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Antibacterial Activity of Senggani (*Melastoma malabathricum* L.) Leaves Extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Abstract:- Ethanolic extract of senggani (*Melastoma malabathricum* L.) leaf contains secondary metabolites such as flavonoids, tannins, steroids, alkaloids, saponins, and triterpenoids. Flavonoids which are dominant among other compounds have shown antibacterial activity, especially in *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The research aims to determine the inhibition zone and minimum inhibition concentration (MIC) of the leaf extract against *P. aeruginosa* and *S. aureus*. The leaf extract is done by maceration utilizing 70 % ethanol (pa) and variation of concentrations viz. 5, 10, and 15 % were treated to *P. aeruginosa* and *S. aureus*. The positive control is ampicillin, and the negative control is the solvent (ethanol 70 %). The results show that the leaf extract at 15% shows the highest inhibition zone against *P. aeruginosa* and *S. aureus* at 0.63±0.18 and 1.48±0.47 cm^2, respectively. The MIC for *P. aeruginosa* is 15% and for *S. aureus* is 10%.

Keywords:- *Melastoma malabathricum, antibacterial activity, MIC, skin infection*

I. INTRODUCTION

Infectious disease is one of the critical diseases that has become a health problem in the world as well as in Indonesia [1]. One of the main causes of infectious diseases is bacteria [2]. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are a few examples of bacteria that mainly infect human skin [3, 4].

Indonesia is known as a megadiversity in terms of plants and some of them have been utilized as traditional medication [5]. One of the plants that have a potency for skin antibacterial activity is senggani (Indonesian, local name (Java); senduduk (Malay); malabar melastome (Singapore, British)) or *Melastoma malabathricum* L. [6, 7]. This plant has also been utilized ethnobotanically by the people in Indonesia as a traditional medicine to cure stomachache, skin infection, skin burn, cough, and toothache [7, 8]. Secondary metabolites were found in the plant leaves such as flavonoids, tannins, steroids, and saponins [9]. These metabolites are predicted to have antibacterial activity that is potentially to be studied further [10].

The occurrence of antibacterial compounds in the plant leaves is expected to inhibit the bacteria that cause skin infection, such as *P. aeruginosa* and *S. aureus*. It has been reported [11] that ethanolic 96% extract of the plant leaves inhibits *Streptococcus mutans* at a concentration of 5% with an inhibition zone of 8.81 mm, a concentration of 10% produces 9.48 mm; and a concentration of 15% with inhibition zone 9.03 mm. The research results of [9] reveal that the minimum inhibitory concentration (MIC) of ethanolic extract of the plant leaves against *E. coli* is 3% and *S. aureus* is 2%.

This present research needs to be done since there is no previous research held specifically in testing the secondary metabolites against skin infectious bacteria and it is critical to determine the antibacterial activity of the plant leaf ethanolic extract. The present research aims to determine the inhibition zone and MIC of the ethanolic extract of *Melastoma malabathricum* leaves against *P. aeruginosa* and *S. aureus*.

II. MATERIALS AND METHODS

A. Materials

Senggani (*M. malabathricum* L.) leaves are obtained from Bogor Agricultural Institute (BAI), Bogor, Indonesia. The chemical materials utilized such ethanol 70% (pa), Nutrient Agar (NA), Nutrient Broth (NB), ampicillin disc 10 mg Oxoid (control positive), aquadestilata, magnesium powder, amyl-alcohol, alcohol 70%, Fe Cl_3 1%, Wagner, Mayer, and Dragendorff reagents, acetate anhydrate, quercetin standard, $AlCl_3$, H_2SO_4, and Na acetate are purchased from bona fide vendor. *P. aeruginosa* and *S. aureus* pure cultures are obtained from Integrated Laboratory Services Universitas Gajah Mada (UGM), Yogyakarta, Indonesia.

The present research applied randomized complete design (RCD) with variations of treatments such as leaves extract concentrations i.e., 5, 10, and 15% with five replications and two test bacteria viz. *P. aeruginosa* and *S. aureus*.

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B. Research Procedures

➢ *Melastoma malabathricum Leaves Extraction*

Five kg of leaves is selected based on freshness, homogen sizes (12-15 x 6-8 cm), and green in color. Leaves are cleansed with water and drained; furthermore, the leaves are dried utilizing an oven for 24 hours at 50 °C to remove the excess water and maintain the chemical compounds in the leaves [12].

