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Testing of Antibacterial Activity and Characterization of Chemical Compounds Composing Essential Oil From Lemo Cuco Fruit Skin (Citrus macroptera Mountrous)

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A B S T R A C T

Lemo Cuco (*Citrus macroptera Mountrous*) is one of the plant species of Rutaceae grown in the districts of Bone and Sinjai, South Sulawesi. This fruit is commonly used for food as scent, cough reliever and as fishy and meaty deodorizing. The peel has a special scent indicating presence of essensial oil components. This study aims to evaluate antibacterial activities and and to characterize compounds in ethanol extract dan n-hexane extract Lemo Cuco (*Citrus macroptera Mountrous*). This study used the soxhlet extraction, and phytochemical test and the characterization of components in extracts with GC-MS and antibacterial activities using the disc diffusion method. The results obtained from the study were testing the antibacterial activities of Lemo Cuco (*Citrus macroptera Mountrous*) peel extract against the *Staphylococcus aureus* and *Salmonella typhi* bacteria. Inhibition of ethanol and n-hexane extract against the bacteria S. aureus included weak, medium, and strong category according to the concentrations (20%, 10%, 5%, 2.5% and 1.25%), whereas inhibitory against *S. typhi* bacteria in n-heksane included weak, medium, and strong category, whereas ethanol extract included medium and weak category, even not active. Based on the results of phytochemical identification of the Lemo Cuco (*Citrus macroptera Mountrous*), peel extract indicated existence of flavonoids, phenolic, steroids, terpenoids, alkaloids and saponins compound. n-heksane exctract contained special fraction saponins, whereas ethanol exctract was nothing. Characterization using GC-MS indicated existence of monoterpenoid and sesquiterpenoid compound. Therefore, the n-hexane extract from the skin of the lemo cuco has potential as an antibacterial.
Keywords: antibacterial acitivity, lemo cuco, essensial oil, *Salmonella typhi*, *Staphylococcus aureus*

INTRODUCTION

Indonesia has abundant natural wealth and biodiversity and is located on the equator with a tropical climate. The diversity of existing plants can be utilized both traditionally and modernly with the isolation of natural compounds in the form of secondary metabolite compounds (1). These secondary metabolite compounds vary greatly in type and quantity in each plant (2). Secondary metabolite compounds can be obtained from parts of the plant starting from the fruit, skin, leaves, stems, and roots (3), in the form of active compounds such as flavonoids, alkaloids (4), saponins, steroids and terpenoids (5). One of the terpenoid compounds is essential oil which can be obtained from several species such as *Compositae, Matricariae, pinaceae, Labiatae, Rutaceae* and etc (6).

One of the *Rutaceae* species found in the citrus family such as lime (*Citrus aurantium* L.), Pontianak orange (*Citrus nobilis* Lour.), Sunkist orange (*Citrus sinensis* L. Osbeck), kaffir lime (*Citrus hystrix* DC) and lemon (*Citrus limon* L.). In the Bugis tribe, especially in the Sinjai and Bone areas, there is a type of orange whose morphology resembles a lemon, the community knows it as "Lemo Cuco". This orange has a distinctive aroma that is commonly used for cooking as a flavoring, cough suppressant and as a fishy odor remover in fish and meat.

The use of orange fruit and leaves has been known by the community since ancient times as a traditional medicine. The leaves are usually used to overcome fatigue and as a food flavoring. While the fruit skin is used as a medicine for boils, internal heat, dermatitis, breast inflammation, scaly skin and peeling skin (7), making cakes and sweets (2). Orange peel contains essential oils that can be extracted so that it has a high selling value (8), which is widely used as a fragrance, soap and

