**Biofarmasetikal Tropis**
*(The Tropical Journal of Biopharmaceutical)* 2025, 8 (1), 44-49
e-ISSN 2685-3167
p-ISSN 2828-6685

# Antibacterial Activity Test of Ethanol Extract of *Sonneratia alba* On *Escherichia coli* And *Staphylococcus aureus*

**Kevin C. D. Wong[1], Jeane Mongi[1*], Sonny D. Untu[2] , Jabes W. Kanter[1] , Hanna M. Rumagit[1], Nerni O. Potalangi[2]**

[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Christian University of Indonesia in Tomohon
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Christian University of Indonesia in Tomohon

*Corresponding author : jeanemongi2@gmail.com
Accepted: 5 April 2024; Approved: 15 April 2025

***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 ekstraks 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 25µg, 50µg, 100µg, dan 150µg. pengujian antibakteri menggunakan metode Kirby-Bauer. Hasil penelitian didapatkan bahwa pada konsentrasi 25µg, 50µg, 100µg, dan 150µg akar mangrove Sonneratia alba mempunyai aktivitas antibakteri. Tetapi yang mempunyai aktivitas antibakteri yang kuat pada bakteri Escherichia coli adalah pada konsentrasi 50µ dengan sedangkan pada bakteri Staphylococcus aureus adalah pada konsentrasi 150µg 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 25µg, 50µg, 100µg, and 150µg. antibacterial testing using the Kirby-Bauer method. The results showed that at concentrations of 25µg, 50µg, 100µg, and 150µg Sonneratia alba mangrove roots had antibacterial activity. But those that have strong antibacterial activity in Escherichia coli bacteria are at a concentration of 50µ with while in Staphylococcus aureus bacteria are at a concentration of 150µg 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

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

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 surface[5].
   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]:

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

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

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

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

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

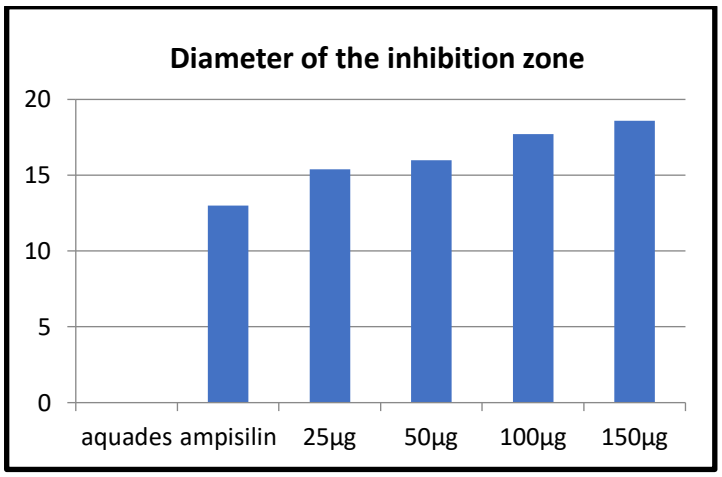


**Figure 1.** Graph of antibacterial activity test against *Escherichia coli*

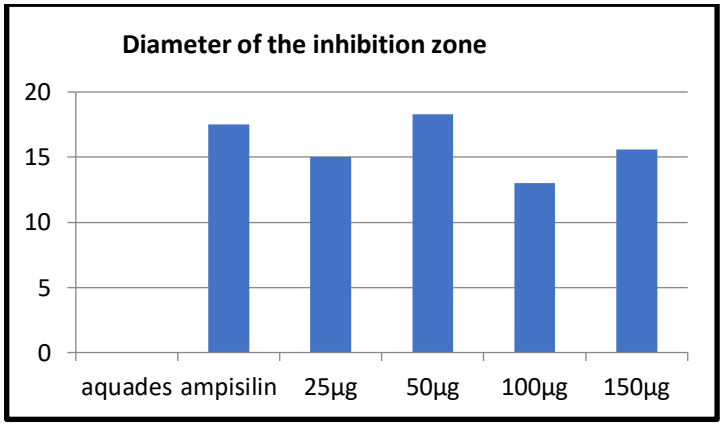


**Figure 2.** Graph of antibacterial activity test against *S. aureus*

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].

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

**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*

*S. aureus*

| 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.

## 5. BIBLIOGRAPHY

1. Abubakar S, Kadir MA, Akbar A, Tahir I. 2018. Asosiasi dan Relung Mikrohabitat Gastropoda Pada
4. Fitokimia dan Uji aktivitas
5. Antioksidan Ekstrak Akar Mangrove Sonneratia alba dengan menggunakan metode DPPH. Jurnal Biofarmasetikal Tropis. Vol. 5. No. 2, Oktober 2022. https://journal.fmipaukit.ac.id/index.php/jbt/article/view/390.
6. Mongi, J. , Karauwan, F. A., & Tuda, A. (2024). Phytochemical screening and anti-inflammatory activity test of *Sonneratia alba* root extract against burns in Rats *(Rattus norvegicus)*. *Journal of Pharmaceutical and Sciences*, 7(4), 730. https://doi.org/DOI:https://doi.org/10.36490/journal-jps.com.v7i4.639
7. Hudzicki, J. (2016). Kirby-Bauer Disk Diffusion Susceptibility Test Ekosistem Mangrove Di Pulau Sibu Kecamatan Oba Utara Kota Tidore Kepulauan Provinsi Maluku Utara. Jurnal Enggano 3 (1): 22-38.
2. Suryano, A. 2013. Sukses usaha Pembibitan Mangrove Sang Penyelamat Laut. Penerbit Pustaka Baru Press. Yogyakarta.
3. Larumpaa, S., Jeane Mongi, Karauwan F., Hariyadi. 2022. Skrining

Protocol. *American Society for Microbiology*, 1–23.
8. Harti, S.A., 2015. *Mikrobiologi Kesehatan*. CV. ANDI OFFSET. Yogyakarta. pp. 3–5.
9. Weinstein, M. P. and, & Lewis, J. S. (2020). The clinical and laboratory standards institute subcommittee on Antimicrobial susceptibility testing: Background, organization, functions, and processes. *Journal of Clinical Microbiology*, *58*(3). https://doi.org/10.1128/JCM.01864-19\
10. Davis, W. W. , and, & Stout, T. R. (1971). Disc Plate Method of Microbiological Antibiotic Assay: I. Factors Influencing Variability and Error. *Applied Microbiology Journal*, *22*(4).