

# Antibacterial Activity Test of *Jatropha multifida* L. sap against *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) in vitro

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## Abstract

**Background:** *Jatropha multifida* L. is a plant that commonly used as a traditional treatment especially for wounds. It has been proven that its sap had an antibacterial activity against *Staphylococcus aureus*. *Staphylococcus aureus* has developed resistant strains named MRSA (Methicillin-Resistant *Staphylococcus aureus*). Due to this resistance development, hopefully the sap of *Jatropha multifida* L. can also performed its antibacterial activity against MRSA. **Objective:** To determine the antibacterial activity of the sap of *Jatropha multifida* L. against *Staphylococcus aureus* and MRSA. **Methods:** A well-diffusion method was performed on several concentration: 100%, 50%, 25%, 12.5%, and 6.25%. From this method, the diameter of the inhibition zone produced by the sap was measured. **Results:** The average of inhibition zone diameter were 11.29 - 16.00 mm for *Staphylococcus aureus* and the bigger zone 12.15 - 17.61 mm for MRSA. **Conclusion:** *Jatropha multifida* L. had antibacterial activity against *Staphylococcus aureus* and MRSA. This plant showed higher antibacterial activity on MRSA than *Staphylococcus aureus*.

**Keywords:** Antibacterial activity, *Jatropha multifida* L., *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus*, well-diffusion method

## Introduction

Wound is a common occurrence among us. A wound is a condition where our skin is exposed, so making it able become a port of entry for bacteria into the body, which can cause an infection. One of the bacteria is *Staphylococcus aureus*. *Staphylococcus aureus* is a Gram positive bacteria shaped like grapes which is the most virulent bacteria in the genus *Staphylococcus*<sup>1</sup>. These bacteria are often normally found on the skin and mucous membranes<sup>2</sup>. These bacteria are bacteria that are of concern at this time because of the many health problems that arise due to these bacteria.

In Indonesia, there are still a lot of ineffective and massive use of antibiotics that cause bacteria to mutate to form multidrug-resistant strains. One of them is *Staphylococcus aureus* which has mutations, one of which is Methicillin Resistant *Staphylococcus aureus* or commonly known as MRSA. Infections caused by resistant bacteria are often a new problem that arises for both patients and their families, because these infections are closely related to increased mortality and morbidity, as well as increased duration of hospitalization which of course requires a lot of money<sup>3</sup>. Asia is a region that has the highest MRSA infection incidence in the world. Most hospitals in Asia are endemic for MRSA with a proportion of about 28% to 70%<sup>4</sup>. Currently, the therapy of choice used to treat *Staphylococcus aureus* infection is first-generation cephalosporins (cefazolin and cephalothin), clindamycin, flucoxacillin, and

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erythromycin, whereas for MRSA therapy, vancomycin is still selected as first-line therapy. In addition, there are alternative agents that may be used for second line therapy of MRSA such as linezolid, telavancin, and ceftaroline<sup>5-7</sup>.

Indonesia is a tropical country with rich biodiversity. One of the uses for biodiversity is the use of plants that can be used as medicine. One such plant is *Jatropha multifida* L. or commonly known as betadine plant. The part that is often used in this plant is the sap which is used for wound treatment. The sap of *Jatropha multifida* L. has antibacterial, antifungal, and antiviral activity. This plant contains several chemical compounds such as alkaloids, steroids, saponins, tannins, etc<sup>8</sup>. The sap of this plant is also known to have antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis*<sup>9</sup>. Therefore, this study will be tested further regarding the antibacterial activity of *Jatropha multifida* L. sap against *Staphylococcus aureus* and MRSA in vitro.

## Material and Methods

### Materials

*Jatropha multifida* L. sap was collected from Mojokerto city area, East Java, Indonesia, and was determined in Purwodadi Botanical Garden, Pasuruan, East Java, Indonesia. *Staphylococcus aureus* and MRSA in this study were inoculated from Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

### *Jatropha multifida* L. sap collection

Sap collection is carried out aseptically. First by cleaning the area of the leaf stems that will be broken using an alcohol swab. Then the sap is taken and collected in a tightly closed sterile container. About 25 ml of the sample is needed, then stored in the refrigerator<sup>10</sup>.

The collected sap is homogenized and then tested for contamination. The contamination test was carried out by rubbing the sample on the nutrient agar using a sterile cotton swab then incubated at temperature 37°C for 24 hours. If there was no microorganisms growth detected, it means the sap is sterile, if it is overgrown with microorganisms it means that the sap isn't sterile.