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cosmetics industry (9). Several studies on citrus have been reported, such as research on lime peel extract (*Citrus aurantium*) which can reduce dental plaque with alkaloid and flavonoid content (10), Pontianak orange peel (*Citrus nobilis* Lour.) with limonene content against subterranean termites (11) and Sunkist orange peel (*Citrus sinensis* L. Osbeck) as a natural styrofoam solvent (12). Other studies have also revealed that lemon peel extract (*Citrus limon* L.) and kaffir lime peel (*Citrus hystrix* DC) have antibacterial activity (13). Research by (10) and (14), revealed that orange peel extract with 70% ethanol solvent has higher effectiveness against microorganism tests than methanol, acetone and dichloromethane solvents. Ethyl acetate extract and kaffir lime peel oil are more potent against *Staphylococcus aureus* than *Escherichia coli* (15). Research by (16) stated that the rutaceae family has high antibacterial activity against 20 serotypes of *Salmonella*. Research by (17) using 100% kaffir lime juice has optimal inhibitory power on the growth of *Salmonella typhi*. This is in accordance with research by (18) that kaffir limes peel has stronger antimicrobial power compared to kaffir lime fruit. The peel of the kaffir lime fruit contains essential oils that have antibacterial effects (19) (20), antifungal (21) (22), antioxidants (23) (24), and fresheners. The antibacterial effect is obtained due to the presence of citronella compounds in it (25). Research by (26) and (27) reported that kaffir lime leaves contain tannins, steroids, triterpenoids, and essential oils of 1-1.5% with a citronellal content of 64.15%, beta citronellal 10.17% and lonalol 5.31%. Other studies such as those reported by (8) revealed that the essential oil content of 2.5% of kaffir lime peel contains the main components of 94% limonene, 0.2% geranial, 0.1% citronellal, 11.93% terpinen-4-ol, 0.5% linalol, 0.4% decanal and 0.5% octanal.

Essential oil extracted from lime peel contains a variety of chemical compounds, predominantly monoterpenes and sesquiterpenes, with limonene, geranial, pinene, neral, and citronellal as the main components, and its yield and composition vary depending on the solvent used (ethanol or n-hexane). Other studies state that the essential oil content of lime peel consists of 16% monoterpene compounds, 6.55% sesquiterpene, aromatic and non-aromatic compounds (28). The main components of lime essential oil are 96% limonene, 97% geranial, 96% pinene and 96% neral (29). (30) reported that the use of ethanol and n-hexane solvents obtained essential oil yields of 13.39% and 10.50% with citronellal content of 65.99% and 97.27%.

Citronellal, found in kaffir lime essential oil, has antibacterial and antifungal properties, making it effective as a botanical pesticide and capable of inhibiting the growth of pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. (31) revealed that citronellal has antibacterial and antifungal properties so that it can be used as a botanical pesticide. This is in accordance with research by (32), (26), and (20) which stated that kaffir lime essential oil can inhibit the growth of *Streptococus mutans* at a concentration of 25%, *Staphylococus aureus* and against *Klebsiella pneumonia* ATCCThis study was conducted to determine the antibacterial activity against *Staphylococcus aureus* and *Salmonella typhi* bacteria and to characterize the components of the essential oil of Lemo Cuco fruit peel (*Citrus macroptera Mountrous*). The reason for using these two bacteria is because bacteria are generally grouped based on their cell wall structure into 2, namely gram-positive bacteria and gram-negative bacteria (33). The cell wall structure of gram-positive bacteria is layered with low lipid content (1-4%) making it easier for bioactive materials to enter the cell, has a peptidoglycan layer on the outside and plays a less effective role as a permeability defense. While gram-negative bacteria contain fewer peptidoglycan layers with a high lipid content (11-22%) whose cell walls have 3 layers (multilayer) consisting of lipoprotein, phospholipid and lipopolysaccharide layers which make the cell wall difficult to penetrate by antibacterial substances.

MATERIALS AND METHODS

Methods

Materials used in this study were gas chromatography-mass spectrometry (GS-MS) Agilant GC Tipe 7890 A MS Tipe 5975, spectrophotometer UV, rotary evaporator Heidolph Vap-Value, oven kirin and memmert, autoclave Gea Yx-280D, incubator Heraeus Thermo Scientific, Laminar Air Flow Cabinet (LAF) ESCO Isocide, easypure II Barnstead D 3750 Thermo Scientific, fume hood Esco Frontier Tm, analytical balancing Electronic Balance Kern ABJ, , Heating Mantel Stirrer B- One, vortekx advanced mixer IR Wizard Velp Scientifica, UV light 254-336 nm, set of soxhlet Pyrex. Sample used was Lemo Cuco (Citrus *macroptera Mountrous*) peel, it was wild plant obtained from Bijinangka Village, Sinjai Borong, Sinjai, South Sulawesi). Chemical material utilized were alcohol 70% Intraco, ampicilin, cefixime, aquadest (H_2O), concentrated sulfate acid (H_2SO_4), boiling stone, white thread, iron (III) chloride ($FeCl_3$) 5% and 1%, dimethyl sulfoxide (DMSO) p.a Intraco, technical ethanol (C_2H_5OH) Duta Gemini, ethyl acetate ($C_4H_8O_2$) Brataco, cotton, disc paper, Duta Gemini filter paper, TLC (Thin Layer Chromatography) plate Silica gel F_{254}, muller hinton agar (MHA), physiological sodium chloride (NaCl) 0.9% p.a, sodium hydroxide (NaOH) 10% p.a, n-Hexane (C_6H_{14}) Brataco, nutrient agar (NA), dragendorff reagent, Lieberman Burchard reagent, mayer reagent, wagner reagent. While the bacteria used were *Staphylocuccus aureus* and *Salmonella thypi*.

