Antibacterial Activity Test of *Sonneratia alba* Mangrove Root Infusa Against *Propionibacterium acnes* and *Salmonella typhi* Bacteria

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ABSTRACT

Penyakit infeksi dapat disebabkan oleh agen mikrobiologi yaitu bakteri. Kemampuan bakteri dalam menginvasi dan menimbulkan infeksi ini disebut sebagai pathogen. Hasil skrining fitokimia menghasilkan senvawa metabolit sekunder alkaloid, flavonoid, tannin, saponin, fenolik berperan sebagai antibakteri. Penelitian ini bertujuan untuk mengetahui aktivitas antibakteri infusa akar mangrove Sonneratia alba terhadap bakteri Propionibacterium acnes dan Salmonella typhi. Jenis penelitian ini merupakan penelitian eksperimental laboratorium, menggunakan metode sumuran dengan melakukan tiga kali pengulangan pada 5 serial konsentrasi, 1 kontrol positif dan 1 kontrol negatif untuk dua jenis bakteri yaitu Propionibacterium acnes dan Salmonella typhi. Hasil yang di dapat dari uji kruskal wallis pada bakteri Propionibacterium acnes vaitu Sig 0.028 < 0.05, dan hasil uji ANOVA pada bakteri Salmonella typhi dari nilai Sig = 0.000 < 0.05. Dan diketahui pada bakteri Propionibacterium acnes bahwa konsentrasi 25% menghambat sebesar 5,83 mm, konsentrasi 50% menghambat sebesar 6,3 mm, konsentrasi 100% menghambat sebesar 9,43 mm. Dan pada bakteri Salmonella typhi bahwa konsentrasi diketahui bahwa konsentrasi 25% menghambat sebesar 9,73 mm, konsentrasi 50% menghambat sebesar 9,43 mm, konsentrasi 100% menghambat sebesar 11,57 mm. Berdasarkan hasil penelitian dapat disimpulkan bahwa infusa akar Sonneratia alba memiliki aktivitas sebagai antibakteri pada bakteri Propionibacterium acnes dan Salmonella typhii.

Kata kunci; Antibakteri, Sonneratia alba, Propionibacterium acnes dan Salmonella typhi

ABSTRACT

Infectious diseases can be caused by microbiological agents, namely bacteria. The ability of bacteria to invade and cause infection is referred to as a pathogenicity. The results of phytochemical screening produced secondary metabolite compounds alkaloids, flavonoids, tannins, saponins, phenolics that act as antibacterial. This study aims to determine the antibacterial activity of Sonneratia alba mangrove root infusa against Propionibacterium acnes and Salmonella typhi bacteria. This type of research is a laboratory experimental research, using the well method by doing three repetitions on 5 concentration series, 1 positive control and 1 negative control for two types of bacteria namely Propionibacterium acnes and Salmonella typhi. The results obtained from the Kruskal Wallis test on Salmonella typhi bacteria from Sig = 0.000 < 0.05. And it is known in Propionibacterium acnes bacteria inhibits 5.83 mm, 50% concentration inhibits 6.3 mm, 100% concentration inhibits 9.43 mm. And in Salmonella typhi bacteria that concentration is known that 25% concentration inhibits 9.73 mm, 50% concentration inhibits 9.43 mm, 100% concentration inhibits 11.57 mm. Based on the results of the study, it can be concluded that Sonneratia alba root infusa has antibacterial activity on Propionibacterium acnes and Salmonella typhi bacteria.

Keywords; Antibacteria, Sonneratia alba, Propionibacterium acnes and Salmonella typhi

1. INTRODUCTION

Infectious diseases can be caused by microbiological agents, namely bacteria. The ability of bacteria to invade and cause infection is referred to as pathogenicity¹. A global study estimates more than 4.9 million people died directly or indirectly from bacterial infections in 204 countries in 2019². Sonneratia alba was chosen as the object of the research sample because there has been no research on Sonneratia alba root samples as an antibacterial treatment for Propionibacterium acnes and Salmonella thypi bacteria.

Acne or acne vulgaris is a chronic inflammatory disease. Microorganisms that play a role in the development of acne include Propionibacterium acnes. A natural ingredient used to treat acne is the root of the *Sonneratia alba* plant. *Propionibacterium acnes* bacteria are normal skin flora, usually found in the sebaceous glands³. The incidence of acne in developing countries ranges from 40 per cent to 80 per cent. The prevalence of acne in Indonesia is 80-85 per cent in adolescents, a study conducted in 2019 showed⁴.

