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Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants

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Abstract

The principal antioxidant components and content of cinnamon (*Cinnamomum cassia*), turmeric (*Curcuma longa*) and golden thread (*Coptidis rhizoma*) extracts were determined using high performance liquid chromatography (HPLC) with UV detection. In general, *C. cassia*, *C. longa* and *C. rhizoma* extracts from domestic Taiwan were rich in cinnamaldehyde, curcumin, and berberin, respectively. The contents of cinnamaldehyde, curcumin, and berberin in the acetone extracts were 1911, 2029, and 840 mg l^{-1}, respectively. The Folin–Ciocalteu method was used to measure the total phenolic concentrations of extracts, which had the content of 9.6 (*C. cassia*), 2.6 (*C. longa*), and 4.3 (*C. rhizoma*) mM l^{-1}. In addition, DPPH radical-scavenging, ferric-reducing antioxidant power (FRAP), and ferric thiocyanate (FTC) assays were employed to measure antioxidant activities. The *C. cassia* fresh extracts had higher antioxidant activities which were 84–90% (DPPH), 17–33 μmol l^{-1} (FRAP), and 53–82% (FTC). The activities of *C. longa* fresh extracts were 22–44% (DPPH), 7–11 μmol l^{-1} (FRAP), and 53–81% (FTC) while *C. rhizoma* were 53–64% (DPPH), 18–26 μmol l^{-1} (FRAP), and 59–82% (FTC).

Keywords: Cinnamaldehyde; Berberin; Curcumin; HPLC; Total phenols; Antioxidant activity

1. Introduction

The active oxygen and nitrogen species may induce some damage to the human body. Over production of various forms of activated oxygen species, such as oxygen radicals and non-free radical species are considered to be the main contributor to oxidative stress (Ellnain-Wojtaszek, Kruczynski, & Kasprzak, 2003; Gulcin, Oktay, Kufrayvioglu, & Aslan, 2002; Yildirim et al., 2000). These oxygen radicals may induce some oxidative damage to biomolecules such as carbohydrates, proteins, lipids and DNA (Lai & Piette, 1977; Wiseman & Halliwell, 1996), thus accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation (Ames, 1983; Stadtman, 1992). The protective action of medicinal plants has been attributed to the presence of antioxidants, especially polyphenolic compounds and antioxidant vitamins, including ascorbic acid, tocopherol, β-carotene, flavonoids, tannins, anthocyanins, and other phenolic constituents (Salah et al., 1995; Saskia et al., 1996; Soong & Barlow, 2004). Food rich in antioxidants plays an essential role in the prevention of cardiovascular diseases and cancers (Gerber et al., 2002; Kris-Etherton et al., 2002) and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (Di Matteo & Esposito, 2003). Thus, it is important to increase the antioxidant intake in the diet and search for natural antioxidant sources among plants used as food additives.

In the past decade, the essential oils and various extracts of plants have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of foods from the toxic effects

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of oxidants (Lis-Balchin & Deans, 1997). Moreover, they offer an effective way to prevent the development of various off-flavors and undesirable compounds that result from lipid peroxidation in foods (Wang et al., 1998). Considering the quality and safety of foods or additives, only selected compounds are allowed (Weng & Wang, 2000). Because of the possible toxicities of the synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), increasing attention has been directed toward natural antioxidants (Naimiki, 1990).

Plants have an almost limitless ability to synthesize aromatic substances, most of which are polyphenolic or their oxygen-substituted derivatives. Epidemiologists have observed that a diet rich in polyphenolic compounds may result in a positive health effect attributed to their antioxidant properties (Frankel, Huang, Aeschbach, & Prior, 1996; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). These phenolic compounds are usually identified by standard HPLC techniques. During the past decades, extensive analytical research has been carried out on the extraction and determination of various plant resources and products. The techniques previously used include UV–Vis spectrophotometry, thin-layer chromatography (Chang, Yen, Huang, & Duh, 2002; Mokbel & Hashinaga, 2006), gas-liquid chromatography (Sacchetti et al., 2005), HPLC (Chen, Zuo, & Deng, 2001; Xiao, Krucker, Albert, & Liang, 2004) and capillary electrophoresis (Peng, Yuan, Liu, & Ye, 2005). In order to determine the antioxidant

