

Identification of Bioactive Compounds and Antibacterial Activity of Papaya Leaf Extract (*Carica Papaya* L.) as an Inhibitor of *Pseudomonas Aeruginosa*

Wd Windi Pratiwi¹, Sogandi²,

^{1,2}Fakultas Farmasi, Universitas 17 Agustus 1945 Jakarta, Indonesia

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ABSTRACT

This study explores the potential of papaya leaf extract (*Carica papaya* L.) as a natural antibacterial agent against *Pseudomonas aeruginosa*. The extraction process was carried out using 96% ethanol through maceration, followed by fractionation with n-hexane and ethyl acetate. Antibacterial activity was assessed using disc diffusion and liquid dilution methods to determine the inhibition zone and the Minimum Inhibitory Concentration (MIC). Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, and tannins in all tested fractions. Among them, the n-hexane fraction demonstrated the strongest antibacterial activity, with an inhibition zone of 15.10 mm at a 10% concentration and a MIC at 6%. Further analysis using Gas Chromatography-Mass Spectrometry (GC-MS) identified 79 compounds in the n-hexane fraction, with several dominant compounds such as hexadecanoic acid methyl ester, pentadecanoic acid 14-methyl methyl ester, and phytol — all known for their antibacterial properties. These results suggest that the n-hexane fraction of papaya leaf extract holds promising potential for development as a natural antibacterial agent, particularly against *Pseudomonas aeruginosa*.

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Corresponding Author:

Sogandi,

Fakultas Farmasi, Universitas 17 Agustus 1945 Jakarta

Jakarta, Jakarta Utara, Indonesia

Email: Sogandi@uta45jakarta.ac.id

1. INTRODUCTION

Indonesia, as a tropical country with abundant biodiversity, possesses numerous medicinal plants that have been widely used in traditional medicine. Among them, papaya (*Carica papaya* L.) leaves are well known for their pharmacological properties, including antimicrobial activity. Recent studies have revealed that papaya leaves contain various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and phenols, which contribute significantly to their antibacterial effects (Rantetampang et al., 2023; Sari & Lestari, 2024).

A study by Gulo and Nainggolan (2024) demonstrated that ethanol extract of papaya leaves exhibited inhibitory effects against *Staphylococcus aureus* with inhibition zones ranging from 6.7 to 11.2 mm. Furthermore, Amalina and Lestari (2023) reported that polar fractions of papaya leaf extract showed inhibitory activity against both *S. aureus* (4.8 mm) and *E. coli* (11.5 mm), confirming its potential as a broad-spectrum antibacterial agent.

Papaya (*Carica papaya* L.) belongs to the Caricaceae family which has therapeutic properties and rich nutritional benefits. Different parts of the papaya plant have been widely used since ancient times for medicinal or therapeutic applications (Nugraha & Leliqia, 2023).

Extracts from papaya leaves (*Carica papaya* L.) have been shown to have antibacterial properties in several studies. One of them was conducted by Herlina et al. (2020) which stated that the antibacterial activity test on papaya leaf extract against *Salmonella thypi* showed significant antibacterial activity with an effective concentration that was able to slow the growth of *Salmonella thypi* bacteria up to 100%, with an inhibition zone area reaching 11.70 mm.

Based on research (Mahatrinny et al., 2014) the results of phytochemical screening show the presence of positive compounds of alkaloids, flavonoids, tannins and glycosides, in line with research (Jati et al., 2019) which isolates and identifies and tests antibacterial activity against *Staphylococcus aureus* and *Esecherichia cole* bacteria from alkaloid compounds in papaya leaf extract have potential antibacterial

activity with an inhibition zone in *Staphylococcus aureus* bacteria of 14.3 mm and *Esecherichia coli* of 16.1 mm.

In a study conducted by Nasri *et al.* (2022) papaya leaf ethanol extract has antibacterial activity against *Pseudomonas aeruginosa* with the largest category inhibition zone diameter value at a concentration of 500 mg/mL.

Based on research (Fauziah *et al.*, 2022). The test results obtained from the n-hexane and ethyl acetate fractions at concentrations of 10%, 15% and 20% with positive control erythromycin 0.375%, and negative control solvents n-hexane nd ethyl acetate) in the form of inhibition zone diameter. The ethyl acetate fraction with concentrations of 10%, 15% and 20% was the most effective fraction with an average diameter of 14.7 ± 6.33 ; 16.4 ± 1.55 ; and 18.7 ± 4.14 . The conclusion obtained from this study is that the ethyl acetate fraction of papaya leaves with a concentration of 15% is the most optimal fraction in inhibiting the growth of *Propionibacterium acnes* bacteria.

