Potential extract of brown algae *Sargassum* sp. and *Padina* sp. as antibacterial *Vibrio harveyi*

To cite this article: M Assegaf and Insafitri 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1251** 012034

Potential extract of brown algae *Sargassum* sp. and *Padina* sp. as antibacterial *Vibrio harveyi*

M Assegaf* and Insafitri

Marine Science Study Program, Faculty of Agriculture, Trunojoyo University Madura. Jl. Raya Telang PO.BOX 2 Kamal, Bangkalan, 69162 Indonesia

*E-mail: maulidiyaassegaff@gmail.com

Abstract. *Vibrio* sp. in shrimp farming ponds cause infection with Vibriosis which results in crop failure. Various efforts have been made, such as the administration of antibiotics derived from chemicals that can cause resistance to bacteria. One of the other efforts that can be done is to use marine organisms as natural antibacterial agents, such as brown algae of the genus *Padina* and *Sargassum* which have antibacterial activity. The purpose of this study was to determine the best concentration of *Sargassum* sp. and *Padina* sp. in inhibiting the growth of *Vibrio harveyi* bacteria and knowing which brown algae extract was more effective in inhibiting the growth of *V. harveyi* bacteria. The extraction process was carried out using the maseration method with methanol as a solvent. Testing of antibacterial activity using agar diffusion method. The results showed that the extract of brown algae that was more effective in inhibiting the growth of *V. harveyi* was *Padina* sp., because the six treatments and positive control at 3 times of observation showed a clear zone that was greater than that of *Sargassum* sp. The best concentration of *Sargassum* sp. and *Padina* sp. in inhibiting the growth of *V. harveyi* bacteria was at a concentration of 2000 ppm for *Sargassum* sp. with the resulting inhibition zone of 18.2 ± 1.15 and 2000 ppm and 3000 ppm for the extract of *Padina* sp. with the resulting inhibition zones of 19.2 ± 2.75 and 19.5 ± 2.35. The resulting inhibition zone falls into the category of strong inhibitory activity.

Keywords: Antibacterial, Brown Algae, *Padina* sp., *Sargassum* sp., *Vibrio harveyi*

1. Introduction

Crop failure during shrimp production in ponds is caused by several factors such as weather, climate, floods and disease-related factors [1]. *Vibrio* is a type of pathogenic bacteria that causes disease in biota in ponds [2]. This bacterium is included in the opportunistic pathogen group which means a type of bacteria that attacks shrimp larvae during stress [3]. Vibriosis is a disease caused by *Vibrio* sp. [4]. Attacks of Vibriosis can cause death up to 100% in larval or juvenile stadia [5]. Clinical symptoms of shrimp affected by Vibriosis are red patches on the pleopod, uropod and abdominal, brownish-red gills and slow swimming [6]. *Vibrio* results in a decrease in the growth of cultivated organisms so that they can experience losses such as the production of shrimp from unsold ponds or the price becomes cheaper [7]. Efforts that can be made to overcome *Vibrio* growth can be done through the administration of antibiotics. The continuous and uncontrolled use of antibiotics provokes bacterial resistance to such drugs. Residues from antibiotics can pollute the waters which results in decreased water quality and causes toxic properties for the consumer's body. Some of the negative impacts caused by the use of these antibiotics resulted in the rejection of fishery products that were suspected to still use chemicals [8].

Another effort that can also be made to prevent *Vibrio* attacks is by giving bacterial cells that have been turned off [9]. This method is usually called immunostimulation. The disadvantage of this immunostimulation is that it is relatively more expensive, so it is necessary to conduct 2 studies to find other alternatives that are environmentally friendly and easily biodegradable in overcoming the outbreak of diseases caused by Vibrio, one of the alternatives that can be done to overcome the problem of disease is the use of seaweed or macroalgae as antibacterial ingredients [10].

