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Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf

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Abstract

Black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf were extracted with 50% acetone and 80% methanol, and evaluated for their radical-scavenging activities against cation ($ABTS^{\cdot+}$), $DPPH^{\cdot}$, peroxyl (ORAC) and hydroxyl ($HO^{\cdot}$) radicals. For each extract, total phenolic content (TPC) and chelating activity were also determined. The extracts of all botanical samples showed significant radical-scavenging capacities, TPC and chelating abilities. The 50% acetone extract of cinnamon had the highest $ABTS^{\cdot+}$-scavenging capacity of 1243 μmol TE/g and the greatest ORAC value of 1256 μmol TE/g on a per weight basis. The 50% acetone extracts of black peppercorn and cinnamon showed higher $ABTS^{+}$-scavenging, ORAC, Fe^{+2} chelating ability and TPC value, but lower $DPPH^{\cdot}$ value than the corresponding 80% methanol extracts. The 80% methanol extract of nutmeg had greater $ABTS^{+}$, ORAC and TPC values than the 50% acetone extract. Electronic spin resonance (ESR) measurements demonstrated that cinnamon had the strongest $HO^{\cdot}$-scavenging activities among all the tested botanical materials. These data indicate that black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf may serve as potential dietary sources of natural antioxidants for improving human nutrition and health. The extracting solvent may alter the antioxidant activity measurement for selected botanicals, including spices and herbs.

Keywords: Antioxidant; Free radicals; Black peppercorn; Nutmeg; Rosehip; Cinnamon; Oregano leaf

1. Introduction

It is widely accepted that imbalance between antioxidants and oxidants results in harmful physiological responses, which may lead to cell damage and has been linked to cancer, aging, atherosclerosis, ischemic injury and inflammation (Mates & Sanchez-Jimenez, 2000). Dietary antioxidants may contribute to antioxidative protection of biologically important cellular components, such as DNA, proteins, and membrane lipids, from reactive oxygen species attacks. Antioxidants may directly react with and quench free oxygen radicals, form chelating complexes with transition metals, act as reducing agents, induce the production of antioxidative enzymes, and/or suppress the generation of oxidative enzymes, such as cyclooxygenase, in the biological systems. Recently, natural antioxidants are in high demand because of their potential in health promotion and disease prevention, and their improved safety and consumer acceptability.

Black peppercorn, *Piper nigrum*, is a commonly used spice. The essential oil of black peppercorn has been shown to possess antimicrobial activity (Dorman & Deans, 2000) and scavenging capacity against cation radical $ABTS^{\cdot+}$ generated by an enzymatic method (Dorman, Surai, & Deans,

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2000). The peppercorn extracts also showed anticarcinogenic (Unnikrishnan & Kuttan, 1990) and antimutagenic (El Hamss, Idaomar, Alonso-Moraga, & Munoz Serrano, 2003) activity, and might be able to suppress lipid oxidation in animal tissues (Vijayakumar, Surya, & Nalini, 2004). Nutmeg is a widely used spice and flavouring ingredient in food products, with possible health beneficial effects, such as anti-inflammatory and antimicrobial activities (Dorman, Deans, Noble, & Surai, 1995; Murcia et al., 2004; Parle, Dhingra, & Kulkarni, 2004). Essential oil of nutmeg was able to suppress lipid peroxidation in chicken tissue homogenates and egg yolk fat (Dorman et al., 1995). Cinnamon is another popular flavouring ingredient, widely used in food products. In addition to its flavouring application, cinnamon has exhibited health beneficial properties, such as antimicrobial activity, for controlling glucose intolerance and diabetes, inhibiting the proliferation of various cancer cell lines, and treating common cold (Anderson & Broadhurst, 2004; Murcia et al., 2004). Cinnamon extracts were able to reduce lipid peroxidation in the β-carotene-linoleic acid system, and the inhibitory effect was comparable to the synthetic antioxidant standard (BHT) at a level of 100 ppm (Mancini-Filho & Van-Koiij, 1998). In another study, cinnamon extracts exhibited a protective capacity against irradiation induced lipid peroxidation in liposomes, and quenched hydroxyl radicals and hydrogen peroxide (Murcia et al., 2004). Rosehip and oregano are two other commonly used botanical materials. A recent study showed that rosehip is rich in phenolics and could inhibit cancer cell proliferation (Olsson & Gustavsson, 2004). Interestingly, the inhibitory capacity against cancer cell proliferation was correlated with its antioxidant capacity (Olsson & Gustavsson, 2004). On the other hand, oregano leaf was used by the Ancient Greeks to treat asthma, indigestion, and headache (http://www.viable-herbal.com/singles/herbs/s793.htm). The action of oregano leaf extracts on lard and vegetable oils demonstrated that they could stabilize lard against oxidation and showed antioxidative properties when tested on vegetable oils during storage or frying conditions (Vekiari, Oreopoulou, Tzia, & Thomopoulos, 1993). These previous studies have suggested that spices and herbs may contain phenolic compounds and contribute to the overall intake of natural antioxidants. Furthermore, the antioxidant activities may be associated with their health beneficial functions.

