International Food Research Journal 23(1): 180-187 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of solvent types and extraction times on phenolic and flavonoid contents and antioxidant activity in leaf extracts of *Amomum chinense* C.

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Article history

Received: 28 January 2015
Received in revised form: 19 June 2015
Accepted: 26 June 2015

Keywords

Antioxidant capacity
Phenolic compounds
Extraction
Amomum Chinense
RP-HPLC

Abstract

The present study determined the phenolic and flavonoid contents in *Amomum chinense* C. leaf extracts obtained using four extracting solvents(distilled water, 80% methanol, 80% ethanol and 80% acetone)with various extraction times (6h, 12h and 24h) at 37°C. All extraction conditions significantly ($p < 0.05$) affected the phenolic contents and antioxidant activity of the leaf extracts. The optimal conditions used for extracting the phenolic compounds from *A. chinense* leaves were 80% methanol for 12h, which provided a higher amount of phenolics and antioxidant activity compared to the other conditions tested. There was a strong correlation between total phenolic content and antioxidant activities based on the three assays with correlation coefficients of 0.805, 0.873 and 0.975 for DPPH, ABTS and FRAP assays, respectively. HPLC analyses of all extracts revealed that the major phenolic acids identified in the tested extracts were chlorogenic acid, cinnamic acid, ferulic acid and caffeic acid, whereas catechin, rutin and quercetin were the major of flavonoids.

Introduction

Herbs and spices are potent plants used as food, which contain antioxidant compounds (Dragland *et al*., 2003).Several studies investigated the phenolic compounds found in herbs and spices, and they showed antioxidant capacities (Shan *et al*., 2005; Norhaiza *et al*.,2009).The herb plants in the family Zingiberaceae are widely used as medicine and food ingredients, and include *Zingiber officinale, Curcuma longa*, *Alpinia galangal* and *Amomum xanthioide* (Wu *et al*., 2012).They contain several phenolic compounds and possess health promoting properties (Elzaawely *et al*., 2007; Chan *et al*., 2008; Ghasemzadeh *et al*., 2010).The properties of the phenolic compounds presented in each plant material showed different antioxidant capacities depending on the factors used in the extraction, such as solvent type, extraction time, solvent concentration, extraction temperature and particle size of samples (Naczk and Shahidi, 2004; Sultana *et al*., 2009; Chew *et al*., 2011).

Amomum chinense Chun ex T.L. Wu., known as Reaw in Thai, is a member of the family Zingiberaceae. It is 1-1.5 m tall, its leaf sheath is conspicuously concave veined and the ligule is purple. It has been used for prevention and treatment of cold, flu and cough. *Amomum* species are well known as medicinal herbs in South-east Asia and China, such as *Amomum tsao-ko* Crevost & Lemarie, *Amomum villosum* Lour., *Amomum krevanh* Pierre ex Gagnep and *Amomum xanthioides* Wall. Several studies have indicated that medicinal *Amomum* plants possess more potent antioxidant activity than common fruits and vegetables (Gan *et al*., 2010; Makchuchit *et al*., 2010). There are some reports in the literature that an essential oil in *A. xanthioides* seeds contain bornyl acetate, camphor, camphorene, limonene, beta-pinene, bitter orange oil alcohols (nerolidol), α-pinene, camphene, eucalyptol essential oils, linalool, alpha-pepper allyl guaiacyl alcohol (guaiaol) and others (Sabulal *et al*., 2006). Recently, a water soluble portion was isolated from the methanolic extract of the *Amomum* seed, which is used as a medicine for stomachic and digestive disorders (Yamazaki *et al*., 2000), and it contains monoterpenoid glucosides (Kitajima and Ishikawa, 2003). In addition, Kikuzaki *et al*. (2001) reported that the seed of *Amomum subulatum* presented an antioxidant activity and have potential health benefits. However, the antioxidant activity and phenolic compounds of crude extract from the leaves of other varieties of *Amomum*, especially *A. chinense* in Thailand, have not been reported. In addition, the

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quantity of phenolic compounds in various plants depends on the type of extraction conditions used (Lapornik *et al.*, 2005; Sultana *et al*., 2009; Chew *et al.,* 2011). Results of previous studies showed that the difference in polarities of the extracting solvents and extraction time might influence the solubility of the chemical constituents and other antioxidants in plants, such as black and black mate and plant by-products (Lapornik *et al*., 2005; Turkmen *et al*., 2006), *Moringa oleifera* leaves (Siddhuvaj and Becker, 2003) and grape marc (Spignov*et al*., 2007). Therefore, in this study we aimed to determine the content and composition of phenolics and flavonoids from the leaves of *A. chinense*, and to evaluate the antioxidant activity of leaf extracts affected by various solvents and extraction times.