Salmonella typhi is a group of bacteria that cause diarrhoea and infections in the human gut. This disease is caused by consuming food or drinks contaminated with the causative bacteria, the Salmonella thypi bacteria. Typhoid fever is an acute infectious disease of the small intestine caused by Salmonella thypi bacteria. The incidence of this disease in Indonesia is still very high at around 21 million cases, of which more than 700 cases result in death⁵.

Research from *Aulia and Sulistiyaningsih Sonneratia alba* mangrove roots contain secondary metabolites with the most compounds, namely phenol compounds, tannins, saponins, and falvonoids⁶. The bioactive compounds in *Sonneratia alba* are antioxidant and antibacterial. There are 202 types of mangroves that grow on the coast of Indonesia, one of which is the *Sonneratia alba* species⁷.

Based on the above background, it is known that *Sonneratia Alba* roots are thought to have potential as antibacterials, therefore researchers are interested in conducting research and testing antibacterial activity on Propinobacterium acnes and *Salmonella typhi* bacteria.

2. RESEARCH METHODS

Place and Time of Research

This research has been conducted in the Microbiology laboratory at the Faculty of Biology. Mathematics and Natural Sciences Universitas Kristen Indonesia Tomohon. Implementation time January 2023 - March 2024.

Toola and Materials

The tools used in this study are: Gloves, masks, lab coats, stationery, cameras, containers, knives, scales, beakers, measuring cups, bunsen, bunsen burners, portable conpores, stirring rod, spatula, thermometer, flannelette, erlenmayer, test tube, test tube rack, cotton swab, sterile gauze, incubator, autoclave, aluminium foil, blender, mortar and pestle, 9 cm petri dish, 9 mm cup cylinder, digital caliper, glass funnel, tweezers, aluminium foil, pipette.

The materials used in the study are: Sonneratia alba roots, Salmonella Typhi and Propionibacterium acnes bacteria, Natrium Broth (NB), Nutrient Agar (NA), distilled water as negative control, ampicillin as positive control.

Research Procedure Tool Sterilisation

The tools to be used were sterilised using an autoclave for 30 minutes at 121°C with a pressure of 1 atm. Sterilisation is an activity whose purpose is to kill microorganisms, in the process of making media, sterilisation of tools is carried out in order to avoid contamination⁸.

Sonneratia alba Mangrove Root Sampling

The samples used in this study were fresh brown *Sonneratia alba* mangrove roots obtained from Tongkaina beach, Manado City, North Sulawesi Province. Samples were taken as much as 500 grams, then the samples were washed thoroughly with running water and drained to reduce the water content, and aerated for 4-7 days. Then cut into smaller parts and blended into smaller parts to facilitate the process of withdrawing active substances during infusion. with the selection of a sample weight of 500 grams so that the stock of roots in case of damage is still sufficient and taken from the remaining 50 grams.

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Compound Group	Reagents	Results	Colour Change
(1)	(2)	(3)	(4)
Alkaloids	Dragendorff		Orange
	Wagner	+	Chocolate
	Mayer	+	White precipitate
Flavonoids	concentrated HCL and Mg	+	Red
Tannins	Ethanol and FeCl ₃	+	Green
Saponins	Aquades	+	Formed bubbles/froth
Steroid	Asam asetat glasial dan asam sulfat pekat	-	No colour formed
Triterpenoid	Asam asetat glasial dan asam sulfat pekat	-	Not formed colour
Fenolik	FeCl ₃ 5%	+	Brown Orange

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

Preparation of Test Bacteria Suspension

Test bacteria were taken and then suspended into an erlenmeyer containing 100 ml of distilled water with 0.8g of sterilised NB and stirred, then the bacterial suspension was incubated at 30°C for 24 hours. Bacterial growth is characterised by turbidity in the media. After incubating for 24 hours, it was taken and shaken until homogeneous. Then the level of turbidity is seen, namely comparing according to the McFarland 0.5 standard solution visually.

Preparation of Positive Control

The positive control solution to be used is ampicillin (500mg), with a concentration of 50μ L/pits. This solution was made by grinding ampicillin tablets using a mortar and pestle and then weighing them to obtain ampicillin powder equivalent to 50 mg, and dissolving it in 50ml of distilled water.

