Journal of Medicinal Plants Research Vol. 5(25), pp. 6067-6075, 9 November, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Preparation of deodorized antioxidant rich extracts from 15 selected spices through optimized aqueous extraction

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Accepted 14 September, 2011

An aqueous deodorized extraction process with the promising yield ranging from 8.2 to 44.8% of antioxidants from 15 most commonly used spices are presented. Total phenolic content (TPC) ranged from 4.4 to 315.3 mg GAE/g extract. EC_{50} by DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) ranged over 0.254 to 19.944 and 0.110 to 15.878 mg/ml, respectively. TPC was found in strong correlation with antioxidant activities of spice extracts. Flavonoids and saponin were detected in cinnamon bark and clove buds extracts through phytochemicals screening tests; both showing the highest TPC and antioxidant activity ($P<0.05$) among all the spices, but lower than standard natural antioxidant (ascorbic acid) and standard synthetic antioxidant (BHA/BHT, 50/50).

Key words: Spices, deodorized aqueous extraction, phenolic content, antioxidant activity, phytochemicals screening.

INTRODUCTION

Lipid peroxidation, induced by reactive oxygen species, is one of the major deteriorations during the processing and storage of food products. Lipid peroxidation affects the nutritional, safety and sensory qualities of food products (Shahidi et al., 1992). Besides deteriorating the qualities of food products, oxidized lipids can increase oxidative stress and potentially contribute to a various disease syndromes (Turek et al., 2003). Oxidized lipids are easily absorbed through the digestive tract (Staprans et al., 1999) and get incorporated into membrane phosphor-lipids, which alter the cell membrane fluidity and may consequently result in cellular damage. Free radical damage to cellular components has been linked to pathogenesis of various diseases, which are including aging, cancers and atherosclerosis (Staprans et al., 1999). Hence, it is essential and crucial to prevent or delay the process of lipid oxidation in food products.

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In order to overcome lipid oxidation in meat products, antioxidants have been widely used since last few decades. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been being commonly used widely; being cheaper and effective at low concentrations. But nowadays, their role in causing number of health risks, including cancer and carcinogenesis is well proven (Iqbal et al., 2005), which has restricted its use in food items. Due to which, the most powerful synthetic antioxidant (TBHQ) is banned as food additive in Japan, Canada and Europe. Similarly, BHA was also removed long ago from the generally recognized as safe (GRAS) list of compounds (Farag et al., 1989). Moreover, nowadays customers also prefer the food free of synthetic additives. This has prompted the food scientists to search some potential alternatives of synthetic antioxidants, which may be cheaper, effective, safer and based on natural origin. As a result, number of botanical materials has been explored so far as potential natural sources of antioxidants (Ismail et al., 2009; Ismail et al., 2010; Iqbal et al., 2007). But still research is going on for searching newer sources of natural antioxidants, more preferably

from routine dietary items (Shahidi et al., 2006). Spices are being used globally as natural flavouring, colouring and preservative agents in food products since ancient times, particularly in India, China and other south-east Asian countries (Srinivasan, 2005). Traditionally, spices are considered as health promoting agent and have been being used in eastern system of medications (Srinivasan, 2005) for multiple medical effects. Now this usage is increasing drastically due to recent research reports highlighting their antioxidative and disease preventive effects. Previous findings show that spices which are commonly found in Malaysian curry powder formulations such as curry leaf, ginger, cinnamon bark and clove buds possess high antioxidant activity (Wangensteen et al., 2004). However, the pungent odor and taste of spice powders, essential oils and organic solvents-extracted oleoresins have limited their direct applications in the food products (Hinneburg et al., 2006).

In order to overcome this limitation, utilization of water as the extraction solvent seems to be a solution in this scenario as water lowers the extractability of pungent flavored compounds from spices in comparison to organic solvents like alcohols (Anderson et al, 2003; Shahidi and Ho, 2005). Thus, the present study was initiated to prepare deodorized extract of spices using hot water as extraction medium and to investigate the phenolic content, radical scavenging activity and anti-oxidant activity of aqueous extracts. For comparison of their effectiveness, ascorbic acid was used as standard natural antioxidant, while a mixture of BHA/BHT (50/50) as standard synthetic antioxidant. To the best of our knowledge, no report discussing the antioxidant potential of deodorized aqueous extracts from spices is presented so far. This report may be useful in applying spices in food items at higher concentrations, to get added advantages, without coming across specific pungent smell.

