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Chemical Composition of Oil Fraction Kaffir Lime (*Citrus hystrix DC*) as Antibacterial Activity of *E.coli*

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ABSTRACT

The purpose of this research is to investigate the composition of oil fraction kaffir lime which is consists as antibacterial activity of *E. coli*. This research was applied a branch kaffir lime to produce oil using fractional distillation (PiloDist 104-VTU) number the stages 120 and reflux ratio 20/10 with 5 mbar pressure. Oil kaffir lime composition was analyzed using GC-MS (type Shimadzu QP 2010S) by helium as a carrier gas with flow rate of 3mL/min. Antibacterial activity assay was employed agar well diffusion which conducted at three concentrations (500, 300, and 100 µL/mL). The result of oil fraction kaffir lime was afforded five fraction oil based on boiling point interval, such as A fraction oil (63.00–70.010^{o}C), B fraction (71.30–70.800^{o}C), C fraction (74.50–74.200^{o}C), D fraction (74.20–74.000^{o}C) and E fraction (72.90–91.100^{o}C). All fractions contained oxygenated monoterpene (MO), except A oil fraction which comprises hydrocarbon monoterpene composition (MH) with a yield of 12.1%. The main components of a fraction which MO compound they are citronella, linalool and isopulegol, while in MH compound they are sabine, β-pinene, β-micrene and limonene. The result of antibacterial activity assay obtained at the highest concentration (500 µL/mL). Antibacterial activity assay also depends on the fraction composition with higher composition of MO. The highest MO component of oil fraction was found on C oil fraction which has MO component such as citronella 74.94%; linalool 20.13%; and isopulegol 3.08%.

Key word: Kaffir lime oil fraction, antibacterial activity, *E. coli*, hydrocarbon monoterpenoid, oxygenated monoterpenoid

INTRODUCTION

Deaths caused diseases via bacterial infections are a serious problem in the world of health. In recent years, the pharmacology industry has produced a number of new antibiotics, however bacterial resistance of drugs are increasing[1]. This situation provides findings to new antimicrobial substances from various sources such as herbs. Antimicrobial compounds derived from plants are capable of inhibiting the growth and proliferation of pathogenic microorganisms[2]. Essential oils can be an alternative option for disease prevention attributed to bacterial infections.

One of oil which has antibacterial activity is kaffir lime (Citrus hystrix DC). Kaffir lime oil can be obtained from some part of a plant. Moreover, this research has applied a branch of the plant to increase the economist value. Some of kaffir lime oil composition which collected from some part has been reported. Essential oil obtained from kaffir lime leaf composed of citronella (23.41%), α-terpineol (5.40%), and linalool (4.36%)[3]. The main

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component of crude kaffir lime is β-pinene (22.54%), d-limonene (22.03%) and terpinene-4-ol (17.37%)[4]. The main component of kaffir lime branch oil such as citronella (46.40%), linalool (13.11%), citronellol (11.03%), and sabinene (5.91%)[5]. The major component of kaffir lime oil is hydrocarbon monoterpene (MH) compound such as β-pinene, d-limonene, and sabinene. Another component such as citronella, β-citronellol, and linalool which is oxygenated monoterpene compound (MO) was contained in kaffir lime oil component. The variation of compound composition MH and MO may influence the biological activity.

Research regarding the activity of citrus oil has been widely reported, one of them about the composition of MO on the kaffir lime oil higher than MH which has twice antioxidant activity [27]. The difference of MH and MO composition can be obtained by fractionation distillation technique which is conducted under pressure. This method affords several fractions of kaffir lime oil which have a composition MH and MO[27]. The fractional oils with various MH and MO compositions were assayed for their activity using *E. coli* bacteria. This research discussed about the composition of fraction oil kaffir lime which consists of antibacterial activity *E. coli*.

EXPERIMENT

Chemicals and instrumentation

The instruments applied in this research are fractional distillation (PiloDist 104-VTU column length 2 m and number the stages 120), and GC-MS (Gas Chromatography-Mass Spectrophotometer) type Shimadzu QP 2010S. Other instruments are Erlenmeyer, and beaker glass, tube reaction, vortex, incubator, Petri dish, spatula, cotton, pin set, coke bore, micro pipet, microscope, autoclave, and hemacytometer.

