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Essential Oil and Antioxidant Activity of Cassumunar Ginger (Zingiberaceae: *Zingiber montanum* (Koenig) Link ex Dietr.) Collected from Various Parts of Thailand

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ABSTRACT

Zingiber montanum (Koenig) Link ex Dietr. is widely found in tropical Asia and is used commonly in folk medicine for the treatment of various diseases. The objectives of this study were to investigate the variation in the essential-oil component and the antioxidant activity of rhizome extracts from *Z. montanum* collected from various regions of Thailand. Essential oils were obtained using a microwave extraction method (ME). Samples collected from western Thailand contained the highest amount of essential oil (11.07 mL/kg), the accession from Kanchanaburi province gave the highest volume (21 mL/kg), whereas those from eastern Thailand contained the lowest volume (4.95 mL/kg). The constituents of the essential-oil samples were determined by GC analysis. This technique identified 15 compounds in *Z. montanum* essential oils. The major constituents of the oil consisted of sabinene, terpinen-4-ol and DMPBD ((*E*)-1(3, 4-dimethylphenyl) butadiene). No significant differences in essential-oil components among the original sources of rhizomes were found. The antioxidant activities of the ethanolic extracts were screened by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Samples from different sources showed significantly different DPPH scavenging activities ($p < 0.05$). The antioxidant activities of the rhizome extract obtained from the north showed the highest activity (80.88%), followed by those from the east (76.47%), the south (72.51%), the northeast (67.38%), the west (66.66%) and the central region (57.63%).

Key words: *Zingiber montanum*, DPPH, essential oil, terpinen-4-ol, sabinene, DMPBD

INTRODUCTION

In recent decades, the phytochemical constituents of plants have received much attention due to their potential utilization in the nutraceutical and drug industries. Spices and herbs are a part of the daily food intake in many regions of the world. They have been used throughout history as natural sources of flavoring and preservatives. Spices possess antioxidant activity because they contain antioxidative phytochemicals such as phenolic compounds, which may reduce the risk of cancer, cardiovascular disease and many other diseases (Robbins and Bean, 2004; Shui and Leong, 2006), as well as reduce the antioxidative damage of cellular components. They play a key role as antioxidants due to the presence of phenolic groups (hydroxyl substituents next to their aromatic structures), which enable them to scavenge free radicals. The essential oils and their components

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in plants have been of great interest as they may be a rich source of antioxidants. Wang *et al*. (2008) inferred *Rosmarinus officinalis* L. essential oils indicating the strong antioxidant activity is the result of positive interactions between the components.

Cassumunar ginger (*Zingiber montanum* (Koenig) Link ex Dietr.) belongs to the Zingiberaceae family. It is probably native to India and is now widely cultivated in tropical Asia. It occurs widely as a home-garden plant in Southeast Asia. The rhizomes are used for food flavoring and are used medicinally in tropical Asia, primarily as a carminative and stimulant for the stomach, and against diarrhea and colic. In Thai traditional medicine, the rhizomes are consumed to relieve asthma, and muscle and joint pain. Dried rhizomes of *Z. montanum* yielded 0.5% essential oil (De Guzman and Siemonsma, 1999), whereas fresh rhizomes contained 3.49% (Aengwanich, 2002) or 13.02 mL/kg essential oil on steam distillation (Manochai *et al*., 2007). The main constituent, terpinen-4-ol and (*E*)-1(3, 4-dimethylphenyl) butadiene (DMPBD) (Figure1), has been found to be effective against a range of pathogenic bacteria and also has anti-inflammatory activity (Poonsukcharoen, 2004). Many pharmacological studies have demonstrated that the rhizome of *Z. montanum* provides antioxidant activity (Jeenapongsa *et al*., 2003; Jitoe *et al*., 1992; Vankar *et al*., 2006; Wongwattananukul *et al*., 2006), anti-inflammatory activities (Masuda and Jitoe, 1994) and anti-allergic activities (Tewtrakul and Subhadhirasakul, 2007) because it has been linked to the presence of curcuminoids. Leelapornpisid *et al*. (2008) reported the essential oil obtained from *Z. montanum* exhibits a high antioxidant activity with an IC_{50} of 1.0599mg/mL. New, complex curcuminoids, cassumunins A, B, C, have been isolated from rhizomes of *Z. montanum* and they possess stronger antioxidant activity than that of curcumin (Masuda and Jitoes, 1994). Phenylbutenoids are typical non-polar substances in the rhizome extracts of *Z. montanum* and recent studies have reported that some phenylbutenoids such as DMPBD provided anti-inflammatory activities (Jeenapongsa *et al*., 2003) as well as exhibitive insecticidal activity in bioassays with brown dog ticks (*Rhipicephalus sanguineus*) (Phonsena *et al*., 2006).

