Chemical Components and Antibacterial Activity of Essential Oil Extracted from *Citrus x aurantifolia* Peel

Elidahanum Husni[1], Sofia Ramadani[1], Suryati[1], and Mesa Irna Suryani[1], Dachriyanus[1*]

[1] *Faculty of Pharmacy, Universitas Andalas, Indonesia*

**Corresponding author. Email: dachriyanus@phar.unand.ac.id*

ABSTRACT

Citrus species are among the plants that generate essential oils, which include a variety of chemical components with antibacterial properties. *Citrus x aurantiifolia* is one of the citrus types developed by a cross between lime (*Citrus aurantifolia*) and lemon (*Citrus hystrix*). The purpose of this study is to compare the chemical component and antibacterial activity of essential oils extracted from Citrus x aurantifolia fruit peel. Hydrodistillation was used to extract the essential oil (EO). Gas chromatography in conjunction with mass spectroscopy were used to analyze chemical composition (GC-MS). D-limonene (44.1%), Terpinen (20.9%), and camphene (14,512%) were the primary chemical components detected in the peel. The dilution method was used to determine the antibacterial activity. The antibacterial test were suppressed the growth of bacteria by this essential oil. *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Escherichia coli*, and *Methicillin Resistant Staphylococcus aureus* all had MIC values of 125 mg/ml, while *Staphylococcus aureus* had MIC values of 62.5 mg/ml.

***Keywords**: Essential Oil, Rutaceae, GC-MS, Antibacterial Activities, Minimum Inhibitory Concentration*

1. INTRODUCTION

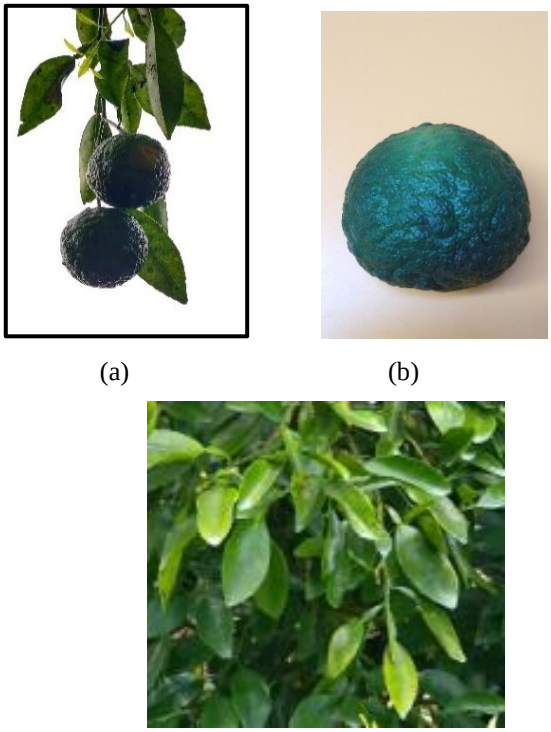
Traditional medicine is currently gaining popularity, and many researchers have investigated the toxicological and pharmacological effects of these natural components. In the medical field, practitioners have begun to use natural components such as plant extracts to prevent and treat disease.

Furthermore, traditional medicine can be used as an alternative to prevent medication resistance. Natural substances for treatment are safer, have more significant therapeutic effects, and have less

negative effects than synthetic medications. One of the potential plants is the Citrus family [1]–[3].

Citrus trees, which belong to the Rutaceae family, are widely grown around the world. Around 150,000 species from 150 genera make up the Rutaceae family, which is extensively dispersed in tropical, subtropical, and temperate climates such as southern Asia, China, Japan, and Indonesia [4]. Citrus essential oil, often known as lime oil, has a long history of usage in traditional medicine. It was made up of hydrocarbons, oxides, lactones, esters, alcohols, phenols, ketones, and aldehyde compounds, among other chemicals [5]. Antibacterial, antioxidant, anticarcinogenic, antitumor, antifungal, anthelmintic, larvicidal, and food preservative are only a few of its biological properties [6]. It has been shown to efficiently suppress the growth of both gram-negative and gram-positive harmful bacteria [4], [5].

Citrus x aurantiifolia is a citrus species that thrives in Indonesia, mainly in West Sumatera.



(a) (b)

(c)

Figure 1. Asam Sundai *(Citrus x aurantiifolia*)

The local community refers to it as "Asam Sundai". *Citrus x aurantiifolia* is a hybrid between lime *(Citrus aurantiifolia)* and *citrus hystrix*. Fruit juice mixed with limewater and coconut oil is traditionally used to alleviate coughs, and it's also used in cooking. However, the use of peels is still limited. Peels are frequently discarded, resulting in trash that contains potential chemical compounds such as essential oils [7]–[9]. Many studies on lime (*Citrus aurantiifolia*) chemical contents and antibacterial action have been published, however there has been minimal research on *Citrus x aurantiifolia* ("Asam Sundai"). As a result, the goal of this research is to identify a chemical content of essential oils extracted from citrus fruit peels, as well as to assess the antibacterial activity.

