Antibacterial Activity of Extract Green Seaweed Ulva intestinalis in North Waters of Aceh

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Abstract. The appearance of bacterial resistance and pathogenic infections makes efforts to find new drugs as natural bacterial agents continue. One of them is through the utilization of marine organisms such as seaweed. Some studies show the content of seaweed bioactive compounds has potential in the pharmacological field. This study aims to determine the type of secondary metabolite compounds and antibacterial activity of ethanol extract of Ulva intestinalis. Seaweed extraction using the maceration method for 3 x 24 hours and antibacterial activities were tested by disc diffusion method. The results showed that there were 5 types of secondary metabolite compounds in the extract, namely alkaloids, flavonoids, glycosides, saponins, and steroids. The antibacterial activity test of seaweed extract against Propionibacterium acnes and Aeromonas hydrophila bacteria in the concentration range of 50% to 100% showed a moderate inhibition zone diameter.

1 Introduction

Indonesian waters have high biodiversity, so they are rich in marine products. One of the marine products that has the potential to be developed both for the food and pharmaceutical industries is seaweed [1]. The prospect of seaweed is supported by many bioactive compounds such as alkaloids, carotenoids, flavonoids, terpenes, tocopherols, peptides, polysaccharides and polyphenols [2]. Several studies reported that seaweed bioactive compound components can be antioxidants, antibacterial, anti-inflammatory, antidiabetic, antidiarrheal, antimicrobial, and antibacterial [3, 4, 5].

Several investigations have revealed that seaweed has antibacterial properties. Red seaweed E. cottoni and Sargassum sp. have antibacterial action against P. acnes [6], as has

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red seaweed *Gracilaria* sp. [7]. Brown seaweed exhibits antibacterial action against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus*, according to [8].

*Ulva intestinalis* is a species of green seaweed found in Indonesian waters that belongs to the *Ulva* sp group [9]. *Ulva intestinalis* bioactive substances include antibacterial, anticancer, and anti-larvicidal properties [10]. According [11] methanol, n-hexane, and dichloromethane extracts of *U. intestinalis* have antibacterial activity against *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, and *Listeria monocytogenes*. *U. intestinalis* from methanol, dichloromethane, and water extracts have antibacterial activity against *Vibrio alginolyticus*, *V. vulnificus*, and *V. parahaemolyticus*, according [12] even though many exhibit antibacterial activity, research articles on their efficiency in suppressing the growth of gram-positive bacteria (*Propionibacterium acnes*) and gram-negative bacteria (*Aeromonas hydrophila*) are scarce. In truth, both bacteria are harmful and could be detected in people and fish. This research aims to discover the bioactive components and antibacterial activity of an ethanol extract of *U. intestinalis* from northern Aceh waters against gram-positive and gram-negative bacteria.

## 2 Materials and Methods

### 2.1 Time and Place

This study was taking place during February and March of 2023. Green Seaweed (*Ulva intestinalis*) were collected at Ulee Lheue Beach in Banda Aceh's Meuraxa District. Extraction activities and phytochemical tests was conducted at Syiah Kuala University's Marine Chemistry and Biotechnology Laboratory, Faculty of Marine and Fisheries, and antibacterial activity tests was conducted at Syiah Kuala University's Microbiology Laboratory, Faculty of Mathematics and Natural Sciences.

![Fig. 1. Map of Sampling Location](image-url)
2.2 Tools and Material

The tools used are glassware, rotary vacuum evaporator, micropipette, analytical balance, UV-Vis spectrophotometer, incubator, hot plate, autoclave, tube rack, light microscope, needle ose, refrigerator, caliper, Hot place, and aluminum foil.

The materials used green seaweed (*Ulva Intestinalis*), ethanol, aquadest, DMSO, Clindamycin, Muller Hilton agar, nutritional agar, and bacterial tests for *P. acnes* and *A. hydrophila*.

2.3 Sample Extraction

A total of 100 grams of dried *U.intestinalis* green seaweed samples were macerated in ethanol for 3 x 34 hours at a sample-to-solvent ratio of 1:5. The extraction products were filtered using filter paper, evaporated using a rotary evaporator at 40 °C to generate a thick extract, and then phytochemical analyses were performed [13].

2.4 Phytochemical Test

Phytochemical examination examined green seaweed *Ulva Intestinalis* for saponins, alkaloids, tannins, phenolic, steroids, terpenoids, and flavonoids. Standardized methodologies were used for phytochemical screening [14].

2.5 Antibacterial Activity

2.5.1. Making agar media

Mixed 2 grammes of powdered nutrient agar (NA) with 100 mL of distilled water in an Erlenmeyer flask. Heat and stir on a hotplate until dissolved. Wrapped in plastic wrap, they were sterilised in an autoclave at 121°C for 15 minutes [15].

