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Inhibitory Test of *Scleria sumatrensis* Stem Extract against *Propionibacterium acnes* Bacteria in Vitro

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ABSTRAK

Penelitian ini bertujuan untuk menguji aktivitas antibakteri ekstrak Scleria sumatrensis terhadap Propionibacterium acnes , bakteri penyebab jerawat. Ekstraksi dilakukan dengan metode maserasi menggunakan etanol 96%, dan uji aktivitas antibakteri dilakukan dengan metode difusi menggunakan konsentrasi 100–500 µg/disc. Hasil menunjukkan bahwa ekstrak memberikan aktivitas antibakteri kategori sedang pada konsentrasi 100 µg/disc dan meningkat menjadi kuat pada konsentrasi 200–500 µg/disc. Ekstrak S. sumatrensis mengandung senyawa metabolit sekunder seperti fenolik, flavonoid, alkaloid, tanin, steroid, dan terpenoid yang berperan dalam menghambat pertumbuhan P. acnes melalui berbagai mekanisme, termasuk kerusakan membran sel, penghambatan sintesis protein, serta gangguan metabolisme bakteri. Berdasarkan hasil penelitian, ekstrak S. sumatrensis berpotensi sebagai alternatif antibakteri alami untuk pengobatan jerawat tanpa efek samping signifikan atau risiko resistensi antibiotik.

Kata kunci: *Scleria sumatrensis*, *Propionibacterium acnes*, *Antibakteri, Jerawat, Senyawa metabolit sekunder.*

ABSTRACT

This study aims to test the antibacterial activity of Scleria sumatrensis extract against Propionibacterium acnes, a bacteria that causes acne. Extraction was carried out using the maceration method using 96% ethanol, and the antibacterial activity test was carried out using the diffusion method using a concentration of 100–500 µg/disc. The results showed that the extract provided moderate antibacterial activity at a concentration of 100 µg/disc and increased to strong at a concentration of 200–500 µg/disc. S. sumatrensis extract contains secondary metabolite compounds such as phenolics, flavonoids, alkaloids, tannins, steroids, and terpenoids that play a role in inhibiting the growth of P. acnes through various mechanisms, including cell membrane damage, inhibition of protein synthesis, and disruption of bacterial metabolism. Based on the results of the study, S. sumatrensis extract has the potential as a natural antibacterial alternative for acne treatment without significant side effects or the risk of antibiotic resistance.

Keywords: *Scleria sumatrensis, Propionibacterium acnes, Antibacterial, Acne, Secondary metabolite compounds*

1. INTRODUCTION

Acne or acne vulgaris is one of the most common skin problems worldwide and is a major cause of dermatological morbidity, especially in adolescents to young adults[1]. The prevalence of acne is reported to reach more than 80% at the age of 12-24 years in various countries, including Indonesia[2]. One of the main pathogenetic factors in the development of acne is the role of *Propionibacterium acnes* bacteria, which plays a role in triggering inflammatory responses and the formation of acne lesions through its activity in breaking down triglycerides in the sebaceous glands[3]. The use of synthetic antibacterial agents to treat acne is

increasingly limited due to increasing antibiotic resistance and long-term side effects. Therefore, the development of natural antibacterial alternatives from plants has great potential as an alternative solution. *Scleria sumatrensis* (Retz.), which is a herbaceous plant from the Cyperaceae family, is known to contain phytochemical compounds such as flavonoids, tannins, and saponins that have the potential to show antibacterial activity4. Several early studies have shown that extracts of similar plants have inhibitory effects on microbial growth, including *P. acnes*. However, information regarding the specific antibacterial activity of *Scleria sumatrensis* against *P. acnes* is still minimal. Therefore, it is necessary to conduct an in vitro inhibition test of *Scleria sumatrensis* extract against *Propionibacterium acnes* bacteria to evaluate its potential as a natural antimicrobial agent in acne management. Considering the high prevalence of acne in the community and the increasing resistance of bacteria to synthetic antibiotics, this study presents an initial effort to explore the pharmacological potential of *Scleria sumatrensis* as a natural antibacterial agent against *P. acnes*. The in vitro approach used will also provide important baseline data for the development of herbal-based anti-acne cosmetic or drug formulations in the future.

2. RESEARCH METHODS

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 study was conducted in September-November 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, *Propionibacterium acnes* 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).

Preparation of S. Sumatresis 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 submerged 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 a refrigerator[5].

Antibacterial Test Using the *Kirby Bauer* Method

Antibacterial testing was conducted in vitro using the agar diffusion method using disc paper against *Propionibacterium acnes* using five concentration variants[6]. This test was conducted using the *S. Sumatrensis* extract compound.

