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Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents

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Total equivalent antioxidant capacity (TEAC) and phenolic content of 26 common spice extracts from 12 botanical families were investigated. Qualitative and quantitative analyses of major phenolics in the spice extracts were systematically conducted by reversed-phase high-performance liquid chromatography (RP-HPLC). Many spices contained high levels of phenolics and demonstrated high antioxidant capacity. Wide variation in TEAC values (0.55−168.7 mmol/100 g) and total phenolic content (0.04−14.38 g of gallic acid equivalent/100 g) was observed. A highly positive linear relationship ($R^2 = 0.95$) obtained between TEAC values and total phenolic content showed that phenolic compounds in the tested spices contributed significantly to their antioxidant capacity. Major types of phenolic constituents identified in the spice extracts were phenolic acids, phenolic diterpenes, flavonoids, and volatile oils (e.g., aromatic compounds). Rosmarinic acid was the dominant phenolic compound in the six spices of the family Labiatae. Phenolic volatile oils were the principal active ingredients in most spices. The spices and related families with the highest antioxidant capacity were screened, e.g., clove in the Myrtaceae, cinnamon in the Lauraceae, oregano in the Labiatae, etc., representing potential sources of potent natural antioxidants for commercial exploitation. This study provides direct comparative data on antioxidant capacity and total and individual phenolics contents of the 26 spice extracts.

KEYWORDS: Spices; antioxidant activity; phenolic compounds; radical scavenging activity; clove; cinnamon; oregano; Labiatae

INTRODUCTION

Spices are common food adjuncts, which have been used as flavoring, seasoning, and coloring agents and sometimes as preservatives throughout the world for thousands of years, especially in India, China, and many other southeastern Asian countries (*1*). Spice plants belong to several main botanical families, such as Labiatae (also called Lamiaceae) (e.g., rosemary, oregano, and sage), Lauraceae (e.g., cinnamon), Peperaceae (e.g., black pepper), Myrtaceae (e.g., clove), Umbelliferae (e.g., cumin), etc. Major spice plants are normally distributed in tropical and temperate areas (*2*).

Not only are spices used as food flavorings and seasonings to improve the flavor, but they may also be used as traditional medicines (*1*, *3*). Many spices have been recognized to have medicinal properties and possess many beneficial effects on health, such as antioxidant activity, digestive stimulant action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic, anticarcinogenic potential, etc. (*1*, *4*−*8*). For example, clove, cinnamon, and Chinese prickly ash are common spices in China and are also used as traditional Chinese medicines.

Spices, like vegetables, fruits, and medicinal herbs, are known to possess a variety of antioxidant effects and properties (*9*−*13*). Phenolic compounds in these plant materials are closely associated with their antioxidant activity. The antioxidant effect of phenolic compounds is mainly due to their redox properties and is the result of various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity (*14*−*17*). They are also known to play an important role in stabilizing lipid peroxidation and to inhibit various types of oxidizing enzymes (*18*, *19*). These multiple potential mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search for health-beneficial phytochemicals and also offer a possibility to use phenolic compounds or extracts rich in them in lipid-rich foods to extend shelf life (*20*). The presence of antioxidative and antimicrobial phenolic constituents in many spices gives food-preserving properties (*1*, *21*).

Spices have been investigated for their antioxidant properties for at least 50 years. As early as 1952, many spices were examined and 32 spices were found to retard the oxidation of lard (*1*). Many studies indicated that rosemary, sage, oregano, and thyme, leafy spices in the family Labiatae, demonstrated high antioxidant activity (*4*, *9*, *22*). Several studies also showed that black pepper, clove, cinnamon, and coriander exhibited

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antioxidant properties (*9*, *23*, *24*). In recent decades, a number of phenolic substances were isolated from a variety of spice sources, including phenolic acids (e.g., gallic acid, caffeic acid, etc.), flavonoids (e.g., quercetin, rutin, myricetin, luteolin, naringenin, and silybin), phenolic diterpenes, and volatile oils (*7*, *22*, *23*, *25*−*29*).

Thus far, numerous studies on antioxidant properties of many spices have been conducted using different assay methods (*9*, *30*−*34*). However, the wide variety of oxidation systems and ways to measure activity used in antioxidant assessment make it difficult to directly compare the results from different studies. Even though intensive studies on the bioactive components and their total content in many spices have been carried out, the phenolic identification data are insufficient and incomplete. In particular, quantitative data on the individual phenolics in the spices are currently lacking. Also, there are few comparisons of phenolic constituents identified in various spices from different spice families. The structure−activity relationships of phenolic compounds in the spices have not been thoroughly discussed and revealed. Moreover, the relationship between total antioxidant activity and phenolic content of a large number of spices was not systematically investigated before. Many researchers claimed that the phenolic compounds in spices were responsible for their antioxidant activity, but few could establish real correlative relationships and provide convincing statistical data to reveal the relationship between the activity and phenolics on the basis of large numbers of spice samples.

The objectives of this study were (1) to evaluate and compare total antioxidant capacity and phenolic content of 26 common spice extracts; (2) to identify and quantify major phenolic constituents present in the tested spices by RP-HPLC; and (3) to establish the relationship between antioxidant activity and phenolic compounds of 26 spice extracts to confirm that the phenolic constituents are responsible for their antioxidant activity.

MATERIALS AND METHODS

Plant Materials. A total of 10 fresh plant materials, i.e., coriander, parsley, mint, sweet basil, dill, rosemary, thyme, sage, oregano, and lemon grass, and 16 dried plant materials, such as nutmeg, green peppercorn, white pepper, black pepper, and Chinese prickly ash, originally from seven Asian countries/regions and four Western countries, were collected and purchased from local supermarkets and drugstores. These plants were distributed in 12 families, mainly Labiatae, Lauraceae, Piperaceae, and Umbelliferae. Edible parts of the 26 spice plants, such as leaves, branches, stems/barks, flowers/buds, fruits/seeds, or whole plants, were used for extraction and analysis in the present study. The scientific names, sources, and tested parts are detailed in **Table 1**.

Chemicals and Reagents. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, and sodium carbonate were purchased from Sigma/Aldrich (St. Louis, MO). Folin−Ciocalteu reagent and HPLC-grade organic reagents were from BDH (Dorset, U.K.). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was from Fluka Chemie AG (Buchs, Switzerland). Authentic standards, such as phenolic acids (e.g., gallic acid, protocatechuic acid, caffeic acid, and rosmarinic acid), flavonoids (e.g., catechin, quercetin, apigenin, kaempferol, naringenin, hesperetin, and quercitrin), volatile oils (e.g., eugenol, carvacrol, thymol, and menthol), and phenolic diterpenes (e.g., carnosic acid and carnosol), were purchased from Sigma/Aldrich.

Sample Preparation. Fresh plant samples were cleaned, freeze-dried, and ground into a fine powder (710 μm) by a Kenwood Multi-Mill (Kenwood Ltd., U.K.) and passed through a sieve (24 mesh). Dried plant samples were further air-dried in a ventilated oven at 40 °C for 24 h and also ground into a fine powder and passed through a sieve as mentioned above. The powdered sample (2 g) was extracted with 50 mL of 80% methanol at room temperature (~23 °C) for 24 h in a shaking water bath (Shaking Bath 5B-16) (Techne, Ltd., U.K.). The extract was filtered by a Millipore filter with a 0.45-μm nylon membrane under vacuum at 23 °C. The filtrate was stored at 4 °C until use within 24 h.