Materials and Methods

Chemicals and plant material

Trolox(6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylicacid),DPPH(2,2'- diphenyl-1-hydrazyl), ABTS(2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate, sodium carbonate, Folin-Ciocalteu reagent, catechin and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO). All chemicals used in the experiments were analytical grade. The leaf of *A. chinense* Chun ex T.L. Wu (local Thai name Reaw or Maak Naeng) was collected from northeastern Thailand and authenticated by Pramote Triboon from the Plant Varieties Protection Division, Bangkok Herbarium, Thailand.

Preparation of leaf extracts

The leaves of *A. chinense* were washed to remove contaminates and dried at 50°C in a hot air oven.Then the dried sample was blended and passed through a 35 mesh sieve to obtain particle size unity. Dried samples were extracted with four solvents (distilled water, 80% methanol, 80% ethanol and 80% acetone) in a flask at a sample to solvent ratio of 1:10 (w/v). The mixtures were extracted using an incubator shaker (NB-205QF) at 200 rpm and 37°C for extraction times of 6h, 12h and 24h. The extracts were filtered and then evaporated to dryness under a reduced pressure and the extraction yields were then measured.

Determination of total phenolic content

Total phenolic contents (TPC) of the extracts were determined using Folin-Ciocalteu reagent according to the method of Butsat and Siriamorpun (2010). Briefly, 0.2 ml of the extract was transferred in triplicate to separate flasks containing of 1.0 ml of a 1/10 dilution of Folin Ciocalteu's reagent in distilled water. Then, 0.8 ml of Na_2CO_3 (10% w/v) was added in each flask and the final volume was adjusted to 5 ml with distilled water. The flasks were kept at room temperature for 60 min and then the absorbance of the extracts was measured at 760 nm in a Perkin-Elmer UV-Visible spectrophotometer (Lambda12). The TPC was expressed as milligram gallic acid equivalent per gram dried sample (mg GAE/g dw).

Determination of total flavonoid content

Total flavonoid contents (TFC) of the extracts were measured according to the method of Sultana *et al*. (2009) with some modifications. The extract (0.5 ml) was transferred to a 5 ml volumetric flask and 0.3 ml of 5% $NaNO_2$ was added to the flask, wait for 5 min, 0.3 ml of 10% $AlCl_3$ was added to the mixture and wait for 6 min then 1 ml of 1M NaOH was added. The mixture solution was adjusted to 5 ml with distilled water and allowed to stand for 30 min. The absorbance of the extract was measured at 510 nm and standard catechin was used to make the calibration curve. The TFC was expressed as milligram catechin equivalents per gram dried sample (mg CE/g dw). All experiments were analyzed in triplicate.

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Butsat and Siriamornpun (2010). Briefly, 0.2 ml of the sample extracts was pipette into a test tube, then 1.8 ml of 0.1 mM methanolic DPPH was added and swirled to mix. The mixture was incubated at room temperature in the dark for 30 min. The absorbance of the sample was measured at 517 nm using a Perkin-Elmer UV-Visible spectrophotometer versus methanol as a solvent blank. Trolox with aconcentration range of 0.05 – 0.40 mM was used as a positive standard. All experiments were performed in triplicate. The result was expressed as micromole Trolox equivalent per gram dried sample (µmol TE/g dw).

