Research Articles

Unveiling the Antibacterial Activity of Petai Seed Ethanol Extract (Parkia speciosa Hassk) with the Kirby-Bauer Method

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Abstrak

Kasus resistensi antibiotik yang meningkat menyebabkan perlu ada alternatif obat lain, salah satunya adalah biji petai (*P. speciosa* Hassk). Biji petai mengandung senyawa kimia seperti tanin, saponin, alkaloid, dan flavonoid yang dikenal sebagai *antibacterial agent*. **Tujuan**: Menentukan aktivitas antibakteri ekstrak etanol biji petai (*P. speciosa* Hassk) terhadap bakteri *Staphylococcus aureus, Pseudomonas aeruginosa*, dan *Salmonella typhi*. **Metode**: Desain penelitian adalah studi eksperimental menggunakan metode *Kirby-Bauer* difusi cakram. Biji petai diekstraksi menggunakan teknik maserasi dengan pelarut etanol. Ekstrak kental dilarutkan menjadi konsentrasi 25%, 50%, 75%, dan 100%. Kertas saring yang telah direndam di dalam konsentrasi uji diletakkan di atas media agar yang telah ditanami bakteri uji. Cawan petri selanjutnya diinkubasi selama 24 jam. Diameter zona hambat diukur menggunakan jangka sorong. Data hasil penelitian dianalisis secara statistik. **Hasil**: Ekstrak etanol biji petai (*Parkia speciosa* Hassk.) memiliki potensi antibakteri yang sedang terhadap pertumbuhan bakteri *Staphylococcus aureus* dan *Pseudomonas aeruginosa* serta memiliki potensi antibakteri yang lemah terhadap pertumbuhan bakteri *Salmonela typhi*. **Simpulan:** Ekstrak etanol biji petai (*P. speciosa* Hassk) memiliki aktivitas antibakteri terhadap masing-masing bakteri uji. Peningkatan konsentrasi berpengaruh nyata terhadap aktivitas antibakteri ekstrak etanol biji petai terhadap pertumbuhan bakteri *Staphylococcus aureus* dan *Pseudomonas aeruginosa*.

Kata kunci: antibakteri, biji petai, ekstrak etanol, metode Kirby-Bauer

Abstract

The increasing number of antibiotic resistance cases have to seek alternative treatments, including the petai plant (Parkia speciosa Hassk). Petai seeds primarily contain chemical compounds such as tannins, saponins, alkaloids, and flavonoids, which are known for their antibacterial properties. ***Objective****: To determined the antibacterial activity of petai seed ethanol extract (P. speciosa Hassk) against Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella typhi.* ***Methods****: This experiment used the Kirby-Bauer Method. Petai seeds were extracted with ethanol using maceration techniques, and the viscous extract was dissolved into concentrations of 25%, 50%, 75%, and 100%. Screening paper soaked in the extract was placed on agar plates inoculated with the test bacteria, and the plates were incubated for 24 hours. The inhibition zones were measured with a caliper, and the data were analyzed statistically.* ***Results****: The study found that the ethanol extract of petai seeds exhibited moderate antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa and weak antibacterial activity against Salmonella typhi.* ***Conclusion****: Increasing the concentration of the extract significantly enhanced its antibacterial effect against Staphylococcus aureus and Pseudomonas aeruginosa, indicating that petai seed ethanol extract could be a potential alternative treatment for bacterial infections.*

Keywords*: antibacterial, extract ethanol, Kirby-Bauer method, petai seed*

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INTRODUCTION

Health issues remain a national concern in Indonesia, particularly in remote areas. One of the contributing factors is the lack of awareness among the Indonesian population about clean and healthy living behavior and personal hygiene. Poor personal hygiene can lead to various health problems, including infectious diseases.[1]

Infectious diseases are among the many health issues that frequently contribute to illness and death. Infection occurs when pathogenic microorganisms invade the body, leading to disease onset.[1] Infections can affect various body organs, including the urinary, digestive, respiratory, and other organ systems. Pathogens that cause infections include protists, fungi, viruses, and bacteria.[2] Some bacteria commonly infect humans are *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Salmonella typhi*. These bacteria are classified as opportunistic, meaning they infect individuals with weakened immune systems. Bacterial infections can be treated with antibiotics, but improper and inadequate use can lead to antibiotic resistance. One way to mitigate these negative effects is to use natural remedies as an alternative treatment.