Estimation of Total Antioxidant Capacity by the $ABTS^{\cdot+}$ Method. Total antioxidant capacity assay was carried out by the $ABTS^{\cdot+}$ method modified by Re et al. (*35*) and Cai et al. (*36*). The $ABTS^{\cdot+}$ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature in the dark for 16 h. The $ABTS^{\cdot+}$ solution was diluted with 80% ethanol to an absorbance of 0.700 ± 0.005 at 734 nm. The filtered sample was diluted with 80% ethanol to give 20−80% inhibition of the blank absorbance with 0.1 mL of the sample. The $ABTS^{\cdot+}$ solution (3.9 mL; absorbance of 0.700 ± 0.005) was added to 0.1 mL of the tested samples and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min, and the absorbance was immediately recorded at 734 nm using a Spectronic Genesys 5 spectrophotometer (Milton Roy, NY). The trolox standard solution (final concentration of 0−15 μm) in 80% ethanol was prepared and assayed at the same conditions. The absorbance of the resulting oxidized solution was compared to that of the calibrated trolox standard. Results were expressed in terms of trolox equivalent antioxidant capacity [TEAC, mmol of trolox equivalents/100 g of dry weight (DW) of the spice powder].

HPLC Analysis. HPLC analysis was performed using a Hewlett−Packard HPLC System (HP 1100 series, Waldbronn, Germany), consisting of a binary pump and a diode-array detector (DAD) and equipped with a Nucleosil 100-C18 column (5 μm, 250 × 4 mm) with a Nucleosil 5 C18 guard column (5 μm, 4 × 4 mm) (Agilent Technologies, Loveland, CO). Phenolic compounds in the spice extracts were analyzed using our previous HPLC method (*36*) with a slight modification. The improved HPLC method was with the following gradient elution program (solution A, 2.5% formic acid, and solution B, 100% methanol): 0 min, 5% B; 15 min, 30% B; 40 min, 40% B; 60 min, 50% B; 65 min, 55% B; and 90−95 min, 100% B. The flow rate was 0.8 mL/min, and the injection volume was 20 μL. Detection was at 280 nm for flavanones, flavanols, hydroxybenzoic acids, tannins, phenolic diterpenes, and volatile compounds, at 320 nm for hydroxycinnamic acids and flavones, and at 370 nm for flavonols.

Quantification of Phenolic Compounds. Individual phenolics identified in the spice extracts, e.g., phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), phenolic diterpenes, flavonoids (flavonols, flavones, flavanols, and flavanones), volatile oils (aromatic compounds and certain monoterpenoids), were quantified using HPLC by comparison with an external standard of corresponding known phenolics and expressed as mg/100 g of DW (*37*). Standard curves were made from each corresponding standards of the known phenolics. The hydrolyzable tannins detected in clove bud extracts actually belong to gallic acid derivatives. The amount of the hydrolyzable tannins was calculated as gallic acid equivalent (mg/100 g of DW). Because the structures of several phenolic diterpenes (carnosic acid, epirosmanol, carnosol, and rosmadial) identified in this study are quite similar, the concentrations of individual phenolic diterpenes were determined with an external standard of carnosic acid and expressed as carnosic acid equivalent (mg/100 g of DW). Because of the limited commercial standards, we could not use HPLC to identify and quantify all peaks of the 26 spice extracts. However, their chemical categories could be identified from their chromatographic behavior and UV spectra. The same categories of phenolics usually have similar chromatographic behavior and UV spectral characteristics (*37*). Therefore, total amounts of unknown/unconfirmed phenolic acids, flavonoids, and their glycosides were quantified and expressed as caffeic acid and quercetin equivalents (mg/100 g of DW), respectively.

Determination of Total Phenolic Content. Total phenolic content was estimated using the Folin−Ciocalteu colorimetric method described previously (*9*, *36*) with a slight modification. Briefly, the appropriate dilutions of the filtered extracts were oxidized with 0.5 N Folin−Ciocalteu reagents, and then the reaction was neutralized with saturated

Table 1. Antioxidant Capacity, Total Phenolic Content, and Major Phenolic Compounds of Methanolic Extracts from 26 Spices[a]

family and scientific name	common name	country/ place	edible parts tested	TEAC (mmol of trolox/ 100 g of DW) [b]	total phenolic content (g of GAE/ 100 g of DW) [c]	major type (representative components) of phenolic compounds
Gramineae						
Cymbopogon citrates Stapf.	lemon grass	Hong Kong	stem	4.41 ± 0.003	0.25 ± 0.009	phenolic acids, volatile oils, flavonoids
Illiciaceae						
Illicium verum Hook. f.	star anise	China	fruit	20.30 ± 0.008	2.02 ± 0.014	phenolic acids (protocatechuic acid), phenolic volatile oils (anethole), flavonoids
Labiatae						
Mentha canadensis L.	mint	Hong Kong	leaf and branch	33.83 ± 0.016	5.15 ± 0.025	phenolic acids (caffeic acid, rosmarinic acid), volatile compounds (menthol), flavonoids
Ocimum basilicum L.	sweet basil	New Zealand	leaf	29.59 ± 0.004	3.64 ± 0.014	phenolic acids (rosmarinic acid, caffeoyl derivatives), phenolic diterpenes, volatile compounds (carvacrol), flavonoids (catechin)
Origanum vulgare L.	oregano	New Zealand	leaf	100.67 ± 0.009	10.17 ± 0.010	phenolic acids (caffeic acid, *p*-coumaric acid, rosmarinic acid, caffeoyl derivatives), volatile compounds (carvacrol), flavonoids
Rosmarinus officinalis L.	rosemary	New Zealand	leaf and branch	37.80 ± 0.021	5.07 ± 0.036	phenolic acids (caffeic acid, rosmarinic acid, caffeoyl derivatives), phenolic diterpenes (carnosic acid, carnosol, epirosmanol), volatile compounds (carvacrol), flavonoids
Salvia officinalis L.	sage	New Zealand	leaf and branch	51.89 ± 0.006	5.32 ± 0.006	phenolic acids (rosmarinic acid), phenolic diterpenes (carnosic acid), volatile compounds, flavonoids
Thymus vulgaris L.	thyme	New Zealand	leaf and branch	38.07 ± 0.003	4.52 ± 0.006	phenolic acids (gallic acid, caffeic acid, rosmarinic acid), volatile compounds (thymol), phenolic diterpenes, flavonoids
mean				48.64	5.65	
Lauraceae						
Laurus nobilis L.	bay	U.S.A.	leaf	34.29 ± 0.001	4.17 ± 0.005	phenolic acids, volatile oils (cinnamic derivatives), flavonoids
Cinnamomum cassia Presl	cinnamon	China	cortex/ bark	61.75 ± 0.014	6.34 ± 0.021	phenolic acids, phenolic volatile oils (2-hydroxycinnamaldehyde, cinnamyl aldehyde derivatives), flavan-3-ols
Cinnamomum zeylanium N.	cinnamon stick	Indonesia	cortex/ bark	107.69 ± 0.010	11.90 ± 0.004	phenolic acids, phenolic volatile oils (2-hydroxycinnamaldehyde, cinnamyl aldehyde derivatives), flavan-3-ols
mean				67.91	7.47	

