Jurnal Ilmiah STIKES Yarsi Mataram
http://journal.stikesyarsimataram.ac.id/index.php/jik
p-ISSN: 1978-89-40 | e-ISSN: 2807-940X
Vol. XV, No. 2, Juli 2025, pp.87-95

Effectiveness Test of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of Papaya Latex Extract (*Carica papaya. L*) Against *Pseudomonas aeruginosa Bacteria*

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Article History:
Received : 16-05-2025
Revised : 03-07-2025
Accepted :14-07-2025
Online : 30-07-2025

Keyword:
Antibacterial; Papaya Latex Extract; *Pseudomonas Aeruginos.*

ABSTRACT

This study aims to test the effectiveness of papaya latex extract (Carica papaya L.) in inhibiting and killing Pseudomonas aeruginosa bacteria using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods. Papaya latex extract was extracted using the percolation method and tested at various concentrations, namely 50%, 25%, 12.5%, 6.25%, and 3.125% and 1,4%. The MIC test results showed that a concentration of 12.5% was able to inhibit bacterial growth, while concentrations of 25% and 50% effectively killed the bacteria completely. The SPSS test showed a significant difference between concentrations ($p < 0.05$) on bacterial growth, with a concentration of 25% as the most effective in killing bacteria. Based on these results, papaya latex extract has good antibacterial potential, especially at concentrations ≥ 25%, and can be used as an alternative in the development of natural antibacterial agents.

ABSTRAK

Penelitian ini bertujuan untuk menguji efektivitas ekstrak getah pepaya (Carica papaya L.) dalam menghambat dan membunuh bakteri *Pseudomonas aeruginosa* dengan menggunakan metode Kadar Hambat Minimum (KHM) dan Kadar Bunuh Minimum (KBM). Ekstrak getah pepaya diekstraksi dengan metode perkolasi dan diuji pada berbagai konsentrasi, yaitu 50%, 25%, 12,5%, 6,25%, 3,125% dan 1,4%. Hasil uji KHM menunjukkan bahwa konsentrasi 12,5% mampu menghambat pertumbuhan bakteri, sementara konsentrasi 25% dan 50% efektif dalam membunuh bakteri secara total. Uji SPSS menunjukkan adanya perbedaan yang signifikan antar konsentrasi ($p < 0,05$) terhadap pertumbuhan bakteri, dengan konsentrasi 25% sebagai konsentrasi yang paling efektif dalam membunuh bakteri. Berdasarkan hasil ini, ekstrak getah pepaya memiliki potensi antibakteri yang baik, terutama pada konsentrasi ≥ 25%, dan dapat dijadikan alternatif dalam pengembangan agen antibakteri alami.

A. INTRODUCTION

Pseudomonas aeruginosa is one of the opportunistic pathogenic bacteria that are often found in nosocomial infections. This bacterium has a high level of resistance to various antibiotics, making infections difficult to treat (Kumari *et al*., 2020). Therefore, The search for alternative antibacterial agents from natural sources is an important effort to overcome this problem. One potential source of natural antibacterial agents is papaya latex (*Carica papaya*). Papaya latex contains various bioactive compounds—including papain, flavonoids, alkaloids, and saponins—that exhibit antimicrobial activity (Aravind *et al*., 2013). These compounds are believed to exhibit antibacterial activity by inhibiting the growth of, and killing, pathogenic bacteria such as *Pseudomonas aeruginosa*

Previous studies have shown that papaya latex extract has antibacterial activity against various types of gram-positive and gram-negative bacteria (Nayak *et al*., 2016). However, the effectiveness of this extract against *Pseudomonas aeruginosa* and the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values specific to this bacteria have not been widely studied. Determination of MIC and MBC of an antibacterial compound is very important in the fields of microbiology and pharmacy. MIC is the lowest concentration of an antimicrobial agent that can inhibit bacterial growth, while MBC is the lowest concentration that can kill bacteria completely (Balouiri et al., 2016). This data is essential for the development of antibacterial agents to ensure both their effectiveness and safety.

This is done as an effort to overcome the increasing antibiotic resistance throughout the world. In addition to its antibacterial potential, papaya latex extract offers other advantages, such as being readily available, cost-effective, and having minimal side effects compared to synthetic antibiotics (Ali et al., 2018). Therefore, this study aims to provide a natural and effective alternative for treating infections caused by Pseudomonas aeruginosa. The use of papaya latex extract is also aligned with current phytopharmaceutical research trends, in which natural ingredients are being explored as substitutes or complements to conventional antibiotics (Sulaiman et al., 2019). This approach aims to address the growing problem of antibiotic resistance worldwide.