### Antibacterial activity test using the well-diffusion method

Bacteria were inoculated in Mueller-Hinton agar using a sterile cotton swab aseptically. Then well was made using 6 mm cork-borer. Various types of sap concentrations were made through serial dilution of pure sap using sterile distilled water four times. A hundred  $\mu$ l of *Jatropha multifida* L. sap with different concentration were added into each well. After that, the media were incubated in 37°C for 24 hours. Then, the antibacterial activity were evaluated by measuring the clean zone around the well for each concentration using digital caliper<sup>11</sup>. In this study, replications were carried out four times according to calculations using the Ferderer formula<sup>12</sup>. The positive control for *Staphylococcus aureus* was erythromycin disk 15mg, while vancomycin disk 30 mg was used in MRSA. Erythromycin was used for *Staphylococcus aureus* because this antibiotic was classified as a group A according to CLSI which mean this antibiotics is a primary testing panel for the specific organism groups. Meanwhile vancomycin was chosen because this antibiotics is still the first line empirical antibiotic for MRSA infection therapy<sup>13,14</sup>.

## Results and Discussion

From the well-diffusion method, the antibacterial activity of *Jatropha multifida* L. sap were able to evaluated. The antibacterial activity can be determined by measuring the diameter of clear diameter zone around the well using digital caliper.

**Table 1. Inhibition zone diameter of *Jatropha multifida L.* against *Staphylococcus aureus***

Concentration	Inhibition Zone Diameters (mm)				Mean (mm)
	1	2	3	4	
100%	14.00	11.67	13.60	9.20	12.11
50%	13.99	17.83	16.76	15.43	16.00
25%	12.50	16.30	15.33	15.27	14.85
12.5%	11.03	15.34	13.55	13.92	13.46
6.25%	8.55	14.19	10.54	11.88	11.29
Erythromycin 15µg	22.44	23.73	23.09	23.14	23.10

**Table 2. Inhibition zone diameter of *Jatropha multifida L.* against MRSA**

Concentration	Inhibition Zone Diameters (mm)				Mean (mm)
	1	2	3	4	
100%	14.35	17.44	15.87	17.75	16.35
50%	16.79	17.42	19.08	17.15	17.61
25%	13.71	14.50	16.28	16.00	15.12
12.5%	12.13	12.16	14.53	15.05	13.47
6.25%	10.80	11.74	12.97	13.08	12.15
Vancomycin 30 µg	16.75	17.42	17.68	15.90	16.94

From Table 1 and Table 2. showed that following an increase of concentration, higher diameter of inhibition zone were produced. The lowest average inhibition zone diameters were found in concentration 6.25% in both *Staphylococcus aureus* and MRSA. The highest concentration were found in 50% in both *Staphylococcus aureus* and MRSA.

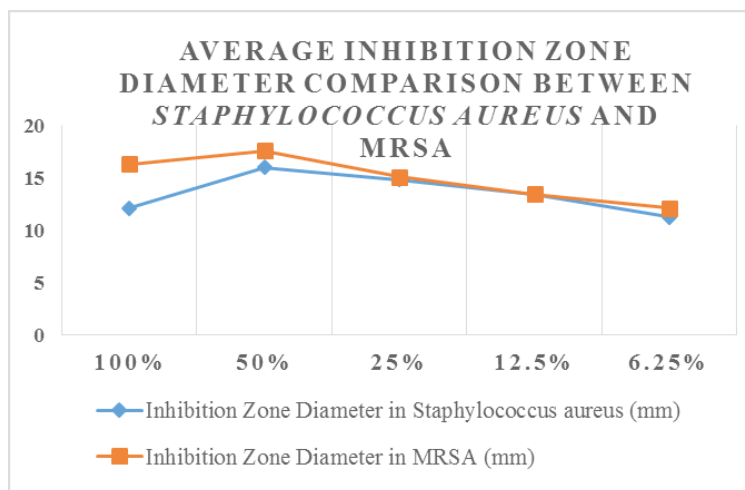
**Figure 1. Comparison of the effect of *Jatropha multifida L.* sap to *Staphylococcus aureus* and MRSA**

Figure 1 shows a comparison chart of the inhibition zone diameter produced by the *Jatropha multifida* L. sap against *Staphylococcus aureus* and MRSA. It can be seen from the graph, that there is a similarity in the pattern of increase in the inhibition zone diameter of the sap for the two bacteria. At a concentration of 100%, the yield is lower than the concentration of 50%. This could be due to the fact that the 100% concentration of pure sap has a lower solubility than the 50% concentration that has been diluted using sterile distilled water. The 50% concentration was diffused completely meanwhile the 100% concentration can't diffused completely<sup>15</sup>.

