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Radical scavenging-linked antioxidant activities of commonly used herbs and spices in Korea

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Abstract

Herbs and spices not only variety and racy flavour to Korean foods, they also are the richest source for antioxidant power. The present study evaluates the radical scavenging-linked antioxidant activities of hot water extracts from commonly used herbs and spices in Korea. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical and superoxide anion scavenging activities of bay extract were 39.5% and 22.1%, respectively. The hydroxyl radical scavenging activity was in order of dill (50.0%) > bay (31.3%) > garlic (27.9%) > white pepper and black pepper (15.1–15.3%) > onion (10.1%) extracts. Bay extract had the highest total phenolic content (17.86 μg CE/g). High correlation coefficients were found between the total phenol content and DPPH radical scavenging activity ($R = 0.9162$). These results indicate that herbs and spices had high antioxidant activity that is partly due to the phenolic compounds and provide basic data for further development of processed food products.

Keywords: *herbs and spices, radical scavenging-linked antioxidant activity, total phenol content, total flavonoid content*

Introduction

Oxidation is one of the major causes of chemical spoilage, resulting in rancidity or deterioration of the nutritional quality, colour, flavour, texture and safety of foods (Frankel and Meyer 2000; Carpenter et al. 2007). It is well known that reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. The oxidative damages caused by ROS on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart disease, atherosclerosis, cancer and aging (Halliwell 1996; Madhavi et al. 1996). Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen (Tachakittirungrod et al. 2007).

Plants, including herbs and spices, have many phytochemicals that are potential sources of natural antioxidants, e.g. phenolics, flavonoids, alkaloids and terpenoids (Moure et al. 2001; Amro et al. 2002; Cai et al. 2004). Plants are major sources of phenolic compounds, which are synthesized as secondary metabolites during normal development in response to stress conditions, such as wounding and UV radiation among others (Stahl and Sies 2003; Close et al. 2005). Plants may contain simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignins and lignans (Naczk and Shahidi 2006). Natural antioxidants are known to protect cells from damage induced

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by oxidative stress, which is generally considered to be a cause of aging, degenerative diseases and cancer (Ringman et al. 2005). These health-promoting effects of antioxidants from plants and spices are thought to arise from their protective effects by counteracting ROS (Kim et al. 2011). Herbs and spices, like turmeric, fenugreek, mustard, ginger, etc., may offer many health benefits and have been proven to counteract oxidative stress *in vitro* and *in vivo* states (Ahmed et al. 2000; Modak et al. 2007). Most of these herbs and spices have been intensely studied only for their active components like phenolic acid and flavonoids (Kikuzaki and Nakatani 1993; Manda et al. 2010). Several assays have been frequently used to estimate antioxidant capacities in medicinal plants vegetables and their products in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al. 1995), hydroxyl radical scavenging activity (HRSA) (Halliwell et al. 1987) and the superoxide anion radical scavenging activity (SRSA) (Liu et al. 2008). Although the efficacy and mechanisms of action of herbs and spices have not been tested scientifically in most cases, simple medicinal preparations from these herbs and spices often mediate beneficial responses due to their active chemical constituents (Park and Pezzutto 2002).

Therefore, the objective of this study is to determine the bioactive compounds (total phenol and total flavonoid contents) and radical scavenging-linked antioxidant properties of commonly used herbs and spices in Korea and to investigate the relationship between bioactive compounds and antioxidant activities.

Materials and methods

Chemicals and spices

Ascorbic acid, potassium persulphate, disodium hydrogen phosphate (Na_2HPO_4), DPPH, deoxyribose, ferric chloride ($FeCl_3$), MEDTA, hypoxanthine, nitroblue tetrazolium (NBT), xanthine oxidase, Folin–Ciocalteu's phenol reagent, (+)-catechin, sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), linoleic acid, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Co. (St. Louis, MO, USA). Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and all solvents used were of analytical grade and purchased from Merck Co. (Darmstadt, Germany). Distilled deionized water (dd. H_2O) was prepared by Ultrapure™ water purification system (Lotun Co., Ltd., Taipei, Taiwan). Dill, bay, mustard, onion, garlic, black pepper and white pepper were purchased from Taewon Food Industry (Seoul, Korea).