Table 1 (Continued)

family and scientific name	common name	country/ place	edible parts tested	TEAC (mmol of trolox/ 100 g of DW) [b]	total phenolic content (g of GAE/ 100 g of DW) [c]	major type (representative components) of phenolic compounds
Myristicaceae						
Myristica fragrans Houtt.	nutmeg	East/West Indies	fruit	20.01 ± 0.017	1.61 ± 0.001	phenolic volatile oils, phenolic acid (caffeic acid), flavanols (catechin)
Myrtaceae						
Eugenia caryophylata Thunb.	clove	Malaysia	bud	168.66 ± 0.024	14.38 ± 0.006	phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetyl eugenol), tannins
Papaveraceae						
Papaver somniferum L.	poppy	Dutch	seed	0.55 ± 0.002	0.04 ± 0.004	not identified
Piperaceae						
Piper nigrum L.	green peppercorn	U.S.A.	fruit	11.15 ± 0.007	0.38 ± 0.003	volatile oils, phenolic amides
Piper nigrum L.	black pepper	U.S.A.	fruit	4.56 ± 0.013	0.30 ± 0.002	volatile oils, phenolic amides
Piper nigrum L.	white pepper	France	fruit	8.97 ± 0.007	0.78 ± 0.004	volatile oils, phenolic amides
mean				8.23	0.49	
Rutaceae						
Zanthoxylum bungeanum Maxim.	chinese prickly ash	China	fruit coat	36.92 ± 0.005	3.13 ± 0.004	phenolic acids, phenolic volatile oils (estragole, xanthoxylin), flavonoids
Solanaceae						
Capsicum annuum L.	chilli	Thailand	fruit	6.05 ± 0.003	0.86 ± 0.004	volatile oils, phenolic acids
Umbelliferae						
Anethum graveolens L.	dill	China	leaf and branch	6.36 ± 0.006	0.98 ± 0.009	phenolic acids (protocatechuic acid), flavonoids (catechin), volatile oils
Carum carvi L.	caraway	U.S.A.	fruit	5.50 ± 0.008	0.61 ± 0.017	volatile oils, phenolic acids, flavonoids (kaempferol), coumarins
Coriandrum sativum L.	coriander	Hong Kong	whole plant	7.02 ± 0.004	0.88 ± 0.007	phenolic acids (caffeic acid), flavonoids, volatile oils
Cuminum cyminum L.	cumin	Turkey	fruit	6.61 ± 0.002	0.23 ± 0.005	volatile oils, phenolic acids, flavonoids (kaempferol), coumarins
Petroselinum crispum L.	parsley	Hong Kong	leaf	6.31 ± 0.005	0.97 ± 0.002	phenolic acids (caffeic acid), flavonoids, volatile oils
mean				6.36	0.73	
Zingiberaceae						
Zingiber officinale Rosc.	ginger	China	rhizome	7.89 ± 0.009	0.63 ± 0.009	phenolic volatile oils (gingerol, shogaol), phenolic acids
Amomum subulatum Roxb.	green cardamom	U.S.A.	fruit	7.53 ± 0.004	0.46 ± 0.009	phenolic acids (caffeic acid), volatile oils
mean				7.71	0.55	
overall mean				31.71	3.26	
LSD ($p < 0.05$)[d]				10.31	0.22	

[a] All values were the mean of three measurements and expressed as mean ± SD. [b] TEAC, trolox equivalent antioxidant capacity. Data expressed as mmol of trolox equivalent/100 g of dry weight (DW). [c] Total phenolic content expressed as g of GAE/100 g of dry weight (DW). [d] LSD ($p < 0.05$), least significant difference, was used for difference comparison among means of various spices.

sodium carbonate (75 g/L). The absorbance of the resulting blue color was measured at 760 nm with a spectrophotometer after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as g of gallic acid equivalent (GAE)/100 g of DW.

Statistical Analysis. All determinations were conducted in triplicate, and all results were calculated as mean ± standard deviation (SD) in this study. Differences between means of data were compared by least significant difference (LSD) calculated using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Comparison of Total Antioxidant Capacity and Total Phenolic Content. Scavenging of different types of reactive oxygen species, mostly free radicals, is thought to be one of the main mechanisms of antioxidant action exhibited by phenolic phytochemicals. The synthetic nitrogen-centered $ABTS^{\cdot+}$ radical is not biologically relevant but is often used as an "indicator compound" in testing hydrogen-donation capacity and thus antioxidant activity (*35*). The total antioxidant capacity assay was conducted to systematically evaluate the ability of 26 spice extracts to scavenge free radicals *in vitro* by the improved $ABTS^{\cdot+}$ method in this study.

Total antioxidant capacity (TEAC) and phenolic content of 26 spice extracts (**Table 1**) indicated very wide variation. Their TEAC mean value was 31.7 mmol/100 g, with clove exhibiting the strongest radical scavenging activity (168.7 mmol/100 g of DW), while poppy demonstrated the lowest activity (0.55 mmol/100 g of DW). Cinnamon stick and oregano also had very strong activity (107.7 and 100.7 mmol/100 g of DW). Other spices with relatively high activity were cinnamon (61.8 mmol/100 g of DW), sage (51.9 mmol/100 g of DW), thyme (38.1 mmol/100 g of DW), rosemary (37.8 mmol/100 g of DW), Chinese prickly ash (36.9 mmol/100 g of DW), bay (34.3 mmol/100 g of DW), and mint (33.8 mmol/100 g of DW). The spices with slightly lower activity than the mean were sweet basil (29.6 mmol/100 g of DW), star anise (20.3 mmol/100 g of DW), and nutmeg (20.0 mmol/100 g of DW). However, coriander, parsley, dill, lemon grass, green cardamom, chilli, caraway, cumin, green peppercorn, black pepper, and white pepper showed quite low antioxidant capacity (between 4 and 11 mmol/100 g of DW).

Total phenolic content of the 26 tested spices also showed significant variation, ranging from 0.04 to 14.38 g of GAE/100 g of DW, with an overall mean of 3.26 g of GAE/100 g (**Table 1**). Clove had the highest level of phenolics, and poppy had the lowest. Cinnamon stick and oregano also contained very high levels of phenolics (11.90 and 10.17 g of GAE/100 g of DW, respectively). Other spices with high levels of phenolics were cinnamon (6.34 g of GAE/100 g), sage (5.32 g of GAE/100 g), mint (5.15 g of GAE/100 g), rosemary (5.07 g of GAE/100 g), thyme (4.52 g of GAE/100 g), bay (4.17 g of GAE/100 g), and sweet basil (3.64 g of GAE/100 g). Star anise and nutmeg contained relatively low phenolics (2.02 and 1.61 g of GAE/100 g), whereas in lemon grass, poppy, green peppercorn, black pepper, white pepper, chilli, dill, caraway, coriander, cumin, parsley, and green cardamom extracts it was quite low (0.04−0.98 g of GAE/100 g of DW). This result basically coincided with those of total antioxidant capacity. In other words, the spice extract samples that had high antioxidant activity showed a tendency to have high phenolic content.

Among 12 families tested in this study, Myrtaceae (with only one tested species, i.e., clove), Lauraceae (three tested species), and Labiatae (six tested species, respectively) showed high mean antioxidant capacity (168.7, 67.9, and 48.6 mmol/100 g, respectively) and contained high levels of phenolics (14.38, 7.47, and 5.65 g of GAE/100 g, respectively). Total antioxidant capacity and phenolic content of Rutaceae (only one tested species) was similar to the mean values of 26 spices (31.7 mmol/100 and 3.26 g of GAE/100 g). However, total antioxidant capacity and phenolic content mean values of the other eight families were significantly lower (**Table 1**). Umbelliferae and Piperaceae include many common spice plants, e.g., dill, coriander, cumin, parsley, and various kinds of peppers. Some previous researchers reported that spices in the Umbelliferae and Piperaceae possessed a strong antioxidant effect (*23*, *38*, *39*). However, they did not compare with spices from other families. In the present study, it was found that all tested spices in the Umbelliferae (five species) and Piperaceae (three species) demonstrated weaker antioxidant capacity (mean = 6.4 and 8.2 mmol/100 g) and lower levels of phenolics (mean = 0.73 and 0.49 g of GAE/100 g) than the spices from Myrtaceae, Lauraceae, and Labiatae.

