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Abstract. The way to prevent or treating bacterial diseases outbreaks with drugs or chemicals is the one of main solutions to solve the problems in aquaculture. The use of antibacterial agents has increased significantly in aquaculture practices, since the antibiotics used in both human as well as veterinary medicines have been tried experimentally to treat bacterial infections of fish. However, the evolving resistance of fish pathogenic bacteria to existing antibiotics has necessitated development of new alternatives. In the last three decades the discovery of metabolites with biological activities from algae has increased significantly. In this study, sixteen extracts from four algae of genus *Eucheuma* were tested by *in-vitro* against five pathogenic bacteria (*Aeromonas salmonicida, Aeromonas hydrophila, Pseudomonas anguilliseptica, Vibrio anguillarum* and *Yersinia ruckeri*). The algae were extracted in Soxhlet apparatus using solvents with increased polarity (hexane, dichloromethane, methanol and water) for 24 h. The extract solutions were then evaporated and liophilized before using for the antibacterial test with agar diffusion method. The results revealed that three of four extracts (hexane, dichloromethane and water) were active against all pathogens, while methanol did not. The highest activity was shown by water extract, followed by the hexane and the dichloromethane extracts with lower activities. The water extract of *Eucheuma spinosum* had a broad activity since it was active against four of five pathogenic strains. *Vibrio anguillarum* and *Pseudomonas anguillaseptica* were the two most susceptible pathogens, while the most resistant was presented by *Aeromonas hydrophila*. The screening results confirm that algae were potential to be developed as a source of antibacterial compounds or as a health-promoting food for aquaculture.

1. Introduction

The intensification of the system in aquaculture has had a significant impact on the spread of disease by pathogenic bacteria. This infectious disease can be due to high population density which results in poor water quality and horizontal transmission of pathogens among dense populations. Prevention efforts can be made by maintaining water quality and enhancing the immune system of cultivated organisms by administering supplements such as probiotics. The problem is, precautions do not always go as planned. Declining water quality can result in a weakening of the immune system of the cultivating organism, which makes it easier for pathogenic bacterial pathogens, which have been living together without any effect on their host, turning to attack their weak host.

Usually a pathogen infection and rapid transmission in a population can only be treated with the use of antibiotics [1]. The method with the use of antibiotics is the only way to overcome the infection of cultivated organisms. However, due to its easy use and farmers' lack of knowledge of its effects, the use of antibiotics is often carried out excessively, not only as a propylaxis effort, but has been used as a prevention effort by giving it through feed. This results in the accumulation of synthetic antibiotics, which are not recycled in the body of cultivated organisms, and accumulates in the area of aquaculture ponds. This problem will certainly become a major problem in the effort to intensify the cultivation system.

The sea occupies a vast area on the surface of the earth, which covers 2/3 of the earth's surface. Besides having a large area, the sea also has a diversity of biological resources that is very high compared to the mainland. About 80% of species on earth are found in the sea. But its potential has not been much studied because of obstacles in getting its source at sea. Scuba equipment and underwater survey vessels are still very limited, so research on the potential of underwater organisms is still experiencing problems.

One type of marine organism whose potential has long been known since ancient times is seaweed. Seaweed has been used for a long time as a treatment tool for various diseases due to its diverse secondary metabolite content. Because it is easily obtained and cultivated, seaweed has long been used as food from the sea, especially for people who live in coastal areas. Its content is rich in vitamins and minerals which has the potential to be used as nutritional supplements in human food and feed, as well as to work to improve the immune system of cultivated organisms. This advantage makes seaweed very potential to be used as a natural antibiotic substitute for synthetic antibiotics that have been used by farmers. Natural antibiotics are very low in side effects, even non-existent, because they are easily recycled in the body of both cultivated organisms and the environment.

Seaweed belongs to a group of macro-sized algae, so it is known as macroalgae. Seaweed or macroalgae consists of 3 classes, namely Chlorophyceae, Rhodophyceae and Phaeophyceae classes. One of the genera of commercial seaweed that has been widely cultivated to produce phycocoloid content that is needed in the world for the food, beverage, pharmacy and cosmetics industries is seaweed from the genus *Eucheuma*. The genus *Eucheuma* belongs to the group of red seaweed (Rhodophyceae) which produces carraginan type phycoloid. The species used in this study are from the genus *Eucheuma* that has been cultivated in South Sulawesi, namely *Eucheuma cottonii* or better known as *Kappahycus alvarezii, Eucheuma denticulatum, Eucheuma spinosum*, and *Eucheuma edule*.

