Journal of Developments in Sustainable Agriculture 7: 89-96 (2012)

Antioxidant Activity and Selected Chemical Components of 10 *Zingiber* spp. in Thailand

Vipada Kantayos and Yingyong Paisooksantivatana*

Department of Horticultural, Faculty of Agricultural, Kasetsart University

Zingiber officinale (ginger) is the one of most commonly used spices and is a traditional herbal medicine in Thailand. Thailand is home to various *Zingiber* wild species. However, the biological activity evidence of these other species is less well studied than that of *Z. officinale*. In this study, we investigated the rhizomes of 10 *Zingiber* species to determine the correlations between total phenolic content, total curcuminoid content, and antioxidant activity, as determined by means of DPPH (2,2-Diphenyl-l-picrylhydrazyl) and ABTS (2,2′ -azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) assays. We also used high-performance liquid chromatography and gas chromatography to determine the quantities of 6-gingerol and terpinen-4-ol, respectively which are the two most important active compounds associated with antioxidant and anti-inflammatory action. Antioxidant activity was highly correlated with total phenolic content. To the best of our knowledge, this is the first report of the antioxidant activities of three of the species (*Z. rubens*, *Z. bisectum*, and *Z. barbatum*). The rhizome extracts of *Z. montanum* showed the highest total curcuminoid content and yielded the highest amount of essential oil and terpinen-4-ol content. 6-Gingerol was detected in only two species: *Z. officinale* and *Z. cornubracteatum*. Our results suggest that *Z. montanum* may be an excellent natural remedy owing to its considerable antioxidant activity and the large amounts of known bioactive chemical constituents it contains. It may be a source of additional bioactive compounds.

Key words: *Zingiber*, antioxidant activity, 6-gingerol, terpinen-4-ol

Introduction

In South and Southeast Asian countries, products obtained from plants of the genus *Zingiber* are used as food, flavorings, and medicines. Common ginger (*Zingiber officinale* (L.) Rosc.), pinecone ginger (*Z. zerumbet* (L.) Roscoe ex Smith), and cassumunar ginger (*Z. cassumunar* (Koenig) Link ex. Dietr.) are part of the daily diet in this region and are well-known for their anti-inflammatory properties, as well as their ornamental uses. Several scientific reports were indicated that various parts of the plants consist of leaves, shoots, flowers, and rhizomes, exhibit various biological effects, including antioxidant activity (Habsah *et al*., 2000). The biological activities of *Zingiber* powder rhizome have been attributed to various chemical constituents. For instance, 6-gingerol most notably inhibitory effects on oxidation and inflammatory activity (Schwertner and Deborah, 2007) and terpinen-4-ol is an anti-inflammatory agent (Sukatta *et al*., 2009).

Antioxidants are chemical compounds that inhibit the oxidation of lipids and other molecules in living cells (Zheng and Shiow, 2001). Antioxidant activity plays a crucial role in many chronic diseases, including cardiovascular disease, diabetes, Alzheimer's disease, and various inflammatory diseases (Gan *et al*., 2010), and interest in obtaining antioxidants from natural sources is rapidly increasing.

Thailand is a rich source of *Zingiber* diversity; at least 50 species have been reported in Thailand (Triboun, 2005). However, the information about the content of antioxidants and other compounds in these species is lacking. Therefore, the objectives of the

Received: July 22, 2011, Accepted: December 7, 2011

* Corresponding author: Department of Horticultural, Faculty of Agricultural, Kasetsart University, 50 Phaholyothin Rd., Ladyao, Jatujak, Bangkok, Thailand 10900.

Tel: +66-2-579-0308, Fax: +66-2-579-1915 (112), E-mail: agryyp@ku.ac.th

present study were to determine the content of antioxidants and related compounds in some *Zingiber* species to identify the potential uses of such compounds in the food and drug industries.

