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Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts *in vitro*

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ABSTRACT

Bitter gourd (*Momordica charantia* L.) has long been regarded as a food and medicinal plant. We investigated the antioxidant activity of the water extract of leaf, stem and fruit fractions by several *in vitro* systems of assay, namely DPPH radical-scavenging activity, hydroxyl radical-scavenging activity, β-carotene–linoleate bleaching assay, ferric reducing/antioxidant power (FRAP) assay and total antioxidant capacity. Total phenolic content was measured by Folin–Ciocalteu reagent. Identification of phenolic compounds was achieved using HPLC with the UV-diode array detection. The extracts of different fractions were found to have different levels of antioxidant activity in the systems tested. The leaf extract showed the highest value of antioxidant activity, based on DPPH radical-scavenging activity and ferric reducing power, while the green fruit extract showed the highest value of antioxidant activity, based on hydroxyl radical-scavenging activity, β-carotene–linoleate bleaching assay and total antioxidant capacity. The predominant phenolic compounds were gallic acid, followed by caffeic acid and catechin. The present study demonstrated that the water extract fractions of bitter gourd have different responses with different antioxidant methods. Total phenol content was shown to provide the highest association with FRAP assay in this present study (R^2 = 0.948).

1. Introduction

Research on relationships between antioxidants and prevention of non-communicable disease, such as cardiovascular disease, cancer, diabetes has been increasing sharply in recent years. Free radicals have been claimed to play a key role, affecting human health by causing severe diseases, such as cancer and cardiovascular diseases by cell degeneration. These free radicals can be generated during normal body function, and can be acquired from the environment. The oxygen radicals are associated with cellular and metabolic injury, and accelerated aging, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation (Ames, 1983; Dasgupta & De, 2006; Stadtman, 1992; Sun, 1990). The oxidative damage might be prevented or limited by dietary antioxidants (Dasgupta & De, 2006). The most extensively used synthetic antioxidants, such as butylated hydroxyl anisole (BHA), and butylated hydroxyl toluene (BHT), have restricted use in food and have been suspected of being responsible for liver damage and carcinogenesis (Grice, 1986; Hettiarachchy, Glenn, Gnanasambandam, & Jonhnson, 1996; Ito, Fukushima, & Tsuda, 1986; Senevirathne et al., 2006; Suja, Jayalekshmy, & Arumughan, 2005; Wichi, 1988). Epidemiological and *in vitro* studies strongly suggest that food containing phytochemicals with antioxidants have potentially protective effects against many diseases, including cancer, diabetes and cardiovascular diseases (Senevirathne et al., 2006). Consumption of fruits and vegetables has contributed to the prevention of degenerative processes caused by oxidative stress (Kaur & Kapoor, 2001; Vinson, Xuehui, Ligia, & Bose, 2001). Foods such as fruits, vegetables and grains are reported to contain a wide variety of antioxidant components, including phytochemicals. Phytochemicals, such as phenolic compounds, are considered beneficial for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (Larson, 1988; Pereira et al., 2007; Pulido, Bravo, & Saura-Calixto, 2000; Silva et al., 2004; Velioglu, Mazza, Gao, & Oomah, 1998). These compounds have been reported to be well correlated with antioxidant potential (Katalinic, Milos, Modun, Music, & Boban, 2004).

Bitter gourd (*Momordica charantia* L.) or Mara (in Thai) has long been used as a food and medicine (El Batran, El-Gengaihi, & El Shabrawya, 2006). Bitter gourd is called by different names since it grows in tropical regions such as India, Malaya, China, tropical Africa, MiddleEast, America (Kirtikar & Basu, 1993) and Thailand. As a medicinal plant, it has been reported to possess antilipolytic, analgesic, abortifacient, antiviral, cytotoxic, hypoglycemic and antimutagenic properties (Singh, Singh, & Bamezai, 1998). Extract powder of fresh and dried whole fruit of bitter gourd lowered blood sugar in diabetic rats, as reported by Virdi et al. (2003). El Batran et al. (2006) reported that bitter gourd extracts showed

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anti-diabetic, hepato-renal protective and hypolipidemic effects in alloxan-induced diabetic rats. Wu and Ng (2007) found that extracts of wild bitter melon grown in Taiwan, possessed potent antioxidant and free radical-scavenging activities. Some researchers have also found that Thai bitter gourd fruit contained anticarcinogens or chemopreventive agent (Kusamran, Tepsuwan, & Kupradinun, 1998; Yasui et al., 2005). However, there has been little information regarding antioxidant activities of different parts of bitter gourd (mostly reported on fruit), especially leaf and stem, which are commonly consumed as vegetable in Thailand. Therefore, the purpose of this present study was to investigate phenolic compounds of bitter gourd fractions and to evaluate antioxidation activity by using different *in vitro* methods. Different fractions of Thai bitter gourd (*M. charantia* L.), namely leaf, stem and fruit fractions, were determined in an attempt to make systematic comparisons among their antioxidant activities and to identify the fractions with high antioxidant activity for further studies. In addition, correlations between total phenol content and antioxidant activity, assessed by different methods, were also evaluated.

2. Materials and methods

2.1. Sample

Bitter gourd (*M. charantia* L.) leaf, stem and fruit were collected from Mahasaraklham, Thailand. The harvest starts from 60–65 days after growing for leaf and stem. Mature (green) and ripe (fully yellow) fruits were harvested, as judged by fully yellow colour. The extracts prepared from the freeze-dried leaf, stem and fruit were made by boiling in distilled water for 5 min. The ratio between sample and extraction medium was 1:25. The mixtures were filtered through a filter paper (Whatman No. 1) (Whatman International Ltd., Maidstone, England) (Dasgupta & De, 2004) and used for analyzing antioxidant activity *in vitro*. All analyses were performed in three replicates.