Spices and herbs are popular ingredients in every cuisine. With the growth in the use of spices, there has been continued research into the active components of spices, not only from a flavour standpoint, but also from functional perspective, to explore the antioxidant properties of botanicals which are essential for preserving foods and offering health benefits for people consuming the botanicals (Noguchi & Niki, 2000; Shobana & Naidu, 2000). Therefore, this study was conducted to investigate black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf for their scavenging activities against $HO^{\bullet}$, $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and oxygen radicals (ORAC), chelating capacities against Fe^{2+} and Cu^{2+}, and total phenolic contents (TPC).

2. Materials and methods

2.1. Materials

Black peppercorn, nutmeg, rosehip, cinnamon and Oregano leaves were provided by Frontier Natural Products Co-op (Norway, IA). Disodium ethylenediaminetetracetate (EDTA), hydroxylamine hydrochloride, 2,2′-bipyridyl, 2,2-diphenyl-1-picryhydrazyl radical ($DPPH^{\bullet}$), fluorescein (FL), 5,5-dimethyl *N*-oxide pyrroline (DMPO), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were obtained from Sigma-Aldrich (St. Louis, MO), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was from Calbiochem (La Jolla, CA), while 2,2′-azobis (2-amino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Methods

2.2.1. Extraction of spice antioxidants

Five grammes of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf were ground to a fine powder using a micro-mill manufactured by Bel Art Products (Pequannock, NJ) and extracted for 15 h with 50 ml of 50% acetone or 80% methanol respectively, at ambient temperature. Both 50% acetone and 80% methanol extracts of black peppercorn, nutmeg, rosehip and cinnamon were used for subsequent testing, while only 50% acetone was utilized for oregano, due to sample availability. The botanical extracts were kept in the dark under nitrogen at room temperature until further evaluation of antioxidant properties.

2.2.2. Radical cation $ABTS^{\bullet+}$-scavenging activity

Radical-scavenging capacities of sample extracts were evaluated against $ABTS^{\bullet+}$ generated by the chemical method according to a previously reported protocol (Miller & Rice-Evans, 1997; Zhou & Yu, 2004a). Briefly, $ABTS^{\bullet+}$ was prepared by oxidizing 5 mM aqueous solution of ABTS, 2, 2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt, with manganese dioxide at ambient temperature for 30 min. The $ABTS^{\bullet+}$ antioxidant reaction mixture contained 1.0 ml of $ABTS^{\bullet+}$ with an absorbance of 0.8 at 734 nm, and 100 μl of sample extracts or 50% acetone or 80% methanol as blank. The absorbance at 734 nm was measured at 1 min of the reaction, and the trolox equivalent was calculated using a standard curve prepared with trolox.

2.2.3. ORAC assay

ORAC assay was conducted using fluorescein (FL) as the fluorescent probe, according to a previously described procedure (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002; Zhou & Yu, 2004a). The final assay mixture contained 0.067 μM of FL, 60 mM of AAPH, 300 μl of sample extracts or 50% acetone or 80% methanol as a reagent blank. The fluorescence of an assay mixture was

determined and recorded every minute. The trolox equivalent was calculated using a standard curve prepared with trolox, and used to compare ORAC of various samples.

2.2.4. Chelating activity against Fe^{2+} and Cu^{2+}

The 2, 2′-bipyridyl competition assay was conducted to measure the Fe^{2+}-chelating activity of each botanical extract, following a previously described method (Yu, Perret, Davy, Wilson, & Melby, 2002b). The reaction mixture contained 0.8 ml of Tris-HCl buffer (pH 7.4), 0.1 ml of 1.8 mM $FeSO_4$ solution prepared using the Tris–HCl buffer, 0.2 ml of botanical extracts, 0.32 ml of 10% hydroxylamine-HCl, and 0.8 ml of 2,2′-bipyridyl solution (0.1% in 0.2 N HCl). The absorbance at 522 nm was measured and used to determine Fe^{2+} chelating activity, using EDTA as a standard.

