



## Original Research

### *In-vitro antibacterial activity of the seed extract of three-member Artocarpus towards Methicillin-Resistant Staphylococcus aureus (MRSA)*

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## HIGHLIGHTS

- The emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections has become a serious health problem.
- Strategy to avoid this is by using alternative therapeutic agents from plants that are effective against MRSA
- Many plants are used as folk medicines to anti-MRSA, one of which is a seed of *Artocarpus*.

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## ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections have created a critical need for the development of natural antibacterials from a biological source. This research aimed to investigate the antibacterial activity of the seed extract of three-member *Artocarpus* (*Artocarpus heterophyllus*, *A. champeden*, and *A. camansi*) against MRSA which are the most prevalent causes of infections in patients. Crude seed extracts of three-member *Artocarpus* were evaluated for their antibacterial activity against MRSA. The antibacterial activity against MRSA of the three extracts was assayed in vitro by the agar well diffusion assay and agar microdilution method and minimum bactericidal concentration. The antibacterial activity, calculated as a zone of inhibition and MIC, MBC values. The Crude seed extracts of three-member *Artocarpus* showed antibacterial activity against the MRSA in the agar well diffusion assay (1.5-9 mm inhibition diameter). The MIC value of extract showed at 15.62 mg/mL and the MBC value of seed extract of *A. heterophyllus* at 62.5 mg/mL, *A. champeden* at 31.25 mg/mL, *A. camansi* at 250 mg/mL. All seed extracts have the potential to be developed as antibacterial agents, particularly against MRSA strain. Studies on the antibacterial activity against MRSA can provide new information about the benefits seed of members of *Artocarpus* as a source of natural antibacterial.

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## 1. INTRODUCTION

*Staphylococcus aureus* is one of the most common food-borne pathogenic bacteria. Toxins produced by it can cause food spoilage and food-borne diseases.<sup>1</sup> Due to the inappropriate use of antibiotics, methicillin-resistant *Staphylococcus aureus* (MRSA) became a new threat in the past decades. MRSA is a major cause of nosocomial infections worldwide including in Indonesia. The prevalence rates of MRSA in hospitals in some Asian countries such as Japan, South Korea, Taiwan, and China are 70–80%.<sup>2</sup> Although the prevalence varies considerably between regions or countries, MRSA has been detected in most countries worldwide.<sup>3</sup>

MRSA results in reduced efficacy of antibacterial drugs methicillin group, making the treatment of patients difficult. One strategy to avoid this is by using natural agents from plants,<sup>4,5</sup> honey,<sup>6</sup> Endophytes,<sup>7</sup> *Streptomyces*,<sup>8,9</sup> marine organisms,<sup>10,11</sup> mushrooms,<sup>12</sup> lichens,<sup>13</sup> lactic acid bacteria<sup>14</sup> and animal,<sup>15</sup> that are effective against antibiotic-resistant bacteria.

Many plants are used as folk medicines to antibacterial,<sup>16</sup> one of them is *Artocarpus*. These genera became one of the plants a great level of scientific interest as they contain important secondary metabolites possessing useful biological activities.<sup>17</sup> Several member *Artocarpus* species are used as food and for traditional folk medicines in South-East Asia, including Indonesia. Species of *Artocarpus* common cultivated in Indonesia are *Artocarpus heterophyllus* (jackfruit), *Artocarpus chempeden* (Chempedak), and *Artocarpus altilis* (breadfruit).

Research related to the antibacterial activity of *Artocarpus* has been reported. Extract from the bark of *Artocarpus rigida* Blume showed antimicrobial activity against *Escherichia coli* and *Bacillus subtilis*.<sup>18</sup> Research on anti-MRSA of seed extract of *Artocarpus* against MRSA has not been reported, so it is necessary to investigate the antibacterial potential of seed extract of *Artocarpus* against MRSA. The study on antibacterial activity against MRSA of seed extract of *Artocarpus* with Methanol solvent is expected to provide new information about the benefits of the seed of *Artocarpus*. Besides, it also can support the seed of *Artocarpus* as a source of natural antibacterial against MRSA. This research aimed to evaluate the antibacterial activity of the seed extract of three-member *Artocarpus* (*A. heterophyllus*, *A. camansi*, and *A. chempeden*) against MRSA which are the most prevalent causes of infections in patients.

## 2. MATERIAL AND METHOD

### Seeds

Fresh seeds (*A. heterophyllus*, *A. camansi*, and *A. chempeden*) were collected from the Bandungan field in Semarang during the rainy season in January 2019 (Figure 1). The seeds were washed with water to remove all unwanted materials. They were then dried under sunlight for 7 days. The dried seeds were then milled into a fine powder using a milling machine and stored in a sterile air-tight container until further use.