2.2. Reagents and standards

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2-deoxyribose, trichloroacetic acid, thiobarbituric acid, β-carotene, Tween 20, linoleic acid, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), Folin–Ciocalteu reagent, ethylene diamine tetraacetic acid (EDTA), ammonium molybdate, phenolic acids (*p*-coumaric, tannic, benzoic, ferulic, gallic and caffeic acids and (+)-catechin) were obtained from Fluka (Neu-Ulm, Germany). HPLC-grade methanol, acetonitrile and other solvents and reagents were purchased from Merck (Darmstadt, Germany).

2.3. DPPHl radical-scavenging activity

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH (Gulluce et al., 2007). The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). Aqueous extract (0.1 ml) was added to 3 ml of a 0.001 M DPPH in methanol. Absorbance at 517 nm was determined after 30 min, and the percent inhibition of activity was calculated as $[(Ao - Ae)/Ao] \times 100$ (Ao = absorbance without extract; Ae = absorbance with extract).

2.4. Hydroxyl radical-scavenging activity

2-Deoxyribose is oxidized by the Fenton reaction and degraded to malondialdehyde (Chung, Osawa, & Kawakishi, 1997): 0.2 ml of $FeSO_4 \cdot 7H_2O$(10 mmol) and EDTA (10 mmol) mixed solution was prepared in a screw-capped test tube, and 0.2 ml of 2-deoxyribose solution (10 mmol), the sample solution and phosphate buffer (pH 7.4, 0.1 mol) were added to give a total volume of 1.8 ml. Finally, 200 μl of H_2O_2 soln. (10 mmol) was added to this reaction mixture and the whole incubated at 37 °C for 4 h. After this incubation, 1 ml each of a trichloroacetic acid solution (2.8%) and thiobarbituric acid solution (1.0%) were added to the reaction mixture, and the whole boiled for 10 min, cooled in ice, and its absorbance measured at 520 nm.

The **.**OH-scavenging activity was calculated as the inhibition rate of 2-deoxyribose

$$\text{.OH-scavenging activity}(\%) = [(1 - (Ab_s - Ab_o)/(Ab_c - Ab_o)] \times 100,$$

where Ab_o is the absorbance with no treatment at 520 nm; Ab_c is the absorbance of treated control at 520 nm; Ab_s is the absorbance of treated sample at 520 nm.

2.5. β-Carotene–linoleate bleaching assay

The antioxidant activity of bitter gourd fraction extract was assayed, based on the β-carotene bleaching method (Othman, Ismail, Ghani, & Adenan, 2007). BHT was used as the standard. β-carotene (0.2 mg in 1 ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2 ml) were transferred into a round-bottomed flask. The mixture was then added to 0.2 ml of bitter gourd fraction extract or standard or ethanol (as control). Chloroform were removed at room temperature under vacuum at reduced pressure using a rotary evaporator. Following evaporation, 50 ml of distilled water was added to the mixture, then shaken vigorously to form an emulsion. Two millilitre aliquots of the emulsion were pipetted into test tubes and immediately placed in a water bath at 50 °C. The absorbance was read at 20 min intervals for 2 h at 470 nm .The antioxidant activity of extracts was based upon two different parameters, namely antioxidant activity (A_A) and the oxidation rate ratio (ROR).

Antioxidant activity (A_A) was expressed as percent of inhibition relative to the control, using the following formula (Suja et al., 2005):

$$A_A = [R_{control} - R_{sample\ or\ standard})/R_{control}] \times 100,$$

where $R_{control}$ and R_{sample} represent the bleaching rates of β-carotene without and with the addition of antioxidant, respectively. Degradation rates (R_D) were calculated according to first-order kinetics:

$$R_D = \ln(A_t/A_x) \times 1/t,$$

where ln is natural log, A_t is the initial absorbance at 470 nm at $t = 0$ and A_x is the absorbance at 470 nm at $t = 20$, 40, 60, 80, 100,120 min.

The oxidation rate ratio (R_{OR}) was calculated as

$$R_{OR} = R_{sample}/R_{Rcontrol},$$

where R_{sample} and $R_{control}$ are as described earlier.

2.6. Ferric reducing/antioxidant power (FRAP) assay

FRAP assay was based on the reduction of Fe^{3+}–TPTZ to a blue-coloured Fe^{2+}–TPTZ (Benzie and Strain,1996). The FRAP assay was adapted from Moyer, Hummer, Finn, Frei, and Wrolstad (2002). The antioxidant potential of the extract sample from citrus peel was determined against a standard curve of ferrous sulphate (FeII, 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM) in Milli-Q water or methanol with 0.1% (v/v) HCl.

The FRAP reagent was freshly prepared by mixing 100 ml of acetate buffer (300 mM, pH 3.6), 10 ml TPTZ solution(10 mM TPTZ in 40 mM/HCl), 10 ml $FeCl_3 \cdot 6H_2O$ (20 nM) in a ratio of 10:1:1 and 12 ml distilled water, at 37 °C. To perform the assay, 1.8 ml of FRAPreagent, 180 μl Milli-Q water and 60 μl sample, standard or blank were then added to the same test tubes, and incubated at 37 °C for 4 min; absorbance was measured at 593 nm, using FRAP working solution as blank. The reading of relative absorbance should be within the range 0–2.0; otherwise, the sample should be diluted. In the FRAP assay, the antioxidant potential of sample was determined from a standard curve plotted using the $FeSO_4 \cdot 7H_2O$ linear regression equation to calculate the FRAP values of the sample.

2.7. Determination of total antioxidant capacity

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Prieto, Pineda, & Aguilar, 1999) and determined by the method described by Dasgupta and De (2004). Aqueous extract (0.3 ml) was added to 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid and BHT.

2.8. Identification and quantification of phenolic compounds

2.8.1. Phenolics extraction

The phenolic compounds in investigated samples were extracted using a modification of the procedure described by Uzelac, Pospišil, Levaj, and Delonga (2005). Each sample (5 g) was mixed with 50 ml of methanol/HCl (100:1, v/v) which contained 2% *tert*-butylhydroquinone, in inert atmosphere (N_2) during 12 h at 35 °C in the dark. The extract was then centrifuged at 4000 rpm/min, and supernatant was evaporated to dryness under reduced pressure (35–40 °C). The residue was redissolved in 25 ml of water/ethanol (80:20, v/v) and extracted four times with 25 ml of ethyl acetate. The organic fractions were combined, dried for 30–40 min with anhydrous sodium sulfate, filtered through the Whatman-40 filter, and evaporated to dryness under vacuum (35–40 °C). The residue was redissolved in 5 ml of methanol/water (50:50, v/v) and filtered through a 0.45 μm filter before injection (20 μl) into the HPLC aperture. Samples were analyzed in triplicate.