Materials and Methods

Plant materials

We used the rhizomes of the following 10 *Zingiber* species: *Z. montanum*, *Z. ottensii*, *Z. rubens*, *Z. cornubracteatum*, *Zingiber* 'Phlai-chompoo', *Z. zerumbet*, *Z. officinale*, *Z. bisectum*, *Z. spectabile*, and *Z. barbatum*. To identify *Zingiber* species in the field, we relied on the taxonomic descriptions of Triboun (2005). Rhizomes were collected from various locations in Thailand and were planted at the Department of Horticulture of Kasetsart University, Thailand. Rhizomes were harvested from 10-month-old plants for ethanol extraction and essential oil distillation.

Preparation of crude extracts

Zingiber rhizomes (ages 10-12 months) were air dried until dry weight stable and milled into crude powder using blender. The crude powder rhizomes were macerated in distilled 95% ethanol (powder/alcohol ratio, 0.025−0.133 w/v) for 24 h at room temperature, and the solids were filtered off with filter paper. The supernatant was kept at −4℃ until it was screened for antioxidant activity.

Determination of antioxidant activity

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of a methanol solution of DPPH and ABTS.

DPPH radical scavenging assay

Radical scavenging activity of *Zingiber* rhizome extracts was determined spectrophotometrically according to the method of Bua-in and Paisooksantivatana (2009). At first, the rhizome powder and ascorbic acid were weight accurately and dissolve in ethanol and distilled water, respectively, to make the different dilutions of ethanol extract (0.05−0.005 g/ml). Here ascorbic acid was taken as positive control. Next, DPPH (Sigma-Aldrich, Lot. D9132) was weight and dissolve in ethanol to make 0.1 mM solution. 3 mL of DPPH solution was applied on test tube with 0.5 mL of the rhizome extracts. The mixtures were kept in the dark for 20 min. at room temperature, and the optical densities were measured at 517 nm.

ABTS radical scavenging assay

The effect of the extracts on ABTS radical was determined by the method of Chaichana *et al*. (2009). Briefly, an $ABTS^{\cdot+}$ (Fluka, ≥99%) stock solution was prepared by mixing 7 mM ABTS and 2.45 mM $K_2S_2O_8$ at a ratio of 8:12 v/v; the mixture was kept in the dark at 4℃ for 12 h for complete reaction. A working solution of $ABTS^{\cdot+}$ was prepared by diluting the stock solution with distilled 95% ethanol at a ratio of 5:100 v/v. The ABTS solution (3 mL) were allowed to react with *Zingiber* rhizome extracts (0.5 mL) and kept in the dark for 20 min., the optical densities of the solutions were taken at 734 nm. An ascorbic acid was use as a positive control an a synthetic antioxidant.

All sample were analyzed in triplicate. The antioxidant activity was expressed as IC_{50} values (the concentration of an antioxidant at which 50% inhibition of free radical activity is observed). The percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_1 - A_0)/A_0)]100$$

where A_0 and A_1 are the absorbance measured for the control reaction and for the sample extracts, respectively. The lower the IC_{50} number, the greater the overall effectiveness of the antioxidant.

Total phenolic content

The total phenolic content was determined with Folin-Ciocalteu reagent according to the method of Chan *et al*. (2007). Briefly, the rhizome extracts (300 μL) were mixed with Folin-Ciocalteu reagent (10 times dilution) and aqueous sodium carbonate (7.5% w/v). The mixture was allowed to stand for 30 min, and then the absorbance at 765 nm was measured. Total phenolic content is expressed in terms of gallic acid equivalents per dry weight (mg GAE/g DW). All samples were run in triplicate.

Total curcuminoid content

Curcumin standard solutions were prepared according to the method of Pothitirat and Gritsanapan (2006) with the following modifications. For preparation of standard solution, standard curcumin (2.00 mg) was accurately weighed and transferred to a 5-ml volumetric flask.

Ethanol was added and adjusted to a final concentration of 400 mg/ml. From this solution, concentrations of 0.005, 0.01, 0.0125, 0.015 and 0.02 mg/mL were prepared and used for preparation of the calibration curve. For preparation of sample solution from *Zingiber* powder rhizome, the powder (100 mg) of each species was added 20 mL ethanol and the absorbance at 420 nm were measured spectrophotometrically.