➢ *Leaves maceration processes*

Dried leaves are powdered utilizing a blender and are filtered with mesh #60; the fine powder is put in the container, weighed, and labeled [13, 14]. As much as 200 g of leaf powder is put into an Erlenmeyer and added with ethanol 70% (pa) with a ratio of 1:5, macerated for five days at 27 °C and shaken at 170 rpm in a shaker incubator JSR JSSI-300C [15]. The result from the maceration is then filtered to separate the residue from the filtrate and subsequently evaporated utilizing a rotary evaporator IKA RV06ML at 65 °C. The rendement yield is calculated with the equation [16]:

$$rendement\ yield = \frac{weight\ after\ extraction\ (g)}{weight\ before\ extraction (g)}\ x\ 100\%$$

➢ *Phytochemical analysis*

- *Qualitative flavonoid*

Leaves extract weighed as much as 1 mg and put into a test tube, diluted in 1 mL ethanol 70%. The sample is added with 5 drops of methanol 30% and then filtered, after that the filtrate is taken. Add the filtrate with 2 drops of concentrated H_2SO_4. The reaction is observed, and the positive reaction is indicated by the red, yellow, or pink color at the amyl alcohol layer [5, 17].

- *Qualitative tannins*

As much as 0.1 g leaf extract is put into a test tube, added with 10 mL aquadestilata, and then shaken. The sample is left for 5 minutes and then filtered. The filtrate is added with 5 drops of $FeCl_3$ 1% and is homogenized. The positive reaction is indicated with a blackish-green color [5, 17].

- *Qualitative saponin*

As much as 0.1 g leaf extract is put into a test tube and added with 10 mL aquadestilata. The extract is homogenized for 30 seconds. The positive reaction is indicated with foam on top of the sample and stands for several minutes with a thickness of about 1 cm [5, 17].

- *Qualitative alkaloids*

As much as 0.1 g leaves extract is put into a test tube, added with 5 mL chloroform and 3 drops of ammonia. The chloroform fraction is separated and then added with 2 drops of H_2SO_4 2M. The acid fraction is divided into three test tubes and each test tube is added with 2-3 drops of Mayer, Wagner, and Dragendorff reagent. The positive reaction for the Mayer reagent is indicated with white precipitate; the positive reaction for the Wagner reagent is brown precipitate, while for Dragendorff is red precipitate [5, 17].

- *Qualitative triterpenoids and steroids*

As much as 0.1 g leaves extract is put into a drop plate and added with 3 drops of acetate anhydrate and 1 drop of H_2SO_4. The positive reaction is indicated by red color for triterpenoids and green color for steroids [5, 17].

- *Quantitative flavonoids*

As much as 10 mg quercetin is weighed and diluted into 20 mL ethanol 70% (500 ppm, standard solution). The standard solution is then diluted into 375, 250, 125, and 50 ppm in 5 mL utilizing the formula [18]:

$$V_1N_1 = V_2N_2$$

Notes:
V_1= volume for solution 1
N_1= concentration for solution 1
V_2= volume for solution 2
N_2= concentration for solution 2

As much as 10 mg of leaf extract is weighed and diluted into 10 mL ethanol 70% and then stirred for 30 minutes and filtered. The test and standard solutions are taken at 0.5 mL each and then added with 1.5 mL ethanol, 0.1 mL $AlCl_3$ 10%, 0.1 mL Na acetate 1M, and 2.8 mL aquadestilata. Solutions are homogenized and incubated in a dark room for 30 minutes and then the absorbances are measured with a spectrophotometer Termo Scientific Genesys 10S UV-Vis at 415 nm wavelengths [13, 19]. Total flavonoid contents are calculated with the equation:

$$TFC = \frac{(R\ x\ N\ x\ V)}{W}$$

Notes:
TFC: Total flavonoid content (mg QE/gram sample)
R: concentration of flavonoid compounds (mg/mL)
N: dilution factor
V: volume of solvent used during extraction (mL)
W: weight of extract used (g)

➢ *Antibacterial activity assay*

- *Well diffusion method*

As much as 0.1 mL of each bacterial culture is inoculated on NA medium in a petri dish with a spread plate technique. Five wells are made in the NA medium with perforator number 3. Leaves extract with concentrations 5, 10, 15%, and ethanol 70% (negative control) each at 20 µL is put into the wells and then ampicillin disc 10 mg (positive control) is placed on the NA using a tweezer. All the petri dishes are incubated for 24 hours at 37 °C. The zone of inhibition area is measured and calculated utilizing the equation [2, 20]:

$$L = 3.14 \times \left[\left(\frac{d_2}{2}\right)^2 - \left(\frac{d_1}{2}\right)^2\right]$$

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Note:
d_1: well diameter (cm)
d_2: zone of inhibition diameter (cm)

- *MIC determination*

The MIC is determined using the dilution method. The leaves extract concentrations are 5, 10, and 15%, each concentration added with 1 mL of bacterial culture and then put into 1 mL NB in the test tubes [21]. The positive control used is ampicillin and the negative control is ethanol 70%. All test tubes are homogenized and then each test tube is taken as much as 100 µL and spread plated on NA in a petri dish. The petri dishes are incubated at 37 °C for 24 hours. The MIC is determined with no bacterial colony growth on the NA [21].