ABTS radical scavenging activity

ABTS radical scavenging activity was measured using the method of Rusak *et al.* (2008) with some modification. Briefly, ABTS radicals ($ABTS^{o+}$) were prepared by mixing 88 ml of 140 mM potassium persulfate solution and 5 ml of 7 mM ABTS, and letting it stand in the dark for 16 h. Freshly-prepared $ABTS^{o+}$ was diluted with distilled water to an absorbance of 0.7 ± 0.05 at 734 nm. The extracts (0.2 ml) were mixed with 1.8 ml of diluted $ABTS^{o+}$ solution and left to stand for 6 min, after which the

absorbance of the mixed extracts was measured at 734 nm. Trolox in the concentration range of 0.05 – 0.5 mM was used as a standard to create a calibration curve. The results were expressed as µmol Trolox equivalent per gram dried sample (µmol TE/g dw).

Ferric reducing ability power (FRAP) assay

The FRAP assay was conducted following Benzie and Szeto (1999), with some modifications. Firstly, the FRAP reagent was prepared by mixing acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ at a ratio of 10:1:1 respectively. Pipette 0.2 ml of extract into a test tube and mix with 1.8 ml of FRAP reagent and incubate at 37°C in a water bath for 30 min. The absorbance of the mixed solution was measured at 593 nm and Trolox (0.10-0.50 mM) was used to make a standard calibration curve. All measurements were performed in triplicate and expressed as µmol Trolox equivalent per gram dried sample (µmol TE/g dw).

Identification of phenolic acids and flavonoids compounds

The identification was done by RP-HPLC according to a modified method described by Sharma *et al.* (2005). Extracted samples were filtered through a 0.45 µm PTFE syringe tip filter using a 20 µl sample loop. The sample was analyzed using a RP-HPLC system equipped with a Shimadzu PDA detector on a reverse phase (RP C-18) column Interstsil ODS-5 µm x 4.6 mm x 250 mm. The flow rate was set at 0.8 ml/min at 37°C. To perform this study a gradient of two mobile phases was used. Solvent A: 1% aqueous acetic acid solution (pH 2.7), and solvent B: acetonitrile. The gradient profile was linearly altered as follows (total run time 65 minutes): 0 min, 95:5;5 min, 91:9; 22 min, 88:12; 38 min, 82:18; 43 min, 77:23;44 min, 10:90; 45 min, 80:20 and hold until 55 min; 60 min, 95:5. After each run, the system was reconditioned for 10 min before analysis of the next sample. Under these conditions, 20 µl of each sample were injected. External polyphenolic standards were used as the external reference. Individual polyphenols in the sample were identified by comparison of their retention times with the external polyphenolic standards. The detection was carried out at 280 nm for hydroxybenzoic acids and flavanols, at 320 nm for hydroxycinnamic acids and 360 nm for flavonols. All sample analyses were done in triplicate.

Statistical analysis

The experiments were carried out in triplicate and the results were expressed as means ± standard deviations. Statistical analysis of variance was performed by ANOVA and significant differences between the means were determined by Duncan's Multiple Range Test. In addition, correlation coefficients were calculated at a significant difference confident limit of 95%.

Results and Discussion

Extraction yield, total phenolic content and total flavonoid content

The difference in polarities of the extraction solvents might influence the solubility of the chemical constituents in a sample and its extraction yield. Therefore, the selection of an appropriate solvent system is one of the most relevant steps in optimizing the recovery of TPC, TFC and other antioxidant compounds from a sample (Ghasemzadeh *et al*., 2011). Table 1 shows a list of the extraction yields, TPC and TFC obtained from the four solvent extractions of *A. chinense* leaves. The 80% methanol extract of *A. chinense* leaves for 12h gave the greatest yield, whereas the water extract in the same extraction time had the lowest yield. However, no significant differences in extraction yield between the 80% methanol and 80% ethanol were detected at all extraction times. Our results are similar to those reported by Ghasemzadeh *et al.* (2011), where the methanol solvent was most effective in extracting phenolic compounds from young ginger. According to some researchers, aqueous methanol and ethanol have been proven as effective solvents to extract phenolic compounds from different plants (Siddhuraju and Becker, 2003; Sultana *et al*., 2009). Our findings are in agreement with a previous investigation by Sultana *et al*. (2009) who reported that the higher extract yields were found in the 80% aqueous methanol of selected medicinal plants, included *M. oleifera, Azadirachta indica, Terminalia arjuna, Acacia nilotica, Eugenia jambolana* and *Aloe barbadenis*.

