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Chemical Content Profile of Essential Oil from Kaffir Lime (*Citrus hystrix* DC.) in Tanah Datar Regency and Antibacterial Activity

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ABSRACT
Essential oil is one type of vegetable oil with many benefits, with the main characteristics of being volatile and having a distinctive aroma. In addition, the essential oil from kaffir lime has been reported to have various bioactivities: antioxidant, antibacterial, antileukemic, and antitussive. This study has several aims: firsts to identify chemical compounds using the GC-MS instrument, secondly to determine the value of Minimum Inhibitory Concentration (MIC) with the agar dilution method, and next to compare chemical compounds and antibacterial activity of kaffir lime peel and leaves (*Citrus hystrix* DC.) from Tanah Datar Regency. The essential oil was isolated by the water distillation method for 4 hours and then analyzed using GC-MS. The results of the identification of the main chemical compounds of kaffir lime peel essential oil are D-Limonene (17.10%), 3-Carene (13.77%), and γ-Terpinene (12.56%) While the essential oil of kaffir lime leaves contains the main chemical compounds are Citronellal (61.31%), Citronellol (10.62%) and 3-Carene (6.61%). The Minimum Inhibitory Concentration (MIC) value of kaffir lime peels essential oil is 50-100 mg/mL, while 25-50 mg/mL from the leaves. The antibacterial activity of kaffir lime leaf essential oil is stronger than that of kaffir lime peel essential oil, which is influenced by differences in the content of its chemical compounds.

Keywords: *essential oil, kaffir lime, peels, leaves, antibacterial*

1. INTRODUCTION

Plant of the genus Citrus with the family Rutaceae are plants mostly distributed throughout the world and have 203 species [1]; one of them is *Citrus hystrix* DC. *C. hystrix* DC. (kaffir lime) is a tropical plant that is widespread in many countries, especially in Southeast Asia (including Indonesia, Malaysia, Philippines, Laos, Thailand, and Vietnam); India, China [2]–[6]. This plant can grow to a height of 3-6 m and has green leaves that are oval-shaped, double-pointed with a fragrant smell, with a length of 7.5–10 cm. It has white fragrant flowers with 4-6 petals and dark green to yellow ellipsoid fruit around 5.0–7.5 cm with a wrinkled fruit surface [4], [7].

The fruits and leaves of citrus species contain various important active compounds, including vitamin C, folic acid, potassium, flavonoids, coumarins, pectin, and dietary fiber [7]. *C. hystrix* Fruit is rich in phenolic compounds. These compounds include flavonoids, glyceroglycolipids, α-tocopherols, limonoids, furanocoumarins, benzenoid derivatives, coumarin quinolinone alkaloids, glycosides, saponins, tannins, hydrocarbons, and fatty acids [4]. Kaffir lime peel contains terpenoids as the main chemical content. The most important volatile substances are β-pinene (30.6%), limonene (29.2%), and sabinene (22.6%). The leaves contain the main chemical content of citronellal (65.4%) and phenolic compounds [2], [8]. The leaves are reported to be rich in phytochemicals containing phenolic and carboxylic functional groups [9]. Citrus family essential oils usually have a volatile fraction of around > 90%. Monoterpenes and sesquiterpenes are in the volatile fraction, with limonene as the main compound [10].

In traditional medicine, this fruit is used for headaches, inflammation, flu, fever, sore throat, bad breath and digestive disorders, hypertension, stomach pain, diarrhea in infants, flavoring, eliminating body odor [5]. The leaves are also used in traditional medicine to maintain healthy teeth and gums and cure scurvy. Kaffir lime peel and leaves are also a source of phenolic compounds and antioxidants. Several bioactive compounds from various

citrus fruits have been shown to inhibit the proliferation of cancer cells [2].

C. hystrix DC is believed to prevent and fight various types of cancer, anti-inflammatory and also used as traditional medicine [4]. Kaffir lime peel and leaves are also a source of phenolic compounds and antioxidants. Several bioactive compounds from various citrus fruits have been shown to inhibit the proliferation of cancer cells. Kaffir lime leaf extract is reported to exhibit antioxidant, anticancer, and anti-inflammatory activities [2]. The findings revealed that *C. hystrix* leaf extracts active compounds (citronellol and citronellal) could strongly inhibit tumor cell growth and trigger apoptosis through overexpression of cleaved caspase-3 and Bax. Next, downregulating anti-apoptotic enzymes Bcl-2 [4].