The method used in this study involved microbiological tests to determine the MIC and MBC of papaya latex extract against *Pseudomonas aeruginosa* . This test was carried out using the micro or macro dilution method to assess the level of effectiveness of the extract in inhibiting growth and killing the bacteria (Wiegand et al., 2008). In addition to effectiveness, other factors such as extract stability, extraction method, and concentration of active substances were also considered in this study. Variations in extraction methods can affect the content of active compounds in the extract, which has the potential to affect the results of the MIC and MBC tests (Khan et al., 2017). Pseudomonas aeruginosa is known to have various resistance mechanisms, such as the production of beta-lactamase enzymes and efflux pumps that can pump antibiotics out of bacterial cells (Poole, 2011). Therefore, finding new antibacterial agents that have different mechanisms of action from conventional antibiotics is a must.

With this research, it is expected to obtain valid data regarding the effectiveness of papaya latex extract in inhibiting and killing *Pseudomonas aeruginosa* . If proven effective, this extract can be further developed as an alternative therapy for bacterial infections that is more natural and safe. This research also has the potential to open up new opportunities in the use of papaya latex as an active ingredient in antibacterial drug formulations. With a more systematic and evidence-based approach, the development of drugs based on papaya latex extract can provide a more sustainable solution in the field of public health. Thus, this study aims to test the effectiveness of papaya latex extract in inhibiting and killing *Pseudomonas aeruginosa* through MIC and MBC tests. The results obtained are expected to be a reference in the development of natural-based antibacterial agents to treat infections caused by this bacteria.

B. METHODS

Titles and authors must be in a single column format and must be centered. This study employed a laboratory experimental design using a *post-test-only control group* to evaluate the effectiveness of papaya latex extract against *Pseudomonas aeruginosa*.

1. Tools and materials

Research tools: glass beaker, test tube, micro pipette, ose needle, Bunsen flame, LAF (Laminar Air Flow), glass bottle, petri dish, autoclave, stirring rod, Erlenmeyer flask, blue tip, incubator, analytical balance

Research materials: papaya latex extract, 96% ethanol, *Pseudomonas aeruginosa isolate* , Nutrient Agar (NA), Muller Hilton Agar (MHA), distilled water, tetracycline, Mg powder, FeCl3, 2N HCl, 1% crystal violet, safranin, alcohol.

2. Research procedures

a) Extraction

A total of 200 g of papaya latex powder was placed into a maceration container and dissolved in 96% ethanol. The mixture was stirred and left to stand for at least 24 hours. The macerated extract was then filtered and concentrated using a rotary evaporator at 50°C until a thick extract was obtained.

b) Ethanol free test

Papaya latex extract of 0.5 g was added with 1 mL of acetic acid and 1 mL of concentrated sulfuric acid, then heated. A positive ethanol-free reaction was indicated by the absence of a distinctive ester aroma.

c) Phytochemical screening

1. Alcaloid

   A total of 0.5 g of extract was dissolved in 96% ethanol, divided into 3 test tubes, and added with 2 N HCl. Dragendorff and Mayer reagents were then added to each tube. A positive alkaloid result is indicated by a red or orange precipitate (Dragendorff) and a white precipitate (Mayer).

2. Flavonoid

   A total of 0.5 g of extract was dissolved in 96% ethanol and transferred into a test tube. Magnesium powder and a few drops of concentrated HCl were then added, and the mixture was homogenized. A positive flavonoid test is indicated by the appearance of a yellow or orange color.

3. Saponins

   A total of 0.5 g of extract was dissolved in distilled water. The solution was heated and shaken vigorously for 10 seconds, then 2 N HCl was added. A positive saponin test was indicated by the formation of stable foam for ± 10 minutes.

4. Terpenoid

   As much as 0.5 g of extract was dissolved in 96% ethanol, then Lieberman-Burchard reagent was added. A positive terpenoid test is indicated by the formation of a purplish-red color, while a positive steroid test is indicated by the formation of a blue-green color.

5. Tannin

   As much as 0.5 g of extract was dissolved in 96% ethanol then added with 1% FeCl3. A positive tannin test is indicated by the formation of a greenish brown or blackish green color.