Antibacterial Test Procedure

In this study, *S. Sumatrensis* extract was weighed as much as 50 mg and then 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 negative control 10 µL / disc and 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

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petri dish and incubated at a temperature of 37^0C for 1 × 24 hours[6].

Preparation of Test Bacterial Suspension

Propionibacterium acnes 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°C[7].

Creating 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[8].

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[8].

Media Creation

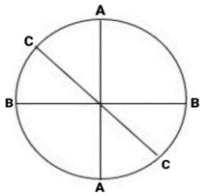
Propionibacterium acnes bacteria, weighed as much as 2.52 nutrient agar in 90 ml of distilled water. then added 0.5 ml of nutrient broth into the container. Then shaken until homogeneous, then sterilized using an autoclave for 30 minutes at a temperature of 121°C[9].

Inhibition Zone Calculation

Calculation of the diameter of the inhibition zone Davis and Stout 1971[9].

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

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



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

Before extraction, the *Scleria sumatrensis* stems were first washed thoroughly using running water to remove any dirt, then cut into small pieces. This cutting was intended to expand the surface area of the sample so that the solvent could more easily extract the active compounds in the next process. The extraction process was carried out using the maceration method because it was considered simple and did not require complex equipment. In addition, this method does not involve direct heating, so the risk of damage to bioactive compounds due to heat can be minimized. In the maceration process, the sample was soaked in 96% ethanol as a solvent. This soaking caused the plant cell walls to rupture due to the difference in osmotic pressure between the inside and outside of the cell. Thus, the secondary metabolites contained in the cytoplasm will dissolve in organic solvents such as ethanol. Maceration was carried out for two times 24 hours with two solvent changes (remaceration) so that all active compounds can be extracted optimally[10]. After the maceration process was complete, the extraction results were filtered and then evaporated using a rotary evaporator at a temperature of 40°C. This temperature was chosen because it is below the boiling point of ethanol (around 60–80°C), so that the solvent evaporation process takes place slowly and selectively without damaging the active compounds extracted[8]. From this process, an extract weighing 13.45 grams was obtained with a green color. This extract was then stored in a freezer at a temperature of -10°C to prevent denaturation or degradation of the bioactive compounds it contains.

Antibacterial Activity Test against *Propionibacterium acnes*

In this study, the antibacterial activity of *Scleria sumatrensis* extract was tested against *Propionibacterium acnes* bacteria. The purpose of this test is to determine the ability of the extract to inhibit the growth of the bacteria. The test was conducted using five variations of extract concentration and two types of controls, namely positive control and negative control. The positive control used was Chloramphenicol at a concentration of 2 µg/disc, while the negative control used a paper disc that was spotted with 70% alcohol as much as 10 µg/disc.

Before testing, all paper discs that have been treated with extracts, positive controls, and negative controls are dried first using a

desiccator. This drying process aims to remove residual alcohol used as a solvent, so that it does not interfere with or affect the results of antibacterial activity during testing.

Table 1. Inhibition Zone Value of *Propionibacterium acnes* Test

Extract Concentration	PI	PII	Avarage
100	10.67	9.49	10.08
200	10.72	10.68	10.70
300	12.25	11.31	11.78
400	13.49	12.17	12.85
500	14.85	13.66	14.25
positive control	31.20	31.20	31.20
Negative control	00.00	00.00	00.00