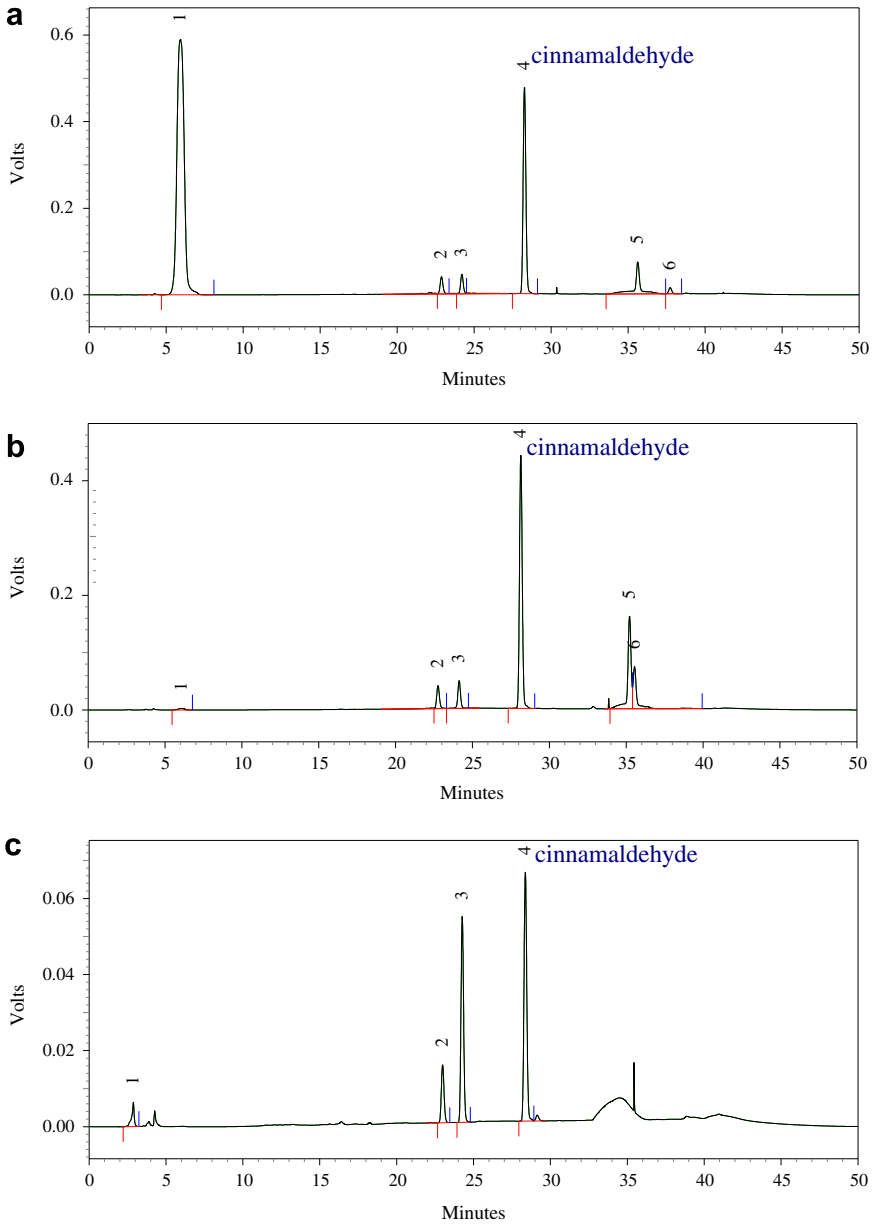

Fig. 1. HPLC chromatograms of cinnamaldehyde extracted from *C. cassia* with various solvents: (a) acetone, (b) methanol, (c) hot water.

activity of medicinal plants, and evaluate the potential use as antioxidant additives in food. The objectives of this work were to determine the principal phenolic phytochemicals, such as cinnamaldehyde, berberin, and curcumin, of cinnamon (*Cinnamomum cassia*), turmeric (*Curcuma longa*) and golden thread (*Coptidis rhizoma*) and study the antioxidant activities of their extracts.

2. Materials and methods

2.1. Herb sample preparation

Three Chinese traditional herbs were chosen based on their reported activities. The medicinal plants used in this study were *C. cassia*, *C. rhizoma*, and *C. longa*. All herbs were purchased from traditional Chinese medicine store. Plant materials were air-dried and then ground into fine powder (less than 20 mesh) using a stainless-steel grinder and stored in pill vials at room temperature.