ESR measurements were carried out to determine the potential Cu^{2+}-chelating capacity of the botanical extracts, according to a previously described condition with slight modification (Antholine, Basosi, Hyde, Lyman, & Petering, 1984). Briefly, 150 μl of sample extracts was mixed with 150 μl of 1 mM copper chloride ($CuCl_2$) solution. ESR spectra were recorded with 40 MW incident microwave power and 100 kHz field modulation of 5 G at 77° K.

2.2.5. Radical DPPH-scavenging activity

Free radical-scavenging capacities of 50% acetone and 80% methanol extracts of samples were determined according to the previously reported procedure, using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH·) (Yu et al., 2002b). The initial DPPH· concentration was 100 μM for all antioxidant–DPPH· reactions. The absorbance at 517 nm was measured against a control of 50% acetone or 80% methanol at 0, 0.67, 3, 5, 10, 20, 40, and 80 min and used to estimate the remaining radical levels. Six levels of botanical extracts were used to examine the time and dose effects of DPPH·–antioxidant reactions for each botanical material. The absorbance measured at 40 min of the antioxidant–DPPH radical reactions was used to obtain the ED_{50} value of DPPH radical-scavenging capacity of each botanical extract. The ED_{50} value is the concentration required for a selected antioxidant to quench 50% free radicals in a radical–antioxidant reaction mixture under the experimental conditions.

2.2.6. Total phenolic contents

The total phenolic contents were determined for the 50% acetone and 80% methanol extracts of each botanical, using Folin-Ciocalteu reagent (Yu et al., 2002b). The reaction mixture contained 100 μl of sample extracts and 500 μl of the Folin-Ciocalteu reagent, freshly prepared in our laboratory, and 1.5 ml of 20% sodium carbonate and 6 ml of pure water. After two hours of reaction at ambient temperature, the absorbance at 765 nm was measured and used to calculate the phenolic contents using gallic acid as a standard.

2.2.7. Hydroxyl radical (HO·)-scavenging activity

Hydroxyl radical (HO·)-scavenging capacities of the botanical extracts were examined by the ESR method. HO· was generated by Fenton reaction, whereas 5,5-dimethyl-*N*-oxide pyrroline (DMPO) was used as the trapping agent (Madsen, Nielsen, Bertelsen, & Skibsted, 1996). The reaction mixture contained 10 μl of 3 mM freshly prepared $FeSO_4$, 80 μl of 0.75 mM PBS, 15 μl of 10 mM H_2O_2, 15 μl of 1 M DMPO and 30 μl of botanical extracts or solvents for the blank. The final concentration was 20 mg botanical equivalents per ml for all botanical extracts. The ESR measurements were conducted at 1 and 10 min of each reaction at ambient temperature with the following spectrometer settings: microwave power of 10 mW, field modulation frequency of 100 kHz, and modulation amplitude of 1 G.

2.3. Statistical analysis

Data were reported as mean ± SD for triplicate determinations. Analysis of variance and least significant difference tests (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL) were conducted to identify differences among means, while a Pearson correlation test was conducted to determine the correlations among means. Statistical significance was declared at $P < 0.05$.

3. Results and discussion

3.1. Radical cation $ABTS^{\cdot+}$-scavenging activity

50% acetone and 80% methanol extracts of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf were evaluated and compared for their $ABTS^{\cdot+}$-scavenging capacities, using a spectrophotometric method. All the tested botanical extracts showed significant $ABTS^{\cdot+}$-scavenging capacity (Table 1). Fifty percent acetone extracts exhibited a $ABTS^{\cdot+}$-scavenging ability range of 1243 μmol trolox equivalents (TE) per gramme of sample for cinnamon to 40 μmol TE/g for black peppercorn, while 80% methanol extracts were from 1064 μmol TE/g for cinnamon to 23 μmol TE/g for black peppercorn. Our results, showing a higher $ABTS^{\cdot+}$-scavenging capacity for cinnamon than for nutmeg, are supported by previous findings of 11.7 and 5.13 μmol TE/ml for water extracts of cinnamon and nutmeg, respectively, by Murcia and others (2004), but cannot be further compared since these previous results were reported as the antioxidant concentration in the testing reaction but not on a per weight basis of the botanical materials. The range of the $ABTS^{\cdot+}$-scavenging capacities observed in the 50% acetone and 80% methanol extracts of the five botanicals are comparable or greater than those of the 8.9–30.8 μmol TE/g detected in cold-pressed black caraway, carrot, cranberry and hemp seed oils (Yu, Zhou, & Parry, 2005), and 17.5–19.7 μmol TE/g in wheat bran (Zhou, Su, & Yu, 2004c), suggesting that black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf may contribute to total dietary natural antioxidants for possible health benefit.