Extraction yield

Herbs and spices were dried for 48 h to about 4% moisture (dry base) in a hot air-dryer at 50°C. After drying, each 40 g of the dried samples was extracted with 400 ml of distilled water at 85°C for 6 h to give an initial extract. The residues were re-extracted with 400 ml of distilled water at 100°C for 3 h. After cooling to room temperature and filtering (Whatman No 2, Whatman Ltd, Maidstone, Kent, England), both the extracts were combined and completely lyophilized in a freeze-dryer (PVTFD10R, ILSIN Bio Co., Kyonggi, Korea) and stored at −20°C until further use.

Measurement of free radical scavenging activity in DPPH assay

The free radical scavenging activity of samples (1 mg/mL in 5% DMSO) was measured using the method of Brand-Williams et al. (1995) with some modification. L-ascorbic acid was used as a positive control. The DPPH (0.15 M) working solution was prepared by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.00 ± 0.02 units at 517 nm using the spectrophotometer (Ultrospec 2100 pro, Amersham Pharmacia Biotech Co., Piscataway, NJ, USA). Sample extracts (150 μL) were allowed to react with 2850 μL of the DPPH solution for 24 h in a dark place. Then the absorbance was recorded at 517 nm. The inhibition percentage was calculated from the following equation: Inhibition % = ([absorbance of control − absorbance of sample]/absorbance of control) × 100.

Measurement of hydroxyl ($OH^{\bullet}$) radical scavenging activity

The scavenging activity of samples at a concentration of 0.5 mg/mL in 5% DMSO on the hydroxyl radical (OH·) was measured by the deoxyribose method (Halliwell et al. 1987) with a slight modification. The reaction mixture consisted of 200 μL $FeSO_4 \times 7H_2O$ (10 mM), EDTA (10 mM) and 2-deoxyribose (10 mM). The sample solution and a phosphate buffer (pH 7.4, 0.1 M) were then added to generate a total volume of 1.8 mL. Finally, 200 μL of H_2O_2 (10 mM) was added to the reaction mixture and incubated at 37°C for 1 h. After incubation, the colour was developed by addition of 0.5% TBA followed by ice-cold 2.8% TCA in 25 mM NaOH and heating for 30 min at 80°C. A control was performed without samples (A1). The sample (A2) was cooled on ice and the absorbance was measured at 532 nm. The HRSA was calculated by the following equation: HRSA% = (A1 − A2/A1) × 100.

Measurement of superoxide anion ($O2^{\bullet -}$) radical scavenging activity

Superoxide radicals were generated by a modified method of Chun et al. (2003). The samples (0.5 mg/mL in 5% DMSO) were added to the reaction solution containing 100 μL of 30 mM EDTA (pH 7.4), 10 μL of 30 mM hypoxanthine in 50 mM NaOH

and 200 μL of 1.42 mM NBT. The solution was pre-incubated at room temperature for 3 min, 100 μL of 0.5 Units/mL xanthine oxidase was added to the mixture and then the volume was brought up to 3 mL with 50 mM phosphate buffer (pH 7.4). After the solution was incubated at ambient temperature (25°C) for 20 min, absorbance of solution was measured at 560 nm. The SRSA was expressed as percent (%) superoxide quenching, which was calculated as $(1 - B/A) \times 100$, where A is the activity of the enzyme without test material and B is the activity of the enzyme with test material.

Measurement of total phenol content using Folin–Ciocalteu assay

Total phenol content of the seven extracts was determined spectrophotometrically according to the Folin–Ciocalteu method (Singleton and Rossi 1965). Because catechin is one of the polyphenol compounds, total phenol content of hot water extract from herbs and spices was expressed as microgram catechin equivalents/gram extract (μg CE/g). The 150 μL of samples at a concentration of 1 mg/mL, 2400 μL of deionized water, and 150 μL of 0.25 N Folin–Ciocalteu reagents were combined in a plastic vial and then mixed well using a vortex mixer. The mixture was allowed to react for 3 min, and then 300 μL of 1 N Na_2CO_3 solution was added and mixed well. The solution was incubated at ambient room temperature (23°C) in a dark place for 2 h. The absorbance was measured at 725 nm using a spectrophotometer (Hewlett Packard 8452A, Diode Array, Santa Clara, CA, USA). Additional dilution was done if the absorbance value measured was over the linear range of the standard curve ($Y = 0.0016X + 0.0424$, $R^2 = 0.9999$).