III. RESULTS AND DISCUSSIONS

A. Leaves Extraction

The leaves extract obtained is characterized by brownish black in color and thick paste with a total weight of 18.18 g from 200 g dry leaves powder. Thus, the extract rendement yield is 9.09%. Similar research utilizing ethanol 96% can gain 12.25% extract rendement yield from 61.28 g extract and 500 g dry leaves powder [22]. The difference between the two results might be caused by different solvents and the total number of leaves powder. Another plausible reason is the origin of the plant used in this research is from Bogor (Java Island), while [22] get the plant from Pangkal Pinang (Sumatera Island). The difference in place of growth can produce different plant yields and metabolites produced.

B. Phytochemical Analysis

➢ *Qualitative Analysis*

The result of qualitative phytochemical analysis is listed in Table 1.

Table 1. Qualitative phytochemical analysis of the leaf extract

Phytochemical Analysis	Standard [17]	Result	Reaction
Flavonoid	Red, yellow, or pink color is formed	Yellow color	+
Saponin	Constant foam is formed, ± 1 cm thick	Constant foam is formed, 1 cm thick	+
Tannin	A blackish-green color is formed	A blackish-green color is formed	+
Alkaloid a. Mayer b. Wagner c. Dragendorff	 White precipitate Brown precipitate Red precipitate	 White precipitate Brown precipitate Red precipitate is not formed	 + + -
Terpenoid	Brown color is formed	Brown layer color is formed	+
Steroid	Green color is formed	Green color is formed	+

Notes:
+ : metabolites present; - : metabolites absent

➢ *Quantitative Analysis*

Quantitative analysis of the ethanol extract of the plant leaves was carried out to determine the value of compounds in the samples using a standard solution, namely quercetin. The analysis was carried out using a spectrophotometric method with a wavelength of 415 nm. The variations of quercetin concentration applied were 500, 375, 250, 125 and 50 ppm, with a sample concentration of 1000 ppm. The result is presented in Figure 1.

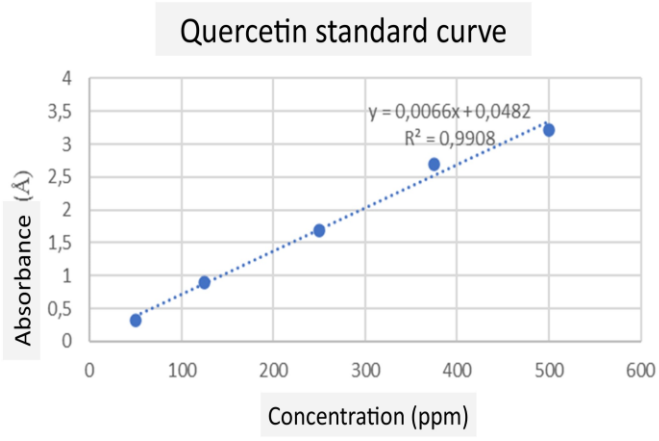

Fig 1. Quercetin standard curve from the leaves extract

With the standard equation y= 0.0066x + 0.0482 and R^2 value equal to 0.99 and the absorbance of flavonoid is 0.27, indicates that the value is inside the standard range. The flavonoid absorbance value as reported by [23] is 0.2-0.8. The flavonoid content of the leaf extract obtained from the present research is equal to 16.73 mg QE/g. Previous research [24] takes note that flavonoid concentrations in the plant leaves extract utilizing ethanol 96% was 4.40 mg QE/g. The difference between the results is due to the total number of the leaves powder and the solvent used. Ethanol 70% is considered a more polar solvent than absolute ethanol (96%), therefore flavonoids that are polar compounds tend to dissolve in a more polar solvent [25].

C. Antibacterial Analysis

➢ *Inhibition Zone*

The leaf extract of *M. malabathricum* shows antibacterial activities against *P. aeruginosa* and *S. aureus* (Table 2).