2. Material and Methods

2.1. Seaweed Preparation

Seaweed samples used in this study consisted of 5 species of the genus *Eucheuma*, which were taken from different locations. Sample of *Eucheuma cottoni* are from Jeneponto, *Eucheuma edule* from Bone, *Eucheuma denticulatum* from Takalar, *Eucheuma spinosum* from Jeneponto. Seaweed samples were obtained in dry form. In the laboratory the sample was then washed again using tap water to remove salt and then rinsed with distilled water. Samples that have been cleaned of impurities and salts were lyophilized until dry and then mashed into powder using a grinder.

2.2. Seaweed Extraction

Sample extraction was done sequentially using soxhlets and solvents of different polarity, starting with hexane, followed by DCM, Methanol and finally with water. The extraction time is 24 hours. After extraction, the extract solution was then filtered using Whatman filter paper No. 1, the filtrate solution was evaporated using a rotary evaporator. The residue was left overnight and then used for extraction with dichloromethane (DCM). The same extraction process was carried out up to the water solvent. Crude extract was put in a vial and stored in cold temperatures until used. For water extract, it was first evaporated with a rotary evaporator, then followed by lyophilization.

2.3. Pathogenic Bacteria Test

The pathogenic species used in this test consisted of 5 species of fish pathogenic bacteria, such as *Aeromonas salmonicida, Aeromonas hydrophila, Pseudomonas anguilliseptica, Vibrio anguillarum,* and *Yersinia ruckeri*. These pathogenic bacteria come from the collection of test cultures of Ernst Moritz Arndt University (EMAU), Greifswald, Germany.

2.4. Antibacterial Activity Test

The antibacterial activity test was carried out using the diffusion method which had been done previously by Zainuddin [2–4]. A total of 2 mg of crude extract was redissolved in 50 µL of each extraction solvent, then dropped on a paper disk with a diameter of 6 mm aseptically, and then left until the solvent dries in a laminar box. Test bacteria were prepared by rejuvenating isolates one day before use. The test bacteria were incubated at 28ºC for 24 hours and then used to test the antibacterial activity by the agar diffusion method. A total of 1 bacterial loop was taken from a Petri dish containing a rejuvenated isolate, and then dissolved in 2 mL of 0.9% NaCl physiological solution. The isolate solution was then taken as much as 200 µL and then placed in a glass baker containing 20 mL of warm TSA media. After mixing evenly, the agar solution contains test bacteria, then poured into a 9 mm diameter petri dish. Petri dishes containing agar solution and bacteria were allowed to cool before being stored in a reversed position in an incubator at 28ºC for 24 hours. As a positive control, 20 µg / disk of oxytetracyclin was used. For negative controls, 50 µL / disk of each solvent was used. The level of antibacterial activity is categorized as follows: very high ≥20 mm, high 15- <20 mm, moderate 10- <15 mm, low> 6 - <10 mm, no activity = 6 mm

3. Results

Seaweed samples used in this study were taken from several seaweed cultivation locations in South Sulawesi, pictures of *Eucheuma* species were performed in the following figure (Figure 1)

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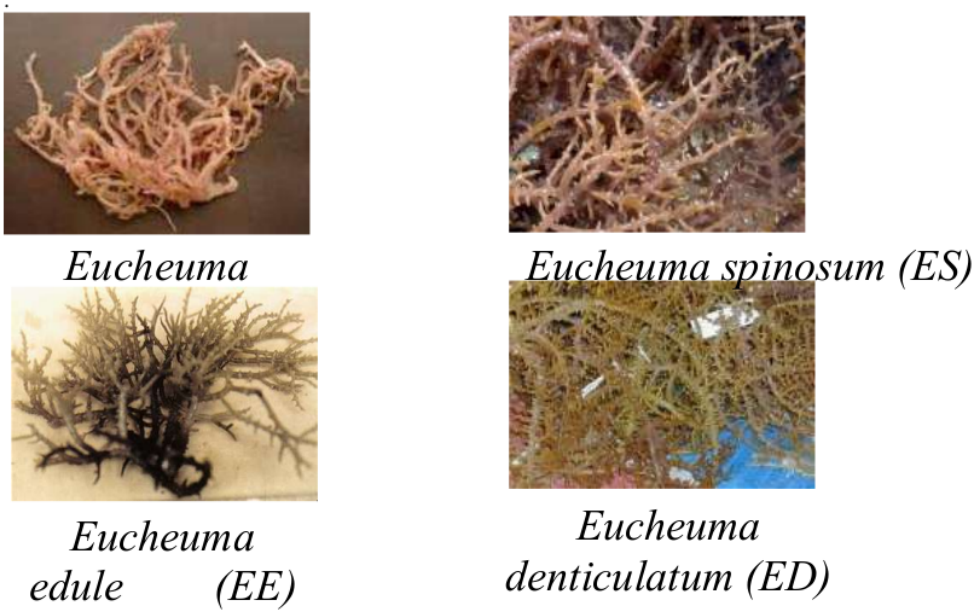