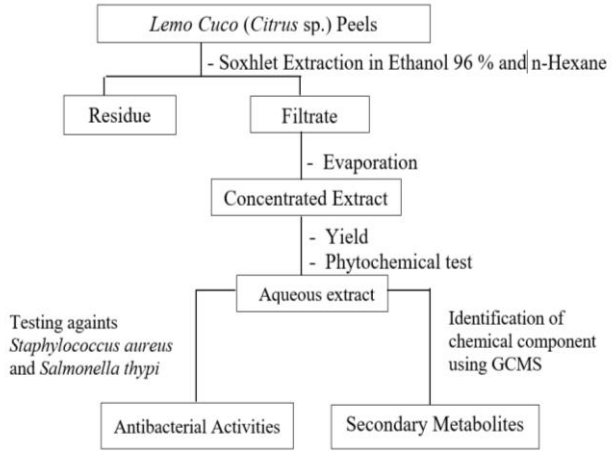

Figure 1. Research Flowchart

Preparation Method of Simplicia

Lemo Cuco fruit samples (*Citrus macroptera Mountrous*) were cleaned then the skin was separated from the flesh. Furthermore, the orange peel was cut into small pieces and dried at room temperature. After that, the Lemo Cuco peel was ground into powder which was then called simplicia.

Extraction

This study was carried out with two variations of solvents, namely n-hexane and ethanol. Weighing 50 grams of simplicia then inserting it into a sleeve made of filter paper. After that, the sleeve was inserted into a soxhlet extractor then extracted with 500 mL of ethanol (C_2H_5OH) 96% at a temperature of 81-96 ºC (heating temperature) until the solvent color returned to its original state. After the extraction process, Lemo Cuco filtrate was obtained. The Lemo Cuco filtrate obtained was then concentrated with a rotary evaporator at a temperature of 50 ºC and 40 rpm until the solvent did not drip, thus producing essential oil. The same treatment was carried out with n-hexane solvent (C_6H_{14}) at a temperature of 72-86 °C. The extract obtained then calculated the yield value and carried out phytochemical tests and characterization of the components of the essential oil of the lemon peel (*Citrus macroptera Mountrous*) with GC-MS.

Phytochemical Test

The content of secondary metabolites in the ethanol and n-hexane extract of lemo cuco peel was tested using various reagents based on standard testing procedures (Harborne, 1996). Phytochemical testing in this study used various reagents to check for the presence of flavonoid, alkaloid, steroid, terpenoid, phenolic and saponin compounds.

Antibacterial Activity Test

Antibacterial activity test using the disc paper diffusion method. The steps in this study include sterilization of equipment, making nutrient agar (na) media, rejuvenating bacteria, diluting essential oils and positive controls, making test suspensions, making test media, soaking disc paper, planting disc paper and measuring inhibition. The disc paper diffusion method was chosen because it is easy and simple to determine the antibacterial activity of the tested extract. Soaking is done for 30 to 60 minutes so that the test solution is completely absorbed into the disc. The absorbed disc paper is aired until no more drips so that when incubated the inhibition zone formed does not spread. The disc paper is planted on a solid medium that has been mixed with the test suspension. The activity test is carried out near the fire so that no other bacteria enter the petri dish which can cause contamination in the media. Furthermore, it is incubated at a temperature of 37 °C for 24 hours. This temperature is used because it is the optimum temperature for bacterial growth (34) (35) (36). Observations are made after incubation to see the clear zone around the disc paper. The measurement of the inhibition zone in this study was carried out for 24 hours to see the response of bacterial growth inhibition by antibacterial compounds in the essential oil of Lemo Cuco fruit skin. The measurement of the inhibition zone used a digital caliper with an accuracy of 0.02 mm per scale. Calculating the diameter of the clear zone formed around the disc paper on 3 sides, namely vertical, horizontal and diagonal, was then averaged as the inhibition zone of the test extract (34) (32).

