

ANTIBACTERIAL ACTIVITY OF ETHYL ACETATE EXTRACT FROM *PARKIA SPECIOSA* PERICARP AGAINST SELECTED BACTERIA

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Abstract: *Parkia speciosa* or *petai* is commonly found in tropical countries such as Thailand, Indonesia, and Malaysia. This plant is usually eaten raw or included in recipes and it is believed that *P. speciosa* can relieve various illnesses. Previous studies have proven that *P. speciosa* has some valuable medicinal properties such as a hypoglycemic agent, antitumor, and antimutagenicity. Usually, only the seeds of *P. speciosa* are consumed while their pericarp is discarded. Thus, in this study, we evaluate the pericarp's potential to be utilised as an antimicrobial agent. The pericarp was extracted using ethyl acetate and the antibacterial activity of the extract was determined by disk diffusion and broth dilution methods against four types of common infectious bacteria: *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella typhimurium*. It was found that the *P. speciosa* pericarp extract exhibited good antibacterial activity toward *S. aureus* and *B. cereus* while *E. coli* and *S. typhimurium* were resistant to the extract. The lowest concentration of extract that has an effect on *S. aureus* and *B. cereus* was 7.81 mg/mL and 1.95 mg/mL, respectively. Thus, the extract may have the potential to be used as a potent antimicrobial agent.

Keywords: *Parkia speciosa*, pericarp, ethyl acetate, plant extract, antimicrobial.

Introduction

Recently, antibiotic resistance has been a growing global concern. The main drivers of resistance to common antibiotics may be due to misuse of broad-spectrum antibiotics, inappropriate prescriptions, and incomplete dosages because of the high cost (Laxminarayan *et al.*, 2013). Antibiotics were the most successful finding in medicinal history (Sen & Batra, 2012).

In 2005, Shah suggested that a new compound that is not from existing classes of synthetic drugs is one of the ways to solve the antibiotic resistance issue. Interestingly, a 2011 article from WHO stated that herbal medicines or preparations from plants are utilised in every country worldwide (WHO, 2011). For thousands of years, people have relied on them to support, promote, maintain, and restore human health. This shows that plants possess biochemicals that bring medicinal benefits to humans in many different ways. Moreover, according to Sen and Batra (2012), plants

produce many types of compounds to protect themselves from pathogens. These compounds may inhibit bacteria via mechanisms that are different from existing antibiotics (Eloff, 1998). Ahmad and Beg (2001) also reported that these plant compounds were believed to target sites on pathogens not exploited by antibiotics. Therefore, plant-derived compounds can combat antibiotic resistance. The active compounds from plants, especially plants that are commonly used in traditional medicine, should be screened and the findings may lead to the discovery of new medicinal drugs from plant sources to reduce antibiotic resistance (Fabricant & Farnsworth, 2001).

In the present study, *petai* or scientifically known as *P. speciosa* has been chosen as the plant of interest. This plant is abundantly found in tropical countries such as Malaysia, Indonesia, Thailand, and the Philippines (Samuel *et al.*, 2010). The seeds were usually

crushed and boiled for stomach pain relief and were also believed to be helpful in treatments for liver disease, diabetes, and worm infestations (Ghasemzadeh *et al.*, 2018). Other than that, previous studies have also documented the anticancer (Ali *et al.*, 2006), antibacterial (Sakunpak & Panichayupakaranant, 2012), antioxidant (Ko *et al.*, 2014), as well as antiangiogenic activity (Kamisah *et al.*, 2013) of *P. speciosa*. The medicinal properties of this species could be attributed to the phytochemicals present. According to Ahmad *et al.* (2019), polyphenols, flavonoids, alkaloids, terpenoids, and tannins are among the phytochemicals found in the extracts of this species. This shows that *P. speciosa* seeds have other potential benefits besides their culinary uses. A previous study by Wonghirundecha *et al.* (2014) found that the pericarps of *P. speciosa* also exhibited antibacterial activity. Hence, the present study focused on the often wasted pericarps of *P. speciosa* as a possible antimicrobial agent against *S. aureus*, *B. cereus*, *E. coli*, and *S. typhimurium*.

Methodology

Preparation of *Parkia speciosa* Pericarp Extract

The *P. speciosa* fruit was bought at the local market around Puncak Alam and Shah Alam and verified by the Forest Research Institute of Malaysia (FRIM). Then, the seeds of *P. speciosa* were separated from the pericarps, which were cut into small pieces, washed with distilled water, and dried under shade for two weeks.