2.2. Extraction of plant material

An aliquot (2 g) of the powdered plant material was extracted with either 45 ml of hot water for 8 h, 3 × 15 ml 80% methanol or 3 × 15 ml acetone for 24 h. After centrifugation at 3800*g* for 30 min, the extracts were collected and evaporated to dryness in vacuum. The concentrate was then diluted to 50 ml with either water or organic solvent as described.

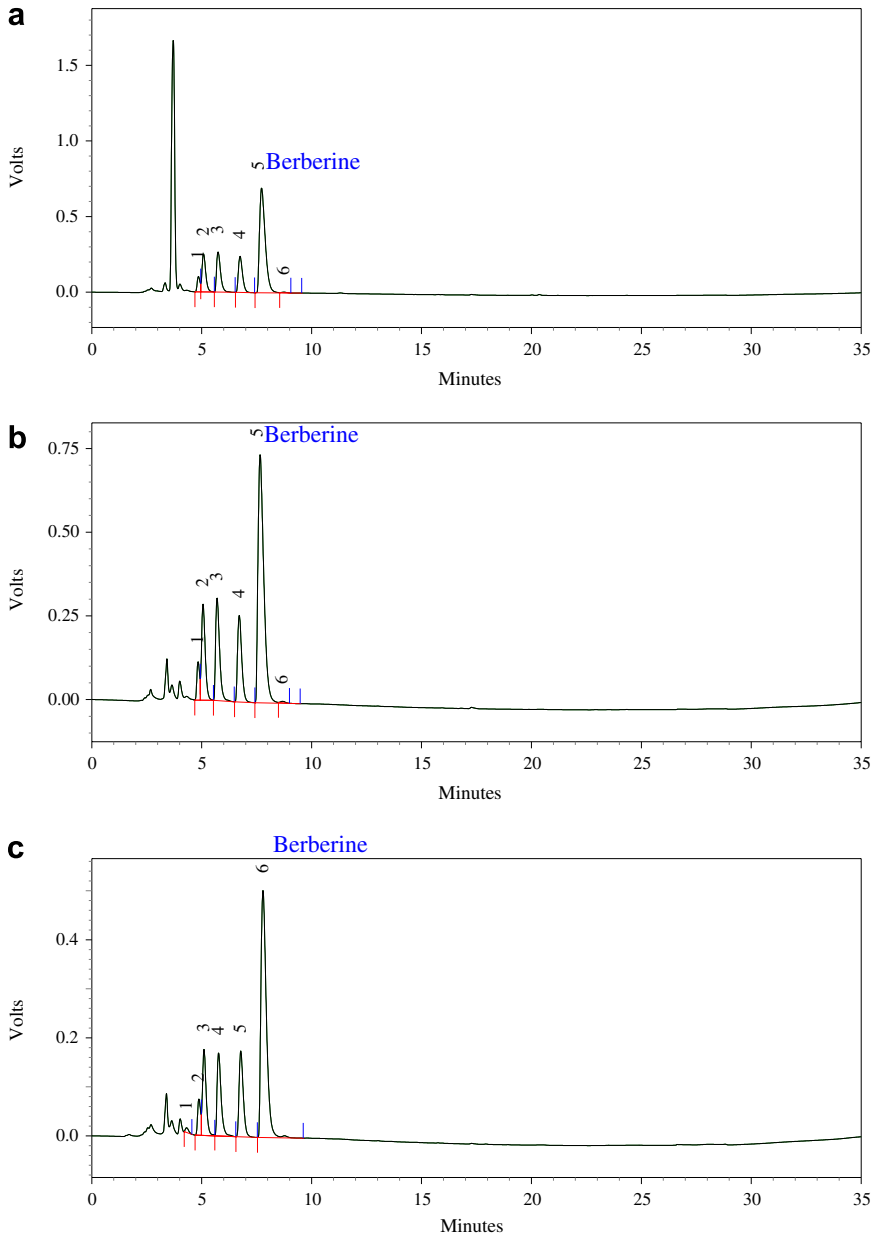

Fig. 2. HPLC chromatograms of berberine extracted from *C. longa* with various solvents: (a) acetone, (b) methanol, (c) hot water.

