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Antioxidant Activities and Antioxidative Components in Extracts of *Alpinia galanga* (L.) Sw.

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ABSTRACT

Galangal extracted using 50% ethanol in water was studied for its antioxidant activity and composition in comparison with two other samples based on a water extract and the essential oil. The antioxidant activities were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) methods. The ethanolic extract showed the highest DPPH free radical scavenging ability as well as the highest ORAC value when compared to the water extract and the essential oil. The IC_{50} values of the galangal ethanolic extract (10.66 mg/ml), water extract (55.48 mg/ml) and essential oil (455.43 mg/ml) were higher than those of α-tocopherol (1.45 mg/ml) and butylated hydroxyanisole (BHA; 0.41 mg/ml). The results indicated that the antioxidant activities of galangal extracts were lower than that of BHA, the commercial synthetic antioxidant generally used in food. The ethanolic extract contained the highest concentrations of total phenolic compounds (31.49 mgGAE/g) and flavonoids (13.78 mgCE/g). The water extract and the essential oil had a total phenolic content of 8.25 and 5.01 mgGAE/g and a total flavonoid content of 1.48 and 0.20 mgCE/g, respectively. Antioxidants in the galangal essential oil, namely, methyl eugenol (4,130.38 μg/g), chavicol (2,390.45 μg/g), and eugenol (728.30 μg/g) were found in its volatile fraction. The water extract contained mainly myricetin (14.60 mg/g extract) and an unknown phenolic compound. The major antioxidants in the ethanolic extract were 1´-acetoxycavichol acetate (10.56 mg/g extract), catechin (1.74 mg/g extract), and three unknown subtances. In addition to the phenolic compounds, 1´-acetoxycavichol acetate (ACA) could play an important role in the antioxidant activity of galangal. The ethanolic extract of galangal was also advantageous as an antioxidant in food due to its mild odor compared with the essential oil.

Key words: *Alpinia galanga,* galangal extract, antioxidants, essential oil, phenolic compounds, 1´-acetoxycavichol acetate

INTRODUCTION

Galangal (*Alpinia galanga*) is used to flavor foods throughout South and Southeast Asia. Its aromatic characteristic is described as woody, minty and floral (Mori *et al.*, 1995). Its rhizome has a wide range of applications in traditional medicine (Yang and Eilerman, 1999), as the essential oil shows an antimicrobial activity against gram-positive bacteria, yeast, and dermatophytes. The most active compound is terpinen-4-ol (Janssen and Scheffer, 1985). In addition, essential oil from galangal was reported as a potential anti-carcinogen (Zheng *et al.*, 1993).

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Several researches reported that 1´-acetoxychavicol acetate (ACA) and 1´-acetoxyeugenol acetate from galangal were anti-tumor substances (Itokawa *et al*., 1987; Kondo *et al*., 1993).

Natural antioxidants from plants have attracted significant interest because of their safety and potential nutritional and therapeutic effects. Several plant materials have been investigated as a potent source of antioxidants. Antioxidants in herbs and spices include: vitamins; phenolic compounds including flavonoids and phenolic acids; and volatile compounds (Carrubba and Calabrese, 1998). The antioxidant activity of phenolic compounds is mainly dependent on their redox properties that allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. Additionally, they have a metal-chelating potential (Rice-Evans *et al*., 1995). Several studies have been conducted on the antioxidant activities of galangal extracts. The antioxidant activity of a 1% (w/v) acetone extract of galangal in 99.5% ethanol was stronger than that of α-tocopherol (Jitoe *et al*., 1992). Zaeoung *et al*. (2005) found the new phenolic compound, *p*-coumaryl-9-methyl ether, in the methanolic extract of fresh galangal. Moreover, two phenolic compounds, *p*-hydroxy cinnamaldehyde and [di-(*p*-hydroxy-*cis*-styryl)] methane, were isolated from the chloroform extract of dry galangal (Barik *et al*., 1987). In food systems, research has mostly been carried out on the ethanolic extract (Cheah and Abu Hasim, 2000; Juntachote *et al*., 2006). The ethanolic extract of galangal acted as a free radical scavenger, exhibiting strong superoxide, anion-scavenging activity, Fe^{2+} chelating activity and reducing power in a concentration-dependent manner (Juntachote and Berghofer, 2005). Although, there were several studies on the antioxidant activities of galangal ethanolic extracts, the information related to their composition was limited. This study aimed to investigate the composition of galangal ethanolic extract with regard to its antioxidant activity in comparison with a water extract and the essential oil.