Chemicals used are kaffir lime oil which obtained from the farmer products in Ngunut, Tulungagung, Indonesia. Some chemicals are sodium sulfate anhydrate (Merck), NA medium (Nutrient Agar) (Merck), NB medium (Nutrient Broth) (Merck), distilled water, alcohol, and bacteria *E. coli* which is collected from Microbiology Laboratory at Faculty of Mathematics and Natural Sciences, Brawijaya University.

Procedure reaction

Kaffir lime oil Fractional Distillation

2L of kaffir lime oil obtained from the fractional distillation of kaffir lime branch was put into round flask then distilled at low pressure until 5 mbar and ratio reflux 20/10. The fractionation result was analyzed by GC-MS.

Analysis GC-MS (Gas Chromatography-Mass Spectrophotometer)

1 µL of kaffir lime oil fraction which collected by syringe then was injected to GC-MS (type Shimadzu QP 2010S) with capillary column (length 30 m, inside diameter 0.25 mm). The operation condition as follows: electron bombardment system was applied in ionization energy of 70eV (injector temperature 300°C; detector temperature 320°C; pressure 12 Pa). The first column temperature was maintained at 50° C then it was increased to 260°C with temperature program 5°C/minute. Helium was used as carrier gas with flow rate of 3 mL/minute. The component was identified by comparing retention time and compound structure which is accordance with mass spectra on the library from system GC-MS [6].

Agar Well Diffusion Assay

Antibacterial activity assay of essential oil was conducted by using agar well assay method [7]. The step was initiated by producing bacteria suspension. The sterile liquid NB

medium was prepared into tube reaction. 1 ose culture from agar miring then was inoculated to NB medium. The next medium is a vortex which was homogenized and incubated for 24 hours at 37°C in the incubator. After that, the Petri dish with NA agar was added bacteria suspension then was assayed using the cell suspension in the amount of 10^6 CFU/mL (100 µL) by using a micropipette, and spread it. Afterward, a hole was fabricated on NA agar medium by using coke bore using the diameter 6 mm. each sample solution was added into the well diffusion hole. Fraction oil sample was maintained in solution concentration of 500, 300, and 100 µL/mL, alcohol as the positive control, and distilled water as the negative control for 24 hours on 37°C. The inhibit zone was measured by using a caliper.

Data Analysis

The inhibition zone data was processed by using Analysis of Variance (ANOVA) by using SPSS 16. The result was determined by significant level 5% (α=0.05).

RESULT AND DISCUSSION

Fraction Oil Composition

Branch kaffir lime oil afforded five oil samples, such as A, B, C, D, and E fraction oil. The five fraction oils was analyzed their composition by using GC-MS. The analysis result of each fraction oil composition was shown in Table 1.

Tabel 1. Composition of Kaffir Lime Oil Fraction

No	Compound Name	Component presentation (%)				
		A Fraction	B Fraction	C Fraction	D Fraction	E Fraction
1.	Sabinene	19.83	9.00	-	-	-
2.	β-Pinene	6.99	2.38	-	-	-
3.	β-Micrene	7.69	4.14	-	-	-
4.	Cis-Ocimene	1.48	1.24	-	-	-
5.	γ-Terpinene	3.00	2.73	-	-	-
6.	Limonene	4.38	3.1	-	-	-
7.	β-Ocimene	7.00	6.92	-	-	-
8.	α-Tujene	0.63	-	-	-	-
9.	α-Pinene	2.27	-	-	-	-
10.	2-Carene	2.18	1.26	-	-	-
11.	Sikloheksanon	-	-	-	2.3	-
12.	α-Terpenilona	1.1	1.23	-	-	-
13.	Linalool	8.17	12.94	20.13	6.13	0.65
14.	Citronella	32.48	50.65	74.94	84.86	36.83
15.	Isopulegol	1.26	1.85	3.08	4.35	2.09
16.	4-Terpeneol	0.64	0.79	1.85	1.97	-
	% Identification	89.99	90.98	100	99.61	39.57

