi) and an L-7455 photodiode array UV–VIS detector (Merck Hitachi). The samples were injected using an L-7200 autosampler (Merck Hitachi). The polyphenols were separated using a LiChroCART® 125-3 Purospher® RP-18 (5 μm) MerckLabs column heated at 30 °C (L-7350 Merck Hitachi).

The mobile phase was composed of solvent A (4.5% formic acid) and solvent B (80% of acetonitrile and 20% of solvent A). The programme began with isocratic elution with 95% A (0–1 min); then a linear gradient was used until 16 min, lowering A to 20%; from 17 min to 24 min A decreased to 0%. The flow rate was 1 ml min^{-1}, and the runs were integrated at 280 and 320, 360 nm for hydroxycinnamic acid and flavonoid derivatives, respectively.

Scanning was performed from 200 to 600 nm. Phenolic compounds were identified by comparing retention times and UV–VIS spectra with those of pure standards to indicate the preparations of standards and the range of calibration curves. The repeatability of the quantitative analysis was ±4%. The analyses were replicated ($n = 3$), and the contents given as mean values, plus or minus the standard deviation. The results were expressed as milligrammes of each compound per 100 g of dry weight (dw) spices.

2.5. Preparation of plant extracts for antioxidant property analysis and total polyphenol content

Ground dry plant materials (1 g) were weighed into a test tube. A total of 10 ml of 80% aqueous methanol was added, and the suspension was stirred slightly. Tubes were sonicated twice for 15 min and one left at room temperature (~20 °C) for 24 h. The extract was centrifuged for 10 min (10 min, 1500*g*), and supernatants were collected at 4 °C prior to use within 24 h.

2.6. Estimation of total polyphenol content

Total polyphenol content was measured using Folin–Ciocalteu colorimetric method described previously by Gao, Ohlander, Jeppsson, Björk, and Trajkovski (2000). Plant extracts (100 μl) were mixed with 0.2 ml of Folin–Ciocalteu reagent and 2 ml of H_2O, and incubated at room temperature for 3 min. Following the addition of 1 ml of 20% sodium carbonate to the mixture, total polyphenols were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue colour was measured at 765 nm with a Shimadzu UV–VIS spectrophotometer. Quantification was done with respect to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), milligrammes per 100 g of dry weight (dw). All determinations were performed in triplicate ($n = 3$).

2.7. Ferric reducing/antioxidant power (FRAP) assay

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma FRAP assay by Benzie and Strain (1996) as a measure of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 μM, pH 3.6), a solution of 10 μM TPTZ in 40 μM HCl, and 20 μM $FeCl_3$ at 10:1:1 (v/v/v). The reagent (300 μl) and sample solutions (10 μl) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of trolox. All solutions were used on the day of preparation. The results were corrected for dilution (e.g. to 1000 ml) and expressed in μM trolox per 100 g dry weight (dw). All determinations were performed in triplicates.

2.8. Free radical-scavenging ability by the use of a stable DPPH radical

The DPPH radical-scavenging activity was determined using the method proposed by Yen and Chen (1995). DPPH (100 μM) was dissolved in pure ethanol (96%). The radical stock solution was prepared fresh daily. The DPPH solution (1 ml) was added to 1 ml of polyphenol extracts with 3 ml of ethanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 10 min. The results were corrected for dilution and expressed in μM trolox per 100 g dry weight (dw). All determinations were performed in triplicate.

2.9. Free radical-scavenging ability by the use of a stable ABTS radical cation

The free radical-scavenging activity was determined by ABTS radical cation decolorization assay described by Re et al. (1999). ABTS was dissolved in water to a 7 μM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 μM potassium persulfate (final concentration) and kept in the dark at room temperature for 12–16 h before use. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of infusion, the samples containing the $ABTS^{\cdot+}$ solution were diluted with redistilled water to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30 °C. A reagent blank reading was taken (A_0). After addition of 3.0 ml of diluted $ABTS^{\cdot+}$ solution ($A_{734\,nm} = 0.700 \pm 0.02$) to 30 μl of polyphenolic extracts, the absorbance reading was exactly 6 min after initial mixing (A_t). The results were corrected for dilution and expressed in μM trolox per 100 g dry weight (dw). All determinations were performed in triplicate.

