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EXTRACTION OF VOLATILE OIL FROM KAFFIR LIME LEAVES (CITRUS HYSTRIX) USING PRESSURISED LIQUID EXTRACTION

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Abstract: *Purpose:* Pressurised Liquid Extraction (PLE) was used to extract volatile oil from kaffir lime leaves.

Methodology: Conventional extraction methods; hydrodistillation and Soxhlet are very time consuming and tedious. Therefore, a rapid extraction method is needed for volatile oil extraction. In this study, volatile flavour components in oleoresin were identified by GCMSD and the main constituents obtained were aldehydes, monoterpene, diterpene, triterpene, sesquiterpene and aromatic hydrocarbons.

Findings: Oleoresin extracted using PLE (100°C, 1000 psi, and 30 min) contained significantly ($p < 0.05$) the highest yield (42.27%, dry weight basis) in comparison to conventional methods; hydrodistillation (0.35%, dry weight basis) and Soxhlet extraction (22.79%, dry weight basis).

Value: Higher quality oleoresin was also obtained with PLE in term of quantitative major compounds; citronellal, linalool and myrcene.

Keywords: *Pressurised Liquid Extraction; Citronellal; Linalool; Myrcene*



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INTRODUCTION

Kaffir lime leaves with antimicrobial properties usually been used for cooking and its essential oil or oleoresin for aromatherapy (Chanthaphon et al., 2008). However, these leaves can't be kept long. The shelf life of the leaves can be extended by transforming fresh leaves into oleoresin, an oil soluble extract. Conventional methods of extraction are hydrodistillation and Soxhlet method. The main weaknesses of conventional method are time consuming, heat instability of the oils, loss of certain water-soluble components and large solvent consumption (Damjanovic, 2003). Pressurised liquid extraction (PLE) is one of the latest techniques developed for the extraction of solid samples and was introduced in 1995. Pressurised Liquid Extraction allows faster extraction than classical methods and requires only small volume of solvent (Hussen et al., 2006). It operates with organic solvents by applying high temperatures (up to 200°C) and pressures (up to 20,000 kPa) to maintain the solvent in a liquid state that close to the supercritical state (Hyötyläinen, 2006). Zaibunnisa et al. (2009) was also able to successfully optimized turmeric (*Curcuma domestica*) oleoresin extraction using PLE. The objective of this paper to compare the quality of the extract obtained using PLE, hydrodistillation and Soxhlet in term of quantity of major compounds (citronellal, linalool and myrcene).

MATERIALS AND METHODS

Sample Preparation

Fresh Kaffir lime leaves (moisture content 65.4%) was obtained from local supplier. The leaves and stem were air-dried at room temperature (22 °C), protected from direct light until moisture content reached 10.4% and 10.2%, respectively. Samples were placed in seal plastic bags and kept at room

temperature until analysed. Prior to extraction, samples were further cut to 3 mm diameter.

Chemicals

Reagent Ph Eur (Merck, Germany) was prepared at a concentration 20mg/L for GC analysis. Anhydrous Na₂SO₄ (HmbG Chemical) and NaCl (Merck, Germany) were use as drying reagent and emulsion breaker, respectively, for liquid-liquid extraction. Diatomaceous earth (DE) non-washed, SiO₂ approximately 90% purity (Sigma,USA) was used as drying agent for PLE extraction.

Extraction Methods

Extractions were carried out using Pressurized Liquid Extraction (PLE), hydrodistillation and Soxhlet extraction following the methods used by Zaibunnisa et al., 2009.

Moisture content and yield

Moisture content and yield of samples were determined following the method used by Zaibunnisa et al., 2009.

Volatile compounds

Volatile compounds were analyzed using Agilent Technologies 6890N Network GC system equipped with Agilent Technologies 5973 Inert Mass Selective Detector and Agilent 7683 Series Injector. A HP-5 MS capillary column (Agilent 19091S-433, 0.25 mm x 30 m 0.25 μm) was used. GC/MS detection was done using an electron ionization system with ionization energy of 70eV in 50-550 a.m.u. mass range. Column temperature was increased from 70-325 °C. Initial and final time was 6 and 20 minutes, respectively. Helium was the

carrier gas, at a flow rate of 1.0 mL/min in splitless mode. Identification of compounds was based on comparison of mass spectra with those of Flavour and NIST library.