2. MATERIALS AND METHODS

2.1 *Sample collection*

Citrus x aurantiifolia fruits were harvested in the Kamang section of the Ampek Angkek District of the Agam Regency in West Sumatra, Indonesia. Botanist Dr. Norainas identified the samples, which were then deposited in the ANDA herbarium at Andalas University's Faculty of Mathematics and Natural Sciences in Padang, Indonesia.

2.2 Test Microorganism

The test microorganisms were *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* FNCC 9027, *Escherichia coli* ATCC 8739, *Streptococcus mutans* ATCC 25175 , and *Meticilin Resistant Staphylococcus aureus* ATCC 43300 which obtained from Research Laboratory, Andalas University.

2.3 Extraction of Essential Oil

The hydrodistillation method was used to extract the peels' essential oil. To remove dirt, the fresh fruits were rinsed with tap water. After gently peeling the orange, both peels were chopped. After the first drop of water vapor, the distillation process was carried out for 4 hours. For future use, the essential oil was preserved in the refrigerator.

2.4 Analysis of Essential Oil Using GC-MS

The essential oils were analyzed for their chemical components using GC Agilent® 7890A instrument couple with MS Agilent® 5975 detector using Agilent® HP-5ms column with a diameter of 0.32 mm, 0.25 m thick, 30 m long. The carrier gas was Helium with flow at a rate of 1mL/min. Temperature conditions was 50-300 °C (temperature of 50 °C was constant for 2 minutes, the temperature was increased to 80 °C with an increase of 2 °C / min, then increased to 150 °C with an increase of 5 °C / min, then increased to 200 °C with an increase of 10 °C/minute and then increased to 300 °C with an increase of 20 °C/min, at a temperature of 300 °C was held constant for 5 minutes. The temperature of the injector and detector were 250 °C and 270 °C, respectively and detecteor energy for 1.25 kV, pressure of 70 kPa. The volume of the injection was 1 µL.

2.5 Antibacterial Activity

The broth microdilution method was used to test antibacterial activity. The cultured bacteria were suspended in saline solution, and the turbidity was measured using the McFarland 0.5 method. The bacterial suspension was then diluted 1:150 in Mueller Hinton Broth (MHB) medium. A total of 50 microliters of MHB media was poured into each well of a 96-well plate. The test solution was added to the first row in 50 microliters (50 mg/ml) increments, and two-fold dilution was conducted until a concentration of 25, 12.5, 6.25, 3.1251, 1.262, 0.781 mg/ml was obtained. Ciprofloxacin, a positive control, was also added to the well. Then, for growth and sterility control, 50 microliters of bacterial suspension was put to the except well. After that, the plate was incubated at 37 0C for 18-24 hours (16). The plate was then incubated at 37^{o}C for 30 minutes with 40 microliters MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide] at a final concentration of 0.5 mg/ml. The color change (from colorless to purple) was noticed after 30 minutes. Where there was no color change, the minimum inhibitory concentration (MIC) was established. The antibacterial test was performed three times [10].

3. RESULTS AND DISCUSSION

3.1 Characterization of Essential Oils

The derived essential oils were pale yellow in color and had a distinct scent. Table 1 shows the percent yield 1.1%, specific gravity 0.857 g/mL, and refractive index 1.469 of essential oils.

The chemical component of essential oils, such as the number of double bonds and carbon chain length, influenced the difference in the value of the refractive index. If the oil contained a chemical with a lot of double bonds, the viscosity of the oil rose, making it difficult to refract light. As a result, the refractive index of the oil increased as the density of the oil increased. The refractive index of essential oils was also altered by their water content. The refractive index value decreases as the amount of water in the oil increases.

3.2 Analysis of Chemical Compounds with GC-MS

The findings revealed that the components of the essential oil of the fruit peel were shown in Table 2. D-limonene (44.1%), Terpinen (20.9%), and Camphene (14.5%) were the significant components in the essential oil of the fruit peel. Citrus essential oil comprises more than 90% volatile components, the majority of which are monoterpenes and sesquiterpenes [6].

3.3 Antibacterial Activity

As seen in Figure 3, the antibacterial activity yielded positive results the essential oil can inbhibit the growth of microorganism.