Figure 1. Photographs seed of plants

Noted: (a) *Artocarpus heterophyllus* (jackfruit); (b) *Artocarpus chempeden* (Chempedak); and (c) *Artocarpus altilis* (breadfruit)

### Preparations of extracts

Seeds of *Artocarpus* extracts were prepared for the method of maceration with Methanol. 100 g powdered seeds were soaked in 300 mL of solvent for 24 h at room temperature and protected from light with shaking. Solvent replacement is done every day. Replacement of solvent is done until the solution becomes clear with the assumption that there is no active compound contained in the dry powder. The supernatant was filtered through filter paper No.1 (Whatman). The maceration solution was concentrated under reduced pressure using a rotary evaporator at 50°C. The crude extracts were collected and allowed to dry at room temperature.

### Bacterial preparation

The MRSA for in vitro antibacterial screening in this study were isolated from patients of the hospital Dr. Kariadi, Semarang City, Indonesia. MRSA isolate was identified and susceptibility patterns were obtained using Vitek<sup>®</sup>MS (bioMérieux). The bacteria were cultured for 24 h at 35±2 °C on 5% sheep blood agar (BAP). The bacteria colonies were homogenized and adjusted to 0.5 McFarland standards (5×10<sup>8</sup> CFU/mL) using spectrophotometry

### Agar well diffusion assay

The antibacterial activity from seeds was evaluated using agar well diffusion assay.<sup>19,20</sup> In this method, 100 µL of MRSA which is equivalent to a 0.5 McFarland standard was inoculated on the MHA. Then it is spread onto the surface of the agar using a sterilized glass spreader. After 10 minutes of inoculation, the wells were prepared using a sterilized steel cork borer (1cm diameter). Wells were made in each plate, out of which five wells were loaded with the extract (100, 200, 500, 700, and 1000 mg/mL). Each test was done in triplicate. All the plates were then incubated aerobically at 35 ± 2 °C for 24 h. Dimethyl sulfoxide (DMSO) was used as a negative control. Vancomycin and oxacillin were applied as positive controls for MRSA. Antibacterial activities were evaluated by measuring the diameters of zones of inhibition (mm) against the test organism.

### Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC).

The MIC and MBC of the extracts were determined using Mueller–Hinton broth microdilution.<sup>21,22</sup> MIC determination was performed by serial dilution technique using 12-well microwell plates. Extract amounts of 100 µL were placed into each well. Then, 100 µL of MRSA suspension (0.5 McFarland) was added to each. Each test was done in triplicate. The seeds extracts were serially diluted to produce final concentrations of 0.24; 0.48; 0.97; 1.95; 3.90; 7.81; 15.62; 31.25; 62.5; 125; 250; and 500 mg/mL. The microwell plates were then incubated for 24 hours at 35 ± 2°C. Dimethyl sulfoxide was used as a control and Mueller–Hinton broth as a negative control. Oxacillin was used as a positive control. The MIC was determined as the lowest concentration of extract that completely inhibited the growth of the MRSA detected by the unaided eye.<sup>23</sup> The MBC was defined as the lowest concentration of the extract that did not any grow.<sup>24</sup> The wells were subcultured using a 10 µL inoculating loop on to a BAP at (35 ± 2)°C for 16–20 hour incubation.

### Phytochemical screening.

The seeds extract were screened for the presence of different classes of secondary metabolites, including alkaloids and flavonoids using previously described methods.<sup>25</sup>

## 3. RESULTS AND DISCUSSION

Extraction is a process that aims to take active compounds from within cell bodies by dissolving active compounds that can then be extracted. The Methanolic extracts of the seed of three-member *Artocarpus* were calculated for the yield ([Table 1](#)), which showed that its

constituents were relatively polar. Methanol has a polarity index of 5.1 and is used for the extraction of polar compounds.<sup>26</sup>

Table 1. The extract yield

Plants	Part of Plants	Solvent	yield (%)
<i>A. heterophyllum</i>	seed	Methanol 96%	5.50
<i>A. champeden</i>	seed	Methanol 96%	6.68
<i>A. communis</i>	seed	Methanol 96%	6.70

The antibacterial activity against MRSA of the three extracts was assayed in vitro by the agar well diffusion assay and agar microdilution method.

