ANTIBACTERIAL TEST OF *ENHALUS ACOROIDES* SEAGRASS AGAINST BACTERIA *SALMONELLA TYPHI*

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ABSTRACT

The use of antibacterial from natural ingredients can as an alternative, one of them is seagrass *Enhalus acoroides* which is known to have inhibiting active compounds bacterial growth. Metabolites from seagrass are known to be biologically active is an important biomedical and can be used as a drug potential. The use of antibacterial from natural ingredients is alternative approach. Bacteria *Salmonella typhi* is a genus of gram-negative enterobacteria sticks that cause typhoid, paratyphoid, and foodborne diseases and resistant bacteria. Therefore, the purpose of this research was to analyze the activity test antibacterial on *Enhalus acoroides* seagrass against *Salmonella typhi* bacteria. The methods used in this study were seagrass extraction, phytochemical tests, and antibacterial activity tests. The results obtained from the research are crude extracts with solvents methanol, ethyl acetate and n-hexane with a concentration of 500 ppm was the most effective extract concentration in inhibiting bacterial growth *Salmonella typhi* with a weak category. Furthermore, characteristic Antibacterial crude extract of seagrass *Enhalus acoroides* was bacteriostatic. This study suggests that the influence of polarity types solvent on antibacterial activity of seagrass extract *Enhalus acoroides* against the growth of *Salmonella typhi* bacteria.

Keywords: *Enhalus acoroides, Antibacterial Activity, Seagrass, Salmonella typhi, Bioactive*

1. INTRODUCTION

Seagrass is an important component of the world's coastal marine environment, offering some of the most commercially and environmentally beneficial ecosystem services of any marine habitat (Lima *et al.,* 2023). The seagrass community plays an important role both physically and ecological, as well as biological in coastal and estuary areas (Veettil *et al.,* 2022**)**. The ecological function of the seagrass ecosystem is as follows primary producer, nutrient recycler, substrate stabilizer, sediment catcher, habitat and food and shelter for other marine organisms (Stankovic *et al.,* 2021**)**. Besides that, Seagrass ecosystems are also closely related to coral reefs and mangroves (Rachel *et al.,* 2021). Therefore, it is important for the management of coastal waters. However, research on seagrass and marine ecosystems have so far focused on exploration activities, cultivation and tourism, but research on the utilization of seagrass for In humans, especially its bioactive components are sparsely explored.

Seagrass has potential utilization which is it contains compounds natural bioactive and antibacterial (Hwan Kim *et al.,* 2021). The active compounds contained in seagrass are flavonoids and steroids (Vincenti *et al.,* 2018). The type of seagrass *Enhalus acoroides* found bioactive compounds as antibacterial, such as flavonoids, phenols, tannins, steroids and saponins (Klangprapun et al., 2018). *Enhalus acoroides* is the most common seagrass species found in reef flat areas and occurs in protected areas mangroves and form a dense and extensive grassland with a canopy closed areas provide important habitat for other species (Waycott, 2004). Enhalus acoroides can grow in various types of sediments ranging from slit to coarse sand and mud to crushed coral and mud to mud-sandy (Supriyadi, 2010).

Metabolites from seagrass are known to be biologically active is an important biomedical and can be used as a drug potential (Punginelli *et al.,* 2023). The use of natural medicine is very important because disease-causing bacteria are resistant to various diseases antibiotics.

Pathogenic bacteria resistance is known not only to one type only antibacterial, but against several types of antibacterial (Murray *et al.*, 2022). The process of extracting the content of metabolites in seagrass depends on the type of solvent used during extraction (Büyükkiraz *et al.*, 2022). Separation compounds can be obtained in the process extraction with organic solvents. Commonly used organic solvents such as methanol, ethanol, ethyl acetate, n-hexane, and chloroform. The solvent will affect the characteristics of the secondary metabolites present on seagrass (Gono et al., 2022). Antibacterial is a compound produced by living things both microorganisms and macroorganisms that can inhibit not only growth but also to break the life cycle of bacteria (Mehari *et al.*, 2019).