Measurement of total flavonoid content

Total flavonoid content was determined using the method of Chun et al. (2003) fwith minor modifications. Exactly, 0.25 mL of sample (1 mg/mL) was added to a tube containing 1 mL of double-distilled water. Then, 0.075 mL of 5% $NaNO_2$, 0.075 mL of 10% $AlCl_3$ and 0.5 mL of 1 M NaOH were added at 0, 5 and 6 min, sequentially. Finally, the volume of the reacting solution was made up to 2.5 mL with double-distilled water. The absorbance of the solution was recorded at a wavelength of 410 nm and was detected using the Ultrospec 2100 pro spectrophotometers (see section 'Measurement of free radical scavenging activity in DPPH assay'). Quercetin, a ubiquitous flavonoid present in many plant extracts, was used as a standard to quantify the total flavonoid content of hot water extract of the spice. Results were expressed as microgram quercetin equivalents/gram extract (μg QE/g).

Statistical analysis

All experiments were repeated six times. The results were reported as mean ± standard deviation (SD). The significance of differences among treatment means was determined by analysis of variance (one-way ANOVA) using SAS version 8.1 (SAS Institute, Cary, NC, USA). Correlation analyses were performed using the Pearson's correlation coefficient (R).

Results

Extraction yield

Table I lists the commonly used herbs and spices in Korea, as well as their scientific names and extraction yields. The differences among the extracts for these parameters were statistically significant ($p < 0.05$). The extraction yields of hot water extracts obtained from the herbs and spices were ranged from 19.73% to 1.85%. The extraction yield can be ranked as dill extract > bay extract > mustard extract > garlic extract > onion extract > black pepper extract > white pepper extract.

DPPH radical scavenging activity

Figure 1 shows that the DPPH radical scavenging activity of samples can be ranked as bay (39.5%) extract > mustard and onion (35.3–34.5%) extracts > dill and garlic (29.5–30.3%) extracts > white pepper (25.2%) extract > black pepper (21.9%) extract. The observed differential scavenging activities of the extracts against the DPPH system could be due to the presence of different bioactive compounds in these extracts.

Superoxide anion radical scavenging activity

The superoxide radical scavenging effects of water extracts from different herbs and spices were compared at the same doses as ascorbic acid (0.5 mg/mL) as shown in Figure 2. The values in superoxide anion radical scavenging activities were in the order of bay (22.1%) extract ≥ mustard (20.4) extract > garlic, white pepper and black pepper (14.4–15.1%) extracts > dill (12.3%) extract > onion (10.1%) extract.

Table I. List of herbs and spices and their extraction yield (%).

Common name	Scientific name	Extraction yield (%)
Dill	*Anethum graveolens*	19.73 ± 0.83[a]
Bay	*Laurus nobilis*	11.92 ± 1.17[b]
Mustard	*Brassica juncea*	8.47 ± 6.46[c]
Onion	*Allium cepa*	6.26 ± 0.06[d]
Garlic	*Allium sativum*	7.57 ± 0.05[e]
Black pepper	*Piper nigrum L.*	4.25 ± 0.43[f]
White pepper	*Piper nigrum L.*	1.85 ± 1.28[g]

[a–g] Values are significantly different among the samples ($n = 6$, error bars represent standard deviation) ($p < 0.05$).

When compared to the activity of ascorbic acid, the superoxide anion radical scavenging activities of these extracts were found to be lower ($p < 0.05$).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activities of seven extracts from herbs and spices at a concentration of 0.5 mg/mL are shown in Figure 3. The result in decreasing order of HRSA was dill (50.0%) extract > bay (31.3%) extract > garlic (27.9%) extract > white pepper and black pepper (15.1–15.3%) extracts > onion (10.1%) extract. HRSA of the dill extract was higher than those of other extracts.

Total phenol and flavonoid contents

Total phenol and flavonoid contents of seven extracts from herbs and spices are shown in Table II. Bay had the highest total phenolic content (17.86 μg CE/g) whereas onion had the lowest value (6.17 μg CE/g), using the standard curve of catechin ($R^2 = 0.9482$). Using the standard curve of quercetin ($R^2 = 0.9825$), the total flavonoid content of herbs and spices varied from 58.92 μg QE/g (dill extract) to 2.29 μg QE/g (garlic extract). These results indicate that total phenol and total flavonoid contents in leaf parts were higher than those in root parts among samples.

