Antibacterial Activity 96% Ethanol Extract of Brown Seaweed (*Padina australis*) from Poteran Island Madura against *Staphylococcus aureus* ATCC 25923

DN Nani Wijayanti*, Giftania Wardani Sudjarwo and Oki Nugraha Putra

Department of Pharmacy, Hang Tuah University, Surabaya, Indonesia.

*Corresponding Author E-mail: nani.wijayanti@hangtuah.ac.id

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(Brown seaweed is one of the natural resources of the sea which is very abundant and grows naturally on the coast of Indonesia, especially in Madura. Lately, disease due to infection is one of the problems in the health sector that continues to grow. Some microorganisms that cause infections include bacteria, viruses, fungi and protozoa. One of the bacteria that can cause infection is Staphylococcus aureus. Staphylococcus aureus is one of pathogenic bacteria that cause abnormalities and infections to skin. The more infections cause an increase in the use of antibiotics, the greater the risk of increasing the incidence of resistance. This encourages the research of sources for antibacterial medicines from natural materials, namely macro algae. One of the macro algae that have antibacterial activity is brown seaweed from Padina australis species. This study uses laboratory experimental methods aimed at proving the antibacterial activity of 96% ethanol extract of brown seaweed Padina australis from the Madura Islands against Staphylococcus aureus ATCC 25923. The antibacterial activity test was carried out by the well diffusion method. The results of the antibacterial activity test showed that ethanol extract of 96% brown seaweed Padina australis with concentrations of 20%, 15%, 10%, and 5% had antibacterial activity against Staphylococcus aureus ATCC 25923 in the medium to strong category. Statistical test results (Kruskal Wallis) showed that there was a significant difference between the concentration and the inhibited zone produced (p = 0.037).

**Keywords:** Antibacterial; Ethanol 96% Extract; Padina australis; maceration; *Staphylococcus aureus*; Well Diffusion Method.

Indonesia is the largest archipelago in the world with 17,504 islands and an area of sea waters of 5.8 million km² (consisting of the territorial sea area of 0.3 million km², area of archipelago waters 2.95 million km², and area of the Indonesian Exclusive Economic Zone (ZEEI) 2, 55 million km²). One of the potential marine biota in Indonesian waters whose existence is very abundant and grows naturally in Indonesian coastal waters, especially in Madura waters, but this potential has not been utilized optimally. In general, brown seaweed contains three types of hydrocolloids, namely: agar (jelly), alginate, and carrageenan. The bioactive content is widely used...
for the development of the pharmaceutical industry as an antibacterial, anti-tumor, anti-cancer and agrochemical industry especially for fungicides and herbicides.² Disease due to infection is one of the problems in the health sector that continues to grow. Infection can be transmitted from one person to another, from animals to humans. Some microorganisms that cause infections include bacteria, viruses, fungi and protozoa. One of the bacteria that can cause infection is Staphylococcus aureus. Staphylococcus aureus is a gram-positive bacteria belonging to the family Micrococaceae, round in diameter 0.7 - 1.2 µm, arranged in irregular groups such as grapes, facultative anaerobes, do not form spores, and immotile. Staphylococcus aureus is often found in human as pathogen causes a variety of clinical manifestation. It also causes multi-drug resistant strains such as MRSA (Methicillin-Resistant Staphylococcus aureus).⁵,⁶ Antibiotic resistance is a growing public health problem throughout the world. The number of resistance events has led to the exploration of new antibiotics as an effort to overcome this resistance problem. Prevention using natural materials is an alternative that can be done considering the World Health Organization (WHO) has recommended the use of natural medicines to deal with the maintenance of public health, prevention and treatment of chronic, degenerative and cancerous diseases.⁷ According to (Salem et al., 2011) several types of macro algae from the Phaeophyta division have antimicrobial activity, including Sargassum sp and Turbinaria sp. According to (Chio-Wei et al., 2011) macro-algae extracts of Padina australis and Laurencia nidifica types have antibacterial potential. Other studies with Padina australis samples from Totok Bay Waters show antibacterial activity against gram-positive Staphylococcus aureus and gram-negative bacteria Escherichia coli.⁸⁹,¹⁰ Rumengan et al., (2018) conducted a study on the antibacterial activity of Padina australis, showing that Padina australis had antibacterial activity against gram-positive bacteria Staphylococcus aureus as indicated by a MIC / inhibitory concentration value of 1210.0 ppm. Potential Padina australis activity as an antibacterial is associated with the content of phenol compounds and their derivatives (flavonoids) steroid compounds, terpenoids, polyphenols and saponins that have the potential as antibacterial.¹¹,¹² It is therefore important to assess the antibacterial activity of Padina australis against Staphylococcus aureus.