2.3. HPLC determinations of antioxidant components

HPLC was conducted with a Shimadzu LC Model system (Shimadzu Co., Japan). The HPLC system consisted of a LC-10AT VP pump, a SIL-10AD VP auto-sampler, a SPD-10A VP ultraviolet-visible detector and a CL-10A VP controller connected to a PC. Peak area was calculated using Shimadzu CLASS-VP v6.12 SP2 software. A Tosoh TSK-GELODS-100S C18 column (250 × 46 mm i.d., 3 μm particle size) was used. The mobile phase for analysis of cinnamaldehyde was a gradient of deionized water and acetonitrile. The gradient was deionized water: acetonitrile

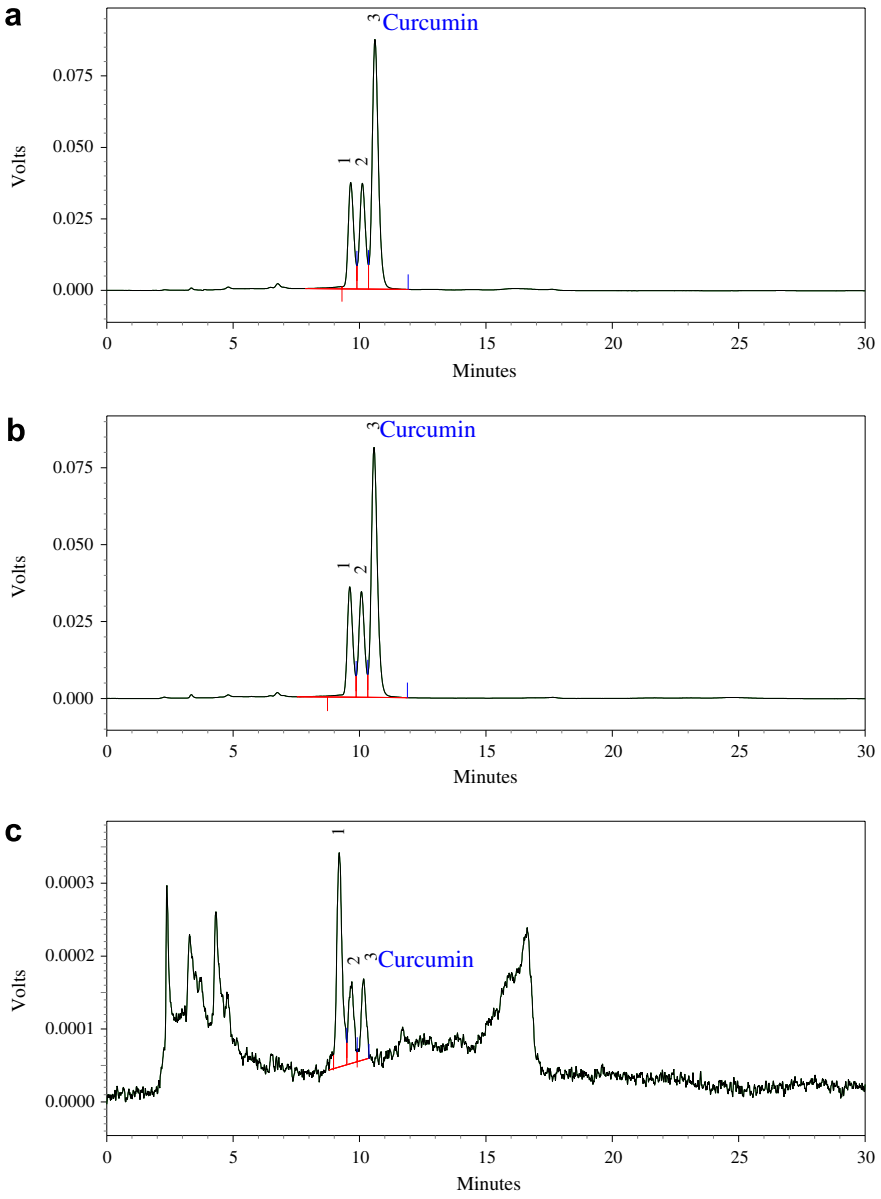

Fig. 3. HPLC chromatograms of curcumin extracted from *C. rhizoma* with various solvents, (a) acetone, (b) methanol, (c) hot water.