RESULTS AND DISCUSSION

Results

Soxhlet extraction is a simple and easy extraction to extract volatile compounds from a sample (37). This study used ethanol and n-hexane solvents. Ethanol solvents have a low boiling point so they are easier to evaporate and can dissolve compounds quickly and are affordable (38). Ethanol solvents have hydroxyl groups that can bind polar compounds such as flavonoids and alkaloids (39) (40) (41). While n-hexane solvents are non-polar solvents that can bind non-polar compounds such as steroids and terpenoids (42). The results of the soxhlet extraction were dark green liquid essential oil which was then concentrated with a rotary evaporator to separate the solvent from the essential oil which was indicated by the evaporation of the solvent (43). The yield of essential oil from Lemo Cuco fruit skin obtained consisted of ethanol extract and n-hexane extract were 25.8% and 3.2%. Ethanol is a polar solvent capable of dissolving polar compounds and some non-polar compounds. In contrast, n-hexane is a non-polar solvent that is more limited in dissolving polar compounds. Since many bioactive compounds in plants, such as phenols and flavonoids, are polar, ethanol is more effective in extracting these compounds. Beside that, ethanol can extract various types of compounds, including monoterpenes, sesquiterpenes, and phenolic compounds, which often have therapeutic effects. n-Hexane, which is more suitable for extracting lipids and non-polar compounds, may not be able to isolate all bioactive compounds present in plant materials. Ethanol can also interact more effectively with the cellular matrix of plants, facilitating the release of compounds from cell walls. This process increases the number of compounds extracted into the solvent, thus enhancing the extract yield. The

results obtained in the ethanol extract were greater while the n-hexane extract was smaller than the study by (30) who obtained the yield of ethanol extract and n-hexane extract with the soxhlet extraction method from kaffir lime leaves of 13.39% and 10.50%. The same study was conducted by (44) who conducted a study on the extraction of 20 grams of orange peel with 200 mL of solvent. Meanwhile, (8) obtained an orange peel extract yield of 1.625% using the steam distillation method and (45) also reported that the yield of essential oil from Citrus nobilis orange peel was 19.383% using the maceration method. The amount of yield produced depends on the solubility properties of the bioactive components and the extraction method used.

Based on Table 1, we can see the groups of compounds found in the essential oil of Lemo Cuco fruit peel.

Table 1. Phytochemical Test of Lemo Cuco Fruit Peel Essential Oil (*Citrus macroptera Mountrous*)

Qualitative Test		Test Sample	
Compounds	**Reagents**	**Ethanol Extract**	**n-Heksane Extract**
Flavonoid	NaOH 10%	+	+
	$FeCl_3$ 5%	+	+
	H_2SO_4 pekat	+	+
Alkaloid	Mayer	+	+
	Wagner	+	+
	Dragendorff	+	+
Steroid	Lieberman Burchard	+	+
Terpenoid	Lieberman Burchard	+	+
Phenolic	$FeCl_3$ 1%	+	+
Saponin	Aquades	-	+

Note: (+) to positive result and (–) to negative result

The identification results obtained are in accordance with several studies such as research by Javed, et al., (2014) which explained the test results on 5 types of oranges containing several groups of secondary metabolite compounds quite high. (5) also reported that the components of lime peel are tannins, flavonoids, polyphenols, steroids and alkaloids. However, it is different from (46) which revealed that orange peel extract contains flavonoids, phenols, seroids, triterpenoids. (47) explained that oranges contain tannins, flavonoids and alkaloids, and (48) reported that kalamodin oranges and kaffir limes contain flavonoids and limonoids. General factors that can affect compound identification tests include differences in growing places and climates that cause different metabolic processes. Antibacterial activity test as a test of a compound used to control the growth of harmful bacteria so that it can prevent the spread of disease and infection and prevent decay or destruction of materials caused by bacteria.