**MATERIAL AND METHODS**

The tools used in this study are analytical balance, a set of glassware, glass jars, grinding tools, rotary evaporators, tweezers, vortex, Petri dishes, incubators, gloves, masks, nurse cap, Laminar Air Flow (LAF), micropipettes and sterile holes. Materials used in this study include samples of Padina australis brown grass, ethanol 96% (for maceration), pure culture suspension of Staphylococcus aureus ATCC 25923 obtained from BBLK (Center of Health Laboratory) Surabaya, NA media (Nutrient Agar), chloramphenicol 0, 1%, and dimethylsulfoxide (DMSO) 0.1%. HCl 2 N, NaCl (Bratachem), Mayer and Wagner reagents, NH4OH 28% (Merck), methanol (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water, ethyl acetate (Merck), Dragendorf reagents, anhydrous acetic acid, H2SO4 (Merck), n-hexane (Merck), water. Rumengan et al., (2018) conducted a study on the antibacterial activity of Padina australis, showing that Padina australis had antibacterial activity against gram-positive bacteria Staphylococcus aureus as indicated by a MIC / inhibitory concentration value of 1210.0 ppm. Potential Padina australis activity as an antibacterial is associated with the content of phenol compounds and their derivatives (flavonoids) steroid compounds, terpenoids, polyphenols and saponins that have the potential as antibacterial.¹¹,¹² It is therefore important to assess the antibacterial activity of Padina australis against Staphylococcus aureus.

**Brown Seaweed Extraction (Padina australis)**

Padina australis simplex powder was extracted using the maceration method. Comparison of samples with solvents is 1: 4, 1: 3, and 1: 3 (w / v) for 3 days, then filtered to get the filtrate. Then the extract was concentrated using a rotary evaporator at 40° C until a thick extract was obtained. Preparation of Staphylococcus aureus ATCC 25923 Suspension

Preparation of bacterial suspension is done by taking the bacteria Staphylococcus aureus and suspended in 5 mL of 0.9% NaCl solution to obtain the same turbidity as Mc Farland 0.5.
Preparation of *Padina australis* Extract Concentration

Ethanol extract 96% was made with a concentration of 20%, 15%, 10% and 5% (w/v). Preparation of a parent standard solution of 20% with 2 grams of extract dissolved in 10 mL 0.1% DMSO. Then prepare work standards with concentrations of 20%, 15%, 10%, and 5% (w/v).

**Antibacterial Activity Test with Wells Diffusion Method**

In this method, a clear area is produced around the well. Antibacterial activity test is done by pouring the base layer media and seed layer into a sterile petri dish. Seed layer was added with 40 µL suspension of Escherichia coli ATCC 25922 which was adjusted to the standard turbidity of 0.5 Mc Farland and according to the transmittance of ± 25%. Then the well was made into the media and filled with 96% ethanol extract *Padina australis* as much as 40 µL with a concentration of 20%, 15%, 10%, and 5%. The same thing was done in the positive control treatment using 0.1% chloramphenicol solution and negative control using 0.1% DMSO solution. Then incubated at 37 °C for 24 hours, a clear zone was observed and measured using calipers.

**RESULTS AND DISCUSSION**

Determination conducted at the Faculty of Fisheries, Airlangga University, Surabaya stated that the sample used was *Padina australis*. *Padina australis* extraction with maceration method yields a yield of 1.23%. The yield value is related to the amount of bioactive content contained in a sample. Variations in the content of bioactive compounds in *Padina australis* can be caused by several factors including light intensity, nutrient concentration, salinity and grazing pressures. The yield of this study is directly proportional to the results of the study on *Padina sp* by 1.30%. According to Sangha et al., (2014), the percentage of yield produced from macro algae extraction using ethanol solvents ranges from 2-3%. The difference in yield of extracts from an ingredient is influenced by the extraction method, the size of the simplicia, the ratio of ingredients and solvents, type of solvent, extraction time, extraction temperature, age of harvest, and differences in habitat.

Phytochemical screening is conducted to determine the content of compounds in the sample. As a result for the screening can be seen in table 1. These results are following other studies.

