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Antioxidant Activity and Total Volatile Oil Content of Cassumunar Ginger (*Zingiber montanum* Roxb.) at Various Rhizome Ages

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Abstract Cassumunar ginger (*Zingiber montanum* Roxb.) was grown in the experimental field at the Department of Horticulture, Kasetsart University, Thailand. The antioxidant activity and volatile oil content of rhizomes of varying age were measured. Antioxidant activity as determined using the DPPH (diphenyl-2-picrylhydrazyl) method differed significantly between samples of different ages. Antioxidant activity and rhizome age were positively correlated, with 22-month old rhizomes showing the highest radical scavenging activity (79.19%). Volatile oil was obtained by steam distillation of fresh rhizomes. The extraction yield of volatile oil was highest in 16-month old rhizomes (13.02 mL/kg). GC-FID data indicated the presence of three major compounds, sabinene, terpinen-4-ol, and (*E*)-1-(3',4'-dimethylphenyl) butadiene (DMPBD), however none of the major components were correlated with the age of rhizome.

Keywords: diphenyl-2-picrylhydrazyl (DPPH), radical scavenging activity, antioxidant, ethanol extract, volatile oil, Cassumunar ginger (*Zingiber montanum* Roxb.)

Introduction

Cassumunar ginger (*Zingiber montanum* Roxb.), known as *phlai* in Thailand, is a perennial rhizomous herb distributed primarily in India and tropical southeast Asia. It is commonly found in both damp, humid, shady environments as well as fully exposed at high elevations (1). All parts of the plant are aromatic with strong camphoraceous odors. *Phlai* is used in Thailand in many products including food coloring and additives, spices, traditional medicines, dyes, perfumes, and cosmetics. The rhizomes also exhibit antimicrobial (2), insecticidal (3), anti-inflammatory (4, 5), cytotoxic (6), anti-allergenic (7), and antioxidant properties (8, 9).

Chavalittumrong and Jirawattanapong (10) reported that turmeric cultivated in different regions contained different curcuminoids and volatile oil content, and that turmeric harvested at 5 months contained a higher concentration of volatile oil than turmeric harvested at other ages. Wisuthipitakkul *et al.* (11) reported that Cassumunar ginger harvested at 10 months was comprised of 1.10% volatile oil based on fresh weight, and that the major components found in the oil were sabinene (45.22-47.86%), terpinen-4-ol (20.51-21.43%), and (*E*)-1-(3',4'-dimethylphenyl) butadiene (DMPBD) (10.12-11.68%). Aengwanich (12) reported that the volatile oil content of 18-month old rhizomes was 3.49% based on fresh weight and that 24.23% of the oil was terpinen-4-ol.

Based on the above reports, we assumed that the age of rhizomes could be related to antioxidant activity and volatile oil content, therefore we conducted this study to investigate the relationships among rhizome ages, antioxidant activity, and volatile oil content.

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Materials and Methods

Plant materials A monospecific stand of *Zingiber montanum* Roxb. was grown under field conditions in the Department of Horticulture's experimental field at Kasetsart University, Kampangsean campus, Thailand. The experiment was conducted from June 2003 to June 2005. Twenty or 30 g or 2-3 buds of rhizome segment were used for propagation. Rhizomes were harvested at 2-month intervals, at which time they were subjected to chemical analysis.

Experimental design A complete randomized block design (RCBD) was used in this experiment. Rhizomes were collected at an interval of 2 months from the age of 2 to 24 months. Three replicate blocks were taken. Rhizomes were washed carefully, separated from roots and chopped, then stored in plastic bags at -20°C until analyzed.

Ethanol extraction Frozen rhizomes from each treatment (50 g) were homogenized with 150 mL of 95% ethanol and allowed to stand at room temperature for 2 days to make ethanol extract, and the insoluble residual was treated again with the same procedure. The extracts were then stored in capped bottles at -20°C until the antioxidant activity assay was conducted.

DPPH radical scavenging activity Radical scavenging activity was assayed using the method described by Blois (13), with the following modifications. The reaction mixture contained 3 mL of 0.1 mM diphenyl-2-picrylhydrazyl (DPPH, in 95% ethanol) and 0.5 mL of *phlai* extract with a predetermined dilution. After allowing the mixture to stand at room temperature for 20 min protected from light, the absorbance was recorded at 517 nm. Deionized water or 95% aqueous ethanol was used as a control. The scavenging activity of DPPH radicals (%) was calculated using the following equation:

DPPH radical scavenging activity (%)
$= [(A_{517\ blank} - A_{517\ sample}) / A_{517\ blank}] \times 100$

Extraction of the volatile oil Steam distillation was used to extract volatile oil from rhizomes. One hundred g of chopped rhizomes were homogenized and placed into a 1-L round bottom flask containing 500 mL of distilled water, which was then boiled, and the steam passed through a condenser into a reservoir flask. Volatile oil was separated from water by collecting upper phase. The oil was then stored in a 5 mL glass bottle at -5°C until subsequent analysis.