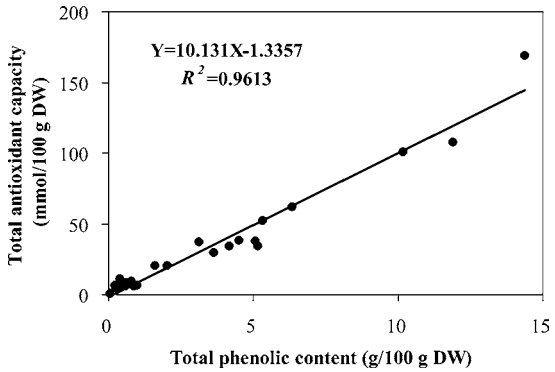

Figure 1. Relationship between the total antioxidant capacity and total phenolic content of methanolic extracts from 26 spices.

There have been extensive studies on antioxidant activity of many spices in the Labiatae (*4*, *9*, *22*, *27*, *30*, *40*). The most common spices in this family are the six (i.e., rosemary, oregano, sage, basil, mint, and thyme) tested in this study. Both our and previous studies demonstrated that spices in the Labiatae overall had very strong antioxidant capacity. Some researchers found rosemary to possess the strongest antioxidant effect, but others found sage or oregano and basil. Our comparative results of the six spices in the Labiatae indicated that their total antioxidant capacity and phenolic content decreased in the following order: oregano > sage > thyme > rosemary > mint > sweet basil. Oregano exhibited the most powerful antioxidant capacity among the five Labiatae spices, over 3-fold greater than sweet basil. The significant differences between different studies were likely due to (1) genotypic and environmental differences within species, (2) choice of parts tested, (3) time of taking samples, and (4) determination methods.

Previous studies also showed that clove (in the Myrtaceae) had a very strong antioxidant activity and a high level of phenolics (*24*, *41*). The various antioxidant mechanisms of clove bud extracts were attributed to a strong hydrogen-donating ability, a metal chelating ability, and their effectiveness as good scavengers of hydrogen peroxide, superoxide, and free radicals. Our results showed that the clove bud extract was the most powerful phenolic antioxidant and exhibited the strongest radical scavenging activity among the 26 spices. Additionally, two cinnamon species in the family Lauraceae were tested in this study, i.e., *Cinnamomum cassia* was from China and *Cinnamomum zeylanium* from Indonesia. These two cinnamon species also had very high antioxidant capacity. However, their total phenolic content and antioxidant capacity existed a significant difference (**Table 1**), which was possibly affected by genetic and environmental differences.

Relationship between Total Antioxidant Capacity and Total Phenolic Content. From **Table 1**, the statistical analysis of 26 spice extracts showed 307-fold (168.66/0.55) and 360-fold (14.38/0.04) differences in total antioxidant capacity (mmol TEAC/100 g of DW) and phenolic content (g of GAE/100 g of DW), respectively. Correlation between total antioxidant capacity (Y) and phenolic content (X) was established as an equation ($Y = 10.131X - 1.3357$) (**Figure 1**), and a highly significant linear correlation ($R^2 = 0.9613$) was obtained. Such high R^2

value suggested that the ABTS$^{\cdot+}$ radical scavenging activity could be credibly predicted on the basis of the Folin−Ciocalteu assay for total phenolic content and directly confirmed that the phenolic compounds in the 26 spices were responsible for their antioxidant capacity. The results emphasized the importance of phenolic compounds in the antioxidant behavior of spice extracts and also indicated that the phenolic compounds contributed significantly to the total antioxidant capacity. The following identification and analysis of phenolic compounds further explained the relationships between structure and activity.

The relationships between total phenolic content and antioxidant properties of many plants (e.g., common vegetables, fruits, and medicinal herbs) were investigated in previous studies (*9*, *10*, *30*, *31*, *36*). Some studies obtained good positive linear correlation, but others got poor linear correlation or even could not explain the relationship between total antioxidant activity and phenolic content. Our experience and studies (*36*) indicated that the correlative relationship was closely associated with the number of the tested samples and the ranges of the values for total phenolic content and antioxidant activity and also influenced by different assay methods. Several tested samples and very small differences between the highest and lowest values obtained were not easy to get good correlations between antioxidant activity and phenolic content. This may partly explain the reason that the poor correlations between antioxidant activity and total phenolics were obtained in certain previous studies.

Qualitative and Quantitative Determination and Analysis of Phenolic Constituents. Different phenolic compounds normally possess specific chromatographic behavior and UV−vis spectral characteristic. Major phenolic compounds in the spice plants tested in this study were preliminarily and systematically identified using RP-HPLC with DAD by comparison with authentic phenolic standards and relative literature data (*36*, *37*, *42*). Major chemical classes and representative constituents of phenolic compounds identified in this study are summarized in **Table 1** and mainly include phenolic acids, flavonoids, phenolic diterpenes, aromatic volatile oils, and tannins. **Figure 2** displays chemical structures of major phenolics identified in the spice plants. **Table 2** shows quantitative analysis results of major individual phenolics identified in different spice extracts.

The spice plants in the Labiatae contain many secondary metabolites, such as flavonoids, phenolic terpenoids, hydroxybenzoic acids, and hydroxycinnamic acids (*22*, *23*, *25*, *30*, *43*, *44*). **Figure 3** displays HPLC profiles of methanolic extracts from six spice plants in the Labiatae. According to the UV spectra, retention time (R_t), and authentic standards, the peaks **1**−**15** in **Figure 3** were identified as gallic acid (**1**), catechin (**2**), caffeic acid (**3**), *p*-coumarin acid (**4**), caffeoyl derivatives (**5** and **6**), rosmarinic acid (**7**), eugenol (**8**), epirosmanol (**9**), carnosol (**10**), thymol (**11**), carvacrol (**12**), rosmadial (**13**), carnosic acid (**14**), and kaempferol (**15**). Although a major peak (oregano) and other minor peaks (after $R_t = 53$ min) were not yet completely identified by relative authentic standards, they might be other phenolic diterpenes, flavonoids (flavonones and flavonols), and aromatic volatile compounds, which were confirmed by our obtained chromatographic and UV spectral data and the analytical characteristics of phenolics reported by Cuvelier et al. (*44*), Cai et al. (*36*), Sakakibara et al. (*42*), and Santos-Buelga and Williamson (*37*).

Figure 3 shows that all six spice extracts in the Labiatae had a large peak (**7**, rosmarinic acid). **Table 2** shows that all tested spices in the Labiatae contained very high concentrations