Information: boiling point interval: A oil fraction (63.00–70.010°C), B fraction (71.30–70.800°C), C fraction (74.50–74.200°C), D fraction (74.20–74.000°C) and E fraction (72.90–91.100°C)

The five oil compositions comprised 16 components with the purity of 89.99%; 90.98%; 100%; 99.61%; 39.57%. All compositions were identified as hydrocarbon (MH) and oxygenized (MO) monoterpenoid compound. MH compound investigated on A fraction oil was presented 10 compounds, such as sabinene (19.83%), β-micrene (7.69%), β-ocimene (7.00%), meanwhile on MO compound was found 6 compounds such as citronella (32.48%), linalool (8.17%), and isopulegol (1.26%). B fraction oil obtained MH compound such as sabinene (9.00%), β-ocimene (6.92%), β-micrene (4.14%) and MO compound which contained citronella (50.65%), linalool (12.94%), isopulegol (1.85%). The C, D, and E fraction oil showed the biggest MO compound such as citronella, linalool, and isopulegol

with the different overflow. The similar composition of kaffir lime oil has been reported, the component of kaffir lime oil which obtained from leaf contains β-citronellol (6.59%), linalool (3.90%) and citronellol (1.76%) [8]. A-E fraction component was obtained slightly because A-E component fraction has the higher boiling point which represented by MO compound (Table 1).

Antibacterial Activity Assay

The *Escherichia coli* antibacterial activity assay results showed inhibition zone at each concentration (100, 300, and 500 µL/mL). The result of inhibition zone on five fraction oil was shown on Figure 1.

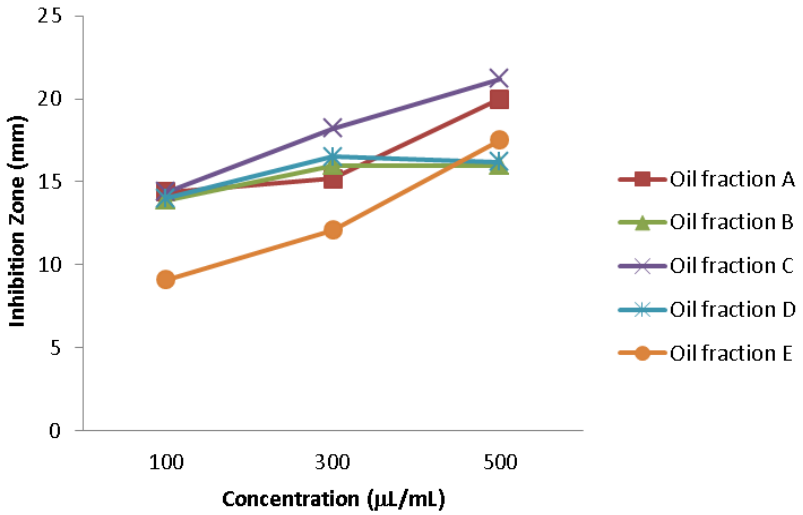

Figure 1. Correlation between inhibition zone with concentration in the five oil fraction

This figure showed that A fraction oil obtained inhibition zone in the amount of 14.4, 15.2, and 20.0 mm, B fraction oil obtained 13.9, 16.0, and 16.0 mm, C fraction oil obtained 14.4, 18.2, and 21.2 mm, D fraction oil obtained 14.0, 16.5, and 16.2 mm, and E fraction oil obtained 9.1, 12.1, and 17.5 mm. This result indicated the greater concentration will increase inhibition zone. Concentration gives effects to the active component of oil[9].

Five fraction oils gave the different result of inhibition zone. The increase of oil component composition is different which can be known as MO compounds such as citronella and linalool that attribute to antibacterial activity and fungus [10,11,12]. Moreover, another component of *W. urticifolia* oil such as β-pinene, limonene, γ-terpinene, α-phellandrene, β-caryophyllene, and germacrene has significant antibacterial activity [13-17]. The relation of MH and MO as main oil component composition in antibacterial activity was shown in Figure 3. All of oil fraction component contain MO compound, therefore the less MO will decrease the antibacterial activity. It was shown on C, D, B and E fraction (Figure 2). MO compound has better antibacterial activity than MH compound due to its hydroxyl group[18], especially the phenolic group which explained into alkylation substitution to the phenol core that can enhance the antimicrobial activity [19]. The component of MO compound has the phenolic group such as citronella which can change the cell morphology by influencing cell osmotic pressure. Therefore, it can disrupt the membrane cytoplasm and caused cell constituent leak [20]. Another hydroxyl group compound such as aldehydes can produce the same activity. It was due to the double bond on carbon which has high electronegativity that can increase antibacterial activity[18,21,22]. The component consist of

the aldehydes group such as citronella can damage the cell membrane, produce permeability change, and release the cell content that also can disrupt the cell metabolism and energy generator [23, 24, 25].