2.8.2. Determination of total phenol content

The total phenolic contents of each extract were determined using the Folin–Ciocalteu reagent (Zhou & Yu, 2006). The reaction mixture contained 1 ml of bitter gourd fraction extract, 0.5 ml of the Folin–Ciocalteu reagent, 3 ml of 20% sodium carbonate and 10 ml of distilled water. After 2 h 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. The total phenolic contents were then expressed as gallic acid equivalents (GAE), in mg/g dry sample.

2.8.3. HPLC–DAD system for analysis of phenolic compounds

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A with diode array detector and chromatographic separations were performed on a LUNA C-18 column (4.6 × 250 mm i.d., 5 μm). The composition of solvents and used gradient elution conditions were described previously by Uzelac et al. (2005) with some modifications. The solvent system used was a gradient of mobile phase A containing 3% acetic acid in water; solution B contained a mixture of 3% acetic acid, 25% acetonitrile and 72% water. The following gradient was used: 0–40 min, from 100% A to 30% A, 70% B with a flow rate 1 ml/min; 40–45 min, from 30%A, 70% B to 20% A, 80% B with flow rate 1 ml/min; 45–55 min, from 20% A, 80% B to 15% A, 85% B with flow rate 1.2 ml/min; 55–57 min, from 15% A, 85% B to 10% A, 90% B with flow rate 1.2 ml/min; 57–75 min 10% A, 90% B with flow rate 1.2 ml/min. Operating conditions were as follows: column temperature, 40 °C; injection volume, 20 μl; UV-diode array detection at 278 nm.

2.9. Statistical analyses

Statistical analyses were conducted using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) version 12.0 for Windows. Analysis of variance (ANOVA) in a completely randomised design, Duncan's multiple range test and Pearson's correlation coefficients were performed to compare the data. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% ($P < 0.05$).

3. Results and discussion

3.1. General

Several methods have been used to determine antioxidant activity of plants. Our present study therefore involved five various established methods to evaluate antioxidative activity of bitter gourd, namely, DPPH radical-scavenging activity, hydroxyl radical-scavenging activity, β-carotene–linoleate bleaching assay, ferric reducing/antioxidant power (FRAP) assay and total antioxidant capacity. Phenolic compounds were identified and total phenol content was also determined. The results obtained from each analysis are considered below (Fig. 1).

3.2. DPPH radical- scavenging activity

DPPH**.** is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples (Hatano, Takagi, Ito, & Yoshida, 1997; Sakanaka, Tachibana, & Okada, 2005; Shimoji et al., 2002). It is a stable free radical with a characteristic absorption at 517 nm, was used to study the radical-scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. To evaluate the scavenging effects of DPPH**.** of water extract of leaf, stem and fruits, DPPH**.** inhibition was investigated. These results are shown as relative activities against BHT and ascorbic acid (Fig. 2a). The activity of 200 mg/ml ascorbic acid was the highest, followed by BHT, green fruit, stem, leaf and ripe fruit, respectively. Antioxidants, on interaction with DPPH**.**, either transfer an electron or hydrogen atom to DPPH**.**, thus neutralizing its free radical character (Naik et al., 2003).The colour changes from purple to yellow and its absorbance at wavelength 517 nm decreases. Different parts of Thai bitter gourd (*M. charantia* L.) extract quenched DPPH**.** (Table 1) in a dose-dependent manner: [$r^2 = 0.8446$] ($P < 0.05$) for leaf; [$r^2 = 0.9963$] ($P < 0.05$) for stem; [$r^2 = 0.8356$] ($P < 0.05$) for green fruit and [$r^2 = 0.9110$] ($P < 0.05$) for ripe fruit. DPPH**.** assay shows that, in this system, the radical-scavenging activities of different parts of Thai bitter gourd are in the order leaf > green fruit > stem > ripe fruit. The relative activities of DPPH**.** inhibition of different parts of bitter gourd against the control (ascorbic acid and BHT) are shown in Fig. 2. The DPPH radical-scavenging activity of 200 mg/ml ascorbic acid was the highest, followed by BHT, leaf, green fruit, stem and ripe fruit

