The antibacterial activity of seagrass *Enhalus acoroides* against *Staphylococcus aureus*

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Abstract. *Enhalus acoroides* is a type of seagrass found in the waters surrounding the Morotai Islands but has not been economically exploited. *Enhalus acoroides* can be used to treat ulcers, acne medications, and cosmetics, among other things, due to their antibacterial activity against a variety of bacteria, one of which is *Staphylococcus aureus*. *Staphylococcus aureus* can cause respiratory and gastrointestinal tract infections. *Staphylococcus aureus* can infect any tissue in the body, causing damage to skin tissue. This study aimed to determine the antibacterial activity of *E. acoroides* against *S. aureus* and characterize the group of bioactive compounds found in the *E. acoroides* extract. This study took place between September and October of 2018. *Enhalus acoroides* samples were collected from the waters surrounding Morotai island. Extraction of *E. acoroides* in maceration using methanol and n-hexane solvents and qualitative identification of bioactive compounds including alkaloid compounds, flavonoids, steroids, and saponins. The results indicated that the yield of *E. acoroides* n-hexane extract was 0.35 percent and Methanol extract was 5.91 percent. Methanol extract *E. acoroides* (5.9 mm in diameter)1000 ppm is more antibacterial than n-hexane extract *E. acoroides* (5.6 mm in diameter). The Methanol extract of *E. acoroides* contains alkaloids, flavonoids, saponins, and steroids, whereas the n-hexane extract of *E. acoroides* contains steroids and flavonoids.

1. Introduction
Seagrass is a pressing plant in coastal areas, breeding and providing habitat for various marine biota, acting as feeding, breeding, and maintenance for numerous marine biota, including rare species[1]. Seagrass is found in shallow marine environments, estuaries with high salt content, constant puddles, and areas with open water at low tide. It is commonly found on sand substrates, muddy sand, mud, and coral shards[2].

Seagrass is frequently used in the surrounding community as compost, webbing, and animal feed. However, seagrass is used as a cosmetic ingredient, medicine and in other pharmaceutical fields in developed countries. It is well established that seagrass contains active compounds that are highly beneficial in the pharmaceutical field, which acts as an antibacterial. Halophila ovalis seagrass extract has antibacterial activity against the bacteria *Staphylococcus aureus*, *Bacillus carreus*, *Escherichia coli*, and *Salmonella paratyphi* [3].
Seagrass Enhalus acoroides have not been widely utilized economically on Morotai Island, for example, for the bioprospecting it contains. Enhalus acoroides extract is antibacterial against Vibrio harveyii, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, and Aeromonas hydrophila[4][5]. Additionally, seagrass methanol extract has been shown to have antifungal and antibacterial activity against various gram-positive bacteria[6].

The antibacterial activity of E. acoroides is due to the presence of bioactive compounds. Environmental conditions affect the production of bioactive compounds (environmental stress). There are differences in the yields of laor extract from three villages on Morotai Island[7]. Organic solvents such as methanol and n-hexane can be used to extract these bioactive compounds[8][9].

Staphylococcus aureus is a bacteria that causes disease in humans by causing damage to skin tissue[10]. Ulcers, acne, impetigo, and wound infections are just a few of the infectious diseases caused by Staphylococcus aureus. Staphylococcus aureus is also the most common nosocomial pathogen and the cause of food poisoning and toxic shock syndrome [11]. As a result, additional research is needed to identify antibacterial candidates for Staphylococcus aureus, particularly those derived from natural materials.

This study aims to determine the antibacterial activity of n-hexane extract and methanol Enhalus acoroides against Staphylococcus aureus and identify the bioactive compound groups contained in n-hexane extract and methanol Enhalus acoroides.

2. Research methods
2.1. Time and place of research
The study took place between July and October 2018. Fresh E. acoroides collection from the waters of Morotai. Bioactive compound extraction and identification of E. acoroides were conducted at Morotai Pacific University's FPIK Laboratory. The laboratory of MIPA University of Papua conducted an antibacterial power test on sea grass against Staphylococcus aureus.

2.2. Research procedure
2.2.1. Seagrass collection
Samples of E. acoroides are taken in Morotai Waters and then washed with clean water; the sample is then dried for one week in the sun until completely dry; the sample is then rendered.

2.2.2. Seagrass extraction
The sample was weighed at 120 grams and then placed in a jar with methanol and n-hexane solvents separately, then extracted for two days, shaking the extract 2-3 times each day. After extracting, the sample was filtered and evaporated and then scraped.

2.2.3. Antibacterial test
Antibacterial tests are conducted using the diffusion method and nutrient agar (NA) media. Up to 20 ml of NA media is poured into a sterile Petri disk. Then, using the spread method, inoculate a Petri dish with up to 75 ml culture bacteria in liquid media incubated for one day. Following that, a few paper disks are placed. On the surface, so that 25 ml of seagrass extract can be tested. After that, the media is incubated at room temperature for two consecutive 24-hour periods. The observations are made by observing the formation of clear zones and quantifying them using the truce period.