Gas chromatography analysis Quantitative analysis was conducted using gas chromatography with a flame ionization detector (model 8000 series; Fisons Instrument, Milano, Italy). The separation was performed on a fused silica capillary DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Two μL of each extract was injected into the column. The inlet temperature was set to 230°C and the oven temperature was programmed to change from 50 to 220°C at a rate of 4°C/min, resulting in an analysis time of 42.5 min per injection. Helium gas (99.995%) was used as the carrier gas at a flow rate of 1.2 mL/min and split ratio of 1/10.

Statistical analyses All results are presented as mean values. Mean differences between treatments were examined by one-way ANOVA followed by Duncan's new multiple range test (DMRT). Correlation between age and major components was conducted using the Pearson product moment correlation coefficient. Sirichae statistics 6.00 and SPSS 13.0 for windows were used for the ANOVA and correlations, respectively.

Results and Discussion

Fresh weight of rhizome yield The production of rhizome based on fresh weight, shown as kg per clump, was significantly different among rhizome ages (Table 1). Fresh weight of 24-month old rhizomes (2.20 kg/clump) was the heaviest among the samples taken, whereas that of 4-month rhizomes was the lightest (0.26 kg/clump), with fresh weight tending to increase as *phlai* aged. At 22 months fresh weight abruptly decreased, then returned to the normal trend at 24 months, however a severe drought in Thailand occurred during month 22, which may be responsible for the weight loss.

Antioxidant activity DPPH radicals are widely used to investigate the scavenging activities of many compounds (14-16). When DPPH radicals are scavenged the color of the reaction mixture changes from purple to yellow and absorbance at 517 nm decreases (13).

The results shown in Table 1 indicate that rhizome antioxidant activity significantly differs between varying ages of rhizome and that it increases linearly with age. The highest antioxidant activity (79.1%) was found in rhizomes from 22-month old *phlai*, while 4-month old rhizomes had the lowest activity (38.40%). These results are similar to those found by Shi *et al.* (17), who studied the content of ginsenoside, an antioxidant compound, in the roots and root-hair of *Panax ginseng*, and found that it increased with age.

Determination of volatile oil The productivity of

Table 1. Means of fresh weight, antioxidant activity, and volatile oil content of rhizome from *Zingiber montanum* Roxb. at various ages[1)]

Age (month)	Fresh weight of rhizome (kg)	Antioxidant activity (%)	Volatile oil			
			Volume (mL/kg)	Percentage		
				Sabinene	Terpinen-4-ol	DMPBD
4	0.26^{d}±0.14	57.12^{ab}±9.24	5.17^{d}±1.02	26.14^{d}±14.61	14.40±0.18	33.20±10.67
6	0.45^{cd}±0.15	47.20^{b}±5.92	9.52^{bc}±1.51	57.22^{a}±5.70	6.88±1.97	19.52±5.49
8	0.38^{cd}±0.17	62.44^{ab}±6.61	6.32^{cd}±1.20	49.07^{ab}±3.72	17.25±1.04	13.23±7.36
10	0.45^{cd}±0.07	51.49^{b}±5.75	9.30^{bc}±1.73	48.06^{ab}±8.10	11.61±2.83	22.48±4.36
12	0.78^{bcd}±0.19	60.14^{ab}±2.27	8.29^{bcd}±1.30	42.14^{bc}±6.79	14.40±0.07	22.89±5.29
14	0.67^{cd}±0.25	58.46^{ab}±3.22	6.22^{cd}±2.50	32.36^{cd}±10.13	17.28±2.91	24.56±3.59
16	1.07^{bcd}±0.38	68.04^{ab}±14.89	13.02^{a}±1.11	41.30^{bc}±0.86	15.10±3.79	23.86±5.08
18	1.35^{abc}±0.39	57.30^{ab}±12.25	8.16^{bcd}±0.34	44.40^{abc}±5.24	18.79±5.13	16.91±1.77
20	1.75^{ab}±0.47	67.94^{ab}±10.38	9.48^{bc}±2.85	34.25^{cd}±2.56	17.46±12.66	30.78±15.29
22	0.97^{bcd}±0.26	79.19^{a}±8.14	11.18^{ab}±1.98	38.15^{bcd}±6.27	18.15±0.04	24.17±5.39
24	2.20^{a}±0.99	78.72^{a}±5.29	11.43^{ab}±1.40	49.69^{ab}±4.25	11.87±2.56	19.56±4.76
F-test[2)]	**	*	**	**	ns	ns
CV	41.71%	21.49%	22.95%	15.37%	32.17%	23.76%

[1)]Means with same superscript within a column are not significantly different based on one-way ANOVA and Duncan' new multiple range test ($p<0.01$). Data are means (±SD) of triplicate measurements.
[2)]*$p<0.05$; **$p<0.01$; ns, not significant.

volatile oil obtained reported in mL per kg based on fresh weight is reported in Table 1. Volatile oils obtained were pale amber in color. Rhizomes from 16-month old had the highest volatile oil yield (13.02 mL/kg), whereas 4-month old had the lowest (3.58 mL/kg). Aengwanich (12) reported that rhizomes harvested at 18 months contained 3.49% volatile oil while Wisuthipitakkul *et al.* (11) found that 10-month old rhizomes had 1.10% volatile oil, therefore by comparing the results of these reports to our findings we conclude that the age of the rhizome affects the volatile oil yield.

