

ANTIBACTERIAL SCREENING OF SARGASSUM SP EXTRACT. OPPOSING VIBRIO PARAHAEMOLYTICUS BACTERIA

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Abstract

Antibiotics cause resistance to pathogenic bacteria, and the emergence of resistance and bacterial pathogenic infections makes scientists try to find new drugs. One of the efforts is to use marine organisms as a natural antibacterial agent, one of them is *Sargassum* sp. *Sargassum* sp contains alginate and iodine which are used in the food, pharmaceutical, cosmetic and textile industries. Apart from that, *Sargassum* sp. contains active compounds of steroids, alkaloids, phenols and triterpenoids which function as antibacterials, can be used to inhibit the growth of *Vibrio* sp bacteria which is the cause of firefly disease which is most dangerous for tiger prawns. The purpose of this study was to determine the ability of the extract *Sargassum* sp. In inhibiting the growth of *Vibrio parahaemolyticus* bacteria with different levels of solvent start with methanol, ethyl acetate, and n-hexane. The rejuvenation of *Vibrio parahaemolyticus* and *Vibrio harveyi* bacteria is carried out in two stages, the first is planting on Triptic Soy Broth (TSB) medium and the second stage is planting on Triptic Soy Agar (TSA) medium. Antibacterial sensitivity test using disc diffusion method. The results of the test showed that 1). *sargassum* sp which is extracted by methanol solvent inhibits the growth of *Vibrio parahaemolyticus* bacteria. 2). *sargassum* sp which extract by ethyl acetate and n-hexane solvent can't inhibit the *Vibrio parahaemolyticus* bacteria.

Keywords : *Sargassum* sp, Antibakteri, *Vibrio parahaemolyticus*

1. Introduction

The most prevalent organism in aquatic settings, bacteria have a wide variety of morphological, ecological, and physiological features. Gram-negative bacteria, which make up the majority of pathogenic bacteria, are characterized by short, rod-shaped cells. Austin (1999) discovered a class of bacteria that are the primary culprits for bacterial infections. One form of bacteria found in aquatic species is the *Vibrio* type. Bacteria of the *Vibrio* type prey on farmed and marine species, including shrimp, fish, and shellfish. This kind of bacteria causes a condition called vibriosis. The condition known as vibriosis typically affects shrimp. When shrimp are weak and the environment is harsh, this particular strain of bacteria infects them and causes sickness (Lopillo, 2000). The research results of Feliatra, (2010) showed that all species of *Vibrio* sp (*V. harveyi*, *V. alginolyticus*, *V. vulnificus*, *V. anguillarum*, *V. parahaemolyticus*) were the pathogens tested, characterized by death of tiger prawn larvae in all treatments. In addition to attacking shrimp, *Vibrio* is a very virulent and hazardous bacteria that affects practically every type of produced marine fish in aquatic fish farming, due to its ability to function as both a primary and secondary pathogen in brackish and seawater. Vibriosis-related mortality of shrimp has happened in hatcheries on Central Java's north coast. Aside from that, ponds in Tarakan City, West Tarakan District, have been reported to harbor *Vibrio* bacteria, which affect shrimp. Other than that, such incidents have happened in Indonesia. and in additional nations including Japan, Thailand, the Philippines, and India (Dhar, 2001). Antibiotics are fed to treat vibriosis, but because they are not ecologically friendly, using antibiotics makes things worse. Antibiotics are fed to treat vibriosis, but because they are not ecologically friendly, using antibiotics makes things worse. These antimicrobial compounds have the potential to make harmful bacteria more resistant (Soemardiharjo, 1999). Because of the emergence of resistance traits brought about by antibiotics and the pathogenicity of bacterial infections, scientists are constantly searching for novel therapeutics. Using marine creatures as natural antibacterial agents is one endeavor