Solid Media Manufacturing

The solid media used is Nutrient Agar (NA). For the Nutrient Agar (NA) solution, as much as 6.92 g dissolved in 300 mL of distilled water was put into an erlenmeyer, then homogenised by shaking, then sterilised using an autoclave at 121°C for approximately 20 minutes.

Antibacterial Test Procedure

Aseptic antibacterial test procedure in laminary air flow, namely:

- 1. Clean the laminary air flow using 70% alcohol
- 2. Provide tools (already sterile) and materials that will be used
- 3. Switch on the bunsen flame so that it is in sterile condition
- 4. Sonneratia alba Infusa roots were weighed as

much as the stock solution needed, namely with a sample weight of 25gr, 50gr, 100gr then dissolved in 100ml of water, then made 3 concentrations of 25%, 50%, 100% with a final volume of 10ml. For positive control, 50mg ampicillin was dissolved in 50ml of aqudest and for negative control, aquadest was used.

- 5. Pour 20ml of Nutrient Agar (NA) media into each of the three sterile empty petri dishes, and place the cylindrical cup on the agar media that has not yet solidified and wait for the media to solidify.
- 6. After the media is confirmed to have solidified, then pour NA (Nutrient Agar) media that has been mixed with 25ml of bacterial suspension each on each Petri dish. After allowing it to solidify, the cup cylinder was removed and the wells were formed.
- 7. Next, Sonneratia alba Mangrove roots with a concentration of 25%, 50%, 100%, were pipetted and bottled into the wells as many as 12 drops adjusted to the capacity of each well concentration. Positive control and negative control were pipetted into the wells with 6 drops and incubated at 37°C for 1x24 hours. Then the clear zone area around the wells was observed and measured using a caliper.

Data Analysis

Observations of the antibacterial activity of *Sonneratia alba* root infusion against salmonella thypi were analysed descriptively in tables and figures⁹. Statistically analysed using Analysis of Variant / Anovaa if the data is normally distributed and homogeneous then the Tukey HSD test is carried out If the data is not normally distributed and not homogeneous then continue with other alternative statistical tests (Kruscal Wallis - Mann Whitney). Inhibition zone diameter calculation according to *Davis and Stout (1971)*¹⁰: Formula : $D = \frac{A+B+C}{3} - ds$

Description : A = vertical diameter

B = horizontal diamete

- C = diagonal diameter
- D = diameter of inhibition zone
- ds = diameter of the well

3. RESULT AND DISCUSSION

Creation Sonneratia Alba

In this study, using Sonneratia alba mangrove root samples taken from Tongkaina beach, Manado City, Bunaken National Park Agency with area SI.717/BTNB/TU/TEK/12/2023 sampling. Samples were then taken to the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, then 500gr samples were wet sorted and dry sorted, after being sorted and then aerated for 5-7 days. Sonneratia alba root samples are cut into small pieces after which the sample will be blended with a sample weight of 25gr, 50gr, 100gr each. The sample will be boiled over a water bath with the sample temperature reaching 90 ° C and start to count for 15 minutes of boiling process, the same is done in each of these concentrations. With 100ml sterile distilled water solvent and produce a final volume of 10ml in a test tube.

The results obtained infusa mangrove roots *Sonneratia alba* blackish brown liquid with a concentrated odour typical of *Sonneratia alba* roots then made dilutions in test tubes as much as 10ml with 3 tubes each for the concentration obtained. For the first tube, 2.5ml of infused extract was pipetted and 7.5ml of distilled water was added until the tube reached 10ml. The infusion is the 25% concentration. A total of 5ml of infusa extract was pipetted and 5ml of distilled water was added until the tube reached 10ml of infusa which is the 50% concentration. Next, 10ml of infusa extract was pipetted and put into a test tube which is 100% concentration.

Three stock solutions of 25g/100ml, 50g/100ml, 100g/100ml, and pipetted into the wells by 12 drops at 3 concentrations of 25%, 50%, 100% according to the capacity of the wells, and for the positive control, 6 drops of negative control were pipetted into the wells and

3 repetitions were made at each concentration.