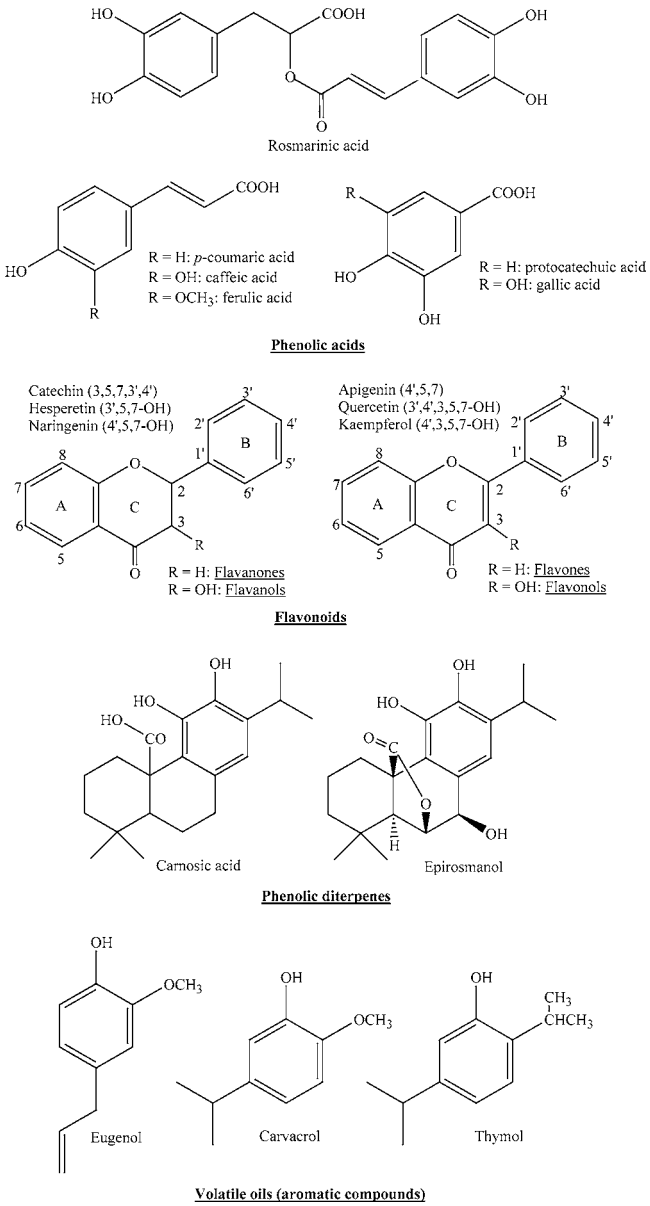

Figure 2. Structures of major phenolic compounds identified in the spices.

of rosmarinic acid, mostly ranging from 1086 to 2563 mg/100 g of DW. This indicated that rosmarinic acid was the dominant phenolic compound in the Labiatae spices. Rosmarinic acid has two *ortho*-dihydroxy groups (catechol structures) (**Figure 2**), which is the most important structural feature for strong antioxidant activity in phenolic compounds. Our results agreed with those of Cuvelier et al. (*44*), Exarchou et al. (*32*), and Zheng and Wang (*9*) who reported that rosmarinic acid was the most abundant phenolic compound identified in the acetone extract of sage, oregano, and thyme. The spice extracts rich in rosmarinic acid had higher radical scavenging activity. In this study, phenolic acids were identified from most of Labiatae spice extracts, such as gallic acid, caffeic acid, and *p*-coumaric acid. We also identified high levels of caffeoyl derivatives (peaks **5** and **6**) in oregano (1324 mg/100 g of DW), sweet basil (380 mg/100 g of DW), and rosemary (278 mg/100 g of DW). Because the structures of caffeoyl derivatives are close to rosmarinic acid, they have similar potent radical scavenging activity. A very high level of caffeoyl derivatives (1324 mg/100 g of DW) and rosmarinic acid (2563 mg/100 g of DW) made oregano exhibit the most powerful activity among the five tested Labiatae spices. Caffeic acid and gallic acid also have

Table 2. Quantitative Analysis of Major Phenolic Compounds Identified in Different Spices (mg/100 g of Dry Weight)[a]

phenolic compounds	mint	sweet basil	oregano	rosemary	sage	thyme	clove	dill	caraway	coriander	cumin	parsley
gallic acid						37.5	783.5					
gallic acid derivatives[b]							2375.8					
protocatechuic acid		41.5						77.9		13.7		
catechin	147.0	107.6		254.9	257.1			144.9				
caffeic acid	27.1	30.4	50.0	40.1	121.5	54.8			16.4	22.2	16.6	103.7
p-coumaric acid		27.9	214.8		40.0	55.9						
caffeoyl derivatives		380.4	1324.2	278.1								
rosmarinic acid	1908.5	1086.1	2562.7	1286.4	2186.1	681.1						
other phenolic acids[c]	84.2	165.9	208.5	98.5	387.4	136.0		332.4	81.1	229.0	61.0	72.5
eugenol		189.6					9381.7					
acetyl eugenol							2075.1					
epirosmanol		142.9		1113.0								
carnosol				801.6								
thymol						591.4						
carvacrol		111.6	108.3									
rosmadial				277.3	462.6							
carnosic acid				655.2	273.8							
quercetin							28.4					
kaempferol			32.9		63.9		23.8		16.4		38.6	
other flavonoids[c]	23.2	21.0	51.3	37.8	20.5	41.3	366.5	241.2	77.2	167.2	171.9	203.0

phenolic compounds	bay	cinnamon	cinnamon stick	chinese prickly ash	nutmeg	star anise	lemon grass	ginger	green cardamom	green peppercorn	black pepper	white pepper
catechin (derivatives)		1057.7	454.4		28.8							
protocatechuic acid						32.2						
caffeic acid		15.3	24.2		16.3	20.2		15.5	15.9			
phenolic amides										385.2	339.6	555.7
other phenolic acids[c]	181.6			190.2			85.3	25.3	22.0			
cinnamyl aldehydes		17 109.1	16 162.3									
anethole						5407.9						
gingerol								187.3				
estragole				5288.3								
other flavonoids[c]	735.8			540.5			31.2					

[a] Major individual phenolics were quantified using HPLC by comparison with external standards of corresponding known phenolics. [b] Data of gallic acid derivatives (hydrolyzable tannins) were expressed as GAE. [c] The trace amounts of known/identified phenolic acids (e.g., chlorogenic acid, ferulic acid, and vanillic acid), flavanones (e.g., hesperetin and naringenin), flavones (e.g., apigenin and luteolin), flavonols (e.g., quercetin and quercitrin), and the large amounts of unknown/unconfirmed phenolic acids and flavonoids were calculated into total amounts of "other phenolic acids" and "other flavonoids" and expressed as caffeic acid and quercetin equivalents, respectively. The content of known/identified volatile oils (e.g., anethole, estragole, and cinnamyl aldehydes) were calculated as eugenol equivalent. Total amounts of other unknown/unidentified volatile oils were not calculated.

catechol structure and can exhibit high activity, but their contents were low, ranging from 27 to 122 mg/100 g of DW (**Table 2**).

Additionally, the total antioxidant capacity of the Labiatae spice extracts should be partly due to the presence of other phenolic compounds, such as phenolic diterpenes and aromatic volatile compounds. According to our chromatographic/UV spectra data and by comparison with authentic standard and literature (*44*), a number of phenolic diterpenes were identified in the Labiatae spices, i.e., carnosic acid, rosmanol, carnosol, and epirosmanol. Especially, rosemary and sage contained high levels of phenolic diterpenes. Total amounts of the phenolic diterpenes in rosemary and sage extracts were 2847 and 736 mg/100 g of DW, respectively (**Table 2**). The presence of a catechol structure (*ortho*-dihydroxy groups) in the aromatic ring of phenolic diterpene skeleton (e.g., carnosic acid and epirosmanol) (**Figure 2**) is also an important structural element for high antioxidant activity of the Labiatae spice extracts. Also, we detected phenolic volatile oils, such as some aromatic compounds (e.g., thymol, eugenol, and carvacrol) in the extracts, especially a high level of thymol (591 mg/100 g of DW) identified in thyme extract (peak **11** in **Figure 3**). The aromatic compounds with only one hydroxyl group (**Figure 2**) had some radical scavenging activity, but their contribution to total antioxidant capacity was limited. The flavonoids identified were also minor components in the Labiatae spices, e.g., flavones (luteolin, apigenin, and its glycosides), flavanones (naringenin