Essential oils are secondary metabolites widely distributed in higher plants; more than 23,000 terpenoid structures have been identified, and these are becoming important. This essential oil has various biological activities such as: fragrance, insect attraction, antiseptic, antioxidant, anticancer, anti-inflammatory, immunomodulatory, antiprotozoal agent, antimicrobial, as well as in the treatment of neurodegenerative diseases, diabetes, and hyperpigmentation, and is also widely used in agriculture, preservation food, skin therapy, aromatherapy, massage and medicine [6], [8], [10]–[12]. In addition, the essential oils from the fruits and leaves of the species *Citrus* are commercially used as a flavoring, fragrance, spices, perfumery, medical treatment, and aromatherapy [7].

C. hystrix essential oil is used as aromatherapy, various cosmetic and beauty products. While in traditional medicine, *C. hystrix* is used to treat flu, fever, hypertension, abdominal pain, and diarrhea in infants. The fruit is used as a digestive stimulant, blood purifier and lowers high blood pressure. In addition, the fruit is used in cooking for flavoring and the production of shampoo as an insecticide for washing heads. The fruit juice is used to soften the skin. The mixture of fruit juice with bath water can be used to get rid of body odor. *C. hystrix* Essential oils have been reported to have various bioactivities such as antioxidant, antibacterial, antileukemic, and antitussive [7].

2. MATERIAL AND METHODS

2.1 Chemical and Microorganism

Chemicals and analytical solvents are used during the study. The test microorganisms used are: *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 25175 represent gram-positive bacteria; Methicillin-Resistant *Staphylococcus aureus* ATCC 43300 represent resistant gram-positive bacteria; *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* FNCC 9027 representing gram-negative. These bacteria were taken from the stock of the Microbiology Laboratory of the University of North Sumatra, Medan, Indonesia, and the Food and Drug Administration. All test bacteria were stored at 37°C on nutrient agar slanted media and used as stock cultures during the experiment.

2.2 Plant Collection

Fresh kaffir lime (*Citrus hystrix* DC) peels and leaves were taken from Nagari Tanjung Barulak, Tanah Datar Regency, West Sumatera, Indonesia in January 2021. Both were identified by botanists Dr. Nurainas in herbarium ANDA, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia.

2.3 Isolation of essential oil

The essential oil was isolated by hydrodistillation method. First, about 6 kg of fresh kaffir lime fruit and 2 kg of fresh kaffir lime leaves were sorted and washed with clean water to remove dirt and soil. Next, the fruit is peeled using a knife from the outside to the inside. As a result, only 2 kg of the skin is obtained. Then the skin of the fruit and leaves are chopped to get smaller pieces and then transferred to a distillation flask. The extraction process was carried out for several 4 hours. Essential oils are stored in dark containers and refrigerators at 4°C.

2.4 Essential Oil Analysis using GC-MS

The volatile oil samples obtained were analyzed using a GC-MS (*Gas Chromatography - Mass Spectrometry*), with the analysis conditions as follows:

Instrument	: GC Agilent® 7890A
Detector	: MS Agilent® 5975C
Column	: HP-5ms (Agilent®), diameter 0.32 mm, thickness 0.25 µm, length 30 m
Gas Flow Rate	: 1 ml/menit
Column Temperature	: 50-300°C (temperature 50°C constant for 2 minutes, the temperature is raised to 80°C with an average increase 2°C/minute, then expanded to 150°C with an average increase 5°C/minute, then raised to 200°C with an increase 10°C/minute and then raised again until 300°C with an average increase 20°C/minute. At temperature 300°C held constant for 5 minutes)

Injector Temperature : 250ºC (constant)

Detector Temperature : 270ºC, energy 1,25 kV Gas

Carrier : Helium

Column Pressure : 70 kPa

Injection Volume : 1µL [13]