RESULTS AND DISCUSSION

The flavour volatiles in the kaffir lime leaves extracted using PLE, hydrodistillation and Soxhlet extraction and analysed using GCMSD. Altogether, 81 compounds were identified as shown in Table 1. The main compounds obtained by using all these methods of extraction were sabinene, myrcene, linalool, citronellal, terpinene 4-ol and citronellol. These results correlate well with the profiles obtained by Fatima *et al.* (2009); marker compounds obtained by these researchers were sabinene and citronellol. However, Ishikama *et al.*, (2005) discovered 90 compounds from kaffir lime (*Citrus hystrix*) peel essential oil and juice from Madagascar. The major components in the peel oil were α -pinene (22.7%), limonene (17.3%), sabinene (11.9%), citronellal (7.8%), terpinen-4-ol (7.2%), citronellol (3.6%), and linalool (2.6%), while in the juice were α -pinene (35.6%), sabinene (7.0%), limonene (5.9%), terpinen-4-ol (19.7%), β -terpinene (4.4%) and linalool (2.8%). Some of the compounds identified in PLE extract cannot be detected in hydrodistillation and Soxhlet extraction due to use of water as extractant and overheating during extraction. The GCMSD chromatogram for the PLE extract is as shown in Figure 1. PLE was able to produce significantly ($p \leq 0.05$) the highest percentage yield (47.27% dry weight basis). According to Ong (2004), extraction efficiencies using water were not satisfactory when compared with conventional methods using solvent. Water gave better extraction of polar compounds but less favorable extraction of non-polar compounds (Marriot *et al.*, 2001) and most of volatile compounds are non-polar. Meanwhile, over-heating during extraction plays the important role that causes the loss of some volatile compounds.

No.	RT	Compounds	PLE ^b		HDF ^c Fresh Leave	Soxhlet ^d Air-dried Leave
			Air-dried Stem	Air-dried Leave		
1	5.48±0.00	Cyclohexene	-	0.14±0.00	-	-
2	5.66±0.24	Sabinene	0.43±0.14	0.18±0.10	-	2.67±1.08
3	5.81±0.28	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	-	0.41±0.00	-	3.00±0.87
4	5.84±0.01	Myrcene	0.10±0.60	0.26±0.10	-	-
5	6.67±0.00	p-Cymene	-	0.48±0.08	-	-
6	6.68±0.00	Benzene	-	0.31±0.00	-	-
7	7.22±0.00	delta-3-carene	0.20±0.13	-	-	-
8	7.51±0.94	α-terpinene	-	0.12±0.09	-	-
9	7.72±0.00	3,7-dimethyl-1,3,6-octatriene	-	-	-	0.70±0.00
10	8.76±0.27	Linalool	2.73±0.59	3.22±0.34	3.77±3.96	4.85±0.15
11	8.66±0.18	Furfuryl alcohol	-	-	2.32±0.00	-
12	9.95±0.30	Isopulegol	0.38±0.03	0.70±0.26	3.63±0.00	-
13	10.24±0.33	Citronellal	13.16±2.46	25.87±3.00	17.51±7.99	36.94±5.06
14	10.31±0.00	Menthone	-	0.15±0.00	-	-
15	10.81±0.02	α-Terpineol	0.04±0.00	0.14±0.00	-	-
16	11.04±0.00	Terpinene-4-ol	-	-	1.42±0.00	-
17	11.11±0.00	n-decanal	-	0.02±0.00	-	-
18	12.04±0.38	Citronellol	5.99±1.03	4.02±0.53	1.33±0.17	3.34±0.16
19	12.66±0.00	Citral	-	0.06±0.00	-	-
20	14.20±1.59	Nerol	0.12±0.01	1.28±0.12	3.46±0.10	2.72±0.30
21	14.09±1.11	Geraniol	1.88±1.55	1.57±0.57	3.53±0.00	2.63±0.37
22	14.21±0.00	Cyclohexane	-	0.77±0.00	-	-
23	14.51±0.01	Citronellyl butyrate	0.65±0.10	1.90±0.83	-	2.45±0.00
24	14.54±0.02	Citronellyl propionate	-	1.88±0.00	-	-
25	14.93±0.00	Decanoic acid	0.05±0.00	-	-	-
26	15.10±0.00	Copaene	-	1.14±0.00	-	-
27	15.11±0.01	α-Cubebene	0.42±0.00	0.95±0.05	-	-
28	15.75±0.30	β-Cubebene	-	1.17±0.05	-	1.67±0.15
29	10.40±0.02	Cyclohexanol	0.81±0.00	0.27±0.03	45.83±0.00	-
30	16.19±0.00	α-Irone (isomer 1) (E-)	-	0.04±0.00	-	-
31	16.38±0.31	Caryophyllene	1.79±0.13	4.04±0.69	2.64±1.49	5.41±0.76
32	16.79±0.00	Geranylacetone	-	0.06±0.00	-	-
33	16.81±0.00	1,4,7-cycloundecatriene	-	0.79±0.03	-	-
34	17.11±0.00	Lauryl alcohol	0.12±0.00	-	-	-
35	17.29±0.00	Bicyclogermacrene	-	0.41±0.00	-	-
36	17.38±0.00	α-Caryophyllene	-	-	-	0.82±0.00
37	17.42±0.00	β-Ionone	-	0.09±0.00	-	-
38	17.79±0.00	1,5,5-Trimethyl-6-methylene-cyclohexene	-	1.56±0.56	-	-
39	17.91±0.00	α-Farnesene	0.94±0.22	2.02±0.06	-	4.44±0.00
40	17.94±0.00	1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene	-	-	-	0.73±0.00