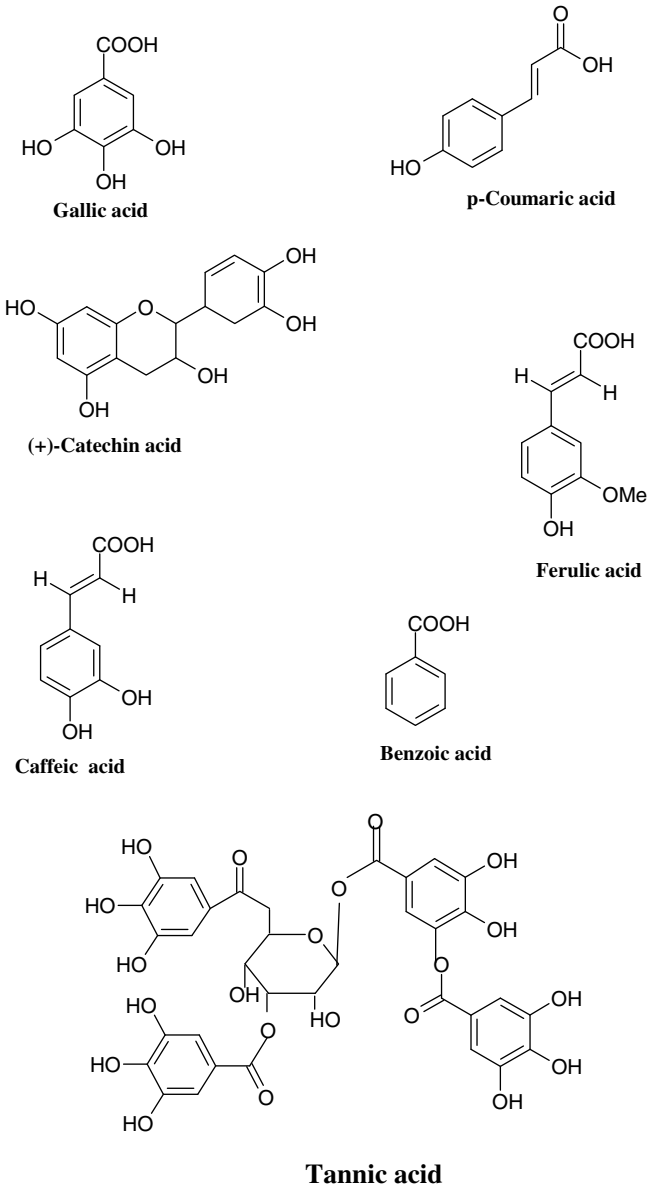

Fig. 1. Structures of the standard phenolic comopounds.

Table 1
DPPH radical-scavenging activities of different parts of bitter gourd (*Momordica charantia* L.)

Sample	Concentration (mg/ml)	% Inhibition ± SD	Logarithm equation (r^2)	IC_{50} (mg/ml)
Leaf	0.1	12.3 ± 1.83	$y = 25.815\ln(x) + 86.513$ ($r^2 = 0.8446$)	9.72 ± 0.25
	0.2	56.8 ± 2.18		
	0.4	74.6 ± 0.39		
	0.8	81.4 ± 1.76		
	1.6	89.3 ± 0.43		
Stem	0.1	10.4 ± 1.22	$y = 26.52\ln(x) + 71.527$ ($r^2 = 0.9963$)	17.8 ± 0.66
	0.2	28.9 ± 2.43		
	0.4	46.3 ± 0.83		
	0.8	68.6 ± 0.90		
	1.6	82.3 ± 0.06		
Green fruit	0.1	7.59 ± 1.26	$y = 23.319\ln(x) + 83.878$ ($r^2 = 0.8356$)	11.0 ± 0.76
	0.2	53.9 ± 0.73		
	0.4	71.2 ± 0.63		
	0.8	80.7 ± 0.41		
	1.6	81.4 ± 0.31		
Ripe fruit	0.1	2.34 ± 0.4	$y = 10.124\ln(x) + 53.746$ ($r^2 = 0.9110$)	27.6 ± 0.23
	0.2	29.3 ± 1.50		
	0.4	50.1 ± 1.83		
	0.8	52.5 ± 1.35		
	1.6	55.5 ± 0.15		

fractions, respectively. Similar results values were also found in IC_{50} values, as shown in Fig. 2. IC_{50} values (concentration of sample required to scavenge 50% free radical or to prevent lipid peroxidation by 50%) were found to be the least in leaf (9.72 ± 0.25), followed by green fruit (11.0 ± 0.76), stem (17.8 ± 0.66) and ripe fruit (27.6 ± 0.23 mg/ml).

3.3. Hydroxyl radical-scavenging activity

Hydroxyl radical is the most reactive among reactive oxygen species (ROS) and it bears the shortest half-life compared with other ROS. Among the oxygen radicals, hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules (Sakanaka et al., 2005). The scavenging abilities of bitter gourd fraction extracts on hydroxyl radical inhibition by the 2-deoxyribose oxidation method are shown in Fig. 2b and Table 2. The results are indicated as the inhibition rate. Different parts of Thai bitter gourd extract showed good hydroxyl radical-scavenging activities (37.1–46.1%) at a concentration of 1.6 mg/ml in

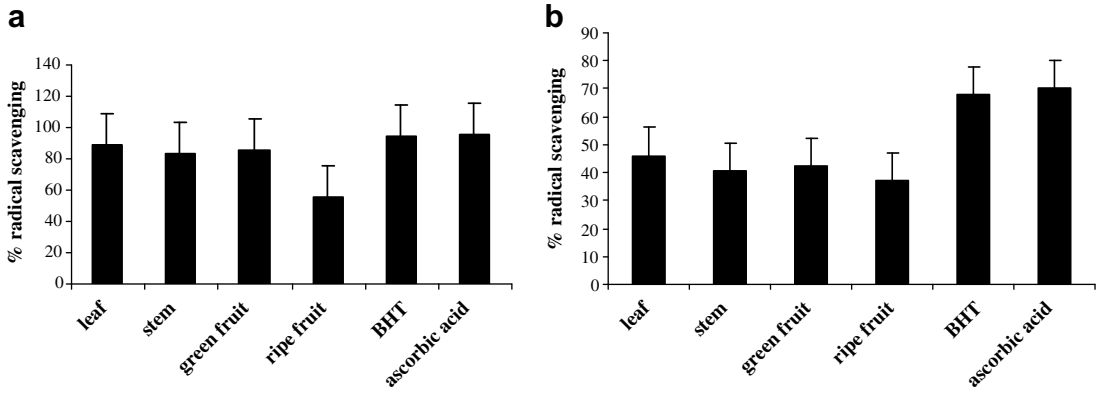

Fig. 2. Radical-scavenging activities of different parts of Thai bitter gourd (*Momordica charantia* L.) extract. Ascorbic acid at 200 ppm was used as positive control. (a) DPPH radical-scavenging and (b) hydroxyl radical-scavenging. Values are means ± standard deviation ($n = 3$).

Table 2
Hydroxyl radical-scavenging activities of different parts of Thai bitter gourd (*Momordica charantia* L.)