6-Gingerol analysis

Zingiber powder (0.50 g) was extracted with 5 mL ethanol, and the mixture was filtered through Whatman No. 4 filter paper. The filtrate was subsequently passed through a 0.20-μm nylon membrane filter. 6-Gingerol content was determined by means of reverse-phase high-performance liquid chromatography (HPLC) on a C_{18} column (250 mm×4.6 mm, Phenomenex, USA) The mobile phase was methanol:water (65:35 v/v) at a flow rate of 1.0 mL/min, the injection volume was 20 μL, and the chromatographic run time was 30 min. A UV-2998 photodiode array detector was used for detection at 281 nm. A calibration curve was prepared by using 20−100 mL/kg solutions of standard 6-gingerol (Sigma-aldrich, Lot. G1046), and peak identification was based on retention time. Analyze of each sample was done in triplicate.

Terpinen-4-ol analysis

Essential oil samples were analyzed on a gas chromatograph (Ultra series, Thermo sciencetific) equipped with a fused-silica capillary column (30 mm×0.25 mm, i.d. 0.32 mm, DB-5, Agilent) and a flame ionization detector. The operating parameters of the instrument were as follows: helium carrier gas at a flow rate 1.0 ml/min; split injection mode; inlet temperature 280℃; oven temperature ramp from 60℃ to 230℃, hold for 10 min. Terpinen-4-ol standard solutions were prepared at concentrations of 0.025–0.1 % v/v in *n*-hexane.

Data analysis

Data were analyzed statistically using analysis of variance (ANOVA), and the differences between samples were determined by Duncan's multiple range test ($p<0.05$). The Pearson's correlations ($p<0.01$ and $p<0.05$) between antioxidant activities determined by means of the two methods, total phenolic content, and total curcuminoid content were evaluated.

Results

Antioxidant activity, Total phenolic content, Total curcuminoid content

The ethanol extracts of the 10 *Zingiber* species showed substantially antioxidant activity in the DPPH and ABTS assays (Table 1). A low IC_{50} value indicates a strong ability to scavenge free radicals. *Z. officinale* exhibited the highest antioxidant activity both assays, with IC_{50} values of 4.26 mg/mL (DPPH) and 7.04 mg/mL (ABTS). The IC_{50} values obtained by the two methods showed positive linear correlation ($r=0.837$, Table 2). However, none of the samples showed antioxidant activities as strong as the activity of L-ascorbic acid (positive control).

Table 1. Antioxidant activities (IC_{50}) and total phenolic contents of 10 *Zingiber* species from Thailand. All measurements were done in triplicate, and all values are means±SD.

Species	IC_{50} (mg/mL)		Total phenolic content (mg GAE/g DW)
	DPPH assay	ABTS assay	
L-ascorbic acid	0.024±0.00	0.071±0.00	—
Z. montanum	4.71±0.15	13.91±0.15	6.53±0.10
Z. officinale	4.26±0.07	7.04±0.01	7.70±0.06
Z. rubens	7.93±0.02	12.81±0.17	4.86±0.10
Z. cornubracteatum	16.3 ±0.31	46.48±1.35	3.31±0.04
Zingiber 'Phlai-chompoo'	13.08±0.96	29.49±0.51	3.88±0.12
Z. zerumbet	16.71±0.44	24.81±0.98	5.90±0.13
Z. ottensii	32.81±0.59	49.10±1.00	2.30±0.16
Z. bisectum	8.89±0.11	9.34±0.34	7.35±0.13
Z. spectabile	39.34±3.84	60.80±13.47	1.34±0.20
Z. barbatum	13.31±0.19	16.52±0.01	2.69±0.01
F-test	*	*	*
CV	3.91%	3.37%	2.14%

* Average value within each column is significantly different at the 95% level of confidence as indicated by Duncan's multiple range test ($p<0.05$).

Table 2. Correlation coefficients between total curcuminoid content, total phenolic content, and antioxidant activity.

	Total curcuminoid content	Total phenolic content	DPPH assay	ABTS assay
Total curcuminoid content	1			
Total phenolic content	0.444*	1		
DPPH assay	0.530**	0.794**	1	
ABTS assay	0.093**	0.832**	0.837**	1

*Correlation coefficient is significant at the 95% level (2-tailed).
**Correlation coefficients are significant at the 99% level (2-tailed).