Table 1 . Chemical composition of the essential oil of *Citrus x aurantiifolia*

No	Compound	(%)
1	γ-Terpinene	20.9
2	D-Limonene	44.1
3	α-Pinene	4.0
5	Athujene	1.6
6	Germacrene D	1.2
7	β- Myrcene	2.0
8	D-sylvestrene	1.6
9	Camphene	14.5
10	alfa terpinolene	2.1
11	α-Terpinene;	1.4
12	cis-Linalool oxide	1.2

The essential oils extracted from the fruit peel is found to prevent the growth of Gram negative, Gram positive, and resistant bacteria. Based on the figure 3 it can be said the essential oils inhibited *P.aeruginosa*, *MRSA*, *S. Aureus* and *E.coli* with the different concentrations. Peel essential oils were more effective at inhibiting *S. aureus* development than *E. coli*. It's possible that this is because Gram positive bacteria have a simpler structure, with peptidoglycan and teichoic acid components. Peptidoglycan, lipoprotein, outer membrane, and lipopolysaccharide make up the Gram negative bacterium's cell Wall [12].

The antibacterial activity of fruit peel due to qualitative and quantitative changes in chemical components. Based on the figure 3. Essential oil of Citrus x aurantifolia Essential oils can control bacterial resistance and can be used as an adjuvant therapy against bacterial resistance to specific antibiotics, in addition to having antibacterial properties [13].

Limonene, with a molecular weight of 136.2340 and a chemical formula of C10 H 16, is a key component in various citrus fruits that efficiently suppresses bacterial development [7].

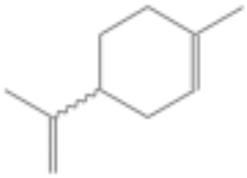


Figure 2. Stucture of Limonene

Limonene has been shown to suppress the development of both Gram positive and Gram negative bacteria, indicating that it has a broad spectrum of action. The mechanism of action of limonene in inhibiting bacterial growth is as follows: I destroying cell walls and membranes, causing protein and bacterial nucleic acid leakage, (ii) inhibiting ATPase activity, thereby inhibiting

ATP synthesis, and (iii) inhibiting ATPase activity, thereby inhibiting ATP synthesis, activity (iii) The respiratory complex's work is inhibited, causing it to be interrupted. bacterial cell metabolic processes Despite the fact that limonene was abundant, it was not the sole source of antibacterial activity.

Other minor components can boost essential oils' antibacterial action by forming a synergistic association between minor and major components at concentrations that result in effective antibacterial activity [14].

Furthermore, monoterpene compounds such as -terpinene, -pinene, and camphene were found to have antibacterial activity against Gram positive and Gram negative bacteria, as well as inhibiting the growth of Mycobacterium TB [6].