Sample	Concentration (mg/ml)	% Inhibition ± SD	Regression equation (r^2)	IC_{50} (mg/ml)
Leaf	0.1	29.8 ± 1.98	$y = 5.3214\ln(x) + 42.385$	167 ± 0.96
	0.2	34.9 ± 0.79	($r^2 = 0.9548$)	
	0.4	37.6 ± 0.25		
	0.8	39.2 ± 0.94		
	1.6	46.1 ± 2.64		
Stem	0.1	20.4 ± 0.11	$y = 6.747\ln(x) + 37.189$	267 ± 0.72
	0.2	28.0 ± 1.74	($r^2 = 0.9633$)	
	0.4	32.0 ± 0.11		
	0.8	34.0 ± 0.68		
	1.6	40.7 ± 1.33		
Green fruit	0.1	18.7 ± 0.30	$y = 8.7705\ln(x) + 40.409$	119 ± 0.34
	0.2	26.5 ± 0.17	($r^2 = 0.9657$)	
	0.4	34.5 ± 0.41		
	0.8	39.7 ± 1.04		
	1.6	42.4 ± 0.71		
Ripe fruit	0.1	24.2 ± 0.29	$y = 4.0798\ln(x) + 38.404$	173 ± 0.23
	0.2	26.1 ± 0.18	($r^2 = 0.8081$)	
	0.4	27.4 ± 0.29		
	0.8	28.6 ± 0.19		
	1.6	37.1 ± 0.75		

the reaction mixture. Each extract showing hydroxyl radical-scavenging activity was increased with increasing concentration of the extract sample. Leaf fraction had higher activity than had other fractions but lower than that of 200 ppm ascorbic acid and BHT (Fig. 2b). Green fruit had the highest value of IC_{50} (119 ± 0.34), followed by leaf (IC_{50} 167 ± 0.96), stem (IC_{50} 267 ± 0.72) and the lowest activity was found in ripe fruit (IC_{50} 173 ± 0.23 mg/ml).

3.4. β-Carotene–linoleate bleaching assay

In the β-carotene bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation at 50 °C. The presence of antioxidants in the extract will minimize the oxidation of β-carotene by hydroperoxides. Hydroperoxides formed in this system will be neutralized by the antioxidants from the extracts. In this study, we evaluated the antioxidant activity of different parts of Thai bitter gourd extracts by the β-carotene–linoleate bleaching method because β-carotene shows strong biological activity and is a physiologically important compound (Kumazawa et al., 2002; Sakanaka et al., 2005; Sarkar, Bishayee, & Chatterjee, 1995).Thus, the degradation rate of β-carotene–linoleate depends on the antioxidant activity of the extracts. There was a correlation between degradation rate and the bleaching of β-carotene; the extract with the lowest β-carotene degradation rate exhibited the highest antioxidant activity. All extracts had lower antioxidant activities than had BHT (Fig. 3). The antioxidant activity of bitter gourd extracts followed the order: green fruit (79.9 ± 0.70) > leaf (63.9 ± 0.71) > ripe fruit (59.0 ± 0.44) > stem (36.2 ± 0.59) as shown in Table 3. There was a significant difference ($P < 0.05$) between the antioxidant activities of difference parts.

Linoleic acid hydroperoxides attack the β-carotene molecule and, as a result, it undergoes rapid decolorization. The corresponding decrease in absorbance can be monitored spectrophotometrically. The presence of antioxidant extracts can hinder the extent of β-carotene bleaching by acting on the free radicals formed in the system (Jayaprakasha, Singh, & Sakariah, 2001; Suja et al., 2005). Oxidation rate ratio bears an inverse relationship with antioxidant activity index. The addition of an antioxidant-containing sample, individual antioxidants (Von, Joubert, & Hansmann, 1997), or plant extracts (Moure et al., 2000; Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2001) retards β-carotene bleaching.

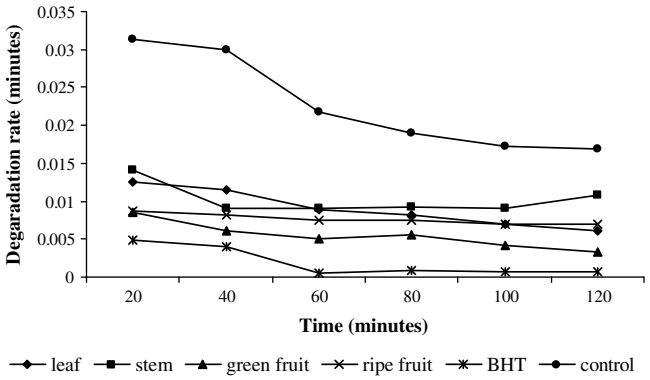

Fig. 3. Degradation rates of different parts of Thai bitter gourd (*Momordica charantia* L.) by β-carotene–linoleate bleaching method ($n = 3$). Concentration sample was 1.6 mg/ml. BHT at 200 ppm was used as the standard.

Table 3
Antioxidant activities of different parts of bitter gourd (*Momordica charantia* L.) by β-carotene–linoleate bleaching method

Sample	A_A[A]	R_{OR}[B]
Leaf	63.9 ± 0.71[b]	0.36 ± 0.00[c]
Stem	36.2 ± 0.59[d]	0.63 ± 0.006[a]
Green fruit	79.9 ± 0.70[a]	0.20 ± 0.007[d]
Ripe fruit	59.0 ± 0.44[c]	0.41 ± 0.004[b]

Concentration sample was 1.6 mg/ml. Values are expressed as mean ± standard deviation ($n = 3$). Means with different letters were significantly different at the level $P < 0.05$.
[A] Antioxidant activity index.
[B] Oxidation rate ratio.

3.5. Ferric reducing activity based on FRAP assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe^{2+}–TPTZ) (Benzie & Strain, 1996).Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom (Duh, Du, &

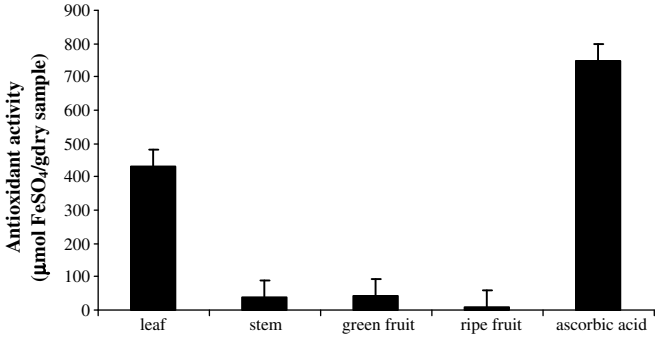

Fig. 4. Antioxidant potentials of different part of Thai bitter gourd (*Momordica charantia* L.) extracts assayed by FRAP assay. Values are expressed as mean ± standard deviation ($n = 3$). Means with different letters were significantly different at the level of $P < 0.05$.