The main components of volatile oil, sabinene, terpinen-4-ol, and DMPBD, were analyzed by GC and reported as a percentage (Fig. 1, Table 1). Sabinene content significantly differed based on the ages of rhizomes. Six-month old rhizomes contained the highest percentage of sabinene (57.22%), whereas 4-month olds had the lowest (26.14%). Terpinen-4-ol and DMPBD were not significantly different between age groups.

Correlation The ages of rhizomes had a positive correlation with fresh weight, antioxidant activity, and volatile oil volume; in contrast, age was not correlated with percentage of sabinene, terpinen-4-ol, and DMPBD (Table 2). In terms of correlation between major components and ages, sabinene and DMPBD had negative correlations. Total volatile oil volume was not correlated with antioxidant activity (Table 3). Since the antioxidant activity assay uses the same volume of essential oil, it only indicates the concentration of antioxidant substances, which may not change significantly. The negative correlation between sabinene and DMPBD indicates that

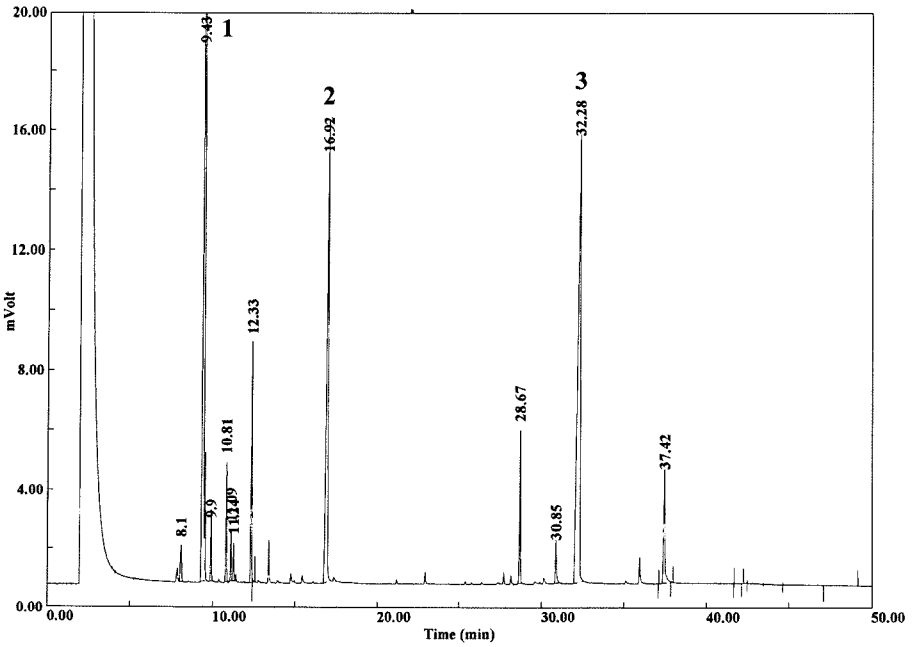

Fig. 1. GC analysis of volatile oil extracted from rhizomes of *Zingiber montanum* Roxb. Volatile oil was obtained from 22-month old rhizomes by steam distillation, then analyzed using a fused silica capillary DB-5 column. Peak 1, sabinene; peak 2, terpinen-4-ol; and peak 3, (*E*)-1-(3',4'-dimethylphenyl) butadiene (DMPBD).

Table 2. Correlation between major components and age of rhizome from *Zingiber montanum* Roxb.

	Fresh weight of rhizome	Antioxidant activity	Volatile oil volume	Sabinene	Terpinen-4-ol	DMPBD
Age[1)]	0.888**	0.801**	0.643*	−0.038	0.405	−0.038

[1)]*Correlation is significant at the 0.05 level (2 tailed), **correlation is significant at the 0.01 level (2 tailed).

Table 3. Correlation between major components of rhizome from *Zingiber montanum* Roxb.

	Antioxidant activity	Volatile oil volume	Sabinene	Terpinen-4-ol	DMPBD
Antioxidant activity	1				
Volatile oil volume	0.493	1			
Sabinene	0.200	0.435	1		
Terpinen-4-ol	0.421	−0.180	−0.528	1	
DMPBD	0.079	−0.183	−0.799**[1)]	0.077	1

[1)]**Correlation is significant at the 0.01 level (2 tailed).

these compounds might share the same metabolic pathway, and it is recommended that this relationship be investigated further.

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