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Table 2. Inhibition zone of the leaf extract of *M. malabathricum*

Treatment	Inhibition Zone (cm^2) ± SD	
	P. aeruginosa	***S. aureus***
Extract 5%	0.12 ± 0.12^{d}	0.24 ± 0.24^{d}
Extract 10%	0.22 ± 0.16^{d}	0.63 ± 0.45^{c}
Extract 15%	0.63 ± 0.18^{c}	1.48 ± 0.47^{b}
Positive control (ampicillin)	0.16 ± 0.16^{d}	4.62 ± 0.11^{a}
Negative control (ethanol)	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}

Notes:

Numbers with the same alphabet indicate no significant difference at a level of confidence of 95%. The experiments were done in quintuplicates for all the treatments.

The leaves extract at 10% and 15% concentrations against *P. aeruginosa* show higher value of inhibition zones compared to positive control treatment (Table 2 and Figure 2). Similar research results reported that piper leaf extract generates a higher diameter of inhibition zone compared to a positive control (ampicillin), i.e., 29 mm to 10 mm [26]. These results are due to the resistance of *P. aeruginosa* to ampicillin as observed by [27]. The resistance of *P. aeruginosa* against ampicillin is caused by the ability of this bacterium to produce β-lactamase enzyme and the enzyme is known to be able to open the β-lactam ring at the ampicillin. Hence ampicillin has no or very low activity to inhibit the growth of *P. aeruginosa* [28, 29, 30].

However, *S. aureus* shows different results in that the bacterium is not resistant to ampicillin and obtains a higher inhibition zone compared to *P. aeruginosa*. This is in accordance with the finding of [4], who stated that Gram-positive bacteria generally have a higher sensitivity to antimicrobial compounds compared to Gram-negative bacteria. The difference in sensitivity of Gram-positive and Gram-negative bacteria to antimicrobials is influenced by differences in cell wall structure in the two groups of bacteria. *S. aureus* (Gram-positive) has thick cell walls consisting of peptidoglycan compared to *P. aeruginosa* (Gram-negative) which has a thin peptidoglycan layer. Even though *S. aureus* has thick peptidoglycan, this bacterium does not have the lipoproteins, outer membrane and lipopolysaccharides that *P. aeruginosa* has [2].

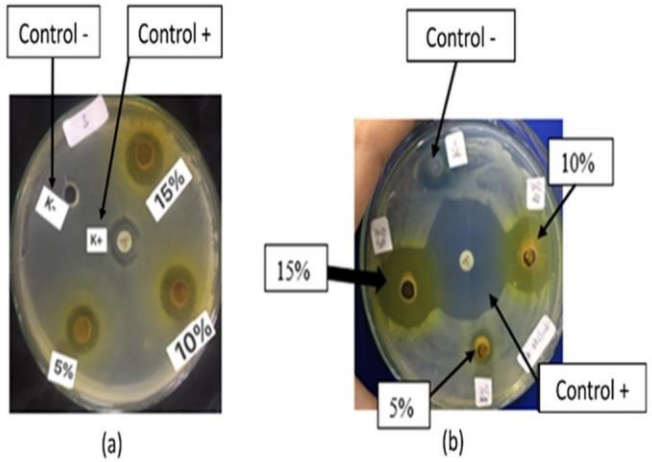

Fig 2. Inhibition zone of the leaf extract in various concentrations against *P. aeruginosa* (a) and *S. aureus* (b). Note: positive control is ampicillin disk and negative control is ethanol.

➢ *Minimum Inhibitory Concentration (MIC)*

The MIC of *M. malabathricum* leaf extract against *P. aeruginosa* and *S. aureus* is shown in Table 3, Figure 3, and Figure 4.

Table 3. The MIC of *M. malabathricum* leaf extract

Treatment	Bacterial Colony Growth	
	P. aeruginosa	*S. aureus*
Extract 5%	+	+
Extract 10%	+	-
Extract 15%	-	-
Ampicillin (Control +)	-	-
Aquadestilata (Control -)	+	+

Note:
+: bacterial colonies present
-: bacterial colonies absent

The MIC of *M. malabathricum* leaf extract to *P. aeruginosa* is at a concentration of 15% (Figure 3) and this result is consistent with the inhibition zone, which is at the same concentration the inhibition zone of the leaf extract to *P. aeruginosa* is the higher (Table 2). But at the concentrations of 5 and 10%, this bacterium still growth after 24-hour incubation period. The ability of *P. aeruginosa* to grow at concentration 10% is allegedly caused by β-lactamase enzyme produced by this bacterium [29].

