Antimicrobial Potential Activity of Extract Selaginella plana (Desv. Ex Poir.) Hieron against the Growth of Staphylococcus aureus ATCC 25922 and Methicillin-Resistance Staphylococcus aureus (MRSA)

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Abstract: For thousands years, medicinal plants have been used as a source of powerful therapeutic agents, and until now, many medicines are used from natural products derived from plants or their derivatives. Plants that contain secondary metabolites can be used as antimicrobials; one of them is Selaginella plana. In this study, there were 8 treatments consisting of 6 treatments of extract concentration, 1 positive control (vancomycin), and 1 negative control (distilled water) with 3 replications. The antimicrobial test used was the Tube Dilution method using Mueller Hinton Broth to determine the MIC and Mueller Hinton Agar to determine the MBC. Selaginella plana extract showed inhibition against Staphylococcus aureus with MIC values of 12.5% and in MRSA with MIC value of 50%. In MBC test, the killing power of Selaginella plana extract against Staphylococcus aureus obtained MBC value of 12.5%. Meanwhile, MRSA bacteria showed negative results, which were indicated by the growth of colonies. Selaginella plana extract (Desv.ex Poir.) Hieron was able to show antimicrobial activity on Staphylococcus aureus with the MIC value of 12.5%, and the MBC value was negative.

1 INTRODUCTION

For thousands years, plants have been used as efficacious therapeutic agents, and until recently, many medicines are used from natural products from plants and its derivatives (Kinghorn et al., 2011; Newman and Cragg, 2012). Almost all ancient findings regarding medicines are sourced from natural ingredients (Quiason, 2011). WHO report in 2014 recorded that in 129 countries and 80% population, natural ingredients were used to meet treatment needs. Similarly, traditional medicines in China contributed approximately 18% of all treatments (WHO, 2014).

It was also discovered that more than a third of medicines (39.1%) authorized by the Food and Drug Administration (FDA) were sourced from natural ingredients (Boy et al., 2018). One of the continuously developed natural molecules is secondary metabolite substances, in which approximately 12,000 have been isolated, and the estimated number is less than 10% (Cowan, 1999).
In Indonesia, the utilization of plant-based medicines is a part of national cultivation and has been existing for centuries. However, its effectiveness and safety have not been supported by a comprehensive study (WHO, 2010). One of the biological resources in Indonesia is Selaginella Pal. Beauv (Selaginellaceae Reichb). Selaginella has been used as an alternative medicine in several traditional treatments, such as to cure injuries, skin diseases, cancers (Chen et al., 2005), anti-inflammation (Raj et al., 2006; Won et al., 2006), rheumatic, and as antimicrobes.

Plants with antimicrobial potential commonly have secondary metabolites. Selaginella has species-dependent molecular bioactivities, such as phenolic (flavonoid), alkaloid, and terpenoid contents. However, bioflavonoids (a dimeric form of flavonoids) are the key bioactive substances of Selaginella, consisting of 13 substances, particularly amentoflavone and ginkgetin (Setyawan, 2011). Antimicrobial substances can be used as a strategy to tackle health problems related to bacteria, fungi, and parasites.

According to (WHO, 2015), one of the current global problems is antimicrobial resistance threatening public health. Hence, the search for effective antimicrobial agents can help prevent and heal the patients. Antimicrobial agent from natural substances is one of the alternative treatments that is continuously developed. The antimicrobial agent is classified into six categories, namely biosynthesis, biological source, biological function, molecular properties, structure, composition, and molecular purpose (Castro-rosas et al., 2017).

From previous studies, it is discovered that only several species had been observed in detail, such as Selaginella uncinata (Zou et al., 2013b; Zou et al., 2014; Taylor et al., 2013; Zou et al., 2016b), Selaginella doederleitii (Li et al., 2016), Selaginella involvens (Long et al., 2015), Selaginella tamariscina (Xu et al., 2011a; Xu et al., 2011b; Xu et al., 2015ab), Selaginella moellendorfii (Zou et al., 2016a; Zeng et al., 2017; Zou et al., 2013a), and Selaginella willdenowii (Chai and Wong, 2012). Meanwhile, the most distributed Selaginella in Indonesia, i.e., Selaginella plana is yet to be observed further.