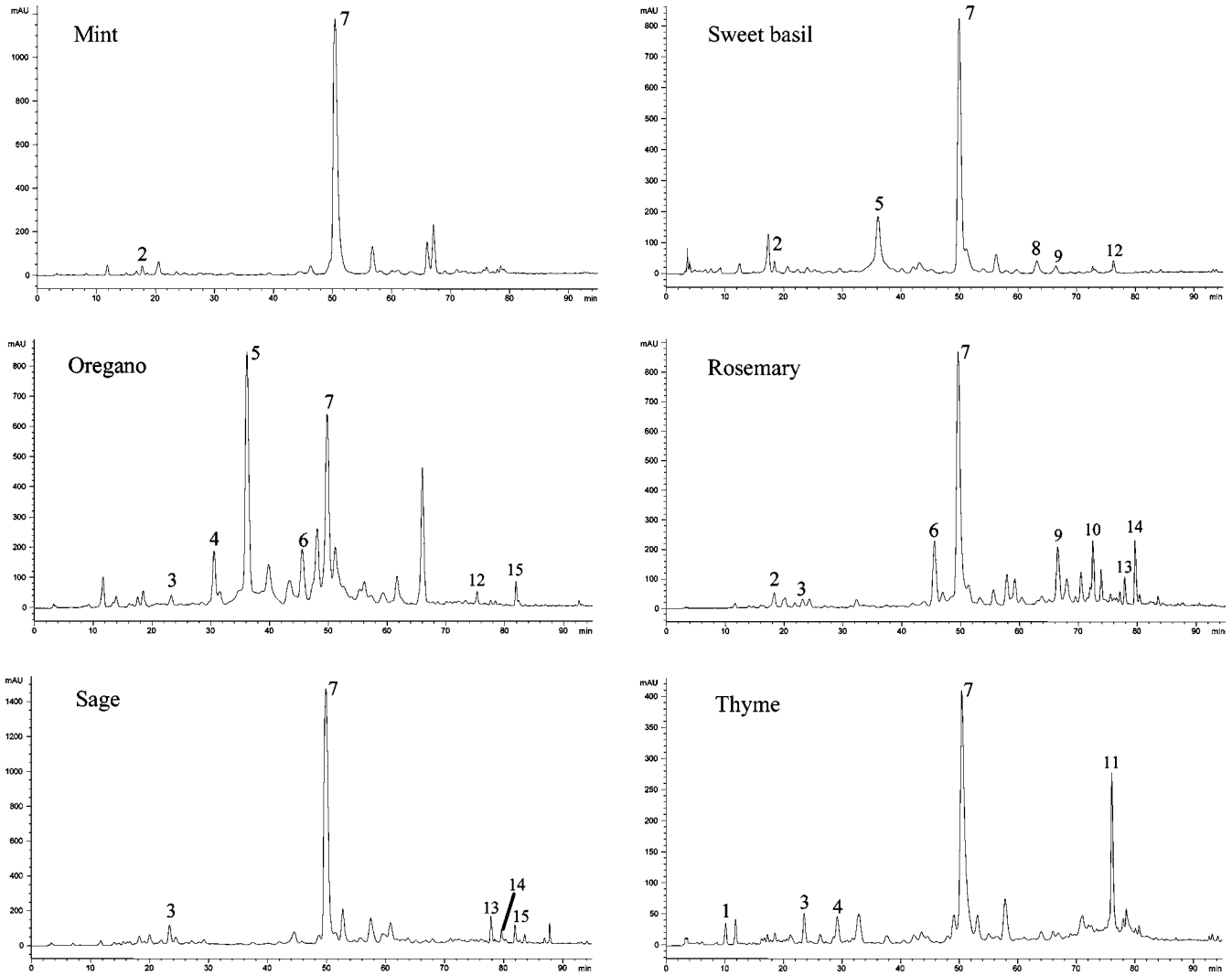

Figure 3. HPLC profiles (280 nm) of methanolic extracts from six spice plants in the family Labiatae. **1**, gallic acid; **2**, catechin; **3**, caffeic acid; **4**, *p*-coumaric acid; **5**, caffeoyl derivative I; **6**, caffeoyl derivative II; **7**, rosmarinic acid; **8**, eugenol; **9**, epirosmanol; **10**, carnosol; **11**, thymol; **12**, carvacrol; **13**, rosmadial; **14**, carnosic acid; and **15**, kaempferol.

and its glycosides), and flavanols (catechin). The concentrations of the flavonoids in most spices were relatively low (**Table 2**). Although some flavonoids (e.g., flavanols) are potent antioxidants, the identified flavonoids had a rather small contribution to the total antioxidant capacity of the spice extracts because of their low concentrations.

Clove belongs to the family Papaveraceae. The HPLC analysis (**Figure 4**) showed that a large number of volatile oils (aromatic compounds) and flavonoids were present in the clove bud extract. Major peaks (**3** and **4**) and minor peaks (**6** and **8**) at 370 nm were identified as flavonol glycosides (**3** and **4**), quercetin (**6**), and kaempferol (**8**). Major peaks (**5** and **7**) at 280 nm were identified as eugenol (**5**) and acetyl eugenol (**7**). In addition, peak **1** was easily identified as gallic acid (**1**) using its authentic standard. Clove buds also contained tannin components. According to our experience and previous study (*36*), many dense peaks between 14 and 25 min (peak **2**) obtained under our chromatographic conditions (at 280 nm in **Figure 4**) were typical traits of gallic acid derivatives, i.e., hydrolyzable tannin components. **Table 2** shows that clove bud extracts had high levels of gallic acid (784 mg/100 g of DW) and its derivatives (tannins, 2376 mg/100 g of DW) and some flavonoids (419 mg/100 g of DW). Molecules of gallic acid, flavonols, and hydrolyzable tannins possess many hydroxyl groups, especially *ortho*-dihydroxy groups (catechol structure) with potent radical scavenging activity. Therefore, they contributed significantly to the highest antioxidant activity of clove buds in this study. Eugenol and its derivative normally contain one hydroxyl group (**Figure 2**) and have relatively lower radical scavenging activity than other phenolics with more hydroxyl groups. However, there were very high levels of volatile oils (eugenol, 9382 mg/100 g of DW; and acetyl eugenol, 2075 mg/100 g of DW) in clove bud extracts (**Table 2**). Thus, eugenol and its derivative also made an important positive contribution to the antioxidant activity of clove buds.

For the Lauraceae family spices, major phenolic constituents in three tested spices were identified as phenolic volatile oils (cinnamaldehyde and its derivatives), flavan-3-ols (catechin derivatives), and phenolic acids, according to literature (*3*) and by comparison with the related UV spectral characteristics and chromatographic behavior. **Table 2** and HPLC profiles (major peaks) of two cinnamon extracts (not shown) revealed that 2-hydroxycinnamaldehyde and cinnamaldehyde derivatives were dominant aromatic components, and phenolic acids were minor components in these two spices. Two cinnamon spices contained much higher levels of volatile oils (aromatic components) and catechin derivatives (mean = 756 mg/100 g of DW) (**Table 2**) compared with bay leaf extract. The identified catechin and

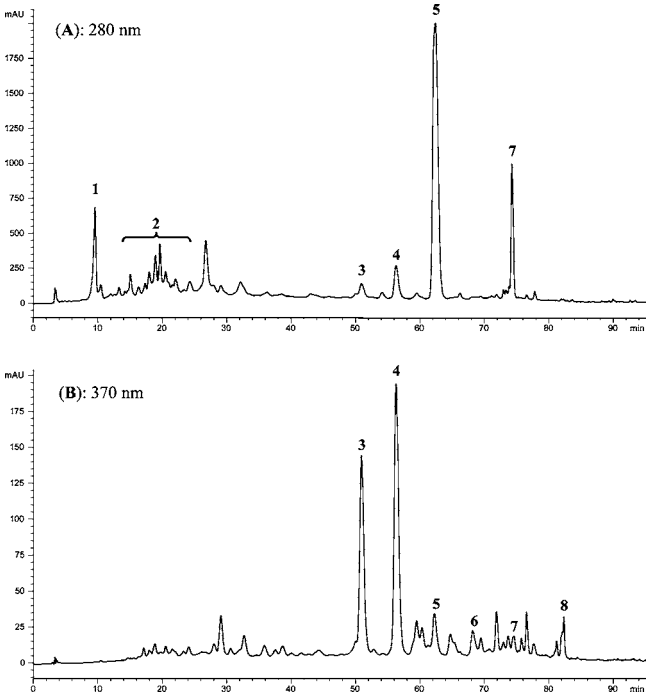

Figure 4. HPLC–DAD profiles of methanolic extract from clove (*Eugenia caryophylata* Thunb.) at 280 and 370 nm. **1**, gallic acid; **2**, tannin constituents; **3** and **4**, flavonol glucosides; **5**, eugenol; **6**, quercetin; **7**, acetyl eugenol; and **8**, kaempferol.