3. Results and discussion

The amount of total phenolics, measured by Folin–Ciocalteu method, varied widely in herb materials and ranged from 0.00 to 15.2 mg GAE/100 g dry weight (dw) (Table 1). The highest level of phenolics was found in *Echinacea purpurea*, while the lowest was in *Carum carvi*.

Melissa officinalis (13.2 mg GAE/100 g dw), *Acorus calamus* and *Taraxacum officinale* (12.6 mg GAE/100 g dw) also had very high levels of phenolics. Other herbs with high levels of phenolics were *Polygonum aviculare* (11.2 mg GAE/100 g dw), and *Valeriana officinalis* (11.1 mg GAE/100 g dw).

Chelidonium majus (2.09 mg GAE/100 g dw), *Petroselinum sativum* (2.02 mg GAE/100 g dw), *Curcuma longa*

(1.72 mg GAE/100 g dw), *Glycyrrhiza glabra* (1.15 mg GAE/100 g dw), *Tanacetum vulgare* (1.68 mg GAE/100 g dw) and *Rosmarinus officinalis* (1.71 mg GAE/100 g dw) had relatively low levels of phenolics, whereas in *Levisticum officinale* (0.72 mg GAE/100 g dw), *Hypericum perforatum* (0.55 mg GAE/100 g dw), *Archangelica officinalis* (0.29 mg GAE/100 g dw), *Juglans regia* (0.24 mg GAE/100 g dw), *Origanum vulgare* and *Cynamonum zeylanicum* (0.13 mg GAE/100 g dw) phenolics were quite low.

Among 19 families tested in this study, Labiatae (six tested spices), Compositae (five tested spices), Umbelliferae (four tested spices), and Asteracae (two tested spices), only Asteracae and Compositae exhibited high levels of polyphenols (12.4 and 5.30 mg GAE/100 g dw, respectively). All Umbelliferae plants were very low in phenolic content (Table 1). Umbelliferae include many common spice plants, e.g. cumin, coriander and various kinds of peppers. Some researchers have previously reported that spices of this family exhibited a strong antioxidant effect (Shan, Cai, Sun, & Corke, 2005). However, they did not compare spices from other families.

There have been extensive studies on antioxidant activity of many spices in the Labiatae family. The most common spices in this family are rosemary, oregano, sage, basil, mint and thyme. The results obtained in the present study showed that the spices were relatively high but not very high in polyphenols. Total phenolic contents of the six spices decreased in the following order: balm > sage > horehound > rosemary > thymus > oregano. Significant differences between the results were likely due to genotopic and environmental differences (namely, climate, location, temperature, fertility, diseases and pest exposure) within species, choice of parts tested, time of taking samples and determination methods (Kim & Lee, 2004; Shan et al., 2005).

Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. Phenolic acids are a major class of phenolic compounds, widely occurring in the plant kingdom especially in fruits and vegetables. Selected phenolics in several species, separated and identified by the RP-HPLC method, are shown in Table 2. Considerable variation was found in phenolic compounds of different species. Because of the diversity and complexity of the natural mixtures of phenolic compounds in hundreds of herb extracts, it is rather difficult to characterize every compound and elucidate its structure, but it is not difficult to identify major groups and important aglycones of phenolic compounds. Many medical herbs and spices have been studied and to some extent their phenolic chemistry is known (Cai et al., 2004). The contents of phenolic compounds, measured by HPLC after enzymatic hydrolysis, were different from those values measured using the Folin–Ciocalteu method. The amount of polyphenols was also dependent on the extraction method. The samples for HPLC were subject to enzymatic hydrolysis which, in contrast to methanol extraction, resulted in specific disruption of linkages and deglycosylation of phenolic compounds. Many authors report that aglycones exhibit higher antioxidant activity than do glycosides (Kim & Lee, 2004; Rice-Evans, Miller, & Paganga, 1997).

It is obvious that the total phenolic content measured by the Folin–Ciocalteu procedure does not give a full picture of the quality or quantity of the phenolic constituents in the extracts (Katsube et al., 2004; Wu et al., 2004).