The phenolic and flavonoid contents in different extracts of *A. chinense* leaves are shown in Table 1. The TPC was between 0.06 to 8.33 mg GAE/g dw. The TFC varied from 0.14 to 9.04 mg CC/g dw. The highest phenolic and flavonoid contents obtained were from 80% methanol, followed by 80% ethanol, 80% acetone and distilled water. In this study the TPC of the 80% methanol extract was found to be higher than *Amomum kravanh* (2.77 mg GAE/g dw) but similar to *A. tsao-ko* (7.15 mg GAE/g dw) and *A. villosum* (9.29 mg GAE/g dw), which were reported by Gan *et al.* (2010). These results are in agreement with the findings of Ghasemzadeh *et al.* (2011) who reported that methanolic extracts from

Table 1. Effect of solvent type and extraction time on antioxidant activity, extraction yield and total phenolic and total flavonoid contents of extracts from leaves of *Amomum chinense* C.

Time (h)	Solvent	Extraction yield (g/100 g dw)	TPC (mg GAE/g dw)	TFC (mg CC/g dw)
6	Distilled water	4.68 ± 0.29 c	0.50 ± 0.05 g	0.47 ± 0.07 f
	80% Methanol	6.27 ± 0.49 b	7.06 ± 0.53 bc	7.37 ± 0.38 b
	80% Ethanol	5.50 ± 0.67 b	5.26 ± 0.36 de	5.45 ± 0.14 de
	80% Acetone	3.75 ± 0.75 c	4.41 ± 0.48 ef	4.51 ± 0.23 e
12	Distilled water	2.48 ± 0.17 d	0.22 ± 0.04 h	0.40 ± 0.04 f
	80% Methanol	7.20 ± 0.42 a	8.33 ± 1.55 a	9.04 ± 0.94 a
	80% Ethanol	6.43 ± 0.24 ab	5.95 ± 0.34 cd	6.42 ± 0.84 c
	80% Acetone	4.95 ± 0.61 c	4.71 ± 0.55 def	5.04 ± 0.22 de
24	Distilled water	2.68 ± 0.25 d	0.06 ± 0.01 i	0.14 ± 0.01 g
	80% Methanol	6.45 ± 0.69 ab	7.63 ± 1.18 ab	8.75 ± 0.97 a
	80% Ethanol	6.12 ± 0.44 b	5.82 ± 0.65 d	5.67 ± 0.63 cd
	80% Acetone	4.41 ± 0.50 c	3.91 ± 0.59 f	5.10 ± 0.18 de

Values are mean ± SD; n =3.
Different letters within the same column denote significant differences ($p < 0.05$)

Table 2. Effect of solvent type and extraction time on antioxidant activity of extracts from leaf of *Amomum chinense* C.

Time (h)	Solvent	DPPH (µmol TE/g dw)	ABTS (µmol TE/g dw)	FRAP (µmol TE/g dw)
6	Distilled water	1.18 ± 0.07 e	1.10 ± 0.06 g	3.93 ± 0.15 f
	80% Methanol	71.93 ± 1.51 a	50.04 ± 3.75 a	82.50 ± 3.39 b
	80% Ethanol	55.35 ± 4.77 d	38.62 ± 1.05 d	54.58 ± 2.29 cd
	80% Acetone	0.97 ± 0.19 e	13.79 ± 1.33 e	49.88 ± 7.93 de
12	Distilled water	0.97 ± 0.11 e	0.43 ± 0.10 h	1.49 ± 0.40 g
	80% Methanol	70.22 ± 1.97 ab	46.27 ± 2.24 b	117.89 ± 10.74 a
	80% Ethanol	65.56 ± 4.28 c	42.64 ± 1.76 c	79.02 ± 1.93 b
	80% Acetone	0.71 ± 0.14 e	6.73 ± 1.02 f	53.27 ± 6.87 cd
24	Distilled water	0.44 ± 0.09 f	0.29 ± 0.06 h	0.09 ± 0.02 h
	80% Methanol	66.37 ± 3.96 bc	46.32 ± 2.86 b	86.54 ± 7.94 b
	80% Ethanol	62.17 ± 2.70 c	37.21 ± 1.84 d	60.79 ± 3.05 c
	80% Acetone	0.75 ± 0.15 e	7.36 ± 1.13 f	43.55 ± 4.01 e