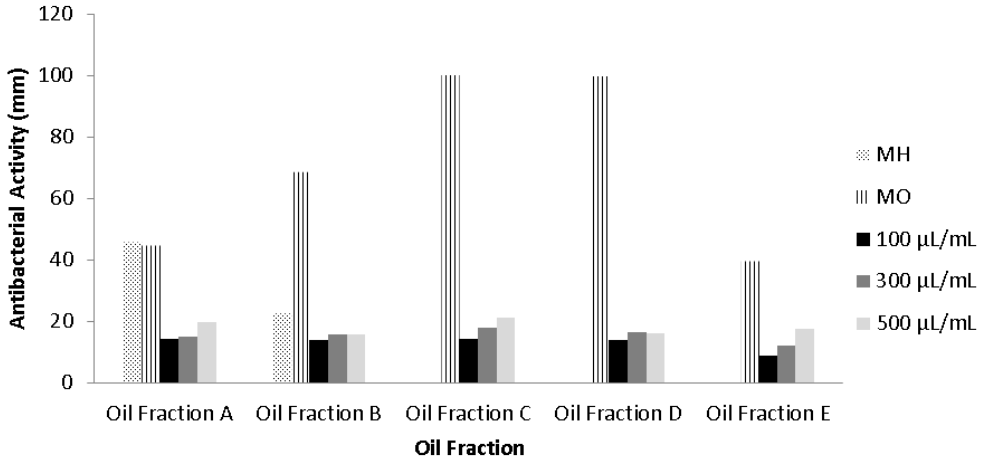

Figure 2. The relationship between monoterpene hydrocarbon (MH) and oxygenized (MO) with antibacterial activity and its concentration

The different result as shown on A fraction, which was compose of MH and MO compound component has better antibacterial activity than E fraction (Figure 2). A fraction component investigated that has MH compound such as sabinene (19.83%), β-pinene (6.99%), limonene (4.38%), and some of MH compound has been known has antibacterial activity. The component such as α-pinene and β-pinene has been reported can destroy cellular integrity, inhibit the respiration, ion transport process, and increase the membrane permeability on yeast cell and mitochondria which have been isolated[26,27]. Other research also reports that limonene oil also has bacteriostatic characteristic on some microorganism[13,14,30]. The result of MH compound with some micro composition shows their contribution to antibacterial activity which gives synergy effect in this research[31].

Antibacterial activity potency on the essential oil generally hard to explain, however in some mechanism possibilities has been proposed such as hydrophobic characteristic of oil which can partition into the fat from membrane cell and caused a leak of cell content[32,33]. The other characteristic of oil such as lipophilic, functional group, and oil volatility which has correlation between bi-layer phospholipid transport electron, translocase protein, gradient ions, phosphorylation and other reaction enzyme, which can increase the bacterial permeability [32,34,35]. Therefore, antibacterial activity not only depends on MH and MO compound composition but also another factor such as volatility, hydrophobic, and lipophilic of the oil itself.

The statistic data showed the result of F arithmetic 1.322 with significance 0.277> 0.05 which indicated no effect of oil fraction composition on antibacterial activity. Antibacterial activity is determined by each oil constituent such as citronella, linalool, and other compounds that determine the amount of antibacterial activity.

CONCLUSION

Kaffir lime oil fraction has been successfully investigated the chemical composition by fractional distillation which afforded five oil fraction that contains MO and MH compound. The majority of component is MO except A fraction. MO compound on five fractions

compose of citronella, linalool, and isopulegol which obtained antibacterial activity. C fractions afforded the highest antibacterial activity with 500 µL/mL concentration.

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