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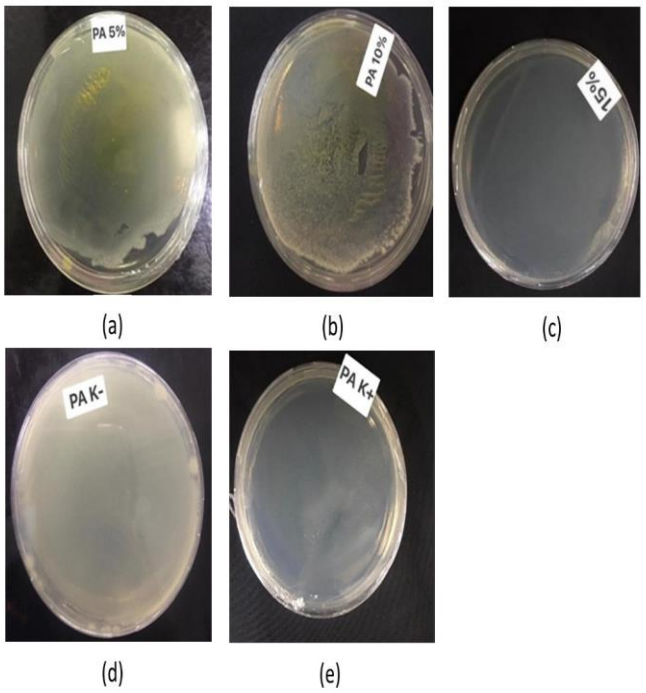

Fig 3. The MIC of the plant Extract at 5, 10, and 15% against *P. aeruginosa* compared to controls treatments. There is the growth of bacterial colonies (a, b, and d) and no growth of colonies (c and e). Note: 5% (a), 10% (b), 15% (c), Control negative, aquadestilata (d), and Control positive, ampicillin (e).

A group of researchers [31] reported that the MIC for *S. aureus* ranges from 25 to 50%, while in this present research, the MIC of *M. malabathricum* leaf extract is 10% (Table 3 and Figure 4). The difference between the results is due to the origin of the plant, the research design, and the solvent utilized. The previous research used methanol as the solvent and ethyl acetate fraction to extract the plant leaves. Methanol is considered more polar compared to ethanol, therefore it can dissolve polar compounds such as phenols. The phenols substances have many hydroxyls functional groups that show antibacterial activities [31].

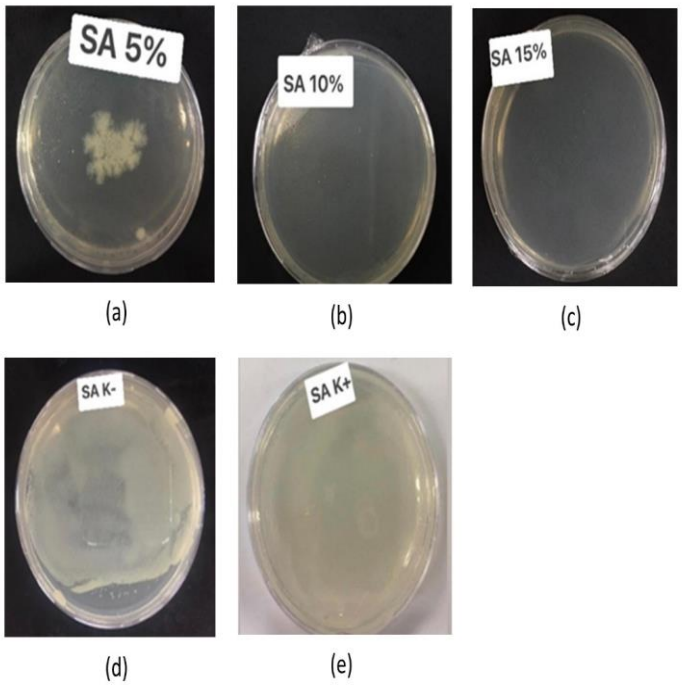

Fig 4. The MIC of the plant Extract at 5, 10, and 15% against *S. aureus* compared to controls treatments. There is growth of bacterial colonies (a and d) and no growth of colonies (b, c, and e). Note: 5% (a), 10% (b), 15% (c), Control negative, aquadestilata (d), and Control positive, ampicillin (e).

IV. CONCLUSIONS AND RECOMMENDATIONS

M. malabathricum leaf ethanolic extracts show the ability to inhibit *P. aeruginosa* and *S. aureus*. The MIC of the plant extracts against *P. aeruginosa* is 15% and *S. aureus* is 10%. Flavonoids are expected to be the active secondary metabolite against both of the bacteria. Therefore, it is recommended to do quantitative flavonoids utilizing GC-MS to disclose the exact values and concentrations of the plant leaf extracts.

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Conflict of Interest

The authors declare that there is no conflict of interest in publishing the research results.

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