Biofarmasetikal Tropis
(The Tropical Journal of Biopharmaceutical) 2025, 8(1), 50-56
e-ISSN 2685-3167
p-ISSN 2828-6685

Antibacterial Activity Test of *Scleria sumatrensis* Leaf Extract *against Staphylococcus* aureus in Vitro

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Accepted: 21 January 2025 ; Approved: 10 April 2025

ABSTRAK

Bakteri Staphylococcus aureus merupakan salah satu patogen penting yang dapat menimbulkan berbagai jenis infeksi, mulai dari yang ringan seperti folikulitis dan impetigo hingga yang dapat mengancam jiwa seperti pneumonia nosokomial, osteomielitis, endokarditis, sepsis, dan sindrom syok toksik. Karena kemampuannya menimbulkan resistensi terhadap antibiotik, diperlukan alternatif pengobatan baru, salah satunya dari bahan alam. Pada penelitian ini, aktivitas antibakteri ekstrak Scleria sumatrensis diuji terhadap Staphylococcus aureus dengan variasi konsentrasi 100 – 500 µg/cakram. Hasil penelitian menunjukkan bahwa pada konsentrasi 100 µg/cakram, ekstrak memberikan aktivitas antibakteri dalam kategori sedang, sedangkan pada konsentrasi 200 sampai 500 µg/cakram, aktivitas yang dihasilkan termasuk dalam kategori kuat. Temuan ini menunjukkan bahwa ekstrak Scleria sumatrensis berpotensi sebagai agen antibakteri alami terhadap Staphylococcus aureus, sehingga layak dikembangkan lebih lanjut sebagai terapi alternatif untuk infeksi yang disebabkan oleh bakteri S. aureus.

Kata kunci: *Scleria sumatrensis, Staphylococcus aureus, antibakteri, Ekstraksi*

ABSTRACT

Staphylococcus aureus bacteria is one of the important pathogens that can cause various types of infections, ranging from mild ones such as folliculitis and impetigo to life-threatening conditions such as nosocomial pneumonia, osteomyelitis, endocarditis, sepsis, and toxic shock syndrome. Due to its ability to develop resistance to antibiotics, new alternative treatments are needed, one of which is from natural ingredients. In this study, the antibacterial activity of Scleria sumatrensis extract was tested against Staphylococcus aureus with a concentration variation of 100 - 500 µg / disc. The results showed that at a concentration of 100 µg / disc, the extract provided antibacterial activity in the moderate category, while at a concentration of 200 to 500 µg / disc, the activity produced was included in the strong category. These findings indicate that Scleria sumatrensis extract has the potential as a natural antibacterial agent against Staphylococcus aureus, so it is worthy of further development as an alternative therapy for infections caused by S. aureus bacteria.

Keywords: *Scleria sumatrensis, Staphylococcus aureus, antibacterial, Extraction*

1. INTRODUCTION

Staphylococcus aureus is a Gram-positive coccus-shaped bacterium that lives as normal flora on the skin and upper respiratory tract of humans, but also has the potential to become an opportunistic pathogen[1]. This bacterium is known to cause various types of infections ranging from mild ones such as folliculitis and impetigo to life-threatening conditions such as nosocomial pneumonia, osteomyelitis, endocarditis, sepsis, and toxic shock syndrome[2]. Its existence as one of the main causes of nosocomial and community infections makes *S.*

aureus an important concern in the medical world[3].

Based on global data from WHO and national surveillance in Indonesia, *Staphylococcus aureus* is one of the top five pathogens causing nosocomial infections, with an incidence rate of around 20–30% of all hospital infection cases[4]. In several referral health centers in Indonesia, isolation of antibiotic-resistant *S. aureus, especially* MRSA (*Methicillin-Resistant Staphylococcus aureus*), has increased sharply in the last decade. Studies show that more than 40% of *S. aureus* isolates in large hospitals in Indonesia are resistant to beta-lactam antibiotics, some of which even show multiclass resistance (MDR: Multidrug Resistant)[5].

The increasing incidence of antimicrobial resistance has limited effective therapeutic options, and increased morbidity, mortality, and medical costs[6]. In addition, the ability of *S. aureus* to form biofilms on the surfaces of medical devices such as vascular catheters and cardiac prostheses makes eradication of infections even more difficult. This condition encourages the need for new antibacterial agents with different mechanisms of action and lower risk of resistance[7].