3. Antibacterial activity test

a. Preparation of test solution

   The concentrations of the test solution used in this study were 50%, 25%, 12.5%, 6.25%, and 3.125%, with tetracycline as the positive control and distilled water as the negative control.

b. Media creation

   Weighed as much as 5.6 g of powder or instant NA powder then dissolved in 200 mL of distilled water. The solution was heated until completely dissolved. Then the NA media and the tools to be used were sterilized using an autoclave at a temperature of 121°C for 15 minutes

c. Bacterial rejuvenation

   A total of 5 mL of NA media was put into a test tube and then left in a tilted position until solidified. Take 1 loop of pure bacterial culture aseptically and then scratch it on the surface of the tilted agar media and incubate at 37°C for 24 hours.

d. Preparation of bacterial suspension

   One loop (ose) of each bacterium was added into a test tube containing 10 mL of 0.9% physiological NaCl solution. The suspension was then shaken until homogeneous and adjusted to match the 0.5 McFarland turbidity standard.

e. Antibacterial activity test

   The dilution method was used to determine the *Minimum Inhibitory Concentration* (MIC) and *Maximum Bactericidal Concentration* (MBC) of papaya latex extract. The dilution method used 1 row of tubes consisting of 5 tubes, with concentrations of 50%, 25%, 12.5%, 6.25%, 3.125% and 1,4% control (+) and control (-). Tube 1, which is a negative control, contains 2 ml of antibiotic solution. Tube 12, as the positive control, contained only the bacterial suspension. All tubes were incubated at 37°C for 24 hours, and turbidity was then observed. The *Minimum Bactericidal Concentration* (MBC) was determined by inoculating the clear tubes onto selective media using the streak method, followed by incubation at 37°C for 24–

48 hours. The presence or absence of bacterial colonies on the plate was then observed.

4. Data Analysis

Data were analyzed using *One-Way ANOVA* with a significance level of $p < 0.05$, followed by Tukey's Post Hoc test.

C. RESULT AND DISCUSSION

1. Result

Phytochemical screening results

Table 1. Results of phytochemical screening of papaya latex extract

No	Compound	Test method	Results	Information
1	Alkaloid	Dragendorff & Mayer	+	Orange/brown precipitate formed
2	Flavonoid	Mg + HCl Reaction	+	Brick red solution
3	Saponins	Foam Test	+	Stable foam ≥ 1 cm is formed
4	Tannin	$FeCl_3$ 1%	+	The solution changes color to blue-black/greenish
5	Terpenoid	Liebermann-Burchard	+	Red-purple/blue-green color

Testing done with method dilution or test serial 7 activity antibacterial

Figure 1. *Minimum Inhibitory Concentration* (MIC)

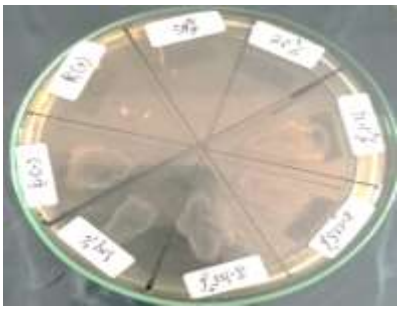

Figure 1. *Minimum Bactericidal Concentration* (MBC)

Table 2. Results of the MIC and MBC tests

Concentration (%)	MIC Results	MBC Results
50%	Not growing	Not growing

25%	Not growing	Not growing
12.5%	Not growing	Grow
6.25%	Grow	Grow
3.125%	Grow	Grow
1,4%	Grow	Grow
Tetracycline	Not growing	Not growing
Media control (BHI)	Grow	Grow

2. Discussion

a. Characterization of Papaya Latex Extract (Carica papaya L.)

Papaya latex extract was obtained through maceration method using 96% ethanol solvent. After the extraction process and solvent evaporation, a thick light brown extract with a distinctive aroma was obtained. Phytochemical tests showed that the extract contains active compounds such as alkaloids, flavonoids, saponins, and tannins, which are known to have antimicrobial activity (Mello *et al.,* 2008).

Phytochemical screening results showed that papaya latex extract contains several groups of secondary metabolite compounds that have the potential to provide antibacterial effects against *Pseudomonas aeruginosa* . These compounds work through various mechanisms against bacterial cells, either by damaging membrane structures, inhibiting protein and enzyme synthesis, or causing cell metabolic disorders.

1. Alkaloids
   The formation of precipitates in the Dragendorff and Mayer tests indicates the presence of alkaloids in the extract. Alkaloids are known to bind to nucleic acids and bacterial proteins, thereby inhibiting DNA or RNA synthesis. In addition, this compound is also able to disrupt the permeability of bacterial cell membranes (Cushnie *et al*., 2011).

2. Flavonoids
   Flavonoids are detected based on the brick red color change after reaction with Mg and HCl. Flavonoids work by damaging the cytoplasmic membrane, inhibiting nucleic acid and energy synthesis, and forming complexes with cell wall proteins that cause bacterial cell lysis (Cowan, 1999).

3. Saponin
   The foam test produces stable foam, indicating the presence of saponin. This compound is a natural surfactant that can reduce the surface tension of microbial cell membranes, causing leakage of cell contents, and ultimately causing cell death. Saponin is also able to increase cell permeability (Sparg *et al*., 2004).

4. Tannin
   Tannin is detected by reaction with $FeCl_3$ which produces a blue-black or greenish color. Tannin works by precipitating proteins, including enzymes important for bacterial metabolism, and inhibiting the growth and division of microbial cells.

