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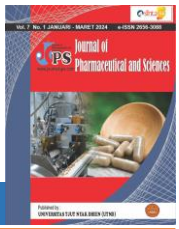

Antibacterial of mangrove root infusion *Sonneratia alba* on *Escherichia coli* and *Staphylococcus aureus*

Antibakteri infusa akar mangrove *Sonneratia alba* pada *Escherichia coli* dan *Staphylococcus aureus*

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Abstract

Antibacterial is a substance that stops and kills the growth of bacteria. Mangrove Sonneratia alba is an exciting plant to use as a medicine because of its antibacterial properties. Secondary metabolite compounds such as alkaloids, phenols, tannins, saponins, triterpenoids, and flavonoids present in the roots are antimicrobial. This study aims to determine the antibacterial activity of mangrove root infusion of Sonneratia alba in Staphylococcus aureus and Escherichia coli. This laboratory study uses the agar diffusion technique with a well to test antibacterial. Using three concentrations, namely 25%, 50%, and 100%, this study found that the infusion of the roots of the Sonneratia alba mangrove 25%, 50%, and 100% showed antibacterial activity against Escherichia coli and Staphylococcus aureus bacteria. The results of the Kruskal-Wallis test showed that Escherichia coli bacteria showed a P value of 0.010<0.05 and Staphylococcus aureus bacteria with a P value of 0.011<0.05, which showed that there was a significant difference between the two types of bacteria because the significant value was less than 0.05.

Keywords: Sonneratia alba, Antibacterial, Escherichia coli, Staphylococcus aureus.

Abstrak

Antibakteri ialah substansi untuk menghentikan dan membunuh pertumbuhan bakteri. Mangrove *Sonneratia alba* adalah tumbuhan yang menarik untuk digunakan sebagai obat karena sifatnya yang antibakteri. Senyawa metabolit sekunder seperti alkaloid, fenol, tanin, saponin, triterpenoid, dan flavonoid yang ada pada akarnya bersifat sebagai antimikroba. Studi ini bertujuan untuk menentukan aktivitas antibakteri infusa akar mangrove *Sonneratia alba* pada *Staphylococcus aureus* dan *Escherichia coli*. Studi laboratorium ini menggunakan teknik difusi agar dengan sumuran untuk menguji antibakteri. Dengan menggunakan tiga konsentrasi, yaitu 25%, 50%, dan 100%, penelitian ini menemukan bahwa infusa akar mangrove *Sonneratia alba* 25%, 50%, dan 100% menunjukkan aktivitas antibakteri terhadap bakteri *Escherichia coli* dan *Staphylococcus aureus*. Hasil uji *Kruskal-Wallis* memperlihatkan bahwa bakteri *Escherichia coli* menunjukkan nilai P sebesar 0,010<0,05 dan bakteri *Staphylococcus aureus* dengan nilai P sebesar 0,011<0,05, yang menunjukkan bahwa adanya perbedaan yang signifikan.

Kata Kunci: Sonneratia alba, Antibakteri, Escherichia coli, Staphylococcus aureus.

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Introduction

Infectious diseases are an essential obstacle to health in community life caused by bacteria. Bacteria infect the body; then, there will be a body response due to immune stimulation. Bacterial infections occur due to various kinds of bacteria [1]. Escherichia coli bacteria cause gastrointestinal diseases in humans and animals, as well as in soil and water [2]. Staphylococcus aureus bacteria, a pathogenic bacterium, is naturally found on the skin's surface and mucous membranes, especially in the genital area, throat, and nose. Various infections can occur due to their existence, ranging from skin infections to respiratory, bone, and blood infections [3].

The wider community still does not know the use of natural resources such as mangroves for bacterial infections. Mangroves are plants that have many benefits for humans, ranging from ecological benefits to benefits as a source of food and medicine. All parts have the potential as medicine from the leaves, stems, fruits, roots, and sap [4]. Sonneratia alba root has the potential to be an antibacterial. Antibacterial is a substance used to stop and kill the growth of bacteria. [5]. Alkaloids, phenols, tannins, saponins, triterpenoids, and flavonoids are secondary metabolite compounds contained in the roots of the mangrove Sonneratia alba. The content of secondary metabolite compounds in mangrove plants has antibacterial, antioxidant, and antifungal properties [6,7].

Given the potential antibacterial properties of the roots of the Sonneratia alba mangrove plant, this study represents a novel exploration of the antibacterial activity of the infusion of these roots in Escherichia coli and Staphylococcus aureus.

