# *In Vitro* Antibacterial Activity Test of Jackfruit (*Artocarpus heterophyllus* Lam.) Leaf Extract against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Abstract: Infectious diseases are still a public health problem in Indonesia. *Methicillin Resistant Staphylococcus aureus* (MRSA) is one of bacteria causing infections that is a concern because of the nature of resistance to various beta-lactam class of antibiotics. Therefore, it is necessary to find an alternative antibiotic for the treatment of MRSA infections. This study aims to determine the antibacterial activity of jackfruit (*Artocarpus heterophyllus* Lam.) leaf extract against MRSA by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) conducted using in-vitro test with broth macrodilution method. Jackfruit (*Artocarpus heterophyllus* Lam.) leaf extract were used in half serial concentration of 1280 µg/ml to 0,625 µg/ml. MIC of jackfruit leaf extract against MRSA was found at concentration of 320 µg/ml which showed by a clear solution in Brain Heart Infusion media. Meanwhile, MBC value of jackfruit leaf extract against MRSA was found at concentration of 1280 µg/ml showed by the absence of MRSA colony growth Mueller-Hinton agar. From this study showed that jackfruit (*Artocarpus heterophyllus* Lam.) leaf extract has anti-bacterial activity against MRSA in-vitro.

## 1 INTRODUCTION

Until now infectious diseases are still a problem in Indonesia (Ministry of Health, 2011; Mardiastuti *et al*, 2007). One approach to manage infectious disease is a rational antibiotic use which in Indonesia misuse and overuse of antibiotics is commonly occur (Mardiastuti *et al*, 2007). This phenomena can lead to new problems of emerging antibiotic-resistant microbial strains (Mardiastuti *et al*, 2007; National Institute of Allergy and Infectious Diseases, 2015).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain of beta-lactam-resistant *Staphylococcus aureus*, including penicillin, oxacillin, methicillin, and so on (National Institute of Allergy and Infectious Diseases, 2015) MRSA resistance to various antibiotics is caused the existence of genetic changes caused by the use of antiobiotics irrationally (Nurkusuma D, 2009). MRSA bacterial transmission is divided into 2 categories, namely Hospital-Acquired (through medical measures such as catheterization or surgery) and Community-Acquired (via direct contact with patients or due to low levels of hygiene) (National Institute of Allergy and Infectious Diseases, 2008). MRSA bacterial infections may cause skin diseases such as eczema, furuncle, impetigo, pyoderma, or clinical manifestations of skin abscesses, venous thrombosis, osteomyelitis or even dissemination resulting in meningitis, pneumonia, endocarditis or sepsis (Brooks GF *et al*, 2016) .

Data on the prevalence of MRSA infections in Indonesia is limited. In 2003 the prevalence of MRSA infections at Atmajaya Hospital Jakarta was recorded at 47%, whereas in 2010 the incidence of MRSA infection in Dr. Moh. Hoesin Palembang reached 46% (Yuwono, 2010). In 2013, research to find the prevalence of MRSA infection was done in ICU and surgical treatment room of RSUD Abdul Moeloek Lampung and got prevalence rate 38,24% (Mahmudah R, 2013). This relatively high prevalence of multiresistant bacterial conditions is one of particular concern as the choice of antibiotics therapy



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becomes more difficult. The drug of choice for the treatment of MRSA infections is vancomycin, but since 1996 MRSA has found a decreased sensitivity to vancomycin (Yuwono, 2010). Even in 2002, clinicians in the United States found a strain of MRSA that was resistant to vancomycin (although the numbers were few) (National Institute of Allergy and Infectious Diseases, 2008).

Jackfruit (*Artocarpus heterophyllus* Lam.) is a dicotyl plant with woody stems that are native to India, Malaysia and Bangladesh, but can also grow well in other tropical-subtropical regions including Indonesia (Manuel NV *et al*, 2012; Orwa *et al*, 2009). In addition to fruit, seeds, or young flowers are useful as a source of food, other jackfruit plant parts are also empirically often used as drugs. Jackfruit root is used to treat skin diseases, asthma, fever, and diarrhea. Jackfruit leaf is believed to cure ulcers. Jackfruit sap is believed to cure abscesses, snake bites, and swollen glands. While the seeds, often used to cure gall disease (National Tropical Botanical Garden, 2015).