Based on the background, the authors were interested in conducting a study regarding the antimicrobial potential activity of Extract Selaginella plana (Desv. Ex Poir.) Hieron against the growth of Staphylococcus aureus ATCC 25922 and Methicillin-Resistance Staphylococcus aureus (MRSA). This study was conducted in the Pharmacology Laboratory, Faculty of Medicine, Universitas Airlangga and Medical Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga since 15 February 2020 to 14 March 2020.

2 MATERIAL AND METHODS

In this section, the authors explain the steps in testing the potential antimicrobial activity of Selaginella plana (Desv. ex Poir.) Hieron extract against the growth of Staphylococcus aureus ATCC 25922 and methicillin-resistance Staphylococcus aureus (MRSA). The solvent used in the extraction process was ethanol 96%. Antimicrobial materials consisted of Mueller Hinton Agar, Mueller Hinton broth, vancomycin, test bacteria Staphylococcus aureus ATCC 25922, methicillin-resistance Staphylococcus aureus (MRSA), suspension of 0.5 McFarland, and distilled water.

Equipment used were autoclave GEA FSF-24LDJ (Hahei, China), oven, refrigerator LG GN-B215SQMT (Taizhou, China), vortex GEMMY VM-300 (Taiwan), incubator Memmert UN 33 53L (Germany), vacuum rotary evaporator Heidolph VV 2000 (Nuremberg, Germany), digital scale FX-300i (Max 320 g), micropipettes, Bunsen, smear loops, and glass equipment such as test tubes, petri dish, Erlenmeyer flask, beaker glass, and volumetric pipettes.

2.2 Plant Extraction Preparation

In this study, Selaginella plana obtained from Kairatu Village, West Seram, Maluku Province, on 10 February 2020. As many as 1 kg of Selaginella plana leaves was washed using running water, dried under shades, chopped to pieces, and dried in the oven. Dried leaves were blended into powders of 600 g. The extraction process used a maceration method with ethanol 96% as a solvent. The 600 g powder was soaked in ethanol of 5400 ml while stirred for 24 hours. The top layer was taken using Whatman paper no. 41, and the soaking process was repeated for three times. The filtrate was dried in the rotary evaporator of 60°C until the ethanol solution was separated from the active substance.

The extract was weighed and calculated using the following formula: Extract % = dried mass / extract.
volume x 1000 ml. The maceration process resulted in *Selaginella plana* extracts of 70 g.

### 2.3 Antimicrobial Activity of the Plant Extracts

This study used Tube Dilution Test method. This method was utilized to determine the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration). The dilution method testing was carried out according to the recommendation of the Clinical and Laboratory Standards Institute for the determination of MIC and MBC.

#### 2.3.1 Bacterial Strain

The antimicrobial activity testing of *Selaginella plana* extracts used two bacteria strains, i.e., *Staphylococcus aureus* ATCC 25922 and methicillin-resistance *Staphylococcus aureus* (MRSA). The *Staphylococcus aureus* ATCC 25922 bacteria strain was obtained from the Health Laboratory Center Surabaya, and the methicillin-resistance *Staphylococcus aureus* (MRSA) bacteria strain was obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya.

#### 2.3.2 Preparation of Bacterial Suspension

The bacteria rejuvenation process used Mueller Hinton Agar. The incubation was carried out for 24 hours at the optimum temperature of 37°C. The bacteria suspension production used Mueller Hinton broth. One smear of microbes was put into 5 ml media in the test tube, vortexed, and adjusted to the standard of 0.5 McFarland (1.5 x 10^8 CFU/ml).