aromatic components contained hydroxyl groups; thus, they played the most important role in enhancing radical scavenging activity of the Lauraceae spices. Furthermore, some flavonoid components (736 mg/100 g of DW) were identified in bay leaf extract, but their structures were not yet elucidated. Cinnamon barks (*Cinnamomum cassia*) usually contained tannin components (*3*), but we could not isolate and identify them under our chromatographic conditions. Because the cinnamon extracts had very high levels of phenolics and strong activity, tannins (the strongest radical scavenger among all natural phenolic compounds) possibly occurred in the cinnamon barks.

For several Chinese traditional spices, star anise, Chinese prickly ash, and nutmeg extracts were also identified to have very high levels of phenolic volatile oils as main active ingredients, such as anethole (5408 mg/100 g of DW) in star anise and estragole (5288 mg/100 g of DW) and other volatile oils in Chinese prickly ash. The flavonoids were clearly detected in Chinese prickly ash extract (541 mg/100 g of DW) but not in star anise and nutmeg extracts. Furthermore, Chinese prickly ash contained more phenolic acids (190 mg/100 g of DW) than star anise and nutmeg. Therefore, Chinese prickly ash extract had a much higher antioxidant capacity (36.9 mmol/100 g of DW) than star anise (20.3 mmol/100 g of DW) and nutmeg (20.0 mmol/100 g of DW).

Black, white, and green peppers in the Piperaceae are three different forms of pepper products. Green pepper is obtained from unripe fully matured berries. Black pepper is produced by conventional sun-drying of mature green pepper berries. HPLC analysis showed that these three spice extracts had similar peak profiles. In comparison with the related UV spectral characteristics and chromatographic behavior, major peaks were identified as volatile compounds (principal components) and minor peaks were identified as a trace amount of phenolic acids, e.g., protocatechuic, vanillic, caffeic, and ferulic acids. **Table 2** shows that some phenolic amides were also identified in three pepper extracts (mean = 427 mg/100 g of DW). From **Table 1**, total phenolic contents of three pepper extracts were relatively lower and their total antioxidant capacities were weaker. This suggested that the principal components in the three extracts belonged to nonphenolic volatile compounds, which had weak radical scavenging activity, not like the phenolic volatile compounds in the clove, cinnamon, star anise, nutmeg, and Chinese prickly ash. According to $LSD_{0.05}$ values, the differences in total antioxidant capacity of the three pepper extracts were not significant but the differences in their total phenolic contents existed. It was also reported that phenolic acid glycosides and flavanol glycosides were isolated and identified in the peppers (*45*, *46*). However, those minor components were not identified from crude methanolic pepper extracts at our chromatographic conditions. Additionally, the volatile oils in the pepper extracts were not well-isolated and identified by RP-HPLC.

Five spice extracts of the family Umbelliferae were identified by cochromatography with authentic standards and by comparison with literature data (*36*, *41*, *42*). It was found that they mainly contained phenolic acids, flavonoids, and volatile compounds. These identified phenolic categories were similar to those in the Labiatae spices. The five Umbelliferae spice extracts contained more flavonoids (mean = 183 mg/100 g of DW) than the six Labiatae spices (mean = 49 mg/100 g of DW) (**Table 2**). However, their total antioxidant capacity and total phenolic contents were significantly lower than those of the Labiatae. It was due to the fact that (1) the five spice extracts in the Umbelliferae were found not to have rosmarinic acid, with very powerful antioxidant activity, which was present in significant amounts in the Labiatae spices, (2) phenolic diterpenes were not detected in the Umbelliferae spices, and (3) the volatile oils identified in the Umbelliferae spices might be nonphenolic volatile compounds, which have no radical scavenging activity or very low activity. Additionally, a trace amount of coumarins were detected in caraway and cumin, but their contribution to total antioxidant capacity was negligible.

Ginger and chilli are popular spices. Low levels of phenolic acids were detected in both their extracts. Major peaks of HPLC profiles were phenolic volatile oils (pungent components). Gingerol and shogaol (gingerol analogues) were the principal pungent components in ginger extract (*36*), while capsaicin and capsaicinol (capsaicinoids) were the main pungent substances in chilli extract (*46*). However, major peaks of chilli extract were not well-separated at our chromatographic conditions.

In comparison with authentic standards and related literature, we used RP-HPLC to simultaneously identify and quantify a limited number of known phenolic compounds and major phenolic categories from 26 crude spice extracts. However, the spice extracts contain complex phenolic compounds, including high levels of volatile oils. Although RP-HPLC can be used for isolation, identification, and quantification of phenolic volatile compounds (e.g., phenolic terpenoids and aromatic constituents), GC and GC–MS are the best analytical tools for identification and quantification of volatile compounds. Thus, further identification and quantification of other unidentified/unknown phenolic constituents (especially volatile oils) in the spice extracts and their structural elucidation are warranted through a multitude of characterization approaches including chromatographic methods coupled with spectroscopic and structural techniques (GC, GC–MS, LC–MS, and NMR).

Our results showed that many spices were rich in phenolic constituents and demonstrated good antioxidant capacity. Qualitative and quantitative analysis of major individual phenolics in the spices could be helpful for revealing the structure–activity relationships of antioxidant phenolics in the spices and also

useful for explaining the relationships between total antioxidant capacity and total phenolic contents in the spices. Through our systematically comparative study of 26 spices, some spices with high level of phenolics, such as clove, cinnamon, oregano, sage, thyme, and rosemary, were screened to use as excellent free-radical scavengers and potent natural phenolic antioxidants for commercial exploration.

LITERATURE CITED

(1) Srinivasan, K. Role of spices beyond food flavoring: Nutraceuticals with multiple health effects. *Food Rev. Int.* **2005**, *21*, 167−188.

(2) Kumar, N. *Introduction to Spices, Plantation Crops, Medicinal and Aromatic Plants*; Oxford and IBH Publishing: New Delhi, India, 1997.

(3) Gao, X. M.; Xu, Z. M.; Li, Z. W. *Traditional Chinese Medicines*; People's Health Publishing House: Beijing, China, 2000; p 2019.

(4) Pizzale, L.; Bortolomeazzi, R.; Vichi, S.; Uberegger, E.; Conte, L. S. Antioxidant activity of sage (*Salvia officinalis* and *S. fruticosa*) and oregano (*Origanum onites* and *O. indercedens*) extracts related to their phenolic compound content. *J. Sci. Food Agric.* **2002**, *82*, 1645−1651.

(5) Dorman, H. J. D.; Deans, S. G. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* **2000**, *88*, 308−316.

(6) Sokovic, M.; Tzakou, O.; Pitarokili, D.; Couladis, M. Antifungal activities of selected aromatic plants growing wild in Greece. *Nahrung* **2002**, *46*, 317−320.

(7) Aruoma, O. I.; Spencer, J. P. E.; Rossi, R.; Aeschbach, R.; Khan, A.; Mahmood, N.; Munoz, A.; Murcia, A.; Butler, J.; Halliwell, B. An evaluation of the antioxidant and antiviral action of extracts of rosemary and provencal herbs. *Food Chem. Toxicol.* **1996**, *34*, 449−456.

