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Investigating the antioxidant potential of Turkish herbs and spices

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RESEARCH ARTICLE

Abstract

Herbs and spices have been used for many purposes including medicine, flavourings, and preservatives, etc. Constituents of herbs and spices can function as natural antioxidants and thus improve human nutrition and health. The main objective of this study was to evaluate the total phenolic and flavonoid contents of 35 different Turkish herbs and spices and to determine their antioxidant activity. Total phenolics, flavonoids, and total antioxidant capacities were analysed by four different methods: 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) assay. The correlation coefficients (R^2) between spectrophotometric assays were calculated. Wide variation in total phenolics (TP; 0.36-104 mg GAE/g), flavonoids (TF; 0.44-53.7 mg CE/g) and antioxidant capacity (TAC; 0.88-1007 mg trolox equivalent/g) was observed. Clove (*Eugenia caryophyllata*), yarrow (*Achillea millefolium*) and rosemary (*Rosmarinus officinalis*) showed the highest TP, TF and TAC values, respectively, whereas mahaleb (*Prunus mahaleb*) showed the lowest TP and TAC. The relationships between TP, TF and TAC generally indicated a weak correlation, ranging from R^2=0.163 (between ABTS and CUPRAC) to R^2=0.760 (between FRAP and CUPRAC). This study provides direct comparative data on TP, TF and TAC of the 35 commonly consumed herbs and spices in Turkey.

Keywords: antioxidants, flavonoids, phenolics, herbs, spices

1. Introduction

Herbs and spices have been used not only as food flavourings and preservatives, but also as traditional medicines for thousands of years (Shan *et al.*, 2005; Yi and Wetzstein, 2011). Since prehistoric times, they have been the basis for nearly all-medicinal therapy until synthetic drugs were developed in the 19th century. Today, herbs are still found in 40% of prescription drugs. In addition, herbs and spices are used for many other purposes including beverages such as tea, dyeing, repellents, fragrances, cosmetics, charms, smoking and industrial uses (Djeridane *et al.*, 2006; Dragland *et al.*, 2003; Zheng and Wang, 2001).

Antioxidants are compounds that can inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Otles and Selek, 2012). There are two basic categories of non-enzymatic antioxidants, namely, natural and synthetic antioxidants (Zheng and Wang, 2001). In the past, synthetic antioxidants have been used widely to prevent oxidation in food products. However, during recent years epidemiological studies have shown the possible health risks related to the consumption of synthetic antioxidants and strict regulations now control their use in foods. Therefore, attention has been directed towards the development/isolation of natural antioxidants from botanical sources, especially edible plants (Hinneburg *et al.*, 2006; Hossain *et al.*, 2008; Konczak *et al.*, 2010; Wangensteen *et al.*, 2004). In this sense, herbs and spices are one of the most important targets to search for natural antioxidants from the safety point of view (Yanishlieva *et al.*, 2006).

Research has shown that many herbs and spices are an excellent source of phenolic compounds that have been reported to show good antioxidant activity (Hinneburg *et* *al.*, 2006; Wojdylo *et al.*, 2007; Zheng and Wang, 2001). In fact, herbs are known to contain large amounts of phenolic antioxidants such as phenolic acids and flavonoids other than well-known vitamin C, vitamin E, and carotenoids (Javanmardia *et al.*, 2003; Pietta *et al.*, 1998; Rodriguez Vaquero *et al.*, 2010).

Turkey is leading producer and exporter of various herbs and spices (Esiyok *et al.*, 2004) and yet information on the total phenolics, flavonoids and antioxidant capacity of traditional Turkish herbs and spices is scarce. So far, no large-scale survey on the total phenolics, flavonoids and antioxidant capacity of these herbs and spices has been made. Therefore, the main objective of this study was to evaluate the total phenolic and flavonoid contents of 35 different Turkish herbs and spices and to determine their antioxidant activity.

2. Materials and methods

Plant materials

The 35 different dried and packaged herb and spice samples (Table 1) were collected at the beginning of 2011 from a local market in Istanbul, Turkey. For each sample, three repetitions were carried out. All samples were ground to a fine powder using a laboratory mill (Moulinex A505; Moulinex, Ecully, France).

