JURNAL SKALA HUSADA: THE JOURNAL OF HEALTH
Available online at: https://ejournal.poltekkes-denpasar.ac.id/index.php/JSH
Vol. 22 No. 1 Juni 2025
P-ISSN 1693-931X I e-ISSN 2580-3700 Halaman: 13-19

Antibacterial Activity of Papaya (*Carica papaya* L.) Seeds Extract on Various Solvent Against *Staphylococcus aureus* Bacteria

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Posted : March 04th, 2025 ; Reviewed : May 22 th, 2025 ; Received: June 30 th, 2025.

ABSTRACT

Staphylococcus aureus is an opportunistic-pathogenic bacteria on the skin's surface that causes impetigo. Treatment therapy for impetigo can be done by administering natural medication from papaya seeds (*Carica papaya* L). This study aims to determine the antibacterial activity of papaya seed extract on various solvent against of *Staphylococcus aureus*. This research is true experimental with a posttest-only control design. The research method used was the disk diffusion method with three treatments of papaya seed extract, namely 96% ethanol extract, ethyl acetate, and n-hexane, positive control using chloramphenicol, and negative control using 96% ethanol, ethyl acetate, and n-hexane. The research showed that each papaya seed extract inhibited *Staphylococcus aureus* with an inhibition zone of 96% ethanol extract of 18.09 mm, ethyl acetate extract of 7.93 mm, and n-hexane extract of 0.36 mm. The One-Way Anova test showed a value of $\rho<\alpha$ (0.05), so there was a significant difference in the inhibition zone diameter in each extract. The Tukey test showed that there were significant differences in the inhibition zone of *Staphylococcus aureus* bacteria with the value of $\rho<\alpha$ (0.05). This research concludes that there is a difference in the antibacterial activity of 96% ethanol extract, ethyl acetate extract, and n-hexane extract of papaya seeds on the growth of *Staphylococcus aureus* bacteria.

Keywords: Antibacterial; Extract; Papaya; *Staphylococcus aureus.*

ABSTRAK

Staphylococcus aureus adalah bakteri patogen oportunistik pada permukaan kulit yang menyebabkan impetigo. Terapi pengobatan impetigo dapat dilakukan dengan pemberian obat alami dari biji pepaya (*Carica papaya* L). Penelitian ini bertujuan untuk mengetahui aktivitas antibakteri ekstrak biji pepaya pada berbagai pelarut terhadap *Staphylococcus aureus*. Penelitian ini merupakan penelitian *true-experimental* dengan rancangan *posttest-only control design*. Metode penelitian yang digunakan adalah metode difusi cakram dengan tiga perlakuan ekstrak biji pepaya, yaitu ekstrak etanol 96%, etil asetat, dan n-heksan, kontrol positif menggunakan kloramfenikol, dan kontrol negatif menggunakan etanol 96%, etil asetat, dan n-heksan. Hasil penelitian menunjukkan bahwa masing-masing ekstrak biji pepaya dapat menghambat *Staphylococcus aureus* dengan zona hambat ekstrak etanol 96% sebesar 18,09 mm, ekstrak etil asetat sebesar 7,93 mm, dan ekstrak n-heksana sebesar 0,36 mm. Uji *One-Way Anova* menunjukkan nilai $\rho<\alpha$ (0,05), sehingga terdapat perbedaan yang signifikan pada diameter zona hambat pada masing-masing ekstrak. Uji *Tukey* menunjukkan bahwa terdapat perbedaan yang signifikan pada zona hambat bakteri *Staphylococcus aureus* dengan nilai $\rho<\alpha$ (0,05). Penelitian ini menyimpulkan bahwa terdapat perbedaan aktivitas antibakteri ekstrak etanol 96%, ekstrak etil asetat, dan ekstrak n-heksana biji pepaya terhadap pertumbuhan bakteri *Staphylococcus aureus*.

Kata kunci: Antibakteri; Ekstrak; Pepaya; *Staphylococcus aureus.*

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INTRODUCTION

Indonesia has a tropical climate, which contributes to the prevalence of skin infections as one of the most common infectious diseases. One such infection is caused by *Staphylococcus aureus*, an opportunistic pathogenic bacterium that resides on the skin and mucous membranes of various human organs.

Impetigo is a skin condition caused by an infection with Staphylococcus aureus. Treatment options for impetigo include synthetic antibiotics and natural remedies. However, the irrational use of synthetic antibiotics can lead to antibiotic resistance, posing significant challenges in the healthcare sector as bacteria develop immunity to the treatments provided[1].