It has been recognized that extraction solvent may significantly alter the antioxidant activity estimation (Zhou & Yu, 2004b). The $ABTS^{\cdot+}$-scavenging capacities of the

Table 1
Antioxidant activities of black peppercorns, nutmeg, rosehip, cinnamon and oregano leaf

	Extraction solvent	$ABTS^{\cdot+}$-scavenging ability (TE μmol/g botanicals)	ORAC (TE μmol/g botanicals)	Fe^{2+} chelating capacity (EDTA Eq mg/g botanicals)	TPC (GE mg/g botanicals)
Black peppercorn	50% Acetone	39.8a ± 1.44	395a ± 36.85	1.09b ± 0.16	1.32a ± 0.00
	80% Methanol	23.3x ± 1.44	363x ± 16.93	0.54y ± 0.11	0.91x ± 0.01
Nutmeg	50% Acetone	168b ± 12.86	398a ± 25.23	1.15b ± 0.02	2.62b ± 0.01
	80% Methanol	191y ± 0.00	1187z ± 8.74	0.48x ± 0.11	2.68y ± 0.12
Rosehip	50% Acetone	379c ± 2.81	838b ± 73.96	3.36d ± 0.07	5.09c ± 0.14
	80% Methanol	190y ± 4.81	1085y ± 24.32	2.11z ± 0.12	2.57y ± 0.14
Cinnamon	50% Acetone	1243d ± 12.22	1256c ± 37.90	0.72a ± 0.08	18.56d ± 0.31
	80% Methanol	1064z ± 12.73	1069y ± 5.47	0.33w ± 0.00	14.82z ± 0.28
Oregano	50% Acetone	337c ± 8.00	1233c ± 41.36	2.93c ± 0.09	5.48c ± 0.34

TPC is the total phenolic content. TE stands for trolox equivalents. GE stands for gallic acid equivalents. EDTA stands for disodium ethylenediaminetetraacetate. Data expressed as mean ± standard deviation ($n = 3$). Values marked by the same letter within a column are not significantly different ($P < 0.05$).

50% acetone and 80% methanol extracts (Table 1) indicated that 50% acetone was a better extraction solvent than 80% methanol for black peppercorn, rosehip and cinnamon, while 80% methanol was better for nutmeg. This suggests that the major antioxidants present in nutmeg are less polar than those in the other botanical materials under the experimental conditions. Results from previous studies of rosehip extracts showed $ABTS^{\cdot+}$-scavenging capacities of 472–625 μmol TE/g (Gao, Bjork, Trajkovski, & Uggla, 2000) versus our findings of 379 and 190 μmol TE/g for 50% acetone and 80% methanol respectively. This difference may be explained by the use of different botanical materials and different antioxidant extraction procedures. Gao and others (2000) used a solid to solvent extraction ratio of 1 g to 20 ml using 50% ethanol, whereas 50% acetone and 80% methanol were employed in the present study at a ratio of 1 g to 10 ml of solvent. It needs to be pointed out that the extraction solvent and the method have significant influence on antioxidant activity estimation, and a generally accepted solvent system and extracting conditions are required to compare the research data across the laboratories.

3.2. ORAC assay

ORAC is a widely used method for investigating antioxidant properties (Huang et al., 2002), although few ORAC assays have been conducted for spice and herb materials. ORAC values were determined for 50% acetone and 80% methanol extracts of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf, and expressed as μmol of trolox equivalent (TE) per gramme of sample. Trolox is a water soluble vitamin E analogue and a commonly used antioxidant standard. All the tested botanical extracts exhibited strong ORAC, with an ORAC value range of 363–1256 TE μmol/g (Table 1). 50% acetone extract of cinnamon showed the greatest ORAC value of 1256 TE μmol/g, followed by that of oregano leaf (1232 μmol/g), rosehip (838 μmol/g), nutmeg (398 μmol/g) and black peppercorn (395 μmol/g). In contrast, the 80% methanol extract of nutmeg had the highest value of 1187 TE μmol/g among all the 80% methanol extracts, and followed by rosehip (1085 μmol/g), cinnamon (1069 μmol/g) and black peppercorn (395 μmol/g). These data suggest that a possible influence of extracting solvent on ORAC estimation for a selected botanical sample.

