Preliminary study: *Padina australis* Hauck's antibacterial activity and phytochemical test against pathogenic shrimp bacteria

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Abstract. The potential of aquaculture organism, Shrimp, need extra watchfulness to prevent it against the pathogenic bacteria attack. The use of synthetic antibiotics in shrimp can make pathogenic bacteria resistant and pollute the environment. Lately, Marine Natural Products (MNP) are other ways to overcome this problem. In this study, the MNP were extracted from macroalga *Padina australis* Hauck. The study aimed to examine the antibacterial activity of n-hexane, ethyl acetate, acetone, and ethanol crude extract against three pathogenic shrimp bacteria (*Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Aeromonas hydrophila*) and phytochemical screening, respectively. Ethyl acetate crude extracts of *Padina australis* Hauck showed that it could act as an antibacterial agent against *Vibrio harveyi* (1.76 mm), *Vibrio parahaemolyticus* (2.3 mm) and *Aeromonas hydrophila* (4.43 mm). The phytochemical tests revealed that *P. australis* Hauck contains a steroid, terpenoid, phenolic, tannin, and alkaloid compounds.

1. Introduction

Shrimp is one of the leading aquaculture organisms in South Sulawesi Province, Indonesia, that was under serious attention due to the prolonged bacterial pathogens in the last decades. Rahman et al. [1] examined types of pathogenic shrimp bacteria from a hatchery, market, and export. Several bacteria were found, i.e., *Escherichia coli*, *Klebsiella* spp., *Vibrio* spp., *Aeromonas* spp., *Pseudomonas* spp., *Listeria* spp., *Staphylococcus aureus*, and *Salmonella* spp. *Vibrio harveyi* bacteria, as high pathogenic bacteria in the vibriosis case, trigger rapid death in shrimp [2].

Prevention or treatment is needed as early as possible to overcome these bacterial problems. The use of synthetic antibiotics has proven to result in some adverse effects. Synthetic drugs are efficient to reform the pathogenic bacteria become resistant to bacteria, poison target species, and pollute the environment [3,4]. Therefore, there are no other ways to overcome them, namely, but utilizing natural products.

Natural products perform a significant role in the development of various drugs, especially from marine natural resources. Marine natural products (MNP) obtained from macroalgae contain a diverse source of secondary metabolites. This compound is beneficial as a popular drug for controlling unknown
diseases or resistant pathogenic bacteria [4]. Marine natural products used in this study are macroalgae, or commonly known as seaweed, a type of 

Padina australis Hauck.

Macroalgae have been shown to have antibacterial (bactericidal or bacteriostatic) agents. These bactericidal or bacteriostatic agents found in algae include terpenoids, phlorotannins, acrylic acids, and phenolic compounds. The others are steroids, halogenated ketones, alkanes, cyclic polysulphides, heterocyclic compounds, sterols, halogen-containing terpenoids, and acetylenes [5,6]. Prior studies, showed that Phaeophyta, 

P. australis Hauck, is potential as an antibacterial agent for shrimp, fish, and humans [7,8,9].

P. australis Hauck, especially in Lae-Lae Island, Makassar, grows in complex environmental conditions. Environmental conditions such as changing salinity of seawater, relatively high salinity, high heavy metal content, and susceptibility the surrounding organisms. The new environment causes seaweed to have the ability to persevere by producing distinctive secondary metabolites. Marine organisms will produce various secondary metabolites in response to environmental pressures, including predation, competition, and tides, that can inhibit or kill other microorganisms [4].

For this reason, it is necessary to evaluate the antibacterial activity of crude n-hexane, ethyl acetate, acetone, and ethanol extracts, along with a phytochemical screening. The crucial information about the antibacterial activity can be used as a source of nutritious chemicals, especially treating diseases caused by pathogenic bacteria in shrimp.

2. Materials and Methods

2.1. Sampling method

Brown algae samples (Padina australis Hauck) were collected by purposive sampling method on Lae-Lae Island, Makassar City, South Sulawesi Province, Indonesia. Samples were washed with sea water, fresh water, and distilled water to clean seaweed from salt, epiphytes, and other impurities. Once clean, samples were weighed in the wet weight, then dried. Powdering used a blender or grinder, and the powder extracts were stored in the freezer until use. The sample was then examined at the Productivity and Water Quality Laboratory, Faculty of Marine Science and Fisheries, Hasanuddin University, South Sulawesi Province.

