Chemical Contents Profile of Essential Oil from Calamansi (*Citrus microcarpa* Bunge) Peels and Leaves and Its Antibacterial Activities

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ABSTRACT

Citrus is a plant that has many health benefits and has been widely reported for its pharmacological effects. One of the citrus that has been reported to have antibacterial activity is calamansi (*Citrus microcarpa* Bunge). This study aims to determine the chemical content profile of the essential oil of fruit peels and leaves of calamansi. The essential oil was isolated by distillation of water and analyzed for chemical compound content by GC-MS. The methods used to determine the antibacterial activity of essential oils are diffusion and dilution methods. GC-MS results show that the main components of essential oil in the peels of the calamansi are D-limonene (29.52%), (R)-(+)-citronellal (13.76%), 3-isopropenyl-5,5-dimethyl-cyclopentene (8.88%), γ-terpinene (7.30%), citronellol (6.90%), and α-terpineol (4.61%) while the calamansi leaves consist of citronellal (25.74%), citronellol (12.94%), 3-carene (8.43), and β-phellandrene (4.89%). The essential oil of the fruit peels and leaves of calamansi showed antibacterial activity against MRSA, *S. aureus, S. mutans, P. aeruginosa* and *E. coli* bacteria. In the antibacterial activity test, the diffusion method showed that the essential oil of the fruit peels had moderate to strong inhibitory power, while the leaves of calamansi had very strong inhibitory power. In the dilution antibacterial activity test, it showed that the smallest MIC in the essential oil of fruit peels was a concentration of 0.39%, while the essential oil for leaves was 0.2%. The diffusion and dilution method of antibacterial activity test showed that the essential oil of the leaves was more potent than the peels of the calamansi.

Keywords: *essential oil, calamansi, GC-MS, antibacterial, terpenes compounds*

1. INTRODUCTION

Citrus was the first fruit crop to be traded internationally due to its high economic and nutritional worth. Citrus is a term that refers to a variety of orange species and hybrids. Lemon (*Citrus lemon*), lime (*Citrus aurantifolia*), sweet orange (*Citrus sinensis*), and other citrus species have considerable commercial value [1], [2].

Citrus are widely traded because they are a rich source of fiber, which can help avoid gastrointestinal disease In Indonesia, citrus is a fruit commodity that is quite profitable with various advantages.. From low-income everyday life. Calamansi (*Citrus microcarpa*) can be used in both food (seasonings, sauces, and other herbal and cholesterol, as well as a supply of carbs including sucrose, glucose, and fructose [1]. Because oranges are low in fat, salt, and cholesterol, they are frequently consumed by obese persons. Carotenoids, folate, flavonoids, limonoids, coumarins, essential oil alkaloids, and minerals are among the secondary metabolites found in oranges [3]–[5]. Flavonoids and carotenoids, which can be employed as antioxidants, are the most frequent compounds found in oranges [4].

to high-income folks, this fruit can be enjoyed [6]. Calamansi is one of the most often utilized oranges in preparations) and drinks (juice) [7]. Calamansi have Been more popular as a refreshing drink in recent years,

particularly in Padang. Its application is confined to the sensation of water, whereas the skin of Calamansi fruit is not well utilized by the community. The Calamansi leaves, like the peels of the Calamansi fruit, is underutilized in the community. Because they contain essential oils, the peel and leaves of Calamansi can be employed as antioxidants and antimicrobials [5], [8], [9].

The essential oil of Calamansi leaves possesses antibacterial action, according to a 2018 study done by Nguyen. *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Aspergillus niger, Candida albicans*, and *Saccharomyces cereviceae* have all been employed as leaf activity tests [8]. Meanwhile, the essential oil found on the fruit's peels has been shown to have antibacterial effect against bacteria such *as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Enterococcus faecalis* [10].