3.3. Radical DPPH-scavenging activity

It is also well known that the radical system used for the antioxidant evaluation may influence the experimental results, and two or more radical systems are required to investigate the radical-scavenging capacities of a selected antioxidant (Yu et al., 2002a). To better examine their antioxidant capacities, the sample extracts were also analyzed for free radical-scavenging activity against stable $DPPH^{\cdot}$.

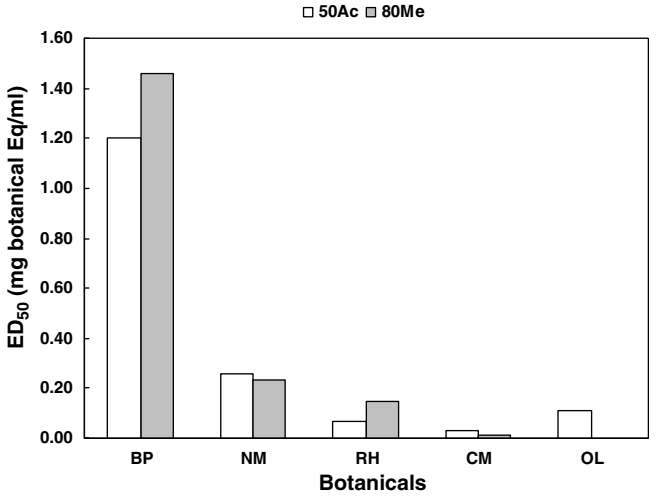

Fig. 1. ED_{50} values of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf against DPPH radicals. ED_{50} is the concentration required for an antioxidant preparation to quench 50% of DPPH radicals in the reaction mixture within 40 min under the experimental conditions. BP, NM, RH, CM and OL stand for black peppercorn, nutmeg, rosehip, cinnamon, and oregano leaf, respectively. 50Ac and 80Me stand for 50% acetone and 80% methanol extracts, respectively.

All extracts were capable of directly reacting with and quenching DPPH· (Fig. 1) The concentration required to scavenge 50% of the free radicals in the reaction mixture within 40 min, the ED_{50} value, was determined (Fig. 1). A higher DPPH radical-scavenging activity is associated with a lower ED_{50} values. The lowest ED_{50} value of 10 μg/ml was detected in 80% methanol extract of cinnamon, while the 80% extract of black peppercorn had the greatest ED_{50} value of 1.46 mg/ml. Overall, the 50% acetone extracts of black peppercorn, nutmeg, rosehip and oregano leaf had lower ED_{50} values than did their corresponding 80% methanol extracts, suggesting that 50% acetone is a better solvent for extracting DPPH radical-scavenging agents from these botanicals. However, in the case of cinnamon, the 80% methanol proved to be a better solvent for extracting DPPH radical-scavengers. Cinnamon had the greatest capacity to quench DPPH·, while black peppercorn had the least ability to quench DPPH·, regardless of extraction solvents (Fig. 1). In addition, DPPH radical-scavenging activities of the tested botanicals were also found to be both time- and dose-dependent. The time and dose effects of the 50% acetone extract of nutmeg are presented in Fig. 2A and those of 80% methanol extract of black peppercorn are presented in Fig. 2B.

