Potential of Seaweed *Gracilaria* sp. As inhibitors of *Escherichia coli*, *Clostridium perfringens* and *Stapylococcus aureus*

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**Abstract.** The problem of resistance and infectious pathogenicity of bacteria to humans is new at now. The search for alternative new drug compounds from seaweed bioactive content as one of the new antibacterial sources. The purpose of this research is to utilize *Gracilaria* sp. on the antibacterial effectiveness of *Escherichia coli*, *Clostridium perfringens* and *Stapylococcus aureus*. The method used in an experimental laboratory. Extraction was done by maceration with n-hexane, ethyl acetate and ethanol. Test antibacterial activity by agar diffusion method. Phytochemical screening based on discoloration. Analysis of bacterial cell leakage based on spectrophotometer results. Yields of 8.08% (ethanol), 5.47% (ethyl acetate) and 1.10% (hexane). Phytochemical screening results contain 6 secondary metabolite compounds in the ethanol and hexane treatment and 7 compounds in the ethyl acetate treatment. The best activity test results on ethyl acetate solvent with inhibition zone of 33.54 mm (*Escherichia coli*), 24.12 mm (*Clostridium perfringens*) and 29.14 mm (*Stapylococcus aureus*). MIC value at 0.51%. The absorbance obtained was 0.178 to 1.898 at a wavelength of 260 nm and 0.149 to 1.328 at a wavelength of 280 nm.

**Key words:** Marine plants, multilevel solvent extraction, yield, inhibition zone, MIC values, *Escherichia coli* bacterial cell leakage, *Clostridium perfringens* and *Stapylococcus aureus*

1. Introduction

The generally, seaweed hydrocolloid compounds have been used as industrial materials such as agar, keraginan, and alginate. Improved function of the benefits of seaweed, in addition to its primary metabolite products, have been studied by secondary metabolite products. Among the secondary metabolites that have been extensively studied are bioactive substances which have the potential to be developed for antimicrobials. Antimicrobials developed include anti-bacterial, anti-fungal and anti-virus.⁹¹ One type of marine plant that is studied and developed for the use of secondary metabolites is *Gracilaria* sp.

The potential of marine plants abundant resources such as *Gracilaria* which is a type of red algae that can be found in almost all perairantropik. This type of seaweed plays a big role in the industrial and pharmaceutical fields, including in making agar. Algae of this type are now cultivated plants in ponds that are often found in Takalar, South Sulawesi, Wakatobi (Southeast Sulawesi) and Saparua (Maluku).⁴¹

From the results that secondary metabolites of *Silpau* seaweed (*Dictyoshaeria versluysii*) with ethanol (alkaloid, steroid, quinon, streptonoid and saponim), 8 secondary metabolite content with ethyl acetate seaweed (alkaloid, flavonoid, phenol), steroids, quinon, tannin, streptonoid and saponin), and 2 secondary metabolite contents of *Silpau* (*Dictyoshaeria versluysii*) by the treatment of hexane (streptonoid and saponim) marine plants that contain secondary metabolites such as footprints, seagrass,
Gracilaria debilis and Silpau. These plants have the potential to produce diverse bioactive metabolites with beneficial activities as antibacterial, antiviral, antifungal and cytotoxic [8].

The secondary metabolite compounds from plants, mainly from phenolic groups and terpenes in essential oils. Secondary metabolites are biosynthesized from many primary metabolites, including; amino acids, acetyl co-A, mevalonic acid, and intermediate metabolites. In addition, some natural antimicrobial compounds derived from plants include phytoalexin, organic acids, essential oils (atsiri), phenolics and several groups of plant pigments or similar compounds [9].