Chemical constituents of essential oils are identified by comparing them with mass spectral database libraries *National Institute of Standards and Technology* (NIST). The composition is reported as a percentage of the total peak area using the following formula:

% Relative of peak area = (Peak area / Total peak area) × 100

2.5 Antibacterial Activity

The dilution test method was carried out using *microtiter plate 96-well* in the *Laminar Air Flow* (LAF). The test sample was dissolved in DMSO at a 1000 mg/mL concentration and diluted with MHB to concentration 100 mg/mL. In rows, B-F each is added 50 µL MHB. As 50 µl concentration test solution with concentration100 mg/mL added to rows A-B in *microtiter plate 96-well*. Then pipetted as much as 50 µL from line B and put in line C. the process is repeated until row F. As 50 µL from row F is discarded until the concentration of 100; 50; 25 12.50; 6.25; and 3.13 mg/mL. As 50 µl of bacterial suspension was added (diluted 1/150 in MHB) and then homogenized. The positive control used was the solubility of ciprofloxacin, and the negative control was DMSO. The positive control ciprofloxacin with a concentration of 3 mg/mL was diluted to a concentration of 0.3 g/mL, and the dilution was carried out in stages at *microtiter plate 96-well* so that the concentration of ciprofloxacin becomes 0.30; 0.15; 0.08; 0.04; 0.02; and 0.01; µg/mL. Then the plate was incubated for 18 hours at 37°C. After 18 hours, 40 µl MTT (methyl thiazolyl diphenyl-tetrazolium bromide)was added to each microtiterplate well *96-well* and incubated again for 30 minutes 37ºC. Observation of the growth of the test bacteria was marked by a color change in the *microtiterplate 96-well* becomes a visually observable blue color. The minimum inhibitory concentration value can be determined by looking at the smallest concentration that does not indicate the occurrence of bacterial growth. The test is carried out with three repetitions [14].

3. RESULT AND DISCUSSION

The isolation of essential oils and the characterization of the test essential oils can be seen in **Table 1.** The percent yield of essential oil of fruit peel is greater than that of the leaves, while the specific gravity and refractive index are not significantly different. This expression appears because their chemical compounds influence specific gravity values and the refractive index of essential oils.

Table 1 Yield result and characterization of kaffir lime essential oil from peels and leaves

Parameter	Peels	Leaves
% Yield	1.74	0.91
Density (g/ml)	0.87	0.86
Refractory index	1.47	1.45

