

Antibacterial Activity of Noni Fruit Extract (*Morinda citrifolia*) against *Staphylococcus aureus* ATCC 25923 and Methicillin Resistant *Staphylococcus aureus* (MRSA)

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Abstract

Background: Noni fruit (*Morinda citrifolia* L.) has long been known by the people of Indonesia, especially Java, Sumatra, and Borneo. Noni fruit (*Morinda citrifolia* L.) is also widely used as traditional medicine in almost all islands in Indonesia. Noni fruit (*Morinda citrifolia* L.) is also known to have antibacterial activity against *Staphylococcus aureus* and MRSA (Methicillin-Resistant *Staphylococcus aureus*). The antibacterial activity of noni fruit is due to the phytochemical compounds found in noni fruit, such as iridoids, flavonoids, fatty acids, lignans, anthraquinone vitamins, and trace elements. **Objective:** This study is aimed to determine the antibacterial activity of the methanol extract of Noni fruit (*Morinda citrifolia* L.) against *Staphylococcus aureus* and MRSA (Methicillin-Resistant *Staphylococcus aureus*). **Methods:** This research was an experiment using a well-diffusion method to determine the antibacterial activity of the methanol extract of Noni fruit (*Morinda citrifolia* L.) against *Staphylococcus aureus* and MRSA (Methicillin-Resistant *Staphylococcus aureus*) in vitro. Minimum Bactericidal Concentration (MBC) values were determined using the diffusion method. The MBC values were determined by suspension streaking Muller Hinton broth on a mac Conkey agar plate. Five concentrations were used for the diffusion method, obtained from serial dilution using distilled water. The concentrations used were 100%, 50%, 25%, 12.5%, and 6.25%, respectively. The positive control of this study for *Staphylococcus aureus* was erythromycin 15 µg, and for MRSA was vancomycin 30 µg. **Results:** The Minimum Bactericidal Concentration (MBC) of methanol extract from Noni fruit (*Morinda citrifolia* L.) on *Staphylococcus aureus* and MRSA (Methicillin-Resistant *Staphylococcus aureus*) in this study was 6.25% and 3.13% respectively. **Conclusion:** This research shows a higher antibacterial activity against MRSA. The average diameter of the inhibition zone in each specific case was 0 – 26.64 mm for *Staphylococcus aureus* and 0 – 24.63 mm for MRSA. Therefore, further research is needed in developing Noni fruit (*Morinda citrifolia* L.) as an oral or topical antibiotic.

Keywords: Antibacterial activity; Noni Fruit Extract, *Morinda citrifolia*, *Staphylococcus aureus* ATCC 25923; Methicillin-Resistant *Staphylococcus aureus*

1. Introduction

Indonesians have widely used herbal plants as traditional medicines. Traditional and non-traditional herbal medicines are currently widely used for preventive, curative or palliative purposes [1] Poverty plays role in switching to the purchase of drugs because they have not been able to fill prescriptions and switch to local or alternative medicines [2]. One of the plants that can be used as herbal medicine is noni fruit or *Morinda citrifolia* L., which has long been a traditional medicine used in Indonesia. The efficacy of noni fruit as a traditional medicine has been proven by its antibacterial, antifungal, and tumor cells suppressing effects [3]. The European

Center for Disease Prevention and Control Antimicrobial Resistance Interactive Database stated that in 2013 alone, in 15 European countries, *Staphylococcus aureus* bacteremia are caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA), with several countries having almost a 50% rate of resistance [4]. This study aims to compare the in vitro antibacterial effect of noni fruit (*Morinda citrifolia* L.) against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus*.

2. Methods and Materials

2.1 Study Materials

Jatropha multifida leaves were extracted at Balai Materia Medika, Batu, East Java, Indonesia. The in vitro antibacterial tests of *Morinda citrifolia* L. extracts against *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) was done at Microbiology Laboratory, Faculty of Medicine, Airlangga University.

