Identification of the potential of brown seaweed 
(phaeophyceae) as an antibacterial against the pathogenic bacterium vibrio spp

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Abstract. The high density of stocking as a consequence of increased aquaculture production turns out to have another effect, namely the disruption of the fish health system. The disruption of the health is due to the limited space and the unconsumed feed. The decline in water quality is followed by a decrease in endurance, making it easier for fish to contract the disease. Various types of antimicrobial, anti-inflammatory, antipyretic, anticoagulant, and other biological activities have been used to increase the survival of fish. Many natural ingredients can be used as antimicrobial, anti-inflammatory, antipyretic, anticoagulant, and other biological activities, including brown seaweed (Phaeophyceae). The study was conducted using the experimental method. Antibacterial testing followed the agar diffusion method. The results showed that there were three (3) types of brown seaweed (Phaeophyceae), which were potential as anti-bacterial against Vibrio alginolticus and Vibrio Harvey. Rosenvingea orientalis failed to show antibacterial capacity.

1. Introduction
An aquaculture is a form of maintenance and breeding of various kinds of aquatic animals or plants, which starts from the maintenance process to increase production, such as regular stocking, feeding, protection against predators against disease, and harvesting [1]. In intensive cultivation, many problems arise, such as the density of population density, the low quality, and quantity of feeding and water quality [2]. Types of diseases caused by pathogenic bacterial infections are vibriosis (Vibrio spp.), Red spot (Pseudomonas spp.), Furunculosis (Aeromonas salmonicida), Motile Aeromonas Septicemia by Aeromonas hydrophila and red-mouth disease by red-mouth disease Yersinia sp. [3].

Fish infected with these diseases can result in substantial economic losses, which, without treatment, can cause mass death of organisms. To overcome this problem, in addition to improving cultivation conditions, the use of antibiotics to treat bacterial infections is highly recommended. Seaweed or macroalgae is one of the marine organisms that play a role in the food chain cycle as a primary producer. Seaweed produces various compounds consisting of primary compounds, which are
compounds produced by living things and are essential for the process of cell metabolisms such as fuchosoids, vitamins, unsaturated fatty acids (UFA), and carbohydrates. Secondary compounds (secondary metabolites) are metabolites that are not essential for the growth of an organism and are found in a unique or different form between one species and another. Each organism usually produces different secondary metabolites such as terpenoids, steroids, coumarin, flavonoids, and alkaloids, the function of secondary metabolites is to defend themselves from unfavorable environmental conditions [4]. Secondary metabolites are a defense mechanism against pathogens, parasites, predators, competitors, and epibiota, and their production is highly dependent on biogeographic conditions [5]. The nature of secondary metabolites as a means of self-defense of marine organisms turned out to have enormous potential as a source of medicinal ingredients for various diseases [6].

One of the efforts undertaken is the use and use of antibacterial naturally, and information from the Indonesian Institute of Sciences has found the efficacy of some red algae that have the potential as pathogenic antibacterials, especially against *Vibrio* spp. Carrageenan from red algae has antimicrobial, anti-inflammatory, antipyretic, anticoagulant, and other biological activities.

In this study, brown algae (Phaeophyceae) were investigated by its antibacterial capacity. The results of this test can be developed to get the right type of seaweed to be used as a model of seaweed polyculture with fish. The presence of seaweed producing antibacterial is expected to reduce the number of pathogenic bacteria, to reduce the possibility of developing diseases that attack fish.

2. Research material

2.1. Place and time of research

This research was conducted in March-May 2018. Brown seaweeds were collected from Probolinggo, East Java, Indonesia. N-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), were analytically grades.

2.2. The procedure of research preparation

Seaweeds were cleaned of gravel and dirt. Before drying, the sample is weighed. During the drying process, seaweed is always turned upside down so that it is exposed to sunlight evenly so that it can dry evenly. The percentage of dry weight / wet weight of each seaweed is calculated by the following formula [7].

\[
D = \frac{T}{W} \times 100\%
\]

Dried seaweed is first mashed with a blender. The refined flour is then filtered to get uniform granules. Extraction is the process of separating solid or liquid organic compounds from their mixtures by utilizing the different solubility properties of each component with the help of particular solvents. The seaweed extraction process uses the maceration method with stirrer for 24 hours [8]. Maceration is a filtering process using the powder soaked in a solvent until it absorbs and softens the cell structure so that that soluble substance will dissolve [9]. Antibacterial activity testing was carried out by the agar diffusion method [8], on a 90 mm petri dish. For the culture of bacterial isolates, the Nutrient Agar II media was used with the following composition: Pancreatic Pepton 10.0 g; NaCl 5.0 g; Agar 12.0 g and 1000 ml distilled water. The making of culture media was 25 g NA dissolved in 1000 ml of distilled water. So that it is mixed with distilled water in an Erlenmeyer flask on a hot plate and then sterilized in an autoclave at 121°C for 20 minutes. Agar was then poured as much as 20 ml on a petri dish, after being solidly etched with as many bacteria as a needle and incubated for 24 hours at 37 °C in an incubator. For antibiotic testing, one bacterial isolate that had been cultured previously was taken as a needle and dissolved in 3 ml of a solution so that NA poured into a 90 mm Petri dish until it hardened.

Sterilized disks of 6 mm diameter filter paper were dropped with a test solution of 2 mg / 50 µL. The test solution is prepared by dissolving each of the crude extracts in the solvent used in the
extraction. Solvent served as a negative control. The solvent was evaporated at room temperature in a sterile state. After the solvent has evaporated, the filter disk is then placed on the agar surface. Before incubation, the plates are first placed in a cold cabinet at 4°C for 3 hours for the pre-diffusion process and then incubated at 37°C for 24 hours for bacteria.