(8) Lampe, J. W. Spicing up a vegetarian diet: Chemopreventive effects of phytochemicals. *Am. J. Clin. Nutr.* **2003**, *78*, 579S−583S.

(9) Zheng, W.; Wang, S. Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **2001**, *49*, 5165−5170.

(10) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.

(11) Shobana, S.; Naidu, K. A. Antioxidant activity of selected Indian spices. *Prostaglandins Leukotrienes Essent. Fatty Acids* **2000**, *62*, 107−110.

(12) Madsen, H. L.; Bertelsen, G. Spices as antioxidants. *Trends Food Sci. Technol.* **1995**, *6*, 271−277.

(13) Kahkonen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J. P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954−3962.

(14) Rice-Evans, C. A.; Miller, J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152−159.

(15) Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Res.* **1995**, *22*, 375−383.

(16) Luiz, M.; Biasutti, A.; Garcia, N. A. Micellar effect on the scavenging of singlet molecular oxygen by hydroxybenzenes. *Redox Rep.* **2002**, *7*, 23−28.

(17) Chen, X. Y.; Ahn, D. U. Antioxidant activities of six natural phenolics against lipid oxidation induced by Fe^{2+} or ultraviolet light. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1717−1721.

(18) Cos, P.; Ying, L.; Calomme, M.; Hu, J. P.; Cimanga, K.; van Poel, B.; Pieters, L.; Vlietinck, A. J.; Vanden Berghe, D. Structure−activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J. Nat. Prod.* **1998**, *61*, 71−76.

(19) Laughton, M. J.; Evans, P. J.; Moroney, M. A.; Hoult, J. R. S.; Halliwell, B. Inhibition of mammalian 5-lipoxygenase and cyclooxygenase by flavonoids and phenolic dietary additives—Relationship to antioxidant activity and to iron lon-reducing ability. *Biochem. Pharmacol.* **1991**, *42*, 1673−1681.

(20) Yanishlieva, N. V.; Marinova, E. M. Stabilisation of edible oils with natural antioxidants. *Eur. J. Lipid Sci. Technol.* **2001**, *103*, 752−767.

(21) Badei, A. Z. M.; El-Akel, A. T. M.; Faheid, S. M. M.; Mahmoud, B. S. M. Application of some spices in flavoring and preservation of cookies: 1-Antioxidant properties of cardamom, cinnamon, and clove. *Dtsch. Lebensm.-Rundsch.* **2002**, *98*, 176−183.

(22) Cuvelier, M. E.; Berset, C.; Richard, H. Antioxidant constituents in sage (*Salvia officinalis*). *J. Agric. Food Chem.* **1994**, *42*, 665−669.

(23) Melo, E. D.; Mancini, J.; Guerra, N. B. Characterization of antioxidant compounds in aqueous coriander extract (*Coriandrum sativum* L.). *Lebensm.-Wiss. Technol.-Food Sci. Technol.* **2005**, *38*, 15−19.

(24) Gulcin, I.; Sat, I. G.; Beydemir, S.; Elmastas, M.; Kufrevioglu, O. I. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb.) buds and lavender (*Lavandula stoechas* L.). *Food Chem.* **2004**, *87*, 393−400.

(25) Aruoma, O. I.; Halliwell, B.; Aeschbach, R.; Loligers, J. Antioxidant and prooxidant properties of active rosemary constituents—Carnosol and carnosic acid. *Xenobiotica* **1992**, *22*, 257−268.

(26) Frankel, E. N.; Huang, S. W.; Aeschbach, R.; Prior, E. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *J. Agric. Food Chem.* **1996**, *44*, 131−135.

(27) Lamaison, J. L.; Petitjeanfreytet, C.; Carnat, A. Medicinal Lamiaceae with antioxidative activity, potential sources of rosmarinic acid. *Pharm. Acta Helv.* **1991**, *66*, 185−188.

(28) Grayer, R. J.; Bryan, S. E.; Veitch, N. C.; Goldstone, F. J.; Paton, A.; Wollenweber, E. External flavones in sweet basil, *Ocimum basilicum*, and related taxa. *Phytochemistry* **1996**, *43*, 1041−1047.

(29) Schwarz, K.; Ternes, W. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. 2. Isolation of carnosic acid and formation of other phenolic diterpenes. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 99−103.

(30) Dorman, H. J. D.; Bachmayer, O.; Kosar, M.; Hiltunen, R. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. *J. Agric. Food Chem.* **2004**, *52*, 762−770.

(31) Dorman, H. J. D.; Kosar, M.; Kahlos, K.; Holm, Y.; Hiltunen, R. Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *J. Agric. Food Chem.* **2003**, *51*, 4563−4569.

(32) Exarchou, V.; Nenadis, N.; Tsimidou, M.; Gerothanassis, I. P.; Troganis, A.; Boskou, D. Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage, and summer savory. *J. Agric. Food Chem.* **2002**, *50*, 5294−5299.

(33) Ninfali, P.; Mea, G.; Giorgini, S.; Rocchi, M.; Bacchiocca, M. Antioxidant capacity of vegetables, spices, and dressings relevant to nutrition. *Br. J. Nutr.* **2005**, *93*, 257−266.

(34) Miura, K.; Kikuzaki, H.; Nakatani, N. Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *J. Agric. Food Chem.* **2002**, *50*, 1845−1851.

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

(36) Cai, Y. Z.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* **2004**, *74*, 2157−2184.

(37) Santos-Buelga, C.; Williamson, G. *Methods in Polyphenol Analysis*; Royal Society of Chemistry: Cambridge, U.K., 2003; p 383.

(38) Duthie, G. G. Parsley, polyphenols, and nutritional antioxidants. *Br. J. Nutr.* **1999**, *81*, 425−426.
(39) Singh, G.; Marimuthu, P.; Catalan, C.; deLampasona, M. P. Chemical, antioxidant, and antifungal activities of volatile oil of black pepper and its acetone extract. *J. Sci. Food Agric.* **2004**, *84*, 1878−1884.
(40) Koleva, I. I.; Linssen, J. P. H.; van Beek, T. A.; Evstatieva, L. N.; Kortenska, V.; Handjieva, N. Antioxidant activity screening of extracts from *Sideritis* species (Labiatae) grown in Bulgaria. *J. Sci. Food Agric.* **2003**, *83*, 809−819.
(41) Lee, K. G.; Shibamoto, T. Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. et Perry]. *Food Chem.* **2001**, *74*, 443−448.
(42) Sakakibara, H.; Honda, Y.; Nakagawa, S.; Ashida, H.; Kanazawa, K. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. *J. Agric. Food Chem.* **2003**, *51*, 571−581.
(43) Masuda, T.; Inaba, Y.; Takeda, Y. Antioxidant mechanism of carnosic acid: Structural identification of two oxidation products. *J. Agric. Food Chem.* **2001**, *49*, 5560−5565.
(44) Cuvelier, M. E.; Richard, H.; Berset, C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *J. Am. Oil Chem. Soc.* **1996**, *73*, 645−652.
(45) Vosgen, B.; Herrmann, K. Flavonol glycosides of pepper *Piper nigrum* L., clove [S*yzygium aromaticum* (L.) Merr. Et Perry], and allspice [*Pimenta dioica* (L.) Merr.]. 3. Phenolics of spices. *Z. Lebensm. Unters. Forsch.* **1980**, *170*, 204−207.
(46) Nakatani, N. Natural antioxidants from spices. In *Phenolic Compounds in Food and Their Effects on Health*; Huang, M. T., Lee, C. Y., Ho, C. T., Eds.; American Chemical Society: Washington, DC, 1992; Vol. 2, pp 72−97.

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