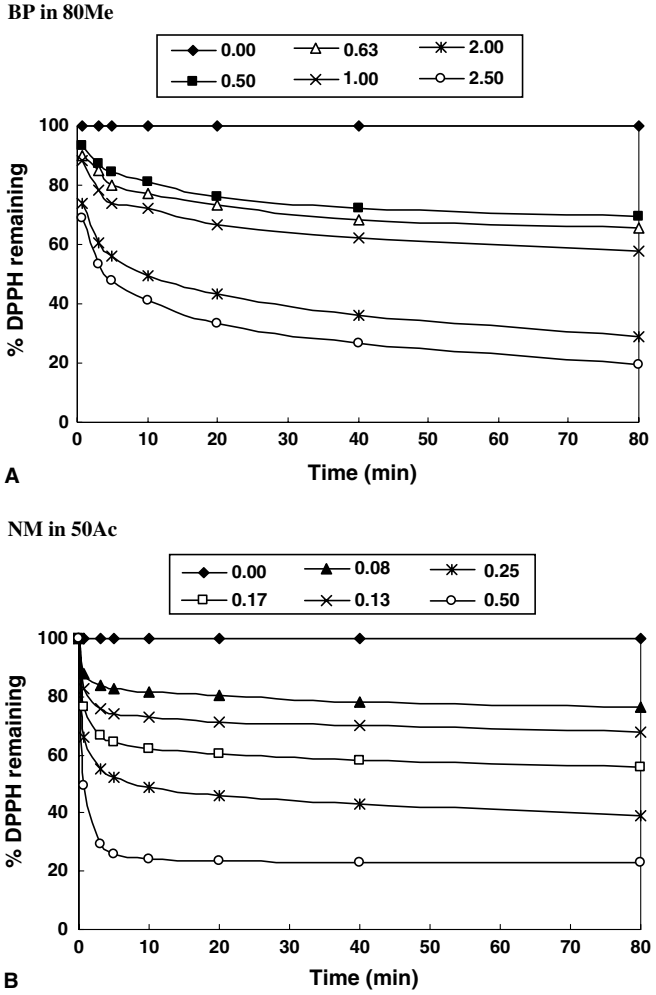

Fig. 2. Dose and time effects of the antioxidant–DPPH· reactions. (A) represents the dose and time effects of 80% methanol extracts of black peppercorn and DPPH· reactions, with 0, 0.50, 0.63, 1.00, 2.00, and 2.50 representing the final concentrations of botanical extracts at 0, 0.50, 0.63, 1.00, 2.00, and 2.50 mg botanical equivalents per ml in the antioxidant–radical reaction mixtures, respectively; whereas (B) represents the dose and time effects of 50% acetone extracts of nutmeg and DPPH· reactions, with 0, 0.08, 0.13, 0.17, 0.25 and 0.50 representing the final concentrations of botanical extracts at 0, 0.08, 0.13, 0.17, 0.25 and 0.50 mg botanical equivalents per ml in the antioxidant–radical reaction mixtures, respectively. The initial DPPH· radical concentration was 100 μM in all reaction mixtures.

3.4. Hydroxyl radical (HO·)-scavenging activity

Electron spin resonance (ESR) spectroscopy determines the presence of unpaired electrons, and is commonly used for free radical evaluations, although it has not been well applied to evaluate the radical-scavenging properties of spice and herbs. The botanical extracts were prepared with 50% acetone, and the ESR examinations were carried out on a similar botanical weight basis, with a final concentration in the reaction mixture at 20 mg botanical equivalents/ml. ESR measurements indicated that all tested botanical extracts had significant HO·-scavenging capacities under the experimental conditions (Fig. 3). Cinnamon had stronger HO·-scavenging capacity than other samples at both 1 min and 10 min.

3.5. Chelating activity against Fe^{2+} and Cu^{2+}

It has been well established that chelating agents stabilize transition metals and reduce their availability as catalysts, to inhibit the production of the first few free radicals and consequently suppress lipid peroxidation in biological and food systems (Yu et al., 2002b). Chelating activity against Fe^{2+} was examined and expressed as EDTA equivalents per gramme of botanical sample (Table 1). Almost all botanical extracts in the two extraction solvents significantly differed in their Fe^{2+} chelating activities, suggesting that solvents might greatly alter the chelating activity estimation of these botanicals. Interestingly, 50% acetone extracts of all samples showed higher chelating abilities than did the corresponding 80% methanol extract, indicating that 50% acetone is a preferred solvent for Fe^{2+}-chelating capacity estimation.

