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Fractionation of Citronella Oil and Identification of Compounds by Gas Chromatography-Mass Spectrometry

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ABSTRACT

Citronella oil is one of the most important essential oils and is widely used in the pharmaceutical, cosmetic and food industries. However, the selling price of citronella oil is still low, so efforts are needed to increase its added value by isolating the active components such as citronellal, citronellol and geraniol. This study aims to isolate the active ingredients of citronella oil that have higher economic added value. Citronella oil was obtained by the process of distillation of *Cymbopogon nardus* (L.) Rendle leaves. The essential oil was then fractionated by a vacuum fractionation process. The essential oil fraction was identified by Gas Chromatography - Mass Spectrometry (GC-MS). Fractions obtained from the fractionation process were identified, and the results were: F1 (D-Limonene: 72.89%), F2 (Citronellal: 50.13%), F3 (Citronellal: 74.89%), F4 (Citronellal: 88.56%), F5 (Citronellal: 84.89%), F6 (Citronellal: 55.38%), F7 (Citronellol: 57.42%), F8 (Citronellol: 44.73%), F9-1 (Geraniol: 65.56%), F9-2 (Geraniol: 64.41%) and residual (Geraniol: 32.04%). Based on these results, several active compounds from citronella oil can be obtained using the vacuum distillation fractionation method.

Keywords: *citronella oil*; *fractionation; gas chromatography-mass spectrometry*

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INTRODUCTION

Indonesia is a tropical country that is rich in natural resources, especially medicinal plants. Until now, Indonesia still plays an important role in the world spices trade, including essential oils and their derivatives. Citronella oil is one of the most important essential oils and is widely used in the pharmaceutical, cosmetic and food industries. However, the value of citronella oil is still low, so efforts are needed to increase its added value by isolating the active components such as citronellal, citronellol and geraniol.

Citronella oil is an essential oil obtained from the steam distillation of *Cymbopogon nardus* (L.) Rendle leaves. Harianingsih et al (2017) identified essential oil from citronella oil using Gas Chromatography – Mass Spectrometry (GC-MS) and obtained 36.11% citronellal, 20.07% geraniol, and 10.82% citronellol. Fractionation of citronella oil has also been carried out with GC-MS analysis, and 80.65% citronellol, 76.63% geraniol, 95.10% citronellal and 75.95% p-menthane-3,8-diol were obtained.

The active compounds found in citronella oil include citral, citronellol, α-pinen, kamfen, sabinen, mirsen, β-felandren, psimen, limonene, cis-osimen, terpinol, citronellal, borneol, terpinen-4-ol, α-terpineol, geraniol, farnesol, methyl heptenone, n-decialdehyde, dipenten, methyl heptenone, bornylacetate, geranylformate, terpinyl acetate, citronellyl acetate, geranyl acetate, β-element, β-cariophylene, β-bergamotene, trans-methylisoeugenol, elemol, and cariophylene oxide (Timung et al., 2016).

Citronella oil has been commonly used for a long time and from several studies it was reported that citronella oil has antibacterial, antiinflammation, diuretics, antioxidants, anticancer, and antipyretics properties. Citronella oil was also used as a mosquito repellent, relaxant, colds and coughs reliever, and treatment for typhus, seizures, rheumatism, food poisoning and body odor (Tsai et al., 2010; Chen & Viljoen, 2010; Lokhande & Lanka, 2009; Ali et al., 2015).

Fractionation was useful for separating essential oils based on their boiling point into several fractions. Several studies related to the isolation of essential oils were conducted by using vacuum fractionation distillation techniques.

Research on the identification of essential oil components using GC-MS has been carried out (Madivoli et al., 2012). Gas chromatography was used

Table 1. Characteristics of citronella oil and SNI quality requirements (Anwar et al., 2019a)

No.	Parameter	Citronella Oil	Testing Method
1	Colour	pale yellow	Visual
2	Density (20°C)	0.8789	SNI 06 – 2385 – 2006 item 5.2
3	Refractive Index (20°C)	1.4629	SNI 06 – 2385 – 2006 item 5.3
4	Solubility in alcohol	1:2 (dissolve)	SNI 06 – 2385 – 2006 item 5.4
5	Optical rotation	-0.05°	SNI 06 – 2385 – 2006 item 5.7

Table 2. The mayor compounds of *Cymbopogon nardus* (L.) rendle (Anwar et al., 2019b)