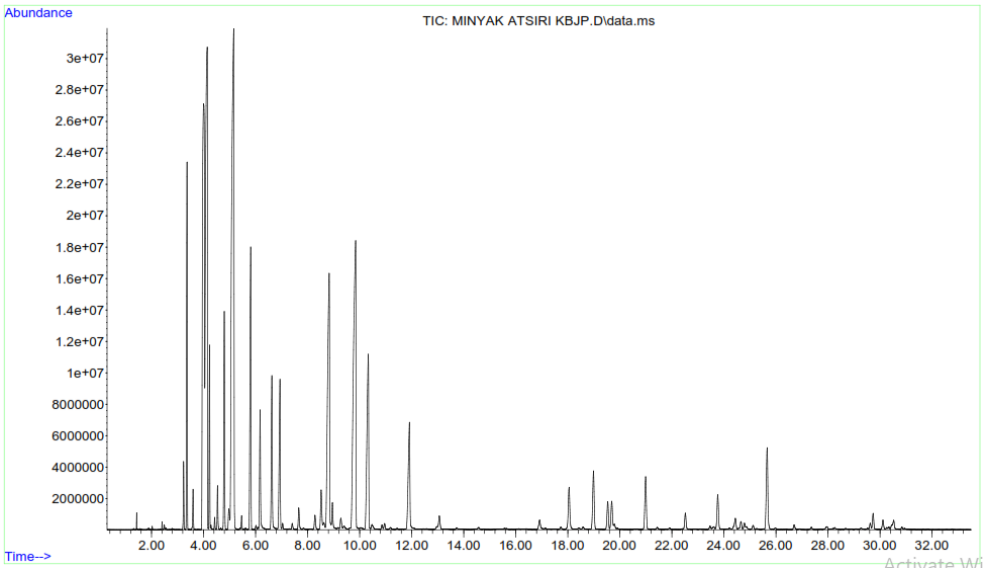
3.1 Analysis of Essential Oil

In the GC-MS instrument, volatile chemical compounds will come out first to the detector so that the retention time is faster. The boiling point of a compound greatly affects the retention time; a compound with a high boiling point has a relatively long retention time compared to a compound with a low boiling point. In addition, the retention time of chemical compounds is also influenced by the interaction between the stationary phase and the compound. The stationary phase used in the GC-MS instrument in this study is non polar so that the more polar compounds will come out first, and the more nonpolar compounds will be retained longer in the column.

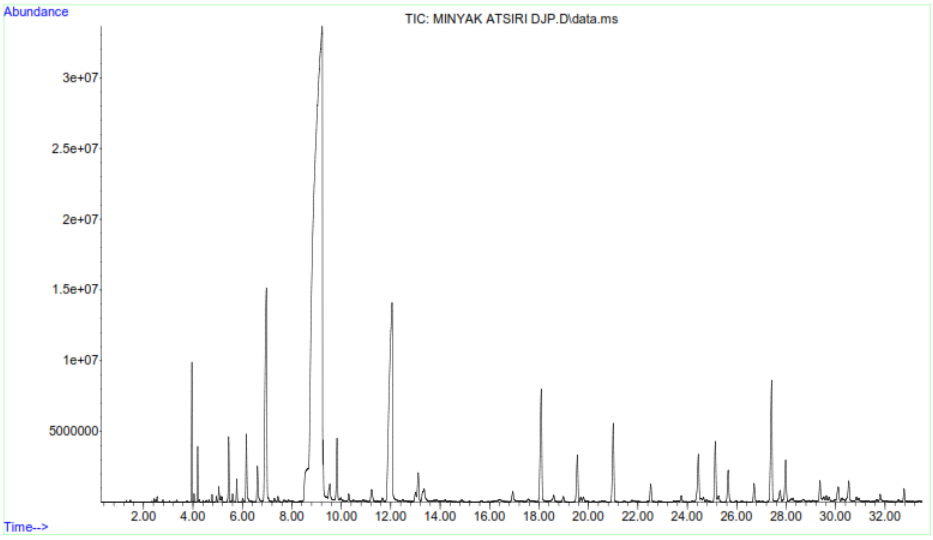
Essential oil chemical compounds consist of terpene group compounds and other volatile chemical compounds. The dominant chemical compounds found in essential oils are the terpenoid group, and the majority are from the monoterpene and sesquiterpene groups. The precursor to the terpenoid group is derived from the isoprene unit (C5). The monoterpene group is derived from two isoprene units while the sesquiterpene group is derived from three isoprene units. Based on the boiling point the monoterpene group (C10) has a lower boiling point than the sesquiterpene group (C15), so it will affect the retention time. In addition, the chemical compounds of essential oils are generally divided into hydrocarbon groups and oxygenated hydrocarbon groups. The hydrocarbon group consists of the elements hydrogen (H) and carbon (C), which are compounds of terpenes

and aromatic hydrocarbons. While the oxygenated hydrocarbon group consists of the elements hydrogen (H), carbon (C) and oxygen (O), namely alcohol compounds, aldehydes, ketones, oxides, esters, and ethers [15].

The results of GC-MS essential oil of fruit peel can be seen in **Figure 1**. the chromatogram of GC-MS results, the higher the peak of a chemical compound, the greater the percentage of its content, and vice versa.