However, currently, there is little information about the antioxidant activity and essential-oil content in *Z. montanum* rhizomes from different sources in Thailand. The aim of this study was to investigate the variation in the essential-oil content and the antioxidant activity of rhizomes from *Z. montanum* collected from various regions throughout Thailand.

MATERIALS AND METHODS

Plant materials

The rhizomes of *Z. montanum* used in this study came from 35 accessions collected

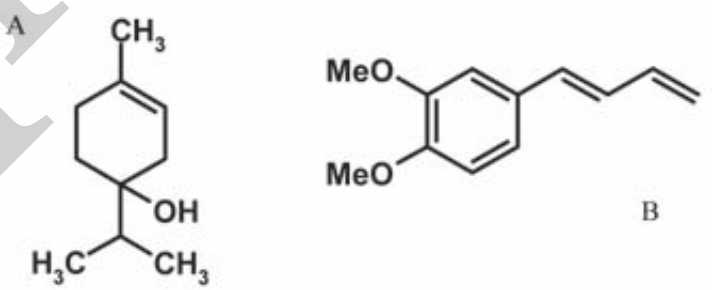

Figure 1 Chemical structure of (A) terpinen-4-ol; and (B) (*E*)-1-(3,4-dimethoxyphenyl) butadiene: DMPBD.

Table 1 Collection locations of *Z. montanum* rhizome accessions from various parts of Thailand.

Region	Accession number	Origin of collectionDistrict, Province
Northeastern	SB 2	Bung Khla, Nong Khai
	SB81	Hua Ta Phan, Amnat Charoen
	SB82	Muang, Amnat Charoen
	SB83	Chanuman, Amnat Charoen
	SB84	Senangkanikom, Amnat Charoen
	SB90	Nong Sung, Mukdahan
	SB91	Nikhom Kham Soi, Mukdahan
	SB92	Khamcha-I, Mukdahan
	SB93	Dong Luang, Mukdahan
	SB94	Nong Sung, Mukdahan
	SB95	Nikhom Kham Soi, Mukdahan
	SB96	Nikhom Kham Soi, Mukdahan
	SB130	Pak Chong, Nakhon Ratchasima
Central	SB3	Muang, Nakhon Sawan
	SB4	Muang, Chai Nat
	SB120	Kampheng Sean, Nakhon Pathom
	SB141	Sa Boat, Lopburi
Northern	SB5	Chiang Dao, Chiang MaiMuang
	SB111	Mae Hong Son
Eastern	SB22	Sa KaeoTha Mai
	SB59	Chanthaburi
Southern	SB31	Chana, Songkhla
	SB32	Nathawi, Songkhla
	SB63	Kanchanadit, Surat Thani
	SB66	Plai Phraya, Krabi
	SB71	Phanom, Surat Thani
	SB73	Yarang, Pattani
	SB74	Huai Yod, Trang
	SB78	Nathawi, Sonhkhla
	SB80	Chana, Songkhla
	SB97	Bang Khum, Songkhla
	SB133	TakBai, Narathiwat
Western	SB58	Srisawatt, Kanchanaburi
	SB61	Pak Tho, Ratchaburi
	SB129	Srisawatt, Kanchanaburi

throughout Thailand (Table 1). These rhizomes were grown at Kasetsart University, Bangkhen Campus, Bangkok. The mature rhizomes were harvested eight months after planting. Whole-rhizome samples were stored in sealed plastic bags at -20°C until use.