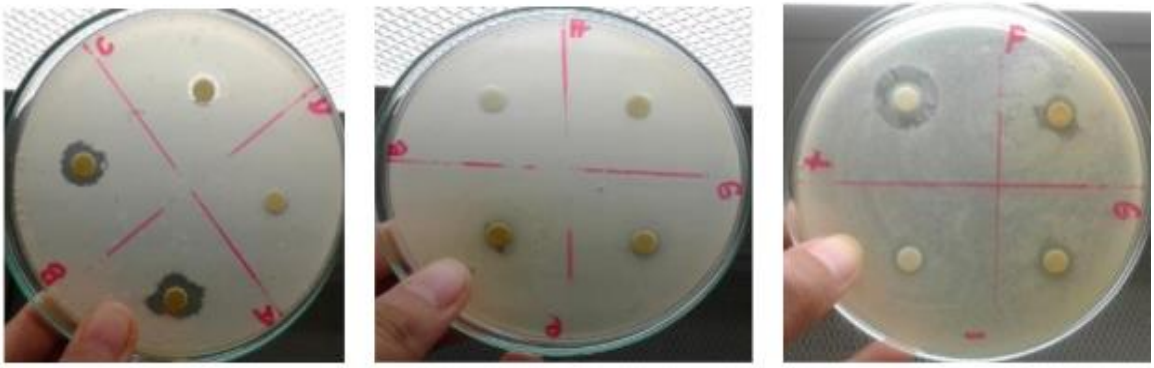

Figure 2. Test against *Staphylococcus aureus* bacteria. A-E (n-Hexane Extract 20%; 10%; 5%; 2.5% and 1.25%), F-J (Ethanol Extract 20%; 10%; 5%; 2.5% and 1.25%), Negative Control (DMSO) and Positive Control (Antibiotic Ampicillin 2%)

Table 2. Antibacterial Activity Test of Essential Oils against *Staphylococcus aureus* Bacteria

Test Sample	Concentration	Diameter of	Inhibition	Category

	(%)	Inhibiton Zone (mm)	Zone (%)	
Extract of n-Heksan	20	16.55	69.79	Strong
	10	12.39	59.65	Strong
	5	9.73	48.62	Medium
	2.5	7.47	33.10	Medium
	1.25	7.17	30.27	Medium
Extract of Ethanol	20	8.39	40.41	Medium
	10	7.68	34.40	Medium
	5	6.62	24.47	Weak
	2.5	5.73	12.74	Weak
	1.25	5.38	7.10	Weak
Control of Negative	0	5.00	0.00	Not Active
Control of Positive	2	14.78	66.17	Strong

Based on the results obtained, it can be said that the essential oil from the n-hexane extract of Lemo Cuco fruit skin from Sinjai Regency has an inhibitory effect on the growth of *Staphylococcus aureus* bacteria. These results are in accordance with the results of research conducted by (49) and (32) that ethanol extract can inhibit the growth of *Staphylococcus aureus* bacteria. The same research was also conducted by (50) who reported that the antibacterial activity of ethanol extract of kaffir lime skin has an inhibition zone of 9 mm against *Staphylococcus aureus* bacteria. This is in accordance with research by (51) reported the best inhibition results at the highest concentration of 25% against *Staphylococcus aureus* bacteria.

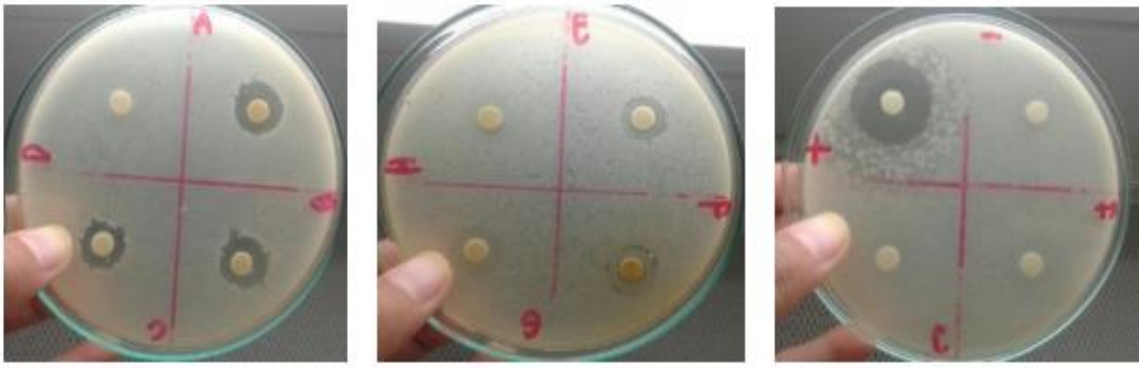

Figure 3. Test against *Salmonella typhi* bacteria A-E (n-hexane extract 20%; 10%; 5%; 2.5% and 1.25%), F-J (ethanol extract 20%; 10%; 5%; 2.5% and 1.25%), negative control (dmso) and positive control (cefixime 2%)

Table 3. Antibacterial Activity Test of Essential Oils against *Salmonella typhi* Bacteria

Test Sample	Concentration (%)	Diameter of Inhibition Zone (mm)	Inhibition Zone (%)	Category
Ekstrak n-Heksan	20	13.08	61.77	Strong
	10	10.72	53.36	Strong
	5	8.61	41.93	Medium
	2.5	6.62	24.47	Weak
	1.25	5.69	12.13	Weak
Ekstrak Etanol	20	9.31	46.29	Medium
	10	5.51	9.26	Weak

