Total Phenolic Content and Antioxidant Activity of Leaves and Rhizomes of Some Ginger Species in Peninsular Malaysia

E.W.C. CHAN, Y.Y. LIM AND T.Y. LIM

School of Arts and Sciences, Monash University Sunway Campus, Bandar Sunway, 46150 Petaling Jaya, Selangor, Malaysia

Abstract

The total phenolic content (TPC) and antioxidant activity (AOA) of leaves and rhizomes of five wild and six cultivated ginger species belonging to seven genera were compared. Altitudinal variation in leaf TPC and AOA of four species of *Etlingera* Giseke was also studied. TPC was measured using the Folin-Ciocalteu method. AOA was measured using the1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and expressed as ascorbic acid equivalent antioxidant capacity (AEAC). Of the 11 wild and cultivated species screened, leaves of *Etlingera* had the highest TPC and AEAC, which were seven to eight times higher than those of rhizomes. Eight species had significantly higher leaf TPC and/or AEAC than rhizomes. Leaves of highland populations of *Etlingera* species had higher values than those of lowland counterparts.

Introduction

Rhizomes of gingers (Zingiberaceae) are widely consumed as spice or condiments (Larsen *et al.*, 1999; Sirirugsa, 1999). Major commercially cultivated species in Peninsular Malaysia are *Zingiber officinale* Roscoe, *Curcuma longa* L. and *Alpinia galanga* (L.) Willd. As traditional medicine, rhizomes are consumed by women during ailment, illness and confinement (Larsen *et al.*, 1999; Ibrahim *et al.*, 2006). They are also taken as carminative for relieving flatulence.

Leaves of gingers have also been used for food flavouring. In Peninsular Malaysia, leaves of *Curcuma longa* are used to wrap fish before steaming or baking (Larsen *et al.*, 1999). The leaves of *Kaempferia galanga* L. and *Curcuma longa* are ingredients of spicy fish and meat dishes. Some tribal natives use leaves of *Elettariopsis slahmong* C.K. Lim to flavour cuisine of wild meat and fish (Lim, 2003). In Okinawa, Japan, leaves of *Alpinia*

zerumbet (Pers.) B.L. Burtt & R.M. Sm. are traditionally used to wrap rice cakes and are commercially sold as herbal tea.

Past studies on the antioxidant activity of wild and cultivated ginger species were confined to rhizomes (Jitoe *et al.*, 1992; Habsah *et al.*, 2000; Zaeoung *et al.*, 2005). Although their leaves have been used for food flavouring, hardly any research has been done on their antioxidant activity.

Antioxidants are molecules that are able to scavenge free radicals or prevent their generation. Phenolic compounds, in general, are able to scavenge free radicals or chelate metal ions to prevent generation of free radicals. Free radicals have been implicated in the pathogenesis of more than 50 diseases (Percival, 1996). Currently, there is much interest in herbs and spices as sources of antioxidants.

In our present study, the total phenolic content (TPC) and antioxidant activity (AOA) of leaves and rhizomes of five wild and six cultivated ginger species were compared. Altitudinal variation in leaf TPC and AOA of species of *Etlingera* was also studied.

Materials and Methods

Species studied

Five wild and six cultivated ginger species were screened for TPC and AOA. Wild species studied were *Etlingera maingayi* (Baker) R.M. Smith, *Alpinia malaccensis* var. *nobilis* (Ridl.) I.M. Turner, *Elettariopsis slahmong* C.K. Lim, *Zingiber spectabile* Griff. and *Scaphochlamys kunstleri* (Baker) Holttum. Cultivated species studied were *Etlingera elatior* (Jack) R.M. Smith, *Alpinia galanga* (L.) Willd., *Zingiber officinale* Roscoe, *Curcuma longa* L., *Curcuma zanthorrhiza* Roxb. and *Boesenbergia rotunda* (L.) Mansf. For each species, leaves and rhizomes of three plants were sampled.

For wild species, leaves and rhizomes of *Alpinia malaccensis* var. *nobilis, Zingiber spectabile* and *Scaphochlamys kunstleri* were sampled from plants growing at Forest Research Institute Malaysia (FRIM) in Selangor, those of *Elettariopsis slahmong* from Bukit Lagong in Selangor, and those of *Etlingera maingayi* from Janda Baik in Pahang. Voucher specimens of wild species studied were deposited at the FRIM herbarium (KEP).