Several studies have been conducted to prove the usefulness of jackfruit plants in the medical field. Jha and Srivastava in his study found that jackfruit seed oil could inhibit the growth of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria with minimum inhibitory concentrations (MIC) between 1.55-5.20 mg / ml (Jha S *et al*, 2013). Other studies have also shown the presence of antibacterial activity from jackfruit extracts to MSSA and MRSA played by the substance artocarpesin (Manuel NV et al., 2012). Silver nanoparticles synthesized from jackfruit leaf extracts have antibacterial activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Aspergillus niger,* and *Pichia pastoris* (Thombre R *et al*, 2012). Testing of jackfruit leaf extracts on MRSA has not been found.

In this study we investigate the ability of jackfruit leaf extracts to inhibit MRSA growth by determining its MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration).

# 2 MATERIAL AND METHODS

## 2.1 Preparation of extracts and MRSA Bacteria

The extract was obtained from Indonesian Institute of Sciences (LIPI) diluted in DMSO (Dimethyl sulfoxide). MRSA bacteria originating from the Microbiology Department Universitas Indonesia culture collection which is stock in cryotube beads and re-growth on blood agar followed by incubation at 37ºC for 18-24 hours. The growing bacteria were identified with Gram staining and biochemical tests using commercial products (Vitex).

## 2.2. Preparation of antibiotic and Jackfruit leaf extract stock solutions

The vancomycin in powder form was diluted in (Brain Heart Infusion) media to reach concentration of 256 µg/ml. Meanwhile, Jackfruit leaf extract was also diluted 10 times higher than vancomycin concentration (2560 µg/ml).

## 2.2 Dilution of antibiotic and Jackfruit leaf extract

Serial dilution of stock solution of antibiotics and Jackfruit leaf extract were done using BHI media until concentration of 0,25 µg/ml antbiotics and 2,5 µg/ml Jackfruit leaf extract were reached.

## 2.3 Determination of MIC (Minimum Inhibitory Concentration)

Suspension of 0,5 McFarland value of MRSA bacteria is inserted into each tube of BHI media containing serial antibiotic and Jackfruit leaf extract solution followed by incubation at 35°C-37°C for 24 hours. After that, an inspection was done on each tube to assess the growth of bacteria characterized by turbidity in the fluid in the tube. MIC value defiened as the smallest extract concentration that inhibits MRSA growth in BHI media.

## 2.4 Determination of MBC (Minimum Bactericidal Concentration)

After MIC is known, 2 tubes at the lower and 2 tubes at the higher concentration of MIC were subcultured into Mueller-Hinton Agar (MHA) media followed by incubation at 37 ºC for 24 hours. MBC value was determined as concentration of MHA plate whose culture results do not show any growth of bacterial colonies.



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Table 1. Result of MIC test of Leaf Jackfruit Leaf Extract (*Artocarpus heterophyllus* Lam.) on MRSA bacteria.

| Treatment | Concentration | Result (Turbidity [+]; Clear [-]) |
|---|---|---|
| Positive control | - | + |
| Negative control | - | - |
| *A. heterophyllus* | 1280 µg/ml | - |
| *A. heterophyllus* | 640 µg/ml | - |
| *A. heterophyllus* | 320 µg/ml | - |
| *A. heterophyllus* | 160 µg/ml | + |
| *A. heterophyllus* | 80 µg/ml | + |
| *A. heterophyllus* | 40 µg/ml | + |
| *A. heterophyllus* | 20 µg/ml | + |
| *A. heterophyllus* | 10 µg/ml | + |
| *A. heterophyllus* | 5 µg/ml | + |
| *A. heterophyllus* | 2,5 µg/ml | + |
| *A. heterophyllus* | 1,25 µg/ml | + |
| *A. heterophyllus* | 0,625 µg/ml | + |