With the development of science and technology and biotechnology, it is known that natural control agents that do not cause adverse impacts on health, namely through the extraction of marine plants such as gracilaria sp. that the antibacterial extract of Gracilaria sp (Seaweed) against Escherichia coli and Staphylococcus aureus bacteria results in inhibition of E.coli bacterial growth of 14.33 ± 3.22 mm and S.aureus of 12.67 ± 2.08 mm with a MIC value of 0.05%[8]. In 2012 that there are 12 seaweed extracts that have antibacterial activity against P. aeruginosa, S.epidermis & M. luteus[13] and 2018 with Silpau with the best test results of the two solvents used namely ethanol and ethyl acetate is ethyl acetate which kills bacteria with the results of the zone of inhibition against E.coli by 2.9 ± 0.20 mm (Horse Tracks), amounting to 2, 8 ± 0.12 mm (Seagrass), amounting to 26 ± 0.16 mm (Gracilaria debilis) and 32 ± 0.08 mm (Silpau). The results of the test of inhibition zone activity against Salmonella bacteria were 30.5 ± 0.10 mm (Horse Tread), amounting to 2.5 ± 0.10 mm (Seagrass), amounting to 27.5 ± 0.16 mm (Gracilaria debilis) and 29 ± 0.18 mm (Silpau). The results of the inhibition zone activity test for Enterococci bacteria were 2.5 ± 0.10 mm (Horse Tread), amounting to 3.4 ± 0.30 mm (Seagrass), amounting to 38 ± 0.10 mm (Gracilaria debilis) and by 42 ± 0.18 mm (Silpau). The test results of inhibition zone activity against S. aureus bacteria were 2.5 ± 0.10 mm (Horse Tread), amounting to 3.4 ± 0.10 mm (Seagrass), amounting to 20 ± 0.12 mm (Gracilaria debilis) and 31 ± 0.10 mm (Silpau). The results of the test of inhibition zone activity against C. perfringens bacteria were 2.9 ± 0.20 mm (Horse Tread), by 2.7 ± 0.12 mm (Seagrass), by 26.5 ± 0.14 mm (Gracilaria debilis) and 32 ± 0.10 mm (Silpau). The results of the test of inhibition zone activity against the Pseudomonas bacteria were 2.7 ± 0.20 mm (Horse Tread), by 2.4 ± 0.14 mm (Seagrass), by 28 ± 0.10 mm (Gracilaria debilis) and by 26 ± 0.22 mm (Silpau).

The occurrence of bacterial inactivation which is the result of the interaction of an antibacterial compound with certain parts of bacterial cells causes a number of changes or damage to bacterial cells which can ultimately affect the function of cell metabolism and at the level of severe damage that can cause death in bacterial cells. This damage can cause damage to cell membrane permeability and cause leakage in intracellular components such as sodium glutamate, sodium hydrogen phosphate, nucleotides, potassium and organic phosphate [8].

With the development of existing research, this study was conducted aiming to utilize the Gracilaria sp. Extract. E. coli inhibitors, C. perfringens and S. aureus.

2. Materials and Method

2.1. Materials

The main ingredient used is seaweed gracilaria sp. The auxiliaries used are n-hexane, ethyl acetate, ethanol, H2SO4, FeCl3 5%, NaOH, amyl alcohol, chloroform, acetic acid, HCl, Meyer reagents, 70% alcohol, filter paper, nutrient agar (NA), nutrient broth (NB), paper discs, bacterial culture of Escherichia coli, Clostridium perfringens and Staphylococcus aureus.

2.2. Equipment

The equipment used is analytical balance, glass jars, mouthpieces, rotary evaporator vacuum brand Buchi, petridish, test tubes, cotton, media bottles, osse needles, tweezer, incubators, hot plates, autoclaves, bunsen, micro pipettes, calipers, colony counters , filter paper, EPPENDORF brand centrifuges, centrifuge tubes, shekers, and UV-VIS spectrophotometer brands Simadzu 2450.

2.3. Method

The method used in this study is the experimental method with the stages of its implementation include; cleaning of raw materials, extraction, phytochemical tests, antibacterial activity tests, determination of MIC values with concentrations of 0.01%, 0.26%, 0.51%, 0.76% and 1.01%. and Escherichia coli,
*Clostridium perfringens* and *Staphylococcus aureus* bacterial cell leak tests with concentrations of 0% MIC, 1% MIC and 2% MIC

### 2.4. Test Parameters

In this research objective observations were made on phytochemical screening, inhibition and leakage of bacterial cells *Escherichia coli, Clostridium perfringens* and *Staphylococcus aureus*

### 3. Results and Discussion

The results of the extraction of ethanol, ethyl acetate and hexane solvents obtained extract extraction, shown in the following table: (Table 1)

**Table 1. Yield of *Gracilaria* sp. Extract Using Ethanol Solvent, Ethyl Acetate and Hexane**

| Solvent Extract | Rendemen, % |
|-----------------|-------------|
| Ethanol         | 8.08        |
| Ethyl Acetate   | 5.47        |
| Hexane          | 1.1         |

Phytochemical screening results obtained as follows: (Table 2)