The phenolic acids and flavonoids such as flavones and flavonols were determined by the HPLC method. The highest concentrations of phenolic acids were found in leaves of *T. vulgare* (1700 mg/100 g dw), *M. officinalis* (969 mg/100 g dw), *L. officinale* (630 mg/100 g dw) and *Thymus vulgaris* (607 mg/100 g dw). The main hydroxycynnamic acids in these plants were caffeic acid, and neochlorogenic acid. However, *p*-coumaric and ferulic acid occurred in small quantities and not in all investigated plants. In herbs, *C. zeylanicum*, *Trigonella foenum-graecum*, *Myristica fragrans*, *Syzygium aromaticum* and *C. longa*, no caffeic acid was detected. Other plants contained caffeic acid (from 2.31 to 858 mg/100 g dw), neochlorogenic acid (from 25.3 to 335 mg/100 g dw), *p*-coumaric acid (from 1.74 to 125 mg/100 g dw) and ferulic acid (from 0.3 to 471 mg/100 g dw). The smallest amounts of phenolic acids were found in *C. zeylanicum* and *M. fragrans* (Table 2). Caffeic acid was found to have high activity, comparable to that of quercetin. Ferulic acid was shown to inhibit the photo-peroxidation of linoleic acid at high concentrations (Wang, 2003). Rosmarinic acid is the main antioxidant constituent in the Labiatae family also containing hydrocaffeic and caffeic acids (Kim & Lee, 2004). Enzymatic hydrolysis resulted in decomposition of rosmarinic acid to hydrocaffeic acid and caffeic acid. For confirmation, a comparative chromatographic analysis was performed for the samples after enzymatic hydrolysis and methanol extraction. Trace amounts of flavonoids and phenolic acids, among others, rosmarininc acid, were identified in the chromatograms, but no hydrocaffeic or caffeic acid was found. Caffeic acid possesses high antioxidant activity but lower than that of rosmarinic acid (Kim & Lee, 2004).

In addition to phenolic acids 32 plants, contain the following flavonoid aglycones: quercetin, kaempferol, luteolin, apigenin and isorhamnetin. Predominant flavonoids in analyzed plants were quercetin, luteolin and apigenin. Nine of the 32 investigated plants were higher in flavonoid content than phenolic acids. The HPLC analysis showed that no flavonoids were present in 13 of the plants under investigation (Fig. 2 and Table 2). However, quercetin (155 mg/100 g dw) was found in one spice, i.e. *S. aromaticum*. On the other hand, flavonoids were found in *Artemisia vulgaris*, *V. officinalis*, *P. aviculare*, in agreement with the data reported by Cai et al. (2004).

Our results are in agreement with those reported by Shan et al. (2005) in that the most common flavonoids are mainly distributed in the Labiatae, Compositae and Umbelliferae. The five analyzed Compositae spice extracts contained more flavonoids (mean = 957 mg/100 g) than the six Labiatae spices (mean = 710 mg/100 g) and

Table 2
Quantitative analysis of major phenolic compounds identified in different 32 selected herbs (mg/100 g dw)

