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Full Length Research Paper

Comparative study of essential oil composition of leaves and rhizomes of *Alpinia conchigera* Griff. at different post- harvest drying periods

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Essential oils from the leaves and rhizomes of *Alpinia conchigera* Griff. dried for different times (0 (fresh), 1, 2, 3 and 7 days of drying, respectively) were isolated using hydrodistillation. The chemical composition of oils was analyzed by using GC and GC-MS. The identified components constituted 95.06, 94.51, 94.64 and 91.60% of the leaf oil and 47.71, 64.19, 49.96, 94.47 and 91.56% of the rhizome oil of fresh, 1, 2, 3 and 7 days drying samples, respectively. Thirty four components were identified, among which 31 had not been detected previously. The major constituents in fresh, one, two and three days dried leaves were cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl), while for leaves dried for seven days were 1, 6, 10-dodecatriene, 7 and 11-dimethyl-3-methylene. β-pinene was the major component for fresh and two days dried rhizomes, while 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) was the major constituent for one, three and seven days dried rhizomes. The post-harvest drying period had a positive effect on the oil yield of both leaf and rhizome. The highest oil yield was obtained from leaves dried for 7 days (0.300 v/w) and rhizomes dried for 3 days (0.162 v/w). This study suggests that the yield and content of essential oils from the leaves and rhizomes of *A. conchigera* could be increased by drying leaves and rhizomes for 3 and 7 days, respectively.

Key words: *Alpinia conchigera* Griff., essential oil, GC-MS, post-harvest drying, Zingiberaceae.

INTRODUCTION

Alpinia conchigera Griff. is a herb commonly found growing in damp, open spaces. It is 0.6 to 1.2 m tall, and is reported to produce the smallest flowers among the *Alpinia* species found in Peninsular Malaysia (Larsen et al., 1999). In Malaysia, *A. conchigera* is known as lengkuas getting, lengkuas ranting, lengkuas padang, lengkuas kecil or chengkenam (Burkill, 1966). The small rhizomes are used as food flavour as well as for treating rheumatism, arthritis and a variety of ailments in native medicine (Perry, 1980). Ibrahim et al. (2000) reported that, in the northern states of Peninsular Malaysia, the rhizome of *A. conchigera* is used as a condiment and occasionally in folk medicine along the east coast to treat fungal infections. In some states of Peninsular Malaysia, the rhizomes are consumed as a post-partum medicine and the young shoots are prepared into a vegetable dish (Ibrahim et al., 2009). The rhizomes of *A. conchigera* are used in Thai traditional medicine to relieve gastro-intestinal disorders and in the preparation of Thai food dishes (Athamaprasangsa et al., 1994). It was reported that the phenyl prepanoid derivatives, chavicol acetate and eugenol acetate are present in the fruit of *A. conchigera*, and have anti-inflammatory activity (Yu, 1988).

There have been a few studies on the chemical constitutes of *A. conchigera*. Yu (1988) reported the presence of nonacosane, β-sitosterol, 10-acetoxychavicol acetate and 10-acetoxyeugenol acetate in the fruit, the two phenylpropanoid derivatives showing anti-inflammatory activity. According to Athamaprasangsa et al. (1994), the fresh rhizomes of *A. conchigera* yielded 0.15% of an essential oil and they reported the presence

