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

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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).[1] One of the potential marine biota in Indonesian waters is macro algae or known as seaweed which is taxonomically grouped into *Thallophyta* divisions.[2] East Java is one of the potential locations for the development of seaweed cultivation, namely in Pacitan, Banyuwangi and Sumenep.[3] Brown seaweed is one of the marine natural resources 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.[4] 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 *Micrococcaceae*, 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*.[8,9,10] 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. Merck), sulfuric acid anisaldehyde, HCl (p), chunks of magnesium, butanol, glacial acetic acid (Merck), 10% NaCl, gelatine, chloroform (Merck), FeCl3 (Merck), toluene (Merck), HNO3 (Merck), NaCl 10%, gelatine, chloroform (Merck), FeCl3 (Merck), toluene (Merck), HNO3 (Merck), chloroform (Merck), technical ethanol.

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^{o} 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 DUSCUSSION

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.[13] 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.[14] The yield of this study is directly proportional to the results of the study on *Padina sp* by 1.30%.[15] 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.[12]

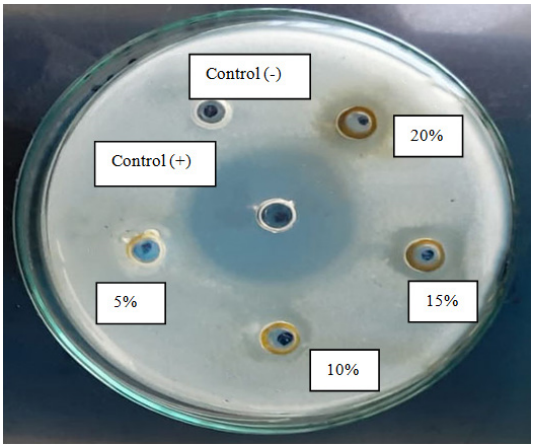
Phytochemical screening is conducted to determine the content of compounds in the sample. As a results for the screening can be seen in table 1. These results are following other studies

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

No.	Test	Reagents	Result	Information
1.	Alkaloid	MayerWagner	++	White sediment formedBrown sediment formed
2.	Flavonoid	Wilstater	+	Red formed
3.	Terpenoid	Liebermann-burchard	+	Formed a red ring
4.	Steroid	Liebermann-burchard	+	Formed a green ring
5.	Saponin	Forth	+	Formed stable foam ± 10 minutes
6.	Polyphenols	$FeCl_3$ 1%	+	Blackish green formed

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

Ethanol Extract Concentration 96% *Padina australis* (% b/v)	Obstacles zone (mm) Replication 1	2	3	Rate ± SD (mm)	Interpretation
5%	10.6	10.6	10.6	10.63 ± 0.15	Moderate
10%	11.1	11.1	11.1	11.73 ± 0.93	Strong
15%	12.2	12.2	12.2	12.07 ± 0.51	Strong
20%	14.8	14.8	14.8	14.37 ± 1.02	Strong
Chloramphenicol0,1% (positive control)	34.4	34.4	34.4	34.30 ± 0.17	Very strong
Ethanol 96% (negative control)	0	0	0	0 ± 0	Weak



Picture 1. Inhibition Zone of 96% Ethanol Extract of *Padina australis* (mm)

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.

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Conflict of Interest

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

REFERENCES

1. Kementrian Kelautan dan Perikanan / KKP. Kelautan Dan Perikanan Dalam Angka 2015. Jakarta : Pusat Data, Statistik dan Informasi Kementrian Kelauatan dan Perikanan (KKP). 2015.
2. Leandro, M, Pereira L, Goncalves, AMM. Diverse Apllications of Marine Macroalgae. *Mar.*

Drug. **18**(1): 17 (2020); https://doi.org/10.3390/md18010017.