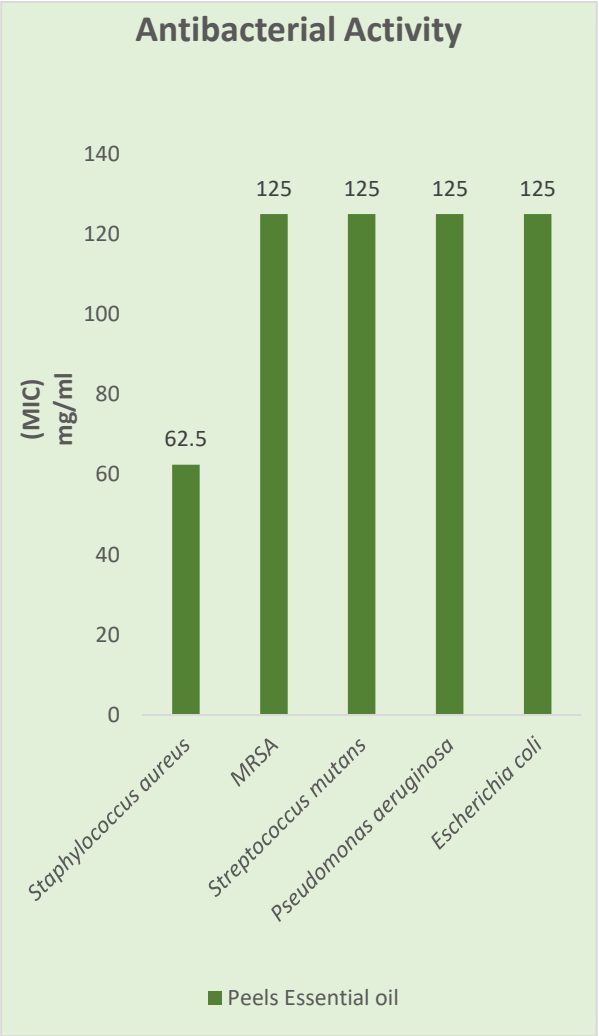


Figure 3. Antibacterial Activity of Essential Oil of *Citrus x aurantiifolia*

4. CONCLUSİON

The chemical component of the *Citrus x aurantiifolia* fruit peel is D-limonene, -terpinene, and Camphene were the most common constituents in the peel essential oils can inhibit the growth of bacteria. *Pseudomonas aeruginosa, Streptococcus mutans, Escherichia coli,* and *Methicillin Resistant Staphylococcus aureus* all had MIC values of 125 mg/ml, while *Staphylococcus aureus* had MIC values of 62.5 mg/ml. *Citrus x aurantiifolia* peels were needed to

test its efficacy against other bacteria and to see if it has the potential to be developed as a treatment.

AUTHORS' CONTRIBUTIONS

Every author contributes to the creation of article.

ACKNOWLEDGMENT

This research was funded by the Directorate of Resources, Directorate General of Higher Education, Ministry of Education, Culture, Research and Technology through the multiple year applied research with main contracts no 266/E4.1/AK.04.PT/2021 and derivative contract T/2/UN.16.17/PT.01.03/PT-Kesehatan/2021

REFERENCES

[1] W. Deng, K. Liu, S. Cao, J. Sun, B. Zhong, and J. Chun, "Chemical composition, antimicrobial, antioxidant, and antiproliferative properties of grapefruit essential oil prepared by molecular distillation," *Molecules*, vol. 25, no. 1, 2020.

[2] B. Satari and K. Karimi, "Citrus processing wastes: Environmental impacts, recent advances, and future perspectives in total valorization," *Resour. Conserv. Recycl.*, vol. 129, pp. 153–167, 2018.

[3] C. E. Palma, P. S. Cruz, D. T. C. Cruz, A. M. S. Bugayong, and A. L. Castillo, "Chemical composition and cytotoxicity of Philippine calamansi essential oil," *Ind. Crops Prod.*, vol. 128, pp. 108–114, 2019.

[4] K. Fisher and C. Phillips, "Potential antimicrobial uses of essential oils in food: is citrus the answer?," *Trends Food Sci. Technol.*, vol. 19, no. 3, pp. 156–164, 2008.

[5] B. S. Jugreet, S. Suroowan, R. R. K. Rengasamy, and M. F. Mahomoodally, "Chemistry, bioactivities, mode of action and industrial applications of essential oils," *Trends Food Sci. Technol.*, vol. 101, pp. 89–105, 2020.

[6] W. N. S. Noura S. Dosoky, "Biological Activities and Safety of Citrus Spp. Essential oils." Molecular Sciences, USA, 2017.

[7] M. A. Raspo, M. B. Vignola, A. E. Andreatta, and H. R. Juliani, "Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States," *Food Biosci.*, vol. 36, p. 100651, 2020.

[8] B. Singh, J. P. Singh, A. Kaur, and N. Singh, "Phenolic composition, antioxidant potential and health benefits of citrus peel," *Food Res. Int.*, vol. 132, p. 109114, 2020.

[9] E. Gómez-mejía, N. Rosales-conrado, M. E. León-González, and Y. Madrid, "Citrus peels waste as a source of value-added compounds : Extraction and quantification of bioactive polyphenols," *Food Chem.*, vol. 295, pp. 289–299, 2019.

[10] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," *J. Pharm. Anal.*, vol. 6, no. 2, pp. 71–79, 2016.

[11] A. K. Chintaluri, A. L. Komarraju, V. K. Chintaluri, and B. Vemulapalli, "Comparative study of antimicrobial activity of essential oils of selected plants of Rutaceae and TLC bioautographic studies for detection of bioactive compounds," *J. Essent. Oil Res.*, vol. 27, no. 1, pp. 9–16, 2015.

[12] D. Benedetto Tiz, D. Kikelj, and N. Zidar, "Overcoming problems of poor drug penetration into bacteria: challenges and strategies for medicinal chemists," *Expert Opin. Drug Discov.*, vol. 13, no. 6, pp. 497–507, 2018, doi: 10.1080/17460441.2018.1455660.

[13] N. Irahal *et al.*, "Chemical composition ,

antibacterial and antioxidant activities of some essential oils against multidrug resistant bacteria," *Eur. J. Integr. Med.*, vol. 35, no. December 2019, p. 101074, 2020.

[14] Y. Han, Z. Sun, and W. Chen, "Antimicrobial Susceptibility and Antibacterial Mechanism of Limonene against Listeria monocytogenes," *molecules*, vol. 25, no. 1, p. 33, 2020.