Table 1
Content of the principal antioxidant compound of plant extracts

Compounds	Concentration (mg l^{-1})		
	Acetone extracts	Methanol extracts	Hot water extracts
Cinnamaldehyde	1911.3 ± 10.3[a]	1496.1 ± 15.0[b]	1425.8 ± 8.2[c]
Curcumin	2028.6 ± 20.0[a]	1890.2 ± 3.0[b]	8.2 ± 0.1[c]
Berberin	840.4 ± 10.0[a]	790.1 ± 8.0[b]	112.9 ± 5.0[c]

Data are expressed as the average of three determinations ± SD. Data with different lower case letters on the individual plant extracts are significantly different ($p < 0.05$).

Table 2
Total phenolic content, DPPH free radical-scavenging activity, and antioxidant activities of *Cinnamomum cassia*, *Curcuma longa* and *Coptidis rhizoma* extracts

Herbs	Item	Phenolic content (mM GAE l^{-1})			Radical-scavenging ability (%)			Antioxidant activity (mmol l^{-1})[A]			Antioxidant activity (%)[B]		
		Acetone	Methanol	Hot water	Acetone	Methanol	Hot water	Acetone	Methanol	Hot water	Acetone	Methanol	Hot water
Cinnamomum cassia	Fresh[C]	9.62 ± 0.34^{a}	7.14 ± 0.11^{b}	2.52 ± 0.08^{d}	89.24 ± 0.16^{a}	89.60 ± 0.25^{b}	84.42 ± 0.16^{c}	33.09 ± 0.07^{a}	31.73 ± 0.31^{b}	16.59 ± 0.6^{c}	78.9 ± 2.1^{a}	81.8 ± 0.5^{a}	53.3 ± 0.9^{b}
	25 °C	1.50 ± 0.03^{e}	0.36 ± 0.06^{g}	1.37 ± 0.05^{f}	75.11 ± 0.73^{d}	35.94 ± 0.51^{g}	44.68 ± 1.10^{f}	2.6 ± 0.17^{g}	0.85 ± 0.05^{h}	3.54 ± 0.08^{f}	77.4 ± 3.7^{a}	37.2 ± 2.4^{d}	16.8 ± 0.5^{e}
	4 °C	3.13 ± 0.23^{c}	1.8 ± 0.13 d^{e}	1.78 ± 0.12^{e}	88.78 ± 0.19^{a}	88.49 ± 0.27^{b}	49.78 ± 1.37^{e}	18.05 ± 1.06^{c}	13.35 ± 0.40^{d}	7.95 ± 0.11^{e}	77.5 ± 2.2^{a}	77.0 ± 1.7^{a}	46.8 ± 2.4^{c}
Curcuma longa	Fresh	1.86 ± 0.04^{b}	2.55 ± 0.05^{a}	1.31 ± 0.03^{c}	37.41 ± 0.86^{b}	40.04 ± 0.41^{a}	22.01 ± 0.69^{e}	10.93 ± 0.12^{a}	9.72 ± 0.91^{b}	6.50 ± 0.88^{c}	80.8 ± 2.5^{a}	73.8 ± 2.0^{b}	52.6 ± 1.6^{d}
	25 °C	1.05 ± 0.01^{f}	0.92 ± 0.02^{g}	0.86 ± 0.02^{h}	24.93 ± 0.44^{d}	20.68 ± 0.49^{f}	16.15 ± 1.59^{g}	3.86 ± 0.13^{f}	2.78 ± 0.33^{g}	3.71 ± 0.10^{f}	38.9 ± 2.3^{f}	63.2 ± 2.9^{e}	30.0 ± 2.1^{g}
	4 °C	1.23 ± 0.04^{d}	1.89 ± 0.04^{b}	1.09 ± 0.03^{f}	34.35 ± 0.51^{c}	33.71 ± 0.92^{c}	20.83 ± 0.38^{f}	5.52 ± 0.20^{d}	7.34 ± 0.33^{c}	5.02 ± 0.16^{e}	51.7 ± 1.9^{d}	64.7 ± 2.8^{c}	44.3 ± 1.2^{e}
Coptidis rhizoma	Fresh	3.48 ± 0.01^{b}	4.26 ± 0.07^{a}	2.69 ± 0.08c^{d}	64.39 ± 3.09^{a}	60.36 ± 1.06^{a}	53.09 ± 2.03^{b}	25.91 ± 1.13^{a}	23.61 ± 1.80^{a}	17.74 ± 0.46^{b}	81.3 ± 1.8^{a}	81.9 ± 1.6^{a}	59.2 ± 0.8^{b}
	25 °C	2.75 ± 0.02^{c}	2.29 ± 0.13^{e}	2.42 ± 0.05^{e}	52.95 ± 1.38^{b}	8.96 ± 0.39^{f}	38.06 ± 2.20^{d}	12.98 ± 0.56^{e}	10.83 ± 0.97^{f}	13.98 ± 0.19^{d}	78.8 ± 2.0^{a}	79.3 ± 3.0^{a}	35.6 ± 1.6^{d}
	4 °C	2.71 ± 0.04^{c}	2.81 ± 0.01^{c}	2.57 ± 0.04^{d}	55.79 ± 0.16^{b}	36.01 ± 0.75^{e}	48.06 ± 0.45^{c}	26.66 ± 0.48^{a}	10.64 ± 1.10^{f}	16.04 ± 0.90^{c}	80.3 ± 3.2^{a}	79.8 ± 1.1^{a}	52.9 ± 2.3^{c}