2.2.4. Identification of bioactive compounds
The identification of bioactive compounds is being conducted to ascertain the number of bioactive compounds present in seagrass. Alkaloid tests, flavonoids, saponins, and steroids are all performed.
2.3. Data analysis
The data obtained in the form of seagrass extract and the results of bioactive compound identification are displayed in drawings and tables and are then analyzed descriptively. The following formula is used to calculate the additional extract weight as a percentage of the yield (percent) extract.

\[
\text{Yield (\%)} = \frac{\text{extract (g)}}{\text{simplicia (g)}} \times 100\%
\]

3. Results and discussions

3.1. Ekstraksi Enhalus acoroides
Enhalus acoroides are extracted using the maceration method. The maceration method was chosen because it avoids high temperatures (heat) during the extraction process, thereby avoiding potential damage to thermolabile bioactive compounds. The extraction process is carried out using n-hexane solvents and methanol. Methanol solvents are most widely used to isolate organic compounds of natural materials [12]. The extraction results of Enhalus acoroides can be seen in Table 1.

| No | Solvent | Yield (g) | Rendemen (%) | Details                |
|----|---------|-----------|--------------|------------------------|
| 1  | Methanol| 7,10      | 5,91         | Dark green color       |
| 2  | n-hexane| 0,42      | 0,35         | Yellowish green color  |

The study's findings indicate that extract yields are higher when methanol solvents are used (5.91 percent) than when n-hexane solvents are used (0.35 percent). They indicate that bioactive compounds in Enhalus acoroides are more polar and thus solvent-soluble. The extract yield will be increased when methanol solvents are used [13][14]. Thalasedendron ciliatum extract yields more in methanol solvents than in n-hexane solvents [15]. They are due to the solvent dissolving / dissolving bioactive compounds from the sample wherever it is. Methanol is a nonpolar (-OH) and polar (-CH3) solvent that attracts various bioactive compounds, particularly polar and nonpolar compounds[8][16].

3.2. Enhalus acoroides test for antibacterial activity
In order to determine the antibacterial activity of Enhalus acoroides against Staphylococcus aureus, the diffusion method is used. Antibacterial activity is determined by the presence of clear or bland zones surrounding the paper disc. The larger the clear zone formed, the stronger the extract's antibacterial activity. Table 2 demonstrates the subtlety of acoroides against Staphylococcus aureus.

| Solvent | Concentration | Clear Zone Diameter (mm) |
|---------|---------------|--------------------------|
| Control | Positif       | 19,5                     |
|         | Negatif       | 0                        |
| Methanol| 1000 ppm      | 5,9                      |
|         | 500 ppm       | 5,2                      |
|         | 250 ppm       | 0                        |
| n-hexane| 1000 ppm      | 5,6                      |
|         | 500 ppm       | 5,2                      |
|         | 250 ppm       | 0                        |

The above antibacterial activity test results indicated that a 1000 ppm methanol extract of Enhalus acoroides has more excellent antibacterial activity against Staphylococcus aureus (5.9 mm) than a 1000 ppm n-hexane extract of Enhalus acoroides (5.6 mm). Enhalus acoroides extract has a negligible antibacterial activity against Staphylococcus aureus. If the diameter of the bland zone is greater than
20 mm, the inhibitory activity is classified as very strong; if the diameter of the bland zone is less than 11-20 mm, the inhibitory activity is classified as vital; if the diameter of the bland zone is between 6-10 mm, the inhibitory activity is classified as moderate; and if the diameter of the bland zone is less than 5 mm, the inhibitory activity is classified as weak[17]. Additionally, other studies have demonstrated that *Enhalus acoroides* possess significant antibacterial activity (15.63 mg/ml) against *Staphylococcus aureus*[18].

The diameter of the formed bland zone also demonstrates the tastelessness of *Enhalus acoroides* extract against *Staphylococcus aureus* (Figure 1). Clear zones form at concentrations of 1000 and 500 parts per million, whereas extract treatments at 250 parts per million do not form clear zones. The water extract of *Enhalus acoroides* possesses no antibacterial activity against *Vibrio harveyii* or *Vibrio alginolitikus* [19]. Methanol extract *Enhalus acoroides* against *Staphylococcus aureus* bacteria due to compounds that inhibit bacterial growth and are also lethal. These compounds are likely to be used as lead compounds for various applications, most notably in the pharmaceutical and chemical industries.

![Figure 1](image.png)

**Figure 1.** By using the diffusion method, determine the antibacterial activity of *Enhalus acoroides* extracts against *Staphylococcus aureus*. The red arrow indicates the direction of the formed clear zone. a) Extract *E. acoroides* in methanol; b) Extract *E. acoroides* in n-hexane.