The 50% acetone and 80% methanol extracts of rosehip had the highest chelating ability of 3.4 and 2.1 EDTAE mg/g, respectively, under the current experimental conditions. These values are comparable to those of 1.0–1.9 EDTAE mg/g detected in wheat bran (Zhou et al., 2004c) and 10.5–25.5 EDTAE mg/g for cold-pressed edible black caraway, carrot and hemp seed oils (Yu et al., 2005). In addition, chelating capacity against Cu^{2+} was evaluated by the ESR method. 50% acetone extract of cinnamon formed strong chelating complexes with Cu^{2+} compared with the control reaction under the experimental conditions

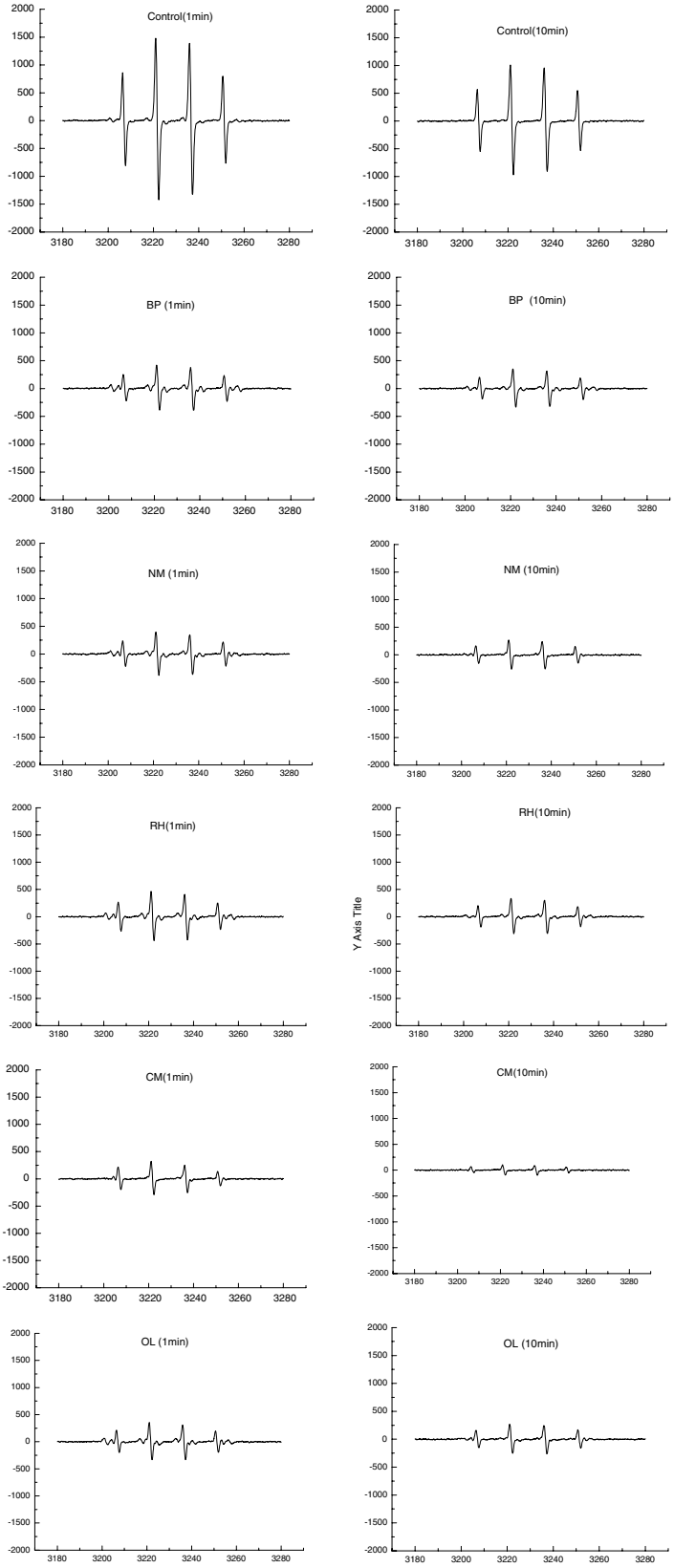

Fig. 3. Hydroxyl radical ($OH^{\cdot}$)-scavenging activity of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. BP, NM, RH, CM and OL stand for black peppercorn, nutmeg, rosehip, cinnamon, oregano leaf, respectively, while the control represents the control reaction containing only 50% acetone. Each reaction mixture contained 10 μl of freshly prepared 3 mM $FeSO_4$, 80 μl of 0.75 mM EDTA, 15 μl of 1 M DMPO, 15 μl of 0.5 mM H_2O_2, and 30 μl of 100 mg/ml spice extracts. ESR signals were recorded at 1 and 10 min of the reaction at ambient temperature.