Kining and Novik (2023) reported that papaya leaf water extract was able to inhibit the early biofilm formation by *Pseudomonas aeruginosa* by up to 40.9% and could disrupt mature biofilm by up to 48.1% at a concentration of 25% with an optimal temperature of 37.5°C for 45 minutes. This antibiofilm effect remained significant even at higher temperatures and longer contact times. In addition, (Sahambangung *et al.*, 2023) showed that ethanol extract of papaya leaves, which is rich in phenolic compounds, had an inhibition zone of 10.87 ± 0.06 mm against *Pseudomonas aeruginosa* at a concentration of 500 mg/mL. The Minimum Inhibitory Concentration (MIC) obtained was 3.125 mg/mL, which is classified as strong activity.

Based on the description that has been stated previously, this research further aims to withdraw the secondary metabolites present in papaya leaf extract (*Carica papaya* L.) so as to find out which solvent has the highest antibacterial activity against *Pseudomonas aeruginosa*.

2. RESEARCH METHODS

This study is an experimental research conducted to analyze the bioactive compounds in the n-hexane fraction of papaya leaves (*Carica papaya* L.). The papaya leaves were collected from a garden located on Jalan Raya Serpong, South Tangerang, Banten. The fractionation and analysis were carried out at the Chemistry Research Center, National Research and Innovation Agency (BRIN), Building 456, BJ Habibie Science and Technology Area, Indonesia, and supported by the Department of Chemical Engineering, Universitas Pamulang, Indonesia.

The identification of chemical compounds was performed using Gas Chromatography-Mass Spectrometry (GC-MS) with an Agilent 7890B GC and 5977A MSD instrument. The column used was Agilent 19091S-433 DB-5ms UI (5% Phenyl Methyl Siloxane), with an injection volume of 1 mL. The compounds were identified by comparing the mass spectra with the NIST 20 database.

3. RESULTS AND DISCUSSION

The chemical content of the N-Hexan fraction of papaya leaves (*Carica papaya* L.) is shown in Figure 1.

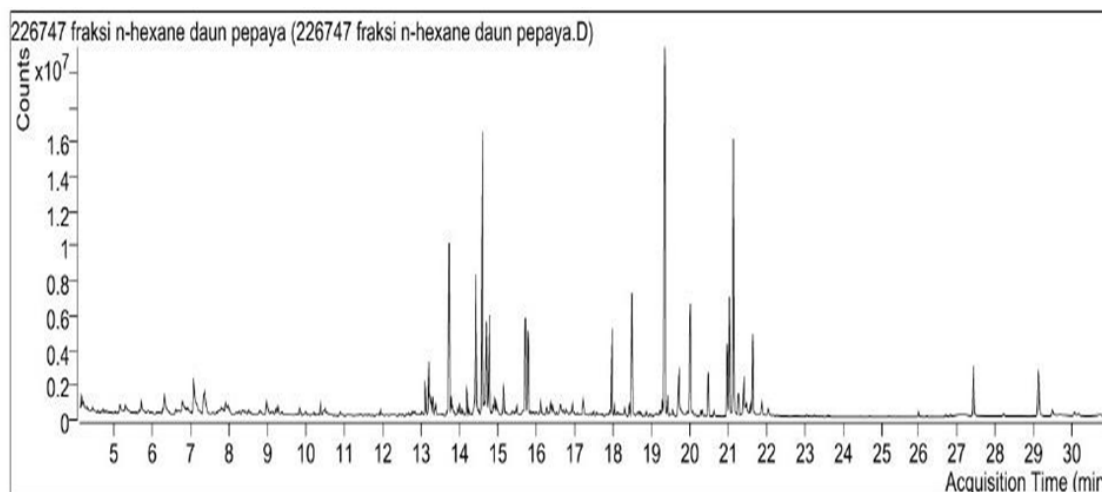


Figure 1. Fractionation chromatography of papaya leaves (*Carica papaya* L.)

Based on the identification in Figure 1, the leaf fraction of papaya leaves (*Carica papaya* L.) is identified to contain 10 compounds and 4 peak compounds that have a large compound content (%) are presented in Table 1, where there are 4 compounds that have the highest content marked with red color,

namely, pentadecane (8.03%) Hexadecanoic acid, methyl ester (11.07%), Pentadecanoic acid, 14-methyl-, methyl ester (11.07%) and phytol (7.22%).