Yen, 1999; Gordon, 1990). According to Benzie and Strain (1996), the reduction of Fe^{3+}–TPTZ complex to blue-coloured Fe^{2+}–TPTZ occurs at low pH. FRAP values of the extracts of bitter gourd factions are shown in Fig. 4. Leaf had the highest FRAP value of 433 ± 0.007 followed by green fruit (43.8 ± 0.008), stem (39.0 ± 0.008), and ripe fruit (9.41 ± 0.007), all as μmol $FeSO_4$/g dry sample. This result indicated that leaf fraction had significantly stronger ($P < 0.05$) reducing power than had other fractions.

Total antioxidant capacity of each different part of bitter gourd is expressed as number of equivalents of ascorbic acid and BHT (Table 4). The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid (Prieto et al., 1999). Green fruit had a higher capacity than had the other parts. The results, from various free radical-scavenging systems, revealed that some parts of bitter gourd had significant antioxidant activity. The extracts were found to have different levels of antioxidant activity in the systems tested. The antioxidant activities of the parts were: leaf > green fruit > stem > ripe fruit.

3.6. Determination of total antioxidant capacity

Total antioxidant capacity of each different part of bitter gourd is expressed as number of equivalents of ascorbic acid and BHT (Table 4). The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid (Prieto et al., 1999). Green fruit had a higher capacity than had the other parts. The results from various free radical-scavenging systems revealed that some parts of bitter gourd had significant antioxidant activity. The extracts were found to have different levels of antioxidant activity in the systems tested. The antioxidant activities of the parts were: leaf > green fruit > stem > ripe fruit.

Table 4
Total antioxidant capacities of different parts of bitter gourd (*Momordica charantia* L.)

Sample	Equivalent to ascorbic acid (mg)/mg part of bitter gourd	Equivalent to BHT (mg)/mg part of bitter gourd
Leaf	0.061[b]	0.0058[b]
Stem	0.044[c]	0.0030[c]
Green fruit	0.073[a]	0.0070[a]
Ripe fruit	0.061[b]	0.0058[b]

Values are expressed as means ± standard deviation ($n = 3$). Means with different letters were significantly different at the level $P < 0.05$.

3.7. Phenolic compounds analysis

3.7.1. Determination of total phenol content

Phenolics or polyphenols have received considerable attention because of their physiological function, including antioxidant, antimutagenic and antitumour activities (Othman et al., 2007).

Plant phenolics present in fruit and vegetables have received considerable attention because of their potential antioxidant activity (Dziedzic & Hudson, 1983; Lopez-Velez, Martinez_Martinez, & Del Valle-Ribes, 2003). Phenolic compounds are widely distributed in plants (Li, Smith, & Hossain, 2006), which have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health (Govindarajan, Singh, & Rawat, 2007; Imeh & Khokhar, 2002; Li et al., 2006; Ross & Kasum, 2002). Total phenol content (TPC) was determined in comparison with standard gallic acid and the results expressed in terms of mg GAE/g dry sample. The TPC values for bitter gourd fractions extract were leaf (474 ± 0.71) green fruit (324 ± 1.63) stem (259 ± 1.2) and ripe fruit (224 ± 0.86), all as mg GAE/g dry sample, respectively (Fig. 5).

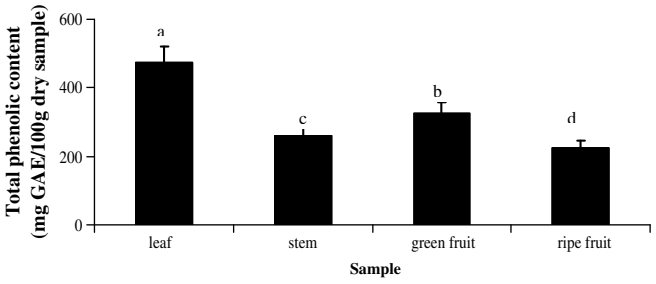

Fig. 5. Total phenolic compounds of Thai bitter gourd (*Momordica charantia* L.) Concentration of sample was 1.6 mg/ml. Results are expressed as gallic acid equivalents. Values are expressed as means ± standard deviation ($n = 3$). Means with different letters were significantly different at the level of $P < 0.05$.

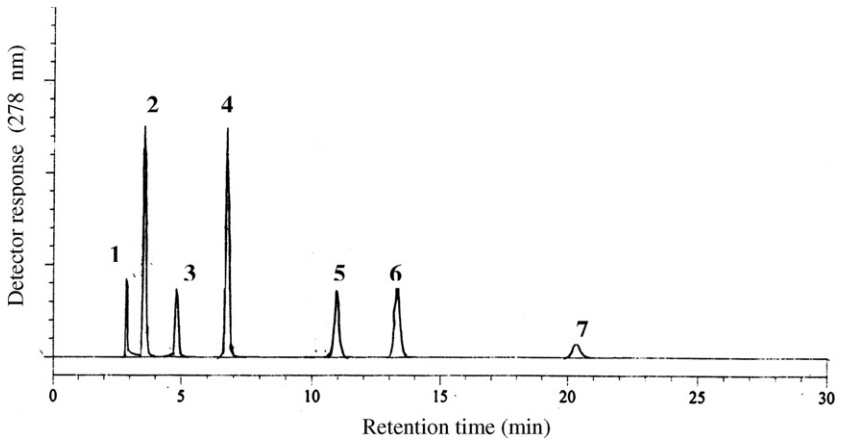

Fig. 6. HPLC–DAD of standard phenolic compounds. (1) Gallic acid, (2) tannic acid, (3) (+)-catechin, (4) caffeic acid, (5) *p*-coumaric acid, (6) ferulic acid, and (7) benzoic acid.