Correlation among the antioxidant characteristics

A regression analysis was used to correlate the results of the five assays (Table III). High correlation coefficients were found between the total phenol content and DPPH radical scavenging activity ($R = 0.9162$, $p < 0.001$). But the HRSA and total phenolic contents exhibited low correlation coefficient ($R = 0.5120$, $p < 0.05$), and the correlation coefficient between total phenolic content and flavonoid content was exhibited low ($R = 0.2864$, $p < 0.01$). However, a non-significant correlation coefficient was found between HRSA and other items. In addition, no significant correlation coefficient between SRSA and other items was found.

Discussion

The antioxidant activity of herbs and spices is mainly contributed by the bioactive compounds present in them. The amount of such compounds deposited in each part of the plant is usually different. Thus, we evaluated the radical scavenging-linked antioxidant activity of hot water extracts from commonly used herbs and spices in Korea. The antioxidant potential was assessed by DPPH radical, superoxide anion radical and hydroxyl radical scavenging activities. We also determined both total phenol and total flavonoid contents using colorimetric methods.

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods (Frankel and Meyer 2000). Superoxide anion ($O_2^{\bullet -}$) is a reduced form of oxygen (O_2) by multiple pathway as oxidation by NADPH oxidase, xanthine or hypoxanthine oxidase. Generally, superoxide anion is converted into hydrogen peroxide by superoxide dismutase (SOD) or reacts with nitric oxide ($NO^{\bullet}$) to form peroxynitrite. Superoxide radicals have been observed to kill cells, inactivate enzymes, and degrade DNA, cell membranes and polysaccharides (Halliwell 1996; Cai et al. 2004). It was, therefore, proposed to measure the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical (Vani et al. 1997). Among the ROS, hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism to initiate cell damage *in vivo* (Mates and Sanchez-Jimenez 2000; Park and Pezzutto 2002). We examined the inhibitory action of tested samples on deoxyribose degradation, which gives an indication of hydroxyl

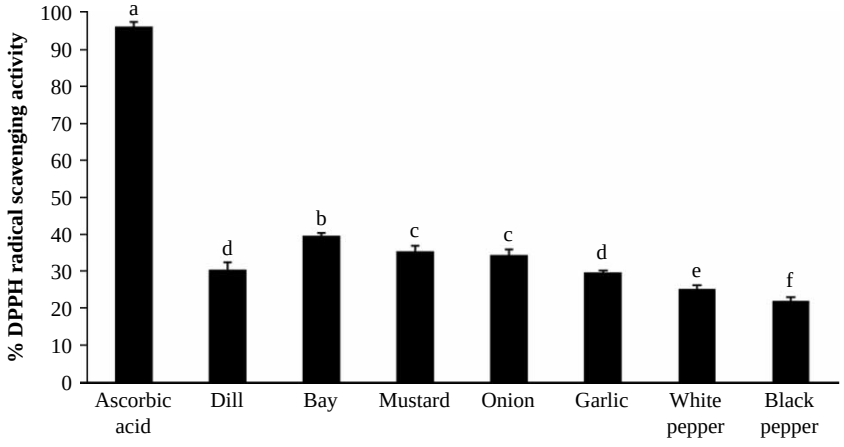

Figure 1. DPPH radical scavenging activities of hot water extracts from herbs and spices at a concentration of 1 mg/mL ($n = 6$, error bars represent standard deviation). Values are significantly different among the samples ($p < 0.05$).