Seaweed is one of the plants from marine waters that has a potential bioactive component as an antibacterial. Green, red, or brown seaweed is a potential source of bioactive compounds that are very beneficial for the development of the pharmaceutical industry such as antibacterial, antitumor and anticancer [11]. Some of the bioactive compounds contained in chocolate algae as the basic ingredients of the pharmaceutical industry include alginate, protein, vitamin C, tannins, iodine, phenols. Brown algae from the genus *Padina* and *Sargassum* are known to have antibacterial activity which indicates that both types of seaweed have the ability to suppress bacterial growth [12]. The potential of both seaweeds, is expected to function as an antibacterial that can control the growth of *V. harveyi* bacteria which are pathogenic bacteria in seawater aquaculture and are recognized as the main causative agent of *Vibrio* which often produces mass mortality in cultivated marine life [10].

Madura Island is an island surrounded by the ocean. Madura Coast is an area that gets very sufficient sunlight so that it can penetrate the depths of the waters. Madura Island is one of the islands that is known to be quite advanced in terms of fisheries and marine potential, both in the business segments of capture fisheries, aquaculture, coral reef ecosystems, mangroves and also the abundance of macroalgae. One of the waters on Madura Island that has a fairly complete marine ecosystem potential is Bangkalan Regency and Sumenep Regency [13]. Marine plants such as algae can grow and develop optimally in Madura waters, especially in Bangkalan Regency and Sumenep Regency, so that not a few brown algae are found in these waters [14].

The number of the two types of seaweed from the Genus *Sargassum* and *Padina* is found, it can be used as an antibacterial against *Vibrio* Bacteria. Based on the description above, it shows that the waters of Madura Island, especially Bangkalan Regency and Sumenep Regency, have great potential in producing *Sargassum* and *Padina*. The abundance of *Sargassum* and *Padina* in these two districts is not as similar to the utilization efforts made by the local community. So, it is necessary to conduct research related to the use of *Sargassum* and *Padina* as antibacterials to prevent the growth of *V. harveyi*. This is the reason behind the research on the Potential of Brown Algae Extract *Sargassum* sp. and *Padina* sp. as An Antibacterial *V. harveyi* was carried out at trunojoyo Madura University. Extract of *Sargassum* sp. and *Padina* sp. is expected to function as an antibacterial that can control the growth of *V. harveyi* bacteria which are pathogenic.

2. Materials and methods

Sampling was carried out at two precise points in the waters of Prancak Village, Sepulu District, Bangkalan Regency and Pak Jebe Beach, East Gedugan Village, Gili Genting District, Sumenep Regency and *Vibrio harveyi* Bacteria obtained from BPBAP Situbondo. Laboratory analysis of the Potential of Brown Algae Extract *Sargassum* sp. and *Padina* sp. as an Antibacterial against *V. harveyi* Bacteria, it is carried out at the Integrated Laboratory, Faculty of Agriculture and the Integrated Service Unit of Trunojoyo Madura University to be precise at the Basic Laboratory of Trunojoyo Madura University.

The extraction process is carried out using Methanol solvent, by introducing *Sargassum* sp. and *Padina* sp. powders that have been mashed into a container of 20 grams, then 100 ml of methanol solvent is added. Then immersion (maceration) is carried out for 3 x 24 hours, then filtration is carried out using whatman paper, so that residue and filtrate are produced [15]. After that, purification of *Vibrio* bacteria is carried out by making a medium to be oblique using Nutrient Agar (NA) as much as 2.3 grams and mixed with NaCl as much as 2.5 grams with 100 ml of aquadest, after that inoculation of *Vibrio* bacterial cultures on the test tube by means of zigzag scratches on the surface of oblique NA which is then incubated for 24 hours.

Next is the dilution of *Sargassum* and *Padina* extracts which is carried out by taking 0.1 g of *Sargassum* sp. extract and *Padina* sp. and mixed it with an aquadest solvent of 10 ml, after which a stratified dilution was carried out with concentrations of 100 ppm, 200 ppm and 300 ppm, 1000 ppm, 2000, 3000 ppm respectively and The positive control used was chloramphenicol, which was made with a concentration of 1 %.