Reagents

Terpinene-4-ol and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich. Commercial grade ethanol (95%) used for all solutions and dilutions was purchased from Delta company and was redistilled before

use. High purity ethanol used for diluting the essential oil for GC analysis was purchased from Duksan Pure Chemical Co. Ltd.

Ethanol extraction

One gram of frozen rhizome was ground with liquid nitrogen using a mortar and pestle. Then, 5 mL of 95% ethanol solution was added and homogenized. The homogenate was collected and centrifuged at 6000 rpm for 10 min, or until a clear supernatant was obtained. Finally, the supernatant was decanted from the centrifuge tube and kept in a capped bottle at -20°C until further use for antioxidant activity assay.

DPPH radical scavenging activity

The following procedure was modified from that described by Blois (1958). A stock solution of the sample (200mg/mL) was diluted to make a dilution series of 1x, 2x, 3x and 4x. Each concentration was tested in triplicate. Proper dilution was done if the absorbance value measured was in the range of the *Z. montanum* standard curve. A portion of the dilution series of cassumunar ginger extract (500 µl) was mixed with 3 mL of 0.1 mM 1,1- diphenyl-2-picrylhydrazyl (DPPH, in 95% ethanol) and allowed to stand at room temperature for 20 min under light protection. The absorbance (A) was then measured at 517 nm. Ethanol (95%) was used as a control. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The results were expressed as a percentage of inhibition (Equation1):

$$\% \text{ Inhibition} = [(A_{517} \text{ control} - A_{517} \text{sample}) / A_{517} \text{ control}] \times 100\% \quad (1)$$

The proper dilution of *Z. montanum* extract was 3x.This dilution was used to evaluate the antioxidant activity of *Z. montanum* with the same method as described above.

Extraction of essential oil

The essential oil was extracted by a Microwave Extraction (ME) method. One hundred grams of frozen rhizomes were chopped into small pieces, homogenized, added with 800 mL of distilled water and distilled in a microwave oven (power 800 watts, frequency 2450 MHz) for 60 min. Distilled water was used as the solvent because polar solvents were proven to absorb microwave energy more readily than non-polar solvents. The oil was stored in a light brown bottle with cap and placed in a refrigerator at -20°C to minimize exposure to oxygen, light and heat.

Gas chromatography analysis

The essential-oil constituents of all samples were analyzed using a GC -2010 gas chromatograph with a flame ionization detector (FID). The DB-5MS column [30 m, 0.25 mm (i.d.), 0.25 µm (film thickness), manufactured by J&W Scientific, Folsom, CA] was used for separation. Two microliters of each sample, dissolved in 100% ethanol (1:100 v/v), was injected into the column automatically. Initially, the column temperature was set at 50°C and then programmed to increase from 50 to 220°C at a rate of 4°C/min and kept at 250°C for 5 min. The inlet temperature was kept at 230°C. Split injection was conducted with a split ratio of 1/10 and high purity helium was used as a carrier gas at 1.2 mL/min flow-rate. The essential-oil components were identified by comparing their relative retention times with a terpinene-4-ol standard solution and with those of the literature data of Manochai (2007). All quantifications were carried out using a built-in data-handling program provided by the manufacturer of the gas chromatograph (GC-2010, Shimadzu Co., Kyoto, Japan). The composition was reported as a relative percentage of the total peak area.

Statistical analysis

This study used three replicates and mean results were calculated. Mean differences between

locations of rhizomes were examined with Duncan's new multiple range test (DMRT). Correlation between antioxidant activity and essential-oil content was performed with the Pearson product moment correlation coefficient.