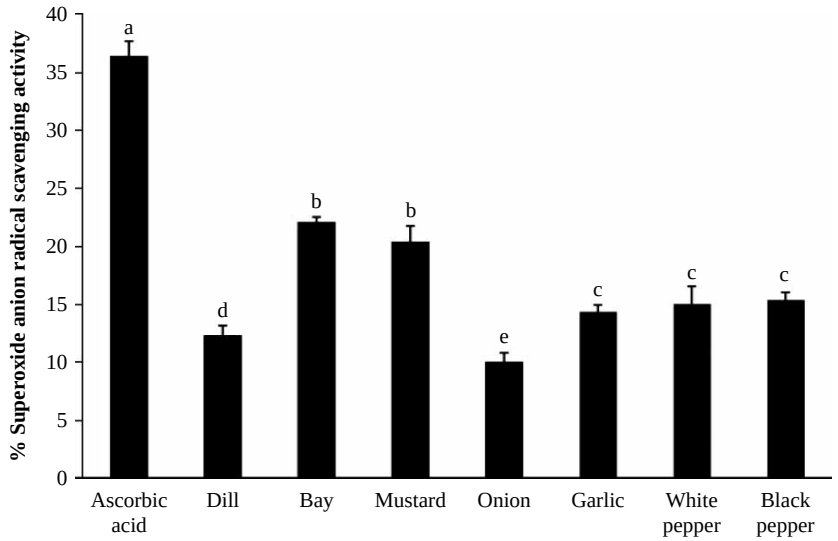

Figure 2. Superoxide anion scavenging activities of hot water extracts from herbs and spices at a concentration of 0.5 mg/mL ($n = 6$, error bars represent standard deviation). Values are significantly different among the samples ($p < 0.05$).

radical scavenging action and iron chelating activity (Halliwell et al. 1987; Lopes et al. 1999).

Our results show that the extraction yield of several herbs and spices was in order of dill extract > bay extract > mustard extract > garlic extract > onion extract > black pepper extract > white pepper extract. In addition, bay extract had the highest total phenolic content (17.86 μg CE/g). Total flavonoid content of herbs and spices varied from 58.92 μg QE/g (dill extract) to 2.29 μg QE/g (garlic extract). These results are in good agreement with the study by Hinneburg et al. (2006) in which water extraction yields of several spices were ranged from 88 mg/g (8.8%) to 422 mg/g (42.2%). Rice-Evans et al. (1996) reported that the antioxidant properties of phenolic acids and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen.

Moreover, DPPH radical scavenging ability of herbs and spices was in order of bay extract > mustard and onion extracts > dill and garlic extracts > white pepper extract > black pepper extract. Although the DPPH radical scavenging activities of the herbs and spices were less ($p < 0.05$) than that of ascorbic acid, the study revealed that most herbs and spices had free radical scavengers or inhibitors, acting possibly as primary antioxidants. A significant and linear relationship existed between the DPPH scavenging activity and phenolic content, indicating that phenolic compounds are major contributors to antioxidant activity. The highly significant correlations obtained in this study support the hypothesis that phenolic compounds contribute significantly to the total antioxidant capacity of medicinal plants (Table III). The good correlation between the results from total phenols analysis and the antioxidative assays has been

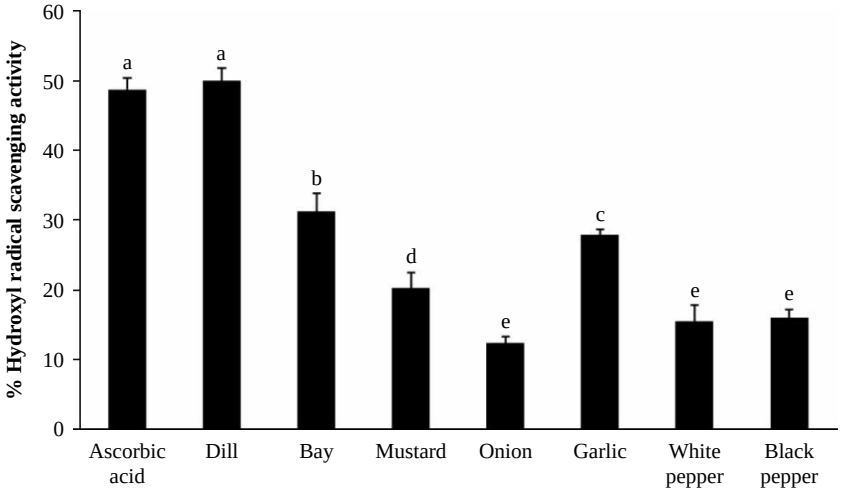

Figure 3. Hydroxyl radical scavenging activities of hot water extracts from herbs and spices at a concentration of 0.5 mg/mL. ($n = 6$, error bars represent standard deviation). Values are significantly different among the samples ($p < 0.05$).