2.5.2. Bacteria regeneration

Isolates of *P. acnes* and *A. hydrophila* were inoculated at a concentration of 1 ose into 25 mL Muller Hinton Agar (MBA), then incubated for 6 hours at 28 °C in an incubator shaker. Fresh bacterial colonies were required for antibacterial action [15].

2.5.2 Antibacterial activity test

The disc method was used in this study to determine antibacterial activity. *Ulva intestinalis* crude extracts were created at concentrations of 25%, 50%, 75%, and 100%. The paper discs were tested for antibacterial activity by soaking them in the crude extract of *U.intestinalis* in each concentration variety and then drying them in the air. Furthermore, the paper disc was placed on the surface of the medium Nutrient agar (NA), which already included the test bacteria, in this example, *P. acnes* (gram-positive bacteria) and *A. Hydrophila* (gram-negative bacteria), and incubated at 37 °C for 24 hours. A caliper is used to measure the diameter of the produced clear space [16].
3 Results and Discussion

3.1 Phytochemical Screening Test

The results of the phytochemical test showed five bioactive compounds contained in the ethanol extract of *Ulva intestinalis*, namely alkaloids, flavonoids, glycosides, saponins, and terpenoids. The test results can be seen in Table 1 below.

<table>
<thead>
<tr>
<th>Metabolite secondary</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Wagner</td>
<td>Positive</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda</td>
<td>Positive</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Keller-Killiani</td>
<td>Positive</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing</td>
<td>Positive</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkowski</td>
<td>Positive</td>
</tr>
</tbody>
</table>

The crude extract of green seaweed (*U.Intestinalis*) showed positive results for the existence of alkaloids and Saponins compounds, as determined by phytochemical testing. According [17] and [18], alkaloid has antibacterial pharmacological effects. Additionally, saponins have antibacterial activity against pathogenic foodborne microorganisms [18].

3.2 Antibacterial Activity

Establish a clear area around the extract-containing paper disc to test antibacterial activity. [19] reported that the diffusion test examined the diameter of the clean zone, which is the extract's antibacterial compound's ability to suppress bacterial growth. The sensitivity of the cultured media organism, incubation circumstances (temperature, duration), and agar dispersion rate (media content and concentration) affect the inhibition zone [20]. Table 2 shows the inhibitory area for each green seaweed extract concentration.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Average Inhibitory Zone (mm) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Propionibacterium acnes</td>
</tr>
<tr>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>6.10</td>
</tr>
<tr>
<td>75</td>
<td>7.20</td>
</tr>
<tr>
<td>100</td>
<td>8.20</td>
</tr>
<tr>
<td>Clindamycin (+)</td>
<td>17.20</td>
</tr>
<tr>
<td>Ethanol (-)</td>
<td>0</td>
</tr>
</tbody>
</table>
Based on Table 2, the antibacterial test on ethanol extract of *Ulva intestinalis* against *P. acnes* and *A. hydrophila* showed moderate antibacterial activity in the concentration range of 50% to 100%. [21] states that the 5-10 mm inhibition zone range offers a moderate inhibitory response. The 25% concentration, the lowest concentration, cannot inhibit the growth of *P. acnes* and *A. hydrophila* bacteria. It is suspected that the active compound content of *Ulva intestinalis* is not strong enough to denature the bacterial cell wall, and the low concentration of the extract carries little active substance. Meanwhile, at concentrations of 50%, 75%, and 100%, a moderate inhibition zone began to form, characterized by an inhibition zone that enlarged as the concentration of the extract increased. [22] found that the suspension of bacterial turbidity and media agar thickness can impact the inhibition zone of bacteria growth.

In green seaweed extracts, bioactive substances like flavonoids, triterpenoids and saponins can also prevent bacterial growth. According [23], triterpenoid compounds have antibacterial effects because they include extensive polysaccharide groups and sulfates that can inhibit transport through bacteria's thicker cell membranes. Triterpenoids prevent the growth of bacteria by inhibiting DNA-binding enzymes [24].

Flavonoid substances affect energy metabolism and hinder bacterial growth. It also denatures protein associations, breaking membranes and cell walls and poisoning bacteria. Flavonoid chemicals' hydroxyl molecules interact with bacterial DNA, supporting their harmful effect [25, 26]. Saponin chemicals inhibit bacteria by lowering lipid tension by binding to lipopolysaccharides on cell walls, inducing lysis and death [27]. The latest research affirmed that the presence of phenolic compounds influences the antibacterial effectiveness of *Ulva sp* [28, 29].
4 Conclusion

The extract of ethanol from green seaweed (*Ulva intestinalis*) contains triterpenoid, alkaloid, glycoside, flavonoid, and saponin. Antibacterial activity assays against *P. acnes*, and *A. hydrophila* exhibited inhibited zone diameters from all moderate concentrations. While this research shows that *Ulva intestinalis* could have potential as a therapeutic agent.

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