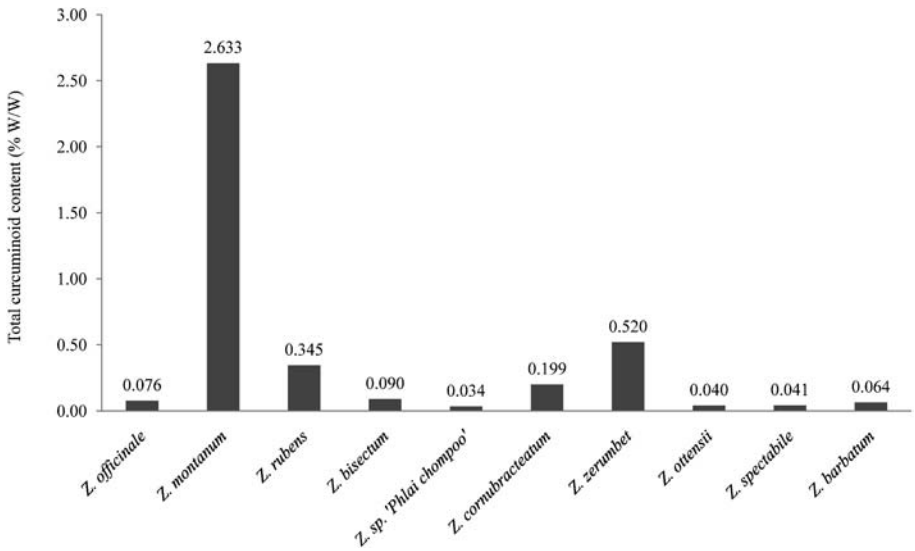

Fig. 1. Total curcuminoid content of *Zingiber* rhizomes percentages of curcumin weight per dry weight of sample.

Zingiber officinale exhibited the highest total phenolic content (7.70 mg GAE/g DW), and *Z. spectabile* exhibited the lowest (1.34 mg GAE/g DW) (Table 1). Positive correlations between antioxidant activity and total phenolic content were observed for both assay methods; the correlation coefficients were $r=0.794$, $p<0.01$, for the DPPH method and $r=0.832$, $p<0.01$, for the ABTS method (Table 2). The total phenolic content varied among the *Zingiber* species, and this result suggests that the phenolic compounds in the ethanol extracts were responsible for the antioxidant activity.

Extracts of *Z. montanum* rhizomes contained the highest curcuminoid content (2.633% w/w), and the lowest curcuminoid content (0.034% w/w) was observed for *Zingiber* 'Phlai-chompoo' (Figure 1; note that there was no significant difference in curcuminoid content between *Zingiber* 'Phlai-chompoo' and *Z. ottensii*). In addition, total curcuminoid content was only weakly correlated with total phenolic content ($r=0.444$; $p<0.05$), although the curcuminoid component is a natural phenolic compound. However, the correlation between total curcuminoid content and the DPPH results was positive ($r=0.534$, $p<0.01$); whereas no correlation between total curcuminoid content and the ABTS results was observed (Table 2).

6-Gingerol

6-Gingerol was quantified by HPLC with methanol: water (65:35, v/v) as the mobile phase. A standard curve was prepared with data from solutions with concentrations ranging from 20 to 100 mL/kg, and the correlation coefficient of 0.999 was indicative of good