MATERIALS AND METHODS

Plant materials

Dried spices, that is clove buds (*Syzygium aromaticum)*, cinnamon bark (*Cinnamomum verum)*, black pepper (*Piper nigrum)*, cumin seed (*Cuminum cyminum)*, fennel seed (*Foeniculum vulgare)*, coriander seed (48 *Coriandrum sativum)*, star anise (*Illicium verum)*, black mustard seed (*Brassica nigra)*, poppy seed (*Papaver somniferum)*, cardamom (*Elettaria cardamomum*) and fenugreek seed (*Trigonella foenum-graecum*) were purchased from Giant Hypermarket, Kajang, Selangor, Malaysia. Turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) rhizomes were purchased from Bintang Supermarket, Sungai Chua, Selangor, Malaysia, whereas curry leaves (*Murraya koenigii*) and fresh red chillies (*Capsicum annum*) were purchased from a wet market in Bandar Baru Bangi, Selangor, Malaysia.

Chemicals and reagents

All the chemicals used were of analytical grade and are listed as follows: Gallic acid (Sigma-Aldrich, Madrid, Spain); Na_2CO_3, $FeCl_3$ (98%), methanol (99.8%) and Folin-Ciocalteu reagent (BDH Laboratory Supplies, Poole, England); 2.2-diphenyl-1-picrylhydrazyl (DPPH) (98.9%), L (+)-ascorbic acid, trichloroacetic acid (99.5%), KH_2PO_4 (99%), ethanol, chloroform (99.4%), ammonia, diethyl ether and anhydrous acetic acid (Merck, Darmstadt, Germany); BHA (90% 3-isomer: 9% 2-isomer) and BHT (99%) (Sigma–Aldrich, Steinheim, Germany); K_2HPO_4 (99%) (Fluka Chemica, Buchs, Switzerland); $K_2[Fe(CN)]_6$ (99%) and HCl (37%) (R&M Chemicals, Essex, UK); KI (99.5%) and petroleum ether (VWR International Ltd., Lutterworth, England); H_2SO_4 (98%), $HgCl_2$ (99.5%)) and magnesium tape (Ajax Chemical Co. Pty., Sydney, Australia).

Preparation of spices extracts

Spices were respectively cleaned using tap water and dried in an oven at 50°C until constant weight was attained; final moisture content being < 5%. Dried spices were separately pulverized into a fine powder by using a stainless steel blender (Waring Commercial, Torrington, CT, USA) and mixed with boiling water (100°C) at 1:20 (w/v). Subsequently, each mixture was stirred by using a magnetic stirrer for 15 min and filtered through Whatman No 1 filter paper. The filtrates were individually pooled and water content was removed from the filtrates under reduced pressure (Rotavapor R210, Buchi, Postfach, Flawil, Switzerland). Finally, the yield of spice extracts was measured and extracts were preserved at -18°C prior to further analyses.

Total phenolic content

Total phenolic contents (TPC) from spices extracts were determined following a previously reported method (Yu et al., 2002) with slight modifications. Briefly, 0.1 ml of spice extracts was respectively mixed with 1 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent followed by vortexing upto 3 min before addition of 1.5 ml of sodium carbonate (20%) and 6.9 ml of distilled water. Subsequently, the mixtures were stirred and incubated at 40°C for 30 min. Finally, the absorbance of the resulting mixtures was measured at 765 nm spectrophotometerically (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). Gallic acid was used as standard and results were expressed as milligram gallic acid equivalent per gram extract (mg GAE/g extract).

DPPH radical scavenging activity

The DPPH radical scavenging activity of extracts was evaluated according to a previously reported method (Iqbal et al., 2005) with slight modifications. In brief, 0.1 ml of each spice extract at different concentrations was respectively reacted with methanolic solution of DPPH (1.4 ml; 0.2 mM) and 1.5 ml of distilled water followed by vigorous vortexing and placing the mixture in dark for 30 min. Finally, the absorbance of resulting mixtures was measured at 515 nm by using a spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). The sample concentration providing 50% of radical scavenging activity (EC_{50}) was obtained through interpolation of linear regression analysis.

Ferric reducing power assay

Ferric reducing power of extracts was determined following a previously reported method described by Oyaizu (1986). In brief, 1.0 ml of spice extracts at various concentrations was respectively added to phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium hexaferricyanide (2.5 ml, 1%) followed by mixing and incubating at 50°C for 20 min. After that, TCA (2.5 ml, 10%) was added to the