As a result, research is needed to determine the chemical profile of essential oils found in leaves and fruit peels, as well as their activity against resistant bacteria like MRSA (methicillin-resistant *Staphylococcus aureus*), gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus mutans*), and gram-positive bacteria. negative (*Pseudomonas aeruginosa* and *Escherichia coli*) so that the primary chemicals found in the leaves and fruit peels that affect their antibacterial activity can be discovered and which essential oils have the greatest antibacterial potential may be determined.

2. MATERIALS AND METHODS

2.1 Material

Calamansi plants were sourced from Padang, West Sumatra's Tunggul Hitam region. The plant's fruit and leaves are the portions harvested. To extract the essential oil, the fruit is cleaned and peeled. The essential oil is then extracted by distilling the leaves after they have been washed and cut.

2.2 Bacteria for Testing

Staphylococcus aureus ATCC 25923, *Pseudomonas aeruginosa* FNCC 9027, *Escherichia coli* ATCC 8739, *Streptococcus mutans* ATCC 25175, and methicillin resistant *Staphylococcus aureus* ATCC 43300 were used, all of which were obtained from the Research Laboratory, Faculty of Pharmacy, Andalas University, Padang.

2.3 Preparation of Samples

The method of distillation utilized is water distillation. Fresh material is cleansed before being cut. After that, a series of modified distillation apparatus is used to complete the process. After the first drop, water distillation was carried out for 4 hours. And obtained distillate, which is an essential oil. The distillate should be kept in a dark, well sealed container. The distillate is kept at a temperature below 4℃.

2.4 Analysis of Essential Oils GC-MS

The chemical components of the essential oil of Calamansi peel and leaves were studied using GC-MS (Gas Chromatography-Mass Spectrometry) under the following analytical conditions [11]: Agilent® 7890A GC instrument, Agilent® 5975 MS detector Column HP-5ms (Agilent®) with 0.25 mm diameter, 0.25 m thickness, 30 m length, gas flow rate 3 ml/min, column temperature 50-250℃ (constant temperature 50℃ for 6 minutes, temperature increased to 80 with 2/min increments, at 80 it was held constant for 1 min, temperature increased to 250℃ in 4/min increments, at 250℃ it was held constant for 8 minutes). The temperature of the injector is kept constant at 225℃, while the temperature of the detector is kept constant at 270℃, and the energy is kept constant at 1.25 kV. Helium, at a column pressure of 70 kPa, was used as the carrier gas 2 μL are used for injection. The computer will be used to read the analysis results.

2.5 Antimicrobial Activity

The antimicrobial test was performed using the agar disk diffusion method. The first step is to make a

bacterial suspension that has been revitalized with physiological NaCl. The suspension's turbidity was compared to the standard 0.5 MC Farland. Take 200 µL of the bacterial suspension and homogenize it with 30 mL of MHA (Mueller-Hinton Agar) media. Place the disc with 10 µg of the sample on top of the solidified media. Before being placed on the medium, the discs were dried. After that, the cells were incubated at 37°C for 24 hours. Examine the inhibition zone on the petri dish by measuring the clear zone on the plate with a caliper. Ciprofloxacin was utilized as a positive control, and DMSO (dimethyl sulfoxide) was used as a negative control.

The 96-well microtiterplate, consisting of the A-H rows of 12 wells each. In the twelfth well, 50 µL of MHB media was added to the B-H row of the second well. On a 96-well microtiterplate, 50 µL of the test solution was added to rows A and B. Row B received 50 µL pipetted and placed into row C, row C had 50 µL pipetted and transferred to row D, and so on until row F received 50 µL pipetted and then discarded. In each row A to F, 50 µL of bacterial suspension was added to achieve sample concentrations of 6.25%, 3.13%, 1.56%, 0,78%, 0,39% and 0,2%. In row G of 50 µL of ciprofloxacin solution, a positive control was added, followed by 50 µL of bacterial suspension. In row H, a negative control was applied, followed by 50 µL of DMSO and 50 µL of bacterial suspension. The plate was then incubated at 37°C for 18 hours.

