In Vitro Study of Antibacterial Activity of Bidara Leaf Extract (Ziziphus mauritiana) against Staphylococcus aureus and MRSA

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Abstract

Staphylococcus aureus can cause various clinical manifestations. Staphylococcus aureus that is resistant to methicillin, related penicillins and cephalosporins is called methicillin-resistant Staphylococcus aureus (MRSA). The widespread of MRSA is troublesome so a new alternative substance is needed. One of the potential alternative substance is bidara leaf (Ziziphus mauritiana) because it contains tannin and flavonoid. This study aimed to determine whether bidara leaf extract has antibacterial activity against Staphylococcus aureus and MRSA or not. The concentration of bidara leaf extract used are 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%. Disc diffusion method was used and all tests were performed in triplicate. The data was obtained in millimeter by measuring the diameter of inhibition zone. The result for Staphylococcus aureus in positive control (23.67 ± 6.61 mm), negative control (0.00 ± 0.00 mm), 10% concentration (0.00 ± 0.00 mm), 20% concentration (9.09 ± 0.45 mm), 30% concentration (8.90 ± 0.06 mm), 40% concentration (9.58 ± 0.11 mm), 50% concentration (9.69 ± 0.52 mm), 60% concentration (9.98 ± 0.34 mm), 70% concentration (10.05 ± 0.92 mm), 80% concentration (9.35 ± 0.26 mm), 90% concentration (9.77 ± 0.69 mm). The result for methicillin-resistant Staphylococcus aureus in positive control (32.26 ± 1.93 mm), negative control (0.00 ± 0.00 mm), 10% concentration (11.25 ± 1.85 mm), 20% concentration (12.83 ± 3.47 mm), 30% concentration (13.37 ± 3.17 mm), 40% concentration (14.42 ± 3.34 mm), 50% concentration (13.44 ± 2.51 mm), 60% concentration (13.52 ± 2.24 mm), 70% concentration (16.03 ± 3.57 mm), 80% concentration (13.62 ± 2.42 mm), 90% concentration (13.06 ± 2.02 mm). Therefore, bidara leaf extract has antibacterial activity against Staphylococcus aureus and MRSA. The largest and smallest inhibition zone produced against both bacteria is seen at 70% concentration and 10% concentration of bidara leaf extract, respectively.

Keywords: bidara leaf extract; Ziziphus mauritiana; antibacterial; Staphylococcus aureus; MRSA

1. Introduction

Staphylococcus aureus is a coccus, Gram positive bacteria that can be found on the skin and mucous membranes of humans [1]. Staphylococcus aureus can cause various clinical manifestations, such as septic arthritis, skin and soft tissue infections, gastroenteritis, bacteremia, pulmonary infections, and urinary tract infections [2]. Staphylococcus aureus that is resistant to methicillin, related penicillins and cephalosporins is called methicillin-resistant Staphylococcus aureus (MRSA) [3]. Antibiotic resistance could lead to longer hospitalizations, increased mortality, and higher medical costs [4].

MRSA infection have spread to various countries in the world. In South Asia, East Asia, Western Pacific, the prevalence of MRSA varies from 2.3% to 69.1%. Furthermore, more than 60% of healthcare-associated Staphylococcus aureus infection in Cyprus, Portugal, Italy and Romania were identified as MRSA [5]. In the United States, MRSA infection causes 11.285 deaths per year [6]. In a study conducted in 19 countries in Asia

Pacific between 2000-2016, the prevalence of MRSA varies from 0% to 73% [7]. A study conducted in Indonesia, from 643 patients admitted to Dr. Soetomo General Hospital in Surabaya 60 MRSA isolates were detected from 52 patients [8]. In Klaten, Indonesia, a study showed that the MRSA prevalence from 2015-2018 increases from 7.69% to 12.94% [9].