Chemicals

For extract preparation and determination of total phenolic, flavonoid and antioxidant activity, gallic acid (≥98%), (+)-catechin (≥98%), ethanol (≥99.8%), Folin-Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and neocuproine were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); methanol (≥99.9%), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), sodium acetate trihydrate ($CH_3COONa.3H_2O$), potassium persulfate ($K_2S_2O_8$), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), copper (II) chloride ($CuCl_2$) and ammonium acetate (NH_4Ac) from Merck KGaA (Darmstadt, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and aluminum chloride ($AlCl_3$) from Fluka Chemie (Buchs, Switzerland); ferric chloride ($FeCl_3$) from Lachema (Czech Republic) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) from Applichem GmbH (Darmstadt, Germany). Water used for all analysis was distilled and purified with a water purification system (TKA GenPure; TKA, Niederelbert, Germany).

Extract preparation

Prior to analyses, a detailed literature research concerning the best extraction solvent was carried out and trials on several herbs and spices were performed. As a result 80% aqueous-methanol was selected as the best solvent

Table 1. Total phenolic (TP) and total flavonoid (TF) contents of 35 selected herbs and spices[1].

Common name	Scientific name	TP (mg GAE/g)	TF (mg CE/g)
Allspice	*Pimenta dioica*	40.1±1.26	7.52±0.79
Basil	*Ocimum basilicum*	13.8±1.09	12.4±2.36
Bay leaf	*Laurus nobilis*	28.3±2.89	13.9±1.25
Black pepper	*Piper nigrum*	4.26±0.43	7.80±1.27
Cardamom	*Elettaria cardamomum*	2.85±0.23	1.83±0.40
Chamomile	*Matricaria recutita*	11.8±1.13	7.03±0.66
Cinnamon	*Cinnamomum zeylanicum*	50.0±2.25	30.6±0.72
Clove	*Eugenia caryophyllata*	104±3.29	25.3±0.94
Coriander	*Coriandrum sativum*	1.69±0.10	1.12±0.21
Cumin	*Cuminum cyminum*	3.89±0.09	1.33±0.02
Dandelion	*Taraxacum officinale*	3.84±0.36	25.2±0.56
Dill	*Anethum graveolens*	13.3±0.98	9.96±2.41
Fennel	*Foeniculum vulgare*	3.72±0.58	2.01±0.42
Fenugreek	*Trigonella foenum graecum*	2.44±0.47	0.49±0.04
Ginger	*Zingiber officinale*	11.4±0.84	1.44±0.14
Hawthorn	*Crataegus oxyacantha*	39.6±2.48	28.6±0.60
Lavender	*Lavandula steoechas*	20.1±0.37	21.9±2.73
Lemon balm	*Melissa officinalis*	18.0±1.10	21.2±1.05
Lemon basil	*Ocimum basilicum*	8.59±0.56	13.5±0.92
Linden	*Tilia argentea*	39.9±2.92	28.1±2.73
Mahaleb	*Prunus mahaleb*	0.36±0.18	0.48±0.02
Milk thistle	*Silybum marianum*	10.1±0.96	27.4±0.71
Mint	*Mentha piperita*	45.2±2.05	41.3±0.96
Nettle	*Urtica dioica*	8.11±0.57	6.09±1.00
Nigella	*Nigella sativa*	13.3±1.06	0.49±0.03
Paprika	*Capsicum annuum*	6.87±0.60	0.80±0.07
Parsley	*Petroselinum crispum*	9.98±1.98	0.44±0.11
Rosehip	*Rosa canina*	19.2±1.71	8.55±1.01
Rosemary	*Rosmarinus officinalis*	40.3±1.16	29.9±2.07
Sage	*Salvia officinalis*	35.8±1.70	32.3±2.10
Sumac	*Rhus coriaria*	41.8±2.60	4.24±0.42
Thyme	*Thymus vulgaris*	16.7±2.28	11.5±2.57
Turmeric	*Curcuma longa*	9.34±1.54	6.94±1.01
White pepper	*Piper nigrum*	2.06±0.18	4.79±0.60
Yarrow	*Achillea millefolium*	11.6±1.25	53.7±1.13
Mean		19.7	14.0
Standard error		2.02	1.32

[1] Data represent average values ± standard deviation of three independent samples.

for extraction. Three independent extractions for each sample were carried out as described previously by Bino *et al.* (2005). One gram of each sample was extracted with 10 ml of 80% aqueous-methanol in an ultrasonic bath (Azakli, Turkey) for 15 min. The treated samples were centrifuged (Hettich Zentrifugen Universal 32R; Hettich Zentrifugen, Tuttlingen, Germany) for 10 min at 4,000 rpm and the supernatant was collected. Another 10 ml of 80% aqueous-methanol was added to the pellet and the extraction procedure was repeated. Two supernatants were combined and adjusted to a final volume of 20 ml. Prepared extracts were stored at -18 °C until analysis.