Data are expressed as the average of three determinations ± SD. Data with different lower case letters on the individual plant extracts are significantly different ($p < 0.05$).
[A] FRAP assay.
[B] FTC assay.
[C] Freshly prepared extract with no storage treatment.

(91:9–80:20 for 0–5 min, 50:50 for 5–35 min, 80:20 for 35–40 min, 91:9 for 40–45 min, and 100:0 for 45–50 min). The mobile phase for analysis of curcumin was acetonitrile: methanol: deioned water: acetic acid (41:23:36:1, v/v/v/v). The solvents used for analysis of berberin was [A] 10 mM K_2HPO_4 (pH 2.5) and [B] acetonitrile. The gradient was [A]:[B] (100:0–70:30 for 0–5 min, 0:100 for 5–20 min, 70:30 for 20–30 min, and 100:0 for 30–35 min). The analyses were carried out at a flow-rate of 0.8 ml min^{-1} with UV detection at 254 nm (for cinnamaldehyde and berberin) and 422 nm (for curcumin). A standard solution containing cinnamaldehyde, curcumin, or berberin was used to calibrate the retention time and standard curve. *n*-Propyl benzoate was used as the internal standard.

2.4. Determination total phenolics content

The Folin–Ciocalteu reagent assay was used to determine the total phenolics content (Singlenton & Rossi, 1965). The sample (0.2 ml) was mixed with 0.5 ml Folin–Ciocalteu reagent previously diluted with 7 ml deionized water. The solution was allowed to stand for 3 min at 25 °C before adding 0.2 ml of saturated sodium carbonate solution. The mixing solution was allowed to stand for another 120 min before the absorbance at 725 nm was measured. Gallic acid was used as standard for the calibration curve. The total phenolics content was expressed as mM gallic acid equivalents (GAE) per liter of sample (mM l^{-1}).

2.5. DPPH radical-scavenging assay

The DPPH radical-scavenging assay was carried out according to the modified method of Su and Silva (2006). Five millimeter of 0.03 g l^{-1} DPPH (2,2-diphenyl-1-picrylhidrazyl) methanol solution was reacted with 0.05 ml sample extract at room temperature. The extraction solvent was used as control. The absorbance was measured at 517 nm after 30 min of reaction in the dark.

2.6. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out by using a modified method of Benzie and Szeto (1999). A 10 μl of the sample extract was mixed with 900 μl of FRAP reagent and 90 μl deionized water. The deionized water was used as control. The absorbance reading at 539 nm was taken after standing for 30 min. Aqueous solutions of 0–5000 μmol l^{-1} ferrous sulfate heptahydrate were used for calibration and antioxidant power was expressed as μmol l^{-1}.

2.7. Ferric thiocyanate (FTC) assay

The assay was performed according to the methods described by Larrauri, Ruperez, and Saura (1997). 0.1 ml of the extract was mixed with 1 ml 50 mM phosphate buffer, 0.4 ml deionized water, 0.5 ml methanol, and 0.5 ml of 2.5% linoleate. One hundred microliters of methanol was

used to replace the plant extracts as a control. The mixture was incubated at 50 °C and analyzed every 24 h interval. 50 μl of the incubated mixture was thoroughly mixed with 9.7 ml of 70% ethanol and 0.1 ml of 30% thiocyanate. The solutions were allowed to stand for 3 min at room temperature before adding 0.1 ml of 20 mM ferric chloride solution. The absorbance was measured at 500 nm.

