

Chemical Composition and Physical Properties of Oil from Plai (*Zingiber cassumunar* Roxb.) Obtained by Hydro Distillation and Hexane Extraction

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ABSTRACT

This research studied essential oils of three native varieties of plai obtained by hexane extraction and hydro distillation and collected from Sa Kaco (S1), Chiang Mai (S2) and Prachuap Khiri Khan (S3) provinces in Thailand. The results showed that the yield of oil obtained from S1, S2 and S3 by hexane extraction was 0.983%, 0.900% and 0.857% (w/w), respectively, while by hydro distillation the yield was 1.137%, 1.262% and 1.373% (w/w), respectively. The chemical constituents of essential oils isolated by hexane extraction and hydro distillation were examined by gas chromatography-mass spectrometry (GC-MS). The results showed that essential oils in the plai rhizomes isolated by hexane extraction and by hydro distillation were rather similar in their major composition, but different in minor components. The plai oil obtained by hexane extraction contained mainly: sabinene (24.05-39.11%), γ -terpinene (6.68-7.74%), terpinen-4-ol (33.11-49.36%) and (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) (5.31-8.28%), whereas that obtained by hydro-distillation contained: sabinene (36.71-53.50%), γ -terpinene (5.27-7.25%), terpinen-4-ol (21.85-29.96%) and DMPBD (0.95-16.16%).

Key words: chemical composition, physical property, plai oil, *Zingiber cassumunar* Roxb., extraction

INTRODUCTION

Zingiber cassumunar Roxb., commonly known as plai, is widely used in folklore remedies as a single plant or as component of herbal recipes in Thailand and many Asian countries for the treatments of conditions, such as: inflammation, sprains and strains, rheumatism, muscular pain, wounds, and asthma, cough and respiratory problems, and as a mosquito repellent, a carminative, a mild laxative and an antidiysenteric

agent, (Wanauppathamkul, 2003; Pithayanukul *et al.*, 2007). The essential plai oil, distilled from rhizome extracts, has proven to be extremely useful for human health. Plai oil has a pale amber color, cool scent and a green peppery odor with a touch of a bite. Plai oil has anti-inflammatory effect and exhibits antimicrobial activity (Wasuwat *et al.*, 1989; Giwanon *et al.*, 2000; Pithayanukul *et al.*, 2007; Tripathi *et al.*, 2008), Active chemicals of plai oil have been identified as sabinene (25-45%) γ -terpinene (5-10%), α -terpinene (2-5%), terpinen-

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4-ol (25-45%), and (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) (1-10%) (Wanauppathamkul, 2003). DMPBD, as a pure compound isolated from plai, has shown anti-inflammatory activity (Ozaki *et al.*, 1991; Jeenapongsa *et al.*, 2003). Terpinen-4-ol and sabinene were found as the major constituents of plai oil and their antimicrobial activities were reported in comparison with commercial terpinen-4-ol (Wasuwat *et al.*, 1989; Giwanon *et al.*, 2000). The rhizome oil of plai was found to exhibit high activity against dermatophytes and yeasts (Pithayanukul *et al.*, 2007). Plai is an important medicinal plant in Thailand and there are many regions where plai is cultivated. The present study aimed to investigate the chemical components in rhizome oil obtained from *Z. cassumunar* by hydro distillation and solvent extraction and to evaluate the physical properties of the plai oil from three native varieties in Thailand.

MATERIALS AND METHODS

Plant materials

Samples of *Zingiber cassumunar* Roxb. were collected by the National Center for Agricultural Biotechnology from rhizomes of native varieties of plai: S1 from Sa Kaco province, S2 from Chiang Mai province and S3 from Prachuap Khiri Khan province. All samples (S1, S2 and S3) were cultivated at the National Corn and Sorghum Research Center, Nakhon Ratchasima province, Thailand for 33 months and then the rhizomes of plai were harvested, cleaned and chopped into small pieces for the experiment.

Preparation of the hexane extract

The fresh rhizomes were chopped into small pieces and 200 g of each sample was extracted with hexane (analytical grade) thrice, at room temperature using the maceration method for three d. The filtrates were pooled and concentrated by rotary evaporator at 40°C. The

crude oils obtained were stored at 4°C until further use. All plai samples were extracted in triplicate.