No.	Mayor Compound	R TIME	AREA	% AREA	SI
1	Beta Myrcene	9.188	3421822	0.09	97
2	D Limonene	10.413	149518476	3.78	96
3	Linalool	12.548	47311656	1.2	97
4	Citronelal	15.164	1413835087	35.72	96
5	Alpha Terpineol	16.458	4265735	0.11	97
6	Citronelol	18.568	597470174	15.09	98
7	Geraniol	19.579	510353648	12.89	93

to identify a compound found in the gas mixture and also to determine the concentration of a compound in the sample. Mass spectrometry is a method for obtaining molecular weight. Alloys of both can produce accurate data in identifying compounds that are covered by their molecular structure. This study aims to identify the compounds of fractionation of citronella oil with vacuum distillation method using GC-MS.

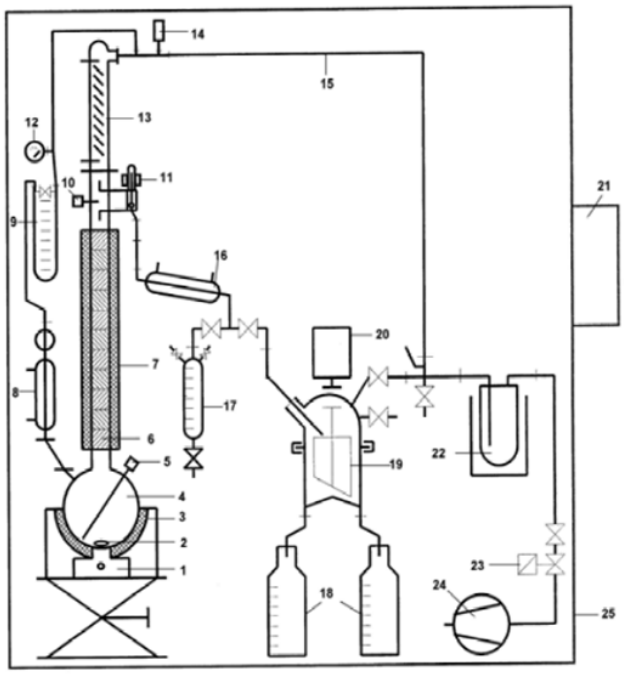
METHODS

Citronella oil was obtained from leaves of *Cymbopogon nardus* (L.) Rendle which was distilled in the experimental garden, Manoko – Lembang, Bandung. Citronella oil has the characteristics as shown in Table 1. From an analysis using GC-MS, the major compounds of citronella oil were characterized, as shown in Table 2. (Anwar et al, 2019a). Citronella oil (1000 mL) was then fractionated in Laboratory of Chemistry at LIPI by vacuum fractionation with packed column and reflux ratio of 10:5 and 5:10, at 0 mbar pressure as shown in Figure 1. The process of fractionation of essential oils was carried out based on the major compounds data and area (%) of the essential oils that had been obtained from GC-MS analysis at 0 mbar pressure (Table 3) (Anwar et al, 2019a). Afterwards, citronella oil fraction was identified by GC-MS.

RESULTS AND DISCUSSION

The fractionation process was carried out using vacuum fractionation at 0 mBar pressure and reflux ratio of 10:5 and 5:10. The results of fractionation in these conditions can be seen in Table 4.

The fractionation process of citronella oil is done by varying the amount of distillate volume. This distillate volume refers to the results of identification by GC-MS of citronella oil. Fraction 1 (F1) has major compound D-Limonene (72.89%), Fraction 2 (F2) has major compound Citronellal (50.13%), Fraction 3 (F3) has major compound Citronellal (74.89%), Fraction 4 (F4) has major compound Citronellal (88.56%), Fraction 5 (F5) has major compound Citronellal (84.89%), Fraction 6 (F6) has major compound Citronellal (55.38%), Fraction 7 (F7) has major compound Citronellol (57.42%), Fraction 8 (F8) has major compound Citronellol (44.73%), Fraction 9-1 (F9-1) has major compound Geraniol (65.56%), Fraction 9-2 (F9-2) has major compound Geraniol (64.41%) and residue has major compound Geraniol (32.04%). The best results of citronellal fraction are obtained by F4 (88.56%). From the five fractions (F2-F6), the fraction F4 has high citronellal area and fraction F5 has high purity (95), the fraction F7 has high citronellol area and same purity (98), fraction F9-1 has high geraniol area and purity (94).