Scientific name of herbs	Phenolic acid				Flavonoids					
	CA	NCA	*p*-CA	FA	QUE	KAEM	LUT	API	I-RHA	MYR
Salvia officinalis	296 ± 0.00	53.1 ± 0.11	10.3 ± 0.17	13.5 ± 0.11	178 ± 1.11		49.6 ± 0.03	22.1 ± 0.03		
Origanum vulgare	649 ± 0.07	96.3 ± 0.21								
Marrubium vulgare	166 ± 0.12		31.6 ± 0.23	15.7 ± 0.87						
Rosmarinus officinalis	406 ± 0.34			36.2 ± 0.20			616 ± 0.43	43.8 ± 0.44		
Melisa officinalis	858 ± 0.01			111 ± 0.15						
Artemisia vulgaris	304 ± 0.11	54.4 ± 0.02		13.8 ± 0.09						
Inula helenium	183 ± 0.14	63.0 ± 0.00		24.5 ± 0.05						
Silybum marianum	92.8 ± 0.00		53.6 ± 0.01	20.7 ± 0.23	2.39 ± 0.00					
Taraxacum officinale	72.6 ± 0.09		2.10 ± 0.09							
Tanacetum vulgare	894 ± 0.04	335 ± 0.05		471 ± 1.15			848 ± 0.11	165 ± 1.00		
Petroselinum sativum	14.4 ± 0.09		11.2 ± 0.05	18.6 ± 0.03				81.1 ± 0.43		
Carum carvi	332 ± 0.15	96.8 ± 0.04		38.3 ± 0.35					33.8 ± 0.00	
Levisticum officinale	390 ± 0.03	164 ± 0.11		76.2 ± 0.45	923 ± 0.65					
Archangelica officinalis	85.3 ± 0.08	25.3 ± 0.14		25.6 ± 0.11	48.6 ± 0.17		96.8 ± 0.43	6.91 ± 0.32	9.85 ± 0.11	
Achillea millefolium	429 ± 0.24	118 ± 0.00		35.0 ± 0.02			103 ± 0.19	84.3 ± 0.17		
Echinacea purpurea	620 ± 0.13	115 ± 0.10	19.5 ± 0.02	17.9 ± 0.03	12.3 ± 0.02					
Acorus calamus	2.31 ± 0.04		4.31 ± 0.23	0.30 ± 0.05	5.00 ± 0.04	1.57 ± 0.00				
Humulus lupulus	38.1 ± 0.08		22.8 ± 0.03	14.3 ± 0.01	47.2 ± 0.04	45.3 ± 0.11			60.8 ± 0.14	
Herniara glebra	78.1 ± 0.07		23.3 ± 0.11	36.9 ± 0.00	228 ± 0.18				162 ± 0.02	
Glycyrrhiza glabra	15.3 ± 0.00		11.9 ± 0.18	19.7 ± 0.14				85.8 ± 0.01		
Hypericum perforatum	229 ± 0.11		32.3 ± 0.16	9.38 ± 0.05	49.7 ± 0.20	5.89 ± 0.03				
Juglans regia	148 ± 0.18	94.7 ± 0.11	125 ± 0.01	35.2 ± 0.02	460 ± 1.01	88.0 ± 0.01				
Thymus vulgaris	517 ± 0.54			90.5 ± 0.11						
Cynamonum zeylanicum			10.3 ± 0.03							
Trigonella foenum-graecum L		53.3 ± 0.23	29.4 ± 0.21		2.95 ± 1.23		512 ± 1.02	731 ± 0.23		
Myristica fragrans				4.91 ± 0.00						
Syzygium aromaticum					155 ± 0.11					
Epilobium hirsutum	23.1 ± 0.03		38.3 ± 0.09	10.9 ± 0.29	214 ± 0.03					191 ± 0.24
Polygonum aviculare	21.5 ± 0.05		14.8 ± 0.03							
Valeriana officinalis	216 ± 0.16		6.20 ± 0.00	18.2 ± 0.34						
Chelidonium majus	186 ± 0.02	167 ± 0.06	71.7 ± 0.11		759 ± 2.01	11.65 ± 0.03		20.0 ± 0.27		
Curcuma longa			5.96 ± 0.10	17.6 ± 0.04						

CA – caffeic acid; NCA – neochlorogenic acid; *p*-CA – *p*-coumaric acid; FA – ferulic acid; QUE – quercetin; KAEM – kaempferol; LUT – luteolin; API – apigenin; IZORHA – isorhamnetin; MYR – myricetin.