Bacteria *Salmonella typhi*, the cause of typhoid fever, is known to be resistant to ampicillin, penicillin, and co-trimoxazole (Saha *et al.*, 2020). Salmonella is the main cause of diseases that are spread through food. Salmonella causes diseases of the digestive organs. Salmonella is a genus of gram-negative enterobacteria sticks that cause typhoid, paratyphoid, and foodborne diseases (Popa *et al.*, 2021). This bacteria are resistant. Salmonella is the main cause of diseases that are spread through food. Salmonella causes diseases of the digestive organs. Salmonella is a genus of gram-negative enterobacteria sticks that cause typhoid, paratyphoid, and foodborne diseases (Mulyatno, 2011). Salmonella species can move freely and produce hydrogen sulfide. Aquatic organisms infected with Salmonella bacteria are usually slimy, decreased appetite, there are spots on the body, usually colored red. If aquatic organisms are exposed to Salmonella and consumed by humans, it will result diarrhea, stomach cramps, and fever within 8-72 hours after ingestion food contaminated with Salmonella. Other symptoms are fever, headache, nausea and vomiting (Mulyatno, 2011). This type of antibacterial is known as Multi Drug Resistant bacteria (Nair et al., 2018). Therefore, it is very important to develop a substance that has potential as an antibacterial by using extract from seagrass (Cristianawati *et al.*, 2019). This study aims to analyze the activity test antibacterial on *Enhalus acoroides* seagrass against *Salmonella typhi* bacteria.

2. MATERIALS AND METHODS

2.1. Extraction of Seagrass *Enhalus acoroides*

Extraction using various solvents, such as methanol, n-hexane and ethyl acetate. Methanol, n-hexane and ethyl acetate solvents used are solutions pro-analyst. Extraction was carried out using the maceration method comparison of sample weight and volume of solvent 1:8 for 2x24 hours (Hidayati, 2022). The extract solution obtained was then filtered using paper Whatman filter No.1. The filtrate is then evaporated and dried at room temperature 55ºC - 60ºC (Apsari, 2021). According to Bakti (2017) evaporation process with the specified temperature was carried out to obtain the extract with paste shape, apart from that the boiling point of each solvent is also a factor affect the temperature during evaporation.

$$Yield = \frac{Final\ Weight}{Initial\ Weight} \times 100\%$$

2.2. Phytochemicals Test

Phytochemical tests were carried out to identify the content of bioactive compounds on seagrass extract. Identification of the nearness of an antioxidant is based on phytochemical approaches which leads to the sedate disclosure alluded as common item screening (Foye *et al.*, 2008). Each portion of the plant may contain dynamic components such as bark, clears out, blossoms, roots, natural products and seeds (Gordon *et al.*, 2001). Phytochemicals are wellknown as bioactive constituents of plants to be specific steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. The stages of the phytochemical test were carried out at the extraction stage in 3 replicate analysis which consisted of Alkaloid test, Flavonoid Test, Saponin Test, Tanin Test, Triterpenoid Test.

a) Alkaloid Test

A 0.05 g of sample was put into a test tube and then added H_2SO_4 and shaken until completely mixed. Then filtered and added Meyer's reagent by looking at the precipitate white is a positive indication.

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b) Flavanoid Test

A 0.05 g of sample was added with 0.05 mg of magnesium powder, then 0.2 ml of amyl alcohol and 4 ml of alcohol were added. Positive test results are indicated by red, yellow, or orange solution on the amyl alcohol layer.

c) Saponin Test

A 0.05 gram sample put in a test tube. Then Hot water was added and the test tube was shaken. After that incubate for 30 minutes and added 1 drop of 2N HCl. Test positive result saponins indicated by the presence of stable foam.

d) Tanin Test

A sample of 0.05 grams is brewed with hot water that has been boiled for 3 minutes. The sample was filtered after it was dripped with FeCl3 1%. The test result is positive if the solution is dark blue or blackish green

e) Triterpenoid Test

A 0.05 gram sample was added with 10 ml of chloroform and sulfuric acid added in the same volume. If a red precipitate forms positive food contains triterpenoids.