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of 12 components: chavicol acetate, cineol, terpinen-4-ol, β-pinene, α-pinene, ρ-cymene, α-terpineol, chavicol and four unidentified components. Sirat and Nordin (1995) reported 34 essential oil components from the rhizomes of *A. conchigera* from the southern region of Peninsular Malaysia, among which β-sesquiphellandrene (20.5%), β-bisabolene (12.1%) and 1,8-cineole (11.6%) were found to be the major components. Wong et al. (2005) stated that fresh rhizomes of *A. conchigera* yielded 0.14% of an essential oil and 50 compounds were identified including β-bisabolene, which was the most abundant component, β-sesquiphellandrene (1.9%), 1,8-cineole (15.3%) and β-caryophyllene (10.0%). Recently, Ibrahim et al. (2009) reported that the rhizomes had more oil (0.32% yield) than the leaves and pseudostems, which yielded (0.30%) and (0.04%) of oil, respectively. Ibrahim et al. (2009) found that the most abundant components in the leaf oil were *β*-bisabolene (15.3%), β-pinene (8.2%), β-sesquiphellandrene (7.6%), chavicol (7.5%) and β-elemene (6.0%), while in the pseudostem were β-bisabolene (19.9%), β-sesquiphellandrene (11.3%), β-caryophyllene (8.8%) and *β*-elemene and in the rhizome were 1, 8-cineole (17.9%), β-bisabolene (13.9%), *β*-sesquiphellandrene (6.8%) and β-elemene (4.0%).

The drying of medicinal plants is necessary for handling and preservation purposes, but drying protocols must be designed to maintain the concentrations of phyto-chemicals (Tanko et al., 2005). Drying of plant materials results in an increase of oil yields and accelerates distillation, by improving the heat transfer (Whish and Willams, 1998). Other advantages are the reduction of microbial growth and the inhibition of some biochemical reactions in the dried material (Baritaux et al., 1992; Combrinck et al., 2006). However, oil may be lost due to volatilization and mechanical damage to oil glands during harvesting and drying (Combrinck et al., 2006). To the best of our knowledge this is the first report on the influence of post-harvest drying periods on the oil yield and the chemical constituents of *A. conchigera*. Hence, the objectives of this study were to optimize the extraction of essential oil from *A. conchigera*, to determine which parts of this species produced the optimum oil yield, to identify the chemical constituents in the essential oils extracted from this species and to predict the optimum post-harvest drying period prior to distillation.

MATERIALS AND METHODS

Plant materials

Leaves and rhizomes of *A. conchigera* were collected from the Agricultural Conservatory Park, Biodiversity Unit, Institute of Bioscience, University Putra Malaysia (UPM). This species was identified by Shamsul Khamis, Biodiversity Unit, Institute of Bioscience. A voucher specimen (SK 1746/10) has been deposited in the herbarium of the Biodiversity Unit, Institute of Bioscience. The fresh plant materials were washed with water and sliced into small portions. Then, the plant materials were dried at room temperature with 76% relative humidity for 6 h (fresh), 1, 2, 3 and 7 days, respectively.

Extraction of essential oils

About 500 g of fresh leaves or 600 g of rhizomes, respectively, were cut into small pieces and submitted to hydrodistillation for 5 h using a Clevenger-type apparatus according to the standard method recommended by European Pharmacopoeia (1983). Extraction of dried material was carried out for each drying period with the same dry weight. The oils obtained were dried over anhydrous sodium sulfate (Merck, AR grade). The experiment was conducted in triplicate.

Essential oils analysis

Gas chromatography-flame ionization detection (GC-FID)

The essential oils (0.01 µml) were analyzed by gas chromatography-flame ionization detector (GC-FID), Shimadzu GC-17A model (Shimadzu Corporation, Kyoto, Japan). The column used was a BP×5 non polar solution (length = 30 m, ID = 0.25 mm, film = 0.25 µm). Helium gas was used as carrier gas together with split injection mode with a split ratio 28:1. Total flow was 25 ml/min and column flow was 0.8 ml/min.

The essential oils were also analyzed by gas chromatography coupled to mass spectrometry (GC/MS), using a Shimadzu CG-17A (Shimadzu Corporation, Kyoto, Japan) chromatograph coupled with a QP-5050A mass selective detector, under the following operational conditions: capillary coated with BP×5 (length = 30 m, ID = 0.25 mm, film = 0.25 µm) with maximum temperature of 270°C. Constituents were identified by matching their mass spectra with those stored in the spectrometer database using the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998), using retention indices as a pre-selection routine (Alencar et al., 1984; 1990). The concentrations of the component of the essential oils were obtained from GC peak area by applying appropriate correction factors.