Research conducted by *Tumangger 2019* showed that *Sonneratia alba* mangrove roots have antibacterial properties¹¹. The fact that a disease can be cured with *Sonneratia alba* plants is revealed by the community and passed down from generation to generation, so that the ingredients and methods of treatment are planted in the area and are still considered correct in that treatment¹².

Preparation Of Bacterial Suspensions

The results of making bacterial suspensions that have been incubated for 24 hours at 27°C show turbidity in Nutrient Broth (NB) media, which means there is bacterial growth in the media. The results of making bacterial suspensions that have been incubated for 24 hours at 27°C show turbidity in Nutrient Broth (NB) media, which means there is bacterial growth in the media.

Antibacterial Aktivity Assay

Antibacterial testing of Sonneratia alba root infusa against Salmonella thypi and Propionibacterium acnes bacteria using agar diffusion method with wells. This method was chosen because the work is very easy and simple and to measure the inhibition zone. In this study using 3 concentrations, namely 25%, 50%, 100% Sonneratia alba with a negative control comparator, namely distilled water and positive control, namely Ampicilin. The choice of ampicilin as a positive control is based on the fact that ampicilin is a β -lactam antibiotic whose mechanism of action inhibits bacterial cell wall synthesis¹³. This antibacterial test was conducted of bacteria, on two types namely Propionibacterium acnes and Salmonella typhi.

NA media a2s much as 20 ml was poured into each of the three Petri dishes and waited for it to solidify. After the media is solid, it is placed on the cup cylinder above it. Then pour NA media that has been mixed with 25ml bacterial suspension each on each Petri dish. After allowing it to solidify, the cup cylinder was removed and the wells were formed. *Sonneratia alba* extract with positive control and negative control were bottled in the wells and incubated at 37°C for 24 hours. Then observed the clear zone area around the wells (Figure 1 and 2).

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Table 2. Calculation result of Sonneratia alba infusa activity test against Propinobacterium acnes bacteria.

Desccription	concentrations	Bacteria	P1	P2	P3	Aerage
(1)	(2)	(3)	(4)	(5)	(6)	(7)
25%	5,75	4,9	6,86	5,83		
Sonneratia alba infusion	50%	Propionibacterium acnes	6,85	5,37	6,78	6,3
Ampicillin	100%		12,31 7,32	8,3 6,17	7,68 17,54	9,43 10,34
Aquadest			0	0	0	0

Table 3. Calculation result of Sonneratia alba infusa activity test against Salmonella thypi bacter

Desccription	concentrations	Bacteria	P1	P2	Р3	Aerage
(1)	(2)	(3)	(4)	(5)	(6)	(7)
	25% 50%	Salmonella typhi	9,34 9,54	10,48 10,77	9,37 7,99	9,73 9,43
infusa <i>Sonneratia</i> alba	100%		12,25	11,52	10,95	11,57
Ampicilin Aquadest			21,63 0	11,72 0	16,47 0	16,60 0

Figure 1. Inhibition Zone Results of Sonneratia alba Infusa against bacteria Salmonella typhi

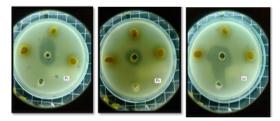
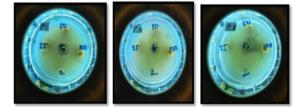


Figure 2. Inhibition Zone Results of Sonneratia alba Infusa against bacteria Salmonella typhi



After obtaining the results of antibacterial testing with the inhibition zone formed, it can be seen in (Figure 4), then measuring the inhibition zone using a measuring tool, with each Petri dish placed on a colony counter with the aim that the inhibition zone obtained can be seen clearly. After obtaining the results of the inhibition zone measurement, calculations were carried out to obtain the average value of the inhibition zone that had been tested for antibacterial activity. Indicates the presence of antibacterial activity produced by *Sonneratia alba* mangrove root infusa, meaning it has the potential as an antibacterial in inhibiting the growth of *Propionibacterium acnes* and *Salmonella typhi* bacteria.