**Table 2. Phytochemical Screening Results of *Gracilaria* sp.**

| No. | Parameter   | n-hexane | Ethyl acetate | Ethanol |
|-----|-------------|----------|---------------|---------|
| 1   | Alkaloids   | +        | +             | +       |
| 2   | Flavonoids  | -        | +             | -       |
| 3   | Steroids    | +        | +             | +       |
| 4   | Phenols     | +        | +             | +       |
| 5   | Saponyms,   | +        | +             | +       |
| 6   | Terpenoids  | +        | +             | +       |
| 7   | Quinones    | +        | +             | +       |
| 8   | Tannin      | -        | -             | -       |

Antibacterial activity test extracts with ethanol, ethyl acetate and hexane solvents against *E.coli, C.perfringens* and *S.aureus* bacteria are shown in Figure 1 below:
**Figure 1.** Antibacterial activity test extracts with ethanol, ethyl acetate and hexane solvents against *E. coli, C. perfringens* and *S. aureus* bacteria.

The minimum value of inhibitory concentration (MIC) of gracilaroa sp. extract are shown in Table 3 below:

| Concentration, % | Inhibition Zone, mm |
|------------------|---------------------|
|                  | *E. coli* | *C. perfringens* | *S. aureus* |
| 0.01             | 0         | 0                | 0           |
| 0.26             | 0         | 0                | 0           |
| 0.51             | 9.62      | 1.03             | 8.04        |
| 0.76             | 9.78      | 1.34             | 8.15        |
| 1.01             | 10.09     | 1.66             | 8.28        |

Bacterial cell leakage test results are shown in Figure 2, 4 and 4 below:

**Figure 2.** *E. coli* bacterial cell leakage
Based on Table 1, the yield of *Graclaria sp.* ending 1.1 - 8.08%. The highest yield (8.08%) was the result of maceration with ethanol, 5.47% with ethyl acetate and the lowest (1.1%) with hexane. The use of different solvents gives different yields. With the differences in the results obtained, it shows that *Gracilaria sp.* soluble in ethanol which is a non-polar solvent, ethyl acetate (semi-polar) and a little soluble in hexane (polar). The nature of each solvent used will affect the solubility of *Gracilaria sp.* so the value of the extract obtained is in accordance with the solubility in the solvent used.

Based on Table 2, there are 6 secondary metabolites of *gracilaria* with ethanol and hexane treatment, namely alkaloids, steroids, phenols, saponyms, terpenoids and quinones. With ethyl acetate treatment, there are 7 content of secondary metabolites in *gracilaria sp.* namely alkaloids, flavonoids, phenols, saponyms, terpenoids and quinines.

In order to obtain earlier information about the content of secondary metabolite compounds group can be identified by phytochemical methods. In line with this, Robison (99) states that this method begins by isolating the content of secondary metabolites using extraction methods such as maceration and partition. To determine the class of compound carried out phytochemical screening is intended as a preliminary examination of the chemical content of plants. Another reason for telling Robinson that phytochemical is to determine the characteristics of active compound that cause toxic effects or beneficial effects shown by crude plants extracts when tested with a biological system. The use of phytochemical processes has an established role in all branches of plants science. Although this method
is important in all chemical and biochemical studies it has also been utilized in high school biology studies.

According to phytochemical analysis is part of the science of pharmacognosy that studies methods or ways of analyzing chemical contents contained in plants or animals as a whole or its parts, including how to isolate or separate them[10].

Inhibition zone for *E. coli* bacteria was 33.54 mm, against *C. perfringens* bacteria was 24.12 mm and *S. aureus* bacteria was 29.14 mm. and has bacteriocidal activity. This shows that there is inhibition of the three bacteria (*E.coli, C. perfringens* and *S. aureus*) in the treatment of ethyl acetate. Bacteria usually live in a semi-polar state. The treatment with ethyl acetate is better than the treatment of ethanol and hexane.

The results of the inhibition zone of *gracilaria* sp. with ethyl acetate solvents against (Fig. 1) that *E. coli* bacteria were 2.9 ± 0.20 mm (Horse tread) and 2.8 ± 0.12 mm (Seagrass) smaller than the results of Melki *et al.,* 2011 from *Gracilaria* sp. amounting to 14.33 ± 3.22 mm. and amounting to 26 ± 0.16 mm (*Gracilaria debilis*) and 32 ± 0.08 mm (*Silpau*) greater than Melki *et al.,* 2011. The results of the study on *S. aureus* bacteria were 2.5 ± 0.10 mm (Horse Tread) and 3.4 ± 0.10 mm (Seagrass) smaller than the results of Melki *et al.,* 2011 study of *Gracilaria* sp. 12.67 ± 2.08 mm and 30 ± 0.12 mm (*Gracilaria debilis*) and amounting to 31 ± 0.10 mm (*Silpau*) is greater than Melki *et al.,* 2011.