Data are expressed as mean ± SD of triplicate experiments. Values in the same column bearing different letters are significantly different at $p < 0.05$.

young ginger parts were more effective compared to those extracted by acetone and chloroform. Similarly, aqueous methanol was found to be more effective in recovering the highest amount of phenolic compounds from *M. oleifera* leaves (Siddhuraju and Becker, 2003). Solvents with different polarities have significant effects on the phenolic content (Turkmen *et al.*, 2006). In addition, the TPC and TFC values in all extracts, except for the water extract, increased when the extraction time was prolonged from 6h

Table 3. Phenolic acid composition of extracted samples from *Amomum Chinese* leaves under different conditions

Solvent	time (h)	ChA (µg/g)	CFA (µg/g)	FA (µg/g)	SnA (µg/g)	EA (µg/g)	CmA (µg/g)	Total PA (µg/g)
Distilled water	6	146.58 ± 5.73 f	20.57 ± 1.68 h	30.61 ± 1.21 g	2.05 ± 0.10 f	6.71 ± 0.47 gh	1.28 ± 0.25 e	207.80 ± 6.12 g
	12	163.37 ± 6.07 f	35.54 ± 3.36 g	15.83 ± 0.63 h	2.08 ± 0.12 f	5.25 ± 0.53 h	0.53 ± 0.03 e	222.60 ± 6.99 g
	24	190.82 ± 4.49 f	37.09 ± 1.56 g	15.14 ± 0.65 h	4.25 ± 0.84 f	3.58 ± 0.39 h	1.99 ± 0.26 e	252.87 ± 4.89 g
80% MeOH	6	1747.59 ± 44.73 a	187.85 ± 8.61 a	285.83 ± 9.60 a	39.78 ± 0.72 a	68.77 ± 4.53 a	711.97 ± 48.64 a	3041.79 ± 67.48 a
	12	1441.21 ± 74.36 b	150.47 ± 6.15 b	241.02 ± 4.90 c	33.97 ± 4.05 bc	50.05 ± 7.42 c	691.38 ± 20.84 a	2608.10 ± 78.08 b
	24	1442.52 ± 57.61 b	150.80 ± 7.53 b	258.53 ± 6.62 b	29.76 ± 0.40 bcd	62.19 ± 2.86 b	620.67 ± 32.76 a	2564.47 ± 67.09 b
80% EtOH	6	1287.41 ± 77.72 c	88.36 ± 7.01 d	224.95 ± 10.72 d	28.84 ± 3.18 cd	29.00 ± 2.48 e	601.92 ± 49.09 b	2260.48 ± 92.90 cd
	12	1214.75 ± 69.73 c	102.41 ± 7.94 c	244.88 ± 7.62 c	29.27 ± 3.80 bcd	37.26 ± 7.21 d	715.79 ± 26.99 a	2344.36 ± 77.13 c
	24	1048.38 ± 57.74 d	103.15 ± 4.09 c	224.12 ± 10.88 d	34.79 ± 8.18 ab	27.85 ± 1.16 e	603.97 ± 45.93 b	2042.26 ± 75.14 d
80% Acetone	6	775.45 ± 59.38 e	56.46 ± 3.84 f	144.14 ± 11.29 f	18.01 ± 0.79 e	19.06 ± 1.02 f	279.24 ± 33.60 c	1292.36 ± 69.27 f
	12	767.84 ± 20.97 e	65.97 ± 6.45 ef	151.60 ± 4.99 f	19.56 ± 0.66 e	12.34 ± 1.35 g	183.33 ± 26.46 d	1200.64 ± 34.76 f
	24	1085.68 ± 57.39 d	72.72 ± 3.62 e	188.21 ± 6.87 e	25.74 ± 1.11 d	8.24 ± 0.65 gh	176.23 ± 3.18 d	1556.82 ± 58.01 e

Values are expressed as means in µg/g ± SD (n=3) of dry sample.
Different letters in the same column show significant differences ($p < 0.05$) by Duncan's multiple range test.
ChA = chlorogenic acid, CFA = caffeic acid, FA = ferulic acid, SnA = sinapinic acid, EA = ellagic acid, CmA = cinnamic acid

to 12h. However, the amount of TPC and TFC in the tested extracts decreased as the extraction time increased to 24h.