	5	5.27	5.12	Weak
	2.5	5.00	0.00	Not Active
	1.25	5.00	0.00	Not Active
Kontrol Negatif	0	5.00	0.00	Not Active
Kontrol Positif	2	14.31	65.10	Strong

Based on Figure 3 and Table 3, the results of the antibacterial activity test of Lemo Cuco fruit peel essential oil obtained against *Salmonella thypi* bacteria in n-hexane and ethanol extracts were able to inhibit bacterial growth. Based on the results obtained, it can be said that the essential oil from the n-hexane extract of Lemo Cuco fruit peel from Sinjai Regency has inhibitory power against the growth of *Salmonella typhi* bacteria. Meanwhile, the results of the measurement of the inhibition zone of essential oil from ethanol extract against *Salmonella typhi* bacteria are in accordance with the results of research conducted by Amin (2012) and Nanasomat (2005) that ethanol extract is able to inhibit the growth of *Salmonella typhi* bacteria.

Research by (17) reported that the juice of purut orange peel (*Citrus hystrix* Dc.) has inhibitory activity at a concentration of 25% to 100% with an average inhibition zone of 3.4 mm. In addition, research of (52) revealed that the decrease in the number of *Salmonella typhi* bacterial colonies was quite sharp after being given a concentration of lime peel extract (*Citrus aurantifolium*) 6.25% which is in line with (53) (54)that lime peel extract can affect the growth of *Staphylococcus* sp, *Salmonella* sp, *Escherichia coli*, *Klebsiella*, *Proteus* sp and *Pseudomonas* sp bacteria.

There is a difference of inhibition zones of the test extract against *Staphylococcus aureus* and *Salmonella typhi* bacteria. This is in accordance with research of (55) which reported that the results of star fruit ethanol extract inhibited gram-positive bacteria more than gram-negative bacteria. This difference is due to the difference in high sensitivity which is indicated by the high level of inhibition produced by a particular antibacterial compound. In addition, it is also influenced by several factors such as the toxicity of the test material, the diffusion ability of the test material in the media, the interaction between the components of the medium and the in vitro microenvironmental conditions. According to (56), the concentration of a test sample as an antibacterial is a determinant of the size of the ability to inhibit the growth of test microbes. The difference is due to differences in cell wall structure between the two bacteria that affect the work of the extract (57). The test results on the positive control using ampicillin antibiotics against *Staphylococcusa aureus* bacteria gave an inhibition zone diameter of 14.78 mm with an inhibition power of 66.17%, in *Salmonella thypi* bacteria using the antibiotic cefixin which gave an inhibition zone diameter of 14.31 mm with an inhibition power of 65.10%.

The results obtained are in line with the research of (58) and (59) who used ampicillin against the activity test of *Staphylococcus aureus* bacteria with an inhibition diameter of 8 mm and 10 mm. Ampicillin as a commercial antibiotic and is one type of penicillin antibiotic that works by inhibiting cell wall synthesis. Research by (60) also reported several antibiotics that are often used against Salmonella thypi bacteria, one of which is the antibiotic cefixime. Cefixime is a third-generation oral cephalosporin antibiotic that has antimicrobial activity including *Enterobacterisceae.* This shows that the antibiotics used are sensitive to both test bacteria. While the test results on the negative control against the two test bacteria did not provide an inhibition zone. This shows that the use of DMSO solvent does not affect the antibacterial test results of Lemo Cuco fruit peel essential oil. the orange peel infusion test had a very strong inhibitory activity level (inhibitory power> 75%), strong (50 <inhibitory power ≤ 75%), medium (25 <inhibitory power ≤ 50%), weak (0 <inhibitory power ≤ 25%) and inactive (0). Based on tables 2 and 3, it can be seen that the inhibitory effect of essential oils from ethanol extract and n-hexane extract of Lemo Cuco fruit skin against *Staphylococcus aureus* bacteria is included in the strong category (concentrations of 20% and 10%) and moderate (5%, 2.5% and 1.25%). While the essential oil from ethanol extract is included in the moderate category (20% and 10%) and weak (5%, 2.5% and 1.25%). The inhibitory power of essential oils from n-hexane extract against *Salmonella typhi* is included in the weak category (1.25% and 2.5%), medium (5%) and strong (10% and 20%). While the essential oil from ethanol extract is included in the moderate category (20%), weak (10% and 5%) and inactive (concentrations of 5%, 2.5% and 1.25%). The difference in the diameter of the inhibition zone produced is due to differences in the components of each part of the plant (20)