2.2 Extraction of Noni Fruit

The noni fruit that meets the criteria is about 2 kg in weight, evenly yellowish-white in color, and the fruit flesh is still hard. The fruit is then washed, drained, and cut into thin pieces. Then it was dried in the sun, shaded with a black cloth to speed up the drying process for two days. 200 g of dried noni fruit is obtained from 2 kg of fresh noni fruit. Then it was made into flour using a blender and 130 g of flour was acquired and ready to be macerated. Before maceration, the distillation of 70% methanol was carried out to produce pure methanol, which would be used as a solvent. Maceration was carried out by soaking 130 g of flour in 650 mL of pure methanol for 24 hours. The ratio of flour and solvent used is 1:5, where one kilogram of flour is dissolved with five liters of methanol and stirred once every 24 hours. The solution is then filtered with filter paper, and the filtrates were macerated again in the same way, up to 3 times filtering. The resulting macerated extracts or filtrates are gathered and evaporated to separate the solvent. Evaporation was carried out using a rotary vacuum evaporator at a temperature of 60-80 °C with a speed of 120 rpm and a pressure of 72 bar until the solvent had evaporated so that 50 grams of noni fruit viscous extract was obtained.

2.3 Evaluation of Antibacterial Activity

Staphylococcus aureus ATCC 25923 standardized pure isolate type and Methicillin-Resistant *Staphylococcus aureus* (MRSA) clinical isolate type were inoculated onto Mueller Hinton agar plates using the scratch method. Each type of bacteria gets five plates, so there are 12 plates with two additional plates for positive control using vancomycin discs for Methicillin-Resistant *Staphylococcus aureus* (MRSA) and erythromycin discs for *Staphylococcus aureus*. Then five wells were made on each plate and filled with noni fruit extract from the diffusion method with five different concentrations (6,25%, 12,5%, 25%, 50%, and 100%). The agar plates were then incubated at 37° C for 24 hours. The disc with no bacterial colony growth was the Minimum Bactericidal Concentration (MBC) value.

3. Results

The Minimum Bactericidal Concentration (MBC) value of Noni Fruit Extract (*Morinda citrifolia* L.) from the experiment between *Staphylococcus aureus* ATCC 26923 and MRSA was then compared. Before the results were analyzed, Levene's test was done and showed that the data were distributed normally and homogenous ($p > 0.05$).

Table 1 : Inhibition zone of noni fruit methanol extract against *Staphylococcus aureus* ATCC 25923

Concentration noni fruit methanol extract	Inhibition Zone (mm)				Mean (mm)
	1	2	3	4	
M1 (100%)	28.27	26.36	25.22	26.71	26.64
M2 (75%)	22.81	16.8	19.1	24.72	20.86
M3 (50%)	15.8	12.1	14	18.12	15.01
M4 (25%)	10.63	10.52	12.93	15	12.27
M5 (0%)	-	-	-	-	0.00
Erythromycin 15 [g	23.88	21.5	24	23.1	23.12

Table 2 : Inhibition zone of noni fruit methanol extract against MRSA

Concentration noni fruit methanol extract	Replication and inhibition Zone (mm)				Mean (mm)
	1	2	3	4	
M1 (100%)	27.96	30.63	18.34	21.6	24.63
M2 (75%)	26.55	20.51	15.36	15.76	19.55
M3 (50%)	15.58	12.89	10.59	11.92	12.75
M4 (25%)	10.04	8.62	9.23	9	9.22
M5 (0%)	-	-	-	-	0.00
Vancomycin 15 [g	17.85	20.04	16.55	19.56	18.5

Based on tables 1 and 2 above, we can compare the antibacterial activity of the noni fruit methanol extract. In the antibacterial activity test of methanol extract from noni fruit against *S. aureus* ATCC 25923 bacteria, the largest average diameter of the inhibition zone was found at M1 concentration (100%), namely 26.64 mm and the smallest diameter at M4 concentration (12.5%), namely 12.27mm. In the antibacterial activity test of methanol extract of noni fruit against MRSA bacteria, the largest average diameter of the inhibition zone was at M1 concentration (100%), namely 24.63 mm, and the smallest diameter at M4 concentration (12.25%). M5 (6.12%) of noni fruit extract no longer produces an inhibition zone, even though M4 (12.5%) has an inhibition zone. However, the inhibition zone is very small or almost insignificant when viewed from standard antibiotic sensitivity.

The inhibition zones produced for each bacteria have a somewhat similar pattern, directly proportional to the concentration of the fruit extract. Noni fruit methanol extract has a more significant inhibition zone on *Staphylococcus aureus* bacteria, with an average of 1 to 2 mm compared to MRSA. The sensitivity of the methanol extract of noni fruit is higher on MRSA when compared with *Staphylococcus aureus* ATCC 25923. At the concentration level M1(100%), methanol extract can produce an inhibition zone with an effectiveness of 115% for ATCC 25923 compared with erythromycin as a positive control. In contrast, with MRSA, the effectiveness level is higher at 133% compared with vancomycin as a positive control.