**Table 1. Phytochemical Screening Ethanol Extract 96% *Padina australis***

<table>
<thead>
<tr>
<th>No.</th>
<th>Test</th>
<th>Reagents</th>
<th>Result</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloid</td>
<td>MayerWagner</td>
<td>++</td>
<td>White sediment formed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brown sediment formed</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoid</td>
<td>Wilstater</td>
<td>+</td>
<td>Red formed</td>
</tr>
<tr>
<td>3.</td>
<td>Terpenoid</td>
<td>Liebermann-burchard</td>
<td>+</td>
<td>Formed a red ring</td>
</tr>
<tr>
<td>4.</td>
<td>Steroid</td>
<td>Liebermann-burchard</td>
<td>+</td>
<td>Formed a green ring</td>
</tr>
<tr>
<td>5.</td>
<td>Saponin</td>
<td>Forth</td>
<td>+</td>
<td>Formed stable foam ± 10 minutes</td>
</tr>
<tr>
<td>6.</td>
<td>Polyphenols</td>
<td>FeCl₃ 1%</td>
<td>+</td>
<td>Blackish green formed</td>
</tr>
</tbody>
</table>

**Table 2. Diameter Data of 96% Ethanol Inhibitory Inhibited Zone**

<table>
<thead>
<tr>
<th>Ethanol Extract Concentration 96% <em>Padina australis</em> (%) b/v</th>
<th>Obstacles zone (mm)</th>
<th>Rate ± SD (mm)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.6 10.6 10.6</td>
<td>10.63 ± 0.15</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>11.1 11.1 11.1</td>
<td>11.73 ± 0.93</td>
<td>Strong</td>
<td></td>
</tr>
<tr>
<td>12.2 12.2 12.2</td>
<td>12.07 ± 0.51</td>
<td>Strong</td>
<td></td>
</tr>
<tr>
<td>14.8 14.8 14.8</td>
<td>14.37 ± 1.02</td>
<td>Strong</td>
<td></td>
</tr>
<tr>
<td>34.4 34.4 34.4</td>
<td>34.30 ± 0.17</td>
<td>Very strong</td>
<td></td>
</tr>
<tr>
<td>0 0 0</td>
<td>0 ± 0</td>
<td>Weak</td>
<td></td>
</tr>
</tbody>
</table>

Chloramphenicol 0.1% (positive control)
Ethanol 96% (negative control)
which state that *Padina australis* contains steroids, terpenoids, polyphenols, saponins, alkaloids, and flavonoids.\(^{11,16,17,18}\)

White sediment the results of the bacterial activity test showed that the chloramphenicol inhibition zone was 34.3 mm which is categorized based on the *Clinical and Laboratory Standard Institute* (CLSI, 2016) which explained the standard of antimicrobial sensitivity testing, it could be included in the sensitive category in inhibiting the growth of *Staphylococcus aureus* bacteria. In this study also obtained a large inhibitory zone of 96% ethanol negative control that is 0 mm which indicates that 96% ethanol does not have antibacterial activity against the *Staphylococcus epidermidis* bacteria. This is a following research that conducted by Chatterjee *et al*., (2006) which shows that 96% ethanol does not impede the testing of antibacterial activity against *Staphylococcus aureus*.\(^{19,20}\) This shows that the inhibition zone formed by *Padina australis* brown algae extract is caused by the content of active compounds in the extract. Besides, *Padina sp.* known to be more effective against gram-positive bacteria compared to gram-negative bacteria.\(^{21,22}\) This is related to the composition of cell walls in gram-positive bacteria more easily experienced because it is made from polysaccharides.\(^{23}\)

From the average diameter data of 96% *Padina australis* extract inhibition zone obtained as listed in table 2 shows that the higher the concentration of 96% *Padina australis* ethanol extract, the active compound content which is antibacterial is higher so that the ability to inhibit the growth of *Staphylococcus aureus* also getting bigger. This shows that the concentration of 96% *Padina australis* ethanol extract 5% (w / v) is in the medium category, while the concentration of 10%, 15%, 20% (w / v) is in the strong category.\(^{24}\) The difference in diameter of the growth inhibition zone of the *Staphylococcus aureus* bacteria obtained as in picture 1 and table 2 is due to the dilution of each concentration series. The higher the dilution, the less active ingredient contained therein so that the smaller the diameter of the inhibitory zone formed.\(^{25}\)

Inhibition zone diameter data obtained by statistical testing (Kruskal Wallis Test) which shows that there are significant differences with the value of \( p = 0.012 \) (\( p < 0.05 \)). Post hoc test was conducted to find out if there were significant differences between concentrations (Mann-Whitnet Test). The results showed that there were no significant differences between concentrations (\( p > 0.05 \)).

**CONCLUSION**

Ethanol extract 96% *Padina australis* can inhibit the growth of *Staphylococcus aureus* bacteria, characterized by increasing concentrations, the greater the diameter of the inhibitory zone produced.

**ACKNOWLEDGMENT**

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**Conflict of Interest**

No conflicts of interest, financial or otherwise, are declared by authors.

**REFERENCES**