1. Stirring motor
2. Magnetic stir bar
3. Heating jacket
4. Feeding pump
5. Feed thermocouple
6. Distillation column
7. Column heating jacket
8. Cooling
9. Pressure difference measuring device
10. Thermocouple peak
11. Reflux unit
12. Manometer
13. Condenser
14. Vacuum sensor
15. Vacuum hose
16. Distillate coolant
17. Container bottle
18. Fraction container
19. Fraction divider
20. Motor dividers
21. Control panel
22. Trap tube
23. Vacuum controller faucet
24. Vacuum pump
25. Aluminium frame

Figure 1. Bench scale fractionation distillation unit (Agustian E, Sulaswatty. A, 2005)

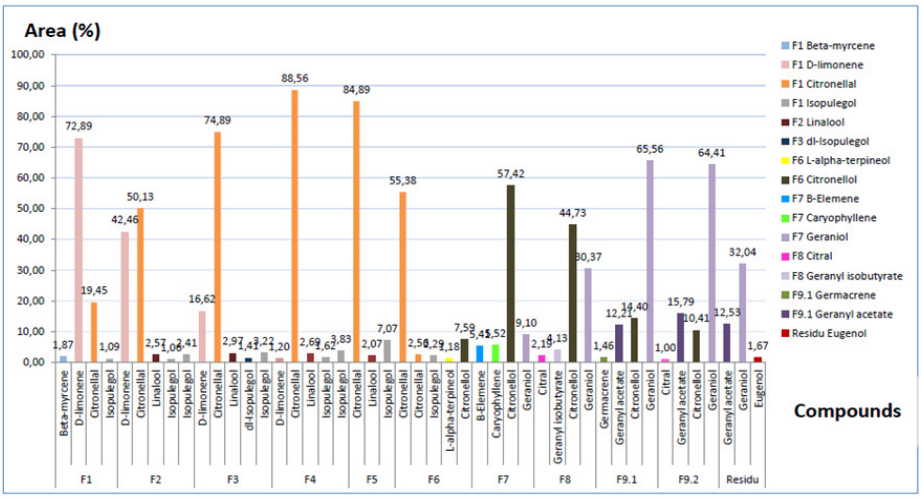


Figure 2. Fraction of *Cymbopogon nardus* (L.) rendle with the highest compounds

Figure 2 shows the fraction with the highest component. The difference in yield obtained is influenced by fractionation time and reflux ratio (whether 10:5 or 5:10). According to Egi et al., one method to improve the efficiency of the separation process is to use a reflux technique, i.e., some products are returned to the system to move from the liquid phase to the vapor phase. (Siwi & Rusli, 2013). The optimum reflux ratio to obtain high D-Limonene, citronellal, citronelol and geraniol purity in this study is 10:5.

CONCLUSION

D-Limonene, citronellal, citronellol and geraniol are the main compounds of citronella oil which can be separated using vacuum fractionation distillation. The difference in the volume of distillate, reflux ratio and the time of fractionation distillation greatly influences the purity of each major compound.

Table 3. The results of fractionation of the essential oil of *Cymbopogon nardus* (L.) rendle (Anwar et al, 2019a)

Name of Fraction	Major Compound	R TIME	% AREA	SI	Volume (ml)
F1	Beta Myrcene	8.063	1.26	96	25
	dl-Limonene	9.387	55.90	99	
	dl-Limonene	9.649	2.96	98	
	Citronelal	22.282	36.38	98	
	L-linalool	25.863	1.51	91	
	Isopulegol	26.863	1.38	99	
F2	D Limonene	9.339	17.17	99	137.96
	Citronelal	22.349	76.00	98	
	Linalool	25.686	2.81	97	
	Isopulegol	26.530	1.22	99	
	Isopulegol	26.878	2.81	99	
F3	dl-Limonene	9.330	1.46	99	12.38
	Citronellal	22.378	91.12	98	
	Linalool L	25.868	2.78	97	
	Neo-isopulegol	26.530	1.37	99	
	Isopulegol	26.873	3.26	99	
F4	dl-Limonene	9.335	1.03	98	348.27
	Citronellal	22.359	90.10	98	
	Linalool L	25.868	1,96	91	
	Neo-isopulegol	26.530	1.85	99	
	Isopulegol	26.878	5.06	99	
F5	Citronellal	22.282	44.77	98	3.41
	Alpha-terpinolene	25.882	1.06	95	
	Neo-isopulegol	26.535	1.89	99	
	Isopulegol	26.882	5.65	99	
	Beta-elemene	28.697	1.87	99	
	Citronellol Acetate	32.268	4.33	94	
	Geranyl Acetate	37.244	1.75	91	
	Citronellol	37.625	25.37	98	
	Geraniol	41.716	13.32	97	
F6	Citronelal	22.206	4.27	98	147.12
	Isopulegol	26.873	1.40	99	
	Beta-elemene	28.735	8.71	99	
	Caryophyllene	29.087	10.92	94	
	Citronellol Acetate	32.287	11.79	94	
	Citronellol	37.692	58.22	98	
	Geraniol	41.692	4.69	97	
F7	Citronellol Acetate	32.302	18.98	94	25
	Geranyl Acetate	37.264	1.29	91	
	Citronellol	37.692	64.84	98	
	Geraniol	41.716	13.77	97	