Based on Table 3, *gracilaria* sp. with ethyl acetate treatment which is more effective to inhibit the growth of *E. coli, C. perfringens* and *S. aureus* bacteria occurs at inhibition with the lowest concentration of 0.51%. The analysis of the obstacle zone data obtained was in accordance with the literature because the higher concentration caused the increase in diameter of the inhibition zone[12][14].

According to said that the antibacterial activity is said to be best if the same concentration test results in better antibacterial activity. The concentration of 100% is the concentration of pure extract so that the results of the inhibition zone diameter obtained is the result of the maximum inhibition zone diameter[12].

The different results are caused by the ability of each bacterium to fight antibacterial activity depending on the thickness and composition of the cell wall. According to there are differences in the composition and structure of cell walls in each bacterium. Gram-negative bacteria contain lipids, fats or substances such as fat in a higher percentage than those contained in Gram-positive bacteria. The cell wall of Gram negative bacteria is thinner than Gram positive bacteria. The structure of Gram negative bacteria has an outer membrane membrane that covers the thin layer of peptidoglycan, the outer structure of this peptidoglycan is a double layer containing phospholipids, proteins and lipopolysaccharides. Lipopolysaccharides are located in the outer layer and are characteristic of Gram negative bacteria[9]. While Gram-positive bacterial cells have a cell wall that consists of a thick layer of peptidoglycan which contains the compounds of the theoicate and lipiteichoic[11].

Strengthening the opinions, the response to the inhibition of microbial growth produced was influenced by the content of active compounds contained in seaweed *Gracilaria* sp. such as alkaloids, steroids, flavonoids, phenols, saponyns, terpenoids and quinons [14]. Obtaining the inhibitory zone values of the three solvents is included in the category of very strong strong inhibition (> 20). As the opinion that 10-20 mm clear zone diameters have strong inhibition, 5-10 mm clear zone diameters have moderate inhibition and <5 mm clear zone diameters have power inhibitory weak [15][14].

Absorbance of *E.coli* bacterial cell leakage with a concentration of 0 MIC at a wavelength of 260 nm by 0.500 and 0.400 at a wavelength of 280 nm, a concentration of 1% MIC was obtained at 0.900 at a wavelength of 260 nm and 0.660 at a wavelength of 280 nm and a concentration of 2 MIC% absorbance was 1,450 at a wavelength of 260 nm and 1,300 at a wavelength of 280 nm. For leakage of *C. perfringens* bacterial cells with a concentration of 0 MIC at a wavelength of 260 nm of 0.200 and 0.149 at a wavelength of 280 nm, a concentration of 1% MIC was 1,201 at a wavelength of 260 nm and 0.961 at a wavelength of 280 nm and a concentration of 2% MIC at 1,898 at a wavelength of 260 nm and 1,469 at a wavelength of 280 nm. For *S. aureus* bacterial cell leakage with a concentration of 0 MIC at a wavelength of 260 nm of 0.178 and 0.155 at a wavelength of 280 nm, a concentration of 1% MIC was obtained with an absorbance of 0.523 at a wavelength of 260 nm and at 0.451 at a wavelength of 280 nm and a concentration of 2 MIC% absorbance was 1,586 at wavelength 260 nm and 1,328 at wavelength 280 nm. wave 280 nm.

Leakage of intracellular components can be observed with spectrophotometers at wavelengths of 260 and 280 nm [4]. The compounds that can be absorbed at 260 nm wavelength are RNA and RNA
derivatives namely nucleotides and at wavelengths of 280 nm are identified as proteins. An increase in absorbance shows an increase in the amount of compounds released by cells. Increasing the amount of cell content found on the outer surface of the cell shows cell membrane damage or changes in cell membrane permeability. Fluid discharge from cells means the cell has leaked. Damage to bacterial cell walls due to interactions with antibacterial compounds can be studied with SEM (scanning electron microscope) [6].

4. Conclusion

Gracilaria sp. best effective on ethyl acetate solvents with inhibition zones of 33.54 mm (Escherichia coli), 24.12 mm (Clostridium perfringens) and 29.14 mm (Staphylococcus aureus) with MIC 0.51% and the absorbance obtained was 0.178 to 1.898 at a wavelength of 260 nm and 0.149 to 1,328 at a wavelength of 280 nm.

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