Table 4. Characterization of Essential Oil Components from Ethanol Extract of Lemo Cuco Fruit Peel (*Citrus macroptera Mountrous*) with GC-MS

No	Retension	%	Molecule	Molecule	Component of

	Time	Area	Formula	Weight	Compound
1	14.301	0.94	$C_{15}H_{24}$	204	β-Elemen
2	15.558	4.69	$C_{15}H_{24}$	204	Germakren
3	15.708	6.49	$C_{15}H_{24}$	204	α-Farnesen
4	16.027	1.94	$C_{15}H_{24}$	204	δ-Kadinen

Table 5. Characteristics of Essential Oil Components from n-Hexane Extract of Lemo Cuco Fruit Peel (*Citrus macroptera Mountrous*) with GC-MS

No	Retension Time	% Area	Molecule Formula	Molecule Weight	Component of Compound
1	13.506	0.51	$C_{10}H_{16}$	136	α-Terpinen
2	14.107	1.28	$C_{15}H_{24}$	204	Kopaein
3	14.747	1.73	$C_{15}H_{24}$	204	Kariofilen
4	15.214	1.27	$C_{15}H_{24}$	204	α-Humulen
5	15.564	6.43	$C_{15}H_{24}$	204	Germakren D
6	16.027	2.86	$C_{15}H_{24}$	204	δ-Kadinen
7	16.778	4.61	$C_{15}H_{22}$	204	Aromandendren
8	17.334	3.33	$C_{15}H_{24}O$	220	Isospathulenol
9	18.585	1.09	$C_{15}H_{26}O$	238	Oplopanon
10	19.355	0.87	$C_{15}H_{22}O$	218	Nutkaton
11	20.262	1.21	$C_{17}H_{34}O_2$	255	Metil Palmitat
12	21.951	0.75	$C_{19}H_{32}O_2$	294	Metil Linoleat

The results obtained based on Table 4 of the compound components contained in the essential oil from the n-hexane extract of Lemo Cuco fruit skin consist of monoterpenoid, sesquiterpenoid and fatty acid compounds. Based on the results of GC-MS characterization of essential oil from ethanol extract and essential oil from n-hexane extract of Lemo Cuco fruit skin, the dominant compounds are monoterpenoid and sesquiterpenoid compounds. The greater variety of mono- and sesquiterpenes identified from n-hexane extracts is primarily due to its non-polar nature, strong affinity for lipophilic compounds, and focused extraction of volatile hydrocarbons. Ethanol, being polar, is more suitable for extracting polar and semi-polar compounds, which limits its ability to capture the diversity of terpenes. Thus, the choice of solvent significantly affects the range of compounds extracted and detected.The results obtained are in accordance with several studies such as the study of (30) which revealed that the components of essential oil are citronellal 65.99%, nerolidol 19.68%, trans caryopilen 8.61%, sabinen 2.07% and decane 0.66%. Other studies such as research by (27) reported that the largest components of essential oil compounds in kaffir lime are citronellal 66.85%, β-spinen 32.967%. The same study was conducted by Javed, et al., (2014) revealing that the largest components of 5 types of citrus are limonene 87.84%, carvone 16.97% and α-terpineol 12.16%. Research by (61) also reported that the components of essential oil compounds in orange peel are limonene, β-pinene, citronellal, α-terpineol, copaene, cadinene, caryophilene and geranyl acetate. The differences in compound components are caused by the place of growth and climate which cause different metabolic processes (Sari, 2013 and Muntaha 2013).