The difference in turbidity in each hole of the plate visually demonstrated the test bacteria's growth inhibition. To clarify the color, MTT dye was applied to each well. The lowest concentration of the test bacterium suspension in the well that appears clear shows that the test microorganisms are not growing and is also the minimal inhibitory concentration (MIC). Three repeats of the test were carried out.

3. RESULTS AND DISCUSSION

The results of the distillation of essential oils from the fruit peel and leaves of Calamansi (*Citrus microcarpa* B.) were physically characterized according to Table 1.

Table 1. The results of determining the specific gravity and refractive index of the essential oil of leaves and rind of Calamansi fruit

Parameter	**Peels**	**Leaves**
Specific Gravity	0.889 g/mL	0.923 g/mL
Refractive Index	1.468	1.460

There are discrepancies in the physical characterisation results between fruit peels and leaves, which is related to the two essential oils' differing compositions. The GC-MS data show that there are 30 compound components in the fruit skin and 47 compound components in the leaves, which can be shown in Tables 2 and 3. These compounds include monoterpenes, monoterpenes-alcohols, sesquiterpenes, aromatics, aldehydes, hydrocarbons, and esters. This indicates that the leaves of the kaffir lime fruit contain more chemical compounds than the peels. This is in line with the findings of Askari et al (2010), who found that the concentration of essential oils from the same plant section can vary [12].

Table 2. The results of the chemical compound analysis of Calamansi peel

No.	Content (%)	Retention time	Compound
1	29.52	14.88	D-Limonene
2	13.76	23.46	(R)-(+)-Citronellal
3	8.88	11.34	3-Isopropenyl-5,5-dimethyl-cyclopentene
4	7.30	24.86	γ-Terpinene
5	6.90	27.84	Citronellol
6	4.61	25.57	α-Terpineol
7	4.27	11.46	γ-Terpinene
8	2.58	19.71	L-β-Pinene
9	2.37	38.29	α-Farnesene
10	2.05	16.66	γ-Terpinene
11	1.82	8.82	(R)-α-Pinene
12	1.66	22.58	(+)-Neoisoisopulegol
13	1.60	28.02	Citronellol
14	1.50	13.74	α-Terpinene
15	1.46	12.36	β-Myrcene
16	1.37	45.33	α-Sinensal
17	1.18	28.05	Citronellol
18	1.17	17.60	trans-Linalool oxide (furanoid)
19	0.79	18.54	Terpinolene
20	0.72	13.07	Octanal
21	0.66	39.67	β-Germacrene
22	0.58	17.75	1-Octanol
23	0.58	18.64	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate
24	0.47	26.44	Decanal
25	0.45	28.95	3-Carene
26	0.39	32.96	2,6-Dimethyl 2,6-octadiene
27	0.38	20.75	γ-Terpinene
28	0.38	16.09	β-Ocimene
29	0.32	32.10	1-Terpinenol
30	0.31	37.26	Germacrene D

Table 3. Results of chemical compound analysis of Calamansi leaves

No.	Content (%)	Retention time	Compound
1	25.74	23.42	Citronellal
2	12.94	27.93	Citronellol
3	8.43	29.19	3-Carene
4	4.89	11.13	β-Phellandrene
5	3.84	19.60	(+)-3-Carene
6	3.23	3971	β-Germacrene
7	3.19	45.36	tricyclo[5,2,1,0 2,6]dec-2-ene
8	3.13	37.82	Bicyclogermacren
9	2.9	35.20	Caryophyllene
10	2.61	16.05	β-Phellandrene
11	2.06	24.88	γ-Terpinene
12	1.92	29.23	3-Carene
13	1.72	34.15	(+)-3-Carene
14	1.72	34.28	Cyclohexane
15	1.25	37.28	Germacrene D
16	1.23	32.21	(+)-4-Carene
17	1.20	24.26	(R)-(+)-Citronellal
18	1.04	54.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
19	0.94	42.57	isoledene
20	0.94	12.29	β-Myrcene
21	0.92	38.07	δ-Guaijene
22	0.92	41.84	L-calamenene
23	0.87	16.53	γ-Terpinene
24	0.80	29.64	Citral
25	0.77	36.34	cis,cis,cis-1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene
26	0.75	40.31	9,10-Dehydroisolongifolene
27	0.71	28.25	1,3,8-p-Menthatriene
28	0.69	38.25	α-Farnesene
29	0.69	14.45	D-Limonene
30	0.63	25.52	α-Terpineol
31	0.60	32.11	(1R,2S,4R)-2,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol
32	0.56	44.44	β-Bisabolene