undertaken (Setyaningsih, 2012). Marine organisms have been widely reported to be antibacterial, one of them has the potential to act as a natural antibacterial agent is macroalgae. Macroalgae have potential active ingredients that can inhibit the growth of bacteria and viruses. The production of active ingredients from secondary metabolites of seaweed such as *Sargassum* sp is expected to be an alternative for disease control in aquaculture in Indonesia. According to Patra, (2008) the methanol extract from *Sargassum* sp shows very strong antioxidant activity. Apart from that, it can also function as an antimicrobial against gram-positive and gram-negative bacteria. *Sargassum* sp. It has been widely used as a raw material in the food, pharmaceutical, cosmetics, feed, fertilizer, textile, paper and so on industries. Apart from that, *Sargassum* sp. contains active compounds such as steroids, alkaloids, phenols and triterpenoids which function as antibacterial, antiviral and anti-fungal (Kusumaningrum, 2007). The results of research by Izzati (2007) who tested the antibacterial activity of extracts of several seaweeds against pathogenic bacteria on tiger prawns showed that *Sargassum* sp seaweed is suitable/can be developed for double cultivation with tiger prawns, because the *Sargassum* extract is active against the two species of *Vibrio* bacteria tested (*Vibrio harveyi* and *Vibrio parahaemolyticus*). The antibacterial capacity of marine species has been investigated in the past (Challouf, 2012). Lestari (2000) discovered that certain chemicals found in microalgae may have antibacterial properties, whereas Umamaheshwari (2009) investigated the antibacterial efficacy of extracts from seagrass. It has been demonstrated that extracts from the seagrass species *Halophila ovalis* and *Halodule pinifolia* exhibit antibacterial activity against *Acinetobacter* Sp *Salmonella typhi*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. Moreover, Lisdayanti (2013) discovered that *Staphylococcus aureus* bacterial growth might be inhibited by a portion of *Enhalus acoroides* seagrass extract sourced from the Spermonde Islands, Makassar. According to Putranti (2013), brown algae, or Phaeophyceae, have more antioxidant activity than Rhodophyceae and Chlorophyceae and create secondary metabolites in tropical climates. As one of the most productive natural synthesis products with a variety of bioactive metabolites, including antibacterial chemicals, *Sargassum* sp. has been shown to provide advantages in several studies. According to Sastry and Rao, 1994 in Bachtiar, 2012, phenol, iodine, and tannin found in *Sargassum* sp. can function as antibacterial agents against a variety of pathogenic bacterial species. According to Widowati (2013), *Sargassum* sp. from Jepara waters can stop the growth of microorganisms like *Staphylococcus aureus* and *Escherichia coli*. Based on a review conducted by Rivai, (2020) seaweed extract *Sargassum* sp. has potential and antibacterial activity against aquaculture pathogenic bacteria. Research conducted by Rahma, (2020) showed that the highest activity was shown by chloroform extract against the three test pathogenic bacteria, namely against *Vibrio harveyi* bacteria with an inhibitory zone diameter of 11.00 mm, *Vibrio alginolyticus* 10.02 mm and *Vibrio parahaemolyticus* 9.33 mm. The minimum inhibitory concentration (MIC) value of chloroform extract against *Vibrio harveyi* is <15.625 ppm. The highest yield was found in the 7.67% methanol extract and the lowest in the 0.18% ethyl acetate extract. From this research it can be concluded that chloroform extract from *Sargassum polycistum* has the potential to be a natural antibacterial against the pathogenic bacteria *Vibrio* spp. The bioactive compounds produced by *Sargassum* sp have known benefits, one of which is antibacterial, seen from various research results that have been carried out. This led to the writing of this scientific research, which investigates the antibacterial potential of *Sargassum* sp. seaweed's crude extract against the *Vibrio parahaemolyticus* bacteria.