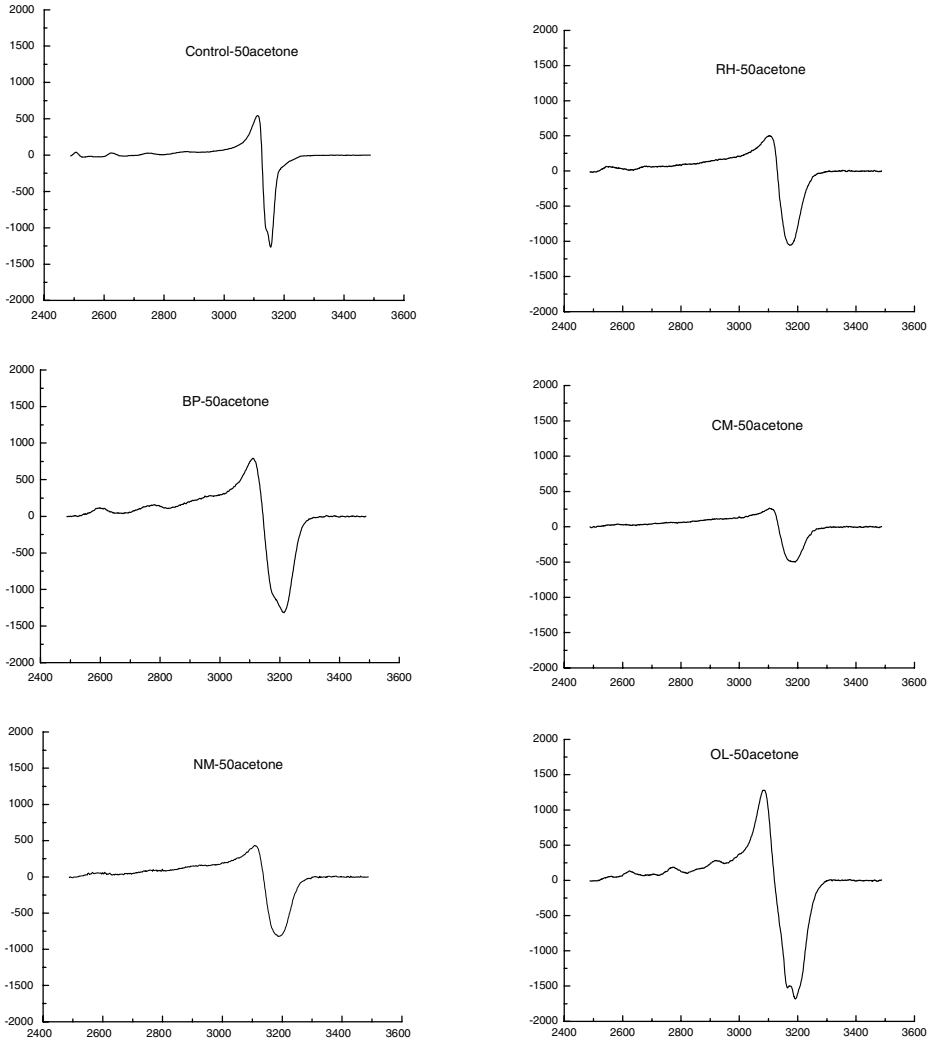

Fig. 4. Interaction between Cu^{2+} and individual botanical extracts measured by ESR. The final concentrations were 50 mg/ml for spice and 0.5 mM for copper chloride ($CuCl_2$). The ESR spectrum was recorded at 1 min of reaction at 77 °K.

(Fig. 4). It needs to be pointed out that ESR results (Fig. 4) are qualitative, not quantitative.

3.6. Total phenolic contents

The total phenolic content of each botanical sample was estimated, since phenolics may significantly contribute to its overall antioxidant activity. Phenolics were detected in all botanical samples (Table 1). The 50% acetone and 80% methanol extracts of cinnamon had the highest total phenolic contents (TPC) of 186 and 148 mg GE/g, respectively, among all tested botanical extracts. The lowest TPC value of 9.12 mg GE/g was detected in the 80% methanol extract of black peppercorn. In addition, the 50% acetone extracts of black peppercorn, nutmeg, rosehip, cinnamon, and oregano leaf all had higher TPC value than their corresponding 80% methanol extracts. These results suggested the possible influence of extracting solvent on total phenolic content estimation.

In conclusion, this research indicates that black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf may serve as potential dietary sources of natural antioxidants for improving human nutrition and health. Cinnamon has the highest natural phenolic contents and has strongest antioxidant properties among the five tested botanicals. In addition, extracting solvent may alter the antioxidant activity measurement for selected botanicals, including spices and herbs.

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