Experimental Section

Materials and Apparatus

The tools used in this study are as follows: Gloves, masks, lab coats, writing stationery, cameras, containers, knives, scales, beaker cups, measuring cups, bunsen, bunsen burners, tripods, stirring rods, spatula, thermometers, flannel cloth, Erlenmeyer flask, test tubes, test tube racks, cotton, sterile gauze, parchment paper, incubator, autoclave, aluminium foil, blender, mortar and pestle, 9 cm petri cup, cup cylinders, digital callipers, glass mouthpieces, tweezers, cling wraps, pipettes and tips.

The material for this study is the roots of the Sonneratia Alba mangrove; acetic acid; H2SO4; 1% and 5% FeCl3; Drangendorff solution; Wagner solution; Mayer's solution; Escherichia coli and Staphylococcus aureus bacteria; Nutrient Broth (NB); Nutrient Agar (NA); alcohol; aqueous as a negative control; ampicillin as a positive control and McFarland's solution 0.5.

Types and Designs of Researchers

This experiment was carried out in a laboratory. In this study, the antibacterial test was carried out using the agar diffusion technique with a well. In this study, three concentrations of mangrove root infusion of Sonneratia alba were used for two types of bacteria: Escherichia coli and Staphylococcus aureus. Ampicillin is used as a positive control, and sterile aquadest as a negative control, with concentrations of 25%, 50%, and 100%.

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Making Mangrove Root Infusion

Sonneratia alba mangrove roots that are still fresh from the Tongkaina beach in Manado, North Sulawesi. After the 500-gram sample is taken, running water is used to clean it, drain it, and dry it for a few days to reduce the amount of water present. After being split into small pieces, the roots of the Sonneratia alba mangrove are weighed in 100 grams and blended before being cooked for fifteen minutes at 90°C while stirring and straining using a flannel cloth.

The boiling results produced a stock solution of Sonneratia alba mangrove roots, which had a concentration of 100%. Pipetted as much as 2.5 ml of the stock solution, then 7.5 ml of aquadest was added to the test tube to a final volume of 10 ml, which is a concentration of 25%. Next, 5 ml is pipetted, and 5 ml of aquadest is added to meet the final volume of 10 ml in the test tube, which is a concentration of 50%. Next, 10 ml is taken to meet the 100% concentration and inserted into a test tube, with a final volume of 10 ml for each concentration.

Manufacture of Test Bacterial Media

1. Test Bacteria Suspension
   The test bacteria are taken and put into a test tube filled with nutrient broth (NB) liquid media. The bacteria are incubated for 24 hours after cleaning. Finally, the turbidity level was compared to McFarland's standard solution 0.5.
2. Solid Media
   Nutrient Agar as a solid medium is used; 6.9 gr is dissolved in 300 ml of aquades in Erlenmeyer and shaken. Then, the autoclave is used for twenty minutes at 121°C.
3. Positive Control and Negative Control
   To make a positive control solution, 500 mg ampicillin tablets are crushed and weighed, and 50 mg of ampicillin powder is taken and mixed with aquadest and aquadest as much as 50 ml.

Antibacterial Test Procedure

The diffusion method is used for this antibacterial test. Pour 20 ml of N.A. media into an unused petri dish. Place the cup cylinder on top of the medium and wait for it to solidify. Once the medium has solidified, pour 25 millilitres of NA media into each petri dish and mix in the test bacteria suspension. When the cup cylinder is removed after compaction, a well hole is formed.

Next, pipette and point the infusion of Sonneratia alba mangrove roots according to the concentration to be used, namely 25%, 50%, and 100%, as well as positive and negative controls, into the well hole as many as 10-12 drops, according to the capacity of each well. The inhibition zone around the well hole can be observed after 24 hours of incubation. The clear area formed can be measured using a calliper. Calculation of the diameter of the resistance zone according to Davis and Staut (1971):

formula: $D = \frac{A+B+C}{3}$ .....(1)

Description: A = vertical diameter
B = horizontal diameter
C = diagonal diameter
D = diameter of the inhibition zone

Determination of MIC and MBC

MBC shows minimal extract concentrations that kill bacteria, whereas MIC inhibits bacterial growth. On the X axis, the linear regression curve (ln M0 = ln Extract Concentration), and on the Y axis, the linear regression curve shows the MIC value. The value of MBC value is multiplied by four while MIC is 0.25 times the value of Mt. [8-10].

