

Antibacterial Activity Test of Ethanol Extract of *Sonneratia alba* On *Escherichia coli* And *Staphylococcus aureus***Kevin C. D. Wong¹, Jeane Mongi^{1*}, Sonny D. Untu², Jabes W. Kanter¹,
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ABSTRAK

Antibakteri merupakan senyawa yang digunakan dalam pengendalian pertumbuhan bakteri berbahaya. Tumbuhan Mangrove yang diduga kuat memiliki kandungan bioaktif, Kandungan bioaktif yang terdapat dalam tumbuhan mangrove bermanfaat di bidang farmasi sebagai bahan obat. Akar *Sonneratia alba* mengandung senyawa metabolit sekunder Flavanoid, tanin dan alkaloid yang berperan sebagai antibakteri. Tujuan dari penelitian ini dalam melihat aktivitas antibakteri ekstrak etanol *S.alba* terhadap bakteri *Staphylococcus aureus* dan Bakteri *Escherichia coli*. Metode ekstraksi melalui metode Maserasi, diperlukan 3 hari untuk ekstraksi. Ekstrak yang diperoleh selanjutnya dievaporasi pelarutnya di rotary evaporator kemudian hasil ekstrak dibuat konsentrasi 25 μ g, 50 μ g, 100 μ g, dan 150 μ g. pengujian antibakteri menggunakan metode Kirby-Bauer. Hasil penelitian didapatkan bahwa pada konsentrasi 25 μ g, 50 μ g, 100 μ g, dan 150 μ g akar mangrove *Sonneratia alba* mempunyai aktivitas antibakteri. Tetapi yang mempunyai aktivitas antibakteri yang kuat pada bakteri *Escherichia coli* adalah pada konsentrasi 50 μ dengan sedangkan pada bakteri *Staphylococcus aureus* adalah pada konsentrasi 150 μ g dengan zona hambat 18,6mm, dan pada bakteri *Escherichia coli* dengan zona hambat 18,3mm.

Kata kunci: *Sonneratia alba*, antibakteri, ekstrak etanol, zona hambat, maserasi**ABSTRACT**

Antibacterial is a compound used to control the growth of harmful bacteria. Mangrove plants are strongly suspected to have bioactive content, the bioactive content contained in mangrove plants is useful in the pharmaceutical field as a medicinal ingredient. *Sonneratia alba* root contains secondary metabolite compounds Flavanoids, tannins and alkaloids that act as antibacterials. The purpose of this study was to determine the antibacterial activity of *S.alba* ethanol extract against *Staphylococcus aureus* bacteria and *Escherichia coli* bacteria. Extraction method by maceration, long extraction time 3 days. The extract obtained is then evaporated solvent in the rotary evaporator then the extract results are made concentrations of 25 μ g, 50 μ g, 100 μ g, and 150 μ g. antibacterial testing using the Kirby-Bauer method. The results showed that at concentrations of 25 μ g, 50 μ g, 100 μ g, and 150 μ g *Sonneratia alba* mangrove roots had antibacterial activity. But those that have strong antibacterial activity in *Escherichia coli* bacteria are at a concentration of 50 μ with while in *Staphylococcus aureus* bacteria are at a concentration of 150 μ g with an inhibitory zone of 18.6mm, and in *Escherichia coli* bacteria with an inhibitory zone of 18,3mm.

Keywords: *Sonneratia alba*, antibacterial, ethanol extract, zone of inhibition, maceration**1. INTRODUCTION**

The mangrove ecosystem is a transition zone between land and sea. The economic

functions of mangroves include household uses and as raw materials for medicines. Mangrove plants are believed to contain bioactive

compounds that are beneficial in the pharmaceutical field as traditional medicine ingredients, particularly for treating illnesses in communities living near coastal areas^{1,2}.

Mangrove roots have potential as antibacterial agents used to inhibit the growth of harmful bacteria. The mechanism of antibacterial compounds involves disrupting bacterial growth by interfering with cell wall formation. This plant is considered a potential traditional source due to its content of biologically active compounds, including tannins, saponins, flavonoids, and triterpenoids. Compounds that have potential as antibacterial agents include flavonoids, alkaloids, and tannins^{3,4}.