2.3. Data analysis
As a positive control, tetracycline antibiotics are used, and for the negative control, appropriate solvent extracts are used. After the incubation period, the diameter of the inhibition zone or the bright area is measured using calipers. Activity calculation is done descriptively by measuring the diameter of the inhibition zone after 24 hours of the incubation period.

3. Result and discussion
The biomass yield of Brown seaweed (Phaeophyceae) is shown in Figure 1 below

![Figure 1](image)

**Figure 1.** The results of the biomass of Brown seaweed (Phaeophyceae) obtained from weighing wet and dry weight. dw=dry weight, ww= wet weight.

Based on Figure 1 above, the highest yields for both wet and dry weight were *Dictyopteris acrostichoides* weighing 973 gr ± 101.7 gr and the lowest *Rosenvingea orientalis* weighing 249.88 gr ± 46 gr. The percentage of dry biomass per wet biomass ranged from 9.37% to 18.41%. The highest percentage was shown by *R. orientalis* seaweed at 18.41%, while the lowest was shown by *Padina boergesenii* seaweed by 9.37%.

**Table 1.** Results of crude extracts from different biomass weights and solvent volumes.

| Species            | Biomass Weight | Solvent  | Solvent volume | Crude Extract |
|--------------------|----------------|----------|----------------|---------------|
| *Dictyopteris acrostichoides* | 25.42          | n-Hexane | 250            | 387.2         | 1.52          |
|                    | 25.42          | DCM      | 250            | 512.3         | 2.02          |
|                    | 25.42          | EtOAc    | 250            | 463.3         | 1.82          |
| *Padina boergesenii* | 12.23          | n-Hexane | 250            | 480.9         | 3.93          |
|                    | 12.23          | DCM      | 250            | 575.1         | 4.70          |
|                    | 12.23          | EtOAc    | 250            | 457.5         | 3.74          |
Based on Table 1. above obtained the highest yield for crude extracts, namely *Padina boergesenii* species with DCM solvent that is equal to 575.1 mg and the lowest in *Sargassum prisnaticum* species is 214.3 mg with n-hexane solvent. The percentage of crude extracts ranged from 1.24% to 4.70%. The highest percentage was shown by *Padina boergesenii* seaweed at 4.70% with DCM solvent, while the lowest was indicated by *Sargassum prisnaticum* seaweed by 1.24% with n-hexane solvent.

The antibacterial activity of Brown seaweed (*Phaeophyceae*) is carried out on two types, namely *Vibrio alginoliticus* and *Vibrio harveyii*. Data on the inhibitory antibacterial activity of Brown seaweed extract against *V. alginolticus* can be seen in Table 2.

**Table 2.** Results of Brown Seaweed Antibacterial Activity Against *V. alginoliticus* dan *V. harveyii*.

| Species Phaeophyceae | Solvent | Inhibition |
|----------------------|---------|------------|
|                      |         | *V. alginoliticus* | *V. harveyii* |
| *Dictyopteris acrostichoides* | n-hexane | 12 | 8 |
|                      | DCM     | 10 | 6 |
|                      | EtOAc   | -  | -  |
| *Padina boergesenii*  | n-hexane | -  | -  |
|                      | DCM     | 23 | -  |
|                      | EtOAc   | -  | -  |
| *Rosenvingea orientalis* | n-hexane | -  | -  |
|                      | DCM     | -  | -  |
|                      | EtOAc   | -  | -  |
| *Sargassum prisnaticum* | n-hexane | 8  | -  |
|                      | DCM     | 27 | -  |
|                      | EtOAc   | 23 | -  |

From Table 2, the above illustrates that of the four n-hexane extracts tested. Three extracts showed low inhibitory activity against *V. alginoliticus*. Meanwhile, six extracts showed inhibition against *V. alginolyticus*. 
Figure 2A showed that two extracts showed low inhibitory activity. The inhibitory activity was shown by the extracts of *Dictyopteris acrostichoides* and *Padina boergeseni*. EtOAc gave a low inhibitory effect. Brown seaweed (*Phaeophyceae*) has potency as a source of antibacterial metabolites. High water content reduces the antibacterial capacity [10]. The results showed that the Brown seaweed (*Phaeophyceae*) extracted using n-hexane had low bacteriostatic activity. While those extracted using DCM, showed excellently antibacterial activity. Bacteriostatic agents work by inhibiting protein synthesis by temporarily binding to an organism's ribosome [11]. The bonds are not so strong that when concentration and stability decrease.

The inhibitory activity of *D. acrostichoides* was recorded on two challenged bacteria. Phenolic compounds, tannins, iodine, auxin, and phenols are present in many extracts. The content of substances in *Sargassum prismaticum* extract, such as iodine, tannin, and phenol, are good in inhibiting bacterial growth, as evidenced by the large inhibitory zone [12].

Gram-negative bacteria contain large amounts of lipoproteins [13]. The presence of layers of cell walls in these bacteria affects the work activity of antibacterial substances. Bacterial cell growth can be disrupted by the phenol component of *S. prismaticum* and *D. acrostichoides* extracts. Phenols can denature proteins and damage cell membranes. Phenol and tannin can denature bacterial cells and inhibit cell membrane function (transporting substances from one cell to another cell). Furthermore, they inhibit acid synthesis nucleate so that bacterial growth can be inhibited [14]. The antibacterial activity was affected by the bioactive compound, the diffusion power, and the types of tested bacteria [15].

4. Conclusion
There are three brown seaweeds (*Phaeophyceae*), which have potency as anti-bacterial. *Dictyopteris acrostichoides*, *Padina boergesi*, and *Sargassum prismaticum*. While *R. orientalis* do not have potency as antibacterial compounds source.

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