MATERIALS AND METHODS

Chemicals and apparatus

2,2-Diphenyl-1-picrylhydrazyl (DPPH), fluorescein sodium salt (FL) and (+)-α-tocopherol were purchased from Sigma-Aldrich (Milwaukee, WI, USA). 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) and 2,2´-azobis (2-methylpropionamidine) dihydrochloride (AAPH) were obtained from Sigma-Aldrich (Munich, Germany), while the gallic acid was sourced from Fluka (Madrid, Spain) and Folin-Ciocalteu's phenol reagent from Fluka (Buchs, Switzerland). Randomly methylated beta cyclodextrin (RMCD) for the ORAC test was purchased from Cyclodextrin Technologies Development (Gainesville, FL, USA). Acetone, ethanol, and methanol came from Merck (Darmstadt, Germany). A fluorescent microplate reader (FLUOstar OPTIMA, BMG Labtech, Offenburg, Germany) and fluorescence filters with an excitation wavelength of 485 nm and emission wavelength of 520 were used. The 96-well black microplates were purchased from BMG Labtech. Volatile compounds were isolated with a high vacuum distillation unit (Edwards, West Sussex, UK) and a spectrophotometer (Spectro 23, LaboMed, Inc., USA) was used for the spectrophotometric assay.

Sample preparation and extraction

Fresh rhizomes of galangal were purchased from local markets in Bangkok and cleaned with tap water. The extracts were prepared from fresh and dried galangal. Dried galangal was prepared by freeze-drying the fresh rhizomes using a freeze dryer (Heto Lab Equipment, Allerod, Denmark). The dried sample was ground to a fine powder using a blender and then passed through a

60-mesh sieve.

Three types of the extracts were prepared: ethanolic extract, water extract and essential oil. The ethanolic extract and water extract were prepared from galangal powder whereas the essential oil was steamed-distilled from fresh galangal.

Ethanolic extract

Galangal powder was extracted using 50% ethanol (v/v) in water at a solid-to-solvent ratio of 1:10. The extraction was performed in a shaking water bath at 50°C for 1 h. The extract was filtered through a No.1 sinter glass funnel. The residue was re-extracted using the same conditions. The two extracts were then combined and concentrated in a rotary evaporator at 40°C. The concentrated extract was dried using a freeze dryer and kept in a glass vial at –40°C.

Water extract

Ten grams of galangal powder were refluxed with 100 ml of distilled water for 3 h at 80°C. The mixture was cooled to room temperature and filtered through a No.1 sinter glass funnel. The residue was then re-refluxed with 100 ml distilled water under the same conditions. The extracts were combined and concentrated in a rotary evaporator at 60°C. The concentrated extract was freeze dried and kept in a glass vial at – 40°C.

Essential oil

The essential oil was obtained from fresh galangal rhizomes by steam distillation using a glass essential oil extractor (Becthai Bangkok Equipment and Chemical, Bangkok, Thailand). The fresh rhizomes were cut into small pieces (1 cm × 1 cm × 0.3 cm) and 320 g of galangal was comminuted with 400 ml water. The slurry was prepared in a one-liter, round-bottomed flask and steam-distilled for 4 h. The essential oil was dried by filtering through anhydrous sodium sulfate and kept in a glass vial at – 40°C.

Antioxidant activity

The antioxidant activity of the water extract, ethanolic extract and essential oil from the galangal rhizome were studied using DPPH and ORAC methods. The use of galangal extracts together with BHA in methanol was also investigated by the DPPH method to study the effects of using the extract with a commercial antioxidant. A comparative study on the antioxidant activities of the samples, BHA and α-tocopherol was also conducted using the DPPH method and reported as IC_{50}.

DPPH assay

The electron-donation abilities of the extracts and commercial antioxidants were measured from the bleaching of purple DPPH in methanol solution.

The DPPH assay was carried out as described by Tepe *et al*. (2005). The ethanolic extract and the water extract were diluted with water and the essential oil was diluted with ethanol to various concentrations. The concentrations of the ethanolic extract were from 0 to1.0% (w/v), the water extract samples were from 0 to 5.0% (w/v), while the essential oil sample ranged from 0 to 25% (w/v). The DPPH studies were conducted with and without the addition of BHA. The ethanolic extract and water extract samples were supplemented with 0.01% BHA (w/v) in methanol, whereas the essential oil samples were supplemented with 0.02% BHA (w/v) in methanol, in order to be able to observe the antioxidant activities.