(a)



(b)

Figure 1 The GC-MS Chromatogram of Essential Oil From Kaffir lime peels (a) and leaves (b)

Based on the GC-MS results, the chemical compounds identified in the kaffir lime peel essential oil contained 26 chemical compounds, while the kaffir lime leaf essential oil contained 13 chemical compounds. The chemical compounds of kaffir lime peel consist of hydrocarbon monoterpenes (74.48%), oxygenated monoterpenes (13.74%), hydrocarbon sesquiterpenes (9.58%), and other compounds (2.20%). While the chemical compounds of kaffir lime leaf essential oil consist of hydrocarbon monoterpenes (14.72%), oxygenated monoterpenes (77.68%), and hydrocarbon sesquiterpenes (7.60%). This research shows that the fruit peel essential oil has a higher hydrocarbon terpene content than the kaffir lime leaf essential oil. While the essential oil of kaffir lime leaves has a higher oxidized terpene content than the essential oil of fruit peel. The difference in chemical content between these two essential oil samples will affect their bioactivity. The difference in the chemical content of these two essential oils can be seen in **table 2**.

Table 2 Analysis Chemical Content of Essential Oil From Kaffir lime peels and leaves

No	Chemical Content	Molecular Weight	Peels (%)	Leaves (%)	Molecular Formula	
1	D-Limonene	136.13	17.10		C10H16	hydrocarbon monoterpenes
2	3-Carene	136.13	13.77	6.61	C10H16	hydrocarbon monoterpenes
3	γ-Terpinene	136.13	12.56	1.16	C10H16	hydrocarbon monoterpenes
4	Neoisoisopulegol	154.14	7.28	1.48	C10H18O	oxygenated monoterpenes
5	d-α-Pinene	136.13	3.94		C10H16	hydrocarbon monoterpenes
6	Terpinolene	136.13	2.68		C10H16	hydrocarbon monoterpenes
7	Citronellol	156.15	2.63	10.62	C10H20O	oxygenated monoterpenes
8	δ-Cadinene	204.19	1.88	0.76	C15H24	hydrocarbon sesquiterpenes
9	β-myrcene	136.13	1.69		C10H16	hydrocarbon monoterpenes
10	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	242.15	1.62		C13H22O4	other compounds
11	Copaene	204.19	1.29		C15H24	hydrocarbon sesquiterpenes
12	Caryophyllene	204.19	1.17	1.94	C15H24	hydrocarbon sesquiterpenes
13	2,6-Dimethyl 2,6-octadiene	138.14	1.00	3.17	C10H18	hydrocarbon monoterpenes
14	α-Amorphene	204.19	0.76		C15H24	hydrocarbon sesquiterpenes
15	cis-muurola-3,5-diene	204.19	0.65		C15H24	hydrocarbon sesquiterpenes
16	3-Thujene	136.13	0.58		C10H16	hydrocarbon monoterpenes
17	α-phellandrene	136.13	0.45		C10H16	hydrocarbon monoterpenes
18	Camphene	136.13	0.40		C10H16	hydrocarbon monoterpenes
19	o-Cymene	134.11	0.37		C10H14	hydrocarbon monoterpenes

No	Chemical Content	Molecular Weight	Peels (%)	Leaves (%)	Molecular Formula	
20	α-Caryophyllene	204.19	0.34		C15H24	hydrocarbon sesquiterpenes
21	β-Maaliene	204.19	0.31		C15H24	hydrocarbon sesquiterpenes
22	α-Gurjunene	204.19	0.28	1.32	C15H24	hydrocarbon sesquiterpenes
23	Cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans-(-)-	136.13	0.27		C10H16	hydrocarbon monoterpenes
24	γ-Muurolene	204.19	0.21		C15H24	hydrocarbon sesquiterpenes
25	Isobornyl alcohol	154.14	0.19		C10H18O	oxygenated monoterpenes
26	α-Muurolene	204.19	0.18		C15H24	hydrocarbon sesquiterpenes
27	Citronellal	154.14		61.31	C10H18O	oxygenated monoterpenes
28	α-Farnesene	204.19		3.16	C15H24	hydrocarbon sesquiterpenes
29	β-Terpinene	136.13		1.40	C10H16	hydrocarbon monoterpenes
30	β-Ocimene	136.13		0.89	C10H16	hydrocarbon monoterpenes
31	β-Pinene	136.13		0.69	C10H16	hydrocarbon monoterpenes

Differences in the chemical structure of the components of essential oils give rise to the names of different chemical compounds eventhougt the same molecular formula. In addition, there are also chemical compounds that are given a Roman number because of the location of the double bond.