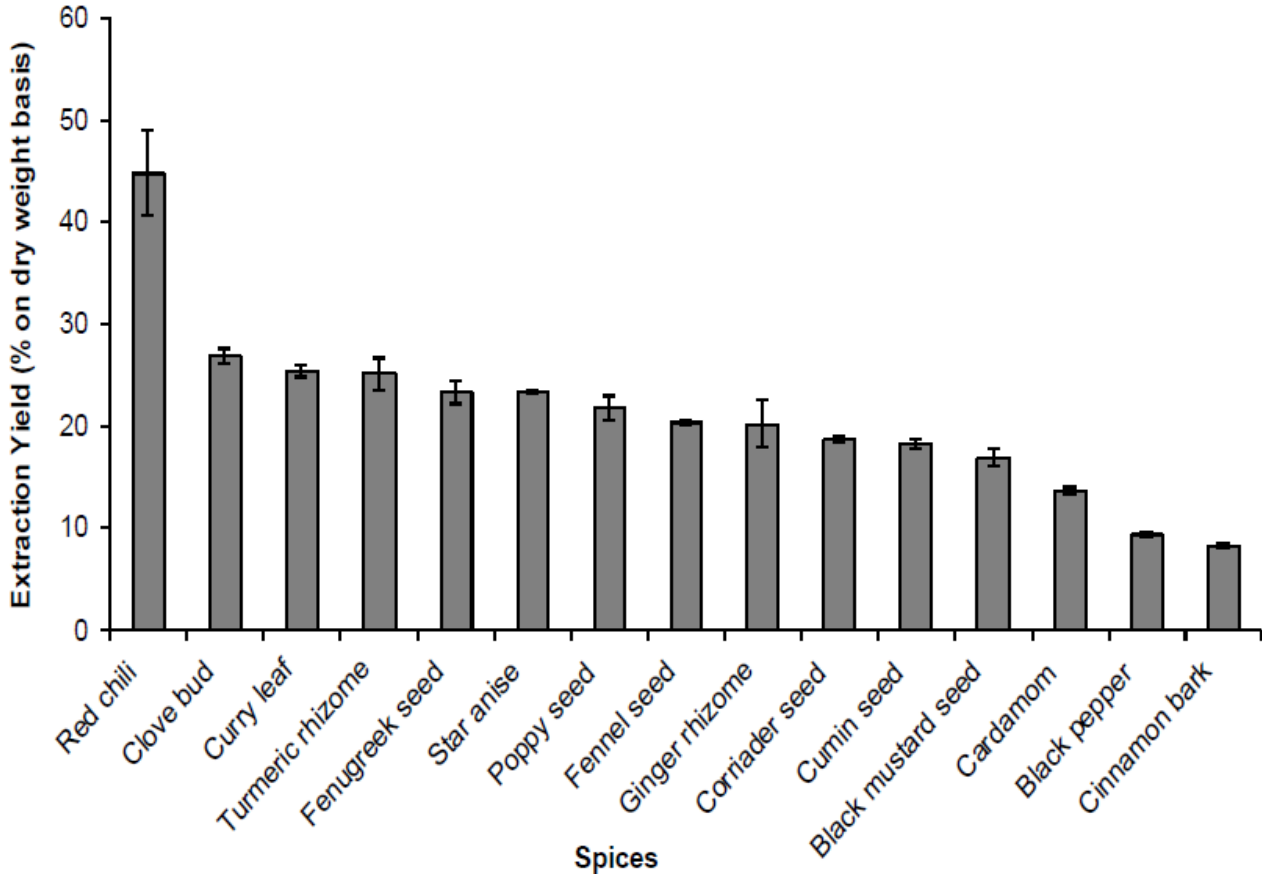

Figure 1. Extraction yield for 15 spices through hot water extraction.

mixtures and mixtures were centrifuged at 3000 rpm for 10 min (Laboratory Centrifuges-Sigma, Deutschland, Germany). In order to determine the ferric reducing power, 2.5 ml of the supernatant was taken out and mixed with 2.5 ml of distilled water followed by addition of ferric chloride (0.5 ml, 0.1%) and vigorous vortexing. Finally, absorbance of the mixtures was measured at 700 nm by using spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). The sample concentration which provides absorbance value of 0.5A (EC_{50}) was calculated through interpolation of linear regression analysis (Mau et al., 2004).

Phytochemical screening tests

Phytochemical screening tests were carried out on two spice extracts exhibiting highest antioxidant activity according to conventional methods described by Guevara and Recio, (1985). The presence of alkaloids, flavonoids, triterpenes/steroids and saponins within the selected extracts was investigated.

Statistical analysis

Pearson correlation test was conducted to determine the correlation between total phenolic content and antioxidant activity of spice extracts. Data obtained from the study was analyzed statistically with the analysis of variance (ANOVA) and Tukey's test by using SAS Version 6.21 (1995) to identify the significant difference between the samples ($P<0.05$).

RESULTS AND DISCUSSION

Extraction yield

Figure 1 shows hot water extracts yield of selected spices. Yield varied significantly among the extracts and ranged from 8.2 to 44.8% (on dry weight basis); suggesting that compounds are of varying nature among different spices. As extraction is strongly dependent on polarity of solvent and extraction temperature, these factors might have contributed significantly on the yield of extracts. Among all, the highest yield was obtained from red chilli (44.8 ± 4.2%), while lowest by cinnamon bark (8.2 ± 0.2 %) ($P<0.05$), others were between them.