2.8. Statistical analysis

A completely randomized design was used. The triplicate data were analyzed using the general linear models (GLM) procedure. The statistical analysis was conducted with SAS program (SAS Inst. Inc., Cary, NC, USA).

3. Results and discussion

3.1. HPLC analyses of the principal phenolic compounds of selected plant extracts

HPLC chromatograms of the principal antioxidant compounds of plant extracts are presented in Figs. 1–3. A good resolution was obtained for identification of cinnamaldehyde (peak 4, Fig. 1). Berberine and curcumin in the extracts were also identified as shown by peak 5 (Fig. 2) and peak 3 (Fig. 3), respectively. However, some reports describe that many phenolic compounds show maximum absorbance between 265 and 335 nm, while excitation and emission spectra are specific (Rodríguez-Delgado, Malovaná, Peŕez, Borges, & García Montelongo, 2001). HPLC determination of phenolic compounds has become one of dominant analytical procedures because of its advantages; e.g. simple sample treatment, possibility to pre-separate and to remove impurities, possibility to change the polarity of mobile phase during analysis, short analysis time, and high reproducibility. Using HPLC analysis, the content of the principal antioxidant compounds of plant extracts are listed in Table 1. The highest concentration of cinnamaldehyde in *C. cassia* (1911 mg l^{-1}), curcumin in *C. longa* (2029 mg l^{-1}) or berberine in *C. rhizoma* (840 mg l^{-1}) was obtained from their acetone extracts.

3.2. Total phenolics

Table 2 shows the total phenolic content of *C. cassia*, *C. longa* and *C. rhizoma* extracts. The extracts of *C. cassia* had higher total phenolics content, followed by *C. rhizoma*, and *C. longa*. The total phenolic content of fresh acetone and methanol extracts *C. cassia* was 9.6 and 7.1 mM l^{-1} GAE. However, after one month of storage at 4 °C and 25 °C,