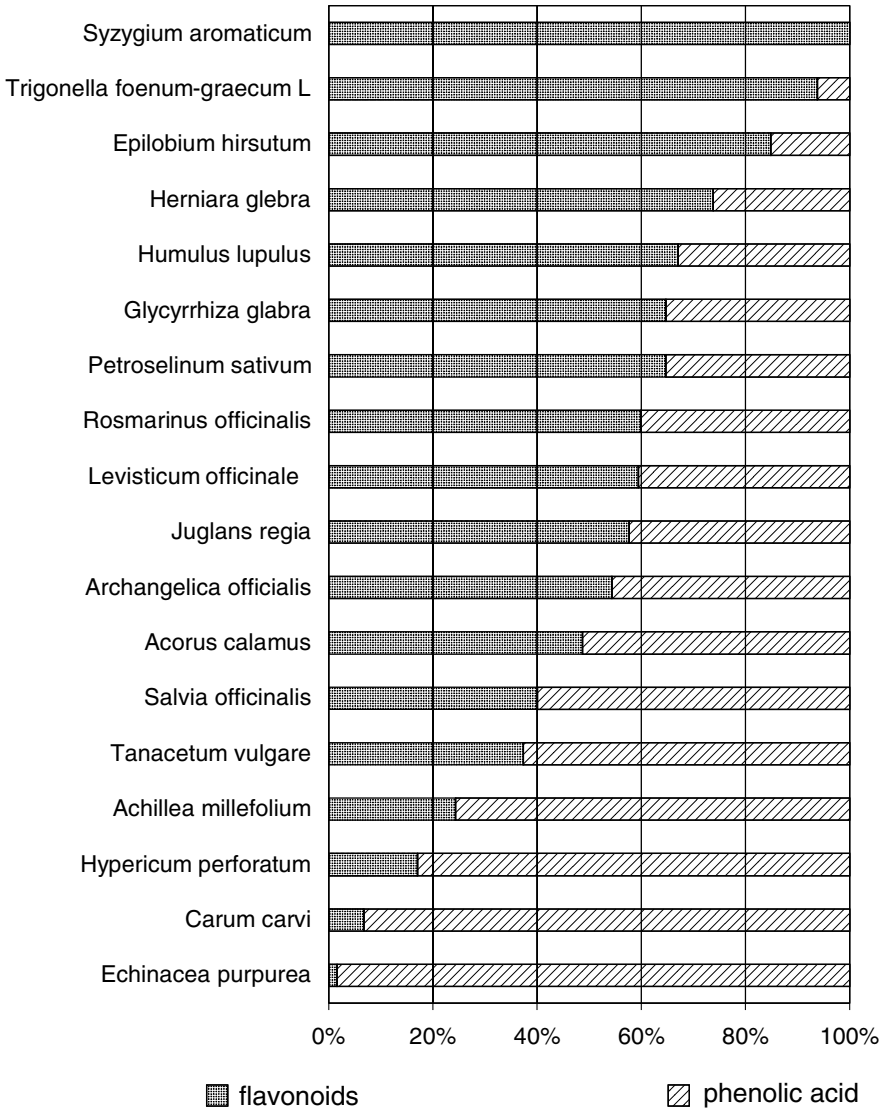


Fig. 2. Proportional relation (%) of flavonoids content to phenolic acids in chosen herbs.

Umbelliferae (mean = 620 mg/100 g). Thyme, rosemary and salvia are known to have high antioxidant capacities. Some methylated flavones and essential oil were isolated from those species. Several phenolic compounds of rosemary, oregano and sage, determined in this study, were similar in content and concentration to those in previous reports. A number of studies have demonstrated that essential oils (e.g. thymol, thyme, rosmanol) were major components that showed high antioxidant and antimicrobial activity (Shan et al., 2005; Wang, 2003).

DPPH radical and ABTS radical cation assays, expressed as TEAC value, were used for evaluation of free radical-scavenging properties of 32 Polish herbs. The results of investigation are shown in Table 1. A total of 32 plant species evaluated as their TEAC values indicated extremely large variation in antioxidant activity. Total antioxidant activity, measured by the $ABTS^{\cdot+}$ method, ranged from 0.45 to 69.6 μM trolox equivalents per 100 g dry weight (μM trolox/100 g dw), and the average TEAC value was 33.2 μM trolox/100 g dw; e.g. *E. hirsutum* from Onagraceae exhibited the highest antioxidant activity (69.6 μM trolox/100 g dw), followed by *H. perforatum* (57.8 μM trolox/100 g dw). It was found that 10 of the 32 species contained more than 25 μM trolox/100 g dw, and 2 species were below 5 μM trolox/100 g dw, while the majority had medium amounts of antioxidants, i.e. 20 species between 5 and 25 μM trolox/100 g dw and 7 species between 25 and 50 μM trolox/100 g dw.

The relatively stable organic radical, DPPH, has been widely used in the determination of antioxidant activity of single compounds, as well as of different plant extracts (Katalinic et al., 2006). Antioxidant activity measured by $DPPH^{\cdot}$ showed the same relationships as did $ABTS^{\cdot+}$ method, but TEAC values were higher. Total antioxidant activity, measured by the DPPH method, ranged from 7.34 to 2021 μM trolox equivalents per 100 g dry weight (μM TEAC/100 g dw), and the average TEAC value was 259 μM TEAC/100 g dw. *E. hirsutum* antioxidant activity,

measured by this method, gave the highest antioxidant concentration (2020.51 μM TEAC/100 g dw). Other species had antioxidant values between 100 and 500 μM TEAC/100 g dw, while only in 14 species were the values lower than 100 μM TEAC/100 g dw, measured by DPPH method.