2.3. Antibacterial Activity Test

Test activity antibacterial using method Disc Diffusion (Kirby-Bauer Test). Suspension 20 μL of test bacteria was added on the media in a petri dish then etched with a sterile cotton swab over the test medium (Difco, 1977). The sterile cotton swab is rotated several times. This procedure is repeated as many times as possibl twice.

Paper discs with diameter measuring 6 mm. Antibacterial activity test was carried out to measure how much potency or the concentration of a compound can have an effect on microorganisms. The antibacterial test in this study used the disc with paper method disc in vitro. The steps taken include the preparation of the test bacteria through media preparation, bacterial rejuvenation and bacterial culture, followed by antibacterial activity testing.

3. RESULTS AND DISCUSSION

3.1 Yield Extraction of Seagrass *Enhalus acoroides*

The extraction of *Enhalus acoroides* seagrass leaves was carried out by the method maceration using a ratio of 1: 8 with different solvents ie methanol (polar solvent), ethyl acetate (semi-polar solvent) and n-hexane (solvent non-polar) and soaked for 48 hours. Extractions with different polarities usually use samples which has been dried (Hamisi *et al.*, 2023). Drying is important preservation method for plant raw materials because it can inhibit enzymatic degradation and limits microbial growth during extraction (Roshanak *et al.*, 2016).

Drying can also reduce volume storage and reduce reactions that can damage the material such as hydrolysis and fat oxidation. The table 1 shows the results of the extract yield with Methanol solvent of 6.24%; Ethyl acetate solvent of 0.76%; Nheksana solvent of 0.50%. Seagrass extraction using a solvent ratio of 1:8 and solvent with different polarities, the highest yield results were obtained from the extract methanol, namely 9.76% followed by the yield of ethyl acetate and n-hexane extracts with successive values of 0.57% and 0.16%.

The yield suggested to be due to differences in the types of seagrass that affect it content of active compounds that can be extracted by each solvent. The extraction performed on seagrass is influenced by the polarity of the solvent used. The more polar the nature of the solvent used, the extraction yield will be higher (Nawaz et al., 2020). This study shows the yield results with more methanol solvent higher compared to ethyl acetate and n-hexane solvents. This showed that the compounds contained in seagrass Enhalus acoroides tend to be more polar.

3.2 Components of Phytochemical Compounds

The bioactive components in *Enhalus acoroides* seagrass can be identified by carrying out a phytochemical test. Phytochemical analysis is an analysis that includes a wide variety of organic compounds formed by plants, viz regarding chemical structure, biosynthesis, metabolism, natural distribution and its biological function. Phytochemical analysis

carried out in this study included alkaloid tests, flavonoids, saponins, tannins and tripertenoids. The content of phytochemical compounds in *Enhalus acoroides* seagrass extract can be seen in Table 2

Table 1. Extraction and yield data

Solvent	Weight (gr)		Solvent Volume (ml)	Time (hours)	Extract (gr)	Yield (%)
	Fresh	Dry				
Methanol	1000	50	400	48	3,12	6,24
Ethyl Acetate	1000	50	400	48	0,38	0,76
N-hexane	1000	50	400	48	0,25	0,5

Table 2. Phytochemical Test Results *Enhalus acoroides*

Bioactive Components	Compound Content		
	Methanol	Ethyl acetate	N-hexane
Alkaloids	+	+	+
Flavanoids	+	+	+
Saponins	+	-	-
Tannins	+	-	-
Triterpenoids	-	+	+