Total phenolic content determination

The total phenolic (TP) content of extracts was determined using Folin-Ciocalteu reagent according to the method modified from Spanos and Wroldstad (1990) using gallic acid as a standard. 100 µl of extract was added to 1.5 ml of freshly prepared Folin-Ciocalteu reagent (1:10, v/v with distilled water). Then 1.2 ml of 7.5% sodium carbonate solution was added to the mixture. After 90 min of incubation at room temperature, absorbance was read at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1700; Shimadzu Corporation, Kyoto, Japan). The TP of extracts was expressed as milligrams of gallic acid equivalent (GAE) per gram sample. Samples of each extraction were analysed in triplicate.

Total flavonoid content determination

The total flavonoid (TF) content was measured colorimetrically as described by Pannala *et al.* (2001) at 510 nm. At time zero, 0.25 ml of sample was mixed with 1.25 ml distilled water and 75 µl 5% $NaNO_2$ solution. After 6 min, 150 µl of 10% $AlCl_3$ was added. At the 11^{th} min, 0.5 ml of 1 M NaOH was added to the mixture. Immediately, distilled water was added to the mixture to reach a final volume of 2.5 ml. The TF of extracts was determined by a (+)-catechin standard curve and expressed as milligrams of (+)-catechin equivalent (CE) per sample. Triplicate samples were analysed for each extract.

Total antioxidant capacity determination

The total antioxidant capacity (TAC) was estimated by four different assays (ABTS, DPPH, ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC)). In all assays, trolox was used as a standard and results were expressed in terms of milligrams of trolox-equivalent (TE) per g sample. Samples were analysed in triplicate for each assay.

Free radical scavenging by the use of ABTS radical

The ABTS assay was performed according to Miller and Rice-Evans (1997) with some slight modifications. ABTS and potassium persulfate solutions were mixed and kept overnight at room temperature in the dark. ABTS stock solution was diluted in 50 mM potassium phosphate buffer (pH 8.0) to an absorbance of 0.90 (±0.20) at 734 nm to prepare the ABTS-working solution. Then, 100 µl of sample extract was mixed with 1 ml of ABTS-working solution and the absorbance was measured at 734 nm exactly 1 min after initial mixing.

Free radical scavenging by the use of DPPH radical

The DPPH assay was performed as described by Kumaran and Karunakaran (2006). 100 µl of each sample extract was mixed with 2 ml of 0.1 mM DPPH in methanol. After 30 min of incubation at room temperature, the absorbance of the mixture was measured at 517 nm against methanol.

Ferric reducing antioxidant power assay

The FRAP assay was carried out according to the procedure of Benzie and Strain (1996). To perform the assay, a 900 µl aliquot of freshly prepared FRAP reagent (a mixture of acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM ferric chloride in proportions of 10:1:1 (v/v/v), respectively) was combined with 100 µl of extract. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min.

Cupric ion reducing antioxidant capacity assay

The CUPRAC assay developed by Apak *et al.* (2004) was used in this study. 100 µl of extract was mixed with 1 ml of 10 mM $CuCl_2$, 7.5 mM neocuproine and 1 M NH_4Ac (pH 7). Immediately, 1 ml of distilled water was added to the mixture so as to make the final volume 4.1 ml. After 60 min of incubation at room temperature, absorbance was read at 450 nm.

Statistical analysis

Data were collected from three independent extractions and reported as mean±standard deviation. Data were subjected to statistical analysis using SPSS software (version 16.0 for Windows, SPSS Inc., Armonk, NY, USA) for the analysis of variance (ANOVA). The correlation coefficients (R^2) for spectrophotometric assays, overall mean and standard error values were calculated by using the Microsoft Office Excel 2011 software (Microsoft Corporation, Redmond, WA, USA).