For cultivated species, leaves and rhizomes of *Etlingera elatior* and *Curcuma longa* were sampled from plants found at FRIM, those of *Alpinia galanga* and *Zingiber officinale* from Bukit Maluri in Kepong, and those of *Curcuma zanthorrhiza* from Damansara Utama in Petaling Jaya. Plants of *Boesenbergia rotunda* were purchased from a nursery in Sungai Buluh in Selangor. Rhizomes of *Alpinia galanga, Zingiber officinale* and *Curcuma longa* purchased from the supermarket were also screened. Voucher

specimens of cultivated species studied were deposited at KEP.

TPC and AOA of leaves of lowland and highland populations of four *Etlingera* species were compared. The species studied were *Etlingera elatior* (Jack) R.M. Sm., *Etlingera fulgens* (Ridl.) C.K. Lim, *Etlingera littoralis* (J. König) Giseke and *Etlingera rubrostriata* (Holttum) C.K. Lim. Their identification was based on taxonomic descriptions and photographic illustrations of Lim (2000 & 2001) and Khaw (2001). Leaves of highland populations were sampled from Janda Baik and Genting Highlands in Pahang and from Ulu Gombak in Selangor, while those of lowland populations were sampled from FRIM. For each location, mature leaves were sampled from three different plants per species. Voucher specimens of *Etlingera* species studied were deposited at KEP. Altitude of locations, where the populations were sampled, was measured using a Casio altimeter (Model PRG-70-1VDR).

Extraction of samples

Fresh leaves and rhizomes (1 g) were powdered with liquid nitrogen in a mortar and extracted by methanol (50 ml), with continuous swirling for one hour at room temperature. Extracts were filtered and stored at -20°C for further use. Analysis of methanol extracts was done in triplicate for each species.

Total phenolic content

Total phenolic content (TPC) was measured using the Folin-Ciocalteu method (Kahkonen *et al.*, 1999). Samples (300 µl in triplicate) were introduced into test tubes followed by 1.5 ml of Folin-Ciocalteu's reagent (10 times dilution) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before absorption at 765 nm was measured. Total phenolic content was expressed as gallic acid equivalent (GAE) in mg/100 g material. The calibration equation for gallic acid was $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$).

Antioxidant activity

Antioxidant activity (AOA) was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay used by Leong and Shui (2002) and Miliauskas *et al.* (2004) with slight modification. Defined amounts of the extract were added to 3 ml of DPPH (3.9 mg/100 ml methanol). The DPPH solution was then allowed to stand for 30 min before absorbance was measured at 517 nm. All spectrophotometric measurements were made with methanol as blank. An appropriate dilution of the DPPH solution was used as negative control, i.e., methanol in place of the sample. Results

were expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg/100 g calculated from the IC_{50} (inhibitory concentration in mg/ml of plant material necessary to reduce the absorbance of DPPH by 50%) using the following formula:

$$AEAC\ (mg\ AA/100\ g) = IC_{50(ascorbate)}/IC_{50(sample)} \times 100{,}000$$

The IC_{50} of ascorbate used for calculation of AEAC was 0.00387 mg/ml.

Results and Discussion

Leaves and rhizomes of wild and cultivated species

Results from screening of five wild species showed that leaves of *Etlingera maingayi* had significantly higher TPC and AEAC than those of *Alpinia malaccensis* var. *nobilis, Elettariopsis slahmong, Zingiber spectabile* and *Scaphochlamys kunstleri* (Table 1). Rhizomes of *Alpinia malaccensis* var. *nobilis* had the highest values. Leaves of *Elettariopsis slahmong*, *Etlingera maingayi* and *Scaphochlamys kunstleri* showed significantly higher values at $P < 0.05$ than rhizomes. Leaves of other wild species were only marginally higher than rhizomes.

For six cultivated species screened, leaf and rhizome TPC and AEAC were highest in *Etlingera elatior* and *Curcuma longa*, respectively (Table 2). In five species, leaves had significantly higher TPC and/or AEAC at $P < 0.05$ than those of rhizomes. Exceptions were AEAC of *Alpinia galanga*, and TPC and AEAC of *Curcuma longa* where rhizomes showed higher values than leaves. The values of *Curcuma longa* were highly variable between rhizomes. For *Alpinia galanga*, *Curcuma longa* and *Zingiber officinale*, differences existed between collected rhizomes and those purchased from the supermarket. This implies that there is variability in TPC and AEAC between different cultivars.