Description: (+) Positive Reaction (-) Negative Reaction

Based on the results of phytochemical analysis it is known that in seagrass extract Enhalus acoroides with methanol solvent contains alkaloid compounds, flavonoids, saponins, and tannins, with ethyl acetate solvent containing compounds alkaloids, flavonoids and triterpenoids, whereas with N-hexane solvent contains alkaloids, flavonoids and triterpenoids. In general, components of phytochemical compounds found in seagrass

Enhalus acoroides most abundant in the extract with methanol solvent. Meanwhile, in ethyl solvent acetate and n-hexane were identified for the absence of tannins and saponins, whereas in methanol solvent found. In contrast to that the solvent methanol did not identify any triterpenoid compounds (Hasballah *et al.*, 2021) which indicates that the phytochemical compounds in seagrass Enhalus acoroides tends to dissolve in polar solvents. This study confirms that the differences in the polarity of the solvents used in the extraction process has a different effect on the content phytochemical compounds in each extract.

3.3 Bacterial Gram Stain

Bacteria are generally divided into two parts based on their properties Gram stains are Gram positive and Gram negative. On Gram stain, the results obtained will be determined from the composition of the cell wall in bacteria. the result of the Gram stain shows that the bacteria Salmonella typhi is rod-shaped and is a Gram-negative bacterium. Salmonella typhi bacteria is a type of Gram-negative bacteria which cannot retain its purple color crystals but the safranin dye can be adsorbed on the cell wall so that when viewed using a microscope will show a red color (Giannella, 1994).

3.4 Antibacterial Activity of Seagrass Enhalus acoroides

This is indicated by the presence of a clear zone as an indicator of the presence of inhibition areas. Compounds that diffuse inward agar from disc paper is able to inhibit bacteria *Salmonella typhi.* The test solution will diffuse from the paper disc to the surface solid agar media that has been inoculated with bacteria. It is influenced by several factors including, such as the thickness of the agar medium, time, density bacterial inoculum, agar media composition and temperature at the time of incubation (Bubonja-Šonje et al., 2020). Antibacterial inhibition mechanism against bacterial growth can be in the form of damage to the cell wall resulting in lysis or inhibition of cell wall synthesis, altering membrane permeability cytoplasm thereby causing the release of food material through the wall cells, cell protein denaturation and destruction of the metabolic system in the cell by inhibiting the

action of intracellular enzymes (McDonnell, 1999).

Antibacterial activity test results of seagrass extract *Enhalus acoroides* (reduced disc paper diameter of 5 mm) against bacteria *Salmonella typhi* showed average results in methanol solvent with range of 4 mm, ethyl acetate solvent of 3.7 mm and nhexane solvent of 3.5 mm. The inhibition zone based on its strength can be categorized namely, weak (<5mm), moderate (5-10mm), strong (>10-20mm), and very strong (>20-30mm) (Detha *et al.*, 2018; Morales *et al.*, 2003). Antibacterial test results data are presented in **Table 3** and **Figure 1.**

The results showed that the extract had antibacterial bioactivity seagrass *Enhalus acoroides* with methanol solvent (polar) against

Table 3. Results of antibacterial activity test results on *Salmonella typhi* bacteria

Solvent	Bacteria	Concentration	Incubation		Zone of Inhibiti Categorize
			24 hours	48 hours	
			Clear zone (mm)		
Methanol	*Salmonella typhi*	125 ppm	2.4 ± 0.4	0	Weak
		250 ppm	2.9 ± 0.9	0	Weak
		500 ppm	2.3 ± 0.10	0	Weak
		Control (+)	9.7 ± 0.11	1.6 ± 0.12	Medium
		Control (-)	0	0	-
Ethyl acetate	*Salmonella typhi*	125 ppm	2.8 ± 0.1	0	Weak
		250 ppm	2.5 ± 0.2	0	Weak
		500 ppm	2 ± 0.6	0	Weak
		Control (+)	10.1 ± 0.2	9.4 ± 0.3	Medium
		Control (-)	0		-
N-hexane	*Salmonella typhi*	125 ppm	2.3 ± 0.4	0	Weak
		250 ppm	1.8 ± 0.5	0	Weak
		500 ppm	2.1 ± 0.6	0	Weak
		Control (+)	9.6 ± 0.7	7.7 ± 0.8	Medium
		Control (-)	0	0	-