Testing of antibacterial activity using the Kirby Baurer method by disc diffusion, which begins with an extract solution that has been made with each positive concentration and control is dripped on the surface of the disc paper by 20 µl and waited for a while for the solution to diffuse into the disc paper. After that, a total of 1 ml of test bacteria suspension was inoculated into a petri dish that had contained NA media, and the bacteria were flattened using a Spreader. Each paper disk is inoculated into a agar medium with a division distance of 4 quadrants, then incubated at a temperature of 37^{o}C for 24 hours. It further observes the diameter of the clear zone formed around the disc paper and measured by a caliper [16].

3. Results and discussion

3.1. Extraction results Sargassum sp. *and Padina* sp.

The yield of the resulting extract is the most important factor to know the number or least of organic compounds that dissolve during the maceration process. The maceration process of *Sargassum* sp. extract. and *Padina* sp. using a 100% methanol solvent. The following is a table of extraction results obtained at the time of the study presented in Table 1 and 2.

Table 1 Yield results *Sargassum* sp.

No	Name	Unit
1	Heavy Dry *Sargassum* sp.	20 grams
2	Extraction Results	5.54 grams
3	Yield	27.7%

Table 2 Yield results *Padina* sp.

No	**Name**	**Unit**
1	Heavy Dry *Padina* sp.	20 grams
2	Extraction Results	4.30 grams
3	Yield	21.5%

From the results obtained, the highest yield value was obtained in the extract of *Sargassum* sp. The yield of the extract of a material can be influenced by several factors, one of which is the concentration of the solvent used, the higher the concentration of the solvent used, the more yields are produced. The duration of maceration has an important role as well as the concentration of the solvent to the level of yield produced.

3.2. Activity antibacterial extract Sargassum sp. *and Padina* sp. *to Bacteria V. harveyi*

Test results of the inhibitory power of the extract *Sargassum* sp. And *Padina* sp. Against *V. harveyi* bacteria using the diffusion method, according to Kirby Baurer, it shows the presence of extract inhibitory activity in test bacteria characterized by the formation of clear zones in the disc paper area. Test results of antibacterial activity of *Sargassum* sp. Extract and *Padina* sp. Against *V. harveyi* presented in Table 3 and 4.

It can be seen in Table 3 of the inhibitory power of the extract of *Sargassum* sp. of 6 different concentrations with each of the 3 repetitions of the bacterium *V. harveyi* resulting in a different

size. Activity of *extract of Sargassum* sp. in the first measurement (1 x 24 hours) obtained the lowest value at a concentration of 100 ppm of 1.0 mm ± 0.00, 200 ppm of 1.0 mm ± 0.00, 300 ppm of 1.5 mm ± 0.87, 1000 ppm of 1.3 mm ± 0.58 and 2000 of 1.0 mm ± 0.00 ppm and obtained the highest value at a concentration of 3000 ppm of 18.1 mm ± 0.86 and a positive control of 18.1 ± 0.86. *Sargassum* sp. extract activity in the second measurement (2 x 24 hours) obtained the lowest value at a concentration of 100 ppm of 1.0 mm ± 0.00, 200 ppm of 1.0 mm ± 0.00, 300 ppm of 1.5 mm ± 0.87, 1000 ppm of 1.3 mm ± 0.58 and 2000 of 1.0 mm ± 0.00 ppm and obtained the highest value at a concentration of 3000 ppm of 1.3 mm ± 0.58 and 2000 of 1.0 mm ± 0.00 ppm and obtained the highest value at a concentration of 3000 ppm of 18.5 mm ± 0.84 and positive control of 18.5 mm ± 0.8.4. The activity of *Sargassum* sp. extract in the third measurement (3 x 24 hours) obtained the lowest value at a concentration of 100 ppm of 1.0 mm ± 0.00, 200 ppm of 1.0 mm ± 0.00, 300 ppm of 3.0 mm ± 0.29, 1000 ppm of 1.0 mm ± 0.00 and obtained the highest value at a concentration of 2000 of 16.5 mm ± 1.32 ppm, 3000 ppm of 18.5 mm ± 0.84 and positive control of 18.5 mm ± 0.8.4.

Table 3. Results of calculation of inhibitory power of *Sargassum* sp. extract during 3 observation times.