The increasing number of MRSA cases and its widespread in various countries are troublesome so a new alternative substance, such as herbal, is needed. Bidara leaf is one of the herbal plants in Indonesia that contain polyphenols, tannins, and flavonoids that have antibacterial effects, such as against Staphylococcus aureus [10-12]. Therefore, This study aimed to determine whether bidara leaf extract has antibacterial activity against Staphylococcus aureus and MRSA or not.

2. Methods

2.1. Plant material

Fresh bidara leaves were collected from a bidara tree at the Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia. After washed with water, collected leaves were dried in shade for about 5 days. Then, the dried leaves were sent to Assessment Service Unit of the Faculty of Pharmacy Universitas Airlangga for the extraction process.

2.2. Extraction

623 g of sample was soaked in 80% methanol with a ratio of 1:2. After 24 hours, the extract was filtered until the first filtrate was obtained. Then, the pulp of the sample was added again with 80% methanol with a ratio of 1:2. After 24 hours, the procedure number 2 and 3 were repeated to get the final filtrate for about 3 days. Then, the final filtrate was evaporated using rotary evaporator to get viscous bidara leaf extract.

2.3. Bacterial suspension

Staphylococcus aureus and MRSA from culture stock were taken by ose and were suspended into Nutrient Broth. Then, the bacterial suspension was homogenized using vortex and adjusted equivalent to 0.5 Mc. Farland.

2.4. Antibacterial activity

The antibacterial activity of bidara leaf extract was determined using disc diffusion method. Bidara leaf extract was dissolved into 100% DMSO then the solution obtained was processed to obtain 9 concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%). Each bacteria were inoculated into 3 Mueller Hinton Agar Plates by sterile swabs. 100% dimethyl sulfoxide was used as negative control and Trimethoprim-Sulfamethoxazole disc (1.25 µg) was used as positive control. At 10 different places, 3 plates were perforated to be used as a place to drop 100 µl of 100% dimethyl sulfoxide (DMSO) and 100 µl of each of 9 concentration of bidara leaf extract. Trimethoprim-Sulfamethoxazole disc was placed in the remaining place of the plate. All tests were performed in triplicate. After that, all plates were incubated for 24 hours at 37°C and evaluated whether there is the inhibition zone or not. Then, the inhibition zone diameter was measured (in mm).

3. Results

3.1. Staphylococcus aureus inhibition zone

Table 1. The mean value of the diameter of inhibition zone (Staphylococcus aureus)

Group	Mean ± SD (mm)
10%	0.00 ± 0.00
20%	9.09 ± 0.45
30%	8.90 ± 0.06
40%	9.58 ± 0.11
50%	9.69 ± 0.52
60%	9.98 ± 0.34
70%	10.05 ± 0.92
80%	9.35 ± 0.26
90%	9.77 ± 0.69
Positive Control	23.67 ± 6.61
Negative Control	0.00 ± 0.00

The result of antibacterial activities of bidara leaf extract against Staphylococcus aureus have shown that the largest and smallest inhibition zone is produced at 70% concentration and 10% concentration, respectively. The diameter of the inhibition zone is measured by observing the transparent area where there is no bacterial growth (Fig. 1). All tests were performed in triplicate and then the mean of the 3 inhibition zone was calculated (Table 1).