Figure 1. *Eucheuma* species commercially cultivated in South Sulawesi

In addition to seaweed sample, bacteria that were used in this study are pathogenic bacteria which cause various diseases in cultivation organisms, as shown in the figure below (Table 1).

Table 1. Fish diseases caused by pathogenic bacteria and their symptoms

Pathogens	Diseases	Symptoms	
Vibrio anguillarum	Vibriosis	haemorrhage erytheme ulcer	
Pseudomonas anguilliseptica	Red spot	dark skin haemorrhage ulcer septicemia	
Aeromonas salmonicida	Furunculosis	haemorrhage lesion ulcer	
Aeromonas hydrophila	MAS (Motile Aeromonas Septicemia	Haemorrhage Septicemie lesion ulcer fin necrosis exophthalmus	
Yersinia ruckeri	Redmouth disease	Hemorrhages on oral cavity discoloration anorexia	

From the results of antibacterial activity tests on 16 extracts obtained from 4 species of the genus *Eucheuma*, it was seen that very high activity was shown by water extracts from *E. spinosum* against *Pseudomonas anguilliseptica* with inhibition zone diameter of 34.73 mm (Figure 2). Also very high activity was demonstrated by water extracts from *E. cottoni* with inhibition zone diameter of 26.37 mm.

The other two extracts, each hexane extract from *E. edule* and *E. denticulatum*, have only low activity, with inhibition zone diameters of 8.0 mm and 8.5 mm respectively.

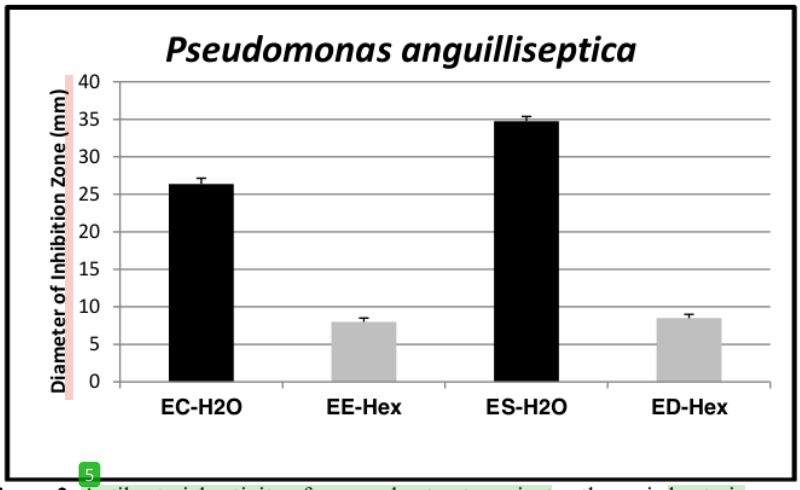

Figure 2. Antibacterial activity of seaweed extracts against pathogenic bacteria *Pseudomonas anguillaseptica* (EC = *Eucheuma cottoni*; EE= *Eucheuma edule*; ES= *Eucheuma spinosum*; ED= *Eucheuma denticulatum*; Hex= n-hexane extract; H_2O= water extract; N= 3, Value = average +SD)

The seaweed extract activity test on *Aeromonas salmonicida* showed very high activity by water extract of *E. denticulatum* with a diameter of inhibition zone of 29.37 mm. A very high activity was also shown by water extracts from *E. edule* with inhibition zone diameter of 21.17 mm and high level activity was shown by water extracts from *E. spinosum* with 16.77 mm (Figure 3). Only low activity was demonstrated by DCM extracts from *E. edule* and *E. denticulatum* with inhibition zone diameters of 8.50 and 8.20 mm, respectively.