Data Analysis

The results of the observation of the antibacterial activity of the mangrove *Sonneratia alba* root infusion against Escherichia coli and Staphylococcus aureus obtained are tabulated in tables and figures [11]. Next, statistics were analysed. The Kruskal-Wallis non-parametric test and Man-Whitney analysis are used if the data are not homogeneous and are not normally distributed.

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Results and Discussion

Phytochemical Screening

The reaction tube test was used to assess the phytochemistry of the root infusion of the mangrove Sonneratia alba. This indicates that the sample is reacted with a specific reagent solution to find out how many secondary metabolites are present. The results of phytochemical screening are shown in the following table:

Table 1. Results of Phytochemical Screening of Mangrove Root Infusion Sonneratia alba.

Compound class	Reagents	Result	Change
(1)	(2)	(3)	(4)
Alkaloid	Dragendorff	+	Orange deposits form
	Wagner	+	produces chocolate deposits
	Mayer	+	There is a white residue.
Flavonoids	HCl and Mg	+	Orange color formed
Tannins	Ethanol and Fecl3 1%	+	Produces bluish-black or green colour
Saponins	Aquadest	+	Bubbles/bubbles form
Steroids	Ethyl acetate and concentrated H_2SO_4	-	No colour change
Triterpenoid	Ethyl acetate and concentrated H_2SO_4	-	No colour change
Phenolic	Fecl3 5% and H_2SO_4 Concentrate	+	The formation of a greenish-black colour

Description: The (+) sign indicates the presence of the compound tested. In contrast, the (-) sign indicates that the compound tested is not present in the infusion of the root of the mangrove Sonneratia alba.

The results of the test of the group of compounds are obtained in Table 1; as a secondary metabolite of the mangrove root infusion, Sonneratia alba contains alkaloids, flavonoids, tannins, saponins, and phenolics. The precipitate formation reaction on two reagents and three reagents used in the test showed positive results from the alkaloid test [12]. The formation of K+ in each of the reagents showed positive results of flavonoid testing, with the samples turning orange after adding Mg and H.C.L. powders. Metal powders and HCL reduce benzopyrone nuclei present in flavonoid structures, resulting in red or orange flavilium salts [13].

The test results showed that the tannins were positive. The addition of 1% FeCl3 to the solution resulted in a blackish-green colour. It is the result of the reaction of the addition of FeCl3 to one of the hydroxyl groups of tannin compounds [14]. The presence of condensed tannins is indicated by the blackish-green colour that appears when FeCl3 is added. The test results showed that the sample contained saponins, which were indicated by the formation of foam after shaking and remained unlost after ten minutes. According to research, these samples can form foam in water that is hydrolysed into glucose and its aglycone compounds [15]. Testing of the triterpenoid compounds and the resulting steroids showed negative results because there was no discolouration; However, testing of phenolic compounds showed blue-black colour changes. This test produces colour due to the reaction of FeCl3 with the sample. Hybridised FeCl3 ions play a role in this [12].

Antibacterial Test Results

In this study, three concentrations of Mangrove root infusion of Sonneratia alba were used, namely concentrations of 25%, 50%, and 100%, with a negative control comparator, namely aquadest and positive control ampicillin.

From the measurement of the average resistance zone in Escherichia coli bacteria in Table 2, the concentration of 25% inhibited by 10.9 mm, 50% inhibited by 12.39 mm, and 100% inhibited by 13.37 mm. The concentration of 25% is considered moderate because it inhibits the growth of Escherichia coli bacteria, and the concentration of 50% and 100% is considered strong as antibacterial in the growth process of Escherichia coli bacteria.

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Table 2. Antibacterial Activity Test Results in Escherichia coli.

Concentration	P.I	P.II	P.III	Average
(1)	(2)	(3)	(4)	(5)
25%	10,66	11,4	10,65	10,9
50%	12,51	12,34	12,34	12,39
100%	13,33	13,19	13,61	13,37
Ampicilin	13,58	16,4	16,16	15,38
Aquadest	0	0	0	0

Furthermore, for the results of the measurement of the inhibition zone and the comparison of each concentration from the concentration of 25%, 50%, 100%, positive control, and negative control for bacteria.

Table 3. Results of Antibacterial Activity Test in Staphylococcus aureus.