One promising approach is the utilization of natural compounds from medicinal plants as an alternative source of antibacterials. *Scleria sumatrensis* (Retz.), a plant native to Southeast Asia including Indonesia, has the potential as a source of bioactive compounds. Although it has not been widely studied, initial phytochemical tests indicate the presence of flavonoids, tannins, alkaloids, and phenolic compounds that are known to have antimicrobial activity[8]. Therefore, testing the antibacterial activity of *Scleria sumatrensis* extract against *Staphylococcus aureus*, especially can provide important information in the development of alternative therapies for bacterial infections that are increasingly difficult to overcome.

2. RESEARCH METHOD

Place and Time of Research

This research was conducted in the microbiology laboratory of the Faculty of Mathematics and Natural Sciences, Christian University of Indonesia Tomohon. The research was conducted in November-December 2024.

Tools and Materials

The tools used in antibacterial testing are gloves, scales, scissors, knives, jars, measuring cups, Erlenmeyer flasks, beakers/chemical glasses, test tubes, filter paper, funnels, pipettes, spatulas, stirring rods, aluminum foil, rotary evaporators, laminar air flow, autoclaves, micropipettes, 10 mm petri dishes, tweezers, microtubes, refrigerators, calipers, ose needles, vials, 8 mm advantec paper discs, McFarland standard set R092-1NO cameras, incubators, Bunsen lamps.

The materials used in antibacterial research are: *S. sumatrensis* plant leaves, Staphylococcus aureus bacteria, 96% ethanol, nutrient agar (Himedia M001-500g), nutrient broth (Himedia M002-500g), distilled water, chloramphenicol, 70% alcohol.

Sampling

S. sumatrensis leaves taken in Kiawa Dua Village, Minahasa Regency, were washed clean, drained, and then cut into small pieces to facilitate the process of extracting active substances during extraction. Then after that, they were put into a vessel containing 96% ethanol solvent and covered with aluminum foil. The sample was then extracted using the maceration method for 2x 24 hours, repeated 2x (2x 24 hours).

Making *S. sumatrensis* Extract

First, *S. Sumatrensis* was weighed as much as 450 grams and extracted using the maceration method for 2x 24 hours. The soaked sample was then filtered using filter paper and a funnel to produce 1 filtrate and 1 residue. After that, residue 1 was soaked again with 96% ethanol until it was completely soaked and macerated again for 2x 24 hours. The same method was repeated until 2 filtrates and 2 residues were obtained, then all filtrates were combined into one. The filtrate was evaporated at a temperature of 40 ° C. to produce a thick extract of *S. sumatrensis* and put into a tube, then weighed and stored in the refrigerator[9].

Antibacterial Test Using the Kirby Bauer Method

Antibacterial testing was carried out in vitro using the agar diffusion method using disc paper against *Staphylococcus aureus* using five concentration variants[10]. This test was carried out using the *S. Sumatrensis* extract compound.

Antibacterial Test Procedure

In this study, 50 mg of *S. sumatrensis* extract was weighed and dissolved in 500 µL of 70% alcohol solution as a standard solution, then five concentration variants were made, namely 100 - 500 µg / disc, as well as a negative control of 10 µL / disc and a positive control using chloramphenicol 2 µg / disc. *S. sumatrensis* extract was spotted onto disc paper and left in a desiccator for 1 × 24 hours. Next, mix 1 mL of bacterial suspension into 90 mL of sterile agar media, then pour 30 mL for each petri dish, then the disc paper was attached to the solid media that had been filled with bacteria in the petri dish and incubated at a temperature of 370 C for 1 × 24 hours[11].

Preparation of Test Bacterial Suspension

Staphylococcus aureus bacteria were grown in liquid media 1.3 grams in 100 mL of distilled water. Furthermore, the bacterial culture was incubated at room temperature 37℃.

Making Positive and Negative Controls

The positive control solution used was chloramphenicol with a concentration of 2 µg/disc. This solution was made by dissolving 5 mg into 5 mL of distilled water. For negative control, 70% alcohol was used as much as 10 µL/disc[12].

Sterilization of Tools

Sterilization is carried out during the media manufacturing process to prevent the spread of microorganisms so that contamination does not occur. The tools used in the study were sterilized for 15-20 minutes at a temperature of 121°C[13].

Media Preparation

For *Staphylococcus aureus* bacteria, weigh 2.52 nutrient agar in 90 ml of distilled water. Then add 0.5 ml of nutrient broth into the container. Then shake until homogeneous, then sterilize using an autoclave for 30 minutes at a temperature of 121℃.