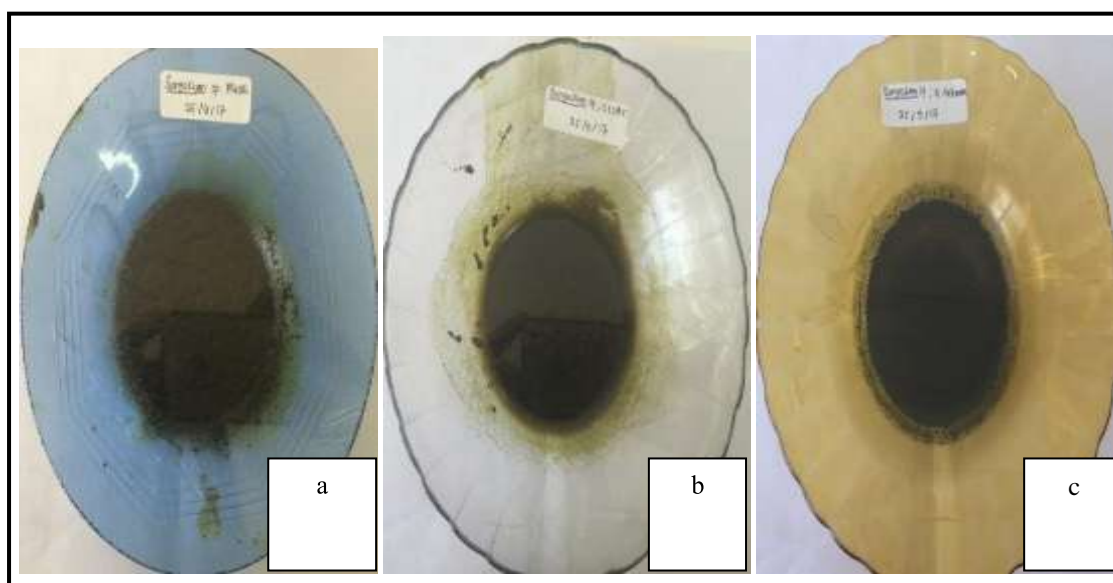
2. Method

In writing this research to produce data, this research uses a process called Experimental design is a method used in scientific research to test hypotheses and evaluate cause-and-effect relationships between specific variables. Experimental research is designed in such a way as to allow the researcher to control certain variables so as to identify the effect of the independent variable on the dependent variable.

The research design used in this study was laboratory experimental, where antibacterial testing was carried out *in vitro* using the agar diffusion method. By observing whether or not a clear zone forms, the bacterial activity test is used to ascertain whether or not bioactive components from an organism are active against harmful bacteria. Three different Pro Analisis solvent types are used by Ekstraksi: n-hexane (a non-polar solvent), ethyl acetate (a semi-polar solvent), and methanol (a polar solvent). To begin the extraction process, 200 g of sargassum sp. powder is added to each container. Next, 600 ml of each solvent is added, with a solvent sample ratio of 1:3. According to Triantono (2001). After two 24-hour soaking times (also known as maceration), the samples in each container were filtered using Whatman paper. The leftover filter dregs from the filtering operation are then retracted into each solvent. Three times the process is carried out. Next, a rotary evaporator is used to vacuum-evaporate the filtering results from multiple solvent levels, producing a concentrated extract. The agar diffusion method with FPD (Flying Paper Disc) is the antibacterial test method for Sargassum sp extract employed in this test. Following the weighing of each extract from the three solvents at concentrations of 2 mg/50 μ l, 3 mg/50 μ l, and 4 mg/50 μ l, the extracts were placed in an Eppendorf tube and dissolved in each solvent. It is then homogenized with the aid of a vortex. The test extract, positive, and negative controls were then spotted onto a paper disc using the FPD (Flying Paper Disc) technique using a micropipette. Once it's totally dry, let it evaporate. Once the disk had dried, it was carefully and aseptically placed on top of the microbe-homogenized agar media. It was then incubated for 24 hours at 30°C. A clear region or halo that developed around the paper disc served as a marker for antimicrobial activity. Using a caliper, the clear zone's area was determined. The positive control in this experiment was the antibiotic ciprofloxacin 40 ppm, while each solvent was employed in the negative control. A descriptive analysis was performed on the antibacterial activity measurement data.

3. Results and Discussion

The maceration method was used for extraction, and the resultant extract in methanol solvent had a weight of 1.05 g/200 g and a greenish-brown color (figure 1a). The solvent for ethyl acetate is greenish colored (Fig. 1b). weighing 0.53 g out of 200 g. The n-hexane solvent weighs 0.16 g/200 g and is yellowish green (Fig. 1c). According to the extract results, the methanol solvent extract is comparatively larger than the extracts from the other two solvents. This indicates that sargassum's active component molecules are polar. According to Nur (2013), polar substances will dissolve in polar solvents.