#### 2.3.3 Antimicrobial Activity Assay

There were 6 *Selaginella plana* (Desv. Ex Poir.) Hieron extracts’ concentrations including 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. One positive control used Vancomycin 30 mg, and 1 negative control used distilled water. The dilution process was conducted in stages, initiated by the treatment 1 (P1) group by putting 1 ml of 100% *Selaginella plana* extract into 1 ml of Mueller Hinton broth and vortexed them to be mixed. The treatment 2 (P2) group was made by putting 1 ml of 50% P1 solution into 1 ml of Mueller Hinton broth and vortexed them to be mixed. The same step was applied to P3 group of 25% sample concentration, the P4 group of 1.25%, the P5 group of 6.25%, and the P6 group of 3.125%. Each group was added with 1 ml of bacteria suspension (1.5 x 10^8 CFU/ml) and repeated three times. Incubations were carried out for 24 hours and 72 hours with a temperature of 37°C in incubators, which were then observed and compared with the positive and negative controls.

#### 2.3.4 Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is the minimum extract concentration to inhibit microbial growth after being incubated for 24 hours. The determination of Minimum Inhibitory Concentration (MIC) was conducted by taking all incubated treatment groups, vortexing each tube of different concentrations, and observing the smallest concentration to inhibit bacterial growth (visually marked by three observers) and determined as the MIC (Brantner and Grein, 1994; Chérigo et al., 2009).

#### 2.3.5 Determination of Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration (MBC) is the minimum concentration of test materials to kill bacteria, measured using the colony counter. The Minimum Bactericidal Concentration (MBC) was conducted by taking samples and smearing them to the Mueller Hinton agar and incubated at 37°C for 24 hours. It was then determined for the smallest concentration where microbial colonies stopped growing on the media.

The colony growth on the Mueller Hinton agar was declared with: (-) if more than 10 colonies were obtained on the Petri dish, (+) if less than 10 colonies were obtained on the Petri dish, and if colonies were grouping, it was counted as one colony.

### 3 RESULTS

#### 3.1 Extraction

*Selaginella plana* with a wet weight of 1 kg was dried to obtain a dry weight of 600 grams. *Selaginella plana* was then extracted with a maceration method using ethanol 96% solvent. Extracts from the maceration process were 70 grams.

#### 3.2 Minimum Inhibitory Concentration (MIC) of the Plant Extract

The microbial activity test was conducted using broth dilution method. Concentrations used were 100%,
50%, 25%, 12.5%, 6.25%, and 3.125%. The negative control was distilled water, while the positive control was vancomycin.

The test results of Minimum Inhibitory Concentration (MIC) of Selaginella plana (desv. ex poir.) Hieron extracts are presented in Table 1. The results of antimicrobial activity testing showed turbidity differences on different concentration levels. Therefore, the Minimum Inhibitory Concentration (MIC) on a particular concentration was determined.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Replication</th>
<th>Test Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25922</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>methicillin-resistant Staphylococcus aureus (MRSA)</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 1: The Minimum Inhibitory Concentration (MIC) of Staphylococcus aureus ATCC 25922.

Figure 2: The Minimum Inhibitory Concentration (MIC) of methicillin-resistant Staphylococcus aureus (MRSA).

The testing of Selaginella plana extract on Staphylococcus aureus ATCC 25922 bacteria was conducted on different concentrations, i.e., 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. The results in Table 1 present that the first (100%), second (50%), third (25%), and fourth (12.5%) tubes showed no turbidity. Therefore, the fourth (12.5%) tube was determined as the Minimum Inhibitory Concentration. On the positive control tube with vancomycin, no turbidity presented. Meanwhile, the negative control tube with distilled water showed turbidity (Figure 1).