Petai, known in Latin as Parkia speciosa Hassk, is a plant belonging to the legume family (Fabaceae). Petai can be found in tropical countries like Indonesia, which is located along the equator. It is commonly grown and cultivated by the people of Indonesia.[3] This plant has seeds that can be consumed raw or after processing. In addition to being used as a side dish, petai seeds have various health benefits, including acting as a remedy for kidney disorders, diabetes, hypertension, headaches, diarrhea, and skin problems.[4] Previous studies have shown that petai seed extract has antibacterial effects against a range of bacteria, from gram-positive bacteria like *S. aureus* to gram-negative bacteria like *E. coli* and *K. pneumoniae*.[5-9] Petai seeds contain chemical compounds such as tannins, saponins, alkaloids, and flavonoids, which are known for their antibacterial properties.

This study aims to examine the antibacterial effect of petai seed ethanol extract (*Parkia speciosa* Hassk) on several pathogenic bacteria using the *Kirby Bauer method.*

METHOD

This study is a true experimental research using a Completely Randomized Design (CRD) and was tested on several pathogenic bacteria, namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* as the research subjects. The Kirby-Bauer method was employed in this study. The research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Andalas University, from November 2022 to December 2023. The research samples included petai seeds and the bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* isolated in the Microbiology Laboratory of the Faculty of Medicine, Andalas University. This study used six different treatment groups: 25% petai seed ethanol extract, 50% petai seed ethanol extract, 75% petai seed ethanol extract, 100% petai seed ethanol extract, a positive control (+) using three antibiotics for each bacterium, amoxicillin for *S. aureus*, ciprofloxacin for *P. aeruginosa*, and chloramphenicol for *S. Typhi*, and negative control (-) using 96% ethanol. A minimum of four experiments were conducted in this research.

Parametric tests using *One-Way* ANOVA and *Post Hoc* cannot be performed if the results are not normally distributed. Alternatively, a non-parametric *Kruskal-Wallis test* was performed to determine whether or not there was a significant difference between the test groups. Finally, a follow-up test using *the Mann-Whitney test* method was continued to find out which groups had significant differences. This study was conducted after ethical clearance approval from the Ethical Commission Faculty of Medicine Universitas Andalas number 324/UN.16.2/KEP-FK/2024.

RESULTS

This study obtained petai seed extract (P. speciosa Hassk) using the maceration method. The solvent was evaporated with a rotary evaporator until all the solvent had evaporated, leaving a thick extract, as shown in Figure 1.

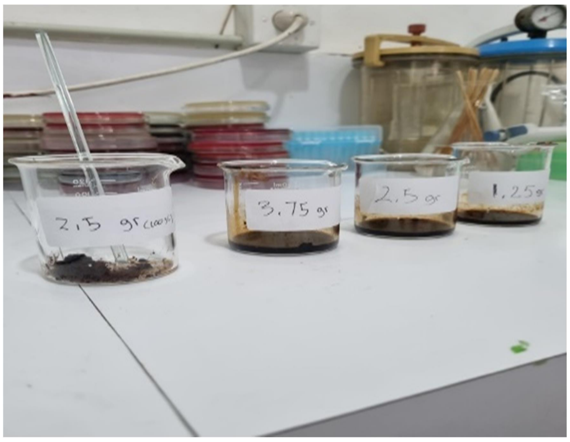

Figure 1. Thick Extract of Petai Seed (*P.speciosa* Hassk)

The purpose of this study was to determine the diameter of the inhibitory zone of each ethanol extract of petai seeds (*Parkia speciosa* Hassk) against *Staphylococcus aureus bacteria, Pseudomonas aeruginosa Salmonella typhi.* After measurements were made using a caliper to determine the diameter of the inhibition zone at each concentration and control, data on the results of inhibition zone measurements in millimeters (mm) were obtained, which can be seen in Tables 1, 2, and 3.

Table 1. Results of measurement of the inhibition zone diameter of Petai Seed Extract (Parkia speciosa Hassk) Against Staphylococcus aureus bacteria

Inhibition Zone Diameter (mm)						
n	**25%**	**50%**	**75%**	**100%**	**K+**	**K-**
1	4,60	6,30	3,30	7,70	14,60	0
2	3,70	5,50	4,60	5,70	17,00	0
3	5,20	5,50	2,50	8,60	16,65	0
4	2,30	5,50	4,00	8,60	15,30	0
Mean	3,95	5,70	3,60	7,65	15,88	0
SD	1,26	0,40	0,90	1,36	1,12	0

n : Repetition
K(+) : Positive control (Amoxicillin)
K(-) : Negative control (Etanol 96%)

The study results demonstrated that petai seed ethanol extract effectively inhibited the activity of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* bacteria. The inhibitory effect increased with higher extract concentrations, with the concentration 100% showing the most potent inhibition across all tested bacteria.