3.7.2. HPLC–DAD system for analysis of phenolic compounds

The HPLC–DAD analysis of bitter gourd fractions aqueous extracts revealed the presence of phenolic compounds. By this means, in the four analyzed fractions, it was possible to identify seven phenolic compounds: *p*-coumaric acid, tannic acid, benzoic acid, ferulic acid, gallic acid, caffeic acid, (+)-catechin (Table 6). Gallic acid was the most predominant phenolic compounds in all parts of bitter gourd, contributing about 72.8 mg/l in stem to 202 mg/l in ripe fruit extract. Caffeic acid showed the highest content in leaf (7.77 ± 1.02 mg/l) and *p*-coumaric acid was the highest content in stem (6.73 ± 0.21 mg/l). Ferulic acid was found only in stem and green fruit while benzoic acid was not present in leaf and stem (see Fig. 6). Chromatographic profiles of phenolic composition of each fraction extract are shown in Fig. 7.

Statistical correlations have been studied between total phenol content and antioxidant activity determined by different assays, as shown in Table 5. Total phenol content was shown to provide the highest association with FRAP assay in the present study (R^2 = 0.948). This supports the results reported by Guo et al. (2003) for different fractions of fruits. This result was also in agreement with Benzie and Stezo (1999), who found a strong positive correlation between total phenolic content and FRAP assay. Similar results were also found for hydroxyl radical-scavenging activity (R^2 = 0.884) and DPPH.?(R^2 = 0.711). Leontowicz et al. (2003) reported a close correlation (R^2 = 0.935) of polyphenol content in fruit extract measured by the Folin–Ciocalteu method and antioxidant activity determined by β-carotene–linoleate bleaching assay. However, there was no significant correlation between total phenol content and total antioxidant capacity and β-carotene–linoleate bleaching assay in our study. Interestingly, a strong correlation (R^2 = 0.982) was obtained between total antioxidant capacity and β-carotene–linoleate bleaching assay. Our results indicated that high total antioxidant activity and β-carotene–linoleate bleaching could not be due to phenolic compounds in the bitter gourd extracts. This may be due to other complex antioxidant compounds in different fractions rather than phenol content. Sun and Ho (2005) reported a significant correlation between total phenolics and scavenging ability of buckwheat extracts on DPPH radicals. By contrast, a study by Yu et al. (2002) found no

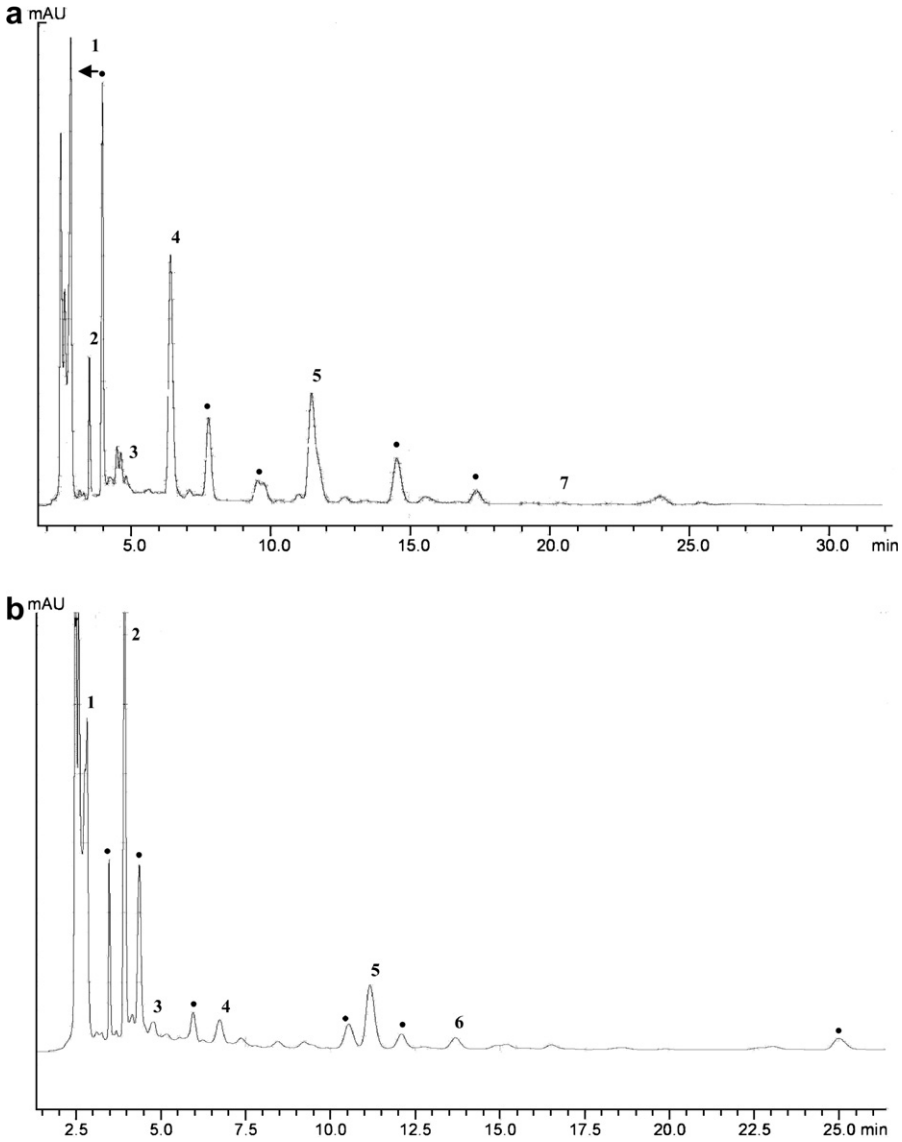

Fig. 7. HPLC–DAD chromatogram of phenolic compounds in bitter gourd fractions. (a) Leaf, (b) stem, (c) green fruit and (d) ripe fruit. Detection was at 278 nm. Peak: (1) gallic acid, (2) tannic acid, (3) (+)-catechin, (4) caffeic acid, (5) *p*-coumaric acid, (6) ferulic acid, and (7) benzoic acid; (●) unidentified.