Table 1. Chemical compounds of the n-Hexan fraction of papaya leaves

No.	Peak	Retention Time (RT)	Area	Compound	Qual
1.	49	19.375	11.07	Hexadecanoic acid, methyl ester	98
2.	49	19.375	11.07	Pentadecanoic acid, 14-methyl-, methyl ester	98
3.	27	14.611	8.03	Pentadecane	97
4.	56	21.152	7.22	Phytol	96
5.	21	13.741	5.37	Caryophyllene	99
6.	26	14.447	4.26	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	99
7.	52	20.030	3.73	Hexadecanoic acid, ethyl ester	96
8.	42	15.732	3.11	Cetene	99
9.	55	21.064	2.97	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	96
10.	29	14.787	2.94	(R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene	99

The thick n-Hexan fraction of papaya leaf leaves (*Carica papaya* L.) was carried out with organoleptic observations to determine the physical texture, odor, color and taste. Based on research conducted by (Aprilia *et al.*, 2023) that the thick n-hexan fraction of papaya leaf leaves (*Carica papaya* L.) has a thick, liquid texture, smells typical of papaya, is greenish black, and tastes bitter.

Phytochemical screening tests include identification of alkaloid, flavonoid, saponin, and tannin compounds. Phytochemical screening is needed to qualitatively identify the class of compounds contained in the n-hexane fraction of papaya leaves. Based on the results obtained that the n-hexane fraction of papaya leaves showed positive results for alkaloids, flavonoids, saponins, and tannins. According to (A'yun & Laily, 2015) in the phytochemical screening of papaya leaves that papaya leaves have alkaloid content, flavonoids, saponins, and tannins. The results obtained have differences due to the nature of the solvent in the ethanol extract of papaya leaves and the n-hexane fraction of papaya leaves.

Table 2. The yield of n-Hexan fraction of papaya (*Carica papaya* L.) leaves

Extract weight (gram)	Fraction weight Fraction	Fraction yield (%b/b)
8 gram	3,2 gram	40%



Figure 2. N-hexane fractionation of papaya leaves (*Carica papaya* L.)

This research began with the determination of the plants used in the Herbarium Depokensis (UIDEP), Biota Collection Room, Faculty of Mathematics and Natural Sciences, University of Indonesia (FMIPA UI). This process was carried out to ensure that the plants used in the study were indeed the species in question, namely papaya leaves (*Carica papaya* L.).

Based on the letter of plant identification results from the Department of Biology FMIPA UI with number 461/UN2.F3.11/PDP.02.00/2024 dated October 10, 2024, the determination results show that the specimen sent with the code [JI24-P-185] is a true species of *Carica papaya* L. from the Caricaceae family. Thus, the plant used in this study has been scientifically verified as (*Carica papaya* L.)

The extraction process was carried out by maceration method using 96% ethanol solvent for 3×24 hours. This method was chosen because it does not involve heating so that it can maintain the stability of active compounds such as flavonoids, triterpenoids, and saponins (Puspitasari, 2018). Ethanol 96% is used because it is universal and efficient in extracting polar to nonpolar compounds, and has a low water content that minimizes contamination (Tiwari *et al.*, 2011).

A total of 300 grams of papaya leaf powder was macerated and produced a thick extract of 43.55 grams with a yield of 14.516%. Evaporation was carried out in an aerated manner for 3 days until a green extract with a distinctive odor of papaya leaves was obtained. The extract was then used for yield analysis and phytochemical screening.

The chemical compounds contained in the ethanol extract, n-hexane fraction and ethyl acetate fraction of papaya leaves are seen from the qualitative screening results shown in table 3.

Table 3. Phytochemical screening results of ethanol extracts and fractions of papaya leaf (*Carica papaya* L.)