Based on the background above, it is known that the roots of *Sonneratia alba* have potential as antibacterial agents. Therefore, in this study, antibacterial activity testing can be conducted against *Staphylococcus aureus* and *Escherichia coli*.

2. RESEARCH METHODS

Tools and Materials

The researcher used tools including test tubes, inoculating loops, tweezers, autoclave, incubator, camera, spatula, scale, caliper, gloves, vacuum evaporator (IKA RV 10 basic), Erlenmeyer flasks, masks, filter paper, spatula, inoculating wire, glass container, micropipette, measuring glass, aluminum foil, analytical balance, glass bottles, incubator, beaker glass, caliper, 9 mm Petri dishes, cuvette, spatula, and funnel.

The materials used were *Sonneratia alba* roots, *Escherichia coli* and *Staphylococcus aureus* bacteria, 95% ethanol, Nutrient Agar (NA), 0.9% NaCl, distilled water as the negative control, and ampicillin as the positive control.

Research Procedure

1. Sample Preparation

The roots used were fresh brown-colored *S. alba* mangrove roots, totaling 4 kg, obtained from Tongkaina beach, Manado City, North Sulawesi Province.

2. Extract Preparation

The fresh samples were sorted (dry and wet), then chopped into small pieces (2 kg) and placed into a transparent container. They were then mixed with 95% ethanol, covered with aluminum foil, and left to stand for 3×24 hours. After that, the mixture was filtered

using filter paper to obtain the filtrate, which was macerated twice. The resulting filtrates were combined to obtain a total filtrate, then concentrated using a rotary evaporator to produce a thick extract. The extract was then weighed and stored in a sealed glass container before use in testing.

3. Antibacterial Testing

Sterilization of Equipment. All tools used in the testing were thoroughly cleaned with soap and dried, then wrapped in aluminum foil. The tools, along with the NA solution, were sterilized using an autoclave at 121°C for 15 minutes.

Preparation of Nutrient Agar (NA) and Slant Media. Mix 5.6 grams of Nutrient Agar (NA) with 200 ml of distilled water in an Erlenmeyer flask, homogenize using a stirrer, and sterilize using an autoclave at 121°C for 15 minutes.

Bacterial Inoculation and Slant Media Preparation. The test bacteria were collected using a sterile inoculating loop and cultured on slant media using the pour method. The cultures were incubated at 37°C for 24 hours.

4. Preparation of Test Bacteria

One inoculating loop of rejuvenated bacteria was added into a test tube containing 0.9% NaCl solution and incubated for one day at 37°C. The turbidity was adjusted to the 0.5 McFarland standard.

5. Antibacterial Test Procedure

Two grams of the thick *Sonneratia alba* root extract were weighed and dissolved in 50 µg of distilled water. Four concentrations were then prepared: 25 µg/ml, 50 µg/ml, 100 µg/ml, and 150 µg/ml. The solutions were vortexed to ensure proper mixing. As the positive control, 50 µg/ml ampicillin was dissolved in 50 µl of distilled water. About 20 ml of NA media was poured into three Petri dishes and allowed to solidify. Once solid, cylinder cups were placed on the surface⁵.

Then, 25 ml of NA media containing bacterial suspension was poured into each Petri dish and left to solidify. After the media solidified and the cylinder cups were removed, wells were formed. The extract was then pipetted into the wells and incubated at 37°C for 24 hours.

Inhibition Zone Measurement

The following formula was used to calculate the diameter of the inhibition zone^{5,6}:

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

Description:

A = vertical diameter

B = horizontal diameter

C = diagonal diameter

D = average inhibition zone diameter

Table 1. Categories of Inhibition Zone Diameters^{7,8}:

Diameter	Inhibition Strength
(1)	(2)
≤ 5 mm	Weak
6–10 mm	Moderate
11–20 mm	Strong
≥ 21 mm	Very Strong

Data Analysis

The results obtained from the antibacterial test were analyzed using SPSS software by calculating the average diameter of the inhibition zones. The non-parametric statistical method used was the Analysis of Variance (ANOVA) test. The requirement for conducting ANOVA is that the sample data must be normally distributed. If there is a significant difference, the testing is continued with the Duncan test.