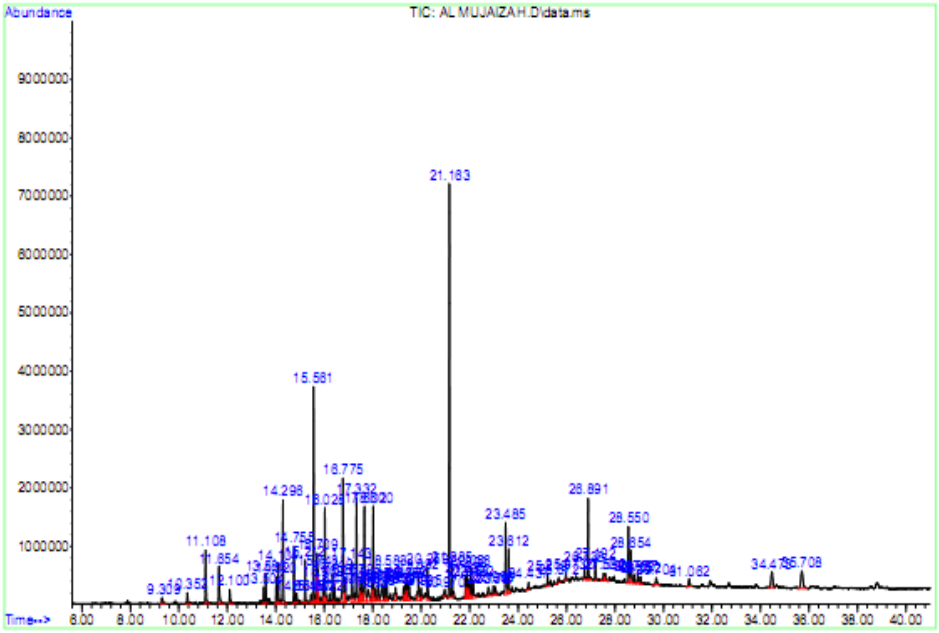

Figure 4. Spectrum of Essential Oils from n-Hexane Extract of Lemo Cuco Fruit Peel (*Citrus macroptera Mountrous*)

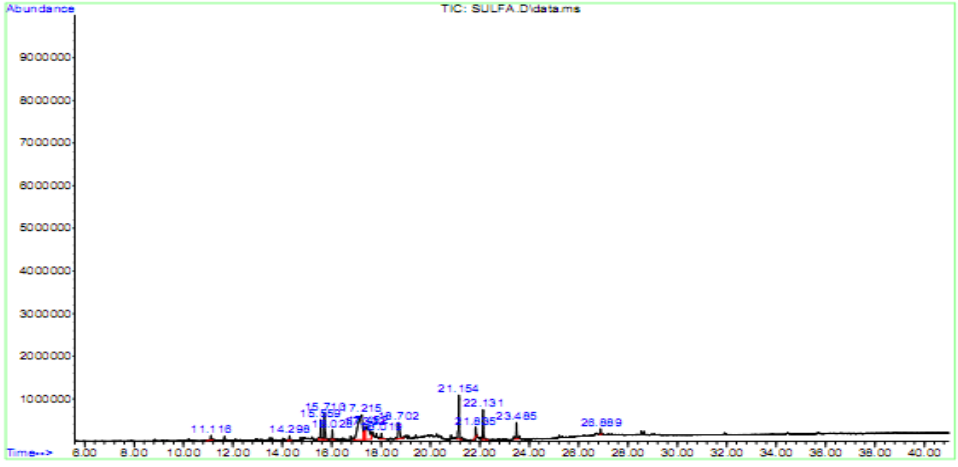

Figure 5. Spectrum of Essential Oils from Ethanol Extract of Lemo Cuco Fruit Peel (*Citrus macroptera Mountrous*)

Based on the characterization of the components of the essential oil of Lemo Cuco fruit skin with GC-MS from ethanol extract and essential oil from n-hexane extract, the dominant compounds are monoterpenoid and sesquiterpenoid compounds. The results obtained indicate that it can be used as an antibacterial.. Research by (62) and (63) also reported that the active antibacterial compounds in lime leaf essential oil are terpene compounds. Terpene compounds work as antibacterials by damaging porins, which are proteins in bacteria so that bacterial growth is inhibited). Research by (64) also explained that essential oils have antibacterial activity because essential oils contain compounds that can inhibit or kill bacterial growth. The difference in the magnitude of the inhibition produced depends on the solubility properties of the bioactive components and the method used (65).

CONCLUSIONS

The antibacterial activity of essential oils from ethanol extract and n-hexane extract of Lemo Cuco fruit peel (*Citrus macroptera Mountrous*) against *Staphylococcus aureus* bacteria is categorized as strong and medium. While from ethanol extract

it is categorized as moderate and weak. The inhibitory power of essential oils from n-hexane extract against *Salmonella typhi* is categorized as weak, moderate and strong. While from ethanol extract it is categorized as moderate, weak and inactive. Bacteria that are more sensitive to lemon coco are *Staphylococcus aureus* which is a Gram-positive bacteria. Ethanol extract is a solvent extract that shows the highest yield percentage and more complex secondary metabolites.

The components of essential oils from Lemo Cuco fruit peel (*Citrus macroptera Mountrous*) from ethanol extract show the presence of α-farnesen, germacrene, δ-cadinene and β-element compounds, while n-hexane extract shows δ-cadinene, germacrene D, aromadendrene, isospathulenol, caryophyllene, copaine, α-humulene, α-terpinene, oplopanone and nutkaton, methyl palmiate and methyl linoleate.

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CONFLICT OF INTEREST

There is no conflict of interest

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