Antibiotic resistance results in bacteria becoming tolerant to antibiotic substances, necessitating the exploration of natural alternatives. One promising natural remedy is papaya fruit seeds (*Carica papaya* L.), which contain secondary metabolite compounds such as flavonoids and alkaloids. Research indicates that the flavonoid content in papaya seeds possesses antibacterial properties. These compounds can damage the integrity of bacterial cell membranes, leading to the leakage of essential metabolites and the activation of bacterial enzyme systems. Alkaloid compounds disrupt the constituent components of bacterial cells, which causes the cell wall layer to be damaged and bacteria to die easily[2,3].

In the antibacterial activity test, the type of solvent used can significantly influence the diameter of the inhibition zone produced. Variations in solvent type and polarity during the extraction process impact the types of secondary metabolite compounds that are generated[4-6].

The 96% ethanol is commonly used as a solvent for extraction due to its polar properties, which allow it to attract a wider range of secondary metabolites from plant extracts. The choice of 96% ethanol is also based on its low cost, easy availability, and safety for use. Ethyl acetate, being a semi-polar and volatile solvent, is non-toxic and not hygroscopic, making it effective for attracting semi-polar compounds found in papaya fruit seeds. N-hexane, with the chemical formula C_6H_{14}, is classified as an alkane hydrocarbon.

Previous studies have shown that extracts using 96% ethanol, ethyl acetate, and n-hexane yield different types of secondary metabolites and exhibit varying degrees of effectiveness in inhibiting the growth of *Staphylococcus aureus* bacteria. [7-9]

Based on this background, it is evident that the choice of solvent influences both the types of secondary metabolites produced and the antibacterial efficacy of papaya fruit seed extract against bacterial growth. Therefore, this study aims to investigate the antibacterial activity of papaya fruit seed extract using different solvents—96% ethanol, ethyl acetate, and n-hexane against *Staphylococcus aureus* bacteria through the disc diffusion method to evaluate their inhibition of bacterial growth.

MATERIALS AND METHODS

The type of this research was True-Experimental, using the Posttest control design to measure the effect of treatment on the experimental group by comparing it with the control group. This research was conducted at the Post Harvest Processing Centre of Medicinal Plants of the Bali Provincial, Chemistry and Toxicology Laboratory, and Bacteriology Laboratory of Poltekkes Kemenkes Denpasar.

The materials used in this study were papaya (*Carica papaya* L.) fruit seeds, taken from the papaya fruit garden in Babahan Village, Penebel, Tabanan by observing the inclusion criteria, namely coming from ripe papaya fruit, oval-shaped seeds, brown-black and not moldy. The solvents used were 96% ethanol, ethyl acetate, and n-hexane, with each extract concentration of 20%. The positive control in this study was chloramphenicol, and the negative control used was 96% ethanol, ethyl acetate, and n-hexane solvents. The antibacterial activity was determined by measuring the inhibition zone of the sample. Data analysis was carried out with the One-Way Anova statistical test to determine whether or not there were significant differences between treatments and Tukey's post hoc test if the data were normally distributed. If the data was not normally distributed, the Kruskal Wallis non-parametric and Games-Howell post hoc tests were used.

Papaya (*Carica papaya* L.) Seed Simplisia Preparation

A total of one kilogram of papaya seeds that meet the inclusion criteria are washed and separated from the seed's mucus membrane. The seeds are then dried in an oven at 50 ºC. Once dried, the seeds are pulverized and sieved using a blender. The water content of the simplisia is calculated and should be less than 10%.

Papaya (*Carica papaya* L.) Seed Extract Preparation

During the extraction phase, a rotary evaporator is used in conjunction with maceration and evaporation methods. Three glass beakers are prepared, each containing 150 grams of simplisia and 750 ml of one of the following solvents: 96% ethanol, ethyl acetate, or n-hexane. The beakers are covered with aluminum foil and allowed to stand for one day, stirring every 8 hours. The re-maceration process is repeated three times for each beaker. The filtrate obtained is concentrated using a rotary evaporator at a temperature of 40-60 ºC. After concentration, the thick extract is weighed, and the extract yield is calculated. A yield value exceeding 10% is considered satisfactory.

Subsequently, the concentration of the papaya seed extract for each solvent is set at 20%. This concentration is prepared using a % b/v ratio. Five grams of extract are measured and added to a volume of 25 ml in a volumetric flask for each solvent.

Phytochemical Screening Test

Alkaloid Test:

Pipette 2 ml of a 20% concentration extract. Add ammonia and a few drops of 2N sulfuric acid (H_2SO_4). Then, introduce 1 ml of Dragendorff's reagent to the solution.

Flavonoid Test:

Combine 1 ml of the diluted extract with 2 ml of a 2% sodium hydroxide (NaOH) solution and a few drops of hydrochloric acid (HCl).

Tannin Test:

Add 1.6 ml of the diluted extract and three drops of ferric chloride ($FeCl_3$) solution. Homogenize the mixture.

Terpenoid Test:

Add 2 ml of chloroform to 5 ml of the diluted extract, then evaporate the mixture in a water bath. After evaporation, add 3 ml of concentrated sulfuric acid (H_2SO_4), which should also be heated in a water bath.