3. RESULTS AND DISCUSSION

The ethanol extraction of *Sonneratia alba* roots weighing 2 kilograms was carried out using the maceration method with 95% ethanol as the solvent. Ethanol 95% is capable of extracting polar compounds. The filtrate was collected three times, then evaporated using a rotary evaporator to obtain a thick extract weighing 61 grams. The results of the antibacterial tests are presented in Tables 2 and 3.

Table 2. Antibacterial Activity Test Results against *Escherichia coli*

No	Concentration	Average Inhibition Zone
(1)	(2)	(3)
1	Distilled Water	0 mm
2	Ampicillin	17.5 mm
3	25 µg/ml	15 mm
4	50 µg/ml	18.3 mm
5	100 µg/ml	13 mm
6	150 µg/ml	15.6 mm

Table 3. Antibacterial Activity Test Results against *Staphylococcus aureus*

No	Concentration	Average Inhibition Zone
(1)	(2)	(3)
1	Distilled Water	0 mm
2	Ampicillin	13 mm
3	25 µg/ml	15.4 mm
4	50 µg/ml	16 mm
5	100 µg/ml	17.7 mm
6	150 µg/ml	18.6 mm

From Tables 2 and 3, it can be observed that the diameter of the inhibition zones produced by the *Sonneratia alba* root extract at various concentrations (25 µg/ml, 50 µg/ml, 100 µg/ml, and 150 µg/ml) against *E. coli* ranged from 13 mm to 18.3 mm, while the positive

control (ampicillin) produced a zone of 17.5 mm. For *S. aureus*, the inhibition zones ranged from 15 mm to 18.6 mm, while the positive control was 13 mm. These results indicate that *Sonneratia alba* root extract is capable of

inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus*.

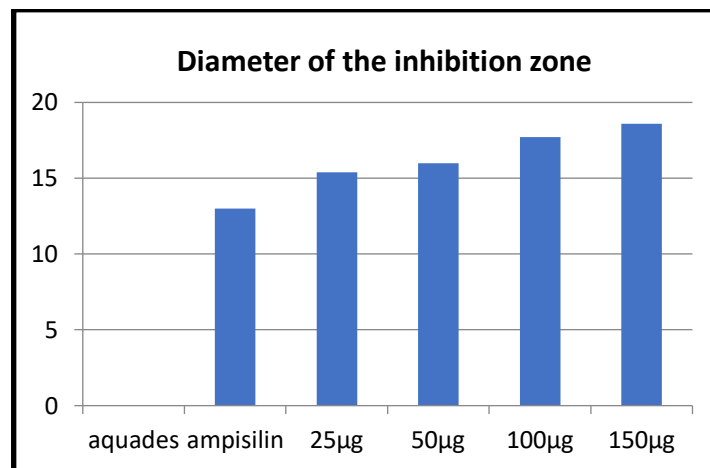


Figure 1. Graph of antibacterial activity test against *Escherichia coli*

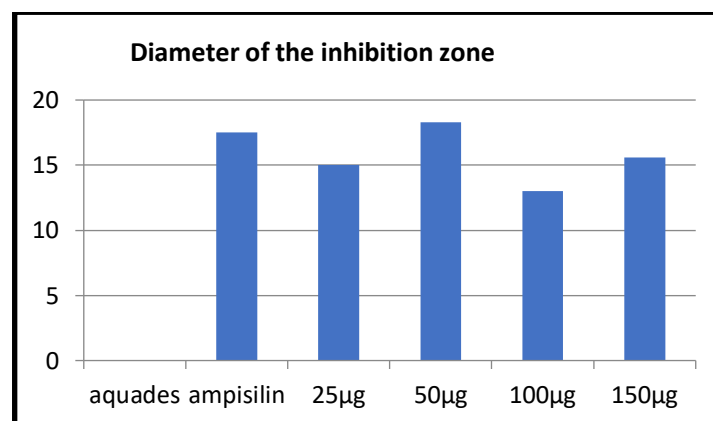


Figure 2. Graph of antibacterial activity test against *S. aureus*

From the results of the antibacterial activity test in Table 2 and Figure 1, it can be seen that all concentrations of *Sonneratia alba* extract exhibit antibacterial activity against *E. coli*. Based on the inhibition zone diameter values and their categorization, the 50 µg/ml concentration falls under the “Intermediate” category with a zone of 17.5 mm. However, the same 50 µg/ml concentration also shows strong antibacterial activity based on the inhibition zone size. The positive control (ampicillin at 50 µg/ml) had an inhibition zone of 17.5 mm, which also falls under the intermediate category. Nevertheless, according to the classification by David and Stout, this value is considered strong^{7,8}.