Source : Personal Documents

Picture 1. Results of the extraction of *Sargassum* sp.: 1a). solvent extraction in methanol. 1b) Solvent extraction of ethyl acetate. 1c). solvent extraction from n-hexane

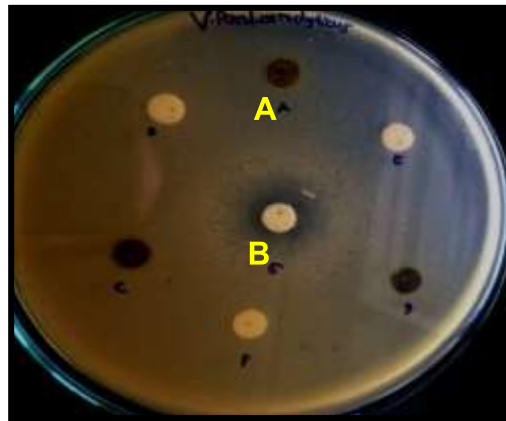
Using the agar diffusion method, the antibacterial activity of *Sargassum* sp. crude extract is tested at three different extract concentration levels: 2 mg/50 μ l, 3 mg/50 μ l, and 4 mg/50 μ l. Methanol, ethyl acetate, and n-hexane were employed as negative controls, and the antibiotic ciprofloxacin was used as a positive control.

Table 1. presents the findings of an antibacterial activity test conducted on *Sargassum* sp. crude extract against *Vibrio parahaemolyticus*.

Solvent	extract concentration <i>Sargassum</i> sp (mg/50 μ l)	zone of impediments (mm) <i>V. parahaemolyticus</i>
Methanol	2	1.43
	3	0.42
	4	0
Ethyl acetate	2	0
	3	0
	4	0
n-hexane	2	0
	3	0
	4	0

Source : Personal Documents

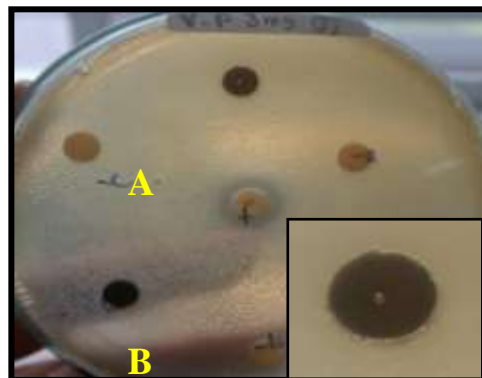
Following two 24-hour incubation cycles, the crude extract in methanol solvent at concentrations of 2 mg/50 μ l and 3 mg/50 μ l demonstrated antibacterial activity against *Vibrio parahaemolyticus*, as evidenced by the formation of halo and clear zones with diameters of 1.43 mm and 0.42 mm, respectively, surrounding the paper disk. Whereas the clear zone suggests that the extract is bacteriocidal, or capable of killing bacteria, the halo zone shows that the extract is bacteriostatic, or limited to inhibiting the growth of germs. According to Madigan (2000), bacteriocidal substances can kill bacteria, whereas bacteriostatic compounds only prevent growth.



Source : Personal Documents

Picture 2. shows the outcomes of an antibacterial activity test conducted on *Vibrio parahaemolyticus* bacteria using a crude extract of *Sargassum* sp. At a concentration of 2 mg/50 μ l and methanol solvent. A.) halo region. B). command plus