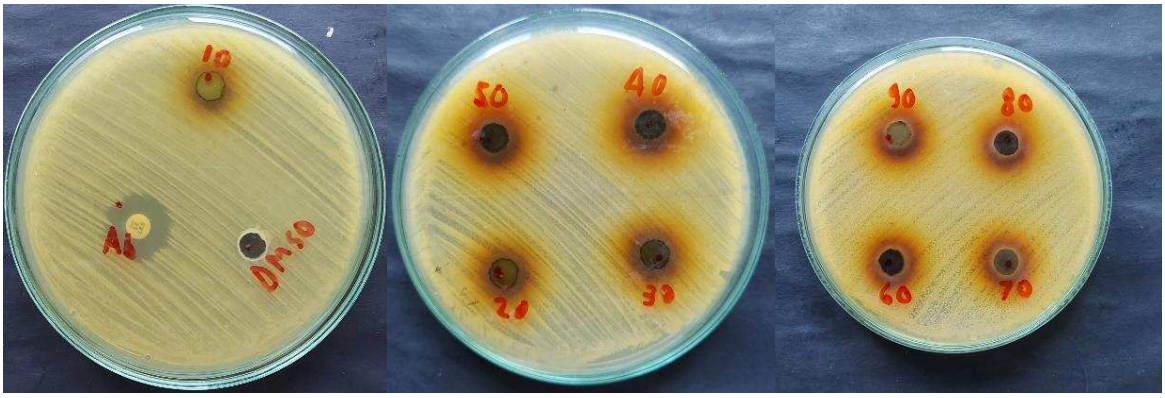

Fig. 1. The inhibition zone diameter (Staphylococcus aureus)

Note: Ab : Trimethoprim-Sulfamethoxazole disc (positive control)
DMSO : Dimethyl Sulfoxide (negative control)
10 : 10% concentration

3.2. Methicillin-resistant Staphylococcus aureus inhibition zone

Table 2. The mean value of the diameter of inhibition zone (MRSA)

Group	Mean ± SD (mm)
10%	11.25 ± 1.85
20%	12.83 ± 3.47
30%	13.37 ± 3.17
40%	14.42 ± 3.34
50%	13.44 ± 2.51
60%	13.52 ± 2.24
70%	16.03 ± 3.57
80%	13.62 ± 2.42
90%	13.06 ± 2.02
Positive Control	32.26 ± 1.93
Negative Control	0.00 ± 0.00

The result of antibacterial activities of bidara leaf extract against methicillin-resistant Staphylococcus aureus have shown that the largest and smallest inhibition zone is produced at 70% concentration and 10% concentration, respectively. The diameter of the inhibition zone is measured by observing the transparent area where there is no bacterial growth (Fig. 2). All tests were performed in triplicate and then the mean of the 3 inhibition zone was calculated (Table 2).

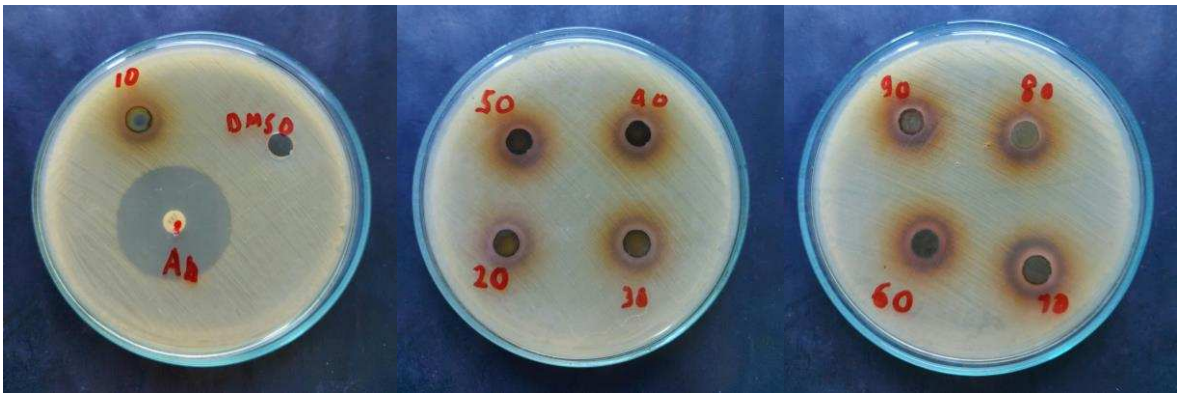

Fig. 2. The inhibition zone diameter (MRSA)

Note: Ab : Trimethoprim-Sulfamethoxazole disc (positive control)
DMSO : Dimethyl Sulfoxide (negative control)
10 : 10% concentration