Concentration	P.I	P.II	P.III	Average
(1)	(2)	(3)	(4)	(5)
25%	11,61	10,56	11,43	11,2
50%	11,67	12,57	12,36	12,2
100%	13,49	13,84	16,31	14,54
Ampicilin	13,83	16,37	15,24	15,14
Aquadest	0	0	0	0

The average results of the measurement of the diameter of the Staphylococcus aureus inhibition zone shown in Table 3 above showed that the concentration of 25% inhibition was 11.2 mm, the concentration of 50% was inhibited by 12.2 mm, and the concentration of 100% inhibition was 14.54 mm. Because the average resistance zone formed at each concentration ranges from 10-20 mm, according to Davis and Staut (1971), they are categorised as strong in preventing the growth of Staphylococcus aureus bacteria.

The average value between ampicillin as a positive control and various concentrations of mangrove root infusion of Sonneratia alba showed not much different values and could be categorised as strong according to Davis and Staut (1971) in stopping the growth of Staphylococcus aureus and Escherichia coli bacteria. The existence of this study shows that the infusion of Sonneratia alba mangrove root has the potential to be antibacterial. The antibacterial potential comes from the presence of active compounds such as alkaloids, flavonoids, saponins, tannins, and phenolics.

Alkaloids have antibacterial pharmacological effects and inhibit enzymes to synthesise proteins. Inhibiting these enzymes interferes with bacterial metabolism. As an antibacterial, alkaloids interfere with the constituent parts of peptidoglycan of antibacterial cells [17, 18].

Flavonoids, secondary metabolite compounds of plants, are found in many plant parts such as fruits, seeds, flowers, leaves, roots, bark, and stems. Flavonoids damage microsomes, lysosomes, and bacterial cell walls when they interact with bacterial DNA. Flavonoids function to interfere with cell membrane function by reducing cell membrane permeability and stopping the binding of enzymes such as ATPase and phospholipase. In addition, these compounds stop bacteria from using oxygen, which inhibits energy metabolism [19, 20].

In addition, the roots of the Sonneratia alba mangrove contain other secondary metabolite compounds, such as phenolics, tannins, and saponins, which have antimicrobial properties. As an antibacterial, saponin compounds denature proteins. The cell wall and permeability of the bacterial membrane will be impaired, which allows proteins and enzymes to escape the cell, causing cell death [21]. As an antibacterial, tannins stop the enzyme, reverse transcriptase, and DNA topoisomerase, preventing bacterial cells from developing [22].

Furthermore, phenolic compounds showed antibacterial activity against gram-positive and gram-negative bacteria. These compounds fight bacteria in various ways, including interacting with proteins and bacterial cell walls, altering cytoplasmic function and membrane permeability, and preventing bacteria from making nucleic acids. This leads to changes in cell structure and morphology or imbalances in bacterial metabolism [23].

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The structure and components of bacterial cells can also affect the results of antibacterial testing of Sonneratia alba mangrove root infusion. Compared to Escherichia coli bacteria, the inhibitory zone of Staphylococcus aureus is larger. The gram-negative bacteria Escherichia coli has a wall which consists of lipoproteins, outer membrane phospholipids, and liposaccharides [24].

Staphylococcus aureus is a gram-positive bacterium composed of peptidoglycans. By inhibiting peptidoglycan on the bacterial cell wall, antibacterial agents facilitate the entry of antibacterial compounds into bacteria with higher osmotic pressure, leading to lysis or damage [25]. The barrier zones formed by Escherichia coli and Staphylococcus aureus bacteria differ due to differences in bacterial cell walls. Secondary metabolite compounds in the roots of the mangrove Sonneratia alba can also cause significantly different resistance.

The comparators used in this test were ampicillin as a positive dick and aqua dest as a negative control. The test results show that in ampicillin, the inhibitory zone formed is larger than the infusion of mangrove roots Sonneratia alba and aquadest. The use of ampicillin compared whether the injection of mangrove root Sonneratia alba at various concentrations had activity comparable to the ampicillin antibiotic or smaller than the antibiotic ampicillin against the test bacteria.

Ampicillin is a broad-spectrum antibacterial of the penicillin group. Ampicillin antibiotics work to prevent the formation of bacterial cell walls by binding to one or more penicillin-protein bonds. This prevents the formation of peptidoglycan in the inhibited cell wall. As a result, bacterial cells break down [26].

Determination of MIC and MBC Values

After the linear regression curve is made, the equation y=bx-a / y=bx+a appears; from this equation, the MIC and MBC values of the infusion of mangrove roots of Sonneratia alba in Escherichia coli and Staphylococcus aureus bacteria are determined [27]. The ln (natural logarithmic) values of each concentration series indicating the inhibitory zones in both bacteria are needed to calculate the MIC and MBC values of a linear curve equation.