Referring to the findings in Table 3 and Figure 2 regarding the antibacterial activity against *S. aureus*, it was found that all tested

concentrations of *Sonneratia alba* extract (25 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml) demonstrated significant inhibition zones. The positive control (ampicillin at 50 µg/ml) produced a smaller inhibition zone of 13 mm compared to all treatment groups, while the negative control (distilled water) showed no inhibition at all against *S. aureus*. Figure 2 indicates that the antibacterial activity of *Sonneratia alba* extract against *S. aureus* is categorized as intermediate, with inhibition zone averages ranging from 15.4 to 18.6 mm for concentrations of 25–150 µg/ml. Meanwhile, the positive control's inhibition zone was smaller at 13 mm, placing it in the “resistant” category. However, according to David and Stout, this inhibition zone diameter is still considered strong^{7,8}.

Table 4. Analysis of Variance (ANOVA) – *E. coli*

Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
(1)	(2)	(3)	(4)	(5)	(6)
Between Groups	68.556	4	17.139	3.845	0.038
Within Groups	44.573	10	4.457		
Total	113.129	14			

Based on the ANOVA (Analysis of Variance) in Table 4, the antibacterial activity data of *Sonneratia alba* against *E. coli* showed no significant difference among the treatments, as the significance value is greater than 0.05, or

the F-value (0.285) is less than the F-table value (3.48 for df = 4:10). Statistical analysis using SPSS showed a significance value of 0.881 > 0.05 and an F-value of 0.285 < F-table value of 3.48.

Table 5. Analysis of Variance (ANOVA) – *S. aureus*

Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
(1)	(2)	(3)	(4)	(5)	(6)
Between Groups	16.417	4	4.104	0.285	0.881
Within Groups	144.007	10	14.401		
Total	160.424	14			

Based on the ANOVA results, the antibacterial activity of *Sonneratia alba* against *S. aureus* showed significant differences among treatments, including the positive control and extract concentrations of 25, 50, 100, and 150 µg/ml. This is indicated by a significance value

of 0.038, which is less than 0.05, or an F-value of 3.845, which is greater than the F-table value of 3.48. This significant difference confirms that at least one group differs meaningfully from the others (p < 0.038).

Table 6. Duncan Test Results – *S. aureus*

<i>S. aureus</i>			
Duncan ^a			
Treatment	N	Subset for alpha = 0.05	
		1	2
(1)	(2)	(3)	(4)
Positive Control	3	13.067	
25 µg/ml	3	15.433	15.433
50 µg/ml	3	15.767	15.767
150 µg/ml	3		18.633
100 µg/ml	3		18.733
Sig.		0.165	,104

The Duncan test results show that in subset 1, the alpha value is 0.165 > 0.05, indicating a significant or meaningful difference. The positive control shows clear differentiation from the extract concentrations, which provided stronger antibacterial activity. The extract concentrations of 25 µg/ml, 50 µg/ml, 100 µg/ml, and 150 µg/ml formed subset 2, displaying relatively similar antibacterial effects. The alpha value in subset 2 is also

above 0.05 (0.104), supporting that there is a meaningful grouping.

The results of this antibacterial activity test indicate that ethanol extract of *Sonneratia alba* root has potential as an antibacterial agent against both *Staphylococcus aureus* and *Escherichia coli*.

4. CONCLUSION

Based on the results obtained, it can be concluded that the root extract of *Sonneratia alba* possesses antibacterial activity. Against

E. coli, the extract demonstrated activity at a concentration of 25 µg with an inhibition zone diameter of 15 mm. Similarly, against *S. aureus*, at the same concentration of 25 µg, the extract exhibited antibacterial activity with an inhibition zone diameter of 15.4 mm.

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