In the DPPH assay, 50 μl of samples at various concentrations were added to 5 ml of 0.004 % DPPH in methanol. After a 30 min incubation period at room temperature, the absorbance was read at 517 nm. The purple color bleaching of the DPPH reagent was evaluated as positive antioxidant activity. The inhibition of free radicals by DPPH in percent (*I* %) was calculated using Equation 1:

$$I\,\% = [(A_B - A_A)/A_B] \times 100 \quad (1)$$

where:

A_B = the absorbance of a blank after 30 min, and

A_A = the absorbance of the sample after 30 min.

For the comparative study on the antioxidant activities of the samples, BHA and a-tocopherol, the results were reported as IC_{50} (the concentration of an antioxidant to achieve 50% inhibition of free radicals by DPPH assay).

$ORAC_{FL}$ method

The ORAC assay in this study followed the method using fluorescence described by Prior *et al*. (2003). The antioxidant activity was quantified by calculating the net protection area under the time-resolved fluorescence decay curve. AAPH, a peroxyl radical generator ($ORAC_{ROO\cdot}$), was used. The data were expressed as an ORAC value (μmol Trolox equivalent (TE)/g extract).

Phenolic compound analysis

Determination of total phenolic compounds

The total phenolic content of the galangal extracts was determined by a modification of the Folin-Ciocalteu method, according to Wolfe *et al*. (2003). Folin-Ciocalteu reagent (0.125 ml) was added to a test tube containing 0.5 ml of deionized water and 0.125 ml of a known dilution of the extract. The reaction was allowed to progress for 6 min, then 1.25 ml of 7% sodium carbonate solution was added to the test tube and the mixture was diluted to 3 ml with deionized water. The mixture was allowed to stand for 90 min. The absorbance was then measured at 760 nm. The content of the phenolic compounds was expressed as mg gallic acid equivalent (GAE)/g sample.

Determination of flavonoid content

The determination of the flavonoid content was performed according to the colorimetric assay described by Wolfe *et al*. (2003). A volume of 0.25 ml of a known dilution extract (5%, 1% and 12.5% for the water extract, ethanolic extract and essential oil, respectively) and 1.25 ml of distilled water were added to a test tube. Then, 0.075 ml of 5% sodium nitrite solution was added to the mixture. The reaction was allowed to progress for 5 min. After that, 0.15 ml of 10 % aluminum chloride was added. After 6 min, 0.5 ml of 1 M sodium hydroxide was added and the mixture was diluted with 0.275 ml of distilled water. The absorbance at 510 nm was read immediately. A calibration curve was prepared with (+)-catechin. The flavonoid content was expressed as mg (+)-catechin equivalent (CE)/g sample.

High performance liquid chromatography (HPLC) analysis

Identification and quantification of the non-volatile phenolic compounds were achieved using an Agilent 1100 HPLC Series system. The ethanolic extract was dissolved in methanol and filtered through a 0.45 μm regenerated cellulose membrane filter. Samples were separated on a reversed-phase Zorbax SB-C18 analytical column (100 × 3.0 nm i.d., 3.5 μm particle). The column was operated at 48°C. A variable UV-VIS detector was used to detect the compounds at 280 nm. The gradient mobile phases were methanol and a potassium dihydrogen phosphate buffer solution. The buffer solution was prepared by dissolving potassium dihydrogen phosphate (40 mM) in deionized water and adjusting the pH to 2.3 with 85% orthophosphoric acid. The binary gradient started with a linear gradient from 5% to 42% methanol in the first 35 min, followed by isocratic elution with 42% methanol for the next 3 min. The flow rate was 1 ml/min and the injection volume was 10 μl. The solutions were filtered through a 0.45 mm nylon membrane filter and degassed in an ultrasonic bath before use. Retention times and spectra were compared to those of the pure standards.

Gas chromatography-mass spectrometry (GC-MS) analysis

For the ethanolic and water extracts, 1 g of the extract was dissolved in 25 ml of deodorized water in a 250 ml glass bottle. The sample was then supplemented with 20 µl of the internal standard (10 µg/ml of 2-undecanol in dichloromethane). The solution was consecutively extracted with 25 ml dichloromethane on a stirring plate for 30 min. The extract was then transferred to a 250 ml round-bottom flask, which was immersed in liquid nitrogen until the extract was frozen. Volatile compounds and the solvent were vacuum-distilled at 10^{-5} Torr at room temperature for 1 h and at 50°C in a water bath for 1 h. The volatile extract was recovered from the first receiving tube and concentrated to 5 ml under a gentle stream of nitrogen. The concentrated extract was then dried over anhydrous sodium sulfate and then further concentrated to 1 ml under a gentle stream of nitrogen. The sample was stored in a glass vial at -40°C until used for analysis. For the essential oil sample, the oil was diluted to 1/100 (v/v) in dichloromethane.