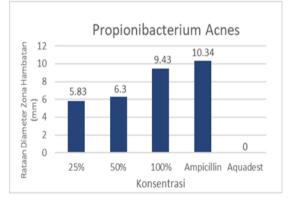


Figure 3. Graph of Antibacterial Activity Testing on Propionibacterium acnes

The results of measuring the average diameter of the inhibition zone on *Propionibacterium acnes* bacteria in table 4 and figure 5 above, it is known that 25%

5.83 50% concentration inhibits mm, concentration inhibits 6.3 mm, 100% concentration inhibits 9.43 mm. Based on the average value of the inhibition zone formed at each concentration, it is categorized as moderate in inhibiting the growth of Salmonella typhi bacteria because it has a medium average value¹⁴.

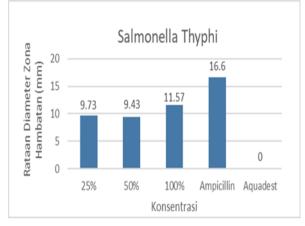


Figure 4. Graph of Antibacterial Activity Testing on Salmonella typhi

The results of measuring the average diameter of the inhibition zone on *Salmonella typhi* bacteria in table 5 and figure 6 above, it is known that 25% concentration inhibits 9.73 mm, 50% concentration inhibits 9.43 mm, categorized as moderate and 100% concentration inhibits 11.57 mm categorized in inhibiting the growth of *Salmonella typhi* bacteria because it has a strong average value¹⁴.

Comparison between ampicillin positive with various concentrations control of Sonneratia alba infusa shows a value that is not much different and can be categorized as strong¹⁵. In inhibiting the growth of Propionibacterium acnes and Salmonella typhi bacteria. The potential antibacterial activity is thought to come from the presence of active compounds such as saponins, tannins. flavonoids, and phenolic compounds that have synergistic work¹⁶.

Saponins are complex glycoside compounds with high molecular weight produced mainly by plants and in some bacteria¹⁷. Saponins, which are widely contained in plants, have long been used for traditional medicine¹⁷.

Sonneratia alba mangrove roots also have other compounds as antibacterials, namely tannins and phenolics. Tannins are polyphenolic compounds present in plants, and water-soluble organic solvents. Tannins can be obtained from almost all types of green plants, low-level and high-level plants with varying content and quality. With the mechanism of action of tannins as antibacterial by inhibiting the enzymes reverse transcriptase and DNA topoisomerase so that bacterial cells are not formed¹⁸.

According 2022, to Murray phytochemical compounds from the flavonoid group in medicine are utilized in medicine, namely as anticoagulation (preventing blood clots². Flavonoid compounds are also often referred to as blood thinners because of their properties that can prevent the collection of blood. Flavonoids show potential as antibacterial compounds, flavonoid compounds are synthesized by plants as a defense system and in response to infection by microorganisms, so it is not surprising that these compounds are effective as antimicrobial compounds.

Phenolic compounds are bioactive secondary metabolite compounds that are widely distributed in plants mainly synthesized by cyclamic acid, pantose phosphate and pheny

lpropanoid pathways (Balasundram et.al., 2016). Structurally, phenolic compounds include compounds that have aromatic rings with one or more hydroxyl groups and can vary from simple molecules to complex polymers¹⁹.

The structure and composition of bacterial cells also play an important role in the antibacterial mechanism. There are 2 types of bacteria in this study, namely Propinobacterium acnes and Salmonella thypi. *Salmonella typhi* is a gram-negative bacterium that has no spores, moves with peritrichic flagellum, is facultative intracellular and facultative anerobic. The cause of typhoid fever is *Salmonella typhi* bacteria, this disease is the largest infectious disease worldwide and until now²⁰. These bacteria are usually found in contaminated food and drinks, besides that these bacteria can also be transmitted from infected people.

Propinobacterium acnes bacteria are gram-positive bacteria that are anarobic and aerotolerant. One of the common bacteria that infect acne is *Propionibacterium acnes*, the cause of acne is so complex that a drug is needed that can overcome all causes of acne²¹. Acne often appears during changes in hormonal levels in preteens; however, the condition is also very common in adults, often associated with (The Tropical Journal of Biopharmaceutical) 2024, 7(2), 15-24

hormonal fluctuations during the menstrual cycle.

MIC and MBC Determination

MIC and MBC can be known by counting the number of colonies that grow on solid media. MIC is the smallest concentration at which the extract is able to inhibit bacterial growth, indicated by clear growth media, but still shows growth on the *mediaMBC* is the smallest concentration that can kill bacteria, characterized by no growth on the media, indicating that the test bacteria died because of the test solution with that concentration.