Extraction of
Volatile Oil From
Kaffir Lime Leaves
(*Citrus hystrix*)
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Liquid Extraction
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Table 1
(continued)

41	18.36±0.27	Naphthalene	0.76±0.15	2.18±0.22	-	5.25±0.00
42	18.74±0.01	Cyclohexyl carbinol	0.44±0.08	0.92±0.05	-	-
43	19.18±0.31	Nerolidol	0.89±0.19	1.93±0.12	3.17±0.59	-
44	19.31±0.01	Germacrene D-4-ol	-	0.32±0.00	-	-
45	21.37±0.01	Dodecyl acrylate	0.64±0.05	0.04±0.00	-	-
46	21.38±0.00	Maleic acid	0.97±0.00	-	-	-
47	21.93±0.00	2-Dodecen-1-yl(-)-succinic anhydric	5.33±0.00	-	-	-
48	21.94±0.00	2,6,10-Dodecatrien-1-ol	-	0.29±0.02	-	-
49	22.05±0.40	Lauric aldehyde	0.11±0.00	-	0.67±0.00	-
50	22.34±0.00	Tetradecanol	-	-	0.67±0.00	-
51	25.86±0.00	Stearic acid	5.47±0.00	0.24±0.00	-	-
52	23.96±0.00	1-Methoxy-3-(2-hydroxyethyl)nonane	-	0.63±0.00	-	-
53	23.73±2.55	Isophytol	-	0.62±0.05	-	-
54	24.95±1.99	Palmitic acid	3.89±3.37	4.43±1.85	-	-
55	26.26±0.03	Lanost-8-en-3-ol, acetate, (3β)-	1.73±1.45	-	-	-
56	26.95±0.00	n-Hexadecanoic acid	-	1.51±1.36	-	-
57	27.03±0.01	14-Methyl-8-hexadecyn-1-ol	-	1.50±0.46	-	-
58	27.04±0.01	Linoleic acid	3.77±1.00	-	-	-
59	27.05±0.34	Phytol	3.50±0.16	9.53±1.65	-	13.03±3.38
60	27.46±0.00	β-Tocopherol	4.00±1.82	-	-	-
61	27.47±0.00	Erucamide	1.83±0.00	-	-	-
62	27.47±0.00	Oleamide	-	1.38±0.00	-	-
63	27.69±0.00	Cetane	-	0.58±0.00	-	-
64	27.69±0.00	Docosane	-	0.54±0.00	-	-
65	27.70±0.00	Heneicosane	1.07±0.00	-	-	-
66	27.80±0.01	Squalene	6.83±0.00	2.77±0.96	-	-
67	17.90±0.00	Tricosane	9.96±0.00	-	-	-
68	27.98±0.00	Stimasterol	5.85±0.00	-	3.63±0.00	-
69	27.98±0.28	Hexacosane	10.79±9.28	0.6±0.00	-	-
70	28.06±0.00	6a, 14a-Methanopicene	5.50±0.00	-	-	-
71	28.25±0.24	Heptacosane, 1-chloro-	0.43±0.00	-	-	-
72	28.14±0.00	Taraxastero	4.32±0.00	-	-	-
73	28.25±0.00	Tritricontane	4.38±0.00	-	-	-
74	28.28±0.86	n-Eicosane	0.68±0.00	0.46±0.29	4.90±0.00	-
75	28.29±0.00	α-Tocopherol	-	0.45±0.00	-	-
76	28.31±0.00	Vitamin E	7.57±3.94	-	-	-
77	28.31±0.00	Heptacosane	0.24±0.00	-	-	-
78	28.72±0.92	Nonacosane	-	1.42±0.89	-	6.98±0.00
79	28.73±0.79	n-Tetracosane	2.73±3.27	0.56±0.52	12.57±0.00	6.98±0.00
80	28.32±0.00	Octacosane	-	-	2.69±0.00	-
81	29.34±0.00	n-Octadecane	-	0.55±0.00	-	-
Yield (% dry weight basis)			14.5±1.24	47.27±0.88	0.35±0.03	22.80±1.85

Table I:
Composition of n-hexane Extract and Essential Oil (% area)^a Obtained Using Pressurised Liquid Extraction, Hydrodistillation and Soxhlet Extraction from the Leaves and Stems of Kaffir Lime. Compounds Identification was Based on Flavor and NIST Library (Resemblance Percentage above 80%).