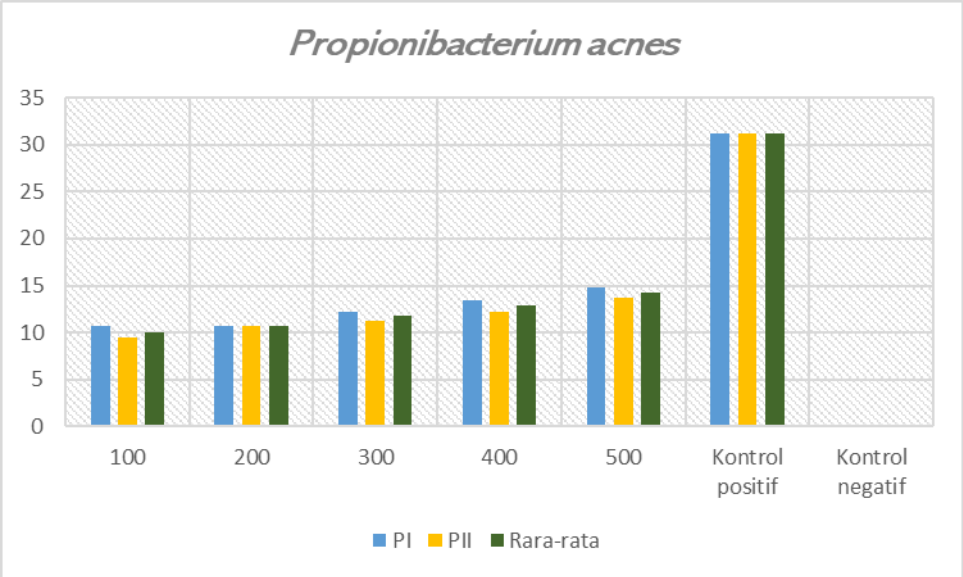


Figure 1. Average Bacterial Inhibition Zone Graph From *S. Sumatrensis* Extract

The results of the antibacterial activity test of *Scleria sumatrensis* extract against *Propionibacterium acnes* bacteria (Table 1), which is a gram-positive bacteria and one of the main causes of acne. The results showed that at a concentration of 100 µg/disc, the extract had moderate antibacterial activity, and increased to a strong category at a concentration of 200 to 500 µg/disc (Figure 1). The increase in activity was in line with the increase in extract concentration, indicating the potential of the extract as a natural antibacterial alternative to treat acne infections caused by *P. acnes*. Research conducted by Wiraswati et al showed that *Scleria sumatrensis* extract contains various secondary metabolite compounds, such as phenolics, flavonoids, alkaloids, tannins, steroids, and terpenoids[4].

Phenolic compounds have effective antibacterial activity against *Propionibacterium acnes* through various mechanisms, such as damaging bacterial cell membranes, inhibiting protein and nucleic acid synthesis, and interfering with metabolic enzyme activity[11]. Phenolic interactions with cell walls and cytoplasmic membranes cause leakage of intracellular components and ultimately bacterial cell death[12]. Phenolic-rich plant extracts have shown significant inhibition against *P. acnes*, making them potential candidates for natural antimicrobial agents for use in acne treatment therapy without triggering antibiotic resistance or severe side effects[13].

Flavonoid compounds show antibacterial activity against *Propionibacterium acnes* through various mechanisms, such as damaging bacterial cell membranes, inhibiting protein synthesis, and interfering with important enzymes in DNA replication such as DNA gyrase[14]. Flavonoids increase the permeability of bacterial cell membranes, causing leakage of ions and intracellular materials[15]. Studies have also shown that flavonoids such as quercetin and

kaempferol are effective in inhibiting the growth of *P. acnes* in vitro[16]. Alkaloid compounds have antibacterial activity against *Propionibacterium acnes* through various mechanisms, such as interfering with cell wall synthesis, inhibiting DNA replication, and meabilizing bacterial cell membranes, causing leakage of intracellular material[17]. Alkaloids also interact with nucleic acids and interfere with the function of important enzymes in bacterial metabolism. Based on research, extracts containing alkaloids showed a significant inhibition zone against *P. acnes* in vitro[18]. Tannin compounds have antibacterial activity against *Propionibacterium acnes* through the mechanism of protein precipitation, damage to bacterial cell membranes, and binding of essential metal ions needed by bacteria to grow. Tannins are able to form complexes with proteins on the surface of bacterial cells, thereby disrupting membrane integrity and inhibiting microbial metabolism. In addition, tannins also have an astringent effect that can reduce sebum secretion, a supporting factor in the development of acne[19]. Steroid compounds show antibacterial activity against *P. acnes* by permeabilizing bacterial cell membranes, causing leakage of intracellular material and cell death[20]. Steroids also interfere with cell wall synthesis and inhibit important metabolic processes such as respiration and energy production[21]. Terpenoid compounds have antibacterial activity against *P. acnes* through the mechanism of cell membrane damage, inhibition of protein synthesis, and interference with metabolic enzymes. Its lipophilic nature allows terpenoids to interact with the lipid layer of bacterial membranes, increasing permeability and causing leakage of intracellular material[22]. In addition, terpenoids are also able to inhibit the formation of biofilms that help bacteria survive[23].

4. CONCLUSION

1. *Scleria sumatrensis* extract showed antibacterial activity against *Propionibacterium acnes*, with increasing effect as concentration increased.
2. The content of active compounds such as phenolics, flavonoids, and alkaloids plays a role in inhibiting bacterial growth through various mechanisms, making it a potential natural anti-acne agent.
3. Suggestion, Further in vivo testing is needed to evaluate the effectiveness and safety of *Scleria sumatrensis* extract as an antibacterial agent in acne treatment. Isolation and identification of specific active compounds in the extract are needed to understand the contribution of each component in antibacterial activity against *Propionibacterium acnes*.

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