Saponin Test:

Combine 2 ml of the diluted extract with 2 ml of distilled water and shake the mixture vigorously for 5 minutes. A foam should form. Finally, add one drop of 2N hydrochloric acid (HCl).

Preparation of Staphylococcus aureus Bacterial Suspension

Take a colony of Staphylococcus aureus bacteria from a pure culture and suspend it in a tube containing 5 ml of 0.9% physiological saline (NaCl). Compare this suspension to the 0.5% McFarland standard. The suspension should then be measured using a McFarland densitometer.

Antibacterial Activity Test

A suspension of Staphylococcus aureus with a concentration of 0.5 McFarland was prepared and streaked onto a Petri dish in a vertical zigzag pattern. After allowing it to stand for 5 minutes, a 6 mm diameter paper disc was soaked with 20 µl of each concentration of the papaya fruit seed extract. Each saturated disc was then placed onto the Mueller-Hinton Agar (MHA) media that had been inoculated with the bacterial suspension, ensuring it was securely attached. Positive and negative controls were also added to the MHA media. The dishes were incubated for 24 hours in an inverted position at 37 ºC. After incubation, the clear zone of inhibition around each disc was measured using calipers.[10]

RESULTS AND DISCUSSIONS

Papaya (*Carica papaya* L.) Seed Simplisia

The papaya fruit seeds used in this study had an initial wet weight of three kilograms. After drying and grinding, the weight reduced to 400 grams. To standardize the simplisia, we calculated its water content, which was found to be 6.443%. This result indicates that the quality of the simplisia meets the required standards, as it is below the acceptable threshold of 10%.[11] This test determines the moisture level in the simplisia, as excessive moisture can lead to a decrease in its quality.

Papaya (*Carica papaya* L.) Seed Extract

Maceration is a commonly used extraction method. The choice of solvent in the extraction process is based on the principle of "like dissolves like," which means that compounds will dissolve in solvents that have similar polarity. In this study, the seeds were extracted using 96% ethanol, ethyl acetate, and n-hexane. The extraction yielded a thick 96% ethanol extract of 45 grams, an ethyl acetate extract of 40 grams, and a n-hexane extract of 35 grams. The yield percentages for the 96% ethanol, ethyl acetate, and n-hexane extracts were found to be 37.5%, 33%, and 29%, respectively. A higher yield value indicates that more active compounds have been successfully extracted.[12]

Phytochemical Screening

The purpose of identifying phytochemical compounds in papaya fruit seed extract is to determine the presence of secondary metabolites that may inhibit bacterial growth. Table 1 illustrates the differences in the types of secondary metabolite compounds found in each extract, depending on the solvent used.

The variation in the content of secondary metabolite compounds is attributed to the difference in the polarity of the solvents. Ethanol (96%), being the most polar solvent, can extract a greater number of active compounds, especially those with similar polar properties. Ethyl acetate, classified as a semipolar solvent, is able to attract both polar and non-polar compounds. In contrast, n-hexane, a non-polar solvent, tends to bind fewer compounds, restricting its extraction to only non-polar substances.

In this study, the 96% ethanol extract was found to contain secondary metabolite compounds such as alkaloids, flavonoids, and tannins. The ethyl acetate extract contained alkaloids and tannins, while the n-hexane extract was limited to terpenoid compounds.

Table 1. The result of phytochemical sreening test

Test	Result		
	96% ethanol extract	Ethyl acetate extract	N-hexane extract
Alkaloid	+++	++	-
Flavonoid	+	-	-
Tannin	++	+	-
Terpenoid	-	-	++
Saponin	-	-	-

Antibacterial Activity of Papaya (*Carica papaya* L.) Seed Extract

An antibacterial activity test of papaya fruit seed extract was conducted using 96% ethanol, ethyl acetate, and n-hexane extracts with 20% concentration each. Chloramphenicol was used as a positive control to ensure the viability of the bacteria used in the test. The use of chloramphenicol as an antibacterial agent is done with consideration of the broad-spectrum nature of chloramphenicol so that it has the potential to fight gram-positive and gram-negative bacteria.[12] In this study, chloramphenicol as a positive control can inhibit Staphylococcus aureus bacteria with an inhibition zone area of 20.13 mm with a powerful category. The negative control in this study is 96% ethanol, ethyl acetate, and n-hexane solvents. The results showed that the negative control used has an inhibition zone area of 0 mm, which means that using solvents without adding papaya fruit seed extract has no effect on the area of the antibacterial inhibition zone of Staphylococcus aureus.