Isolation of essential oils

Two hundred grams of each freshly chopped rhizome sample were distilled in triplicate using water distillation with a Clevenger-type apparatus for about 6 h. The essential oils were separated from the aqueous solution, dried over anhydrous Na₂SO₄, then transferred into an amber glass bottle and stored at 4°C until use.

Analysis of physicochemical parameters

Specific gravity was measured with a density meter (DA-100M, METTLER TOLEDO Switzerland). Optical rotation was recorded using a polarimeter (POLAX-2L, ATAGO, Japan). The refractive index was determined using a digital refractometer (RX-5000a, ATAGO, Japan) at 20°C.

Analysis of volatile compounds by GC/MS

Oil sample analysis was performed using a Shimadzu QP5050A gas chromatography interfaced to a mass spectrometer (GC-MS) employing the following conditions: column DB5 (60 cm × 0.25 mm i.d., composed of 5% phenylmethylpolysiloxane), connected to an ion trap detector operating in electron impact mode at 70 eV; helium as the carrier gas, flow rate of 1.2 ml min⁻¹; split mode, ratio of 1:7; injection volume of 1 µl (in C₅H₁₂); injector temperature 250°C; detector temperature 250°C. The oven temperature was programmed from 60°C (isothermal for 3 min), with an increase of 1°C min⁻¹, to 80°C and then an increase of 3°C min⁻¹ to 120°C, ending with 4°C min⁻¹ to 220°C. The compounds were identified by matching their mass spectral fragmentation patterns with those stored in the spectrometer database, using the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998).

RESULTS AND DISCUSSION

The yields of oil obtained from fresh plai rhizome samples of S1, S2, and S3 by hydro distillation were 1.137%, 1.262% and 1.373% (w/w), respectively, while the yield from hexane extraction was 0.983%, 0.900% and 0.857% (w/w), respectively (Table 1). The results showed that yields were significantly different among the six treatments. The higher yield from hydro distillation than solvent extraction suggested that conventional solvent extraction is likely to involve losses of more volatile compounds during the removal of the solvent (Presti *et al.*, 2005). There was no significant difference among the oil yield of samples from S1, S2 and S3 obtained by the hydro distillation or hexane extraction method. The water distillation of the rhizome of plai yielded clear, low viscosity liquids and yellowish essential oils, while oils from the hexane extraction were yellow-orange in color and had high viscosity. This was due to the ability of hydro distillation to extract only the volatile compounds, whereas solvent

extraction of plant materials may produce oleoresin, which contains not only the volatile compounds but also waxes, color and pigments (Ibrahim, 1997). The physicochemical parameters of plai oil obtained by hydro distillation and hexane extraction are shown in Table 1. Optical rotations of rhizome plai oil at 20°C were found to be in the range 27.5500° to -21.5333° for hydro distillation and -11.7833° to -9.6833° for the hexane extraction method. The difference between these values indicated a change in chemical composition. Refractive indices of oil were found to be in the range 1.5386 to 1.5169 and 1.5477 to 1.5567 for hydro distillation and hexane extraction, respectively. The range of the refractive index values indicated that the components were neither degraded nor polymerized and remained as mono- or sesqui terpenoids and their derivatives. (Behera *et al.*, 2004) The values of the oil specific gravity ranged from 0.9360 g/cm³ to 0.9743 g/cm³ and 1.0243 g/cm³ to 1.0450 g/cm³ for hydro distillation and hexane extraction, respectively. This result can be explained by the fact that oil obtained from

Table 1 Volatile oil yields obtained by hexane extraction and hydro distillation and their physicochemical properties.

Method	Sample	Yield (%w/w)	Specific gravity (g/cm ³)	Refractive index	Optical rotation (°)
Hexane extraction	S1	0.983 ^{bc}	1.0450 ^a	1.5567 ^a	-11.7833 ^c
	(Sa Kaeo)	(0.130)	(0.0000)	(0.0005)	(0.3819)
	S2	0.900 ^{bc}	1.0243 ^b	1.5506 ^b	-10.3833 ^b
	(Chiang Mai)	(0.108)	(0.0006)	(0.0014)	(0.2082)
	S3	0.857 ^c	1.0253 ^b	1.5477 ^b	-9.6833 ^a
Hydro distillation	(Prachuap Khiri Khan)	(0.034)	(0.0006)	(0.0007)	(0.6171)
	S1	1.137 ^{ab}	0.9743 ^c	1.5386 ^c	-21.5333 ^d
	(Sa Kaeo)	(0.149)	(0.0006)	(0.0006)	(0.1041)
	S2	1.262 ^a	0.9463 ^d	1.5226 ^d	-25.1167 ^e
	(Chiang Mai)	(0.058)	(0.0006)	(0.0036)	(0.2021)
	S3	1.373 ^a	0.9360 ^e	1.5169 ^e	-27.5500 ^f
	(Prachuap Khiri Khan)	(0.108)	(0.0010)	(0.0002)	(0.1323)