Total phenolic content

Figure 2 shows total phenolic content of aqueous extracts from 15 spices, which ranged from 4.4 to 315.3 mg GAE/g extract. Extracts of cinnamon bark and clove buds possessed the highest total phenolic content as shown in the following order: cinnamon bark ≥ clove buds > curry leaf > star anise > fennel seed > cumin seed > black

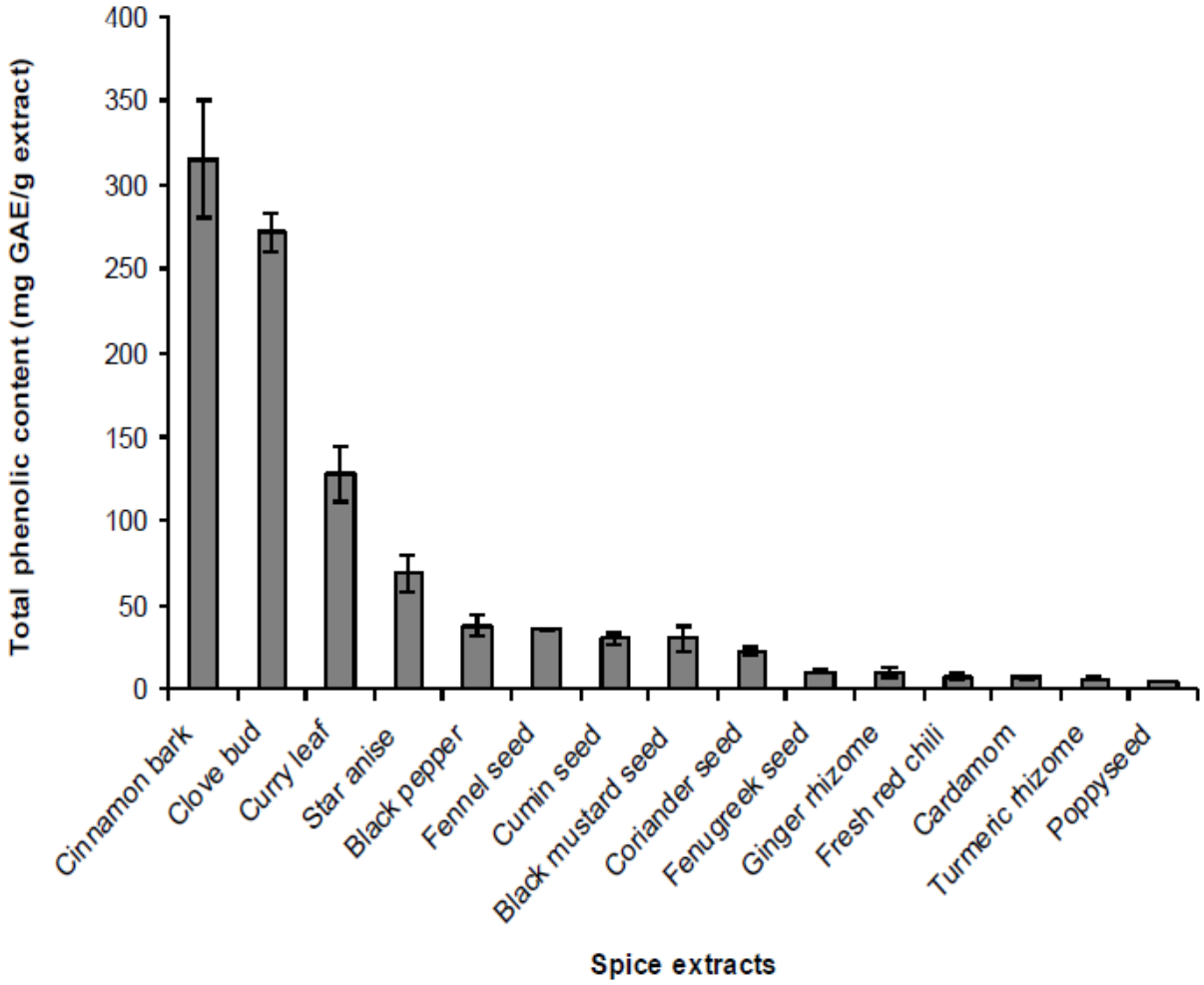

Figure 2. Total phenolic content of aqueous extracts from 15 spices.

mustard seed > coriander seed > fenugreek seed > ginger rhizomes > red chilli > cardamom > turmeric > poppy seed ($P<0.05$). Results are supported by findings of Shan et al. (2005), who reported that among all 26 methanolic spice extracts, the highest TPC were detected in clove buds and cinnamon bark extracts whereas the lowest content in poppy seed extracts. This indicates that hot water might be considered as an appropriate solvent in extracting phenolic compounds from spices, with reduced level of pungency. Content of phenolic in hot water extract was found lower than reported earlier from methanolic extracts (Shan et al., 2005), which might be attributed to the differences in polarity of solvents, extraction techniques as well as other environmental factors such as climate, sun exposure, soil composition, which may alter the phenolic metabolism of spice plants (Djeridane et al., 2006). Besides higher heating temperature, duration of vacuo-water removal process might accelerate the polyphenols oxidation and hence result in lower TPC in aqueous extracts as compared to methanolic extracts. These observations are highlighted in report of Xu et al. (2007), who reported that heating process is capable to cleave the ester and glycosidic bonds of phenolic compounds, subsequently leading to destruction of compounds.