 Table 4. Ln squared values of the zone of inhibition of Propionibacterium acnes

value X	value Nilai Y
	Propionibacterium acnes
0.91	33.98
1.60	39.69
2.30	88.92

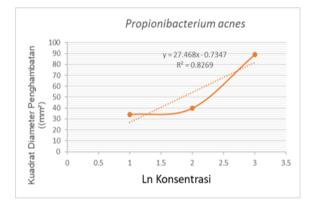


Figure 5. Antibacterial testing curve against *Propionoibacterium acnes*

Table 5. MIC and MBC Calculation Results of
Sonneratia alba Mangrove root infusa
against *Propionibacterium acnes*

Infusa akar Mangrove	Propionibacterium
Sonneratia alba	acnes
MIC	0,25 uL/mm
MBC	1 uL/mm

Based on table 4, it can be seen that the MIC MBC value of Sonneratia alba Mangrove root infusa which can inhibit bacteria is 0.25 uL/mm, and for the MBC value that can kill bacteria is 1uL/mm.

Table 6.	Ln Value and Quadratic Value of Zone
	of Inhibition

value X	value Y
	Salmonella typhi
2.28	94.67
2.24	88.92
2.45	133.86

Based on the values in table 7, the MIC and MBC values can be determined.



Figure 6. Antibacterial Testing Curve against Salmonella typhi

Table 7. MIC and MBC Calculation Results of
Sonneratia alba Mangrove root infusa
against Salmonella typhi

Mangrove root infusa Sonneratia alba	Salmonella typhi
MIC	0,74 uL/mm
MBC	2,96 uL/mm

Based on table 4, it can be seen that the MIC MBC value of *Sonneratia alba* Mangrove root infusa which can inhibit bacteria is 0.74 uL/mm, and for the MBC value that can kill bacteria is 2.96 uL/mm. Because the results of antibacterial testing of *Salmonella thypi* have a better inhibition zone value compared to the results of testing *Propionibacterium acnes* bacteria.

Table 8. Kruskal-WallisTestResultsofPropionibacterium acnesBacteria

Test Statistics				
Daya Hambat Propionibacterium acne				
Chi-Square	10.911.			
Df	4			
Asymp. Sig.	.028			

The results of data analysis from the observation of antibacterial activity on Propionibacterium acnes and Salmonella typhi bacteria seen from the clear zone or the inhibitory effect of the infusion are analyzed statistically, the results of the analysis using parametric and non-parametric statistical methods used are one-way variant (ANOVA) and Kruskall Wallis test. The requirements in ANOVA testing are that the data to be analyzed must meet the normality test and homogeneity test, if these requirements can be met, then it can proceed to the ANOVA test, while the Kruskal Wallis test requirements ignore the two conditions above.

The results of the analysis for clear zone research data from Propionibacterium acnes bacteria using another alternative test, namely the Kruskal wallis non-parametric statistical test, table 6 (attachment 14) shows that the data entered are not normally distributed and not homogeneous. The Kruskal wallis test requirement is a test to see if there is a significant difference or a significant difference in the average diameter of the inhibition zone between the treatment of Sonneratia alba infusa with a concentration of 25%, 50%, 100 positive and negative controls, the results obtained from the Kruskal wallis test are Sig 0.028 < 0.05 or chisquare value> r.table, namely 10.911> 9.488 which means there is a significant difference or there is a significant difference from the treatment of the concentration of antibacterial activity of P. acne. This significant difference or there is a meaningful difference between the concentration of Sonneratia alba infusa against the growth of P.acne bacteria.

At concentrations (25% and positive control) has a Sig value of 0.127 > 0.05, (50% and positive control) has a Sig value of 0.275 > 0.05, (100% and positive control) has a Sig value of 0.513 > 0.05, and concentration (25% and 50%) has a Sig value of 0.827 > 0.05, meaning that there is no significant difference or meaningful difference in inhibiting the growth of P.acne bacteria.

Based on the results of the Mann Whitney test, it can be seen that all the results of the combination between concentration treatments with positive controls get P > 0.05 or do not have significant differences compared to the results of the combination between concentration treatments with negative controls which get P < 0.05 or have significant differences in inhibiting the growth of P. acne bacteria.