In the essential oil of kaffir lime peel, there are main chemical compounds: D-Limonene (17.10%), 3-Carene (13.77%), and γ-Terpinene (12.56%), where all three are monoterpene hydrocarbon groups with double bonds or known as alkenes. Kaffir lime leaves contain main chemical compounds: Citronellal (61.31%), Citronellol (10.62%), and 3-Carene (6.61%), where citronella is an oxygenated monoterpene group with an aldehyde functional group. Citronellol belongs to the group of oxygenated monoterpenes with an alcohol functional group. And 3- Carene belongs to the group of hydrocarbon monoterpenes with double bonds, known as alkenes. Warsito, in his study (2017), concludes that kaffir lime essential oil contains the main components β-pinen (21.44%), citronellal (20.91%), limonene (12.59%), and terpinene-4-ol (11.93%), While the essential oil of kaffir lime leaves has the main component of citronellal (85.07%), linalol (3.46%) and sabinene (2.79%) [16]. There are differences in the main chemical compounds of essential oils in the study with the literature results, and this is due to differences in the area where plants grow so that the chemical compounds contained are also different. The chemical content of essential oils in a plant is influenced by the type of plant, climate, growing area, season, soil type, extraction method, and the part of the plant from which the oil is extracted [17], [18].

3.2 Antibacterial Activity

There are differences in chemical compounds in essential oils that can affect their antibacterial activity, as shown in **table 3**.

Table 3. Antibacterial Activity Essential Oil of Kaffir lime peels and Leaves

Strain	MIC		
	Peels (mg/mL)	Leaves (mg/mL)	Ciprofloxacin (µg/ml)
Staphylococcus aureus ATCC 25923	50	25	0.30
Methicillin Resisten *Staphylococcus aureus* ATCC 43300	50	25	0.04
Streptococcus mutans ATCC 25175	100	50	0.08
Escherichia coli ATCC 8739	100	50	0.04
Pseudomonas aeruginosa FNCC 9027	50	25	0.04

Based on the functional group, the antibacterial activity rating shows that the phenol group > aldehyde > ketone > alcohol > ester > hydrocarbon. In addition, this antibacterial activity can also be influenced by the synergy between the chemical compounds it contains [19]. The kaffir lime peel essential oil has the main chemical compounds of the hydrocarbon monoterpene group. In contrast, the kaffir lime leaf essential oil contains the chemical compounds of the oxygenated monoterpene and the hydrocarbon monoterpenes groups. The main chemical compounds of kaffir lime peel are D-Limonene, 3-Carene, and γ-Terpinene consists only of a hydrocarbon group and a double bond. Compared with the main chemical compounds of kaffir lime leaves, namely Citronellal, Citronellol, and 3-Carene, Citronellal has an aldehyde functional group, Citronellol has an alcohol functional group, and 3-Carene has a hydrocarbon group. It can be known and proven the difference in antibacterial activity between the rind of the fruit and the leaves of this kaffir lime. Kaffir lime peel has weaker antibacterial activity than kaffir lime leaves because the main chemical compounds have hydrocarbon groups. Kaffir lime leaves have antibacterial activity twice as strong as kaffir lime leaves because the main chemical compounds have aldehyde and alcohol, functional groups, with high antibacterial activity. In addition, antibacterial activity can also be influenced by the synergy between the chemical compounds it contains.

4. CONCLUSION

The main chemical compounds of kaffir lime peel essential oil are D-Limonene (17.10%), 3-Carene (13.77%), and γ-Terpinene (12.56%). In comparison, the essential oil of kaffir lime leaves contained the main chemical compounds Citronellal (61.31%), Citronellol (10.62%), and 3-Carene (6.61%). Minimum Inhibitory Concentration (MIC) value of kaffir lime peels essential oil 50-100 mg/ml while kaffir lime leaves essential oil 25-50 mg/ml. The aldehyde and alcohol functional groups of the main chemical compounds of kaffir lime leaf essential oil resulted in its antibacterial activity twice as strong as that of the kaffir lime peel essential oil with a hydrocarbon functional group in the main chemical compound.

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