Results			
Testing	96% Extract Ethanol	Fraction n-hexan	Ethyl acetate fraction
Alkaloid	+	+	+
Saponin	+	+	+
Tannin	+	+	+
Phenolic	+	-	+
Flavonoid	+	+	+
Glycoside	+	-	-
Triterpenoid	+	+	-

The minimum inhibitory concentration (KHM) of ethanol extracts and fractions of papaya leaves against *Pseudomonas aeruginosa* bacteria is presented in table 2. This anti-bacterial activity test is determined by the agar diffusion method by measuring the inhibition zone. The results of the inhibition zone diameter are presented in table 4

Table 4. Value Observation Results (KHM)

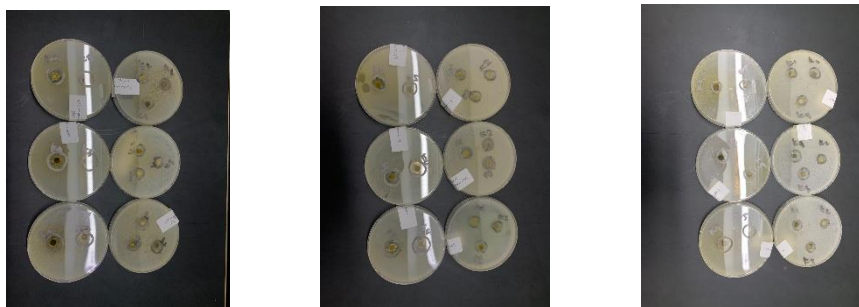
Sample	Concentration (%)				
	2	4	6	8	10
Ethanol	+++	+++	+	-	-
Ethyl Acetate	+++	+++	+	-	-
N-hexane	+++	+++	-	-	-

Description:

- : no bacterial growth
- + : there is weak intensity bacterial growth
- ++ : there is moderate intensity bacterial growth
- +++ : there is a lot of intensity bacterial growth

Table 5. Zone of Inhibition of Ethanol Extracts and Fractions of Pepeya Leaves (*Carica Papaya* L.)
Against the Growth of *Pseudomonas aeruginosa*

Concentration	Extract	N-hexane fraction	Ethyl acetate fraction
2	7,94	10,38	8,09
4	13,24	12,08	7,65
6	9,53	13,01	8,79
8	9,84	14,37	9,53
10	10,44	15,10	10,23
KN	3,95		
KP	18,87		



Inhibition zone results of 96% ethanol extract and fractionation of papaya leaves (*Carica papaya* L.)

Extraction

Noni leaf simplisia is extracted by maceration method. Maceration is one of the cold extraction methods with the aim that no active substances are damaged by the heating process. This process is very beneficial in the isolation of natural materials because the maceration process using 96% ethanol will break the cell wall caused by the pressure difference between inside and outside the cell, so that secondary metabolites in the cytoplasm will be dissolved in organic solvents. The selection of solvents for the maceration process can provide high effectiveness by paying attention to the solubility of natural material compounds in the solvent. (Banu & Cathrine 2015). The solvent used for extraction is ethanol solvent which has polar properties so that it can attract secondary metabolite compounds which are also polar.

Ethanol Free Test

The results of extraction using ethanol solvent obtained in this study were carried out ethanol free test with the aim of ensuring that the thick extract of maceration results which will be used as samples in testing antibacterial activity is free from residual ethanol solvent, because ethanol is antibacterial and antifungal so it will not cause false positives in the sample treatment later. From the test results, it is known that the sample is free of ethanol marked by the absence of ester odor after reacting the extract with sulfuric acid and heated acetic acid. (Sogandi *et al.*, 2020).

Minimum Inhibitory Concentration (KHM) of Ethanol Extract and Fraction of Papaya Leaf (*Carica papaya* L.)

The minimum inhibitory concentration (KHM) of ethanol extract and fraction of papaya leaf against *Pseudomonas aeruginosa* bacteria is presented in Table 4. This antibacterial activity test was determined by agar diffusion method by measuring the inhibition zone. The observation results of KHM values are presented in Table 4.

Based on the data in Table 4, the ethanol extract and ethyl acetate fraction are known to have a minimum inhibitory concentration at a concentration of 8%, which is characterized by the absence of bacterial growth (-) starting from that concentration. Meanwhile, the n-hexane fraction has a lower minimum inhibitory concentration, which is at a concentration of 6%. The lowest minimum inhibitory concentration is found in the n-hexane fraction, because at a concentration of 6% it can inhibit bacterial growth as indicated by the absence of bacterial growth. The difference in the ability to inhibit bacterial growth of each fraction is thought to be caused by differences in the content of compounds extracted in each solvent. Based on phytochemical screening data (Table 3), the compounds extracted in the n-hexane fraction are thought to have non-polar components with higher antibacterial activity against *Pseudomonas aeruginosa* than the ethyl acetate fraction and ethanol extract.