The species *E. hirsutum* showed the highest antioxidant values in both methods of analysis while total phenolics was not high with the Folin–Ciocalteu method (4.03 mg GAE/100 g dw) and phenolic acids, flavones and flavonols with the HPLC method. This plant was additionally analyzed by acidothiolysis HPLC methods (Oszmiański & Wojdyło, 2005). Catechins and procyanidins were found in *E. hirsutum* at a concentration of 102 mg/100 g dw (data not shown). This result may explain the high antioxidant activity of this species. It is known that procyanidins have the strongest radical-scavenging power among all natural phenolic compounds (Shan et al., 2005). The procyanidins were found also in another species, i.e. *J. regia*, but at smaller concentrations (15.9 mg/100 g dw).

The *A. officinalis*, *A. vulgaris*, which had a low content of total phenolics measured by Folin–Ciocalteu, and low content of phenolic acids and flavonoids, estimated by HPLC method (total 298 mg/100 g dw), also had a very low TEAC value (Table 1). These results are in agreement with the data reported by Cai et al. (2004).

The species *E. purpurea*, with the highest content of total phenolics and high content of phenolic acids (especially caffeic acid derivatives), showed low antioxidant activity, measured by $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. Similar results were obtained by Pietta, Simonetti, and Mauri (1998).

Extensive studies have been carried out on antioxidant activity of many species of the Labiatae family (Lamaison & Petitjeanfreytet, 1996; Shan et al., 2005; Zheng & Wang, 2001). They demonstrated that this family species had a very strong antioxidant capacity. Some of them found that rosemary had the strongest antioxidant effect, but others found this with sage or oregano and basil. Our comparative study has shown that, among 32 species under investigation, Labiatae had on average antioxidant activity. Low antioxidant activity of Labiate species was likely due to unsuitable drying methods used by the manufacturer.

Table 1 shows great differences in total antioxidant capacity measured by the FRAP method between the species. The FRAP value was found within the range 13.8–693 (mean 229) μM trolox/100 g dw. There are many methods that differ in terms of their assay principles and experimental conditions and particular antioxidants have varying contributions to total antioxidant potential (Cao & Prior, 1998). In this study, we used the FRAP assay because it is quick and simple to perform to measure the antioxidant capacity of pure compounds and not only fruits, wines, and animal tissues. According to their reducing ability/antioxidant power, the 32 species infusions can be divided in five groups: (a) very low FRAP (<10 μM/100 g), $n = 0$; (b) low FRAP (10–50 μM/100 g), $n = 4$; (c) good FRAP (50–100 μM/100 g), $n = 10$; (d) high FRAP (100–500 μM/100 g), $n = 13$; very high FRAP (>500 μM/100 g), $n = 2$. The strongest antioxidant properties, measured by FRAP assay, were in two species of Labiatae herb (*T. vulgaris* and *R. officinalis*). All plants from this family exhibited higher capacity in reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) than to scavenging free radicals. Katalinic et al. (2006) showed that *Melissae folium*, out of 70 tested medicinal plants, exhibited the highest antioxidant activity measured by the FRAP method. In our research, we did not find such a high activity of *M. officinalis*. Most of the herbs reduced ferric ion (Fe^{3+}) to 100–500 μM trolox/100 g. In contrast, the weakest abilities to reduce ferric ion were exhibited by *T. foenum*, *T. officinale*, *P. sativum* and *A. officinalis*, as in previous $DPPH^{+}$ and $ABTS^{\bullet+}$ methods. It was interesting that, among 32 plants analyzed, no herbs were able to reduce ferric ion below 10 μM trolox/100 g.

E. hirsutum, which exhibited the highest scavenging of $DPPH^{+}$ and $ABTS^{\bullet+}$ did not show ferric ion reduction ability.

In general, antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups. The essential requirement for effective radical scavenging is the 3′,4′-orthodihydroxy configuration in ring B and 4-carbonyl group in ring C. The presence of 3-OH group or 3- and 5-OH groups, giving a catechol-like structure in ring C, is also beneficial for the antioxidant activity of flavonoids. The presence of the C2–C3 double bond configured with a 4-keto arrangement is known to be responsible for electron delocalization from ring B and it increases the radical-scavenging activity. In the absence of the *o*-dihydroxy structure in ring B, a catechol structure in ring A can compensate for flavonoid antioxidant activity.