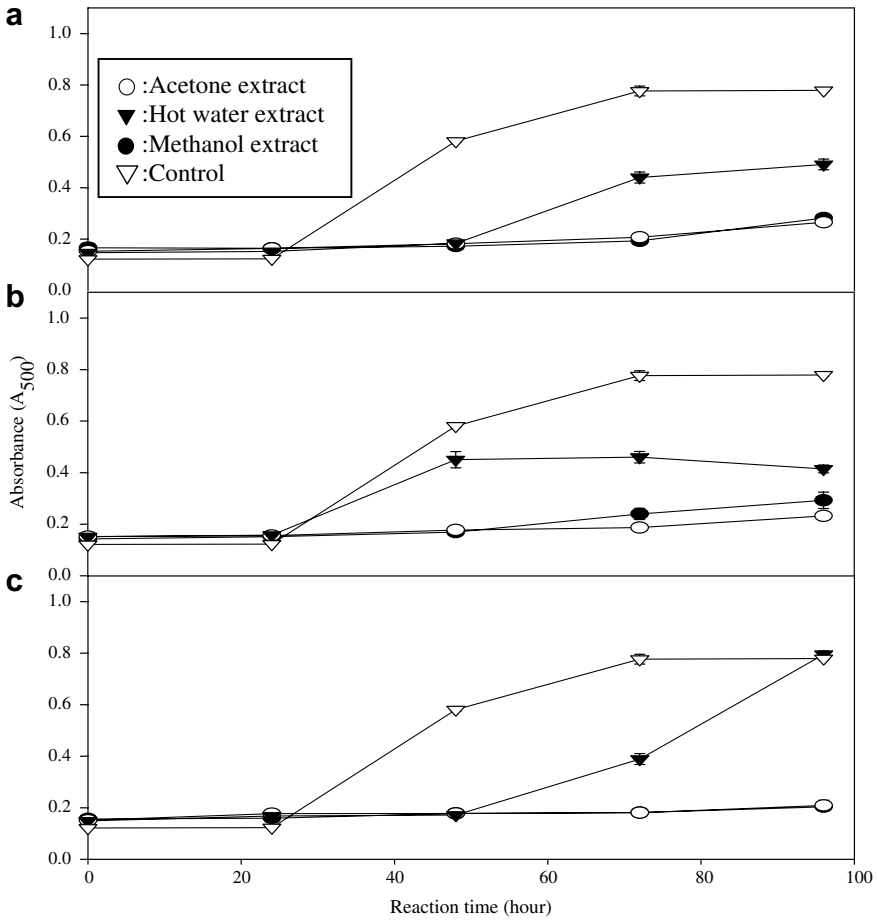

Fig. 4. The antioxidant activities of: (a) *C. cassia*, (b) *C. longa* and (c) *C. rhizoma* fresh extracts were measured using FTC assay.

the total phenolics content of extracts of *C. cassia* decreased to 1.8–3.1 and 0.4–3.5 mM l^{-1} GAE, respectively. The total phenolic content of *C. longa* or *C. rhizoma* extracts also showed the same decreasing tendency during storage. An elevated storage temperature will lead to decrease of the total phenolic content of plant extracts. The period of storage affects stability of antioxidant compound might due to chemical and enzymatic decomposition. These have been suggested to be the main mechanisms causing the degradation of phenolics. However, further investigation is needed to explain this phenomenon.

3.3. The DPPH radical-scavenging ability of selected plant extracts

DPPH is a free radical compound that has been widely used to determine the free radical-scavenging ability of various samples (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004; Hatano, Kagawa, Yasuhara, & Okuda, 1988). The DPPH free radical-scavenging activity of plant extracts is presented in Table 2. The results are shown as relative activities against the control. The relative activities of freshly prepared *C. cassia* extracts were between 89% and 84%, which were higher than those of *C. longa* or *C. rhizoma* extracts. After one month of storage at 4 °C and 25 °C, the activities of *C. cassia* extracts also showed a better stability than those of *C. longa* or *C. rhizoma*. In addition, relative activities of acetone or methanol extracts were significantly higher than those of hot water extracts. Due to different antioxidant potentials of different compounds, the antioxidant activity of extract strongly dependents on the extraction solvent. The DPPH radical-scavenging activity of *C. cassia* or *C. rhizoma* extract was close to the results reported by Steenkamp, Grimmer, Semano, and Gulumian (2000) and Tomaino et al. (2005). Furthermore, The DPPH radical-scavenging activity of *C. longa* extract was about 15% higher than the result reported by Mau et al. (2003).

3.4. Antioxidant activity

The antioxidant activities of herb extracts using FRAP and FTC assay are shown in Table 2. Among all plant extracts investigated, the extracts of *C. cassia* had higher FRAP value followed by *C. rhizome*. There was more than 5-fold difference between FRAP values in various freshly prepared plant extracts. Comparing all the acetone, methanol and hot water extracts, there were four kinds of extracts in which the FRAP values were over 20 mmol l^{-1}. The fresh acetone extracts of *C. cassia* had the highest FRAP value (33.1 mmol l^{-1}) followed by the methanol extract and the hot water extract. Using a different solvent, the results of the *C. longa* fresh extracts were significant different. The acetone extracts and methanol extracts of *C. longa* or *C. rhizoma* also showed stronger antioxidant activities than the hot water extracts. Thus, it is apparent that fresh extracts in this study have strong effects against the oxidation of linoleic acid. Solvent (acetone or methanol) extracts showed a good inhibition of linoleic acid peroxidation as compared with the control (Fig. 4). The absorbance of the control increased that it had a faster rate from 0.16 to 0.7, whereas, the acetone or methanol fresh extracts of *C. cassia* had lower rate maintaining 0.16 and 0.20, respectively.

4. Conclusion

Acetone or methanol extracts show a high capacity to inhibit of linoleic acid peroxidation. The total antioxidant capacity and phenolics content vary considerably from one kind of plant to another in this study. In addition, they are found to be different for different kinds of solvent extracts. After storage at 25 °C, the total phenolics and antioxidant activities of the extracts were considerably decreased. The extracts of *C. longa* exhibited less antioxidant activity than those of *C. cassia* and *C. rhizoma* in this study. However, a large amount of curcumin was determined in the *C. longa* extract. Therefore, other antioxidant compounds in the extracts of *C. longa* should be further studied.

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