In general, leaves of wild and cultivated *Etlingera* species contain the most antioxidants by having the highest TPC and AEAC. Values were 1110 mg GAE/100 g and 963 mg AA/100 g for *Etlingera maingayi* (Table 1), and 2390 mg GAE/100 g and 2280 mg AA/100 g for *Etlingera elatior* (Table 2) respectively. The outstanding leaf TPC and AEAC of both *Etlingera maingayi* and *Etlingera elatior* were seven to eight times higher than those of rhizomes.

There are very few studies comparing between the AOA of leaves and rhizomes of ginger species. Agnaniet *et al.* (2004) reported that essential oils extracted from leaves of *Aframomum giganteum* K. Schum. had higher AOA than rhizomes. Contrary to our results, Katsube *et al.* (2004) reported higher TPC and AOA in rhizomes of *Zingiber officinale* than leaves. It is

Table 1. Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves and rhizomes of five wild ginger species.

Species and location	Voucher number	Plant part	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
Alpinia malaccensis var. *nobilis* - FRIM	EC01	Leaves	744 ± 61 [a]	800 ± 62 [a]
		Rhizomes	564 ± 209 [a]	745 ± 342 [a]
Elettariopsis slahmong - Bukit Lagong	EC02	Leaves	346 ± 45 [a]	269 ± 67 [a]
		Rhizomes	219 ± 57 [b]	197 ± 76 [a]
Etlingera maingayi - Janda Baik	EC06	Leaves	1110 ± 93 [a]	963 ± 169 [a]
		Rhizomes	160 ± 52 [b]	122 ± 53 [b]
Scaphochlamys kunstleri - FRIM	EC08	Leaves	203 ± 21 [a]	171 ± 33 [a]
		Rhizomes	73 ± 3 [b]	14 ± 2 [b]
Zingiber spectabile - FRIM	EC09	Leaves	242 ± 7 [a]	121 ± 24 [a]
		Rhizomes	157 ± 100 [a]	124 ± 109 [a]

Values of TPC and AEAC are means ± SD (n = 3). For column of each species, values followed by the same letter (a-b) are not significantly different at $P < 0.05$ measured by the Tukey HSD test. ANOVA does not apply between species.

not known whether their comparisons were based on samples from same or different plants.

This is probably the first study where TPC and AOA of leaves and rhizomes of gingers have been systematically compared. For most of the species screened, TPC and/or AEAC of leaves were significantly higher than rhizomes.

Antioxidants are secondary metabolites, which form part of the plant's protective mechanism against free radicals. In Zingiberaceae, it is generally believed that antioxidants and other secondary metabolites are transported to the rhizomes where they are accumulated. This implies that rhizomes would have higher AOA than other plant parts. However, results of this study showed that this might not be the case.

Photosynthesis and respiration are physiological processes comprising several free radical intermediates. Exposure to sunlight can also increase the amount of free radicals. Leaves therefore require much more free radical scavengers than other plant parts. Similarly, Frankel and Berenbaum (1999) found that foliage of tropical forest plants produced more antioxidants when exposed to elevated light conditions. This observation may also apply to species of *Etlingera*, which have the highest leaf TPC and AEAC. *Etlingera* plants grow in gaps of disturbed forest and are continually

Table 2. Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves and rhizomes of six cultivated ginger species.

Species and location	Voucher number	Plant part	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
Alpinia galanga - Bukit Maluri	EC10	Leaves	366 ± 15 [a]	72 ± 4 [a]
		Rhizomes	150 ± 22 [b]	96 ± 6 [b]
Supermarket		Rhizomes	214 ± 20	168 ± 13
Boesenbergia rotunda - Sungai Buluh	EC11	Leaves	260 ± 8 [a]	157 ± 2 [a]
		Rhizomes	197 ± 50 [a]	89 ± 7 [b]
Curcuma longa - FRIM	EC12	Leaves	230 ± 19 [a]	113 ± 18 [a]
		Rhizomes	534 ± 205 [b]	390 ± 127 [b]
Supermarket		Rhizomes	386 ± 219	275 ± 183
Curcuma zanthorrhiza - Damansara Utama	EC13	Leaves	503 ± 57 [a]	287 ± 39 [a]
		Rhizomes	250 ± 52 [b]	134 ± 21 [b]
Etlingera elatior - FRIM	EC14	Leaves	2390 ± 329 [a]	2280 ± 778 [a]
		Rhizomes	326 ± 76 [b]	295 ± 96 [b]
Zingiber officinale - Bukit Maluri	EC15	Leaves	291 ± 18 [a]	96 ± 7 [a]
		Rhizomes	157 ± 18 [b]	84 ± 3 [a]
Supermarket		Rhizomes	184 ± 11	107 ± 9