Statistical analysis

Collected data were analyzed using the SPSS statistical package software version 17.0. (Chicago, USA). Analysis of variance (ANOVA) was used to determine the significance of variation among the different treatments.

RESULTS AND DISCUSSION

Five samples (fresh, 1, 2, 3 and 7 days, respectively) of each part of *A. conchigera* (leaves and rhizomes) were subjected to hydrodistillation for 5 h for essential oil extraction. Light yellow oils with strong and fresh fragrance were obtained.

Effect of drying on the yield of leaves: The initial oil yield of fresh leaves was 0.120% (v/w). By day one after harvest, the oil yield was decreased to 0.086%. After day two, the oil yield was increased to 0.220%, and by day three, decreased to 0.160%. The oil yield reached the highest level at day seven (0.300%). Effect of drying on

Table 1. Essential oils constituents of the leaves of *A. conchigera* at different post-harvest air drying periods.

Compounds	RI [a]	Area (%) [b]				
		fresh	1 day	2 days	3 days	7 days
Bicyclo(3.1.1)hept-2-ene, 2,6,- trimethyl	923	1.06	3.05	2.84	4.68	2.49
β- pinene [c]	976	3.63	10.32	9.83	14.78	7.41
Cyclohexene, 4-ethyl-3-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)	1305	0.65	0.93	1.13	0.66	
Phenol,4-(2-propenyl)-acetate	1314		0.44	0.37		
dodecanal	1352		0.63			
Bicyclo(7.2.0)undec-4-ene,4,11,11-trimethyl-8-methylene	1362	16.92	19.76	18.32	16.42	13.49
Germacrene D 1,6-cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)	1375	2.36	2.48	2.50	1.62	1.13
1,6,10-dodecatriene,7,11-dimethyl-3-methylene	1377	1.90	1.80	2.16	1.74	54.79
α-caryophyllene	1378	1.07	1.59	1.30	1.47	1.27
Cyclohexene,1-methyl-4-(5-methyl-1-methylene-4-hexenyl)	1407	61.19	47.89	50.47	46.58	
Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	1411	4.32	3.94	4.96	3.33	4.68
1,6,10-dodecatriene-3-ol,3,7,11,trimethyl	1424	0.40	0.61		0.32	0.37
α-bisabolol	1481	0.72	1.07	0.76		
Phytol	1622	0.84				
Total		95.06	94.51	94.64	91.60	85.63
Yield (%v/w)		0.120	0.085	0.220	0.160	0.300

[a] Retention index, [b] Oil abundance in percentage (%), [c] Previously reported in *A. conchigera* rhizome oil by Sirat and Nordin (1995) and Ibrahim et al. (2009).

the yield of rhizomes: The initial oil yield of fresh rhizomes was 0.078%. At day one, the oil yield was increased to 0.112%. By day two after harvest, the yield was decreased to 0.078%. This yield was almost the same as the initial yield. The oil yield reached a maximum at day three after harvest (0.162%), before declining back to 0.145% by day seven. Generally, the dried plant material yielded more essential oils than did the fresh materials (Tables 1 and 2).

In previous studies, Ibrahim et al. (2009) reported that the essential oil content of *A.conchigera* was 0.32 and 0.30% from rhizomes and leaves, respectively, while, Wong et al. (2005) reported 0.14% oil yields from rhizomes and Athamaprangsa et al. (1994) found 0.15% of an essential oil from fresh rhizomes. While in the present study oil yields were in range of 0.086 to 0.30% for leaves and 0.076 to 0.162% for rhizomes. The different oil yields in the present study compared with those obtained in previous studies may be ascribed to the different types of extraction techniques used. In this study, hydrodistillation was employed for 5 hours, while in the previous studies of Athamaprangsa et al. (1994) and Ibrahim et al. (2009) steam-distillation was used for 8 h. Generally oil yields were higher from leaves than from rhizomes. For rhizomes, the highest essential oil yield was obtained from rhizomes dried for three days. This is likely due to the loss of volatile terpenoids, during drying as reported by Combrinck et al. (2006) conducted for *Lippia scaberrima.*