Based on Table 2, the results of the 96% ethanol extract antibacterial activity test obtained the area of the Staphylococcus aureus antibacterial inhibition zone of 18.09 mm (strong category) in line with the research of Ahmad, Jangga, and Hasnaeni (2023) where the 96% ethanol extract of papaya seeds at a concentration of 20% chose an inhibition zone of 16.6 mm against Propionibacterium acnes bacteria. Ethyl acetate extract from papaya fruit seeds produces an inhibition zone area of 7.93 mm (medium category) when compared to the research of Fauzan, Lasmini, and Marietta (2017), where 75% concentration of ethyl acetate extract has an inhibition zone area of 27.86%, then the ethyl acetate extract in this study has a smaller inhibition zone area because of the difference in concentration used, namely 20%. The n-hexane extract of papaya fruit seeds has an antibacterial inhibition zone area of Staphylococcus aureus of only 0.36 mm (weak category) in accordance with the research of Lingga, Pato, and Rossi (2015) with n-hexane extract of kecombrang stems in inhibiting Staphylococcus aureus bacteria at a concentration of 100% has an inhibition zone area of 1.8 mm.

Table 2. Antibacterial activity of papaya (*Carica papaya* L.) seed extract

Sample	Inhibition zone (mm)	Category
Chloramphenicol	20.13	Very strong
96% ethanol	0	-
Ethyl acetate	0	-
N-hexane	0	-
96% ethanol extract	18.09	Strong
Ethyl acetate extract	7.93	Moderate
N-hexane extract	0.36	Weak

The results indicate that the polarity of the solvent used in the extraction process affects the diameter of the antibacterial inhibition zone against Staphylococcus aureus bacteria. Specifically, as the polarity of the solvent increases, the size of the inhibition zone also increases. This is attributed to the interaction between the solvents and the compounds in the extracts that serve as natural antibacterial alternatives[13]. Bioactive compounds, including polyphenols, flavonoids, and tannins, act as antibacterial agents and show higher solubility in relatively polar solvents, such as ethanol, compared to ethyl acetate and n-hexane[14].

A wider antibacterial inhibition zone produced by an extract suggests that a larger quantity of secondary metabolite compounds can be extracted. For instance, 96% ethanol, which is highly polar, contains secondary metabolite compounds including alkaloids, flavonoids, and tannins. In contrast, ethyl acetate extracts contain only alkaloids and tannins, while n-hexane extracts are limited to terpenoid compounds. Terpenoids, which fall into the non-polar compound group, function as antioxidants and may help prevent cancer.

Alkaloids inhibit bacterial growth by disrupting the structural components of bacterial cells, making them more vulnerable to death. Flavonoids also exhibit antibacterial properties; they act by breaking down proteins in bacterial cells and damaging cell membranes, leading to cell lysis and toxic effects on the bacteria[15]. Additionally, flavonoids can inhibit the activity of reductase enzymes during the electron transfer stages in bacteria, further hindering their growth[16]. Tannins can damage bacterial cell walls by targeting cell wall polypeptides, resulting in flawed bacterial cell walls and ultimately causing bacterial cell death[17].

Analysis of Differences in Antibacterial Activity of Papaya Fruit Seed Extract (Carica papaya L.)

The antibacterial activity of 96% ethanol extract, ethyl acetate extract, and n-hexane extract from papaya fruit seeds against *Staphylococcus aureus* was analyzed using statistical tests performed with SPSS software. The normality of the data was assessed using the Shapiro-Wilk test, which yielded the following probability values (p): positive control $p = 0.686$, ethanol extract $p = 0.288$, ethyl acetate extract $p = 0.221$, and n-hexane extract $p = 0.087$. These results indicate that the data for the positive control, 96% ethanol extract, ethyl acetate extract, and n-hexane extract were normally distributed ($p > 0.05$).

To determine differences among the groups, a One-Way ANOVA was conducted, resulting in a p-value of 0.000 (< 0.05), which indicates a significant difference in the inhibition zones of Staphylococcus aureus across the various extracts of papaya seeds.

The homogeneity of variances was tested using the Levene Statistic, which revealed a p-value of 0.081 ($p > 0.05$), confirming that the data are homogenous. Moreover, a post hoc Tukey test demonstrated significant differences in the inhibition zones of Staphylococcus aureus ($p < 0.05$) between the positive control and 96% ethanol extract, the positive control and ethyl acetate extract, the positive control and n-hexane extract, as well as between the 96% ethanol extract and both the ethyl acetate and n-hexane extracts.

CONCLUSIONS

The results demonstrate that the 96% ethanol extract of papaya seeds contains alkaloids, flavonoids, and tannin compounds, while the ethyl acetate extract contains alkaloids and tannins. In contrast, the n-hexane extract only contains terpenoid compounds. The antibacterial activity of the three papaya seed extracts shows significant differences, as indicated by the mean diameter of the inhibition zones. The 96% ethanol extract falls into the strong antibacterial activity category, the ethyl acetate extract shows moderate activity, and the n-hexane extract displays weak activity.

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