Qualitative and quantitative analyses of the extracts were performed using an HP 6890 gas chromatograph equipped with an HP 5973 mass selective detector (MSD, Agilent Technologies, Palo Alto, USA). The MSD was operated at 70 eV, with a scanning speed of 1 s over a 40-300 amu range with an ion-source temperature of 180°C. An HP-5 and an HP-FFAP column were used for separation of the volatile compounds. The HP-5 capillary column (60 m × 0.25 mm × 0.25 μm) was used under the following conditions: inlet temperature, 200°C; detector temperature, 280°C; ans an oven temperature program with an initial temperature of 35°C heated to 250°C at a rate of 10°C/ min and held for 10 min. Helium was used as the carrier gas at the flow rate of 1.5 ml/min. The split ratio was 1:5. The HP-FFAP capillary column (60 m × 0.25 mm × 0.25 µm) was used under the same GC conditions, except for the oven temperature program, which commenced with an initial temperature of 40°C heated to 210°C at a rate of 10°C/ min and held for 30 min.

For the essential oil, the analysis was modified due to the differences in solubility and the profile of the volatile compounds in the sample. One microliter of diluted essential oil was injected with the split ratio of 1:5. Then 2-Undecanol solution (10 mg/ml dichloromethane), an internal standard, was added to the sample at the concentration of 10 μl/ml. The HP-5 capillary column was used under the conditions previously described. The HP-FFAP was used under the following conditions: split-splitless inlet, 200°C; detector temperature, 280°C; and an oven temperature program with an initial temperature of 40°C, heated to 210°C at a rate of 6°C/ min and held for 10 min. Helium was used as the carrier gas at the flow rate of 1.3 ml/min.

Compounds were identified using authentic standards, matching mass spectra with the Wiley275 Mass Spectra Database and by comparison of the retention indices (RIs) related to the retention times of the *n*-alkane series. Relative concentrations were determined using the MS response factors for each component related to the internal standard.

Statistical analysis

The data were subjected to analysis of variance (ANOVA). The significance of the difference between means was determined by Duncan's multiple-range test ($p \leq 0.05$) using SPSS version 12 (SPSS Inc., Chicago, IL). The correlations between the phenolic content, flavonoid concentrations and antioxidant activity were performed using Pearson's correlation coefficient. For GC and HPLC analysis, the experiments were carried out in duplicate. The other experiments were performed in triplicate.

RESULTS AND DISCUSSION

Three extract samples of galangal were used in this study: water extract, ethanolic extract and essential oil. The water extract was a light brown powder that was almost odorless. The ethanolic extract was a light brown powder with a mild galangal aroma. The essential oil was a colorless, clear liquid with a pungent and spicy odor. The strong odor of the essential oil could limit its use to only specific foods in which the galangal aroma is acceptable.

Antioxidant activities of galangal extracts

The antioxidant activities of the samples were studied using the DPPH and ORAC methods. To obtain the ethanolic extract, 50% ethanol in water was used. It was the lowest concentration of ethanol that produced the extract with high antioxidant activity in the preliminary study. The preliminary experiment showed that galangal extracts obtained by using 50 and 75% ethanol at 30°C and 50°C exhibited similarly high antioxidant activities at 33.58-36.63% inhibition. The extract that used 95% ethanol exhibited a lower percent inhibition at 11.41-14.41%.

The data from the DPPH test showed that ethanolic extract had the highest antioxidant activity followed by the water extract and the essential oil (Figure 1). The antioxidant activities in all samples were concentration dependent. This agreed with the work on galangal extracts and essential oil by Zaeoung *et al.* (2005). In the current study, galangal extracts were also mixed with BHA to observe the effect when used together. The results in Figure 1 indicate that the mixture had merely an add-on effect on antioxidant activity. The ethanolic extract of galangal had the potential to be used as an antioxidant and could partially replace BHA to reduce the use of BHA but it did not have a synergistic effect.

The ORAC values of the galangal water extract, ethanolic extract and essential oil were 6845.40, 8114.43 and 1730.32 μmol TE/g, respectively (Table 1). The results from the ORAC test showed a similar trend as previously described in the DPPH test.