Positive control = sterile BHI and MRSA bacterial inoculum; Negative control = BHI sterile

## 3 RESULT AND DISCUSSION

The experiments performed by finding MIC and MBC value of jackfruit leaf extract (*Artocarpus heterophyllus* Lam.) against MRSA bacteria in vitro. The research was conducted by broth macrodilution method . The extract used was a crude extract in a DMSO solvent with a potential of 100,000 µg / mg obtained from LIPI, a total volume of ± 235 µL extract. Based on research that has been done, obtained MIC jackfruit leaf extract to MRSA bacteria of 320 µg/ml and MBC of 1280 µg/ml. In this experiment we found different turbidity levels on test tubes after incubation for 1x24 hours. Basically, the addition of stock extract on sterile BHI will produce a turbid liquid. Nevertheless, the researchers compared the turbidity of each tube with positive control and negative control. The tubes contain a jackfruit leaf extract in a serial concentration of 0.625 µg/ml to 160 µg/ml were having similar turbidity with positive controls (BHI and MRSA bacteria without extract). Meanwhile, tubes containing jackfruit leaf extract with concentrations of above than 320 µg/ml resulting clearer turbidity. The results obtained can be seen in the table 1.

Cultures on MHA medium were carried out to confirm the results of MIC and determine the MBC. In this experiment, culture of MHA medium was performed for all extract concentrations. The results of culture can be seen in the figure 1.

MBC is the lowest concentration in which no bacterial colony growth is found in culture. Thus, the MBC for jackfruit leaf extract (*Artocarpus heterophyllus* Lam.) is 1280 µg/ml.

In addition, tubular macrodilution testing was also performed to determine MIC and MBC vancomycin against MRSA bacteria. In this study vancomycin antibiotics were used as a comparison. From the results of MIC and MBC vancomycin tests on MRSA, 2 µg/ml result was obtained for both. Based on the Clinical Laboratory Standards Institute (CLSI), there are three classifications of *S. aureus* based on their susceptibility to vancomycin, ie sensitive (<2 µg/ml), intermediates (4-8 µg/ml), and resistant (> 16 µg/ml) . Thus it can be concluded that the MRSA used in this study is sensitive to vancomycin.

Test method of jackfruit leaf extract in macrodilution was chosen because the result obtained is quantitative, that is by finding MIC and MBC. The macrodilution method is less used than the disc diffusion method. However, with the consideration that the extract is a crude extract that still contains solids material and easily settles, it is feared the extracts are not spread evenly if the researchers use disc diffusion method.



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However, the weakness of the macrodilution method is manual preparation that often occurs errors, requiring more places and reagents, and generally MIC or MBC is found to be the range between the lowest concentrations with no bacterial colonies and the nearest concentration below (Jorgensen JH *et al*, 2009).

In this study, we used dimethylsulfoxide (DMSO) as a solvent of jackfruit leaf extract. DMSO is a very polar liquid that is generally used as a solvent. DMSO was chosen because it does not affect the antibacterial activity of the extract. Another study show that DMSO has bacterial inhibition but is weaker than methanol or ethanol (the solvent commonly used to dissolve the extract), so the use of DMSO is more recommended (Wadhwani T et al, 2008).

Jackfruit (*Artocarpus heterophyllus* Lam.) Has active ingredients, ie phytosterol and terpenoids. Jackfruit leaves contain flavonoids, terpenoids, steroids, phenols, glycosides, and saponins (Sivagnanasundaram P *et a*l, 2015). Jackfruit leaf extraction with silver nitrate can also produce silver nanoparticles (Thombre R *et al*, 2012). These active ingredients are believed to have antibacterial and antioxidant effects. The content of the active ingredient in jackfruit leaves is consistent with the results of a study conducted by Thombre et al. which shows that the use of jackfruit leaf extract can inhibit the growth of *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* with inhibition zones of 20 mm, 20 mm, and 17 mm, respectively. The zone of inhibition was obtained by adding 20 μL of extract with concentration of 40000 μg/ml into each diffusion well.