Treatment	Inhibition Zone Diameter (mm)					
	24 hours	Category	48 Hours	Category	72 Hours	Category
100 ppm	1.0 ± 0.00	Weak	1.0 ± 0.00	Weak	1.0 ± 0.00	Weak
200 ppm	1.0 ± 0.00	Weak	1.0 ± 0.00	Weak	1.0 ± 0.00	Weak
300 ppm	1.5±0.87	Weak	1.5±0.87	Weak	3.0±0.29	Weak
1000 ppm	1.3±0.58	Weak	1.3±0.58	Weak	1.0 ± 0.00	Weak
2000 ppm	1.0 ± 0.00	Weak	1.0 ± 0.00	Weak	18.2±1.15	Strong
3000 ppm	16.2 ± 0.76	Strong	16.2 ± 0.76	Strong	16.5±1.32	Strong
Positive Control	18.1±0.86	Strong	18.5±0.84	Strong	18.4 ± 1.02	Strong

The value shown in the table is the average value of the ± the standard deviation from the repetition three times.

It can be seen in Table 4 that the inhibitory power of *Padina* sp. extracts from 6 different concentrations with 3 repetitions of *V. harveyi* bacteria each resulted in different sizes. The activity of *the extract of Padina* sp. in the first measurement (1 x 24 hours) obtained the lowest value at a concentration of 200 ppm of 1.0 mm ± 0.00, 300 ppm of 1.0 mm ± 0.00, 1000 ppm of 1.2 mm ± 0.29 and obtained the highest value at a concentration of 2000 ppm of 12.3 ± 0.41, 3000 ppm of 17.3 mm ± 2.03 and a positive control of 19.3 mm ± 0.82. The activity of *Padina* sp. extract in the second measurement (2 x 24 hours) obtained the lowest value at a concentration of 200 ppm of 1.0 mm ± 0.00, 300 ppm of 2.0 mm ± 0.00, 1000 ppm of 2.0 mm ± 0.50 and obtained the highest value at a concentration of 2000 ppm of 12.3 ± 0.41, 3000 ppm of 19.8 mm ± 2.02 and a positive control of 19.6 mm ± 1.39. *Padina* sp. extract activity in the third measurement (3 x 24 hours) obtained the lowest value at a concentration of 200 ppm of 2.0 mm ± 0.00, 300 ppm of 2.7 mm ± 0.58, 1000 ppm of 2.0 mm ± 0.00 and obtained the highest value at a concentration of 2000 ppm of 12.7 ± 0.41, 3000 ppm of 19.2 mm ± 2.75 and a positive control of 19.5 mm ± 2.35.

The degree of effectiveness of the extract to inhibit the growth of test bacteria is seen from how much the extract is used and how large a clear zone is produced. Comparison of extracts *of Sargassum* sp. and *Padina* sp. at 1 x 24 hours observation is an extract of *Padina* sp. more effective in inhibiting the growth of *V. harveyi* because the clear zone formed is greater than *that of Sargassum* sp. extract, comparison of *Sargassum* sp. extract and *Padina* sp. at 2 x 24 hours observation was an extract of *Padina* sp. is more effective in inhibiting the growth of *V. harveyi* because the clear zone formed is larger than the extract of *Sargassum* sp. and comparison of *Sargassum* sp. extracts. and *Padina* sp. at 3 x 24 hour observations was an extract of *Padina* sp. is more effective in inhibiting the growth of *V. harveyi* because the clear zone formed is larger than the extract of *Sargassum* sp.

Table 4. Results of Calculation of inhibitory power of *Padina* sp. extract during 3 observation times.