Table 4. Ln Value and Quadratic Value of the Average Inhibition Zone.

Nilai X	Nilai Y^2	
	E.C	S.A
0,92	118,81	125,44
1,61	153,51	148,84
2,3	178,76	211,41

Remarks: X = ln Extract Concentration
Y = square value of the inhibition zone

Based on the test results of Escherichia coli and Staphylococcus aureus bacteria, the MIC and MBC values of the samples can be determined.

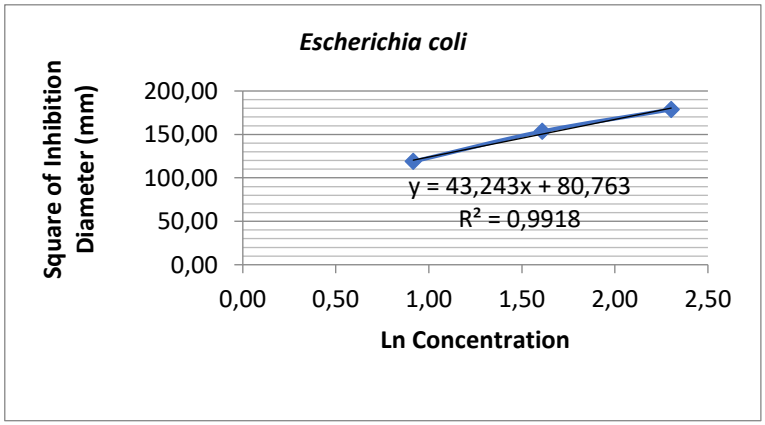

Figure 1. Antibacterial Activity Test Curve in Escherichia coli

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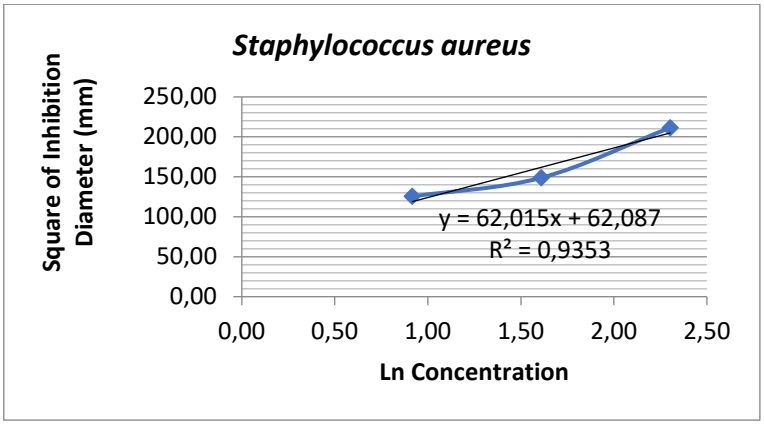

Figure 1. Antibacterial Activity Test Curve in Staphylococcus aureus

Table 5. MIC and MBC Values from Mangrove Root Infusion Sonneratia alba

Sonneratia alba	Nilai Y^2	
	E.C	S.A
MIC	1,60	0,68
MBC	6,4	2,72

Based on the data from Table 5, it can be seen that the smallest MIC and MBC values from the mangrove root infusion of Sonneratia alba are 0.68 mg/ml and 2.72 mg/ml. Meanwhile, the largest MIC and MBC values were 1.60 mg/ml and 6.4 mg/ml. The MIC value is the lowest concentration value needed by a subject to inhibit the growth of a bacterium. In contrast, the MBC value is the lowest value required to kill a bacterium.

Data Analysis

To check the date of the results of the antibacterial test, the Kruskal-Wallis non-parametric statistical test is used to find out if there is a significant difference between each group, and the Man-Whitney test will be used to determine the difference between each group.

The results of data analysis using the Kruskal-Wallis test are shown in Table 6. The P value was 0.010<0.05, indicating a significant difference because the set significance value is lower than 0.05, with a 95% confidence level for Escherichia coli.

Table 6. Kruskal-Wallis Test Results of Escherichia coli Bacteria

Test Statistics [a,b]	
Inhibition of Escherichia coli	
(1)	(2)
Chi-Square	13.353
Df	4
Asymp.S	.010

Furthermore, the antibacterial result data was analysed using the Kruskal-Wallis test in Table 7. The P value is 0.011<0.05, which is smaller than the specified significant value of 0.05. Thus, it can be concluded that the inhibition zone of the root infusion of the mangrove Sonneratia alba significantly stops the growth of Staphylococcus aureus bacteria.