In the analysis of fresh leaves extracts, the essential oil contained 12 chemical constituents (about 95.06% of the oils). The most abundant chemical compound found in this oil sample was cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) (61.19%). Other constituents are listed in Table 1. Thirteen components were identified in the analysis of 1 day dried leaves sample (about 94.51% of the oils). The major component found was cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) (47.89%). Other chemical constituents identified in this essential oil are listed in Table 1. Eleven chemical constituents were identified from the essential oil of 2 days dried leaves (about 94.64% of the oils). Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) was found to be the most abundant constituent in this oil at concentration of 50.47%. Other major compounds are presented in Table 1. For 3 days dried leaves, 10 chemical constituents were identified from the extracted essential oil (about 91.60% of the oils). Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) was the most abundant with a concentration of 46.58%. Other compounds are listed in Table 1. The essential oil extracted leaves dried for 7 days showed the presence of 8 chemical constituents (about 85.63% of the oils). 1, 6, 10-dodecatriene, 7, 11-dimethyl-3-methylene (54.79%) was found as the major component. Other constituents are summarized in Table 1. Fourteen components (about 47.71% of the oils) were identified in the analysis of the fresh rhizome sample. The most abundant component in this sample was β-pinene (18.44%). Table 2 shows all of the chemical constituents identified in the essential oil of this sample. The analysis of one day dried rhizomes showed the presence of 11 chemical constituents (about

Table 2. Essential oils constituents of the rhizomes of *A. conchigera* at different post-harvest air drying periods.

Compounds	RI [a]	Area (%) [b]				
		fresh	1 day	2 days	3 days	7 days
Bicyclo(3.1.1)hept-2-ene, 2,6,- trimethyl	924	4.92	3.69	4.75	6.26	5.57
β- pinene [c]	978	18.44	18.70	15.74	17.09	16.99
β-mycrene	986	1.25			1.62	1.23
Benzene,1-methyl-3-(1-methylethyl)	1027		1.54	5.28	0.35	
Cyclohexene,1-methyl-4-(1-methylethynyl)	1030	1.13	2.38		1.46	0.67
Eucalyptol	1044				42.70	45.07
1,4-cyclohexadiene,1-methyl-4-(1-methylethyl)	1063	0.86			0.61	0.91
Bicyclo(3.1.1)heptan-3-ol,6,6-dimethyl-2-methylene	1153			4.56		
Bicyclo(3.1.1)hept-2-ene-2-methanol,6,6-dimethy	1176			3.34		
3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	1192	2.25				1.80
α-terpinol(p-menth-1-en-8-ol)	1207					0.99
3-cyclohexeno-1-methanol,alpha,alpha 4-trimethyl	1208	1.80			0.61	
Bicyclo(3.1.1)hept-3-en-2-one,4,6,6-trimethyl	1210			6.76		
Cyclohexene, 4-ethyl-3-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)	1304					0.28
Phenol,4-(2-propenyl)-acetate	1314		0.83		1.90	2.92
2,6-octadien-1-ol,3,7-dimethyl-acetate	1331				0.42	0.25
2,6,10-dodecatrien-1-ol,3,7,11-trimethyl	1346		5.05			0.41
Bicyclo(7.2.0)undec-4-ene,4,11,11-trimethyl-8-methylene	1360	5.64			4.92	4.53
α-caryophyllene	1379	0.57	0.99		0.56	0.43
1-dodecanol	1383				0.32	
1,6,10-dodecatriene,7,11-dimethyl-3-methylene	1384	7.67		1.23	0.40	0.24
1H-cycloprop(e)azulene,decahydro-1,1,7-trimethyl-4-methylene	1395				0.94	
Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)	1396	1.25	1.11	0.80		0.73
n-hexadeconoic acid	1399			4.61		
Cyclohexene,1-methyl-4-(5-methyl-1-methylene-4-hexenyl)	1402		28.44	2.89	11.51	6.70
Phenol,2-methoxy-4-(2-propenyl)-acetate	1406					0.31
Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	1408	0.63	1.01		0.82	0.62
T-muurolol [c]	1470	0.72				0.50
α-bisabolol [c]	1479	0.58	1.05		0.51	0.41
α-cadinol	1480				0.47	
Total		47.71	64.79	49.96	93.47	91.56
Yield (%v/w)		0.078	0.112	0.078	0.162	0.145