Information :

- The above values are mean ± SD
- SD is the standard deviation
- 5 ± 0 = no clear zone

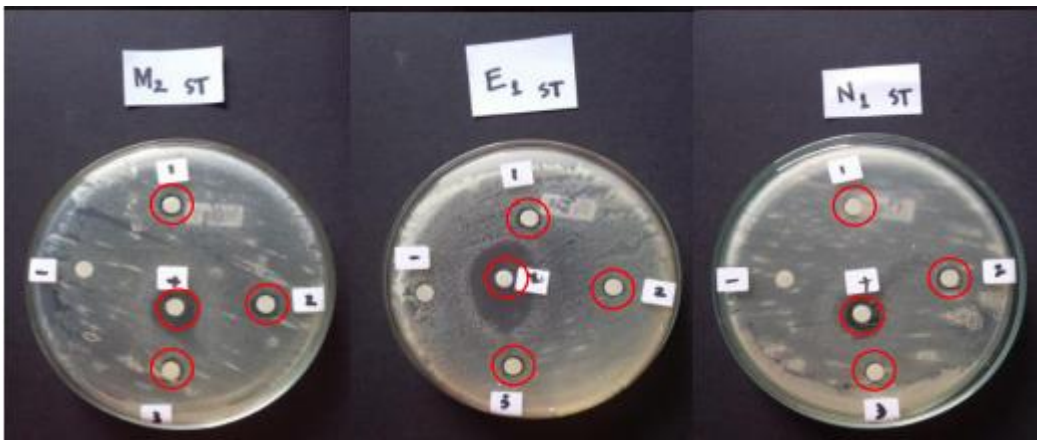

Figure 1. Results of the Anti-Bacterial Activity Test of Salmonella typhi
M = Methanol; E = Ethyl acetate ; N = N-hexane
1=125 ppm ; 2 =
250 ppm ; 3=500ppm
ST=S. typhi

bacteria *Salmonella typhi.* This can be seen with the formation of a clear zone around the disc at a concentration of 125; 250; 500 ppm. The formation of an inhibition zone is due to the crude extract of seagrass *Enhalus acoroides* has an active compound that acts as an antibacterial. The inhibition of the bacteria occurs due to the reaction of a compound chemical as an antibacterial. Existence chemical compounds of the class of flavonoids, alkaloids and steroids in crude extracts *Enhalus acoroides* showed that seagrass has potential as a natural chemical antifouling, antibacterial, antifungal, and pharmaceutical raw materials. The results of the antibacterial activity test showed that the diameter of the inhibition zone which is formed at a concentration of 500 ppm is greater than 125 ppm and 250 ppm. According to Alqadeeri (2019) the greater extract concentration, the greater the resulting inhibition due to The higher the concentration, the higher the activity of the antibacterial compound. The more the higher the concentration of the extract, the active compounds the bacteria contained the ability to inhibit increased microbial growth. Furthermore, the average of *Enhalus acoroides* seagrass extract with methanol solvent (polar) showed higher yields compared to average clear zone diameter of *Enhalus acoroides* seagrass extract in ethyl solvent acetate (semi polar) and n-hexane (non polar). This is caused by differences in the polarity of the solvent in the adsorbing compounds on seagrass *Enhalus acoroides*. The choice of solvent matters the effectiveness of the content of bioactive compounds that can be extracted from seagrass during extraction. Based on the selective toxicity of antibacterials divided into two, antibacterial which inhibits the growth of bacteria and antibacterial which kills bacteria. Antibacterial as bactericidal and very bacteriostatic depending on the dose/active power of the given antibacterial concentration. Furthermore, antibacterial can be increased from bacteriostatic becomes bactericidal when the level of antibacterial is increased (Jonathan *et al.,* 2019). The inhibition zone formed on an incubation time of 48 hours has a smaller diameter than an incubation time of 24 hours. This shows that the antibacterial activity of seagrass extract *Enhalus acoroides* against *Salmonella typhi* bacteria bacteriostatic (inhibits bacterial growth).