From Figure 1, it can be seen that the average diameter of the inhibition zone of *Jatropha multifida* L. sap tends to be higher in MRSA when compared to *Staphylococcus aureus*. This is in line with previous research which showed that the results of the antibacterial activity of some natural ingredients had a higher effect on resistant bacteria, namely MRSA compared to *Staphylococcus aureus*<sup>16</sup>. The cause or mechanism of the effectiveness of this *Jatropha multifida* L. sap is better in resistant bacteria is still not known for certain, therefore further research is needed to find out the mechanism.

Based on research conducted by Trisia et. al.<sup>17</sup> the antibacterial activity against *Staphylococcus aureus* is classified as very high if the inhibition zone diameter is > 20 mm, high if the inhibition zone diameter is 16-20 mm, moderate if the inhibition zone diameter is 10-15 mm, and low if the inhibition zone diameter is <10 mm. In this study, the inhibition zone produced by the *Jatropha multifida* L. with 50% concentration against *Staphylococcus aureus* was classified as high, while at concentrations of 100%, 25%, 12.5%, and 6.25% the antibacterial activity was classified as moderate. The inhibition zone produced by *Jatropha multifida* L. sap with 100% and 50% concentrations towards MRSA was high, while at concentrations of 25%, 12.5% and 6.25% the antibacterial activity was moderate.

*Jatropha multifida* L. sap has several compounds such as alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides<sup>18</sup>. These compounds have various types of mechanisms which create the antibacterial activity of this sap.

Alkaloids are organic compounds that are found in nature. Many pharmacological effects possessed by

these alkaloid compounds such as anti-inflammatory, hepatoprotective, anticancer, increase the cell's antioxidant effect, and alkaloids also have antimicrobial properties<sup>19</sup>. The first mechanism is by inhibiting cell division. This occurs through the inhibition of DNA synthesis which inhibit the cell division. In addition, alkaloids can also damage the bacterial cell membranes, both towards Gram positive and negative bacteria<sup>20</sup>.

Saponins are secondary metabolites which are found in many plants. According to research conducted by Maatalah et al.<sup>21</sup>, saponins have higher antibacterial activity compared to alkaloids. Saponins work on bacterial cell walls by increasing cell permeability, so the bacteria will easily lysis and die<sup>22</sup>.

The mechanism carried out by tannins was through inhibiting of the bacterial growth in several pathways, namely by inhibiting nucleic acid synthesis, disrupting protein transport and can also damage bacterial cell walls which leads to bacterial death. In addition, tannins also have the ability to activate enzymes from bacteria and interfere with the passage of proteins in the inner layers of cells. The mechanism of flavonoids as antibacterial washthrough restricting the energy metabolism of bacteria by inhibiting the use of oxygen by bacteria<sup>23,24</sup>. In addition, *Jatropha multifida* L. sap also contains phenols and triterpenoids which also posses antibacterial activity. It was reported that phenol can interfere with the growth of *Staphylococcus aureus* through their acidic properties which allows them to denature proteins and can damage cell membranes<sup>25</sup>. Triterpenoids act on the cell wall by damaging the wall structure and can interfere with active transport in the cytoplasm of bacteria<sup>26</sup>.

From the results of this study as well as several reasons that have been mentioned, *Jatropha multifida* L. sap can be considered as an alternative antibiotic especially for *Staphylococcus aureus* and MRSA. It is also necessary to do further research on safety and side effects as well as in vivo research for *Jatropha multifida* L. sap in the future.

## Conclusion

*Jatropha multifida* L. sap had an antibacterial activity against both *Staphylococcus aureus* and MRSA. From this study *Jatropha multifida* L. can be considered as a candidate for new antibiotics against those bacteria. This

sap had a higher antibacterial activity against MRSA than *Staphylococcus aureus* with the highest average inhibition zone are 16 mm for *Staphylococcus aureus* and 17.61 mm for MRSA.

**Ethical Clearance:** This experimental study protocol had been approved by the Faculty of Medicine, Airlangga University, Surabaya, East Java, Indonesia.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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