Antioxidant activity

Figure 3 shows DPPH radical scavenging activity of aqueous extracts from 15 spices. EC_{50} values of spice extracts, calculated through DPPH radical scavenging activity test, ranged from 0.254 to 19.944 mg/ml. DPPH radical scavenging activity of spice extracts is presented in the following descending order: cinnamon bark > clove buds > curry leaf > star anise > fennel seed > coriander seed > black mustard seed > black pepper > cumin seed > ginger rhizome > cardamom > red chilli > fenugreek seed > turmeric rhizome > poppy seed ($P<0.05$).

In general, all the spice extracts investigated in this study showed good antiradical activity except extracts of cardamom, red chilli, fenugreek seed, ginger rhizome and

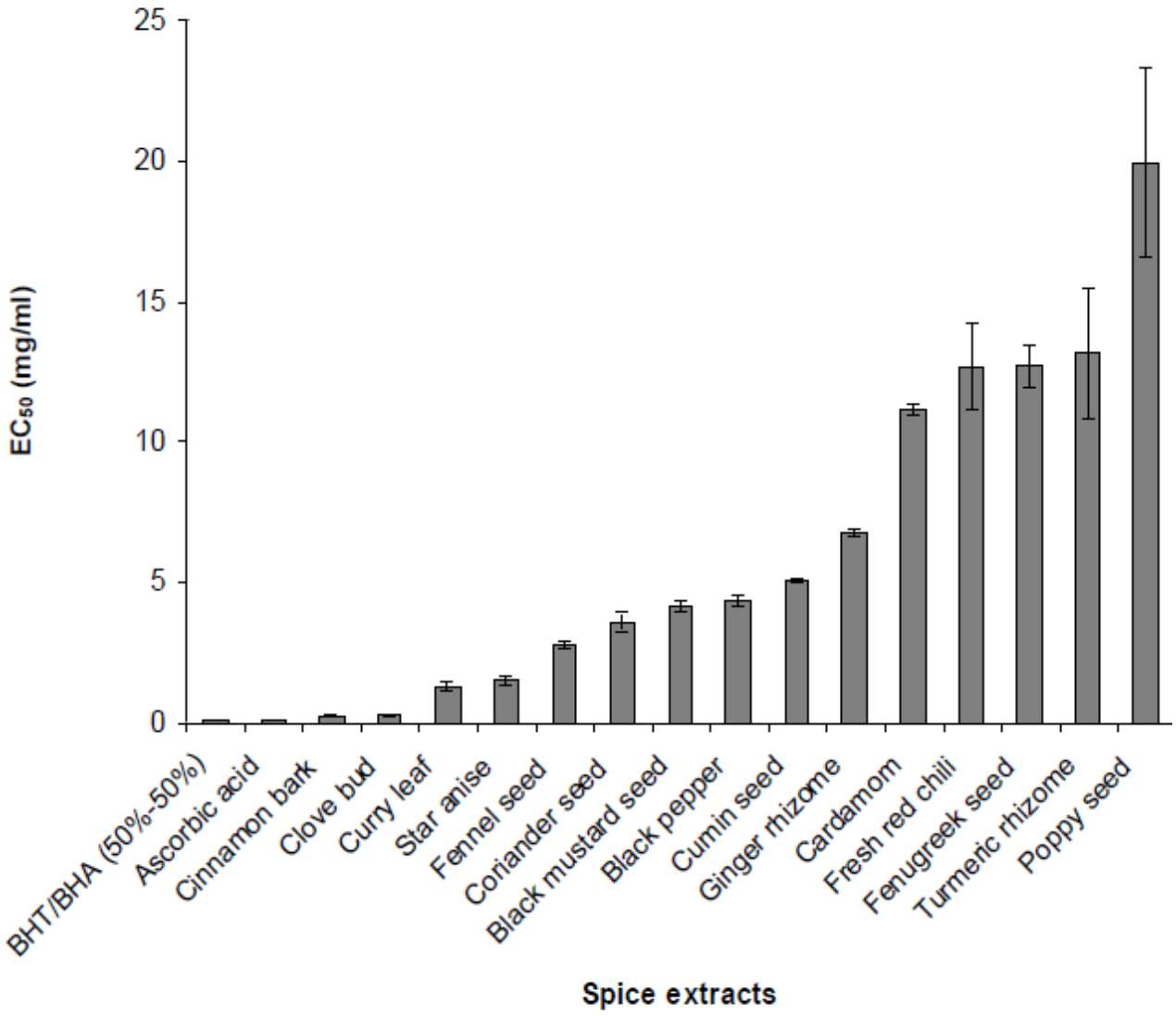

Figure 3. DPPH radical scavenging activity of aqueous extracts from 15 spices.