The results of measuring the KHM value are different from the research conducted by (Nasri *et al.* 2022) that the ethanol extract of papaya leaves has a MIC value of 0.3125% (3.125 mg/mL) against the growth of *Pseudomonas aeruginosa* bacteria. Meanwhile, according to (Torar *et al.* (2017), papaya seed ethanol extract only shows antibacterial activity against *Pseudomonas aeruginosa* at concentrations $\geq 20\%$, which is categorized as moderate activity. Meanwhile, according to (Nuralifah *et al.* 2022), a cream formulated with young papaya seed extract at a concentration of 10% was able to produce an inhibition zone of 19.3 mm against *Pseudomonas aeruginosa*.

Antibacterial Activity

Antibacterial activity of ethanol extract and fraction of noni leaves against *Pseudomonas aeruginosa* bacteria using disc diffusion method. Determination of this activity is seen from the magnitude of the inhibition zone formed from each test sample with ciprofloxacin as a positive control and sterile distilled water as a negative control.

The results of the antibacterial activity test of ethanol extracts and papaya leaf fractions (*Carica papaya* L.) against *Pseudomonas aeruginosa* showed a variation in the inhibition zone which was influenced by the type of fraction and concentration used (Table 5). In general, an increase in concentration tends to cause an increase in antibacterial activity. The n-hexane fraction showed the highest antibacterial activity against *Pseudomonas aeruginosa*, with a maximum inhibition zone of 15.10 mm at 10% concentration, followed by 8% concentration at 14.37 mm. These results indicate that the n-hexane fraction is more effective than the ethanol extract and ethyl acetate fraction, but the difference is not too far from the positive control (ciprofloxacin 19.87 mm), thus indicating strong antibacterial potential but still within reasonable limits for natural compounds.

The ethanol extract and ethyl acetate fraction showed inhibition zones of 10.44 mm and 10.23 mm at 10% concentration, respectively. This indicates that the non-polar compounds in the n-hexane fraction are more dominant in providing antibacterial effects against Gram-negative bacteria such as *Pseudomonas aeruginosa*. As a reference, the positive control using ciprofloxacin is known to produce an inhibition zone of 19.87 mm, while negative controls such as distilled water generally show no real antibacterial activity, with an inhibition zone of 3.95 mm. Thus, the inhibition shown by the extracts and fractions can be assumed to come from the active compounds in papaya leaves, not from the solvent or media.

Despite the relatively low concentration of extracts and fractions used (2-10%), the test results showed strong inhibition, indicating the potential of papaya leaf active compounds at low doses. This effectiveness is likely due to the high content of bioactive compounds and the efficiency of the extraction method used.

The difference in sensitivity of bacteria to antibacterial compounds is strongly influenced by their cell wall structure. Gram-positive bacteria have a wall consisting of a thick peptidoglycan layer without an outer membrane, making it easier for antibacterial compounds to enter the cell. In contrast, Gram-negative bacteria such as *Pseudomonas aeruginosa* have an additional outer membrane containing lipopolysaccharide (LPS), which serves as a strong barrier against the penetration of antibacterial compounds. This structure is also supported by the presence of porins and active efflux systems, which contribute to natural resistance to various antibiotics (Silhavy *et al.*, 2010; Therien *et al.*, 2021).

The antibacterial activity of various parts of the papaya plant (*Carica papaya* L.) both in the form of extracts and fractions, has been widely studied to determine its effectiveness against pathogenic bacteria, including *Pseudomonas aeruginosa*. Research (Dagne *et al.* 2021) shows that the ethanol extract of papaya leaves has good antibacterial activity against *Pseudomonas aeruginosa*, with an inhibition zone of 13.2 ± 2.3 mm and a Minimum Inhibition Concentration (KHM) of 12.5 mg/mL. These results indicate that the active compounds in papaya leaves can significantly inhibit the growth of Gram-negative bacteria.

Another study by (Alo *et al.* 2021) used ethanol extract from papaya seeds and produced an inhibition zone of 14.3 mm against *Pseudomonas aeruginosa*. Although the plant parts used were different, their effectiveness still showed moderate antibacterial potential. In contrast to these two studies, a study by (Torar *et al.* 2017) found that papaya seed extract only showed antibacterial activity at relatively high concentrations, i.e. $\geq 20\%$, with MIC reaching 25 mg/mL, indicating lower effectiveness when compared to leaf extract.