Antioxidant activities of A. chinense *leaf extracts*

Antioxidant activities in plant extracts were measured by well-known methods, such as DPPH, ABTS and FRAP due to their simplicity, stability, accuracy and reproducibility (Stratil *et al.*, 2006). According to the statistical results of the antioxidant activity based on the three assays, significant differences were found between the values obtained in all extracts. For antioxidant activity, most results were also shown to be influenced by the extracting solvent and time.

As indicated in Table 2, the antioxidant activity of the 80% methanol extract exhibited the greatest antioxidant activities in all tested assays, followed by 80% ethanol, 80% acetone and distilled water extracts. The 80% methanol for 6h gave the highest antioxidant activity based on DPPH and ABTS radical scavenging ability, whereas the highest FRAP value was found in the 80% methanol for 12h.

The DPPH radical scavenging activity of the 80% methanol extract ranged from 66.37 to 71.93 µmole TE/g dw, with the highest activity being found at 6h; however, there were no significant differences between extracts at 6h and 12h with 80% methanol. Whereas, the leaf extracts with 80% acetone and distilled water for all extraction times were the least reactive with values ranging from 0.71 to 0.97 µmole TE/g dw and 0.44 to 1.18 µmole TE/g dw, respectively.

The ABTS radical scavenging activity was expressed a TEAC value with the range of 46.27 to 50.04 µmole TE/g dw in the 80% methanol extract, 37.21 to 42.64 µmole TE/g dw in the 80% ethanol extract, 6.73 to 13.79 µmole TE/g dw in the 80% acetone extract and 0.29 to 1.10 37.21 to 42.64 µmole TE/g dw in the water extract.

For the FRAP value, the 80% methanol extracts showed the highest reducing ability with FRAP values ranging from 82.50 to 117.89 µmole TE/g dw, followed by 80% ethanol, 80% acetone and water extracts. The present results are similar to those of Ghasemzadeh *et al.* (2010) who observed that the leaves of ginger with high TFC and TPC also had high antioxidant activities.

The results in Tables 1 and 2 indicated the influence of the extracting solvent on the phenolic and flavonoid contents as well as antioxidant activities of *A. chinense* leaf extracts. There were strongly positive correlations between the concentration of the phenolic compounds and the antioxidant activities. The correlation coefficients (r) calculated from linear regression analysis between TPC and DPPH, ABTS and FRAP assays were 0.805, 0.873 and 0.975 ($p<0.01$), respectively. The correlations between TFC and antioxidant activities based on the three assays were r = 0.784 for DPPH, r = 0.853 for ABTS and r = 0.969 for FRAP assays. This finding was similar to those reported by Kubola *et al.* (2008) who studied bitter gourd fractions and Alothman *et al.(*2009) who reported that there was a good correlation between the TPC and antioxidant capacity of fruits, include pineapple, banana and guava.

Table 4. Flavonoid contents in extracted samples from *Amomum Chinese* leaves under different conditions