How to calculate the inhibition zone

Calculation of the diameter of the inhibition zone[11].

$$Formula : D = \frac{A + B + C}{3}$$

Information:
D = average inhibition zone diameter
A= vertical diameter
B= horizontal diameter
C= diagonal diameter

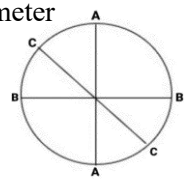

Table 1. Antibacterial Categories[11].

Inhibition Zone Diameter(mm)	Antibacterial Activity
<5	Very Weak
5-10	Medium
10-20	Strong
≥20	Very Strong

Data Analysis

Antibacterial data analysis was carried out by measuring the inhibition zone in each petri dish and then tabulated. Observations were made for 1×24 hours. Measurement of the diameter of the inhibition zone using a vernier caliper. The data obtained were analyzed descriptively in the form of tables and images.

3. RESULTS AND DISCUSSION

***S. sumatrensis* Leaf Extraction Process**

S. sumatrensis leaves are first cleaned with running water before being extracted and then cut into small pieces, this aims to ensure that the surface area of the sample is in direct contact with the solvent during the extraction process. In the process of extracting S. Sumatrensis leaves, the maceration method is used. The maceration method was chosen because the process is simple and does not require sophisticated equipment. The maceration method does not require a heating process that can damage the active substances contained in the sample. In the maceration process, the sample soaked in ethanol will break down the cell wall due to the difference in pressure inside and outside the cell, so that the secondary metabolites in the cytoplasm dissolve in organic solvents such as ethanol and the extraction of the compound will be perfect. The maceration process is carried out 2x 24 hours with two remacerations or replacement of new solvents which aim to ensure

that the compounds in the sample can be completely extracted. The solvent used in the extraction process is 96% ethanol. Ethanol solvents have the ability to bind polar, semi-polar and non-polar compounds[14]. The maceration results are evaporated with a rotary evaporator at a temperature of 40 ^{0}C. A temperature of 40 0C is a lower temperature than the boiling point of the solvent. Ethanol, which ranges from 60-80 ^{o}C. The use of lower temperatures allows the solvent to evaporate more slowly and more selectively, thereby reducing damage to the extracted compounds[15]. The extract obtained from the evaporation process with a rotary evaporator was 13.45 grams green in color. The extract was then stored in a refrigerator at -10 ^{0}C, this aims to avoid denaturation of bioactive compounds contained in the extract.

Antibacterial Activity Test of Staphylococcus aureus

In this study, a test was conducted on *Staphylococcus aureus* bacteria. This was done with the aim of determining the potential of *S. Sumatrensis* extract in inhibiting bacterial growth by using five concentration variants and two controls. The positive control used was Chloramphenicol with a concentration of 2 µg/disc. The negative control used a paper disc that had been spotted with 70% alcohol as much as 10 µg/disc, but before testing, the paper disc that had been spotted with the extract as well as the positive and negative controls were evaporated using a desiccator. This aims to evaporate the alcohol used when dissolving the extract and negative control so as not to affect antibacterial activity.

Table 2. Test Inhibition Zone Values *Staphylococcus aureus*

Extract Concentration	PI	PII	Average
100	9.34	9.41	9.37
200	10.68	10.75	10.71
300	12.46	11.33	11.89
400	13.72	12.39	13.05
500	14.67	13.65	14.16
Positive control	31.12	31.12	31.12
Negative control	00.00	00.00	00.00

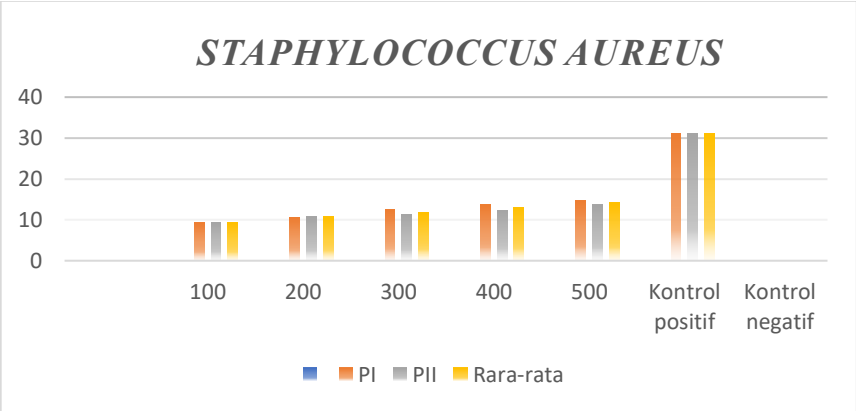

Figure 1. Average Graph of Bacterial Inhibition Zone from *S. sumatrensis* Extract