3. Results

Total phenolic content

TP of all herbs and spices are expressed in Table 1. Among the 35 extracts, clove (*Eugenia caryophyllata*) showed the highest TP (104 mg GAE/g), which was approximately twice the amount of cinnamon (*Cinnamomum zeylanicum*), which had the second highest TP. On the other hand, mahaleb (*Prunus mahaleb*) showed the lowest TP (0.36 mg GAE/g), which was ~4 fold lower than coriander (*Coriandrum sativum*), which had the second lowest TP. Previous studies also confirmed that clove (*E. caryophyllata*) had a high level of phenolics (Gulcin *et al.*, 2004; Jayakumar and Kanthimathi, 2012; Shan *et al.*, 2005). In our study, approximately 40% lower results were reported for clove (*E. caryophyllata*) compared to the study carried out by Shan *et al.* (2005) despite the fact that in both studies 80% methanol was used as an extraction solvent. On the other hand, in contrast to our results, Dudonne *et al.* (2009) obtained a higher level of TP in cinnamon (*C. zeylanicum*) compared to clove (*E. caryophyllata*).

Total flavonoid content

The amount of TF ranged from 0.44 to 53.7 mg CE/g (Table 1). The highest level of TF was found in yarrow (*Achillea millefolium*), while the lowest was in parsley (*Petroselinum crispum*). Mint (*Mentha piperita*), sage (*Salvia officinalis*) and cinnamon (*C. zeylanicum*) also had high levels of TF (30.5 to 41.3 mg CE/g), whereas in mahaleb (*P. mahaleb*), fenugreek (*Trigonella foenum graecum*), nigella (*Nigella sativa*) and paprika (*Capsicum annuum*) TF levels were quite low (0.48 to 0.80 mg CE/g). The flavonoid content of yarrow (*A. millefolium*) has been investigated before and main flavonoids are determined as apigenin-7-O-glucoside, luteolin-7-O-glucoside and rutin (Benedek *et al.*, 2007).

Total antioxidant capacity

TAC values (Table 2), measured by using four different methods (ABTS, DPPH, FRAP, CUPRAC), indicated extremely large variations (greater than 1000 fold difference) ranging from 0.88 to 1,007 mg TE/g. Moreover, for each assay, ranking of the samples based on their TAC values was found to be different from each other. According to ABTS method, cinnamon (*C. zeylanicum*) showed the highest TAC, which was ~190 fold higher than mahaleb (*P. mahaleb*), which had the lowest TAC at 1.10 mg TE/g. In agreement with TP results, TAC was found to be the highest in clove (*E. caryophyllata*) based on DPPH and FRAP methods. Despite being half the amount of clove (*E. caryophyllata*), dandelion (*Taraxacum officinale*), sumac (*Rhus coriaria*) and milk thistle (*Silybum marianum*) also exhibited high DPPH radical scavenging activity (52.5 to 53.8 mg TE/g), whereas in fenugreek (*T. foenum graecum*), white pepper (*Piper nigrum*) and fennel (*Foeniculum vulgare*) TAC was pretty low (1.84 to 2.79 mg TE/g). As judged by FRAP method, following clove (*E. caryophyllata*), rosemary

Table 2. Total antioxidant capacity (TAC) of 35 selected herbs and spices[1].