The antibacterial activities against MRSA of the three seed extracts were assayed in vitro by agar diffusion method. The zones of inhibition are presented in [Figure 2](#). All seeds demonstrated the zones of inhibition against MRSA. The diameters of the zones of inhibition with various concentrations *A. heterophyllum* and *A. champeden* (100-1000 mg/mL) and *A. communis* (500-1000 mg/mL) are presented in [Figure 3](#). All extract showed antibacterial activity against MRSA in the agar well diffusion assay (1.5-9 mm inhibition diameter). This showed that all seed had the potential to be developed as antibacterial agents for MRSA strains. These results are similar to another previously obtained result, which indicated the efficacy of seed extracts with methanolic solvent.<sup>27</sup> Another study showed that the crude methanol extracts of the *Artocarpus heterophyllum* seed exhibited a minimum (<12 mm) antibacterial activity against MRSA.<sup>28</sup>

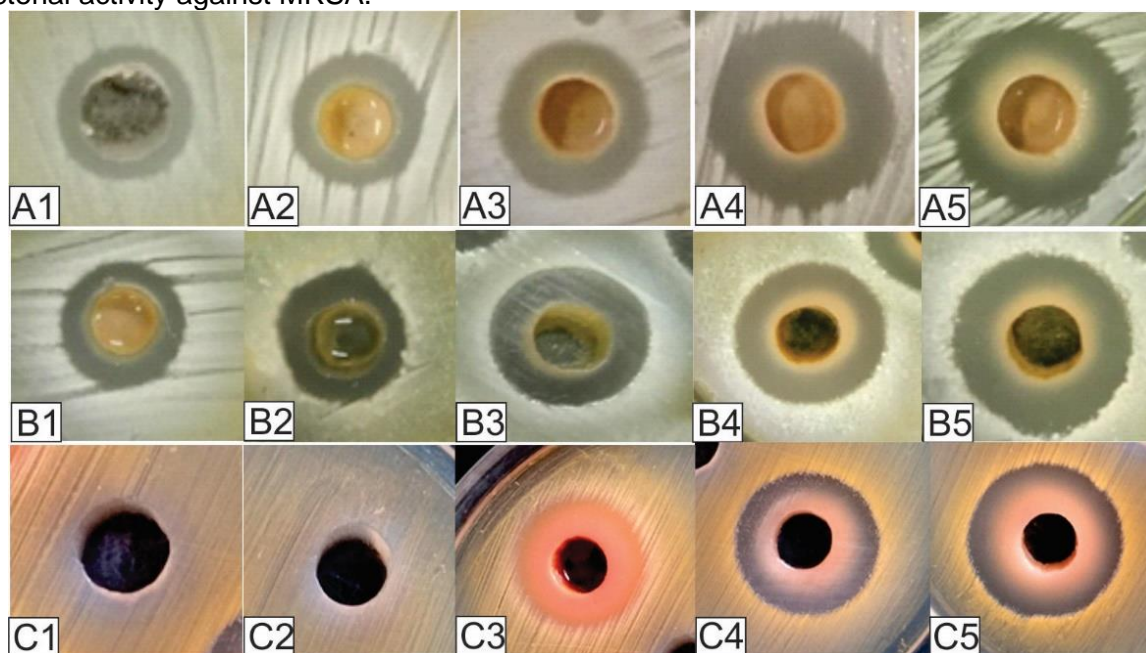


Figure 2. Zone of the inhibition of seeds extracts against MRSA

Note:

(A) *A. heterophyllum*

(B) *A. champeden*

(C) *A. communis*

- (1) with concentration 100 mg/mL
- (2) with concentration 200 mg/mL
- (3) with concentration 500 mg/mL
- (4) with concentration 700 mg/mL
- (5) with concentration 1000 mg/mL



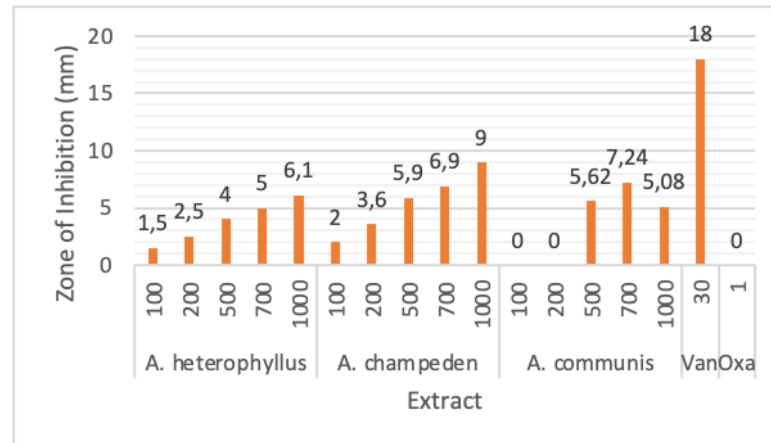


Figure 3. Graphical representations are indicated a zone of inhibition of seed extracts of *Artocarpus* against MRSA

Noted:

Van: Vancomycin

Oxa: Oxacillin

The antibacterial activity of the seed of *Artocarpus* was assayed in vitro by the agar microdilution method against MRSA. Table 2 shows all seeds exhibited the value of MIC against MRSA at 15.62 mg/mL. Figure 3, 4 and 5 shows value of MBC at seeds of *A. heterophyllum* (62.5 mg/mL), *A. champeden* (31.25 mg/mL) and *A. camansi* (250 mg/mL). This showed that seeds of *A. heterophyllum*, *A. champeden*, and *A. camansi* had the potential to be developed as antibacterial agents for MRSA strains. Plants extracted in methanol provide more consistent antibacterial activity compared to other solvent extracts of the same plants.<sup>29</sup> Most antimicrobial active compounds from plants that have been identified were soluble in methanol solvents.<sup>30</sup> Extraction techniques are also important to separate the active compounds, because some active compounds may be destroyed by heat. Seeds of *Artocarpus* extracts were prepared for the method of maceration with methanol this method does not use heat, so the active compound is not broken.