The relationship between the chemical structure of flavonoids and their radical-scavenging activities was analyzed by Bors, Heller, Michael, and Saran (1990). Quercetin has a catechol structure in ring B, as well as a 2,3-double bond in conjunction with a 4-carbonyl group in ring C, allowing for delocalization of the phenoxyl radical electron to the flavonoid nucleus. The combined presence of a 3-hydroxy group with a 2,3-double bond additionally increases the resonance stabilization for electron delocalization; hence it has a higher antioxidant value. Quercetin and luteolin have identical numbers of hydroxyl groups, with 3′,4′- and 5,7-dihydroxyl groups, in rings B and A, respectively. Flavonols (quercetin, myricetin, kaempferol and isorhamnetin) have a hydroxyl group at position 3. Kim and Lee (2004), which suggests a structurally important role of the 3-OH group of the chroman ring responsible for enhancement of antioxidant activity. In our research, the plants with high contents of quercetin, kaempferol and luteolin had high antioxidant activity (namely, *S. aromaticum*, *T. foenum*, *Herniara glebra*, *R. officinalis*). Among the flavonoids identified in our study, only myricetin had a galloyl structure in ring B and appeared to be a better antioxidant than quercetin, with a catechol moiety (Kim & Lee, 2004). Myricetin was

the principal compound, close to quercetin, a component in *E. hirsutum*, which accounted for high activity by $DPPH^{+}$ (Table 1).

Antioxidant activity of *E. purpurea*, *T. vulgare*, *Achillea millefolium*, *Hypericum performatum* and *O. vulgare* is a result of phenolic acid, especially caffeic and *p*-coumaric, acid content. The 3,4-position of dihydroxylation on the phenolic ring in caffeic acid showed increased antioxidant activity as compared to *p*-coumaric acid (Kim & Lee, 2004). Caffeic acid is expected to have higher antioxidant activity because of additional conjugation in the propenoic side chain, which might facilitate the electron delocalization, by resonance, between the aromatic ring and propenoic group.

Some authors (Cai et al., 2004; Djeridane et al., 2006; Katalinic et al., 2006; Katsube et al., 2004) have demonstrated a linear correlation between the content of total phenolic compounds and their antioxidant capacity, while others (Czapecka et al., 2005; Wong et al., 2006) show poor linear correlation or report total antioxidant activity and phenolic content with no comment. The results obtained in our study show good correlation within one family. Similar results were reported by Wu et al. (2004) who found no correlation between fruit, vegetable, nut and grain foods, but only within one group.

Total phenolic compounds, determined using HPLC and antioxidant activity in Labiatae and Compositae, showed a good correlation. The correlations with Labiatae were: $R = 0.9263$ between $ABTS^{\bullet+}$ and total phenolics, $R = 0.8352$ between $DPPH^{\bullet}$ and total phenolics and $R = 0.9100$ between FRAP and total phenolics. The correlation coefficients for the species of the Compositae family were: 0.9620, 0.6709 and 0.9193, respectively. In addition, a significant linear relationship was found between the antioxidant activity, especially with $ABTS^{\bullet+}$ and FRAP, while phenolic compounds were major contributors to antioxidant activity. The results prove the importance of phenolic compounds in the antioxidant behaviour of spice extracts and also that they contribute significantly to the total antioxidant capacity.

Our results showed that Polish species were rich in phenolic constituents and demonstrated good antioxidant activity measured by different methods. These plants, rich in flavonoids and phenolic acids could be a good source of natural antioxidants. Therefore, qualitative and quantitative analysis of major individual phenolics in the spices could be helpful for explaining the relationships between total antioxidant capacity and total phenolic contents in the species. A positive and significant correlation existed between antioxidant activity and total phenolics, measured by HPLC analysis in some selected family herbs, revealing that phenolic compounds were the dominant antioxidant components. Through our systematically comparative study of 32 selected Polish herbs, some herbs, especially belonging to the Labiate family, were excellent free-radical-scavengers and a potent natural phenolic antioxidant for commercial exploration.

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