Treatment	Inhibition Zone Diameter (mm)					
	24 hours	Category	48 Hours	Category	72 Hours	Category
100 ppm	9.8±7.5	Currently	10.0 ± 7.21	Currently	10.5±6.87	Strong
200 ppm	1.0 ± 0.00	Weak	1.0 ± 0.00	Weak	2.0 ± 0.00	Weak
300 ppm	1.0 ± 0.00	Weak	2.0 ± 0.00	Weak	2.7 ± 0.58	Weak
1000 ppm	1.2±0.29	Weak	2.0 ± 0.50	Weak	2.0 ± 0.00	Weak
2000 ppm	12.3 ± 0.41	Strong	12.3 ± 4.01	Strong	12.7 ± 4.01	Strong
3000 ppm	17.3±2.03	Strong	19.8 ± 2.02	Strong	19.2±2.75	Strong
Positive Control	19.3 ± 0.82	Strong	19.6±1.39	Strong	19.5±2.35	Strong

The values indicated in the table are the average values ± the standard deviation from the three-time repetition.

Generally, the diameter of the inhibitory zone tends to increase in proportion to the increase in the concentration of the extract, but in this study it occurs unlike in general and the resulting inhibitory zone is unstable at some concentrations. The diameter of the inhibition zone formed does not always rise in proportion to the increase in the concentration of the extract, this is due to the difference in the diffusion speed of antibacterial compounds in the agar medium and the different types and concentrations of antibacterial compounds also give different inhibitory zone diameters at a certain time. In addition, the difference in the diameter of the inhibitory zone in each treatment is influenced by several factors. These factors include the concentration of antibacterial compounds, the number of bacteria, the type of bacteria, and the temperature [17].

Research conducted shows that antibacterial activity is bacteriostatic, that an antimicrobial is bacteriostatic if the antimicrobial compound is only able to inhibit bacterial growth if the administration of the compound continues to be carried out and if it is stopped or exhausted, the growth and propagation of the bacteria will increase again which is characterized by a decrease in the diameter of the resistance zone [18]. The results of the study [19] showed that the mechanism of action of the antibacterial compound *Sargassum* sp. it is suspected by disrupting the peptidoglycan component in bacterial cells, so that the layers of the cell wall are not formed intact and cause the death of these cells. According to research [20] secondary metabolite compounds in marine algae that have the potential to be antibacterial are flavonoids, tannins, steroidal saponins. Flavonoid compounds are thought to have their mechanism of action to denature the proteins of bacterial cells and damage cell membranes irreparably. Flavonoids are also lipophilic which will damage microbial membranes. Inside flavonoids contain a phenol compound. The growth of *V. harveyi* bacteria can be disturbed due to phenol compounds. Phenol is an alcohol that is acidic so it is also called carbolic acid. Phenols have the ability to denature proteins and damage cell membranes. Acidic conditions by the presence of phenols can affect the growth of bacteria. Flavonoids can function as antibacterials because they can damage the cell walls of bacteria.

The most optimal result in *Padina* sp. was found at a concentration of 3000 ppm with an observation time of 72 hours, which was 19.8 ± 2.02. *Padina* sp. extract was more optimal at the time of the observation, it is suspected because at this concentration the content of antibacterial compounds that have the potential to be antibacterial is sufficient. An observation time of 72 hours indicates more effective inhibition zone activity compared to a 24- and 48-hour embedding time. *Padina* sp. contains bioactive compounds that have the potential to be antibacterial. The results of phytochemical test studies with acetone extract obtained that *Padina* sp. contains steroid compounds, terpenoids, polyphenols and saponins that have the potential to be antibacterial [21].

Data testing is carried out using the Mann Whitney Test, this test is another alternative to the T test because the requirements for using the T test are not met, that is, the data is not distributed normally. This test is used to find out if there is a noticeable difference between the 3 observation times and also the difference in clear zones produced by *Sargassum* sp. and *Padina* sp. The results obtained showed that

the clear zones produced at all three observation times had no difference and the clear zones produced by *Sargassum* sp extract. and *Padina* sp. has differences. The difference can be seen in the results of the Sig (2-tailed) values shown in Table 5.

Table 5 Mann Whitney Test Results 1 x 24 hours *Sargassum* sp. and *Padina* sp .