Extraction of *Parkia speciosa* Pericarp

The dried pericarps were ground into a fine powder using a blender. Approximately 164.05 g of the pericarp powder was soaked in 500 mL of ethyl acetate in a ratio of 1:3 for three days. The solvent was then filtered using cotton gauze and Whatman No. 1 filter paper. The process was repeated until the solvent was clear as the exhaustive extraction technique was applied in this study. The extract obtained was then

concentrated using a rotary evaporator (Buchi, Switzerland) under reduced pressure at 50°C to obtain the crude extract. The crude extract was then stored at 4°C until further analysis.

Preparation of Different Concentrations of *Parkia speciosa* Pericarp Extract

To prepare the stock extract (1000 mg/mL), 1 g (1000 mg) of *P. speciosa* crude extract was added to 1 mL of 10% dimethylsulfoxide (DMSO). Then, serial dilution was performed with 10% DMSO to get concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL of extract.

Preparation of Disk Embedded with Extract

Six mm disks of Whatman No.1 filter paper were prepared and autoclaved before use. After that, 10 µL of different concentrations (1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL) of *P. speciosa* extract were dispensed onto the filter paper using a micropipette and then dried at room temperature for 15 minutes. The negative control used in this study was 10 µL of 10% DMSO while the positive control used was the ampicillin 10 µg/disk.

Disk Diffusion Method

The antibacterial activity of *P. speciosa* pericarp extract prepared using ethyl acetate was screened using the Kirby-Bauer disk diffusion method (Reller *et al.*, 2009). The microorganisms used were *S. aureus*, *B. cereus*, *E. coli*, and *S. typhimurium*. All bacteria were incubated in Tryptic Soy Broth (TSB) at 37°C for 24 hours and the turbidity of the prepared inoculum of bacteria was adjusted to 0.5 McFarland standard. Sterile swabs were used to swab the surface of Mueller Hinton (MH) agar by rotating the plate at 60°C to make sure an even distribution of bacterial suspension. Subsequently, the 1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL of *P. speciosa* pericarp extract disks, as well as positive and negative control disks were placed on the MH

agar plate. Then, the MH agar was incubated in at 37°C for 24 hours. The AST was performed in duplicates and the zones of inhibitions were measured using a ruler in millimetres (mm).

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is defined as the minimum concentration of antimicrobial agents that can inhibit the visible growth of a microorganism after 24 hours of incubation (Andrews, 2001). In this study, the antimicrobial agent used was the ethyl acetate extract from the pericarp of *P. speciosa* and the microorganism used were bacteria that showed sensitivity against *P. speciosa* pericarp extract obtained from the Disk Diffusion assay. MH broth was used as a medium to cultivate the organism.

Preparation of Bacterial Suspension and Plant Extract

In this test, a two-fold serial dilution of *P. speciosa* extracts ie. 1000 mg/mL until 0.49 mg/mL was made by diluting the extract with MH broth. The suspension of bacteria was incubated according to the time required by the respective organism to reach its exponential phase.

Determination of MIC Value

After 24-hour incubation, wells of the microtiter plates were observed for any changes in turbidity. Turbidity signified the presence of bacterial growth while a clear well indicated the absence of bacterial growth. The first well that was clear was selected as the MIC value.

Minimum Bactericidal Concentration (MBC)

Inoculums from the MIC test were subcultured onto MH Agar and incubated for 18-24 hours. MBC values were taken by selecting the lowest concentration of extract that showed no bacterial growth on the MH agar. This test was also performed in triplicate.

Results and Discussion

Preparation of *Parkia speciosa* Pericarp Extract

The crude extract obtained after the concentration process using a rotary evaporator was in semi-solid form, greasy and greenish. The extraction yield was defined as the extract obtained mass compared with the initial amount of raw material. In this study, the crude extract obtained was 4.00 g while the initial pericarp powder used was 164.05 g. Thus, the percentage of extraction yield obtained was approximately 2.50%.