[a] Retention index,[b] Oil abundance in percentage (%), [c] Previously reported in *A. conchigera* rhizome oil by Athamaprasangsa et al. (1994), Sirat and Nordin (1995), Wong et al. (2005) and Ibrahim et al. (2009).

64.79% of the oils). Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) (28.44%) was found as the major component. Other constituents are presented in Table 2 Concerning 2 days dried rhizomes, 10 chemical components (about 49.96% of the oils) were identified in the analysis of this sample. The major component in this sample was β-pinene (15.74%). Table 2 shows all other chemical constituents. With regard to 3 days dried Rhizomes, 19 chemical constituents (about 93.47% of the oils) were identified. Eucalyptol was the most abundant with concentration 42.70%. Other compounds are listed in Table 2. With regard to 7 days dried rhizomes, 21 components (about 91.56% of the oils) were detected. Eucalyptol was found as the most abundant compound with the percentage of 45.07%. In this study, there are differences in oil yields and their chemical constituents between leaves and rhizomes, also as a function of the duration of drying period. Bicyclo(3.1.1)hept-2-ene, 2,6,-trimethyl, β- pinene, cyclohexene, 4-ethyl-3-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl, phenol,4-(2-propenyl)-acetate, cyclohexene,1-methyl-4-(5-methyl-1-methylene-4-hexenyl), cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene and α-bisabolol were detected in the oils of the leaf and rhizome.

Thirty one of the components had not previously been detected, namely, bicyclo (3.1.1) hept-2-ene, 2,6,-trimethyl, cyclohexene; 4- ethyl-3-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl; phenol, 4-(2-propenyl)-

acetate; dodecanal; bicycle (7.2.0) undec-4-ene,4,11,11-trimethyl-8-methylene; germacrene D 1,6-cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl); 1,6,10dodecatriene,7,11-dimethyl-3-methylene; α-caryophyllene; cyclohexene,1-methyl-4-(5-methyl-1-methylene-4-hexenyl); cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene; 1,6,10-dodecatriene-3-ol,3,7,11,trimethyl; phytol; β-mycrene; benzene,1-methyl-3-(1-methylethyl); cyclohexene,1-methyl-4-(1-methylethynyl); eucalyptol; 1,4-cyclohexadiene,1-methyl-4-(1-methylethyl); bicyclo(3.1.1)heptan-3-ol,6,6-dimethyl-2-methylene; bicyclo(3.1.1)hept-2-ene-2-methanol,6,6-dimethy; 3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl); α-terpinol (p-menth-1-en-8-ol); 3-cyclohexeno-1-methanol,α,α 4-trimethyl; bicyclo(3.1.1)hept-3-en-2-one,4,6,6-trimethyl; 2,6-octadien-1-ol,3,7-dimethyl-acetate; 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl; 1-dodecanol; 1H-cycloprop(e)azulene,decahydro1,1,7trimethyl4methylene; naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl); n-hexadeconoic acid; phenol,2-methoxy-4-(2-propenyl)-acetate and α-cadinol.