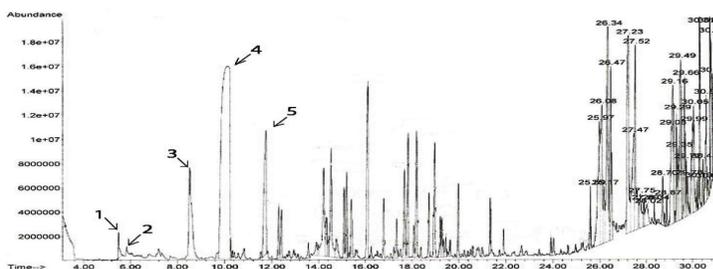
^a Percent of component based on area normalization. Each value is express as means ± S.D. (n=3)

^b Pressurised liquid extraction conditions: sample, 2 g; solvent, n-hexane; temperature, 100 °C; pressure, 1000 psi; static time, 30 min

^c Hydrodistillation condition: sample, 300 g; time 8 hr

^d Soxhlet extraction condition: sample, 2 g; solvent, n-hexane; time, 16 hr

Figure 1:
GC Chromatogram of
Volatile Compounds
Obtained Using PLE
(100°C, 1000 psi, and
30 min) from Air-
Dried Kaffir Lime
Leaves. Marker and
Major Compounds
Peaks: Sabinene (1),
Myrcene (2), Linalool
(3), Citronellal (4) and
Citronellol (5).



Soxhlet extraction with long time of progression (16 hr.) and exposure to light resulting in some compounds were lost during extraction.

These results indicate that PLE was able to extract efficiency with low solvent consumption. High efficiency of PLE was due to high pressure that was applied during extraction which results in extracting solvent at subcritical state. Soxhlet extraction, moderate extraction yield (22.80% \pm 1.85) was obtained. However, this method uses large amount of solvent and also a time consuming process.

The concentrations of major compounds are as shown in Table 2. Myrcene was unidentified in hydrodistillation and Soxhlet extraction due to use of water as extractant, overheating and long extraction time. There were also no significant ($p \leq 0.05$) different in the concentration of linalool in

	PLE ¹ Air-dried Leave	Hydrodistillation ² Fresh Leave	Soxhlet extraction ³ Air-dried Leave
Myrcene	0.02 ^a ±0.00	-	-
Linalool	0.72 ^a ±0.10	0.49 ^a ±0.13	0.65 ^a ±0.04
Citronellal	5.00 ^a ±0.42	1.70 ^b ±0.76	5.06 ^a ±0.06

Mean value with different alphabets are significantly different ($p \leq 0.05$) according to Duncan's Multiple Range test.

¹ Pressurised Liquid Extraction conditions: sample, 2 g; solvent, *n*-hexane; temperature, 100°C; pressure, 1000 psi; static time, 30 min.

² Hydrodistillation conditions: sample, 300 g; time 8 hr.

³ Soxhlet extraction conditions: sample, 2 g; solvent, *n*-hexane; time, 16 hr.

Table 2:
Major Volatile Compounds Concentration (g/100g) in Kaffir Lime Essential Oil Extracted Using PLE in Comparison to Conventional Techniques; Hydrodistillation and Soxhlet Extraction.

all methods of extraction. There was no significant ($p \geq 0.05$) difference in term of concentration of citronellal in the extract obtained from PLE and Soxhlet extraction. According to Bautz *et al.* (1998), PLE was able to give better extraction efficiency of polychlorinated dibenzo-p-dioxins and dibenzofurans when compared with Soxhlet extraction.

CONCLUSION AND RECOMMENDATION

PLE was able to produce significantly ($p < 0.05$) higher yield than Soxhlet extraction and hydrodistillation. The PLE has more advantage over other conventional methods; shorter extraction time (30 min compared to 8 hours for hydrodistillation and 16 hours for Soxhlet extraction), lower solvent consumption and more compounds can be extracted out. With the addition of small amount of oleoresin, flavour and aroma of kaffir lime leaves can be introduced in food products.

BIOGRAPHY

Zaibunnisa had obtained her B.Sc from University of Nottingham,UK and MSc in Food Science from Universiti Putra Malaysia. She earned her PhD in Food Science (Flavour Chemistry) from Universiti Kebangsaan Malaysia. She has received several awards (2nd Runner up ASEAN Best Graduate Research and Excellent Service Award 2010) and had presented several papers on volatile oil extraction and stabilization technology on several conferences. She has supervised students for Master's and Doctorate degrees at UiTM. Dr. Zaibunnisa is also the author and co-author of a number of journal articles.

Chutima had obtained her B.Sc from Mae Fah Luang University, Thailand. She did her research attachment at Universiti Teknologi MARA under the supervision of Dr. Zaibunnisa Abdul Haiyee.

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