Common name	TAC (mg TE/g)			
	ABTS	DPPH	FRAP	CUPRAC
Allspice	135±2.66	50.9±2.28	46.3±1.39	114±6.53
Basil	29.1±2.53	35.0±1.12	44.8±1.60	315±1.46
Bay leaf	57.2±2.56	31.9±0.31	54.9±2.01	86.7±0.50
Black pepper	18.8±1.83	3.09±0.25	6.49±0.03	9.38±0.05
Cardamom	5.69±0.61	2.96±0.43	6.42±1.00	18.2±0.90
Chamomile	17.5±1.33	43.4±1.42	22.4±1.92	139±0.68
Cinnamon	211±2.56	51.6±1.54	36.2±2.29	171±2.01
Clove	75.7±0.80	113±2.38	244±3.19	579±31.1
Coriander	3.27±0.54	3.65±0.22	11.8±0.31	26.5±0.33
Cumin	14.4±0.19	4.64±0.63	3.65±0.03	5.05±0.65
Dandelion	9.94±2.59	53.8±0.78	9.72±0.47	46.9±2.03
Dill	29.7±0.45	41.6±0.74	22.0±2.95	136±2.58
Fennel	11.8±0.59	2.79±0.46	5.09±1.33	34.7±2.71
Fenugreek	22.2±0.20	1.84±0.39	1.34±0.07	4.52±1.51
Ginger	21.0±1.26	9.51±0.94	28.5±0.83	27.4±1.41
Hawthorn	79.6±3.62	41.0±0.73	97.7±2.92	645±2.71
Lavender	35.0±2.80	17.6±1.46	84.4±1.35	566±1.98
Lemon balm	32.1±1.73	31.3±0.68	61.7±2.58	424±2.81
Lemon basil	37.9±2.24	17.9±0.97	41.2±1.11	26.6±0.30
Linden	58.7±2.27	28.0±0.62	190±2.15	726±2.08
Mahaleb	1.10±0.39	5.73±0.07	0.88±0.26	6.32±0.46
Milk thistle	17.4±1.42	52.6±0.65	19.5±1.87	96.4±1.67
Mint	92.7±1.53	50.5±3.27	53.4±3.61	592±6.53
Nettle	14.6±1.13	46.1±2.89	18.4±1.78	95.1±1.88
Nigella	18.0±0.33	5.53±0.87	1.82±0.06	22.6±1.10
Paprika	17.3±0.82	3.57±0.27	2.49±0.05	9.31±0.05
Parsley	22.4±0.08	6.73±0.39	6.59±0.35	21.0±1.71
Rosehip	36.4±1.74	31.3±2.78	106±1.13	329±1.88
Rosemary	97.9±2.33	34.0±2.11	199±0.79	1007±2.08
Sage	63.2±1.39	47.7±2.64	146±2.34	811±2.53
Sumac	88.0±1.47	53.6±3.54	41.0±2.43	152±7.53
Thyme	86.8±1.51	26.2±2.26	27.4±1.25	62.3±2.31
Turmeric	26.9±0.68	7.45±0.18	5.20±0.15	19.5±1.05
White pepper	12.4±0.53	1.85±0.16	3.11±0.07	1.46±0.00
Yarrow	16.8±0.18	47.8±1.85	42.7±1.58	147±1.73
Mean	43.3	28.8	48.3	214
Standard error	4.22	2.35	5.90	26.6

[1] Data represent average values ± standard deviation of three independent samples.
ABTS = 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; DPPH = 1,1-diphenyl-2-picrylhydrazyl; FRAP = ferric reducing antioxidant power; CUPRAC = cupric ion reducing antioxidant capacity; TE = trolox equivalent

(*Rosmarinus officinalis*), linden (*Tilia argentea*) and sage (*S. officinalis*) had the highest TAC levels (146 to 199 mg TE/g), while the lowest TAC was observed in mahaleb (*P. mahaleb*) and fenugreek (*T. foenum graecum*) (0.88 and 1.34 mg TE/g, respectively). In general, CUPRAC assay gave the highest TAC values with rosemary (*R. officinalis*) being the highest among all 35 herbs and spices (1,007 mg TE/g). The high antioxidant capacity of rosemary (*R. officinalis*), clove (*E. caryophyllata*) and cinnamon (*C. zeylanicum*) has been reported before (Dragland *et al.*, 2003; Hossain *et al.*, 2008; Proestos *et al.*, 2013; Shan *et al.*, 2005). Dragland *et al.* (2003) and Su *et al.* (2007) measured similar TAC values in cinnamon compared to our ABTS results. On the other hand, higher FRAP results have been reported in clove by Hossain *et al.* (2008). As far as it is known, no previous study evaluated the antioxidant capacity of rosemary (*R. officinalis*) using CUPRAC method.