Table II. Total phenol and flavonoid contents of seven extract from herbs and spices at a concentration of 1 mg/mL.

Herb and spices	Total phenol content (μg CE/g)	Total flavonoid content (μg QE/g)
Dill	10.44 ± 0.71^{b}	58.92 ± 4.94^{a}
Bay	17.86 ± 0.48^{a}	9.02 ± 0.15^{d}
Mustard	9.31 ± 0.31^{c}	3.24 ± 0.62^{e}
Onion	6.17 ± 0.35^{d}	10.21 ± 4.67^{c}
Garlic	6.19 ± 1.24^{e}	2.29 ± 0.55^{e}
White pepper	6.81 ± 0.38^{d}	10.61 ± 2.28^{c}
Black pepper	9.17 ± 0.28^{c}	16.05 ± 0.52^{b}

[a–e] Values are significantly different among the samples ($n = 6$, error bars represent standard deviation) ($p < 0.05$).

previously reported (Zheng and Wang 2001). Liu et al. (2008) have reported that the clove extract was significantly higher in the total phenol content and DPPH radical scavenging activity than other Chinese herbal plants. Although it is possible that the DPPH radical scavenging activity of herbs and spices could be mediated by individual phenolic acids, the overall antioxidant potential of herbs and spices is likely exhibited by the synergistic effect of the combinations of total phenolic acids and other antioxidant components including antioxidant vitamins considering the wide mixture of phenolic antioxidants present in herbs and spices extract.

We also found that both bay and mustard extracts had stronger superoxide anion scavenging activities than other herbs and spices extracts. These results suggest that phenolic compounds in both bay and mustard possess strong antioxidant effects due to superoxide anion radical scavenging in the cellular level. Furthermore, HRSA of the dill extract was higher than those of other extracts. However, HRSA of dill extract showed a different pattern with other methods used such as DPPH and superoxide anion scavenging abilities. Bioactive compounds in various herbs and spices are a complex mixture of compounds. Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans et al. 1996). Flavonoid as one of the most diverse and widespread group of natural compounds is probably the most important natural bioactive compound. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Foti et al. 1996; Miliauskas et al. 2004). The concentration of the phenolic and flavonoid compounds in various herbs and spices varies depending on their cultivar and climate. In addition, Yoo et al. (2007) reported that there are many methods for the determination of antioxidant capacities and each method has its own limitation. It was shown that some antioxidant assays gave different antioxidant activity trends. Taken together, our results show that the antioxidant activities of various herbs and spices have different tendency, which may depend on the methods used and/or the profile of phenolics.

Table III. Correlations (R) between different antioxidant capacity parameters (by DPPH, hydroxyl, and superoxide radical scavenging activity) and total phenol or total flavonoid contents of hot water extracts from the herbs and spices.

	TPC	TFC	DPPH	HRSA	SRSA
TPC		$0.2864^{\dagger}$	$0.9162^{\ddagger}$	−0.3655	−0.1773
TFC			$0.5120^{\star}$	−0.3074	0.4432
DPPH				−0.4360	−0.0487
HRSA					−0.1617

Note: R, correlation coefficient; TPC, total phenol content; TFC, total flavonoid content; DPPH, DPPH radical scavenging activity; HRSA, hydroxyl radical scavenging activity; SRSA, superoxide anion radical scavenging activity. Significance level at $^{\star}p < 0.05$, $^{\dagger}p < 0.01$ and $^{\ddagger}p < 0.001$.

Total phenol and total flavonoid contents have been reported to be responsible for the antioxidant activities of botanical extracts. DPPH radical scavenging activity, HRSA and SRSA have been used to measure antioxidant activity, and these results should correlate with those of total phenol and flavonoid content. Do et al. (2004) demonstrated that some bioactive compounds present in medicinal plant possessed high antioxidant activity, which was due to the presence of phenolic, carotenoids and flavonoids. Liu et al. (2008) reported a negative correlation between flavonoid content and antioxidant activity. High correlation coefficients were found between the total phenol content and DPPH radical scavenging activity ($R = 0.9162$).

In conclusion, hot water extracts of several herbs and spices had a high antioxidant activity which is partly due to the bioactive compound such as phenolic compounds. This provides basic data, having implications for further development of processed food products.

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