Table 2 compares the effectiveness for DPPH radical scavenging of galangal extracts with the commercial antioxidants: BHA and α-tocopherol. These two were the most effective antioxidants with IC_{50} = 0.41 and 1.45 mg/ml, respectively. Among the galangal extracts, the ethanolic extract was the most effective radical scavenger with IC_{50} = 10.5 mg/ml. From the IC_{50} data, the extract samples had lower antioxidant activities than BHA and α-tocopherol. This was different from what was reported by Jitoe *et al.* (1992), who found that the antioxidant activity of an acetone extract of fresh galangal was higher than that of α-tocopherol. This could have been due to the use of a different solvent as well as differences in the raw material preparation. In this study the galangal was dried before the extraction, but Jitoe *et al.* (1992) extracted it directly from the fresh rhizomes.

Total phenolic compounds and total flavonoids

Phenolic compounds especially flavonoids including catechin are superoxide scavengers and show a strong antioxidant activity (Hanasaki *et al.*, 1994). In this experiment, galangal extracts were analyzed for their total phenolic compounds using Folin-Ciocalteu reagent (FCR) and total flavonoids were analyzed by spectrophotometric assay.

The ethanolic extract had the significantly highest total phenolic content, whereas in the water extract and essential oil samples this was not significantly different ($p > 0.05$). The total phenolic compounds in the water extract, ethanolic extract and essential oil were 8.25 ± 0.78, 31.49 ± 4.09 and 5.01 ± 0.14 mg GAE/g extract, respectively (Table 3). Antioxidant activity (ORAC method) was well correlated with total phenolic content (r = 0.800,