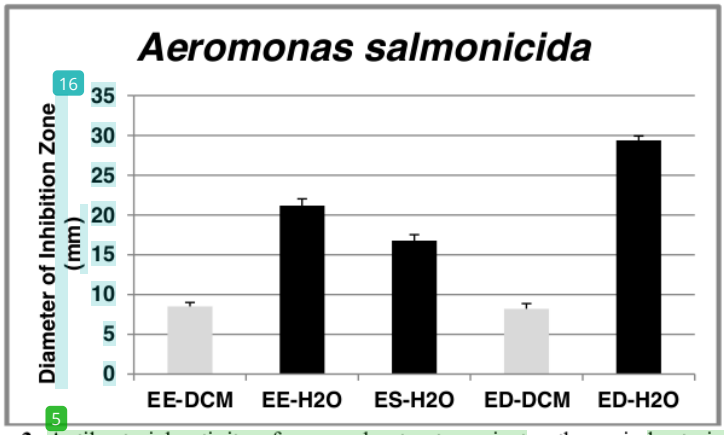

Figure 3. Antibacterial activity of seaweed extracts against pathogenic bacteria *Aeromonas salmonicida* (EE = *Eucheuma edule*; ES= *Eucheuma spinosum;* ED= *Eucheuma denticulatum*; DCM= dichloromethane extract; H_2O= water extract; N= 3, Value= average +SD)

The inhibition assay of *Eucheuma* extract on the bacterium *Yersinia ruckery* showed a very high activity on the water extract from *E. denticulatum* with inhibition zone diameter of 25.83 mm. Followed by high activity of water extract from *E. spinosum* with inhibition zone diameter of 17.0 mm. The water extract of *E. edule* showed a moderate activity with a diameter of inhibition zone of 12.50 mm. Both dichloromethane extracts of *E. edule* and *E. denticulatum* only showed low activities with each inhibition zone diameters of <10 mm (Figure 4.).

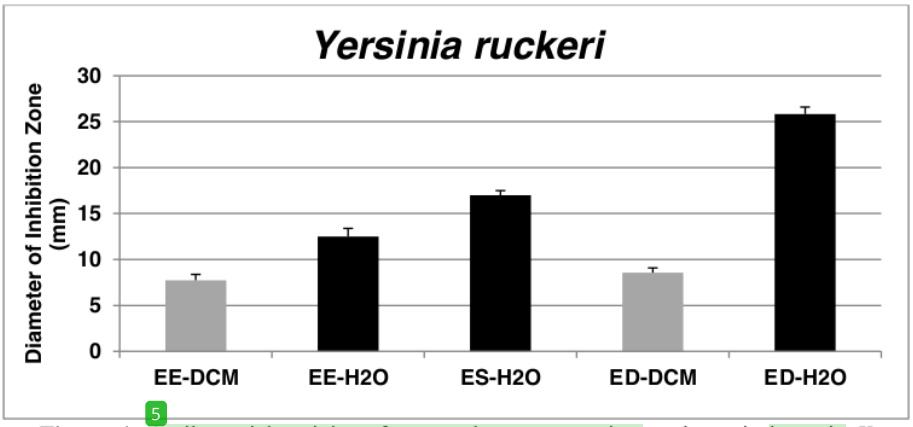

Figure 4. Antibacterial activity of seaweed extracts against pathogenic bacteria *Yersinia ruckeri* (EE = *Eucheuma edule*; ES= *Eucheuma spinosum*; ED= *Eucheuma denticulatum*; DCM= dichloromethane extract; H_2O= water extract; N= 3, Value= average +SD)

A moderate activity against *Vibrio anguillarum* was shown by water extract of *E. spinosum* with inhibition zone diameter of 15.9 mm, followed by hexane extract from *E. denticulatum* with a low activity (8.4 mm diameter), and hexane extract of *E. edule* with diameter of 7.0 mm (Figure 5).