poppy seed; due to their EC_{50} values lesser than 10 mg/ml. According to Mau et al. (2004), extracts with EC_{50} value lower than 10 mg/ml through are ranked among having high antiradical activity. Cinnamon bark extract (EC_{50} = 0.254 ± 0.001 mg/ml) showed the highest, whereas the poppy seed extract (EC_{50} = 19.944 ± 3.399 mg/ml) demonstrated the lowest antiradical activity as compared to other extracts ($P<0.05$).

Order of radical scavenging potential among the extracts is well in agreement with TPC; suggesting that phenolics are mainly responsible for free radical scavenging (Iqbal et al., 2007).

Ferric reducing power refers to the ability of a reductone to donate electrons to reactive radicals and convert them into more stable and unreactive species (Dorman et al., 2003). Figure 4 shows EC_{50} values for spices extracts from 15 different spices, standard (ascorbic acid) and synthetic (BHA/BHT, 50/50) antioxidants. Antioxidant activity varied widely among the extracts ranging from 0.110 to 15.878 mg/ml. Standard and synthetic antioxidants @ 200 ppm exhibited higher ferric reducing power than spice extracts ($P<0.05$); while among the extracts, clove buds (EC_{50} = 0.236 ± 0.004 mg/ml) exhibited the highest reducing power while poppy seed showed the lowest (EC_{50} = 15.878 ± 2.029) through FRAP. With the exception of cardamom and poppy seed extracts, remaining 13 spice extracts exhibited excellent reducing power as is evident from their EC_{50} values, which are less than 10 mg/ml (Mau et al., 2004).

Again the findings for ferric reducing power of spice extracts are in good agreement to their DPPH radical scavenging activity as well as TPC. Highest FRAP was observed for synthetic antioxidant that is, BHT/BHA (50 to 50%) followed by standard that is ascorbic acid, while