In addition to crude extracts, several studies have also evaluated the effectiveness of more specific solvent fractions such as n-hexane and ethyl acetate. Research conducted by (Auwal *et al.* 2018) reported that the n-hexane fraction of papaya leaves was able to produce an inhibition zone of 18.23 ± 1.12 mm, while the ethyl acetate fraction produced an inhibition zone of 15.35 ± 1.04 mm against bacterial isolates including *Pseudomonas aeruginosa*. These results indicate that the non-polar compounds contained in the n-hexane fraction have higher inhibitory potential than the semi-polar compounds in the ethyl acetate fraction. This difference is thought to be related to the type of bioactive compounds extracted, where non-polar compounds such as terpenoids and alkanes are known to have strong antibacterial activity through the mechanism of bacterial cell membrane damage.

Based on the results of this study, it can be seen that the activity of each fraction is different and the fraction that has the greatest inhibition is the n-hexane fraction, so that the n-hexane fraction of papaya leaves (*Carica papaya* L.) is continued to the stage of identifying bioactive compounds using GCMS. (Sogandi *et al.*, 2020).

The difference in the diameter of the inhibition zone is likely due to the content of different antibacterial compounds and the presence of active substances containing antibacterial compounds from the n-hexane fraction of papaya leaf extract (*Carica papaya* L.) containing compounds of Pentadecane, Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-, methyl ester and phytol. Identification of bioactive compounds

The results of the identification of secondary metabolite compounds from the n-hexane fraction of papaya leaves using GC-MS instruments showed that the main compound groups were alkanes and terpenoids, with the highest abundance being hexadecanoic acid, methyl ester compounds at 11.07%. The next highest content was pentadecanoic acid, 14-methyl-, methyl ester compound at 11.07%, followed by

pentadecane compound at 8.03%, and phytol at 7.22% which is a diterpenoid compound. The pentadecane compound is a saturated aliphatic hydrocarbon compound with a straight chain structure, which was identified with a relative abundance of 8.03% in the n-hexane fraction of papaya leaves. This compound is found in many plant essential oils and is known to have potential as an antimicrobial. The mechanism of action is thought to be through disruption of the bacterial cell membrane, which causes damage to the structure and function of the membrane. In a study conducted by (da Silva *et al.*, 2018), pentadecane showed significant inhibitory activity against *Leishmania infantum*, which suggests that this compound could also have antibacterial effects against other microorganisms.

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The compound hexadecanoic acid, methyl ester or also known as methyl palmitate belongs to the class of saturated fatty acid esters. This compound is known to have strong antibacterial activity through the mechanism of disruption of the bacterial cell membrane, causing leakage of cell contents and microbial death. (Khan *et al.* 2021) showed that this compound is effective against pathogenic bacteria such as *Pseudomonas aeruginosa*, even against antibiotic-resistant strains. This activity suggests its potential as a natural antibacterial agent.

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The last compound was found in an amount of 7.22%, and belongs to the class of alcoholic diterpenoids. Phytol is widely recognized as a bioactive compound that has antibacterial, antioxidant, and anti-inflammatory activities. Based on a study by (Kono *et al.* 2016), phytol has the ability to inhibit the growth of *Pseudomonas aeruginosa* through the induction of oxidative stress and increased free radical production (ROS), which ultimately leads to bacterial cell death. This ability supports its role in the antibacterial activity of the n-hexane fraction of papaya leaves.

4. CONCLUSION

This study revealed that ethanol extract and n-hexane and ethyl acetate fractions of papaya leaves (*Carica papaya* L.) have antibacterial activity against *Pseudomonas aeruginosa*. The n-hexane fraction showed the highest activity with a Minimum Inhibitory Concentration (KHM) value of 8%, followed by the ethanol extract and ethyl acetate fraction with a KHM of 10% each. The maximum inhibition value obtained at 10% concentration was 15.10 mm for the n-hexane fraction, 10.44 mm for the ethanol extract, and 10.23 mm for the ethyl acetate fraction. These values indicate that the three samples have good antibacterial potential, although they are still below the positive control (clindamycin) which showed an inhibition zone of 19.87 mm.

GC-MS analysis of the n-hexane fraction of papaya leaves identified the main bioactive compounds as hexadecanoic acid, methyl ester, pentadecanoic acid, 14-methyl-, methyl ester, phytol, and pentadecane. These compounds are thought to contribute to antibacterial activity against the test bacteria, mainly by the mechanism of damaging cell membranes or inhibiting bacterial protein synthesis.

5. ACKNOWLEDGEMENT

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