Antibacterial Sensitivity Test (AST)

The disk diffusion method was applied to determine the antibacterial effect of *P. speciosa* pericarp extract against *S. aureus*, *B. cereus*, *E. coli*, and *S. typhimurium*. Antimicrobial Sensitivity Testing (AST) was performed using 6 different concentrations of *P. speciosa*: 1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.50 mg/mL and 31.25 mg/mL against the selected bacteria. 10% DMSO was used as a negative control while ampicillin was a positive control. To gain reliable results, the test was performed in duplicates, and the mean value was calculated. The results of the mean zone of inhibition for all bacteria are tabulated in Table 1.

As summarised in Table 1, only two species were susceptible to *P. speciosa* pericarp extract: *S. aureus* and *B. cereus*. *S. aureus* showed a higher inhibition zone value at 18 mm, compared to *B. cereus* at 12.50 mm. Both gram-negative bacteria, *E. coli*, and *S. typhimurium* were resistant to *P. speciosa* pericarp extract and showed no zones of inhibitions.

Minimum Inhibitory Concentration (MIC)

Based on the results shown in Table 2, for *S. aureus*, wells 1-8 showed no visible growth. Starting from well 9 (at *P. speciosa* pericarp extract concentration of 3.91 mg/mL), the wells showed turbidity. Thus, the lowest concentration

Table 1: Mean zones of inhibition of *P. speciosa pericarp* extract against tested bacteria

The concentration of Extract (mg/mL)	Zones of Inhibition (mm)			
	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>S. typhimurium</i>
1000	18	12.50	0	0
500	17	14	0	0
250	16	12.50	0	0
125	14.50	12	0	0
62.50	12	10	0	0
31.25	9.50	8.50	0	0
Positive control (ampicillin 10 μ g)*	34	9.75	14.50	23
Negative control (10% DMSO)	0	0	0	0

*Diameter of zone of inhibition: Resistant \leq 11mm; Intermediate 12-13 mm; Susceptible \geq 14 mm

Table 2: Mean MIC of *P. speciosa pericarp* extract against *S. aureus* and *B. cereus*

Well	Dilution Factor	The Concentration of Extract (mg/mL)	Result	
			<i>S. aureus</i>	<i>B. cereus</i>
1	1	1000	C	C
2	2	500	C	C
3	4	250	C	C
4	8	125	C	C
5	16	62.50	C	C
6	32	31.25	C	C
7	64	15.63	C	C
8	128	7.81	C	C
9	256	3.91	T	C
10	512	1.95	T	C
11	1024	0.98	T	T
12	2048	0.49	T	T
13	-	Control	T	T
14	-	Positive	C	C
15	-	Negative	T	T

C: Clear; T: Turbid

of extract that could inhibit the growth of *S. aureus* was 7.81 mg/mL. For *B. cereus*, wells 1-10 showed no visible growth until well 11 which showed turbidity at *P. speciosa pericarp* extract concentration of 0.98 mg/mL. Therefore, the lowest concentration of extract capable of inhibiting the growth of *B. cereus* was 1.95 mg/mL.

Minimum Bactericidal Concentration (MBC)

Based on the results shown in Table 2, for *S. aureus*, there was no growth observed with inoculums from wells 1-8 until well 9 with *P. speciosa pericarp* extract at a concentration of 3.91 mg/mL. Thus, the MBC value for *P. speciosa pericarp* extract against *S. aureus* is

7.81 mg/mL. For *B. cereus*, there was no growth observed with inoculums from wells 1-10 but growth was observed with inoculums tested with *P. speciosa* pericarp extract at a concentration of 0.98 mg/mL, therefore, the MBC value for *P. speciosa* pericarp extract against *B. cereus* is 1.95 mg/mL.

In the present study, ethyl acetate extract from *P. speciosa* pericarp was compared with an existing antimicrobial agent (ampicillin). The *P. speciosa* pericarp extract was tested for its antibacterial activity against *S. aureus*, *B. cereus*, *E. coli*, and *S. typhimurium*. The resulting antibacterial activity of the extract may be due to the presence of various bioactive compounds available in the *P. speciosa* pericarp. A study by Hasim et al. (2015) showed the presence of alkaloids, saponins, flavonoids, tannins, and triterpenes in the ethyl acetate extract of *P. speciosa* pericarps. All these phytochemicals may have contributed to the killing of the bacterial strains that were tested against the extract. However, from the AST result, it was shown that the *P. speciosa* pericarp extract exhibited antibacterial activity only against *S. aureus* and *B. cereus*.