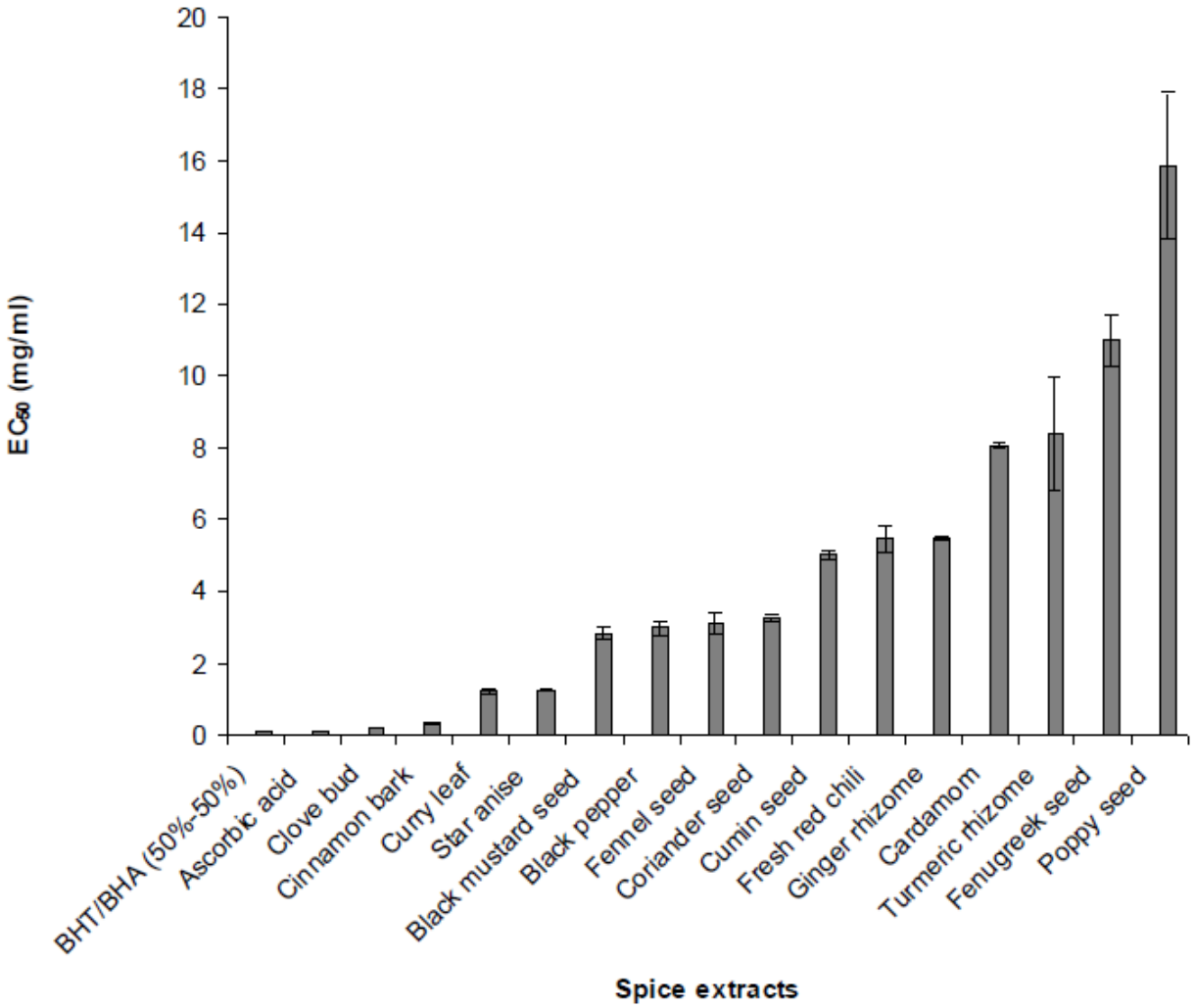

Figure 4. FRAP of aqueous extracts 15 spices.

among the spice extracts order was like: clove buds > cinnamon bark > curry leaf ≥ star anise > black mustard seed ≥ black pepper ≥ fennel seed ≥ coriander seed > cumin seed ≥ fresh red chilli ≥ turmeric rhizome > cardamom≥ ginger rhizome > fenugreek seed > poppy seed ($P<0.05$).

Higher antioxidant activity for cinnamon bark and clove buds extracts may be attributed to the presence of high amounts of phenolic compounds. Previous reports revealed that clove bud methanolic extracts contain high levels of gallic acid and tannins besides some flavonoids (419 mg/100 g of DW), whereas cinnamon bark methanolic extracts contain considerable amounts of flavan-3-ols (catechin derivatives), and caffeic acids (Shan et al., 2005). These phenolic compounds possess catechol (*o*-dihydroxyl group) structures in the aromatic ring, a very important structural element that forms intramolecular hydrogen-bonds with the free radicals and contributes to the high antioxidant activity of both these extracts.

However, contrary to some previous studies (Ajith et al., 2007; Amin and Hamza, 2006), ginger and turmeric rhizomes extracts did not exhibit high antioxidant activity in current study. This is due to the reason that curcumin and gingerol-derivatives, the major antioxidant compounds from turmeric and ginger respectively, are thermally labile compounds (Bhattarai et al., 2001; Kshirsagar et al., 2009). Hence, extraction by hot water might have destroyed these compounds, resulting in lower antioxidant activity for both these extracts. On the other hand, antioxidant activity of some oilseed spices such as black mustard seed and poppy seed might also be underestimated in the present study as hot water has minimized the extractability of lipophilic antioxidants from oilseed spices, for instance α-tocopherol. According to earlier reports, oils extracted from oilseeds such as sesame seed and kenaf are high in antioxidant activity (Shahidi et al., 2006; Chan and Ismail, 2009).