RESULTS AND DISCUSSION

Chemical composition of essential oil

The microwave-extracted essential oil of *Z. montanum* was a pale amber color with an aromatic-spicy odor. The essential-oil yields are presented in Table 2 based on fresh weight (mL/kg). Rhizomes from the western part of Thailand gave the highest essential oil yield (11.07 mL/kg), whereas those from the east gave the lowest yield (4.95 mL/kg). Yields obtained from the northeastern (6.38 mL/kg), the southern (5.79 mL/kg) and the central parts (5.74 mL/kg) were similar to those of Manochai *et al*. (2007) who reported that for *Z. montanum* grown in Nakhon Pathom province (the central part of Thailand) the yield of essential oil from rhizomes harvested at 8 months was 6.32 mL/kg. The variation in essential-oil yield suggests that there is genetic diversity among accessions and it is possible to select suitable rhizome accessions for commercial planting.

The chemical components of *Z. montanum* essential oil from various regions are listed in Table 3 in the order of their elution on the DB-5 MS column. The table also includes their retention time and the average percentage composition. The retention times of all components in this study are slightly different from those reported by Manochai (2007). The main components determined in the essential oil of *Z. montanum* rhizomes were sabinene, terpinen-4-ol and DMPBD ((*E*)-1(3, 4-dimethylphenyl) butadiene) (Figure 2), with no significant differences in components of essential oil between locations.

DPPH free radical-scavenging activity

The DPPH method has been widely applied for estimating the antioxidant activity of various natural products in recent years (Molyneux, 2004). This method has the advantages of being a stable, easy and rapid way to study the antioxidant activity of food items (Brand-Williams *et al*., 1995; Villa~no *et al*., 2007), which act as free radical- scavengers or hydrogen donors *in vitro*. (Wu *et al*. 2007). When DPPH reacts with an antioxidant compound, the color changes from deep violet to light yellow and the absorbance at 517 nm on a UV/visible light spectrophotometer decreases (Blois, 1958). In this study, the DPPH absorption inhibition ranged from 57.63% for *Z. montanum* rhizomes from the central part to 80.88% for rhizomes from the northern part of

Table 2 Average antioxidant activity, essential oil yield and major components of Thai cassumunar ginger from each region.

Region	DPPH inhibition (%)	Essential oil			
		Volume (mL/kg)	Relative percentage		
			Sabinene	Terpinen-4-ol	DMPBD
Northeastern (n=13)	67.38^{ab} ± 14.44	6.38^{ab} ± 2.17	38.52 ± 2.20	19.98 ± 1.74	21.24 ± 1.50
Central (n=4)	57.63^{a} ± 27.60	5.74^{ab} ± 1.93	42.17 ± 4.44	17.76 ± 2.30	18.52 ± 2.49
Northern (n=2)	80.88^{b} ± 12.08	9.74^{bc} ± 6.43	33.99 ± 2.77	24.36 ± 4.90	21.49 ± 4.72
Eastern (n=2)	76.47^{b} ± 16.85	4.95^{a} ± 4.31	38.20 ± 9.99	11.50 ± 2.45	27.54 ± 5.24
Southern (n=11)	72.51^{ab}± 13.94	5.79^{ab} ± 2.59	36.25 ± 2.80	22.03 ± 2.83	20.40 ± 1.52
Western (n=3)	66.66^{ab}± 11.30	11.07^{c} ± 8.06	47.54 ± 11.82	15.54 ± 3.28	19.66 ± 8.21
F-test	*	*	ns	ns	ns
CV	23.85%	53.98%	30.82%	46.16%	38.87%

* Means within each column followed by the same superscript are not significantly different at the 95% level of confidence based on Duncan's New Multiple Range Test ($p<0.05$); ns, not significant.