Table 1 demonstrates the susceptibility of both *S. aureus* and *B. cereus* toward the ethyl acetate extract of *P. speciosa* pericarps. Among the two gram-positive organisms, *S. aureus* was found to be more susceptible with an inhibition zone measured at 18 mm compared to *B. cereus* with an inhibition zone of 12.50 mm. However, none of the gram-negative bacteria was found to be susceptible to the extract. The difference in the antimicrobial activity was probably due to the difference in the membrane structure and composition of the organisms. Gram-negative bacteria have a more complex membrane structure consisting of lipopolysaccharide, lipoprotein, peptidoglycan, and porins proteins, thus making them more resistant to penetration of the extract molecules (Silhavy et al., 2010). Meanwhile, gram-positive organisms, only have a peptidoglycan layer and this eases the penetration of extract into the bacterial cell (Nostro et al., 2000). The tougher cell membranes make the gram-negative organism more robust

in surviving any other environmental substances including the antibacterial agent (Sanchez et al., 2005).

Even so, Hasim et al. (2015) reported that the extract of *P. speciosa* pericarps was sensitive against *S. aureus* and *E. coli*. The activity on *S. aureus* was similar to our finding but was in contrast to the result on *E. coli*. This discrepancy may be due to the different origins of the *P. speciosa* pericarps which may influence their phytochemical constituents. It has been suggested that even different soils will influence the phytochemical constituents of plants (Mudau et al., 2022). Besides, the method of drying may also affect the antibacterial activity due to the changes in chemical constituents within the plant sample (Parekh & Chanda, 2007). Hasim et al. (2015) used the oven to dry the pericarps of *P. speciosa* at 52°C but our study dried the pericarp at room temperature. In addition, another similar study by Wonghirundecha et al. (2014) reported that *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Vibrio cholera*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes* showed susceptibility towards the *P. speciosa* pericarps extract. Differences in the results obtained may be due to differences in solvents used during the extraction process. Wonghirundecha et al. (2014) used 50% (v/v) ethanol for the extraction. Since ethanol is more polar than ethyl acetate, the phytochemical compounds within their extract may differ from ours, hence leading to the difference in the antibacterial activities of the extract. It has been proven that the difference in the polarity of the solvent used may affect the compound extracted from the plant (Sunder et al., 2011). Moreover, the presence of water in the 50% ethanol increases the polarity compared to absolute alcohol, and this also allows the extraction of bioactive compounds with a broad range of polarity (Sun et al., 2015; Hikmawanti et al., 2021). The result in Table 2, shows that the MIC value of *B. cereus* was 1.95 mg/mL which was lower than *S. aureus* (at 7.81 mg/mL). Correspondingly, the MBC value for *B. cereus* was also lower than *S. aureus* as shown in Table 3. Thus, both bacteria were sensitive to the

Table 3: Mean MBC of *P. speciosa* pericarp extract against *S. aureus* and *B. cereus*

Well	The concentration of Extract (mg/mL)	Result	
		<i>S. aureus</i>	<i>B. cereus</i>
1	1000	No growth	No growth
2	500	No growth	No growth
3	250	No growth	No growth
4	125	No growth	No growth
5	62.50	No growth	No growth
6	31.25	No growth	No growth
7	15.63	No growth	No growth
8	7.81	No growth	No growth
9	3.91	Growth	No growth
10	1.95	Growth	No growth
11	0.98	Growth	Growth
12	Control	Growth	Growth
13	Positive	No growth	No growth
14	Negative	Growth	Growth

extract of *P. speciosa* pericarp prepared using ethyl acetate. However, when comparing *S. aureus* and *B. cereus*, ethyl acetate extract from *Parkia speciosa* pericarp was more effective for antibacterial activity toward *B. cereus*.

Conclusion

P. speciosa pericarps extract has been shown to exhibit antimicrobial activity against *S. aureus* and *B. cereus*. The extract may have a better effect on *B. cereus* than ampicillin. Thus, *P. speciosa* has potential as an antimicrobial agent. Further studies should be performed on several other gram-negative and gram-positive bacterial and fungal strains. The effect or mechanism of actions of the extract on morphological changes in the bacteria should be observed. Phytochemical compounds present in the extract should also be identified and quantified. The toxicity levels of the extract should also be performed before being used as an antimicrobial agent for human use.

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