 Table 9. Bacterial Mann Whitney Test Results

 Propionibacterium acnes

Konsentrasi	25%	50%	100%	Positif
25%	-			
50%	0,827	-		
100%	0,050	0,050	-	
Positif	0,127	0,275	0,513	-
Negatif	0,037	0,037	0,037	0,037

Furthermore, the Salmonella thypi bacteria use the parametric statistical method test used, namely one-way variance (ANOVA). The requirements in ANOVA testing are that the data to be analyzed must go through a normality test and homogeneity test, if these requirements can be met, then it can be continued.

Table 10 in appendix 14, homogeneity test The data entered is accepted or meets the homogeneity test, it can be seen from the significant value. in the table, Sig 0.065 > 0.05 so it can be said that the data entered is homogeneous. Thus the data entered has met the requirements of the homogeneity test, then proceed to use the ANOVA test to see if there is a significant difference between the 25%, 50%, 100% concentration treatments.

Tabel 10.Uji ANOVA Bakteri Salmonella typhi

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	435.318	4	108.829	19.893	.000
Within Groups	54.707	10	5.471		
Total	490.024	14			

From Table 10, it can be seen that the data from the ANOVA test results from the Sig value = 0.000 < 0.05 or Fcount> F.Table (4; 10), namely 19.893> 3.48, meaning that this states that the data tested has a significant difference in each treatment concentration of 25%, 50%, 100%, that is, sennoratia alba infusa can inhibit the growth of Salmonella typhi bacteria Because there is a significant difference, the test is continued with the Tukey HSD (Honest Significance Difference) Test. Tukey test is a test to see if there is a significant difference in the average diameter of the inhibition zone between the treatment of sennoratia alba infusa (concentration 25%, 50%, 100%), positive and negative controls.

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Tabel 11. Tukey HSD to	est for Salmonella typhi	
bacteria		

Konsentrasi	N	Subset for alpha = 0.05		
		1	2	3
Negatif	3	.0000		
50%	3		9.4333	
25%	3		9.7300	
100%	3		11.5733	11.5733
Positif	3			16.6067
Sig.		1.000	.793	.136

Based on the results of the Tukey HSD test in *Table 12*, it can be seen that there are significant differences or meaningful differences between sennoratia alba infusa against salmonella typhi bacteria. There are 3 treatment subsets, namely subset 1 by negative control treatment, subset 2 by 50% and 25% concentration treatment, subset 3 by 100% concentration and positive control treatment, meaning that concentrations with the same subset have almost the same inhibition and do not have significant differences or meaningful differences because they are in the same subset.

Propionibacterium acnes bacteria cause acne by producing lipase enzymes that break free fatty acids in skin lipids. These fatty acids can cause tissue inflammation when associated with the immune system and then *acne*. The public health impact of *Salmonella typhi* is further exacerbated by the emergence of antimicrobial resistance, especially to the highest priority clinically important antimicrobials, such as quinones and 3rd and 4th generation cephalosporins.

Based on the results of the study, it can be seen that the bacteria Salmonella typhi and Propionibacterium acnes provide inhibitory activity seen from the clear zone formed, because in the infusion of *Sonneratia alba* roots there are secondary metabolites or bioactive compounds that can have an influence on the growth of the two bacteria.

4. CONCLUSIONS

Based on the research conducted, it can be concluded that the infusa of *Sonneratia alba* mangrove roots has inhibition or activity as an antibacterial against Propinobacterium acnes bacteria and Salmonella typhi bacteria. The results of the analysis showed concentrations of 25%, 50%, 100%, with an average diameter sequentially for Propinobacterium acnes bacteria 5.83 mm; 6.3 mm; 9.43 mm; categorized as moderate and for Salmonella typhi bacteria 9.73 mm; 9.43 mm; categorized as moderate, for 11.57 mm; which is categorized as strong as antibacterial. categorized as moderate, for 11.57 mm; which is categorized as strong as antibacterial.