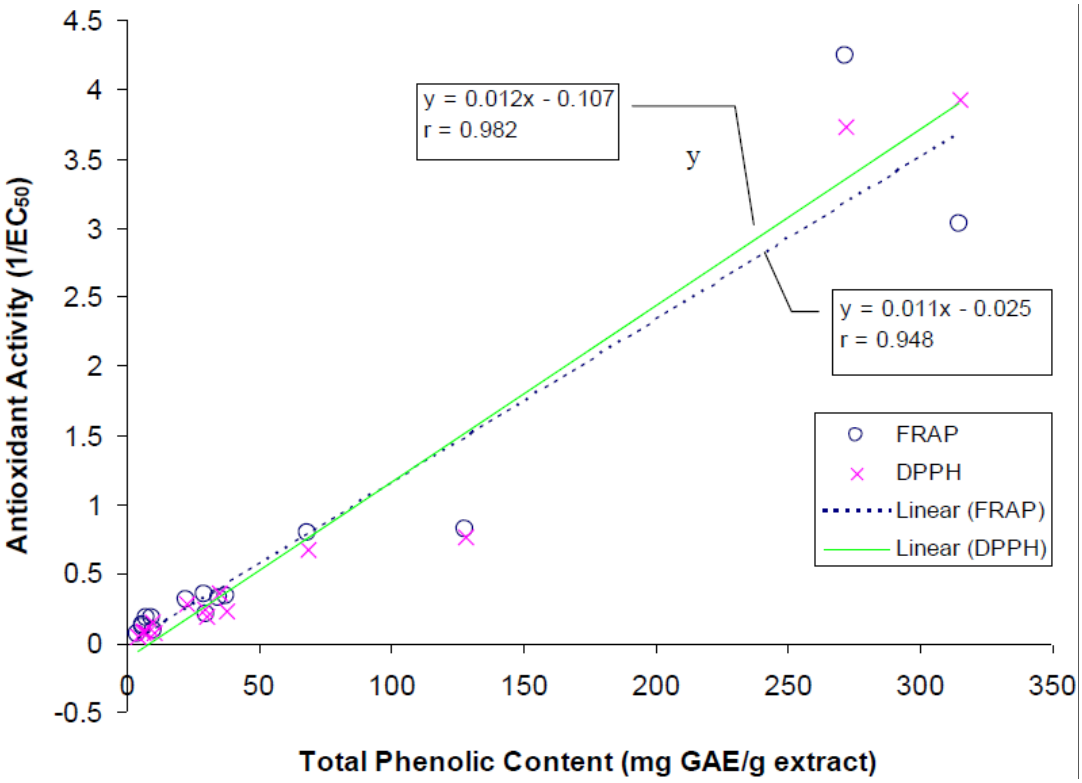

Figure 5. Correlation between total phenolic content and antioxidant activity of 15 spices extracts.

Table 1. Phytochemical screening for selected spice extracts.

Phytochemical compounds	Cinnamon bark extract	Clove buds extract
Alkaloids	-	-
Flavonoid	+	+
Saponin	+	+
Triterpene/steroid	-	-

+ = Presence of phytochemicals; - = Absence of phytochemicals.

Correlation

Figure 5 shows the correlation between TPC and antioxidant activity of aqueous extracts from 15 spices. A strong positive correlation was found between TPC and antioxidant activity, measured by DPPH test (r = 0.982) and FRAP test (r = 0.948), respectively. This indicates that phenolic compounds are one of the dominant antioxidant components contributing to the antioxidant activities of spice aqueous extracts. The results are in agreement to the findings of Shan et al. (2005), who showed that TPC of 26 spice methanolic extracts is well correlated with their antioxidant activity. The current study demonstrate that water might serve as an alternative solvent to alcohols in extracting phenolic compounds from the spices with promising yield; if the pungent taste and odour of the spices are undesired in the particular food products. Aqueous extracts have the advantage that the essential oils which carry the intrinsic flavour of the spice is removed and phenolics compounds, as the main compounds responsible for antioxidant activity are concentrated (Hinneburg et al., 2006).

Phytochemical screening tests

Since cinnamon and clove extracts exhibited the highest antioxidant activity through multiple antioxidant assays, which prompted the researchers to conduct phyto-chemical screening tests for these. Table 1 shows that both cinnamon bark and clove buds aqueous extracts contained high concentrations of flavonoids and

saponins. It is believed that these two types of compounds might have actively contributed to their antioxidant activity, which is proven through DPPH scavenging activity and ferric reducing power tests.

Flavonoids are the major group of phenolic compounds covering 50% of over 8000 phenolic compounds identified so far (Harborne et al., 1999). Contrary to tocopherol, which is fat soluble antioxidants, flavonoids are common natural antioxidants, which are sufficiently effective in both hydrophilic and lipophilic systems. According to Shan et al. (2005), the major flavonoid in polar extracts (methanol) from cinnamon bark extracts is flavan-3-ol at 452.2 mg/100 g (dry weight basis), while those from clove bud methanolic extracts are quercetin, kaempferol and some unidentified flavonoids at level of 28.4, 23.8 and 366.5 mg/100 g (dry weight basis), respectively. Saponins are surface active sterols or triterpene glycosides, which are widely found in plants and some marine organisms such as sea cucumber and star fish. Commonly used edible saponins are found in soybeans, chick peas, peanuts and spinach. As far as authors are concerned, there are no reports showing the presence of saponins in clove and cinnamon aqueous extracts. Some saponins play important role in exerting the antioxidant activity of botanical extracts. For instance, saponins from *Platycodon grandiflorum* (traditional Chinese herbs), ginseng and soybeans have shown high antioxidant activity through various *in vitro* and *in vivo* models (Lee et al., 2004).

Conclusion

In conclusion, it is found that these 15 selected deodorized aqueous extracts from spices exhibit antioxidant activity due to their high phenolic contents. Data obtained from this study shows that cinnamon bark and clove buds extracts have the potential, comparable to BHA/BHT and ascorbic acid, to serve as effective, economical natural antioxidants in food products with lower level of unfavorable pungency. However, more work needs to be done on isolation and purification of bioactive compounds from these spices. Their effectiveness or toxicity also needs to be investigated using *in vitro* and animal models.

ACKNOWLEDGEMENT

The authors are grateful to the National University of Malaysia, Universiti Putra Malaysia, Malaysian Ministry of Agriculture and Agro-based (MOA) as well as Malaysian Ministry of Science, Technology and Innovation for the financial and technical supports.

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