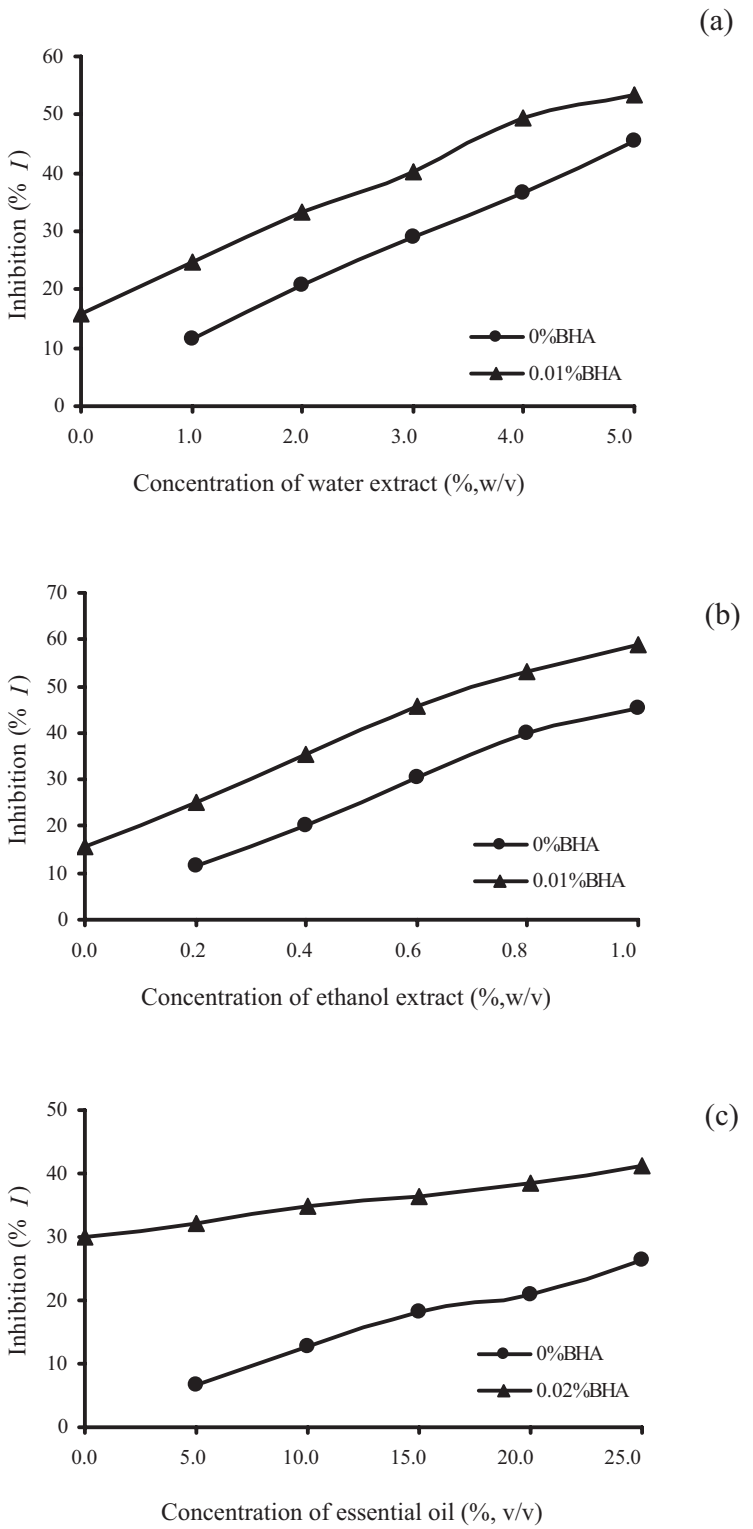

Figure 1 Antioxidant activities at different concentrations, with and without BHA of (a) galangal water extract, (b) ethanolic extract and (c) essential oil.

Table 1 The ORAC values of water extract, ethanolic extract and essential oil.

Sample	ORAC value [1/] (μmol TE/g extract)
Water extract	6845.40 ± 89.19 [b]
Ethanolic extract	8114.43 ±718.48 [c]
Essential oil	1730.32 ± 80.73 [a]

[1/] Different letters within the same column indicate significant differences between treatments based on one-way ANOVA ($p \leq 0.05$).

Table 2 The IC_{50} of BHA, α-tocopherol, water and ethanolic extracts and essential oil of galangal.

Sample	IC_{50} (mg/ml) [1/]
BHA	0.41[a]
α-Tocopherol	1.45 [a]
Water extract	54.39 [c]
Ethanolic extract	10.50 [b]
Essential oil	431.69 [d]

[1/] Different letters within the same column indicate significant differences between treatments based on one-way ANOVA ($p \leq 0.05$).

$p \leq 0.01$). On a dry weight basis, the essential oil fraction of galangal contributed much less phenolic compounds in comparison to the ethanolic and water extracts.

The amounts of total flavonoids in the extracts are shown in Table 3. The flavonoid content of the water extract, ethanolic extract and essential oil was 1.48 ± 0.02, 13.78 ± 0.60 and 0.20 ± 0.01 mgCE/g extract, respectively. The antioxidant activity (ORAC method) of galangal extract correlated well with the flavonoid content ($r = 0.720$, $p \leq 0.05$).

Although the water extract and essential oil contained approximately the same amount of total phenolic compounds (Table 3), the essential oil was lower in total flavonoids. This indicated that some phenolic compounds in the essential oil probably belonged to groups other than flavonoids.

HPLC analysis

The phenolic compounds in the samples were further analyzed using HPLC. The water and ethanolic extracts were rich in phenolic compounds but the essential oil contained only a trace amount when analyzed with HPLC.

The results in Table 4 and Figure 2 show that the water extract contained mainly myricetin (14.60 mg/g extract) and an unknown substance, A (13.79 mgCE/g extract). The ethanolic extract had the unknown substance A (2.32 mgCE/g extract) and catechin (1.74 mg/g extract) as its major phenolic compounds. The extract also

Table 3 Total phenolic and flavonoid content of water extract, ethanolic extract and essential oil of galangal.

Sample	Concentration [1/]	
Total phenolics	mg GAE/g extract	mg GAE/g dry galangal
Water extract	8.25 ± 0.78 [a]	2.94 ± 0.28 [b]
Ethanolic extract	31.49 ± 4.09 [b]	10.28 ± 1.33 [c]
Essential oil	5.01 ± 0.14 [a]	0.10 ± 0.00 [a]
Total flavonoids	mg CE/g extract	mg CE/g dry galangal
Water extract	1.48 ± 0.02 [b]	0.53 ± 0.08 [b]
Ethanolic extract	13.78 ± 0.60 [c]	4.50 ± 1.74 [c]
Essential oil	0.20 ± 0.01 [a]	0.004 ± 0.00 [a]

[1/] Different letters within the same column of the same analysis indicate significant differences between treatments based on one-way ANOVA ($p \leq 0.05$).

contained *p*-coumaric acid and two other unknown substances. Several minor compounds in the ethanolic extract were unidentified in this experiment (Table 4, Figure 3). These compounds could have been *p*-coumaryl-9-methyl ether (Zaeoung *et al*., 2005), curcuminoids (Jitoe *et al*., 1992), *p*-hydroxy cinnamaldehyde and [di-(*p*-hydroxy-*cis*-styryl)] methane as reported in a study on the chloroform extract of galangal rhizomes by Barik *et al*. (1987).