2.2. Preparation of extraction

Samples, in the powder form, were extracted in various solvents based on the level of polarity. The extraction of secondary metabolites was carried out using n-hexane, ethyl acetate, acetone, and ethanol. The extraction procedure was conducted according to El Shafay et al. [10], with a ratio of powder extract and solvent to 1:8 (w:v).

2.3. Antibacterial analysis

Bacterial species were donated by Center for Brackish Water Aquaculture, Takalar, and Maros, South Sulawesi Province, Indonesia. This analysis used Vibrio harveyi (M-120), Aeromonas hydrophilla, and Vibrio parahaemolyticus (T-170). The turbidity of bacterial suspension was measured using the Chong et al. method [7]. Turbidity of the suspension was standardized against 0.5 McFarland using a spectrophotometer at a wavelength of 600 nm. The bacterial inoculum was 10⁸ cfu/ml.

Antibacterial activity tests included positive, negative control and antibacterial activity tests for seaweed extract. The positive control test was carried out using ciprofloxacin antibiotics, and the negative control test used solvents (DMSO). The method applied in this test is the agar diffusion method, according to Bauer, K. [11] with a little modification. Sterile paper discs with a diameter of 6 mm were soaked in extracts with various concentrations. The concentrations are 0.05 gr, 0.03 gr, and 0.02 gr (50µl /disc). The samples were incubated for 24 hours at 30°C. The distinct zone around the paper disc is a sign of bacterial activity. Every experiment was conducted three times. Inhibition zones >15 mm were declared as strong, from 8 to 15 mm as moderate, and 1 to 8 mm as weak activities [3].
2.4. Phytochemicals assay

A phytochemical identification test was used to determine the chemical content in a material qualitatively. Identification was carried out the alkaloid, flavonoid, saponin, steroid, terpenoid, tannin, and phenolic test based on the Harborne method [12] with little modification.

3. Result and Discussion

Results of the phytochemical tests (Table 1) showed that the crude extracts of Padina australis Hauck have several compounds. They are predominated by steroid, terpenoid, and phenolic compounds, followed by alkaloids and tannins. This result is in agreement with prior studies, that P. australis Hauck contains steroid compounds, terpenoids, phenols, and tannins. These compounds allow these plants to inhibit various pathogenic bacteria [13,14]. Following this outcome, the antibacterial activity of four crude extracts against Gram-negative bacteria has been performed (Table 2).

### Table 1. Results of phytochemicals test.

| No | Phytochemicals | Padina australis Hauck Extracts |
|----|----------------|---------------------------------|
|    |                | N-hexane | Ethyl acetate | Acetone | Ethanol |
| 1  | Alkaloid       | +        | +            | +       | +       |
| 2  | Flavonoid      | -        | -            | -       | -       |
| 3  | Steroid        | ++       | ++           | ++      | ++      |
| 4  | Terpenoid      | ++       | ++           | ++      | ++      |
| 5  | Phenol         | ++       | ++           | ++      | ++      |
| 6  | Saponin        | -        | -            | -       | -       |
| 7  | Tannin         | +        | +            | +       | +       |

Note: (-) Negative, (+) Weak positive, (++) Strong positive

The crude ethyl acetate extract of P. australis Hauck can inhibit all three Gram-negative bacteria. The highest inhibition value is found in Aeromonas hydrophilla compared to other bacteria, which is 4.43 mm at a concentration of 0.05 gr. While the highest inhibition values of Vibrio harveyi and Vibrio parahaemolyticus at a concentration of 0.03 gr were 1.76 mm and 2.30 mm. Crude acetone extract also showed antibacterial activity against V. parahaemolyticus with inhibition zone 1.07 mm at a concentration of 0.03 gr. The value of the inhibition zone P. australis Hauck obtained when adjusted to Bansemir et al. [3], was belong to the category of weak.

P. australis Hauck from brown algae is familiar as an active antibacterial, because of the presence of phenolic compounds and tannins. Polyphenols or phenolic compounds have antimicrobial potential against several Gram-positive and Gram-negative bacterial pathogens. Polyphenols are divided into phloroglucinols and phlorotannins. Phlorotannin is an oligomeric polyphenol of the phloroglucinol class compounds, with the addition of halogen or hydroxyl groups [4].