Values of TPC and AEAC are means ± SD (n = 3). For column of each species, values followed by the same letter (a-b) are not significantly different at $P < 0.05$ measured by the Tukey HSD test. ANOVA does not apply between species.

exposed to direct sunlight (Poulsen, 2006). Furthermore, leaves of *Etlingera* are long lasting and do not abort. This may be due to an efficient protective mechanism delaying senescence in leaves, which is partly attributed to oxidative stress.

Altitudinal variation in leaves of Etlingera *species*

Leaves of all four species of *Etlingera* sampled from highland populations were found to have higher TPC and AEAC than lowland counterparts (Table 3). Leaves of *Etlingera rubrostriata, Etlingera elatior* and *Etlingera fulgens* showed significantly higher values at $P < 0.05$, while *Etlingera littoralis* was marginally higher. Highest TPC and AEAC were found in the leaves of highland populations of *Etlingera elatior* with values of 3550 mg GAE/100 g and 3750 mg AA/100 g, and of *Etlingera rubrostriata* with values of 3480 mg GAE/100 g and 3540 mg AA/100 g, respectively.

Table 3. Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves of four *Etlingera* species sampled from highland and lowland locations.

Species and location	Voucher number	Altitude (m asl)	Moisture content (%)	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
Etlingera elatior - Janda Baik FRIM	EC03	400 100	66.1 ± 2.0	3550 ± 304 [a] 2390 ± 329 [b]	3750 ± 555 [a] 2280 ± 778 [b]
Etlingera fulgens - Janda Baik FRIM	EC04	400 100	74.3 ± 0.1	2270 ± 31 [a] 1280 ± 143 [b]	2030 ± 126 [a] 845 ± 158 [b]
Etlingera littoralis - Genting Highlands FRIM	EC05	800 100	71.2 ± 0.8	2810 ± 243 [a] 2340 ± 386 [a]	2930 ± 220 [a] 2220 ± 913 [a]
Etlingera rubrostriata - Ulu Gombak FRIM	EC07	300 100	71.6 ± 2.8	3480 ± 390 [a] 2430 ± 316 [b]	3540 ± 401 [a] 2640 ± 508 [a]

Values of TPC and AEAC are means ± SD (n = 3). For columns of each species, values followed by the same letter (a-b) are not significantly different at $P < 0.05$ measured by the Tukey HSD test. ANOVA does not apply between species.

Higher altitudes seem to trigger an adaptive response in the species of *Etlingera*. The higher leaf TPC and AEAC of highland populations over lowland counterparts might be due to environmental factors such as higher UV-B radiation and lower air temperature. Plants protect themselves from oxidative damage due to UV exposure by producing antioxidative compounds (Larson, 1988). Enhanced UV-B radiation induces greater production of phenolic compounds (Bassman, 2004). Enzymes associated with the synthesis of phenolics are produced in greater quantities or show increased activity (Chalker-Scott & Scott, 2004). Phenylalanine ammonia lyase (PAL) of the phenylpropanoid pathway is up-regulated resulting in the accumulation of flavonoids and anthocyanins, which have free radical scavenging ability (Jansen *et al.*, 1998). Low temperatures have also been shown to enhance PAL synthesis and activity in a variety of plants, leading to an increase in flavonoids and other phenolics (Chalker-Scott & Scott, 2004).

Conclusion

Based on five wild and six cultivated ginger species belonging to seven genera, highest TPC and AOA were found in the leaves of *Etlingera*. For

most species screened, leaf TPC and/or AEAC were significantly higher than those of rhizomes. Rhizomes from different cultivars showed variability in TPC and AEAC. Leaves of highland populations of *Etlingera* had higher values than lowland counterparts. Of the four species studied, highest TPC and AEAC were found in the leaves of highland populations of *Etlingera elatior* and *Etlingera rubrostriata.*

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