Solvent	time (h)	CC (µg/g)	EGCG (µg/g)	Ru (µg/g)	QCT (µg/g)	Total (µg/g)
Distilled water	6	111.15 ± 1.86 h	5.19 ± 0.56 h	166.27 ± 9.65 g	1.78 ± 0.04 g	284.39 ± 9.85 f
	12	148.63 ± 3.45 h	9.79 ± 0.31 h	243.65 ± 8.95 h	1.25 ± 0.05 g	403.31 ± 9.60 f
	24	182.59 ± 4.96 h	8.51± 0.72 h	145.64 ± 8.65 i	1.24 ± 0.11 g	337.98 ± 9.99 f
80% MeOH	6	2337.36 ± 57.88 a	155.99 ± 3.38 b	1748.21 ± 21.43 a	52.67 ± 9.60 bcd	4294.23 ± 62.55 a
	12	1899.07 ± 30.68 d	93.55 ± 1.35 d	1583.41 ± 47.55 b	53.31 ± 1.16 bc	3629.35 ± 56.62 bc
	24	2019.74 ± 55.62 bc	100.37 ± 7.22 d	1571.10 ± 55.15 bc	48.90 ± 3.19 cde	3740.12 ± 78.73 b
80% EtOH	6	2091.20 ± 60.63 b	217.19 ± 6.75 a	1361.62 ± 50.96 e	58.79 ± 4.67 ab	3728.80 ± 79.63 b
	12	2089.10 ± 53.05 b	215.26 ± 18.10 a	1506.69 ± 41.20 c	64.57 ± 4.46 a	3875.62 ± 69.71 b
	24	1954.93 ± 49.08 cd	117.09 ± 6.20 c	1432.17 ± 47.97 d	62.72 ± 9.89 a	3566.91 ± 69.62 c
80% Acetone	6	1159.11 ± 59.38 e	34.71 ± 1.49 g	726.85 ± 50.97 g	32.01 ± 5.53 f	1952.67 ± 78.45 d
	12	749.05 ± 19.94 g	58.34 ± 0.77 f	990.50 ± 38.42 f	44.19 ± 1.50 de	1842.08 ± 43.32 e
	24	894.22 ± 22.28 f	77.03 ± 1.72 e	1041.14 ± 40.82 f	40.83 ± 2.27 e	2053.22 ± 46.59 d

Values are expressed as means in µg/g ± SD (n=3) of dry sample.
Different letters in the same column show significant differences ($p < 0.05$) by Duncan's multiple range test.
CC = catechin, EGCG = epigallocatechin gallate, Ru = rutin, QCT = quercetin