Notes: Data are means of three replicates, the values in brackets are standard deviations.

Mean values followed by different superscripts within a column are significantly different using Duncan's multiple range test (P < 0.05).

hexane extraction contained oxygenated compounds, pigments and colors, which have higher molecular weight (Gamarra *et al.*, 2006) resulting in a higher specific gravity of oil obtain from hexane extraction than hydro distillation.

The GC-MS chromatograms of the hexane extracted and hydro distilled oils revealed the presence of several components (Table 2) that were identified and compared with the fragmentation patterns using the mass spectral data base of the gas chromatograph computer (NIST-MS, 1998). Twenty-two phytochemicals were identified as constituents of the essential oils of *Zingiber cassumunar* Roxb. These components and their retention times are summarized in Table 2 and Figures A-F. The main constituents of the essential oils from the fresh rhizome part of plai obtained from hexane extraction were: sabinene

(24.05-39.11%), γ -terpinene (6.68-7.74%), terpinen-4-ol (33.11-49.36%) and DMPBD (5.31-8.28%), whereas the main compounds of oil from hydro distillation were: sabinene (36.71-53.50%) γ -terpinene (5.27-7.25%), terpinen-4-ol (21.85-29.96%), DMPBD (0.95-16.16%). The main composition of plai oil from samples of S1, S2 and S3 obtained from hydro distillation was sabinene, while terpinen-4-ol was the main compound of plai oil from samples of S2 and S3 obtained from hexane extraction. The results suggested that the essential oils of the plai rhizome isolated either by hexane extraction or by hydro distillation were rather similar in their major composition, but different in minor components. Moreover, it was found that hexane extraction yielded a higher amount of oxygenated compounds than hydro distillation, except in the oil from

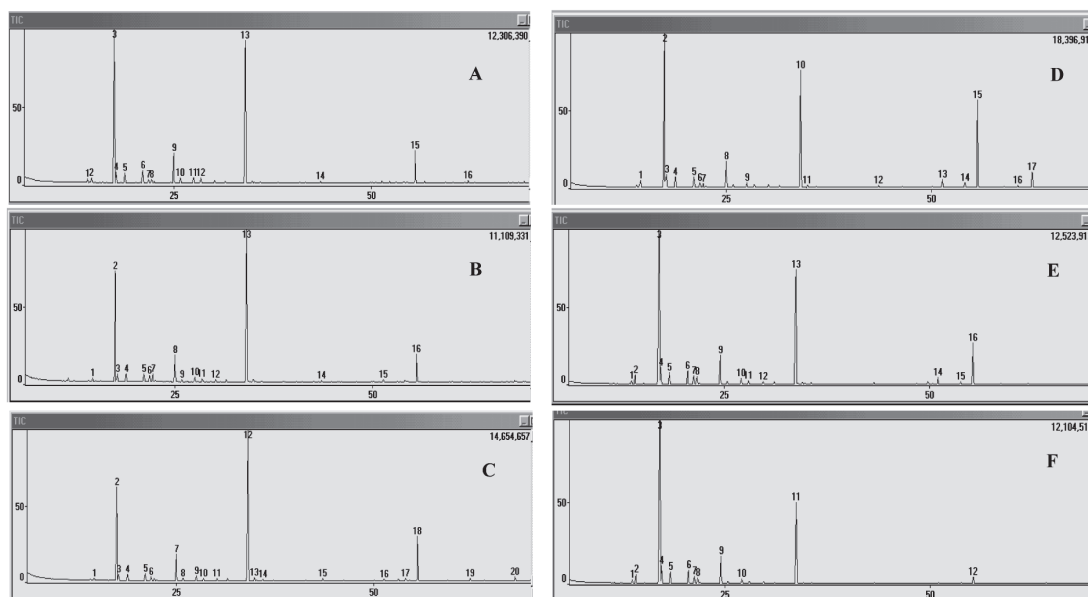


Figure A-F Gas chromatograms of the oil of *Zingiber cassumunar* Roxb. On DB-5 capillary column.