The methanolic extract of *Artocarpus* seed showed activity against MRSA. The antibacterial activity of the methanol, ethanol, acetone, chloroform, and petroleum ether extracts of the *A. heterophyllum* seed powder against MRSA strain by the disc diffusion method. It was observed that methanol extracts possessed good activity. The methanolic extracts were active against all the twelve isolates. The antibacterial effects of the methanolic extract were also better than that of methicillin (5 µg) used as the positive control.<sup>28</sup>

Table 2. MIC value of seed extract of *Artocarpus* against MRSA

Well	Concentrations(mg/mL)	<i>A. heterophyllum</i>	<i>A. champeden</i>	<i>A. camansi</i>
1	500	-	-	-
2	250	-	-	-
3	125	-	-	-
4	62.5	-	-	-
5	31.25	-	-	-
6	15.62	-	-	-
7	7.81	+	+	+
8	3.90	+	+	+
9	1.95	+	+	+
10	0.97	+	+	+
11	0.48	+	+	+
12	0.24	+	+	+

Noted: (+): Present; (-): Absent



Figure 4. MBC value of seed extract of *A. heterophyllus* against MRSA at 62.5 mg/mL (d), the arrow points to the growing bacterial colony

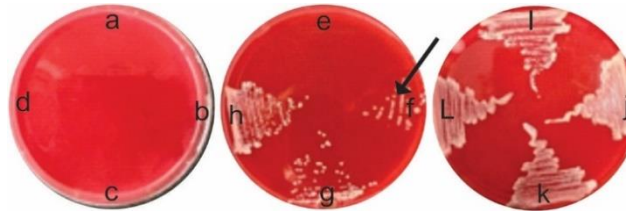


Figure 5. MBC value of seed extract of *A. champeden* against MRSA at 31.25 mg/mL (e), the arrow points to the growing bacterial colony

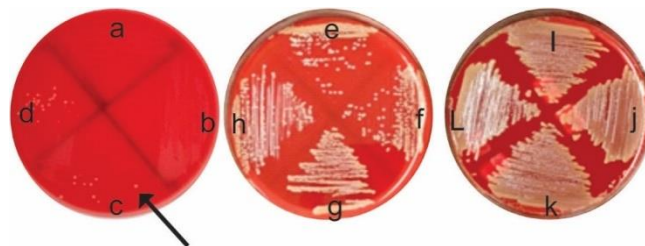


Figure 6. MBC value of seed extract of *A. camansi* against MRSA at 250 mg/mL (b), the arrow points to the growing bacterial colony

The screening of the phytochemical composition was conducted all for the seeds because they have the potential to be developed as antibacterial agents. The secondary metabolites are shown in [Table 3](#) flavonoids were present in the all seed extract.

Table 3. The results of the phytochemical analysis of seeds extracts of the *Artocarpus*

Seed extracts	Secondary metabolites	
	Alkaloid	Flavonoid
<i>A. heterophyllus</i>	-	+
<i>A. champeden</i>	-	+
<i>A. camansi</i>	-	+

+: Present; -: Absent.

Flavonoids were present in all seed of *Artocarpus*. These bioactive compounds have been reported to be used by plants for protection against bacterial.<sup>31</sup> The mechanism of antibacterial activity flavonoids compound in the study still unknown, but many studies have shown that flavonoids have antibacterial activity. Flavonoid expressed a stronger antibacterial effect against *Escherichia coli*.<sup>32</sup> Antibacterial activities against *E. coli* (ATCC 25922), *Salmonella* (ATCC 51812), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus* (ATCC 49619), of flavonoids fraction, were also proved to be stronger.<sup>33</sup>

The seeds of *Artocarpus* were shown to be potentially developed as an antibacterial against MRSA strains. This study can provide new information about the benefits of *Artocarpus* seed as a source of natural antibacterial against MRSA. Further in vivo research

and discovery of action modes are needed to shed light on their antibacterial effects, so that potential antibacterial products could be developed.

#### 4. CONCLUSION

All seed extracts have the potential to be developed as antibacterial agents, particularly against MRSA strain. Further in vivo research and discovery of mode action are needed to shed light on their antibacterial effects.

#### DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

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## SHORT BIOGRAPHY

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