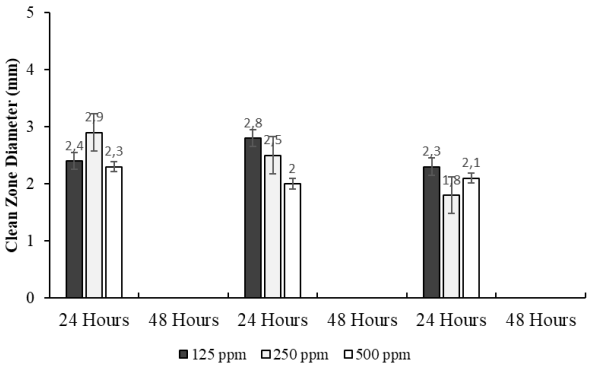

Figure 2. Antibacterial of seagrass extract *Enhalus acoroides* against *Salmonella typhi*

The antibacterial properties of *Enhalus acoroides* seagrass extract are obtained from several sources compounds, such as alkaloids, flavonoids, saponins, tannins and triterpenoids. Alkaloids has the ability as an antibacterial. Mechanism of action of alkaloids as antibacterial activity is interfering with the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed completely and cause the death of the cell (Hidanah *et al.,* 2022).

Mechanism of action of flavonoids in inhibiting bacterial growth by inactivation of proteins (enzymes) in the cell membrane resulting in causing the protein structure to be damaged. Instability of the cell wall and the bacterial cytoplasmic membrane causes a selective permeability function, active transport function, controlling the arrangement of proteins from bacterial cells to disturbed which will result in the loss of macromolecules and ions from the cell that the bacterial cells lose their shape and lysis occurs (Nourbakhsh *et al.*, 2022). Saponin compounds can carry out inhibition mechanisms by how to form complex compounds with cell membranes, it can destroy the permeability properties of the cell wall and can eventually cause cell death. Damage to the cell membrane is very disturbing continuity living bacteria (Campos *et al.,* 2009). Tannins have antibacterial activity associated with its ability to inactivate microbial cell adhesin, inactivates enzymes, and interfere with protein transport in the inner lining of cells. Tannins has a target on the cell wall polypeptide so that the formation of the wall cells become imperfect. This causes the bacterial cell to lyse due to

osmotic and physical pressure so that the bacterial cells will die (Wong *et al.*, 2019). The mechanism of triterpenoids as an antibacterial is to react with transmembrane protein on the outer membrane of the bacterial cell wall, forming bonds strong polymer that results in damage to the transmembrane protein. Damage to the transmembrane protein which is the entry and exit point compound will reduce the permeability of the bacterial cell wall and result in Bacterial cells will lack nutrients, so that bacterial growth is inhibited (Cowan M, 1999).

4. CONCLUSION

This study concluded that the influence of polarity types solvent on antibacterial activity of seagrass extract *Enhalus acoroides* against the growth of *Salmonella typhi* bacteria shows the composition of the bioactive compounds contained in *Enhalus acoroides* seagrass extract such as, alkaloids, flavonoids, saponins, tannins and triterpenoids. The biggest Zone of Inhibition is formed in seagrass extract with solvent methanol (polar) compared to ethyl acetate (semi polar) and n-hexane (non polar). Moreover, the zone of Inhibition occurs in seagrass extract *Enhalus acoroides* against *Salmonella typhi* bacteria showed that the antibacterial bioactives are bacteriostatic

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