A = Plai oil obtained from Sample S1 using the hexane extraction method.

B = Plai oil obtained from Sample S2 using the hexane extraction method.

C = Plai oil obtained from Sample S3 using the hexane extraction method.

D = Plai oil obtained from Sample S1 using the hydro distillation method.

E = Plai oil obtained from Sample S2 using the hydro distillation method.

F = Plai oil obtained from Sample S3 using the hydro distillation method.

sample S1. Oxygenated compounds contribute to the fragrance of the essential oil and are highly odoriferous (Bousbia *et al.*, 2009). Hydro distillation could be a good method for the isolation of essential oils from plai rhizomes because of high yield, low production cost and good characteristics of the oil.

CONCLUSION

The yield of oil obtained from fresh plai

rhizome samples S1, S2, and S3 by hydro distillation was 1.137%, 1.262% and 1.373% (w/w), respectively and by hexane extraction was 0.983%, 0.900% and 0.857% (w/w), respectively. The results showed that hydro distillation gave higher oil yields than solvent extraction. The main constituents of the essential oils from the fresh rhizome part of plai obtain by hexane extraction were sabinene (24.05-39.11%), γ -terpinene (6.68-7.74%), terpinen-4-ol (33.11-49.36%) and DMPBD (5.31-8.28%), whereas those obtained

Table 2 Percentage of constituents in plai essential oils as identified by GC-MS analysis.

RT	Possible compound ^a	% peak area					
		Hexane extracted plai oil			Distilled plai oil		
		S1	S2	S3	S1	S2	S3
14.047	α -thujene	0.70	-	-	-	0.44	0.60
14.534	α -pinene	0.58	0.69	0.34	1.15	1.53	2.00
17.423	sabinene	39.11	30.39	24.05	36.71	41.39	53.50
17.678	β -pinene	2.43	1.96	1.36	2.25	2.77	3.78
18.772	β -myrcene	2.13	2.03	1.63	1.83	2.15	2.65
21.031	α -terpinene	3.18	2.16	1.83	2.04	2.60	3.39
21.761	p-cymene	0.87	1.66	0.77	0.74	1.63	1.71
22.157	β -phellandrene	0.84	2.34	-	0.74	1.69	1.24
24.986	γ -terpinene	7.54	7.74	6.68	5.27	6.31	7.25
25.829	(Z)-sabinene hydrate	1.25	0.67	0.61	-	-	-
27.495	terpinolene	1.11	0.87	1.00	0.84	0.94	1.09
28.393	(E)-sabinene hydrate	1.07	0.56	0.54	-	0.49	-
30.137	(Z)-p-menth-2-en-1-ol	-	0.54	0.62	-	0.41	-
34.094	terpinen-4-ol	33.11	42.23	49.36	27.00	29.96	21.85
34.898	α -terpineol	-	-	0.76	0.29	-	-
35.958	myrtenol	-	-	0.27	-	-	-
43.570	β -terpinyl acetate	0.21	0.31	0.46	0.29	-	-
51.353	β -sesquiphellandrene	-	0.53	0.27	1.03	0.83	-
54.114	Unknown ¹	-	-	0.26	0.54	0.32	-
55.605	DMPBD	5.52	5.31	8.28	16.16	6.54	0.95
62.309	4-(2,4,5-trimethoxyphenyl)but-1,3-diene	0.34	-	0.28	0.21	-	-
67.999	Unknown ²	-	-	0.64	1.91	-	-
Total oxygenated compounds		40.25	48.95	60.57	43.66	37.40	22.80
Total non-oxygenated compounds		59.75	51.05	38.53	52.60	62.28	77.20
Unknown		-	-	0.90	2.54	0.32	-
Total		100	100	100	100	100	100

Notes: RT = Retention time; ^a = Compounds are listed in order of their elution from a DB-5 column.

from hydro distillation were sabinene (36.71-53.50%), γ -terpinene (5.27-7.25%), terpinen-4-ol (21.85-29.96%) and DMPBD (0.95-16.16%).

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