Table 2. Results of measurement of the inhibition zone diameter of Petai Seed Extract (Parkia speciosa Hassk) Against Salmonella typhi bacteria

Inhibition Zone Diameter (mm)						
n	**25%**	**50%**	**75%**	**100%**	**K+**	**K -**
1	2,00	2,00	3,30	3,40	21,80	0
2	1,00	1,00	1,20	1,80	23,00	0
3	0,00	0,00	1,00	1,00	22,50	0
4	0,00	0,50	1,00	1,80	22,50	0
Mean	0,75	0,87	1,62	2	22,45	0
SD	0,95	0,85	1,12	1	0,49	0

n : Repetition
K(+) : Positive control (Kloramfenikol)
K(-) : Negative control (Etanol 96%)

Statistical tests were conducted to assess the effect of increasing concentrations on the inhibition zone diameter of petai seeds (*P. speciosa* Hassk) against the growth of each bacterium. A data normality test was performed using the Shapiro-Wilk test ($p > 0.05$). The data were not normally distributed, as the Shapiro-Wilk test yielded a p-value of 0.000. Consequently, the non-parametric Kruskal-Wallis test was used to determine whether there were significant differences between the test groups with concentrations of 25%, 50%, 75%, 100%, positive control, and negative control.

Table 3. Results of measurement of the inhibition zone diameter of Petai Seed Extract (Parkia speciosa Hassk) Against Pseudomonas aeruginosa Bacteria

Inhibition Zone Diameter (mm)						
n	**25%**	**50%**	**75%**	**100%**	**K+**	**K-**
1	3,70	6,50	7,50	9,30	34,00	0
2	2,30	4,50	4,50	7,50	34,48	0
3	2,00	6,80	7,80	6,60	33,00	0
4	0,80	7,80	8,40	7,00	34,10	0
Mean	2,20	6,40	7,05	7,60	33,90	0
SD	1,19	1,38	1,74	1,19	0,63	0

n : Repetition
K(+) : Positive control (Siprofloksasin)
K(-) : Negative control (Etanol 96%)

The results of the data analysis using the Kruskal-Wallis non-parametric test for each test group showed a p-value of <0.05, indicating a significant difference ($p < 0.05$) between the concentrations of

25%, 50%, 75%, 100%, positive control, and negative control. Each group was then analyzed using the Mann-Whitney test to identify significant differences. The results of this analysis were presented in Table 4.

Table 4. Mann-Whitney test analysis results inhibition zone diameter

Test Bacteria	Research Group		P
Staphylococcus aureus	25%	50%	0,018
		75%	0,663*
		100%	0,020
		K+	0,021
		K-	0,014
	50%	75%	0,018
		100%	0,037
		K+	0,018
		K-	0,011
	75%	100%	0,020
		K+	0,021
		K-	0,014
	100%	K+	0,020
		K-	0,013
	K+	K-	0,014
Psedomonas aeruginosa	25%	50%	0,021
		75%	0,021
		100%	0,021
		K+	0,021
		K-	0,014
	50%	75%	0,381*
		100%	0,248*
		K+	0,021
		K-	0,014
	75%	100%	0,885*
		K+	0,021
		K-	0,014
	100%	K+	0,021
		K-	0,014
	K+	K-	0.014
Salmonella typhi	25%	50%	0,765*
		75%	0,234*
		100%	0,186*
		K+	0,019
		K-	0,131*
	50%	75%	0,237*
		100%	0,189*
		K+	0,020
		K-	0,047
	75%	100%	0,372*
		K+	0,019
		K-	0,013
	100%	K+	0,019
		K-	0,013
	K+	K-	0,013

Ket: * There was no significant difference in the groups

DISCUSSION

This study obtained petai seed extract (P. speciosa Hassk) using the maceration method. The solvent was evaporated with a rotary evaporator until all the solvent had evaporated, leaving a thick extract, as shown in Figure 1.

This study aimed to determine the diameter of the inhibitory zone of each ethanol extract of petai seeds (*Parkia speciosa* Hassk) against *Staphylococcus aureus bacteria, Pseudomonas aeruginosa Salmonella typhi.* After measurements were made using a caliper to determine the diameter of the inhibition zone at each concentration and control, data on the results of inhibition zone measurements in millimeters (mm) were obtained, which can be seen in Tables 1, 2, and 3.

The study results demonstrated that petai seed ethanol extract effectively inhibited the activity of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* bacteria. The inhibitory effect increased with higher extract concentrations, with the concentration 100% showing the strongest inhibition across all tested bacteria.

Statistical tests were conducted to assess the effect of increasing concentrations on the inhibition zone diameter of petai seeds (*P. speciosa* Hassk) against the growth of each bacterium. A data normality test was performed using the Shapiro-Wilk test ($p > 0.05$). The data were not normally distributed, as the Shapiro-Wilk test yielded a p-value of 0.000. Consequently, the non-parametric Kruskal-Wallis test was used to determine whether there were significant differences between the test groups with concentrations of 25%, 50%, 75%, 100%, positive control, and negative control.