Phlorotannins consist of phloroglucinol (1), eckol (2), fucofuroeckol-A (3), phlorofucofuroeckol-A (4), dioxinodehydroeckol (5), 8,8'-bieckol (6), 7-phloro eckol (7), and dieckol (8) (Figures 1 and 2), have shown their activity as antibacterial [15]. Besides, Peng et al. [16], stated that phlorotannin contains phloroglucinol polymer and is only found in brown algae. This compound is divided into six main subclasses, including eckols, fuhalols, fucophloretol, fucols, phloretol, and isofohalos, which have biological activities as bactericidal.
Table 2. Average zone of inhibition for crude extracts *Padina australis* Hauck (paperless disc).

| Test samples          | Concentration (g/50 µl) | Inhibitory Zone (mm) |
|-----------------------|-------------------------|----------------------|
|                       |                         | Ah<sup>a</sup> | Vp<sup>b</sup> | Vh<sup>c</sup> |
| N-hexane              | 0,05                    | -          | -          | -          |
|                       | 0,03                    | -          | -          | -          |
|                       | 0,02                    | -          | -          | -          |
| *Ciprofloxacin*       | 40 ppm                  | 8.20       | 7.0        | 5.8        |
| Ethyl acetate         | 0,05                    | 4.43       | -          | -          |
|                       | 0,03                    | 2.06       | 2.30       | 1.76       |
|                       | 0,02                    | 1.54       | 1.23       | 1.23       |
| *Ciprofloxacin*       | 40 ppm                  | 9.41       | 6.0        | 9.3        |
| Acetone               | 0,05                    | -          | -          | -          |
|                       | 0,03                    | -          | 1.07       | -          |
|                       | 0,02                    | -          | -          | -          |
| *Ciprofloxacin*       | 40 ppm                  | 9.25       | 4.63       | 6.3        |
| Ethanol               | 0,05                    | -          | -          | -          |
|                       | 0,03                    | -          | -          | -          |
|                       | 0,02                    | -          | -          | -          |
| *Ciprofloxacin*       | 40 ppm                  | 8.70       | 8.5        | 8.0        |

<sup>a</sup> *Aeromonas hydrophilla*  
<sup>b</sup> *Vibrio parahaemolyticus*  
<sup>c</sup> *Vibrio harveyi*

Dieckol compound and phlorofucofuroeckol-A (Figure 3) are identified to be the most active as an antibacterial against several pathogenic bacteria [15,17,18]. Therefore, phlorotannin is considered to be a candidate drug for several pathogenic bacteria. The interactions of phlorotannins with bacterial protein pathogens have an essential role as the bactericidal agents. Besides phlorotannin, brown algae, also, contain fucoxanthin compounds, sulfated polysaccharides, sterols, polyunsaturated fatty acids, and soluble fibers that have antibacterial activity against certain bacteria [18,19].

![Chemical structures of phlorotannins 1-4](image-url)

Figure 1. Chemical structures of phlorotannins 1-4 [9].
There are some reasons that crude extracts of the *Padina australis* Hauck showed a weak inhibition. First, Gram-negative bacteria are insusceptible to compounds in crude extracts. Then, bacteria have a lipopolysaccharide (LPS) in hydrophilic cell wall structures. It blocked the permeation of hydrophobic oils/steroids, also prevented the aggregation of oils/steroids and extracts in cell membrane targets. Furthermore, crude extracts are yet composed of various compounds, however, that the active compounds are weak [3,20]. If the isolation of secondary metabolites is made possible, it can increase its bioactivity as antibacterial.

![Chemical structures of phlorotannins 5-8](image1)

**Figure 2.** Chemical structures of phlorotannins 5-8 [15].

![Chemical structures of (a) dieckol, and (b) phlorofucofuroeckol-A](image2)

**Figure 3.** Chemical structures of (a) dieckol, and (b) phlorofucofuroeckol-A [17].

The data can be used as initial information. Then, it can illustrate how much the potential of secondary metabolites from macroalgae in Lae-Lae Island as an antibacterial agent. The use of algae in
the future can represent as an alternative to conventional antibiotics in aquaculture. Furthermore, algae can be an answer to unsolved infectious diseases by discovering new drugs.

4. Conclusion

*Padina australis* Hauck crude extracts showed antibacterial activities using multilevel extraction. Ethyl acetate crude extract has the highest inhibition zone among other extracts against 3 Gram-negative bacteria. It should be mentioned that the phytochemical test showed that extracts consist of steroid, terpenoid, phenolic, tannin, and alkaloid. However, prior further antibacterial investigations regarding solvent, concentration extracts, and the component of the extracts.

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