Table 3. continued

Name of Fraction	Major Compound	R TIME	% AREA	SI	Volume (ml)
F8	Citronellol Acetate	32.282	11.82	94	125.68
	Geranyl Acetate	37.263	6.03	91	
	Citronellol	37.654	37.42	98	
	Geraniol	41.773	44.72	96	
F9	Geraniol	41.754	41.37	96	198.4
	Geranyl Acetate	37.249	14.83	91	

Table 4. The results of fractionation of the essential oil of *Cymbopogon nardus* (L.) rendle

Name of fraction	Mayor Compounds	Area (%)	SI	T head	T flash	Vol.theoritical (Vol. Real) (ml)	Reflux
F1	Beta-myrcene	1.87	96	67.0	97.0	10.285 (9)	10 : 5
	D-limonene	72.89	99				
	Citronellal	19.45	93				
	Isopulegol	1.09	94				
F2	D-limonene	42.46	99	80.4	100.3	42.79 (45)	10 : 5
	Citronellal	50.13	90				
	Linalool	2.57	97				
	Isopulegol	1.06	95				
	Isopulegol	2.41	97				
F3	D-limonene	16.62	99	81.3	99.2	11.455 (13)	10 : 5
	Citronellal	74.89	94				
	Linalool	2.97	96				
	dl-Isopulegol	1.41	96				
	Isopulegol	3.22	99				
F4	D-limonene	1.20	99	80.0	106.6	441.99 (301)	10 : 5
	Citronellal	88.56	94				
	Linalool	2.69	96				
	Isopulegol	1.62	93				
	Isopulegol	3.83	99				
F5	Citronellal	84.89	95	84.8 - 85.7	116.7	441.99 (143)	5 : 10
	Linalool	2.07	96				
	Isopulegol	7.07	99				
F6	Citronellal	55.38	95	87.5 - 89.7	115.5 - 116.7	2.63 (2.9)	10 : 5
	Citronellal	2.56	58				
	Isopulegol	2.29	96				
	L-alpha-terpineol	1.18	95				
	Citronellol	7.59	98				
F7	B-Elemene	5.41	91	89.8 -105.2	89.8 - 125.5	206.19 (206)	5 : 10
	Caryophyllene	5.52	99				
	Citronellol	57.42	98				

Table 4. continued

Name of fraction	Mayor Compounds	Area (%)	SI	T head	T flash	Vol.theoritical (Vol. Real) (ml)	Reflux
	Geraniol	9.10	94				
F8	Citral	2.19	97	105.5 - 130.7	105.5 - 125.5	3.225 (4)	10 : 5
	Geranyl isobutyrate	4.13	90				
	Citronellol	44.73	98				
	Geraniol	30.37	94				
F9.1	Germacrene	1.46	99	130.7 - 150.5	125.5 - 165.9	192 (192)	5 : 10
	Geranyl Acetate	12.21	91				
	Citronellol	14.40	98				
	Geraniol	65.56	94				
F9.2	Citral	1.00	94	101 - 101.6	193 -326	88 (3)	5 : 10
	Geranyl Acetate	15.79	91				
	Citronellol	10.41	98				
	Geraniol	64.41	94				
Residue	Geranyl Acetate	12.53	91				
	Geraniol	32.04	94				
	Eugenol	1.67	98				

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