3. Indriani H dan Suminarsih E. Budidaya, Pengolahan, dan Pemasaran Rumput Laut. Penebar Swadaya. Jakarta. (2003).
4. Bixler, H.J., & Porse, H. A decade of change in the seaweed hydrocolloids industry. *J. Appl. Phycol.,* (2010) DOI 10.1007/s10811-010-9529-3.
5. Spicer, WJ. Clinical Microbiology and Infectious Diseases (2nd ed.). Philladelphia : Elsivier, 2008.
6. Taylor, TA, Unakal, CG. Staphylococcus aureus. Bethesda: StatPearls Publishing., 2019.
7. WHO. Worldwidwide Situation Analysis Response to Antimicrobial Resistance. USA: Worl Health Organization, 2015.
8. El-Fatimy, ES., Said, AA. Aktivitas antibakteri ekstrak metanol pada alga laut (Padina Pavonia) dari Tolmeta Pesisir, Libya. *Journal of American Science,* **7**(4): 745-751 (2011).
9. Dulger, G dan Dulger, B. Aktivitas Antibakteri dari dua alga coklat (Cystoseira compressa dan Padina pavonica) Melawan Methicilin-Resistant Staphylococcus aureus. *Journal British Microbiology Research,* **4**(8): 918-923 (2014).
10. Ismail, A., Leilaktari, Mehboobahmed, Henkbolhuis. Aktivitas Antimikrobakteri diasosiasikan dengan alga coklat (Padina pavonica). *Journal Frontiers In Microbiology.* **7**(1027): 1-13 (2016).
11. Ke-Xin, Y, Norhisham, SN, Chean, HN. Antimicrobial Potetial of Padina australis and Sargassum polycystum against Respiratory Infections Causing Bacteria. *International Journal of Medical Toxicology and Legal Medicine;* **22** (2019). DOI: 10.5958/0974-4614.2019.00030.5.
12. Kumar A, Kumari SN, Bhargavan D. Evaluation of in vitro antioxidant potential of etanolic extract from the leaves of Achyranthes aspera. *Asian Journal of Pharmaceutical and Clinical Research.* **5**(3): 146-148 (2012).
13. Wijaya dkk. Perbandingan Metode Ekstraksi Terhadap Rendemen Ekstrak Daun Rambai Laut (Sonneratia caseolaris L.Engl). *Jurnal Ilmiah Manuntung,* **4**(1): 79-83 (2018).
14. James, BM, and Bill, JB. Marine Chamical Ecology. CRC press, Boca Raton London New York Washington, D.C., (2001)
15. Nuzul, P, *et al*. Uji Aktivitas Antibakteri Alga Coklat Jenis Padina sp. Dari Pantai Sorido Biak Terhadap Bakteri Staphylococcus aureus dan Shigella dysenteriae. Program Studi Farmasi, FMIPA Universitas Cenderawasih, Jayapura-Papua, (2018).
16. Imas, S, dkk. Efektivitas Ekstrak Padina australis Sebagai Antibakteri Echerichia coli Penyebab Penyakit Diare. Program Studi Biologi FMIPA Universitas Pakuan, (2014).
17. Latifah, LA, Soekamto, NH, Tahir, A. Preliminary study : Padina australis Hauck's antibacterial activity and phytochemical test against pathogenic shrimp bacteria, (2019) https://iopscience.iop.org/journal/1742-6596.
18. Maharany, F., Nurjanah, Suwandi, R., Anwar E., Hidayat T. Kandungan Senyawa Bioaktif Rumput Laut Padina Australis dan Eucheuma Cottonii Sebagai Bahan Baku Krim Tabir Surya. *Jurnal Jphpi.,* **20**(1):11-18 (2017).
19. Kandhasamy, M, Arunachalam, KD. Evaluation of in vitro antibacterial property of seaweeds of southeast coast of India. *African Journal of Biotechnology,* **7**(12): 1958-1961 (2008).
20. Dyrda, G, Boniewsk-bernacka, E, Man, D, Barchiewicz, K, Slota, R. The effect of organic solvents on selected microorganisms and model liposome membrane. 2019: https://link.springer.com/article/10.1007/s11033-019-04782.
21. Wadhwani, T, Desai, K, Patel, D, Lawani, D. Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobial. *Internet Journal of Microbiology,* **7**(1) (2009).
22. Taherpour A. dkk. Screening of marine algae (Padina sp.) from the Lengeh Port, Persian Gulf for antibacterial and antifungal activities. *Journal of Coastal Life Medicine,* **4**(9): 698-702 (2016).
23. Beeby, M, Gumbart, JC, Roux, B, Jensen, GJ. Architecture and assembly of the Gram-positive cell wall. *Mol Microbiol.,* **88**(84): 664-672 (2013). doi: 10.1111/mmi.12203.
24. Balouiri, M, Sadiki, M, Ibnsouda, SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.,* **6**(2): 71-79 (2016). doi: 10.1016/j.jpha.2015.11.005
25. Mastoda, AA, Bakri, MM. Antimicrobial activity od some plant extracts against bacterial strains causing food poisoninh disease. *Saudi Journal of Biological Sciences,* **25**(2): 361-366 (2018). https://doi.org/10.1016/j.sjbs.2017.02.004.
26. Salem, WM. Galal, H. Nasr, EF. Screening for antibacterial activities in some marine algae from The Red Sea (Hurghada, Egypt). *African Journal of Microbiology Research*, **5**(15): 2160-2167 (2011).
27. Chio-Wei, C, Ling, HS, Wong, CL. Antibacterial activity of Sargassum polycystum C. Agardh and Padina australis Hauck (Phaeophyceae). *African Journal of Biotechnoloy,* **10**(64) (2011). DOI: 10.5897/AJB11.966.
28. Rumengan *et al*. Antibacterial Analysis of Alga Padina australis Hauck in Totok Bay

and Blongko Waters. Manado: Program Studi IlmuKelautan, Fakultas Perikanan dan Ilmu Kelautan, Universitas Sam Ratulangi, (2018).

29. Sangha, J, Kelloway, S, Critchley, AT, Prithiviraj, B. Seaweeds (Macroalgae) and Their Extracts as Contributors of Plants Productivity and Quality : The Current Status of Our Understanding. New York: Elsivier Ltd., (2014): DOI: 10.1016/B978-0-12-408062-1.00007.
30. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. Clinical and Laboratory Institute, 2016.
31. Chatterjee, I, Somerville, GA, Heilmann, C, Sahl, HS, Maurer, HH, Herrmann, M. Very Low Ethanol Concentration Affect the Viability and Growth Recovery in Post-Stationary-Phase Staphylococcus aureus Populations. *App Enviro microbial.,* **72**(4): 2627-2636 (2006). doi: 10.1128/AEM.72.4.2627-2636.2006.