5. LITERATURE

- Belon, C., & Blanc-Potard, A. B. (2016). Pathogenicity of extracellular bacteria: Immune evasion strategies and implications in human disease. *Frontiers in Microbiology*, 7, 1235.
- Murray, C. J. L., Ikeda, C., & Shirodaria, C. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10338), 1204-1222.
- 3. Ravikumar, M., et al. (2021). "Antibacterial activity of garlic extract on Propionibacterium acnes and its formulation in a gel." *International Journal of Creative Research Thoughts*, 9(7), 1123-1129.
- Zhu, Z., Chen, H., Wu, M., Wang, F., & Li, Y. (2024). Global, regional, and national burdens of acne vulgaris in adolescents and young adults aged 10-24 years from 1990 to 2021: A trend analysis. British Journal of Dermatology.
- Yatnita, C. P. (2018). Bakteri Salmonella Thypi Dan Demam Tifoid. Andalas Journal Of Publik Health, 6 (1):42-46.
- 6. Aulia, N. R, & Sulistiyaningsih, R. (2020). Kanduangan Metabolit Sekunder Dan Aktivitas Senyawa Bioaktif Tumbuhan Mangrove Perepat (Sonneratia alba) (hlm. 151–155). Farmaka 17
- Harti, S.A. (2015). Mikrobiologi Kesehatan. CV. ANDI OFFSET. Yogyakarta.Berita Biologi. Jurnal Ilmu Hayati, 19(2) pp. 3-5.
- Istini. (2020). Pemanfaatan Plastik Polipropilen Standing Pouch Sebagai Salah Satu Kemasan Sterilisasi Peralatan Laboratorium. Indonesian. *Journal Of Laboratory*, 2 (3): 41-46.
- Kusumadewi, T, S, Khotimah, A.H, & Yanti. (2014). Ekstrak Metanol Buah Sonneratia alba J.E.Sm sebagai Penghambat Pertumbuhan Helminthosporium sp. Yang diisolasi

dari Daun Jagung. *Pontianak: Universitas Tanjungpura*, 3 (2) : 149-154.

- Novitasari, I. W. (2015). Uji aktivitas antibakteri infusa daun mangga bacang (Mangifera foetida L.) terhadap pertumbuhan Salmonella typhi. Jurnal Mahasiswa PSPD FK Universitas Tanjungpura, 3(1).
- Tumangger, B.S, & Fitriani. (2019). Identifikasi dan Karakteristik Jenis Akar Mangrove Berdasarkan kondisi Tanah dan Salinitas Air Laut di Kuala Langsa. Jurnal Biologica Samudra, 1 (1): 13
- 12. Parubak, A. S. (2019). Senyawa flavonoid yang bersifat antibakteri dari akway (Drimys becariana. Gibbs). *Chemistry Progress*, 6(1).
- Alouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). "Methods for in vitro evaluating antimicrobial activity: A review." Journal of Pharmaceutical Analysis, 6(2), 71-79.
- Muhtadi, M., Murni, A., & Syafii, W. (2024). Medicinal Potential of Mangrove Species in Indonesia: A Review of Pharmacological Activities. *International Journal of Applied Pharmaceutics*, 16(Special Issue 5), 1-8.
- Pikhtirova, A., Pecka-Kiełb, E., & Zigo, F. (2023). "Antimicrobial activity of saponin-containing plants: review."

Journal of Dairy, Veterinary & Animal Research, 12(2), 121-127.

- Putri, P. A, Chatri, M, & Advinda, L. (2023). Karakteristik Saponin Senyawa Metabolit Sekunder pada Tumbuhan. Jurnal Serambi Biologi, 8(2), 252–256.
- Wijaya, M. D., & Indraningrat, A. A. G. (2021). Antibacterial Activity of Mangrove Root Extracts from Ngurah Rai Mangrove Forest, Denpasar-Bali. *Biology, Medicine, & Natural Product Chemistry.*
- Balasundram, N., Sundram, K., & Samman, S. (2016). Phenolic Compounds in Plants and Agriindustrial By-products: Antioxidant Activity, Occurrence, and Potential Uses. Food Chemistry, 99(1), 191-203.
- Fairuza, I. (2020). The cause of typhoid fever is Salmonella typhi bacteria, this disease is the largest infectious disease worldwide and until now. Dalam Prosiding Seminar Nasional Biologi di Era Pandemi COVID-19. Universitas Islam Negeri Alauddin Makassar. Diakses dari journal3.uinalauddin.ac.id
- Bjourson, A.J., Barnard, E., & McDowell, A. (2019). "Propionibacterium acnes and Acne Vulgaris: New Insights from the Integration of Population Genetic, Multi-Omic, Biochemical and Host-Microbe Studies." *Microorganisms*, 7(5), 12