Concentration	Mann-Whitney Test Sig Value		
	24 hours	48 Hours	72 Hours
100 ppm	0.037	0.037	0.037
200 ppm	1	1	0.025
300 ppm	0.317	0.48	0.043
1000 ppm	0.796	0.178	0.025
2000 ppm	0.037	0.037	0.046
3000 ppm	0.513	0.051	0.184
Control (+)	0.042	0.118	0.198

Based on Table 5 the results obtained from the Mann Whitney Test in time 1 x 24 hour observation shows that at concentrations of 100 ppm, 2000 ppm and control positive extract *Sargassum* sp. and *Padina* sp. show that sig value (2-tailed) 0.037 < 0.05 equal with Reject H0, which means there is the difference in the diameter of the clear zone produced in the extract *Sargassum* sp. and *Padina* sp., the results obtained from the *Mann Whitney* Test on time 2 x 24 hour observations show that at concentrations of 100 ppm and 2000 ppm extract *Sargassum* sp. and *Padina* sp. show that sig value (2-tailed) 0.037 < 0.05 equal with Reject H0, which means there is the difference in the diameter of the clear zone produced in the extract *Sargassum* sp. and *Padina* sp. and the results obtained from the *Mann Whitney* Test on time 3 x 24 hour observations show that at concentrations of 100 ppm, 200 ppm, 300 ppm, 1000 ppm, and 2000 ppm extract *Sargassum* sp. and *Padina* sp. show that sig value (2-tailed) 0.037 < 0.05 equal with Reject H0, which means there is the difference in the diameter of the clear zone produced in the extract *Sargassum* sp. and *Padina* sp. Conclusions that can be taken from results obtained is more brown algae extract effective in hinder growth bacteria *V. harveyi* is *Padina* sp., due to the sixth treatment and control positive at 3 times observation show more clear zone results big compared with extract *Sargassum* sp.

According to study [22] written compound there are active in extract *Padina australis* that is compound phytol have ability for hinder growth *Vibrio cholerae*. *Padina australis* also contains steroid compounds, terpenoids, polyphenols, and saponins, alkaloids, flavonoids, triterpenoids, saponins, phenolhidroquinone and tannins. [23] Report that results measurement in research show that the average total flavonoid content of sample *Padina* sp. more tall compared to contained in *Sargassum* sp. so brown algae type *Padina* sp. suspected have activity more analgesic effective compared with *Sargassum* sp. Flavonoid compounds have mechanism work with denature cell proteins bacteria and damage membrane cell without could repaired again. Flavonoids are also lipophilic that will damage membrane microbes. The flavonoids contain something compound phenol. Growth bacteria *V. harveyi* could disturbed caused compound phenol. Phenol is something alcohol which is sour so that also called acid carbohydrates. Phenol have ability for denatures proteins and destroys cell membrane. Condition sour by presence phenol could take effect to growth bacteria. Flavonoids can working as antibacterial because could damage wall cell bacteria. Happening inhibition growth bacteria could caused because damage to components structural membrane cell bacteria. Membrane arranged cells on proteins and lipids are very susceptible to substance chemistry that can lower voltage surface. Damage membrane cell cause disruption of nutrient transport (ionic compounds) so that cell bacteria experience deficiency necessary nutrients for its growth. Inhibition bacteria by compound bioactive could caused by several factors, such

as interference with compounds composer wall cell and increasing permeability membrane cells that cause damage or Dead cell [24].

4. Conclusion

The brown algae extract that is more effective in inhibiting the growth of *Vibrio harveyi* bacteria is *Padina* sp., because in all six positive treatments and controls at 3 observation times showed greater clear zone results compared to *Sargassum* sp. extract. The best concentration of *Sargassum* sp. Extract. and *Padina* sp. to inhibit the growth of *V. harveyi* bacteria is at a concentration of 2000 ppm for *Sargassum* sp extract. and 2000 ppm and 3000 ppm for *Padina* sp. extract, because it produces a large inhibition zone so it is categorized into strong inhibitory activities.

5. Acknowledgement

The author would like to thank those who have contributed so that this research can be completed. First of all, the author would like to thank Trunojoyo Madura University for giving the author the opportunity to complete his education so that he could finally complete this research well. Second, the author would like to express his deep and sincere gratitude to Mrs. Insafitri for providing guidance, support, comments, suggestions, and provisions that helped in the completion and success of this research and paper. It is a great honor to work and study under her guidance.

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