The results of the data analysis using the Kruskal-Wallis non-parametric test for each test group showed a p-value of <0.05, indicating a significant difference ($p < 0.05$) between the concentrations of 25%, 50%, 75%, 100%, positive control, and negative control. Each group was then analyzed using the Mann-Whitney test to identify significant differences. The results of this analysis were presented in Table 4.

This research tested the antibacterial activity of petai seed ethanol extract (*P. speciosa* Hassk) against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* using the disc diffusion method (Kirby-Bauer method). The research measured the diameter of the inhibition zones produced by the extract for each bacterial strain. The tests were performed four times using four different concentrations. An inhibition zone around the bacterial growth demonstrated the extract's effectiveness in preventing bacterial growth.

The ethanol extract of petai seeds effectively inhibits *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* bacteria, as demonstrated by inhibition zones on petri dishes. Increasing the extract concentration results in a larger inhibition zone. This is due to the increased amount of active compounds in the extract enhancing the antibacterial effect. However, the size of the inhibition zone is not always directly proportional to the extract concentration, as the diffusion rate of the antibacterial compounds in the agar medium can vary. Factors influencing diffusion speed include microorganism concentration, medium composition, temperature, and incubation time.[11]

At a concentration of 100%, the average diameter of the most significant inhibition zones was 7.65 mm for *Staphylococcus aureus*, 7.60 mm for *Pseudomonas aeruginosa*, and 2 mm for *Salmonella typhi*. Antibacterial activity is categorized as weak if the inhibition zone diameter is less than 5 mm, moderate between 5-10 mm, strong between 10-20 mm, and very strong if over 20 mm. According to these criteria, the petai seed ethanol extract (*P. speciosa* Hassk) shows moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However, its effect on *Salmonella typhi* is relatively weak.

Antibacterial activity can generally be an early indication of how effective an antibacterial substance is. The way to determine the antibacterial effectiveness of an extract is to compare the diameter of the inhibitory zone of the extract with the diameter of the Antibacterial activity, which often provides an initial measure of how effective an antibacterial agent is. To evaluate the effectiveness of an extract, the size of the inhibition zone produced by the extract was compared to that of the positive control. In this study, the inhibition zone diameter of petai seed ethanol extract (*Parkia speciosa* Hassk) was compared to that of the positive control to determine how effective the petai seed ethanol extract is against bacteria.

In this study, three antibiotics were used for each test bacterium, namely amoxicillin (*S. Aureus*), ciprofloxacin (*P. Aeruginosa*), and chloramphenicol (*S. Typhi*). Amoxicillin has an average inhibition zone diameter of 15.88 mm, ciprofloxacin of 33.90 mm and chloramphenicol of 22.45 mm. According to the classification by the Clinical and Laboratory Standards Institute (CLSI), antibiotics are categorized as sensitive or susceptible if the inhibition zone diameter is ≥18 mm, as moderate or intermediate if the diameter is between 13-17 mm, and as resistant if the diameter is ≤12 mm. Based on these criteria, ciprofloxacin and chloramphenicol are classified as sensitive or susceptible to *P. aeruginosa* and *S. typhi* bacteria, respectively. Meanwhile, amoxicillin was classified as intermediate against *S. aureus* bacteria.

The antibacterial activity of petai seed ethanol extract (*P. speciosa* Hassk.) is linked to active compounds in petai seeds, including saponins, tannins, flavonoids, and alkaloids, all of which have antibacterial properties. Saponins interact with porins on the outer membrane of the bacterial cell wall, forming strong polymer bonds that damage these porins and disrupt bacterial cell membrane permeability. Tannins form hydrophobic complexes with proteins, inactivating enzymes and cell wall transport proteins, thereby hindering bacterial growth.[12] Flavonoids denature bacterial cell proteins and damage cell membranes, leading to bacterial cell lysis. Alkaloids interfere with the peptidoglycan formation process in bacterial cells, resulting in an incomplete bacterial cell wall.[13]

The findings of this study show that petai seed ethanol extract (*P. speciosa* Hassk.) exhibits moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but weak activity against *Salmonella typhi*. Statistical analysis demonstrated a significant relationship between the concentration of the petai seed extract and the size of the inhibition zones for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This indicates that higher concentrations of the extract notably enhance its antibacterial effectiveness against these two bacterial strains.

CONCLUSION

1. As the concentration of petai seed ethanol extract increases, its inhibitory effect on bacterial growth increases.
2. The ethanol extract of petai seeds (*Parkia speciosa* Hassk.) demonstrates moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. At the same time, its effect on *Salmonella typhi* is comparatively weak.
3. Increasing concentration significantly enhances the antibacterial effectiveness of petai seed ethanol extract against Staphylococcus aureus and Pseudomonas aeruginosa.

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