Table 4 Phenolic compounds in galangal extracts, analyzed by HPLC.

compound	Water extract		Ethanolic extract	
	mg/g extract	mg/g dry galangal	mg/g extract	mg/g dry galangal
Catechin	0.73 ± 0.29	0.26 ± 0.10	1.74 ± 0.26	0.57 ± 0.08
p-Coumaric acid	-	-	0.07 ± 0.00	0.02 ± 0.00
Myricetin	14. 60 ± 2.15	5.21 ± 0.77	0.45 ± 0.03	0.15 ± 0.01
Unknown A 1/	13.79 ± 1.33	4.92 ± 0.47	2.32 ± 0.15	0.76 ± 0.05
Unknown B 1/	0.47 ± 0.05	0.17 ± 0.02	1.35 ± 0.08	0.44 ± 0.03
Unknown C 1/	0.06 ± 0.01	0.02 ± 0.00	1.52 ± 0.10	0.50 ± 0.03

1/ Concentrations of the unknowns are reported as relative concentrations to that of the catechin standard.

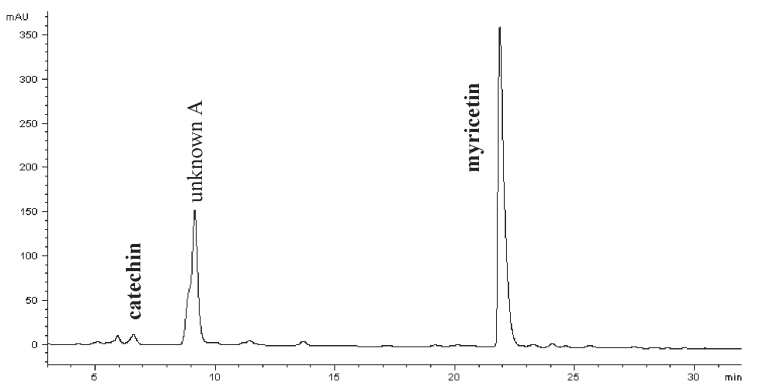

Figure 2 HPLC chromatogram of the water extract of galangal rhizomes.

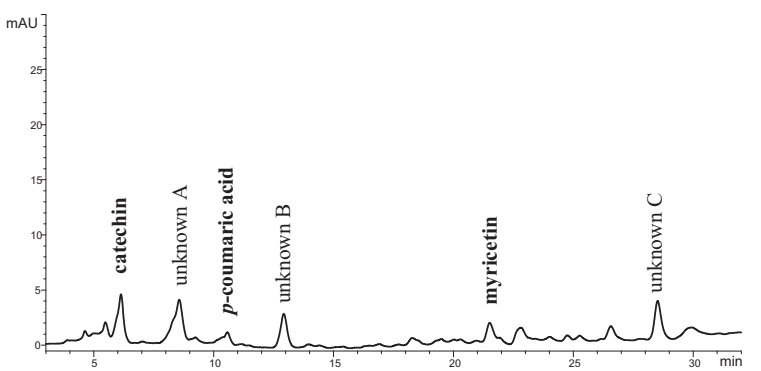

Figure 3 HPLC chromatogram of the ethanolic extract of galangal rhizomes.

GC analysis

The volatile phenolic compounds and their derivatives were analyzed by GC-MS (Table 5). It was clear that most phenolic compounds in the essential oil were volatile components, being: chavicol, methyl eugenol, thymol and eugenol. The major phenolic compounds in the essential oil of galangal were methyl eugenol (4130.38 μg/g extract) and chavicol (2390.45 μg/g extract). Essential oils from other spices that showed high antioxidant activity usually contained high eugenol (Tomaino *et al*., 2005). The essential oil of galangal in this study had a low concentration of eugenol and low antioxidant activity. Most compounds in the essential oil were terpenes, with Dorman *et al*. (1995) reporting that there were antioxidant properties in: α-pinene, camphene, sabinene, β-pinene, myrcene, ρ-cymene, 1,8-cineole, limonene, γ-terpinene, linalool, borneol, terpinene-4-ol, α-terpineol, geranyl acetate, methyl eugenol and β-caryophyllene. All of these compounds were detected in the essential oil from this study (data not shown).