The testing for MIC was also applied to the methicillin-resistance Staphylococcus aureus (MRSA) bacteria with the same concentration of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. The results in Table 1 show that the 100% and 50% concentrations had abilities to inhibit MRSA’s growth, marked by no turbidity in tubes. Therefore, the 50% concentration was considered as the MIC. However, the inhibitory potential of Selaginella plana extract was considered weak because the lower concentrations of 25%, 12.5%, 6.25%, and 3.125% showed turbidity and thick lumps. The positive control tube with vancomycin showed no turbidity, and the negative control tube with distilled water showed turbidity (Figure 2).

However, due to the incomplete screening of Selaginella plana extraction results, it may leave dregs that pose bias in determining the MIC. Therefore, the microbes’ growth inhibition was also tested using selective growth media for each microbe. It aimed to confirm the presence or absence of microbes’ growth in a particular concentration showing the Minimum Inhibitory Concentration (MIC). The result obtained was determined as the Minimum Bactericidal Concentration (MBC).
3.3 **The Minimum Bactericidal Concentration (MBC) of the Plant Extract**

The test results of Minimum Bactericidal Concentration (MBC) of *Selaginella plana* (desv.ex poir.) Hieron extracts are presented in Table 2.

The Minimum Bactericidal Concentration (MBC) test to *Staphylococcus aureus* ATCC 25922 bacteria on the concentrations of 100%, 50%, 25%, and 12.5% showed positive results, marked with zero growth of *Staphylococcus aureus* ATCC 25922 colony on all test concentrations (Figure 3).

The Minimum Bactericidal Concentration (MBC) test to MRSA bacteria on the concentrations of 100% and 50% showed negative results, marked with MRSA colony growth on all concentrations. It shows that *Selaginella plana* extracts are incapable to kill MRSA (Figure 4).

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Replication</th>
<th>Test Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 25922</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>methicillin-resistant <em>Staphylococcus aureus</em> (MRSA)</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Minimum Bactericidal Concentration of *Selaginella plana*.

**DISCUSSION**

4.1 **Minimum Inhibitory Concentration (MIC) of *Selaginella plana***

*Staphylococcus aureus* and methicillin-resistance *Staphylococcus aureus* (MRSA) bacteria are pathogenic microorganisms commonly infecting humans, and many researchers suggested that these microbes are resistant to medicines (Kumar, 2016; Passàli et al., 2007; Ksiezopolska, 2018; Onanuga, 2011). It was discovered that secondary metabolite substances in plants play a vital role as antimicrobial, especially phenolic (Geechev et al., 2014).

On the MRSA bacteria, the MIC test showed positive results, marked by the absence of turbidity in a high concentration. The previous study discovered that a flavonoid substance of 7-O-Butyl Naringenin had activity against MRSA strains on a lower MIC than natural flavonoids (Lee et al., 2013a). Glabrol elements in flavonoids disturb membrane potentials and permeability, hence, potential to be used as an anti-microbe against MRSA (Wu et al., 2019).

The study by (Cao et al., 2010b) on active substances in *Selaginella pulvinata* have good and significant inhibitory activity against *Staphylococcus aureus* with a MIC value of 9.6 μg/ml. According to (Zou et al., 2016a), flavonoid compounds can inhibit *S.aureus* growth with a MIC value of 12.5 μg/ml.

Flavonoids ability as anti-microbes depends on the aromatic ring structure (Xie et al., 2014).
Flavonoid activities disturb membrane integrity due to an interaction with phospholipids that change the membrane protein’s structure and function, adhere to the membrane’s hydrophobic and hydrophilic sides, and cause dysfunction of plasma membrane’s works (Górniak et al., 2019). It also causes cell agglutination (Babii et al., 2016), energy metabolism disruption, nucleic acid synthesis, coenzyme metabolism, and cell leaking (Cushnie and Lamb, 2011).