e-ISSN 2685-3167
p-ISSN 2828-6685

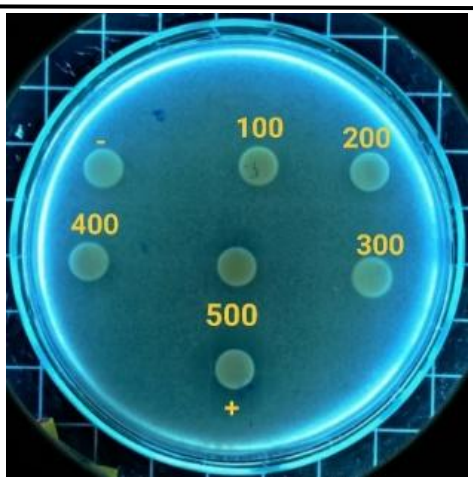

Figure 2. Inhibition zone of extract *S. sumatrensis*

Table 2 shows the results of the activity test of Staphylococcus aureus bacteria, which is a gram-positive bacteria. The results showed antibacterial activity with a moderate category at a concentration of 100 µg/disc, and had antibacterial activity with a strong category at a concentration of 200 to 500 µg/disc. Wiraswati et al's research shows that S. Sumatrensis extract contains secondary metabolite compounds such as phenolics, flavonoids, alkaloids, tannins, steroids and terpenoids[8].

Molecularly, phenolic compounds inhibit bacterial growth by interacting directly with the bacterial cell membrane, damaging the lipid bilayer structure through the surfactant effect. This interaction causes leakage of ions (such as K^+ and H^+), nucleotides, and important cytoplasmic proteins, thus disrupting cell homeostasis and ultimately causing lysis[16]. In addition, some phenolics can inhibit key enzymes in bacterial metabolism such as dehydrogenase and ATP synthase enzymes[17]. Some compounds also reduce the expression of virulence genes such as toxin-encoding genes (eg α-hemolysin in *S. aureus*), thereby reducing bacterial pathogenicity. Flavonoid compounds work through various mechanisms[18]. One of the most significant is the inhibition of the DNA gyrase enzyme (subunits A and B), which interferes with bacterial DNA replication and stops cell proliferation[19]. Flavonoids also interfere with protein synthesis by binding to the 30S or 50S ribosomal subunit, inhibiting mRNA-tRNA translocation[20]. Alkaloid compounds themselves have a high affinity for nucleic acids (DNA/RNA) and enzymatic proteins in bacterial cells. Some alkaloids, such as berberine, bind to DNA through intercalation, disrupting replication and transcription. Alkaloids also inhibit topoisomerase enzymes and metabolic enzymes such as enolase and pyruvate kinase, thereby affecting energy production (glycolysis)[21]. Tannin compounds precipitate enzymatic and structural proteins on the surface of bacterial cells through the formation of tannin-protein complexes. This process interferes with the function of adhesin proteins and hydrolytic enzymes such as proteases and lipases required for tissue colonization and invasion. Tannins also inhibit the expression of virulence genes responsible for biofilm formation and toxin production. In *S. aureus*, tannins can reduce the expression of spa genes (which encode protein A) and hla (α-hemolysin)[22]. Natural steroids such as steroid saponins interact with sterols in the bacterial cell membrane. This interaction forms transmembrane pores (pore-forming), causing the loss of ions and intracellular materials. At the molecular level, steroids also interfere with the synthesis of long-chain fatty acids and cell wall biosynthesis. Some steroids also inhibit active transport systems and respiratory enzymes such as NADH dehydrogenase[23]. Volatile terpenoids such as thymol and carvacrol interact with membrane lipids through hydrophobic effects, causing destabilization of membrane structure and increased permeability. At the molecular level, they interfere with the function of membrane proteins such as ATPase enzymes and ion pumps, inhibiting energy production and causing osmotic imbalance. Terpenoids also inhibit cell wall synthesis by interfering with transglucosylase and transpeptidase enzymes. Some terpenoids are even able to inhibit the quorum sensing system in *S. aureu*s biofilms[24].

e-ISSN 2685-3167
p-ISSN 2828-6685

4. CONCLUSION

The results of this study indicate that *Scleria sumatrensis* Retz. Leaf Extract has antibacterial activity against *Sthaphylococcus aureus* bacteria at a concentration of 100 - 500 µg / disc. And the inhibitory concentration that falls into the strong category is at 200-500 µg / disc. Further research is needed to characterize compounds that have potential antibacterial activity.

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