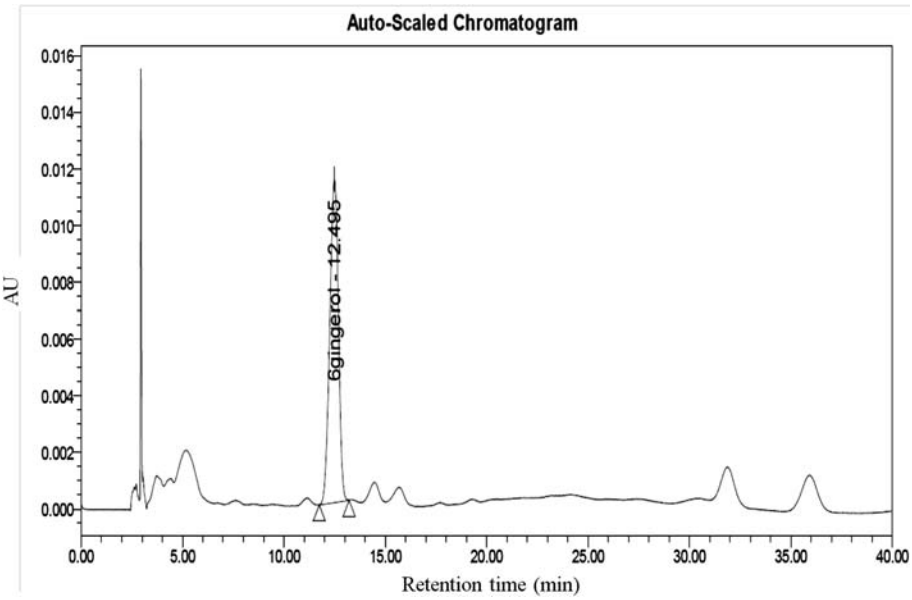

Fig. 2. HPLC chromatogram of a *Z. officinale* extract with UV detection at 281 nm. The peak with a retention time of 12.495 min corresponds to 6-gingerol.

Table 3. Volatile oil yields and terpinen-4-ol contents of some *Zingiber* species.

Species	Oil yield (%, v/w)	Terpinen-4-ol content (%)
Z. ottensii	0.26±0.07	5.5±0.21
Z. officinale	0.13±0.02	trace
Zingiber 'Phlai-chompoo'	0.10±0.04	4.36±0.34
Z. zerumbet	0.08±0.004	trace
Z. montanum	0.89±0.14	14.51±2.59
Z. rubens	0.33±0.05	trace

* Average value within each column is significantly different at the 95% level of confidence as indicated by Duncan's multiple range test ($p<0.05$).

linear dependence of peak area on concentration. 6-Gingerol was detected in only two species compose of : *Z. officinale* (Figure 2) and *Z. cornubracteatum*. The content of 6-gingerol was in the range of 255.35−291.78 mL/kg of *Z. officinale* rhizome extract, as measured for a 10-fold-diluted extract, and *Z. cornubracteatum*, 6-gingerol content was in the range of 63.032−68.418 mL/kg.

Essential oil yield and terpinen-4-ol content

We analyzed the essential oils from only five of the 10 *Zingiber* species, because the oil yields from some of the species were too low for gas chromatographic analysis. The oil yields from the five *Zingiber* species ranged from 0.08 to 0.89% (v/fresh weight), as indicated by peak area (Table 3). *Zingiber montanum* gave the highest yield, in the form of a yellowish oil. The terpinen-4-ol contents of the oils were determined by means of gas chromatography. Terpinen-4-ol (retention time approximately 12 min, Fig. 3) was detected in *Z. ottensii* (5.5% v/w), *Zingiber* 'Phlai-chompoo' (4.36% v/w), and *Z. montanum* (14.51% v/w).

Discussion

This is the first report of the antioxidant activity of extracts of *Z. rubens*, *Z. bisectum*, and *Z. barbatum* from Thailand. These data may serve as the basis for future studies of the uses of these species for medicinal purposes. The DPPH and ABTS assays of the *Zingiber* extracts had highly correlation IC_{50} values for all