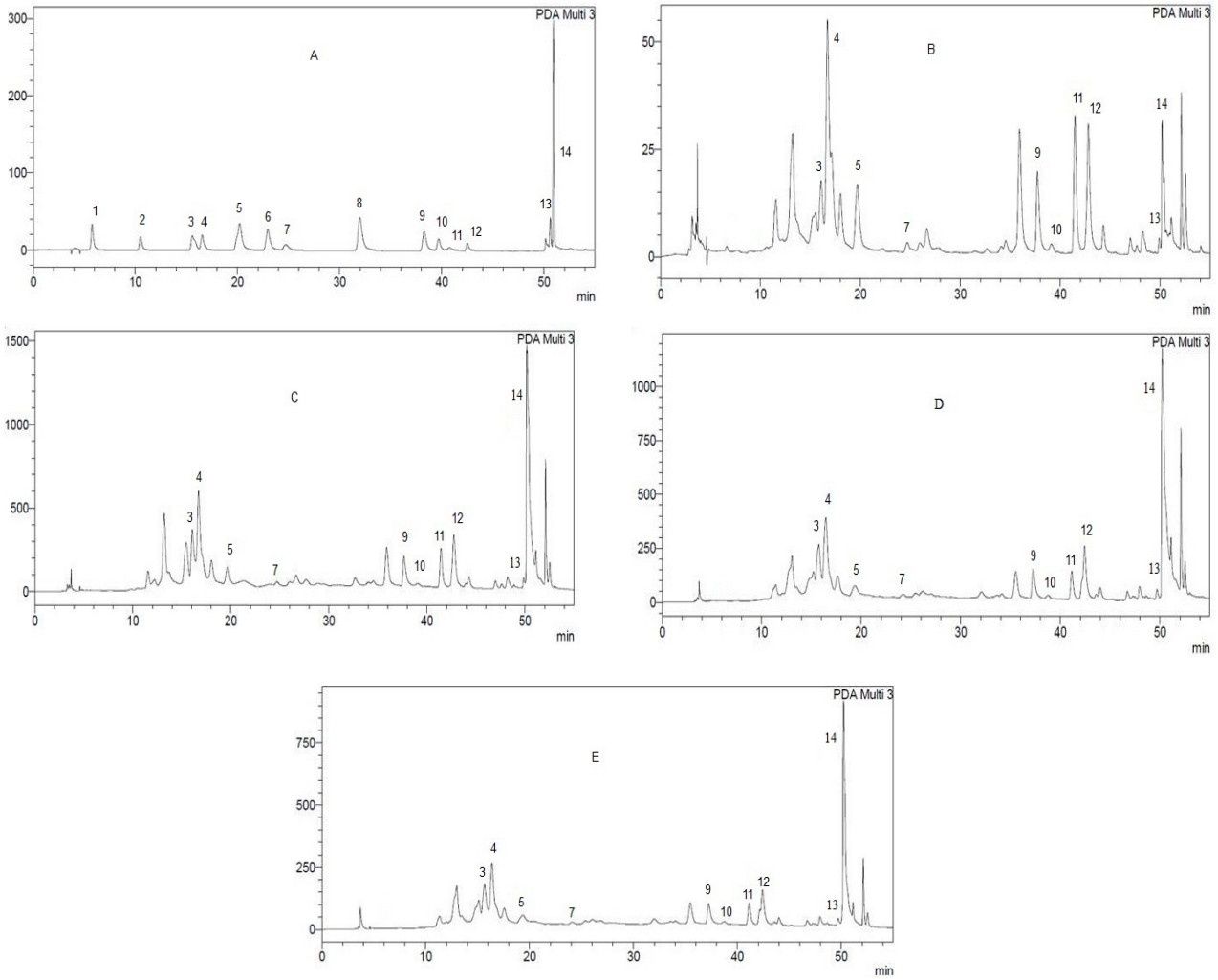

Figure1. HPLC chromatogram of a mixture of phenolic standards (A), extracts of *Amomun Chinense* leaves in water (B), 80% methanol (C), 80% ethanol (D) and 80% acetone (E) obtained using a gradient elution system by PDA detector at 280 nm
1 = gallic acid, 2 = protocatechuic acid, 3 = catechin, 4 = chlorogenic acid, 5 = caffeic acid,
6 = syringic acid, 7 = epigallo-catechin gallate, 8 = p-coumaric acid, 9 feluric acid,
10 = sinapic acid, 11 = ellagic acid, 12 = rutin, 13 = quercetin, 14 = t-cinnamic acid

Qualitative and quantitative analysis of phenolic acids and flavonoids compounds

The HPLC chromatograms of standard phenolic compounds and the extracts of *A.chinense* leaves from different extraction solvents and extraction times are shown in figure 1. There were numerous peaks that were not identified due to a lack of suitable standards. The samples were analyzed from at least three replications at 280 nm for hydroxybenzoic acids and flavanols, 320 nm for hydroxycinnamic acids and 360 nm for flavonols.

Quantitative analysis of individual phenolic compounds by HPLC showed that the 80% aqueous methanol was the best extraction solvent for phenolic compounds. These results showed that the most important compounds identified in the 80% methanol extract were hydroxycinnamic acids, including chlorogenic acid followed by trans-cinnamic acid, ellagic acid, ferulic acid and caffeic acid (Table 3). Whereas, hydroxybenzoic acids were found in small quantities and are not shown. High contents of catechin, rutin, epigallocatechin gallate and quercetin were obtained from 80% methanol followed by 80% ethanol, 80% acetone and distilled water, respectively (Table 4).This finding showed that the 80% methanol extract of A. chinense leaves had a higher content of catechin (2.33 mg/g dw) and rutin (1.75 mg/g dw) than reported by Ghasemzadeh *et al*. (2010), who reported that the methanol extract of Malaysian young ginger had high contents of catechin (0.56 mg/g DW) and rutin (0.32 mg/g dw).

Conclusion

These results clearly demonstrated that the solvent type is important in the extraction of antioxidant compounds from *A. chinense* leaves, whereas the extraction times slightly affected the amount of phenolics and flavonoids as well as antioxidant activities. 80% methanol showed potentially for extracting phenolic compounds from *A. chinense* leaves by having the greatest antioxidant activity, followed by 80% ethanol, 80% acetone and distilled water. Strong correlations were found between the phenolic and flavonoid contents and their antioxidant activities. Overall, *A. chinense* leaves contain chlorogenic acid, cinnamic acid, catechin and rutin that possess antioxidant activity; therefore, they could be a useful source of natural antioxidants.

Acknowledgements

The authors are thankful to Rajabhat Mahasarakham University and Thailand Research Fund (MRG5480177) for providing financial support for this research and other facilities. We also wish to thank Dr. Jolyon Dodgson, Faculty of Science, Mahasarakham University for his help improving the English.

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