The water and ethanolic extracts contained only small amounts of volatile phenolics. However, there was a phenylpropanoid, namely 1´-acetoxychavicol acetate (ACA), which could have contributed to the antioxidant activity of the ethanolic extract (Table 5). In this study, ACA was present at a high concentration (10 563.30 mg/g extract) and was found only in the volatile fraction of the ethanolic extract (Table 5). It was identified by matching the mass spectrum (Figure 4) with that of ACA from galangal seed (Mitsui *et al*., 1976). ACA was also isolated from galangal oleoresin extracted with pentane (Yang and Eilerman, 1999) and from the dried galangal rhizome extracted with *n*-pentane/diethyl ether (Janssen and Scheffer, 1985). The antioxidant

Table 5 Relative concentrations of volatile-phenolic compounds and phenylpropanoids in the extracts from rhizomes of galangal, analyzed by GC-MS.

Compound	RI		ID [1/]	Concentration	
	HP-5	FFAP		μg/g extract	μg/g dry galangal
Water extract					
Chavicol (4-allylphenol)	1259	2359	a, b, c1	21.24 ± 1.85	7.56 ± 0.66
Vanillin	1417	-	a, b	2.92 ± 0.60	1.04 ± 0.21
Ethanolic extract					
Chavicol (4-allylphenol)	1260	2359	a, b, c1	257.39 ± 43.25	84.11 ± 14.13
Methyl eugenol	1411	2022	a, b, c1	87.63 ± 13.37	28.64 ± 4.37
Eugenol	-	2195	a, b	13.32 ± 2.92	4.35 ± 0.95
1'-Acetoxychavicol acetate	1655	2505	c3	10 563.30 ± 3238.97	3452.06 ± 1058.49
Essential oil					
Thymol	1289	1903	a, b, c2	48.56 ± 7.16	1.00 ± 0.15
Chavicol (4-allylphenol)	1256	2350	a, b, c1	2390.45 ± 1387.35	49.28 ± 28.60
Methyl eugenol	-	2022	a, b	4130.38 ± 139.07	85.16 ± 2.87
Eugenol	-	2183	a, b	782.30 ± 36.65	16.13 ± 0.76

[1/] ID = Identification by a = comparison of MS data with Wiley 275 library; b = comparison of RI with those in the ESO 2000 database of essential oil (Boelens Aroma Chemical Information Service, 1999)9; c = comparison of RI with literature data; c1 = Adams (1995); c2 = Lorjaroenphon (2004); c3 = Mitsui *et al*. (1976).

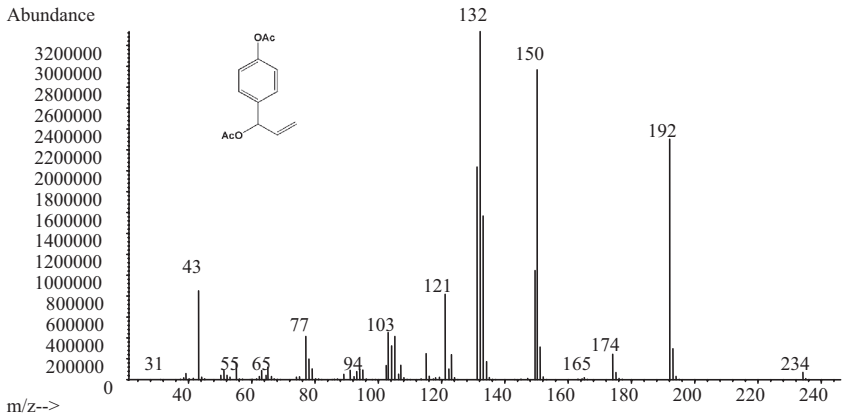

Figure 4 Mass spectra and structure of 1´-acetoxychavicol acetate from the ethanolic extract of galangal rhizomes.

activities of ACA and its related compounds that formed during heating were studied by Kubota *et al*. (2001). Their data from ferric thiocyanate and TBA assays indicated that ACA had a higher antioxidant activity than α-tocopherol and was close to that of butylated hydroxytoluene (BHT). In this study, ACA was one of the major peaks in the GC-MS chromatogram of the ethanolic extract. It could be extrapolated that the highest antioxidant activities of the ethanolic extract were a result of its phenolic compounds and ACA.

CONCLUSIONS

The galangal ethanolic extract in this study showed potential to be used as a natural antioxidant in food products. Having phenolic compounds and ACA with a milder odor makes it advantageous for use with more varieties of food products when compared to other herbs and spices that have pungent terpenes as their antioxidative components.

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