Comparison of the results shows that the different drying periods had an effect on the percentage of the main components. Increased concentrations of various volatile substances with air-drying have been observed for numerous spices and are probably caused by the breakdown of glycosylated forms, dehydration reactions, or oxidation reactions (Baritaux et al., 1992; Bartley and Jacobs, 2000) or due to the rupture of the plant cells in which the volatiles are stored.

Some essential oil components could arise from the dehydration of oxygenated compounds, such as eucalyptol, bicylo (3.1.1)heptan-3-ol,6,6-dimethyl-2-methylene, phenol,4-(2-propenyl)-acetate, 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl, α-cadinol and phenol, 4-(2-propenyl)-acetate. This might be due to some chemical transformations during the process of drying. The inability of the GC-MS to detect the presence of eucalyptol in the fresh rhizomes and the quantity of the component and its sudden appearance on drying from day 3 to day 7 (42.70 to 45.07%) is noteworthy. On the other hand, a chemical compound like phytol was not detected upon drying process. This compound might be stored on or near the leaf surfaces and vaporized upon drying (Moyler, 1994) or it may have been converted to other compound upon drying.

In comparison to previous studies on *A. conchigera*, The GC-MS was unable to detect the presence of 1′-acetoxychavicol acetate, 1′-hydoxychavicol acetate, 4-acetoxycinnamyl alcohol and 4-acetoxycinnamyl acetate, as reported by Athamaprasangsa et al. (1994), or α-thujene, δ-2-carene, (Z)-β-ocimene, *p*-mentha-1,5,8-triene, p-cymenol, *trans*-carvyl acetate, neryl acetate, methyl eugenol, δ-selinene, farnesol, γ-elemene, 2,4-di(tert-butyl)phenol, 1-hexadecene and heptadecane, as reported by Sirat and Nordin (1995), or β-sesquiphellandrene (20.5%), β-bisabolene (12.10%), 1,8-cineole (11.56%), β-caryophyllene (4.39%) from rhizomes as reported by Sirat and Nordin (1995) or β-bisabolene (28.9%), 1,8-cineole (15.3%), β-caryophyllene (10.0%),) camphene, *α*-phellandrene, (*E*)-β-ocimene, p-cymenene, bornyl acetate, tridecane, thymyl acetate, pentadecane, t-muurolol, α-bisabolol, heptadec-1-ene, (E,E)-farnesyl acetate and nonadec-1-ene, as reported by Wong et al. (2005), or β-bisabolene (15.3 %), β-sesquiphellandrene (7.6 %), chavicol (7.5 %) from leaves as reported by Ibrahim et al. (2009). 1, 8-cineole (17.9 %), β-bisabolene (13.9 %), β-sesquiphellandrene (6.8 %) from rhizomes as reported by Ibrahim et al. (2009). This difference might be due to different extraction techniques, genetic factors, different chemotypes and the nutritional status of the plants as well as other environmental factors.

Comparison of chemical constituents obtained from this study with the ones obtained from other species in the same genus such as: *A. calcarata*, *A. galangal*, *A. hainanensi*, *A. katsumdai* and *A. latilabris*. The comparisons are based on previous studies by Rout et al. (2005) for *A. calcarata*, Wong et al. (2005) for *A. latilabris*, Nan et al. (2004) for *A. hainanensi* and *A. katsumdai* and Ibrahim et al. (2004) for *A. galangal*. β-Pinene has also been isolated from these species. This indicated that the species in the same genus showed similarity in some of their chemical constituents.

Conclusion

The essential oils are very important and widely used in pharmaceutical, food industries, aromatherapy and the perfume industries. Therefore, many studies, such as this study is very crucial in order to identify what type of chemical constituents existing in the materials that we want to use, before the essential oil can be utilized in those industries.

In the present study we found that the essential content was significantly affected by post-harvest period and showed that 7 day drying period is the best one for oil yield from leaf and 3 drying period is the best one for rhizome. This study highlights the influence of the post-harvest period on providing the components of the essential oil.

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