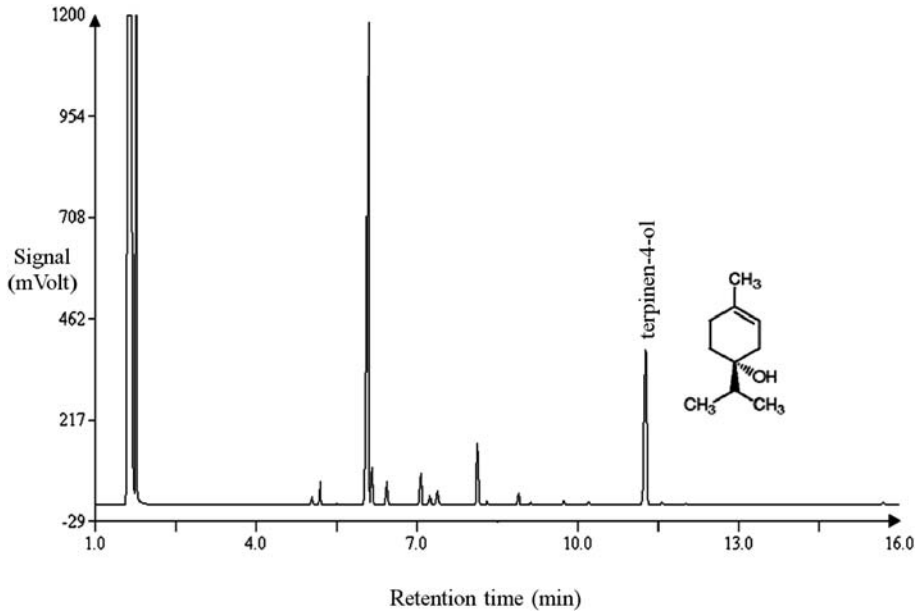

Fig. 3. GC chromatogram of essential oil of *Z. montanum*, showing the peak for terpinen-4-ol.

of the *Zingiber* species tested. This is in agreement with results obtain later (Tang and Liu, 2007). The antioxidant activity of the 10 species ranged from 4.26 to 60.80 mg/mL, and *Z. officinale* showed the highest activity at 4.26 mg/mL (DPPH method). This result is in agreement with previously published results indicating that *Z. officinale* has strong antioxidant activity (Ghasemzadeh *et al*., 2010). The values obtained from the DPPH assay were lower than those obtained from the ABTS assay, perhaps owing to the different mechanisms of the assays (Khomsug *et al*., 2010). Other factors that affect a compound's capacity to scavenge free radicals are the stereostructure of the radical and the solubility of the rhizome extracts in dissimilar testing systems also related to the ability of plant extracts to respond and scavenge various radical (Yu *et al*., 2002). The antioxidant activity of a plant extract also depends on the extraction solvent and procedure (Gan *et al*., 2010). For instance, a methanol extract of *Z. officinale* shows an IC_{50} of 65.1 μg/mL (Khalaf *et al*., 2008), whereas a carbon dioxide extract shows an IC_{50} of 0.64 μg/mL (Stoilova *et al*., 2007). The relationship between antioxidant activity and total phenolic content was found strong correlation. It has been reported that phenolic compounds are responsible for antioxidant activity due to their capacity by donating electrons or a hydrogen atom to free radical (Huda *et al*., 2009).

Among the most commonly use natural antioxidants for dietary supplement are the curcuminoids, which are the yellow substances in turmeric (*Curcuma longa*) as well as *Zingiber montanum* has been reported to contain curcuminoids (Masuda and Jitoe, 1994). In this study, we found the correlation between total curcuminoid content and total phenolic content was weak. It is likely that each species of *Zingiber* rhizome extracts have various phenolic compound except curcuminods for instance, anthocyanins, tannin, vanillic acid, caffeic acid. Several compounds show different response with folin-ciocalteu reagent may contribute to the total phenolic content. In addition, Ghasemzadeh *et al*. (2010) reported that antioxidant activities in various ginger could be the synergistic effect of more than two compounds that may be present in the plant.

6-Gingerol is the major bioactive compound in the rhizome of *Z. officinale*; it shows antioxidant activity as well as antibacterial and anti-inflammatory properties (Young *et al*., 2002). Using ethanol as an extraction solvent, we quantified the amounts of 6-gingerol in the *Zingiber* species by means of gas chromatography. We detected 6-gingerol in only two of the species: *Z. officinale* and *Z. cornubracteatum*. The 6-gingerol content may also decreases during postharvest storage, maturation, and thermal processing (Puengphian and Sirichote, 2008) due to gingerol become dehydrated in order to produced shogaol

(Bailey-Shaw *et al*., 2008). Our results indicate that *Z. cornubracteatum* is the new record species that contains 6-gingerol.