In this study the obtained concentration of jackfruit leaf extract is smaller to inhibit bacteria compared with the concentrations obtained from research Thombre, et al. MIC jackfruit leaf extract was obtained at concentration of 320 μg/ml and MBC at a concentration of 1280 μg/ml. This difference may be due to Thombre, *et al* in his research specifies the active substance used, ie only using silver nanoparticles. While in this study the extract used is a whole extract without extraction or separation of further compound fractions. There may be contributions from other antibacterial substances other than silver nanoparticles in jackfruit leaf extract used by researchers, so the use of the required concentration becomes smaller to obtain MBC.

Until now, vancomycin is still the preferred antibiotic for MRSA infections. Based MIC and MBC values, there is a considerable concentration difference between vancomycin and jackfruit leaf extract against MRSA bacteria. Vancomycin has MIC and MBC = 2 μg/ml, while jackfruit leaf extract has MIC = 320 μg/ml and MBC 1280 μg/ml. This may be due to differences in the mechanism of vancomycin or jackfruit leaf extract in inhibiting growth or killing MRSA bacteria.

In this study, we use crude extract of jackfruit leaf which not yet purified for its active compound towards MRSA or other bacteria, yet may become one of the reason of high value of MIC and MBC. Other active compound of jackfruit against MRSA can be further analyzed as Manuel NV et al, 2012 found substance artocarpesin is the main compound against MRSA, Furthermore, our MIC and MBC is relatively low compare to study performed by Jha S *et al*, 2013 which obtain 1.55-5.20 mg/ml of MIC towards *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria.

However, with increasing MRSA resistance to various antibiotics including vancomycin (Tenover FC *et a*l 2006), the use of jackfruit leaf extract as an alternative antibiotic for MRSA infections may be considered.

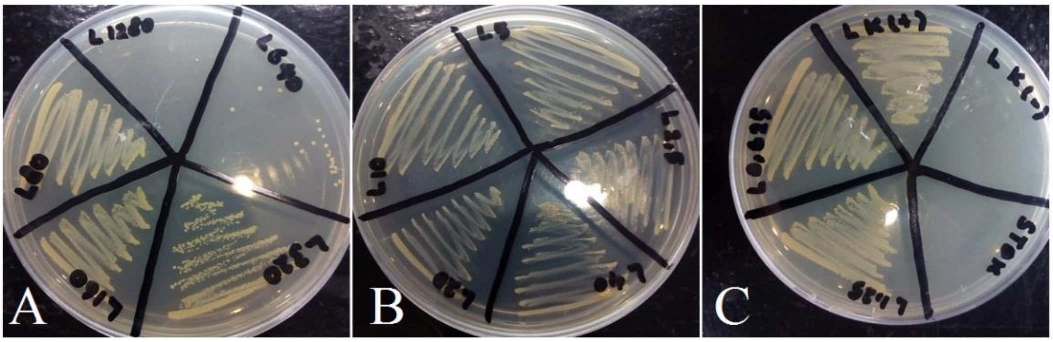


Figure 1. The results of the culture of jackfruit leaf extract (*Artocarpus heterophyllus* Lam.) With MRSA Bacteria on Mueller-Hinton Agar medium. At concentrations of 0.625 μg/ml extract, 1.25 μg/ml, and so on up to 320 μg/ml there is a solid colony growth that can not be counted. In extracts with concentrations of 640 μg/ml there is still a growth of 22 colonies of bacteria.



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While in the extract with a concentration of 1280 µg/ml no bacterial growth of MRSA was found.

## 4 CONCLUSION

Based on our study, jackfruit leaf extract is having anti MRSA properties with MIC and MBC value were 320 µg/ml and 1280 µg/ml respectively.

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