Thailand. No significant difference was found among the rhizomes from the northeastern, southern and western areas, nor between the northern and eastern parts of Thailand (Table 2). These results are similar to those reported by Venskutonis *et al*. (2006), who studied the radical-scavenging activity and composition of raspberry leaves (*Rubus idaeus*) from different locations in Lithuania, and also found that all extracts were active over a wide range in DPPH reaction systems. The result from this study was similar to that detected in bran extracts of Platte wheat obtained from different growing locations in Colorado that showed a significant difference

Table 3 Percentage of essential oil constituents in fresh rhizomes of *Z. montanum* collected in Thailand*.

Components[A]	RI[B]	Relative percentage					
		Northeastern	Central	Northern	Eastern	Southern	Western
1. α-thujene	7.70	0.72 ± 0.04	0.89 ± 0.14	0.53 ± 0.53	tr	0.75 ± 0.03	0.39 ± 0.39
2. α-pinene	7.98	1.11 ± 0.05	1.18 ± 0.06	1.13 ± 0.17	0.78 ± 0.78	0.94 ± 0.04	1,37 ± 0.08
3. sabinene	9.27	38.52 ± 2.20	42.17 ± 4.44	33.99 ± 2.77	38.20 ± 9.99	36.25 ± 2.80	47.54 ±11.82
4. β-mycrene	9.45	2.23 ± 0.08	2.46 ± 0.13	2.20 ± 0.20	1.76 ± 0.46	2.12 ± 0.06	2.21 ± 0.55
5. α-terpinene	9.75	1.53 ± 0.05	1.70 ± 0.05	1.47 ± 0.09	1.31 ± 0.32	1.46 ± 0.04	1.52 ± 0.31
6. ρ-cymene	10.78	2.49 ± 0.19	2.88 ± 0.43	2.59 ± 0.51	1.32 ± 0.35	2.51 ± 0.23	2.01 ± 0.14
7. β-phellandrene	11.06	1.10 ± 0.12	1.15 ± 0.18	1.36 ± 0.37	tr	1.19 ± 0.23	tr
8. γ-terpinene	11.31	1.46 ± 0.15	1.15 ± 0.06	1.34 ± 0.36	1.55 ± 0.38	1.38 ± 0.20	0.85 ± 0.02
9. sabinene hydrate	12.28	5.22 ± 0.39	5.81 ± 0.86	5.55 ± 0.93	2.70 ± 0.55	5.39 ± 0.52	4.57 ± 0.80
10. terpinolene	13.30	1.11 ± 0.07	1.34 ± 0.12	1.12 ± 0.17	0.41 ± 0.41	1.89 ± 0.76	0.98 ± 0.20
11. terpinen-4-ol	16.94	19.98 ± 1.74	17.76 ± 2.30	24.36 ± 4.90	11.50 ± 2.45	22.03 ± 2.83	15.54 ± 3.28
12. terpinyl acetate	29.05	2.00 ± 0.57	1.72 ± 0.50	1.74 ± 0.84	2.93 ± 1.19	2.78 ± 0.81	1.62 ± 0.90
13. β-sesquiphellandrene	31.09	1.18 ± 0.41	1.03 ± 0.30	1.26 ± 0.19	1.45 ± 0.54	1.13 ± 0.23	1.48 ± 0.60
14. DMPBD	32.29	1.24 ± 1.50	18.52 ± 2.49	21.49 ± 4.72	27.54 ± 5.24	20.40 ± 1.52	19.66 ± 8.21
15. Unknown1	37.55	1.12 ± 0.42	1.19 ± 0.67	2.18 ± 0.94	1.34 ± 0.52	1.51 ± 0.62	1.99 ± 1.63

*Data represent average percentages of accessions from each location.
tr =Trace (<0.05%).
[A]Compounds are listed in the order of elution from a DB-5MS column; Identification based on retention index and pure reference chemical.
[B]Retention indices on the DB-5MS column.