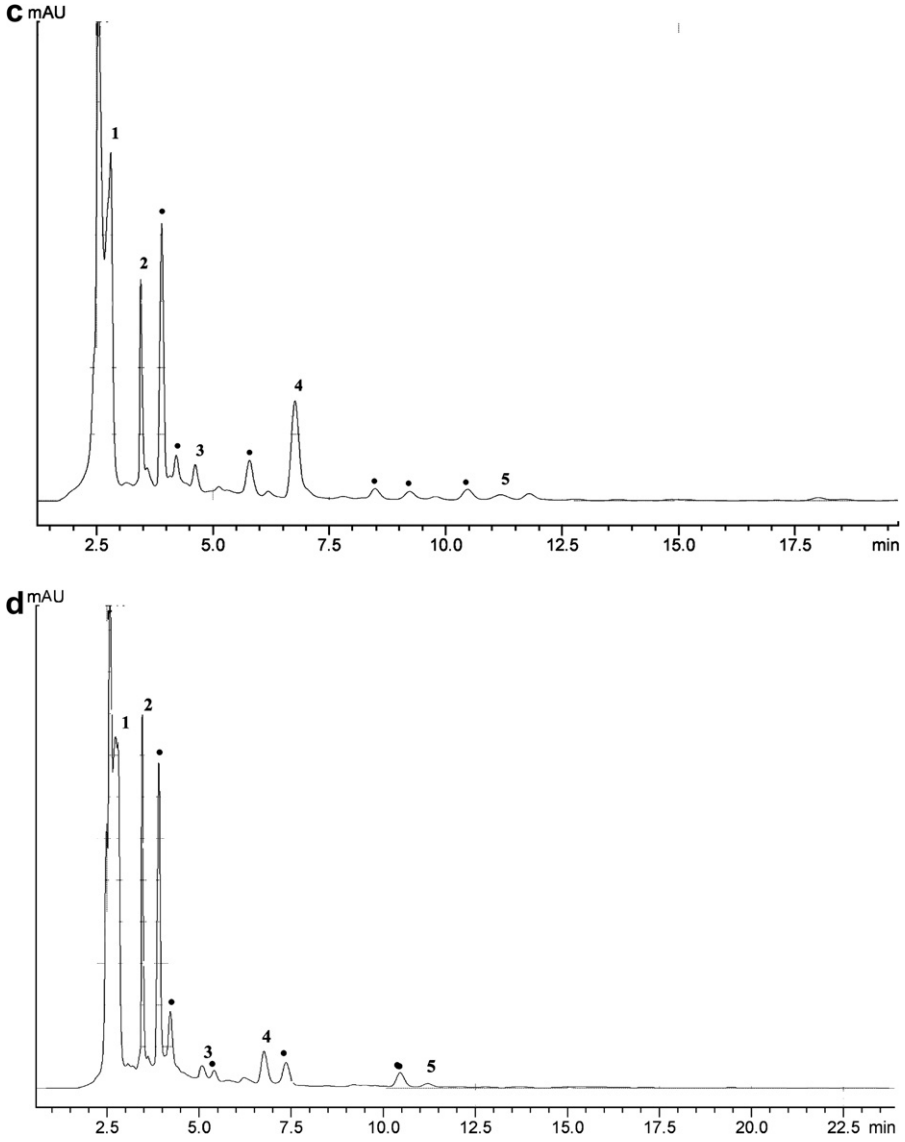

Fig. 7 (*continued*)

Table 5
Correlation between total phenol content and antioxidant activity determined by different assays

	TPC	DPPH	Hydroxyl	FRAP	CLBA	TAC
TPC	1.00					
DPPH	0.711**	1.00				
Hydroxyl	0.884**	0.827**	1.00			
FRAP	0.948**	0.546	0.778**	1.00		
CLBA	0.374	0.109	0.272	0.159	1.00	
TAC	0.269	-0.71	0.131	0.091	0.982**	1.00

TAC: total antioxidant capacity.
TPC: total phenol content.
CLBA: β-Carotene–linoleate bleaching assay.
** Significantly correlated at $P < 0.01$, $n = 12$.

Table 6
Phenolic compound content (mg/l) in bitter gourd fractions

Compounds	Bitter gourd fractions			
	Leaf	Stem	Green fruit	Ripe fruit
Gallic acid	95.8 ± 0.31	72.8 ± 0.89	95.6 ± 0.97	202 ± 1.60
Tannic acid	2.13 ± 0.67	2.40 ± 0.20	1.08 ± 0.81	1.41 ± 0.34
(+)-Catechin	4.39 ± 0.80	4.68 ± 0.35	3.95 ± 0.52	4.54 ± 0.65
Caffeic acid	7.77 ± 1.02	1.03 ± 0.64	3.35 ± 0.62	1.62 ± 0.84
p-Coumaric	0.36 ± 0.32	6.72 ± 0.21	0.56 ± 0.93	0.16 ± 0.51
Ferulic acid	ND	0.70 ± 0.51	ND	ND
Benzoic acid	0.10 ± 0.42	0.004 ± 0.32	0.01 ± 0.09	ND

Values are means ± SD ($n = 3$), and they are given as mg/l of investigated bitter gourd fractions.
ND: not detected.

correlation between scavenging activity and the total phenolic content (Othman et al., 2007). Our present study demonstrated that the antioxidant activity could be determined by using several test systems. However, there are several methodological limitations for antioxidant determination (Kaur & Kapoor, 2001). To measure the antioxidant capacity of bitter gourd, we suggest that FRAP is an appropriate method.

In conclusion, the present study has demonstrated that bitter gourd fractions are rich in phenolics and have a strong antioxidant activity and a radical-scavenging action in all of the tested methods.

This suggests that bitter gourd is a good source of natural antioxidants. Thai bitter gourd might have health benefits for consumers as a potential functional food or value-added ingredient.

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