4. Discussion

Noni fruit has many active metabolite compounds, for example, fatty acids, fatty acid esters, flavonoids, iridoids, vitamins, natural elements, anthraquinones, and lignans which have many benefits such as antifungal, antioxidant, anticancer and anti inflammatory [5]. The flavonoid found in noni fruit itself is

Quercetin, a flavonoid that belongs to the type of *flavonols* [5], [6]. Quercetin, in particular, has antibacterial activity by increasing the permeability of the bacterial cell membrane and interfering with the energy potentiation of the cell membrane. Research conducted by Mirzoeva et al. also showed that Quercetin and naringenin significantly inhibited bacterial motility and strengthened the conclusion that the proton pump in the cell membrane was disrupted [7].

The high antibacterial activity of the methanol extract of noni fruit exceeding 100% of the effectiveness of the selected antibiotic for each bacteria can be elicited because it uses methanol for the extract. Although methanol has been evaporated with a rotary vacuum evaporator, the antibacterial properties of the alcohol itself may remain. However, it does not invalidate the antibacterial activity of the noni fruit's methanol extract; instead, it provides evidence of the antibacterial activity and that its greatest potential can be extracted using methanol extract.

The other compound abundant in noni fruit is vitamin C [8]. Vitamin C's antibacterial activity functions by lowering the pH around bacteria and increasing the oxidative stress activity of bacteria, thereby preventing bacterial growth. However, recent research has proven that even in neutral pH conditions, vitamin C can inhibit the growth of *S. aureus* bacteria. Moreover, recent research has shown that vitamin C's antibacterial activity synergizes when combined with specific artificial or natural antibiotic agents; for example, antibacterial activity increases in MRSA with co-administration of vitamin C with Quercetin [9], [10].

Research conducted by De La Cruz-Sánchez et al. (2019) showed that the antibacterial activity using several noni seed extracts, namely n-hexane, dichloromethane, and methanol, against MRSA, *S. aureus*, *S. haemolyticus*, and *S. epidermidis* using several extracts, the methanol extract was the most active against all bacteria. The study proved that methanol extract had stronger antibacterial activity against MRSA, possibly due to the interaction between lignan compounds and coumarins found in noni seeds. Lignans act on the cell wall of MRSA bacteria and inhibit protein transport, while Coumarins work by inhibiting Penicillin Binding Protein (PBP) in MRSA. Coumarin in noni seeds and fruit also inhibits DNA gyrase (in vitro) from competing with ATP in the β subunit enzyme [11]. The antibacterial activity of coumarin seems to be due to its basic structural form, namely benzopyrone or benzo-2-pyrone having a structure like benzopyridone or antibiotics belonging to the quinolone group [11], [12]. The straight structure of coumarin, which has similarities to antibiotics, is used to develop a new type of antibiotic, namely Novobiocin (aminocoumarin) from the by-product of actinomycete *Streptomyces nivosus* by inhibiting bacterial DNA synthesis targeting DNA gyrase and restraining the activation of adenosine triphosphatase (ATPase) [13].

Based on the discussion, the higher antibacterial activity of noni fruit against MRSA is possibly due to coumarin, which inhibits the efflux pump of multi-drug resistant (MDR) bacteria wall. Another reason was the combination of co-administration of the active compounds on noni fruit: quercetin and vitamin C. Further research is needed to determine the secondary metabolites that work and synergize in methanol extract against MRSA and *S. aureus*.

5. Conclusion

In conclusion, methanol extract of noni fruit (*Morinda citrifolia* L.) has antibacterial activity against *Staphylococcus aureus* ATCC 25923 and Methicillin-Resistant *Staphylococcus aureus* (MRSA) in the diffusion test, which shows more significant antibacterial activity against MRSA than *S. aureus* with an average diameter largest 26.64 mm in *S. aureus* and 24.63 mm in MRSA.

6. Recommendations

This study still has some limitations, and the researcher suggests further study about the mechanism of antibacterial activity of noni fruit extract against gram-positive bacteria, especially *Staphylococcus aureus* ATCC 25923 and Methicillin-Resistant *Staphylococcus aureus* (MRSA), the toxicity and safe doses on humans for pharmaceutical purposes.

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