Relationship between TP, TF and TAC

The correlation coefficients (R^2) for spectrophotometric assays ranged from 0.163 (between ABTS and FRAP) to 0.760 (between FRAP and CUPRAC). FRAP and CUPRAC are electron transfer (ET)-based assays, and ABTS is a mixed-mechanism (i.e. a mixture of hydrogen atom transfer- and ET-based mechanisms) assay (Apak *et al.*, 2007); therefore such weak correlations are rather rare in literature as similar mechanism assays generally yield high correlation coefficients between their results. The extremely low correlation of ABTS and FRAP may be attributed to the slow reaction kinetics of the FRAP chromophore with certain phenolic acids, rendering completion of the concerned redox reactions impossible within the protocol time period of the FRAP assay (Apak *et al.*, 2007). Correlation coefficients above 0.4 are shown in Figure 1. TP and TF of herbs and spices showed a low correlation with a correlation coefficient of R^2=0.234. The highest correlation between TP and TAC was demonstrated in case of FRAP (R^2=0.627), followed by DPPH (R^2=0.610), ABTS (R^2=0.485) and CUPRAC (R^2=0.404) (Fig. 1). The lowest correlation coefficients were obtained for TF and TAC ranged from 0.168 (between TF and ABTS) to 0.410 (between TF and CUPRAC). The reason that TF correlated weakly with other assays is that flavonoids constitute a relatively small percentage of the whole antioxidants present in these samples whereas TP and TAC assays measure an overwhelmingly larger portion of such compounds. The $AlCl_3$-$NaNO_2$ test measures only flavonoids with the aid of a complexion reaction whereas both TP and TAC tests are based on redox reactions (Apak *et al.*, 2007).

4. Discussion

Herbs and spices can act as natural antioxidants and thus improve human nutrition and health. In the literature, there are several reports investigating the antioxidant capacity of various herbs and spices. In general, studies report similar rankings among the individual herbs and spices, however because of differences in extraction, bioassay techniques and growth conditions of the plants, the values reported in those studies are not always comparable with the values observed in this study.

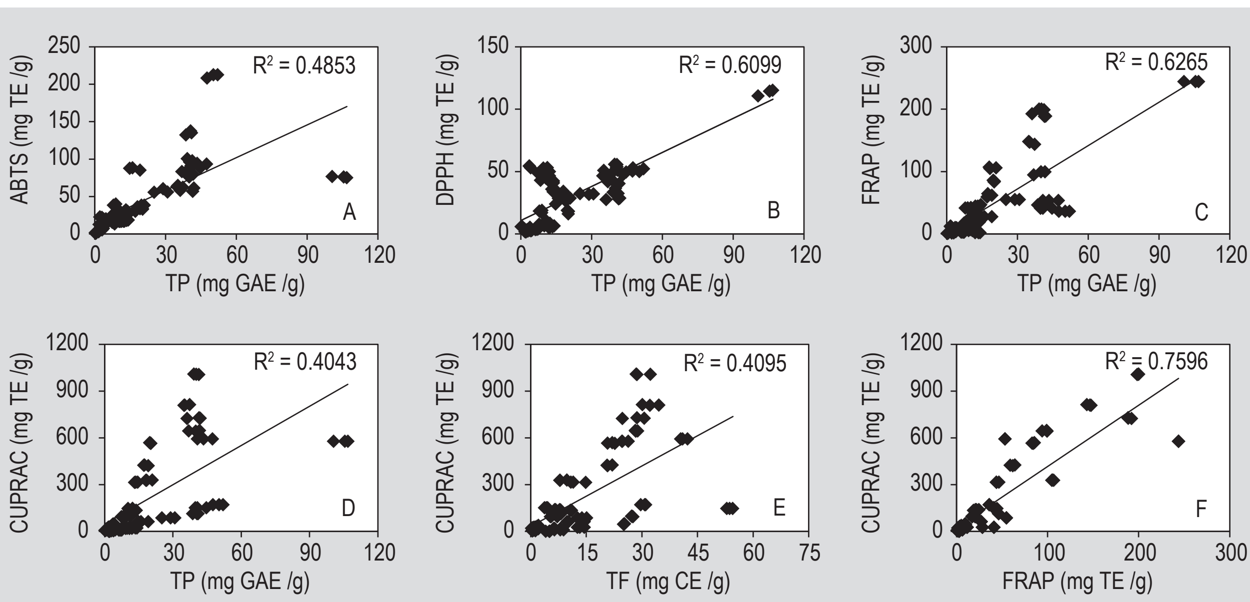

Figure 1. Correlation coefficients (R^2) between (A) total phenolic content (TP) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS); (B) TP and 1,1-diphenyl-2-picrylhydrazyl (DPPH); (C) TP and ferric reducing antioxidant power (FRAP); (D) TP and cupric ion reducing antioxidant capacity (CUPRAC); (E) total flavonoid content (TF) and CUPRAC; and (F) FRAP and CUPRAC. TE = trolox equivalent.