33	0.56	32.98	3,7-Dimethyloct-6-enyl ethyl carbonate
34	0.55	14.21	o-Cymene
35	0.54	38.66	(+)-δ-Cadinene
36	0.49	42.21	t-Cadinol
37	0.46	26.82	Cyclopentene
38	0.45	22.45	(+)-Neoisoisopulegol
39	0.44	40.47	Aromandendrene
40	0.43	51.66	1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane
41	0.36	11.20	β-Pinene
42	0.36	17.48	trans-Linalool oxide (furanoid)
43	0.33	8.78	1R-(+)-α-Pinene
44	0.30	38.59	α-Terpinene
45	0.29	22.84	Isopulegol
46	0.28	41.57	γ-Muurolene
47	0.28	39.13	β-Himachalene

The amounts and compositions of the chemical components of the calamansi peel and leaves varies. D-limonene (29.52%), (R)-(+)-citronellal (13.76%), 3-isopropenyl-5,5-dimethyl-cyclopentene (8.88%), γ-terpinene (7.30%), citronellol (6.90%), and α-terpineol (6.90%) are the primary chemical components in calamansi peel (4.61%). Citronella (25.74%), citronellol (12.94%), 3-carene (8.43%), and β-phellandrene (4.89%) were the primary chemical components in calamansi leaves. The antibacterial activity of each sample's essential oil was regulated by differences in the principal chemical components of the fruit peel and leaves. There are variances in minor chemicals that are exclusively found in the essential oil of the fruit peel or solely in the essential oil of the leaves, in addition to differences in the primary content between calamansi peel and calamansi leaves. The antibacterial activity of essential oils can be affected by differences in chemical component changes between them. The diffusion test was performed with a 50 percent (w/v) concentration, and the results are shown in the following figure 1.

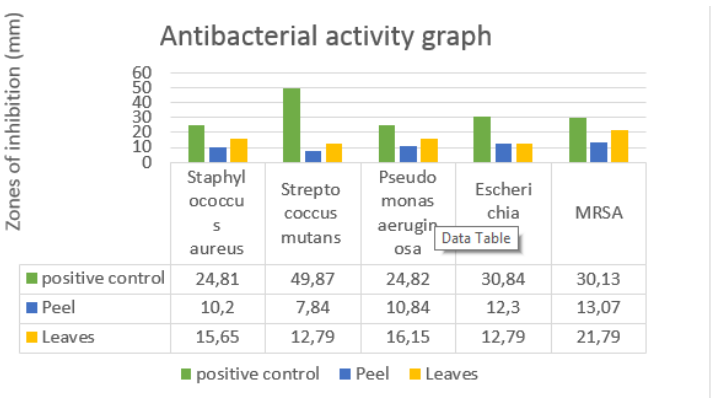

Figure 1. Graph of antibacterial activity of essential oil of calamansi fruit peel and leaves

The essential oil of calamansi leaves had a strong inhibitory effect on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Streptococcus mutans* bacteria based on the measurement of the inhibitory diameter, while methicillin-resistant *Staphylococcus aureus* had a very strong inhibitory power. Compounds with a high degree of reactivity The essential oil of calamansi leaves had a strong inhibitory effect on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and *Streptococcus mutans* bacteria based on the measurement of the inhibitory diameter, while methicillin-resistant *Staphylococcus aureus* had a very strong inhibitory power. According to research conducted by Ouchari et al (2019), compounds with a strong inhibitory power have an inhibitory diameter of 10-20 mm, whereas compounds with a very strong inhibitory diameter have an inhibitory diameter of more than 20 mm [13]. While *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* germs are all strongly inhibited by the essential oil of calamansi peel, *Streptococcus mutans* is somewhat inhibited [13]. Table 4 shows the results of the essential oil activity test.