The quantity of essential oil from *Zingiber* rhizome varied in species and is considerably affected by factors including extraction parameters (e.g., extraction fibers, extraction time, and extraction temperature; Yang *et al*., 2009), location of harvest (Bua-in and Paisooksantivatana, 2009), rainfall amounts, rhizome age (Manochai *et al*., 2007), and genetic factors (Devarenne, 2006). We determined the terpinen-4-ol contents in the essential oils of six of the 10 *Zingiber* species and genetic factors (Devarenne, 2006). We determined the terpinen-4-ol contents in the essential oils of six of the 10 *Zingiber* species by means of gas chromatography (Table 3). Terpinen-4-ol is the main component of the essential oil of *Z. montanum* (Pithayanukul *et al*., 2007). Terpinen-4-ol, which has been shown to have anti-inflammatory activity (Hart *et al*., 2000), was found in tea tree (*Melaleuca alternifolia*; Brophy *et al*., 1989) and *Zanthoxylum piperitum* pericarp (Hieu *et al*., 2010). We detected terpinen-4-ol in *Zingiber* 'Phlai-chompoo' and *Z. ottensii*, which were other species not have reported which contain terpinen-4-ol.

Conclusion

Most of the *Zingiber* rhizome extracts showed high antioxidant activity and high total phenolic content. This is the first report that *Z. cornubracteatum* ethanol extract contains 6-gingerol as well as *Zingiber* 'Phlai-chompoo' and *Z. ottensii* contain terpinen-4-ol in the essential oils. Furthermore, our investigations of the antioxidant activity and bioactive chemical constituents of the *Zingiber* rhizome extracts is an alternative evidence to support the traditional usage of these medicinal plants.

Acknowledgements

The authors acknowledge financial support from the Graduate School of Kasetsart University, Bangkok, Thailand.

References

Bailey-Shaw, Y., Lawrence, A.D.W., Junor,G.A.O., Green, C. E., Hibbert, S.L., Salmon, C.A.N., Smith, A.M., 2008. Changes in the contents of oleoresin and pungent bioactive principle of Jamaican ginger (*Zingiber officinale* Roscoe.) during maturation. J. Agric. Food Chem. 56, 5564−5571.

Brophy, J. J., Davies, W. N., Southwell, A. I., Stiff, A. I., Williams, R.L., 1989. Gas chromatographic quality control for oil of *Melaleuca* terpinen-4-01 type (Australian Tea Tree). J. Agric. Food Chem. 37, 1330−1335.

Bua-in, S., Paisooksantivatana, Y., 2009. Essential oil and antioxidant activity of Cassumunar ginger (Zingiberaceae: *Zingiber montanum* (Koenig) Link ex Dietr.) collected from various parts of Thailand. Kasetsart J. (Nat Sci) 43, 467−475.

Chaichana, J., Niwatananun, W., Vejabhikul, S., Somna, S., Chansakaow, S., 2009. Volatile constituents and biological activities of *Gardenia jasmonoides*. J. Health Res. 23(3), 141−145.

Chan, E.W.C., Lim, Y.Y., Chew, Y.L., 2007. Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. Food Chemistry. 102, 1214−1222.

Devarenne, A., 2006. Olive oil yield factors affecting production: Newsletter of olive oil production and evaluation 2 (1), 1−4.

Gan, R.Y., Xu, X.R., Song, F.L., Kuang, L., Li, H.B., 2010. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. J. Med. Plant. Res. 4(22), 2438−2444.

Ghasemzadeh, A., Jaafar, Z. E. H., Rahmat, A., 2010. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). Molecules 15, 4324−4333.

Habsah, M., Amran, M., Mackeen, M.M., Lajis, N.H., Kikuzaki, H., Nakatani, N., Rahman, A.A., Ghafar, D., Ali, A.M., 2000. Screening of *Zingiber*aceae extracts for antimicrobial and antioxidant activities. J. Ethnopharmacol. 72, 403−410.

Hart, P.H., Brand, C., Carson, C.F., Riley, T.V., Prager, R.H., Finlay-Jones, J.J., 2000. Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. Inflamm. Res. 49, 619−626.