Considering the TP results, herbs and spices had high phenolic contents, some of which are comparable to the commonly consumed fruits and vegetables in Turkey including blueberry (Koca and Karadeniz, 2009), mulberry (Ercisli and Orhan, 2007) and tomato (Capanoglu *et al.*, 2008). However, Folin–Ciocalteu method was found to be insufficient to reflect the amount of phenolics in herbs and spices, hence, is not recommended for determining phenolic compounds in herbs and spices as a single method. Due to the lack of specificity of this method for phenolic compounds, the presence of other non-phenolic reducing compounds may affect the accuracy of the assay because both the composition and redox potential of the Folin reagent is not exactly known, and this reagent is capable of oxidizing some common sugars, amino acids, and citric acid in addition to phenolic antioxidants (Apak *et al.*, 2007). Therefore, to obtain more accurate results, in further studies high-performance liquid chromatography analysis of individual phenolic compounds should also be performed.

Many *in vitro* and *in vivo* antioxidant capacity assays have been developed, but only a few are rapid and reliable methods that are applicable for a large number of plant extract samples (Cai *et al.*, 2004). Yet, the measurement of antioxidant activities, in the case of multifunctional or complex multiphase systems, such as herbs and spices, cannot be evaluated satisfactorily by a single method. The principles of the methods vary greatly depending on the radical that is generated or the time of reaction. Even the methods based on the same principle such as ABTS and DPPH can show several important differences in their response to antioxidants. Moreover, various different mechanisms may contribute to oxidative processes, such as in Fenton reactions, where transition metal ions play a vital role, different reactive oxygen species might be generated and various target structures such as lipids, proteins and carbohydrates, can be affected (Hinneburg *et al.*, 2006). Therefore, it is highly recommended to apply several test procedures to evaluate antioxidant activities to obtain the full picture. All antioxidant assays applied in this study have been widely used to determine the antioxidant capacities of plant extracts as they require relatively standard equipment and deliver fast and reproducible results. The ABTS assay is particularly interesting in plant extracts because the wavelength absorption at 734 nm eliminates colour interference (Dudonne *et al.*, 2009).

The relationships between TP, TF and TAC of many herbs and spices were investigated in previous studies (Cai *et al.*, 2004; Hinneburg *et al.*, 2006; Javanmardia *et al.*, 2003; Miliauskas *et al.*, 2004; Niciforovic *et al.*, 2010; Shan *et al.*, 2005; Wojdylo *et al.*, 2007). Some studies obtained good positive linear correlation, but others got poor linear correlation or even could not explain the relationship. Our experience and studies carried out by Miliauskas *et al.* (2004) and Niciforovic *et al.* (2010) indicated a weak correlation, especially between TF and TAC. It is known that only flavonoids of a certain structure and particularly hydroxyl position in the molecule determine antioxidant properties and in general these properties affect the ability to donate hydrogen or electron to a free radical (Miliauskas *et al.*, 2004). Studies that found a direct relationship were generally based on the determination and statistical analysis of a quite homogeneous or small number of samples. It would have been possible to find a stronger correlation between assays especially if the plants were from the same genus or family, thus containing similar chemical constituents. In our study, the plants were from different families which makes it difficult to establish a strong correlation. Also, it should be taken into consideration that different phenolic compounds may show different antioxidant activities, depending on their structure, as well as synergistic or antagonistic effect of other compounds, which are present in the crude extract (Niciforovic *et al.*, 2010). Several other factors including the ranges of the tested values, different antioxidant assay methods and specific conditions of samples all could greatly influence the correlative relationship (Shan *et al.*, 2005).

Besides investigating the antioxidant capacity of herbs and spices, it is also important to evaluate the bioavailability of the health associated compounds present. This will provide valuable data for elucidating the true biological relevance of these compounds in the context of nutrition and human health. Very limited information is available on the bioavailability of phytochemicals in herbs and spices. Without a doubt, *in vivo* animal or human studies will provide more solid evidence in this area since phenolic compounds may act differently under *in vivo* conditions (Yi and Wetzstein, 2011).

In conclusion, our work has focused on the antioxidant capacity of traditional Turkish herbs and spices. Although herbs and spices contribute little weight on our dish, they might still be important to our daily antioxidant intake, especially in dietary cultures where they are used regularly. In fact, in a normal diet, intake of 1 g of these herbs and spices may make a relevant contribution to the total intake of plant antioxidants, and be an even better source of dietary antioxidants than many other food groups.

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