5. Terpenoids

   Positive results in the Liebermann-Burchard test indicate the presence of terpenoids. These compounds are able to disrupt the integrity of cell membranes and reduce transmembrane potential, which ultimately causes bacterial death (Bakkali *et al*., 2008).

b. Antibacterial activity test results

Testing the effectiveness of papaya latex extract (Carica papaya L.) against *Pseudomonas aeruginosa bacteria* was carried out using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods using five concentrations: 50%, 25%, 12.5%, 6.25%, and 3.125%. Based on the results obtained, it is known that at concentrations of 50% and 25%, there was no bacterial growth either in liquid media (MIC test) or in solid media (MBC test). This shows that both concentrations are able to effectively inhibit and kill *P. aeruginosa bacteria* .

The concentration of 25% is determined as the MBC (Minimum Killing Concentration) value, because it is the lowest concentration that is still able to eliminate bacteria completely, indicated by the absence of colony growth on Nutrient Agar (NA) media after incubation. This indicates that at this concentration, the extract has bactericidal activity. This effectiveness can be attributed to the content of active compounds in papaya latex such as alkaloids, flavonoids, and tannins, which are known to have a working mechanism in damaging cell membranes and disrupting bacterial metabolism.

At a concentration of 12.5%, the test results showed that there was no turbidity in the liquid media, indicating that bacteria did not grow or were inhibited, but colony growth was still found on solid media. Therefore, this concentration is categorized as MIC (Minimum Inhibitory Concentration), which is the lowest concentration that can prevent bacterial growth, but is not strong enough to kill bacteria completely. This shows that at a concentration of 12.5%, the extract is only bacteriostatic. Meanwhile, at lower concentrations, namely 6.25% and 3.125%, both liquid and solid media showed bacterial growth. The turbidity of the media and the presence of bacterial colonies on solid media indicate that the concentration is not enough to inhibit or kill Pseudomonas aeruginosa . Thus, the antibacterial effectiveness of papaya latex extract began to decrease significantly at concentrations below 12.5%.

The control results support the validity of the test: the positive control (without extract) showed normal bacterial growth, while the negative control (without bacteria) showed no growth, proving that the medium was not contaminated. Overall, these results indicate that papaya latex extract has significant antibacterial potential against P. aeruginosa , with optimal activity at a concentration of ≥25%. This activity is very likely influenced by the presence of secondary metabolite compounds that have been identified in the phytochemical screening results.

The results of statistical analysis using the One-Way ANOVA test on the Minimum Inhibitory Concentration (MIC) data showed that there was a significant difference between various concentrations of papaya latex extract on the growth of Pseudomonas aeruginosa ($p < 0.05$). The Optical Density (OD) value as an indicator of bacterial growth showed a significant decrease with increasing extract concentration. Concentrations of 12.5%, 25%, and 50% showed very low to zero OD, indicating strong inhibitory activity. Tukey's post-hoc test

showed that a significant difference occurred between low concentrations (3.125% and 6.25%) compared to concentrations ≥12.5%, but there was no significant difference between concentrations of 25% and 50%, indicating that the maximum inhibitory effect had been achieved at a concentration of 25%.

Meanwhile, the results of the MBC test were also analyzed using One-Way ANOVA on the number of bacterial colonies growing on solid media after treatment. The results showed a significant difference between concentration groups ($p < 0.05$), where the number of colonies drastically decreased at concentrations ≥25%, and no colony growth was found at all at concentrations of 25% and 50%. This indicates that a concentration of 25% is the lowest concentration that can effectively kill bacteria (MBC). Statistically, the post-hoc test also supports these results, with a significant difference between the 12.5% and 25% groups, reinforcing that 25% is the critical point of the extract's effectiveness as a bactericidal agent against *Pseudomonas aeruginosa* .

D. CONCLUSION AND SUGGESTIONS

Papaya latex extract (Carica papaya L.) showed significant antibacterial activity against *Pseudomonas aeruginosa* , as evidenced by the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests. The results showed that a concentration of 12.5% papaya latex extract was effective in inhibiting bacterial growth, while concentrations of 25% and 50% were able to kill bacteria completely. Statistical tests using SPSS with One-Way ANOVA and Tukey's post-hoc tests showed significant differences between concentrations, with 25% as the most effective concentration in killing bacteria. Therefore, papaya latex extract has the potential as an antibacterial agent that can be used as an alternative natural treatment for infections caused by *P. aeruginosa* .

E. ACKNOWLEDGEMENT

We express our gratitude to Kadiri University for supporting us in carrying out this study to look for an antimicrobial agent to treat various types of diseases by taking advantage of natural materials from the surrounding environment.

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