The diameter of the clear zone was used by Davis and Stout (1971) to categorize the inhibitory zone response to bacterial growth. These categories included an inhibitory zone diameter of less than 5 mm, which was classified as weak, an inhibitory zone diameter of 5–10 mm, which was classified as moderate, an inhibitory zone of 10–20 mm, which was classified as strong, and an inhibitory zone diameter of more than 20 mm, which was classified as very strong. This criterion is utilized in studies to categorize an extract's inhibitory power. The test findings indicate that *Sargassum* sp. has comparatively limited antibacterial power based on these parameters. The extract's ability to suppress the growth of *Vibrio parahaemolyticus* test bacteria was demonstrated by the halo zone observed at a concentration of 2 mg/50 μ l (Fig. 2). As a result, the extract is bacteriostatic, meaning it can only stop germs from growing. A similar result was achieved by Alamsyah (2014), who observed a halo zone surrounding the disc paper in the crude extract of *Sargassum cinerum* that was extracted using methanol solvent. A halo zone in the antibacterial activity test indicates that the extract is a static antibacterial; bacteriostatic substances can only impede the growth of bacteria by blocking the synthesis of proteins by momentarily attaching themselves to the ribosomes of an organism. This bond is not so strong that the antimicrobial will release the ribosomes to allow the bacteria to grow again when the concentration and stability drop. The antibacterial effect can be mediated through several different mechanisms, such as blocking the production of the cell wall, blocking the permeability of the cell wall, blocking the synthesis of cell wall proteins, blocking the synthesis of nucleic acids, and blocking the metabolism of microbial cells (Jawetz, Melnick, & Adelberg, 2005).



Source : Personal Documents

Picture 3. shows the outcomes of a test conducted on *Vibrio parahaemolyticus* bacteria to determine the antibacterial activity of a crude extract of *Sargassum* sp. in methanol solvent. A.) halo region. B). command plus

A clear zone measuring 0.42 mm is present at an extract concentration of 3 mg/50 μ l (Fig. 3). The presence of a clear zone suggests that the extract can prevent the growth of *Vibrio parahaemolyticus* bacteria, indicating the antibacterial activity of the chemicals attracted to polar solvents. This is consistent with the claim made by Trono and Ganzon (1988) in Kadi (2008) that the extract of *Sargassum* sp. contains substances like iodine, tannin, and phenol that are quite effective at inhibiting bacterial growth. The method of action of tannin and phenol chemicals in inhibiting bacterial cells was later added by Purwanti (2007). This mechanism involves denaturing bacterial cell proteins, which inhibits the function of cell membranes, which is the transfer of substances from one cell to another. others) and prevents the synthesis of nucleic acids, which stops the growth of bacteria. The results of research conducted by Izzati (2007) showed that crude extracts of sargassum using water and methanol solvents were able to inhibit the growth of *Vibrio parahaemolyticus* bacteria. Akbari (2015) found that extracting sargassum using methanol as a solvent can inhibit the growth of *E. Coli* bacteria. These and other studies have demonstrated the effectiveness of polar solvents in extracting sargassum using water or methanol in inhibiting bacterial growth. When applied to *Vibrio parahaemolyticus* bacteria, sargassum sp crude extract in ethyl acetate and n-hexane solvents at doses of 2 mg/50 μ l, 3 mg/50 μ l, and 4 mg/50 μ l does not exhibit strong antibacterial action. Research using mangrove leaf extract by Trianto (2014) revealed the opposite outcomes. Riniatsih (2009) discovered that *Thalassia empiric* seagrass extract using water and methanol solvent was able to suppress the growth of *Vibrio harveyi* bacteria, while *Aegiceras corniculatum* using methanol solvent was able to inhibit the growth of *Vibrio parahaemolyticus* bacteria. It is suspected that both test bacteria are more sensitive to extract compounds that dissolve in polar solvents than semi- and non-polar compounds because research findings indicate that extract compounds that dissolve in polar solvents, such as methanol and water, are effective at inhibiting both test bacteria. This is consistent with a study by Renhoran (2012) that found gram-negative bacteria typically exhibit polar antimicrobial sensitivity.

4. Conclusions

It may be inferred from the study findings that *Vibrio parahaemolyticus* and *Vibrio harveyi* are susceptible to the extract in methanol solvent. *Vibrio parahaemolyticus* was not susceptible to the antibacterial properties of extracts containing ethyl acetate and n-hexane. Since methanol solvent extracts have antibacterial properties, polar solvents can be used in subsequent studies to evaluate the antibacterial properties of these extracts against different harmful microorganisms.

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