Hieu, T.T., Kim, I.I.S., Kwon, H.W., Ahn, Y.J., 2010. Enhanced repellency of binary mixtures of *Zanthoxylum piperitum* pericarp steam distillate or *Zanthoxylum armatum* seed oil constituents and *Calophyllum inophyllum* nut oil and their aerosols to *Stomoxys calcitrans*. Pest Manag. Sci. 66, 1191−1198.

Huda, F.N., Noriham, A., Norrakiah, A.S., Babji, A.S., 2009. Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr. J. Biotechnol. 8(3), 484−489.

Khalaf, A.N., Shakya, A.K., Othman, A.A., Agbar, Z.E., Farah, H., 2008. Antioxidant Activity of Some Common Plants. Turk. J. Biol. 32, 51−55.

Khomsug, P., Thongjaroenbuangam, W., Pakdeenarong, N., Suttajit, M., Chantiratikul, P., 2010. Antioxidative activities and phenolic content of extracts from Okra (*Abelmoschus esculentus* L.). J. Biol. Sci. Res. 5(4), 310−313.

Manochai, B., Paisooksantivatana, Y., Kim, M.J., Hong, J.H., 2007. Antioxidant activity and total volatile oil content of Cassumunar ginger (*Zingiber montanum* Roxb.) at various rhizome ages. Food Sci. Biotechnol. 16(2), 290−293.

Masuda, T., Jitoe, A., 1994. Antioxidative and anti-inflammatory compounds from tropical gingers: Isolation, structure determination, and activities of cassumunins A, B, and C, new complex curcuminoids from *Zingiber cassumunar*. J. Agric. Food Chem. 42, 1850−56.

Pithayanukul, P., Tubprasert, J., Wuthi-Udomlert, M., 2007. *In Vitro* Antimicrobial activity of *Zingiber cassumunar* (Plai) Oil and a 5% Plai Oil Gel. Phytother. Res. 21, 164−169.

Pothitirat, W., Gritsanapan W., 2009. Variation of bioactive components in *Curcuma longa* in Thailand. Current Science 91 (10), 1397−1400.

Puengphian, C., Sirichote, A., 2008. [6]-gingerol content and bioactive properties of ginger (Zongiber officinale Roscoe) extracts from supercritical CO_2 extraction. As. J. Food Ag-Ind. 1 (1), 29−36.

Schwertner, H. A., Deborah, C. R., 2007. High-performance liquid chromatographic analysis of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogoal in ginger-containing dietary supplements, spices, tea and beverages. J. Chromatograph. B 856, 41−47.

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P., Gargova, S., 2007. Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chem. 102, 764−770.

Sukatta, U., Rugthaworn, P., Punjee, P., Chidchenchey, S., Keeratinijakal, V., 2009. Chemical composition and physical properties of oil from Plai (*Zingiber cassumunar* Roxb.) obtained by hydro distillation and hexane extraction. Kasetsart J. (Nat. Sci.). 43, 212−217.

Tang, Y.Z., Liu, Z.Q., 2007. Free-radical-scavenging effect of carbazole derivatives on DPPH and ABTS Radicals. J. Am. Oil Chem. Soc. 84, 1095−1100.

Triboun, P. 2005. Biogeography and biodiversity of the genus *Zingiber* in Thailand. KKU. Res. J. (Graduate studies) 5 (1), 23−32.

Yang, Z., Yang, W., Peng, Q., He, Q., Feng, Y., Luo, S., Yu, Z., 2009. Volatile phytochemical composition of rhizome of ginger after extraction by headspace solid-phase microextraction, petrol ether extraction and steam distillation extraction. Bangladesh J. Pharmacol. 4, 136−143.

Young, H.Y., Chiang, C.T., Huang, Y.L., Pan, F.P., Chen, G.L., 2002. Analytical and stability studies of ginger preparations. J. Food Drug Anal. 10(3), 149−153.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., Qian, M., 2002. Free radical scavenging properties of wheat extracts. J. Agric. Food Chem. 50, 1619−1624.

Zheng, W., Shiow, W.Y., 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric Food Chem. 49(11), 5165−5170