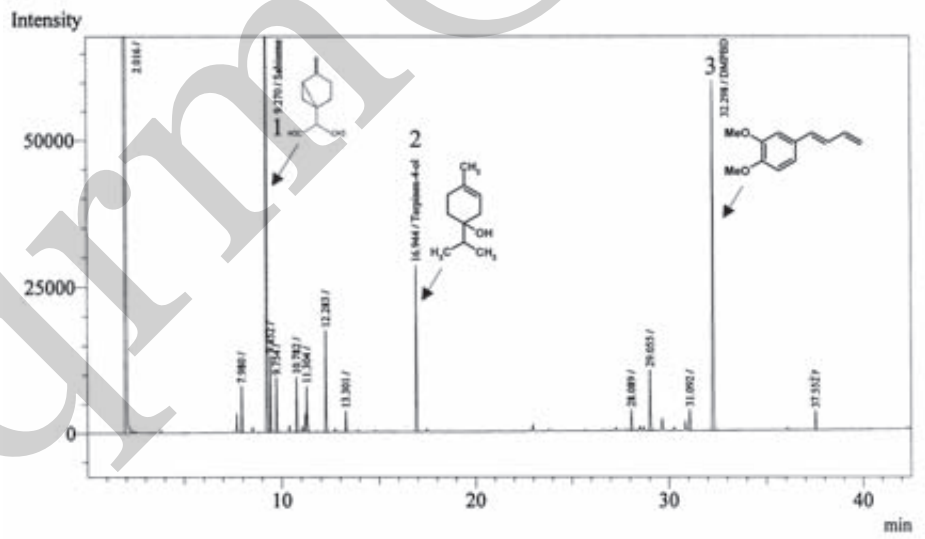

Figure 2 GC chromatogram for oil obtained using the microwave extraction method from accession number SB22 *Z. montanum* rhizome. Peak 1= sabinene; peak 2= terpinen-4-ol; and peak 3= DMPBD.

Table 4 Correlation between major components and antioxidant activity of *Zingiber montanum* rhizome collected in Thailand.

	DPPH inhibition	Essential oil volume	Sabinene	Terpinen-4-ol	DMPBD
DPPH inhibition	1				
Essential oil volume	0.00	1			
Sabinene	0.143	0.011	1		
Terpinen-4-ol	-0.329**	-0.060	-0.625**	1	
DMPBD	0.126	-0.143	-0.059	-0.249	1

** Correlation is significant at the 0.01 level (2-tailed).

($P < 0.05$) in their capacities to react with and quench DPPH radicals (Yu and Zhou, 2004). This study suggests that individual *Z. montanum* genotypes may respond to environmental changes differently for a particular antioxidant property. Interactions between genotype and environmental factors may also contribute to the antioxidant properties of *Z. montanum*.

Correlation

The results shown in Table 4 indicate that the percentage of DPPH inhibition of rhizomes is not correlated with essential-oil volume and percentage of DMPBD, while the percentage of DPPH inhibition has a negative correlation with the percentage of terpinen-4-ol, and the percentage of Terpinen-4-ol has a negative correlation with the percentage of sabinene.

CONCLUSION

Ethanol extracts of *Z. montanum* rhizomes from various parts of Thailand significantly differed in their scavenging activity against DPPH. The rhizomes of *Z. montanum* from different locations contained significantly different amounts of essential oil. The essential-oil yield of the rhizomes from the west was higher than that from the other locations**.** The main components of the essential oil were sabinene, terpinen-4-ol and DMPBD. However, there was no significant difference in the components of the essential oil. This study indicated that *Z. montanum* from the northern and eastern parts of Thailand may be considered as a good source of natural antioxidant to be used in medicinal and food products to promote human health and prevent diseases. There is also a high possibility of producing *Z. montanum* rhizomes rich in natural antioxidant by optimizing the selection of rhizomes for planting from good sources. More research is needed to: investigate the genetic diversity within *Z. montanum* and among species of *Zingiber,* to evaluate the biological activity of essential oils, and to identify and characterize the compounds in rhizome extracts.

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