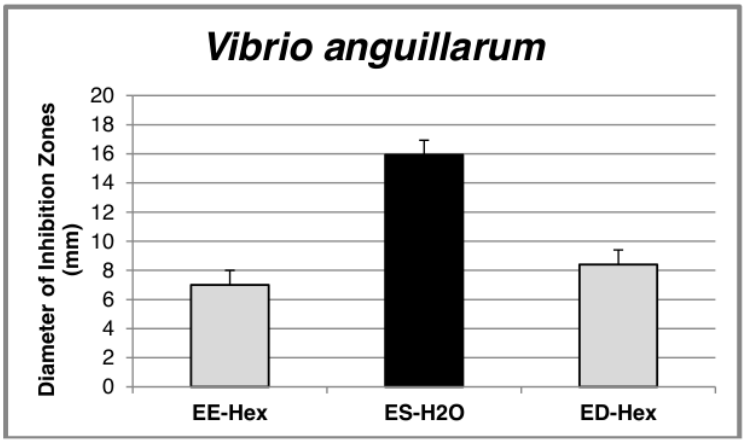

Figure 5. Antibacterial activity of seaweed extracts against pathogenic bacteria *Vibrio anguillarum* (EE = *Eucheuma edule*; ES= *Eucheuma spinosum*; ED= *Eucheuma denticulatum*; Hex= n-hexane extract; H_2O= water extract. N= 3, Value= average +SD)

4. Discussion

Eucheuma seaweed is commercial seaweed cultured in tropical climates to supply the world's carrageenans needs. The genus *Eucheuma* is a member of the red algae group or Rhodophyceae which is easy to cultivate and has economic value for countries such as Indonesia, the Philippines, Malaysia, Tanzania and Madagascar [5]. In addition to its benefits to produce carrageenan, *Eucheuma* seaweed has also been widely studied for its antimicrobial potential, especially as an antibacterial agent [6].

The content of carrageenan phycocoloid produced by the red algae group of the genus *Eucheuma* is suspected to be an anti-infective agent against pathogenic bacteria. From the results of this study it was seen that generally polar water extracts, which have high activity in inhibiting all test pathogenic bacteria except *Aeromonas hydrophila*. Carrageenan is a polysaccharide that has sulfate bonds, dissolves in water and easily binds water at room temperature. In this study *E. cottoni* which has the kappa-carrageenan shows very high activity only to one pathogenic bacterium *Pseudomonas angguillaseptica*. Utilization of k-carrageenan as an antibacterial revealed the damage of bacterial cell wall and cytoplasmic membrane and suppress the growth of both Gram-positive and Gram-negative bacteria [7].

Very high activity of water extracts of various species of *Eucheuma* against *Pseudomonas angilaseptica, Aeromonas salmonicida* and *Yersinia ruckeri*, indicates that secondary metabolites that have antibacterial activity found in water extracts are a group of very polar metabolites. Unfortunately the test results against *Aeromonas hydrophila* showed the presence of resistance from this bacterium to all extracts of all species of seaweed tested. From various studies generally antibacterial activity against fish pathogens varies and the activity is highly dependent on the method of extraction, solvent used in extraction and season at which samples are collected [8]. Several different organic solvents have been used for extraction and screening of macroalgae for antibacterial activity in fish pathogens such as methanol [9,10], acetone [11], and ethyl acetate [12]. In our study, from the screening of four extracts of *Eucheuma* spp., only water extracts showed high activity, while hexane and DCM extracts showed only low activity and no activity was detected in the methanolic extracts of all the species. The lower activity was also showed by methanolic extract of *Kappaphycus spicifera* compared to isopropanol and diethyl ether extracts against *V. alginolyticus*, with diameters of inhibition zone of 1.16, 1.33 and 1.36 mm, respectively [13].

Species of *Eucheuma* which have very high activity from the results of this study are *E. spinosum* and *E. denticulatum*. Water extract from *E. spinosum* has the highest activity in inhibiting *Vibrio anguillarum* and *Pseudomonas anguillaseptica*. A very high activity was demonstrated by aqueous extracts of *E. spinosum* against *Pseudomonas anguilliseptica*. Aside from being an antibacterial pathogen in fish, inhibition of ethanol extract from *E. spinosum* was also seen against *P. gingivalis* with MIC of 3.5% [14], and ethanol extract from *E cottoni* against the growth of *Streptococcus mutans* with MIC 6 , 25% [15].