Several antimicrobial compounds found in seagrass include tannins, saponins, terpenes, alkaloids, and glycosides[20]. Numerous studies have established that alkaloids [21][22] and saponins [23] possess antibacterial bioactivity. The inability of an extract to inhibit some strains of test bacteria indicates the presence of resistance mechanisms to enzymatic inactivation, modification of the target site, or insufficient compound construction to produce inhibition [24]. Additionally, variations in extract activity can be attributed to the species-specific distribution of antimicrobial compounds.

Compounds from the alkaloid family are used as antibacterial and analgesic agents [25]. Compounds in this class are thought to be capable of interfering with the constituent components of peptidoglycans, resulting in the breakdown of bacteria's cell walls, which then results in death. Extracts containing steroid group compounds have been shown to have antibacterial and antifungal properties through the mechanism of damaging bacteria's cell membranes, thereby inhibiting their growth [26].

### 3.3. Identification of groups of bioactive compounds

The group of seagrass bioactive compounds is qualitatively identified by adding chemical reagents to the extract. When a specific group of compounds is added, the appropriate reaction produces a specific color, allowing the compounds to be identified. Alkaloids, flavonoids, saponins, and steroids have all been identified as bioactive compounds. Table 3 summarizes the results of the identification of the compound group *Enhalus acoroides*.

*Enhalus acoroides* contain alkaloid compounds, flavonoids, saponins, and steroids (methanol solvents) in addition to flavonoids and steroids (n-hexane solvents). *Enhalus acoroides* are a source of
alkaloids, flavonoids, steroids, saponins, phenols, and tannins. The number and composition of secondary metabolite compounds vary according to the type of extraction, the duration of the extraction, the temperature, the natural state of the solvent, the solvent construct, and the polarity of the solvent. As demonstrated in this study, the polarity of the solvents had an effect on the number and type of secondary metabolite compounds obtained. Additionally, the composition of compounds in plants can be influenced by various factors, including climate variations, habitats, soil nutrients, and the time of plant harvesting. The solvent used in the extraction process and the conditions under which the extract is prepared can then affect the compounds in the extract being tested.

### Table 3. Bioactive compounds identified *Enhalus acoroides*

| No | Bioactive Compounds | Solvents |
|----|---------------------|----------|
|    |                     | Methanol | n-hexane |
| 1  | Alkaloids           | +        | -        |
| 2  | Flavonoids          | +        | +        |
| 3  | Saponins            | +        | -        |
| 4  | Steroids            | +        | +        |

Alkaloids are the most abundant class of bioactive compounds with toxic properties, frequently used in medicine. The compound possesses antibacterial and anti-infective properties. Alkaloids exhibit a wide range of pharmacological properties, including antihypertensive activity (many in indole alkaloids), antiarrhythmic activity (quinidine, sparteine), antimalarial activity (quinine), and anticancer activity (many in indole dimer, vincristine, and vinblastin).

Flavonoids have been shown to have a variety of beneficial properties, including anti-inflammatory activity, enzyme inhibitory activity, antimicrobial activity, estrogenic activity, antiallergic activity, antioxidant activity, vascular and cytotoxic activity, and antitumor activity. Flavonoid compounds can inhibit bacterial growth by reducing the permeability of cell walls, improving liver function, and enabling them to act as antimicrobial and antiviral agents.

Saponins are soap-like surface-active compounds that can be detected by forming foams and exhale blood. The identification method of shuffling and observing the formation of stable foam is used because it is simple, quick, and does not require complex equipment or materials. Saponins are amphibian; these characteristics contribute to saponins forming foam, as saponins can form lipid bonds with cell membranes. Saponins can be found in methanol extract but not in n-hexane extract, as saponins dissolve in alcohol and water but not in nonpolar organic solvents such as n-hexane.

The positive reaction of triterpenoid and steroid tests is initially indicated by a red solution for a positive triterpenoid reaction and a blue and green solution for a positive steroid reaction. Water extracts, ethanol, methanol, chloroform, and ether all contain terpenoid compounds. Steroids, sterols, and glycosides are triterpenoids that exhibit anti-inflammatory, sedative, insecticide, and cytotoxic activity. Steroids are frequently used as primary ingredients in the manufacture of drugs to increase stamina. Additionally, steroid compounds have antibacterial and antifungal properties. The compound damages bacteria’s cell membranes and inhibits their growth.

### 4. Conclusion

The research results indicate that the yield of *Enhalus acoroides* extract in methanol solvent (5.91 percent) is significantly higher than that in n-hexane (0.35 percent). Methanol and n-hexane extract with a bland zone diameter of 5.9 mm and 5.6 mm, *Enhalus acoroides* have antibacterial activity against *Staphylococcus aureus*. *Enhalus acoroides* methanol extract contains alkaloids, flavonoids, saponins, and steroids, whereas *Enhalus acoroides* n-hexane extract contains steroids and flavonoids.

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