4. Discussion

In this study, the inhibition zone produced against Staphylococcus aureus in positive control (23.67 ± 6.61 mm), negative control (0.00 ± 0.00 mm), 10% concentration (0.00 ± 0.00 mm), 20% concentration (9.09 ± 0.45 mm), 30% concentration (8.90 ± 0.06 mm), 40% concentration (9.58 ± 0.11 mm), 50% concentration (9.69 ± 0.52 mm), 60% concentration (9.98 ± 0.34 mm), 70% concentration (10.05 ± 0.92 mm), 80% concentration (9.35 ± 0.26 mm), 90% concentration (9.77 ± 0.69 mm). As a comparison, a study using bidara leaf extract with 20% DMSO as a solvent could form inhibition zone with diameter of 12 mm [13]. Other study conducted in Indonesia using bidara leaf extract and 96% ethanol as a solvent tested against Staphylococcus aureus produced inhibition zone diameter at 1% concentration (0.00 ± 0.00 mm), 10% concentration (0.92 ± 0.056 mm), 20% concentration (1.22 ± 0.021 mm), 30% concentration (1.36 ± 0.017 mm), and 40% concentration (1.68 ± 0.03 mm) [12].

In a study of bidara leaf extract conducted in Malaysia using hexane, chloroform, and methanol as a solvent, the diameter of inhibition zone against Staphylococcus aureus were 15 mm, 5 mm, and 9 mm, respectively [14]. Then, in a study using silver nitrate ($AgNO_3$) as a solvent with concentration of 100 g/ml bidara leaf extract, from a volume of 7 µl, 14 µl, and 28 µl, the diameter of inhibition zone produced against Staphylococcus aureus were 8 mm, 16 mm, and 28 mm, respectively [15]. Another study of bidara leaf extract using 80% methanol as a solvent tested against Staphylococcus aureus produced inhibition zone diameter at concentration of 400 mg/ml (7.25 ± 0.75 mm) and 200 mg/ml (6.50 ± 0.50 mm) [16]. Then, the study conducted in Tunisia using bidara leaf extract with 70% methanol as a solvent resulted in the inhibition zone diameter of 14.2 ± 0.27 mm against Staphylococcus aureus [10].

In this study, the inhibition zone produced against methicillin-resistant Staphylococcus aureus in positive control (32.26 ± 1.93 mm), negative control (0.00 ± 0.00 mm), 10% concentration (11.25 ± 1.85 mm), 20% concentration (12.83 ± 3.47 mm), 30% concentration (13.37 ± 3.17 mm), 40% concentration (14.42 ± 3.34 mm), 50% concentration (13.44 ± 2.51 mm), 60% concentration (13.52 ± 2.24 mm), 70% concentration (16.03 ± 3.57 mm), 80% concentration (13.62 ± 2.42 mm), 90% concentration (13.06 ± 2.02 mm). As a comparison, in a study in India, the ethanolic extract of bidara leaf tested against Staphylococcus aureus which was resistant to several antibiotics (one of its resistance was to the methicillin). From 13 resistant Staphylococcus aureus isolates, the inhibition zone diameter for bidara leaf extract ranged from 10.00-12.66 mm [17]. These results were quite different from this study, in this study almost all concentrations (except at a concentration of 10%) had larger diameter.

So, the results of this study which showed that bidara leaf extract has antibacterial activity against Staphylococcus aureus and MRSA are supported by the various studies mentioned above. The variety of antibacterial activity produced by other studies can be attributed to the the type of solvent used, the extraction procedure, type of media used, the incubation temperature and duration, the part of the plant used and the time it was taken, the size of the inoculum, the antibacterial activity test method, and the bacterial strain used [17].

5. Conclusion

Bidara leaf extract has antibacterial activity against Staphylococcus aureus and MRSA. The largest and smallest inhibition zone produced against both bacteria is seen at 70% concentration and 10% concentration of bidara leaf extract, respectively.

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