Water extract of *E. denticulatum* has the highest activity against *Aeromonas samonicida* and *Yersinia ruckery*. Methanolic extract of *E. denticulatum* showed a higher inhibitory effect than the methanolic extract of *K. alvarezii/E. cott*onii against *A. hydrophila* (19.43 ± 0.55 mm) and *V. harveyii* (19.85 ± 0.23 mm) [6]. Choudhury [30] reported three marine algae extracts (*G. corticata, U. fasciata, and Eucheuma compressa*) extracted using hexane, chloroform, ethyl acetate, and methanol, showing inhibition against the pathogenic bacteria *E. tarda, V. alginolyticus, P. fluorescens, P. aeruginosa* and *A. hydrophila*. Unfortunately in our study no activity was found from all species of the genus *Eucheuma* against *Aeromonas hydrophila*.

Although there are many obstacles encountered in the isolation of polar compounds, there are also advantages in using polar extract, which is easily applied as a dietary supplement. It make easier for the farmers to use it as a substitute of synthetic antibiotics in feed. According to Hanin and Pratiwi [17], ethanol solvents have the ability to attract all polar compounds. This is consistent with the results of Sulistyaningsih et al [18] who reported that ethanolic extract of *E. cottonii* was active against *S. aureus, B. subtilis* and *S. epidermidis* at a concentration of 30, 40, 50 and 60% (w/v). Ethanolic extract of *E. spinosum* at concentrations of 1%; 2%; 5%, and 10% (v / v) resulting in inhibition zones of 1.98; 4.14; 7.42; and 10.27 mm, respectively. The MIC value of *E. spinosum* extract against *S. aureus* was 10%

(v/v), and MBC was 15% (v/v). The phytochemical analysis showed that alkaloids and saponins were the main components detected in that extract [19].

The low potential of n-hexane and DCM extracts from the genus *Eucheuma* in our study, is in accordance with the results of Fattah [20], where hexane extract of *E. spinosum* did not inhibit *Vibrio cholerae* and *Staphylococcus aeureus*. Methanol and ethanol extracts had activity in concentrations of 0.4%, 4% and 40% against *Vibrio cholerae and Staphylococcus aeureus*. A higher inhibition zone was found in methanolic extract with a concentration of 40% against *Staphylococcus aeureus* [20]. The inhibition zone of methanolic extract of *E. spinosum* against *Staphylococcus aureus* and *Escherichia coli* at concentrations of 80 mg/mL was 4 mm and 3 mm, respectively. Phytochemical analysis of methanol extract of *E. spinosum* revealed flavonoids, triterpenoids and ascorbic acid as main compounds [21]. Siregar et al. [22] also reported that methanol extract from *Euchema* sp. contains alkaloid compounds, while n-hexane extract contains steroid compounds.

Activities from *Eucheuma serra* which was collected from Warambadi seashore of Sumba Island were also reported by Anggadiredja [23]. *E. serra* produced a variety of compounds with antibacterial activities which consist of 3 fatty acids, 3 steroids, and 2 aldehydes. All compounds were active against Gram-positive bacteria *Bacillus subtilis* and 2 compounds showed active against Gram-negative *Escherichia coli*. No activity was detected in all compounds against *Pseudomonas aeruginosa* and *Salmonella typhimurium*.

Overall, the content of bioactive compounds in macroalgae has the potential to be developed into antibiotic drugs, because these compounds can inhibit the growth of pathogenic microorganisms [24]. Steroids or triterpenoids also have the ability to inhibit bacterial growth through the mechanism of inhibiting protein synthesis that results in changes in the components of the bacterial cell [22]. Terpenoid compounds which tend to dissolve in lipids will more easily penetrate the cell walls of Gram positive and negative bacteria [25]. Phenolic compounds are compounds that are known to have antimicrobial, anti-inflammatory, antiviral and anticancer activities [26,27]. Saponin has been exploited biologically as an antioxidant, antidiabetic and antiobesity, antibacterial and anticancer [28].

5. Coclusion

Besides having a high commercial value of the phycocoloid content, especially the carrageenan, red seaweed of the genus *Eucheuma* has also been proven to be potentially used as a natural antibiotic. The application can be by immersion method or oral administration as a dietary supplement for cultivation organisms.

Its activity is very high in polar extracts or water extracts, very beneficial and easy for farmers to use in the field. Water is a safe solvent and does not cause side effects like other extracts, especially in its application in the field. In addition, the cost is cheap when compared to the price of synthetic antibiotic drugs or the price of organic solvents. To use it in the form of natural antibiotic preparations, further research is needed to isolate the secondary metabolite compounds by guided isolation method and identification of the pure compounds with various methods of chromatography and NMR.

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