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Table 7. Results of the Kruskal-Wallis Test of Staphylococcus aureus Bacteria

Test Statistics [a,b]	
Inhibition of Staphylococcus aureus	
(1)	(2)
Chi-Square	12.993
Df	4
Asymp.S	.011

Table 8. Mann-Whitney Test Results of Escherichia coli Bacteria

Concentration	25%	50%	100%	Positive
(1)	(2)	(3)	(4)	(5)
25%	-			
50%	.046	-		
100%	.050	.046	-	
Positive	.050	.046	.127	-
Negative	.037	.037	.037	.037

The results of the Mann-Whitney test in Table 8 for Escherichia coli bacteria can be seen where the statistical values are between concentrations 1-2 (25% and 50%); concentrations 2-3 (50% and 100%); concentrations 2-4 (50% and positive control) had Sig values of 0.046 < 0.050. Concentrations 1-3 (25% and 100%) and concentrations 1-4 (25% and positive control) had a Sig value that had the same value as the set significant value of 0.050. Concentrations 1-5 (25% and negative control); concentration 2-5 (50% and negative control); concentration 3-5 (100% and negative control), concentrations 4-5 (positive control with negative control) have a Sig value of 0.037

< 0.050, which means that there is a significant difference or a significant difference between the concentration of Mangrove root infusion of Sonneratia alba against Escherichia coli bacteria. It is said that there is a significant difference because the P value < 0.050.

Concentrations 3-4 (100% and positive control) have a Sig value of 0.127 > 0.050, which indicates that there is no significant difference, as the P value is greater than 0.050.

Based on the results of the Mann-Whitney test, it was obtained that all the results of the combination between the concentration treatment with the positive control obtained a P value of > 0.050 or did not have a significant difference compared to the results of the combination between the concentration treatment and the negative control which had a P value of < 0.050 or had a significant difference or significant difference in inhibiting the growth of Escherichia coli bacteria.

Table 9. Mann-Whitney Test Results of Staphylococcus aureus Bacteria

Concentration	25%	50%	100%	Positive
(1)	(2)	(3)	(4)	(5)
25%	-			
50%	.050	-		
100%	.050	.050	-	
Positive	.050	.050	.513	-
Negative	.037	.037	.037	.037

Results of the Mann-Whitney test in Table 10 concentrations 1-2 (25% and 50%); concentrations 1-3 (25% and 100%); concentrations 2-3 (50% and 100%); concentrations 1-4 (25% and positive control) concentrations 2-4 (50% and positive control) obtained a Sig value of 0.050 equal to the set significant value of 0.050.

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Concentrations 3-4 (100% and positive control) obtained Sig values of 0.513 > 0.050, which means that there were no significant differences or meaningful differences.

Next, concentrations 1-5 (25% and negative control) concentration 2-5 (50% and negative control) concentration 3-5 (100% and negative control), concentrations 4-5 (positive control and negative control) were obtained with Sig values of 0.037 < 0.050 which means that there is a significant difference or a significant difference between the concentration of infusion of Sonneratia alba mangrove and the growth of Staphylococcus aureus bacteria. It is said that there is a significant difference because the P value < 0.050.

Conclusions

Based on the research conducted, it can be concluded that the infusion of mangrove root Sonneratia alba shows antibacterial activity against Escherichia coli and Staphylococcus aureus bacteria at all concentrations, namely 25%, 50%, and 100%. With a sequential average diameter for Escherichia coli bacteria of 10.9 mm, 12.39 mm, and 13.37 mm and for Staphylococcus aureus bacteria of 11.2 mm, 12.2 mm, 14.54 mm, which is categorised as strong as antibacterial. From the results of data analysis using the Kruskal-Wallis test for Escherichia coli bacteria, 0.010< 0.05 for Staphylococcus aureus bacteria 0.011< 0.05, which means that there is a meaningful difference because it is smaller than the set significant value of 0.05 with a confidence level of 95%.

Conflict of Interest

The authors declare that they hold no conflicts of interest that could compromise the objectivity or credibility of this study. They confirm the absence of any financial, commercial, or other affiliations that might be seen as influencing the interpretation of the findings or the conclusions drawn. This disclosure is provided to maintain complete transparency and accountability, adhering to the highest ethical standards in this academic publication.

Acknowledgement

Supplementary Materials

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