Bacteria used in this study were gram-positive bacteria, i.e., *Staphylococcus aureus* and methicillin-resistance *Staphylococcus aureus* (MRSA), which are also influenced by flavonoids. The reason is because positive gram bacteria cell’s walls contain a high amount of peptidoglycans. On the outer cell part, phosphate groups contain ≥ 80% negative charges (Cha et al., 2006). It causes interaction between negative and positive charges on the carbon atom of 1,3-dithiolium flavonoid ring (Bahrin et al., 2014). As a result, an intracellular leak. Another study also found that a particular dosage of saponins was effective in damaging *Staphylococcus aureus* cell walls (Khan et al., 2018). However, terpenoids do not pose activities on *Staphylococcus aureus*, while on methicillin-resistance *Staphylococcus aureus* (MRSA), terpenoids posed activities as anti-MRSA, although not effective as standard medicines (Nzogong et al., 2018).

### 4.2 Minimum Bactericidal Concentration (MBC) of *Selaginella plana*

The MBC value test in Table 2 shows that *Selaginella plana* extracts on the concentrations of 100%, 50%, 25%, 12.5%, and 6.25% had positive results against *Staphylococcus aureus*.

Different results were presented by methicillin-resistance *Staphylococcus aureus* (MRSA) bacteria. *Selaginella plana* extracts had no ability as bactericidal, marked by bacteria colony growth on Petri dishes. Phytochemical substances such as tannin and polyphenol are major contributors to inhibit methicillin-resistance *Staphylococcus aureus* (MRSA) bacteria. Therefore, these substances’ absence affects the non-synergized multi-target effects against methicillin-resistance *Staphylococcus aureus* (MRSA) bacteria (Chew et al., 2018). The mixture of constituents may act on several antibacterial targets concurrently, i.e. depolarizing the cell membrane, inhibiting the efflux pump, disintegrating the genetic materials (Coutinho et al., 2009; Effert and Koch, 2010).

A study conducted by (Chew et al., 2018) found that tannins in plants could contribute to MRSA inhibitory activity. The potency of the phytochemical compound can be increased if it is combined with other medicines since it has different targets in MRSA. Phytochemical compounds can change the permeability of the outer cell membrane, inhibit the efflux pump, change the active site, and β-lactamase inhibitors (Kubo et al., 2003).

Multi-target effects of phytochemical substances are known to act as anti-MRSA by depolarizing cell membranes, inhibiting efflux pumps, and damaging genetic materials (Coutinho et al., 2009; Effert and Koch, 2010). Methicillin-resistant *Staphylococcus aureus* (MRSA) resistance towards extracts is caused by mucosa layer thickness surrounding cell walls. The cell wall layer produced by resistant isolates is thicker than the sensitive walls of strains (Amira, 2016). It was caused by the decreased *penicillin-binding proteins* (PBP) activity affecting the cross-link in peptidoglycan and an increase in gene expression related to cell wall synthesis caused an increase in the production of teichoic acid in the cell wall (Garcia, et al., 2017).

A quantitative study against the inhibitory of phytochemical compounds needs to be conducted in determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) by observing the Optical Density (OD) value in each tested concentration and further investigation is conducted against the specific phytochemical compound in preventing, inhibiting, and degrading the biofilm growth in each microbe.

### 5 CONCLUSION

Based on the study results, conclusions can be drawn as follow:

1. *Selaginella plana* (Desv.ex Poir.) Hieron extracts have the potential as anti-microbes on the Minimum Inhibitory Concentration (MIC) test with the concentrations of 100%, 50%, 25%, and 12.5% could inhibit *Staphylococcus aureus*’ growth, and the concentrations of 100% and 50% can inhibit MRSA’s growth.

2. *Selaginella plana* (Desv.ex Poir.) Hieron extracts have the potential as bactericidal on the Minimum Bactericidal Concentration (MBC) test with the concentrations of 100%, 50%, 25%, and 12.5% can kill *Staphylococcus aureus*’ growth. However, the results are negative against MRSA with colony growth on the concentrations of 100% and 50%.
REFERENCES


uncinata. (October), 37–41.
https://doi.org/10.1080/10286020.2012.745515

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