Table 4. Activity Test Results of Kasturi Citrus Fruit Peel and Leaves Essential Oil with Dilution Method

Sample	Bacteria	Concentration (mg/mL)							
		12,5	6,25	3,13	1,56	0,78	0,39	0,20	0,10
Peels	*S. aureus* ATCC 25923	-	-	-	+	+	+	+	+
	P. aeruginosa FNCC 9027	-	-	-	-	-	-	+	+
	E. coli ATCC 8739	-	-	+	+	+	+	+	+
	S. mutans ATCC 25175	-	-	+	+	+	+	+	+
	MRSA ATCC 43300	-	-	-	+	+	+	+	+
Leaves	*S. aureus* ATCC 25923	-	-	-	-	+	+	+	+
	P. aeruginosa FNCC 9027	-	-	-	-	-	-	-	+
	E. coli ATCC 8739	-	-	-	-	+	+	+	+
	S. mutans ATCC 25175	-	-	-	-	+	+	+	+

	MRSA ATCC 43300	-	-	-	-	-	+	+	+
Positive control (+)		30	15	7.5	3.75	1.88	0.94	0.20	0.10
		-	-	-	-	-	-	-	-
Negative control (-)		Steril			Grow				
		-	-	-	+	+	+		

Table 4 also shows the MIC of the essential oil of calamansi plants. The essential oil of calamansi leaves exhibits MIC with varying quantities of each bacteria, as shown in the table. At a concentration of 1.56%, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans* have the same MIC. The MIC of the essential oil of calamansi leaves against *Pseudomonas aeruginosa* bacteria was the smallest of all the bacteria tested, with a concentration of 0.20%. The MIC of the essential oil of calamansi leaves at a concentration of 3.13% was found in resistant bacteria, MRSA, which was employed as the test bacteria. The inhibition zone of the antimicrobial disc in the diffusion method is inversely proportional to the MIC, which is the link between the diffusion and dilution methods. The smaller the minimum inhibitory concentration of MIC, the wider the inhibition zone [14].

Because essential oils are made up of terpene compounds, compounds that act as antibacterials in essential oils, both fruit peels and leaves, are terpenoid compounds. Terpenoids have antibacterial activity, and their mechanism of action is to disrupt bacteria's cell walls because they are lipophilic. Terpenoids can also react with porins (transmembrane proteins) on the bacterial cell wall's outer membrane, forming strong polymer bonds and damaging the porin, reducing bacterial cell wall permeability. Bacterial cells will be deprived of nutrition as a result, and their growth will be inhibited or they will die [15]. According to the findings, the essential oil of kaffir lime fruit peel and leaves inhibited Gram-negative bacteria better than Gram-positive bacteria. Gram-negative bacteria's cell walls are made up of lipids and a thinner peptidoglycan, allowing essential oils to diffuse more easily and enter the cell wall [16].

4.

CONCLUSION

The main chemical compounds found in the peels of the Calamansi were D-Limonene, (R)-(+)-Citronellal, 3-Isopropenyl-5, 5-dimethyl-cyclopentene, γ-Terpinene, Citronellol, and α-Terpineol. The main chemical compounds found in the leaves of the Calamondin were Citronellal, Citronellol, 3-Carene, and β-Phellandrene. The antibacterial activity test of the diffusion and dilution methods showed that the essential oil of the leaves was more potent than the peel of the Calamansi

AUTHORS' CONTRIBUTIONS

Every author contributes to the creation of articles

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