Uji Aktivitas Antibakteri Ekstrak Kasar Alga Merah *Eucheuma spinosum* Terhadap Pertumbuhan Bakteri *Staphylococcus Epidermidis*

Antibacterial Activity Of Crude Extract Red Algae *Eucheuma spinosum* Against *Staphylococcus Epidermidis* Bacteria Growth

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Abstract

Eucheuma spinosum is a seaweed that has not been widely used as a source of bioactive material from algae, which has a high content of carrageenan and secondary metabolites which are thought to have the potential to have antibacterial activity. *Eucheuma spinosum* has been tested by phytochemical screening with UV-Vis spectrophotometer showing that *Eucheuma spinosum* contains active compounds including flavonoids, triterpenoids, alkaloids and ascorbic acid.

The working method used is an experimental and qualitative research design. Extraction of *Eucheuma spinosum* using maceration with 99% ethanol solvent and evaporated using a *Rotary Evaporator* to obtain a crude extract yield of 1.54%. The phytochemical screening test used thin layer chromatography and precipitation tests. The results of the antibacterial test using the disc diffusion method (Kirby-Bauer test) with positive control clindamycin 10µl/ml as comparison and aquadest negative control obtained through a literature study. This study uses data analysis in the form of descriptive analysis and content analysis.

The results of the phytochemical screening test of *Eucheuma spinosum* extract and the results of testing the antibacterial activity of *Staphylococcus epidermidis* based on research data that have been carried out by several previous researchers through journal reviews are suspected to be effective for tackling acne-causing bacteria, *Staphylococcus epidermidis*. The antibacterial activity is because seaweed contains secondary metabolites such as flavonoids, alkaloids, terpenoids, and saponins. The need for herbal therapy to treat diseases caused by *Staphylococcus epidermidis* bacteria to reduce bacterial resistance to antibiotics.

Keywords : Antibacterial Activity, *Eucheuma spinosum*, *Staphylococcus epidermidis*

1. Introduction

Indonesia is an archipelagic country where 75% of its territory is an ocean that is rich in marine product diversity, especially seaweed. *Eucheuma spinosum* contains Iota Carrageenan which can function as a stabilizer, thickener, emulsifer, gelling agent, thickener. In addition, seaweed contains secondary metabolites that have the potential as a producer of bioactive metabolites which these metabolites can be produced because of his extreme environments such as high salinity or used to defend themselves from the threat of predators. The chemical compounds contained are groups of polysaccharides, lipids, proteins, alkaloids and phenolic and triterpenoid components (Nurjanah *et al*., 2017).

Eucheuma spinosum comes from the *Rhodophyceae* class where the utilization of its bioactive compounds has not been widely used. In addition, there are not many types of research on in-vitro antibacterial activity on this type of seaweed. *Eucheuma spinosum* has been tested by phytochemical screening with

UV-Vis spectrophotometer showing that *Eucheuma spinosum* contains active compounds including flavonoids, triterpenoids, alkaloids and ascorbic acid (Mardiyah *et al*., 2014).

The production of this type of seaweed is quite abundant, especially in the Sumenep Regency area with a total of 116 thousand tons worth IDR 466 million (Sumenep District Fisheries Service, 2017). The use of natural ingredients, especially from marine products *Eucheuma spinosum* as a natural antibacterial in the treatment of acne. Acne is a disease that affects many people, especially teenagers. This disease can be caused by bacteria such as *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis.*

Until now the use of antibiotics is one way in the treatment of acne, but the use of antibiotics as the primary choice should be reviewed because of the use of antibiotics in the long term it will lead to resistance. The use of traditional medicine using natural ingredients is considered to have fewer side effects than the use of drugs derived from chemicals.

2. Research Methodology

2.1 Research Material

The research material used was the red marine macroalga *Eucheuma spinosum* obtained from seaweed farmers in Sumenep Regency, Madura, East Java Province. The sample was taken in a wet state of seaweed and then stored in a Polypropylene (PP) sack to the sample drying location in the Mulyorejo area, Surabaya. Then stored in a closed plastic from the drying location to the extraction location at the Chemical and Analysis Laboratory, Faculty of Fisheries and Marine Affairs, Airlangga University, Surabaya, *Staphylococcus epidermidis* and using a comparison antibacterial as a control, clindamycin with a standard of 10µl. The chemicals used for the extraction and phytochemical screening test of *Eucheuma spinosum* seaweed were distilled water, methanol (Merck, Indonesia) alcohol (Alcohol for Analysis Emsure 1.00975.1000 1L, Merck, Indonesia), HCl (HCL 37%, Merck, Indonesia), Meyer's reagent (Merck, Indonesia) Dragendroff's reagent (Merck, Indonesia) Anisaldehyde-H2SO4 (Merck,Indonesia), N-hexane reagent (Merck, Indonesia) chloroform (Merck, Indonesia), NaOH (Merck, Indonesia) H2SO4 (Merck,Indonesia), acetic acid (Merck, Indonesia) Ammonia vapor staining (Merck, Indonesia) 99% ethanol pro analysis. (Merck, Indonesia)

The equipment used in the manufacture of *Eucheuma spinosum* seaweed extract and this phytochemical screening test are analytical balance (ABS 220-4N, Kern, Germany), *grinder* (CG9100, Klaz, South Korea), *beaker glass* (Iwaki, Indonesia), test tube (Iwaki, Indonesia), test tube rack (HS-059 ori stainless rack, Iwaki, Indonesia), Erlenmeyer (Erlenmeyer Flask, IWAKI, Indonesia), petridish (90x15mm, Iwaki, Indonesia), *rotary evaporator* (RE-1000 HN, B-ONE, China), hot plate (IKA C-MAG HP 10 IKATHERM® Hotplate, IKA, Germany), *orbital shaker* (MI010302 Shaker, Four E's Scientific, China), ruler, Thin Layer Chromatography (TLC) plate (10x10cm GF254, YUBAO, China) for phytochemical screening, refrigerator (Laboratory Refrigeration Equipment 588L Medical Stainless Steel Refrigerator, Biobase, China), measuring cup (Iwaki, Indonesia), drying oven (UF110 Oven Lab 108 L UF 110 Universal Oven Drying oven, Memmert, Germany), Filtering Flask (Glass Ware Filtering Flask Heavy Wall Thickness 5340FK500R, Iwaki, Indonesia), *Buchner funnel* (L 127C/2, Haldenwanger™ Porcelain Buchner Funnel, Sweden).

2.2 Research Methods

2.2.1 Sampling and Handling

Eucheuma spinosum seaweed was collected in Sumenep Regency, Madura, East Java Province. Samples of semi-wet seaweed *Eucheuma spinosum* were taken as much as ± 10 kilograms and placed in a sack made of Polypropylene. Samples were taken in a state of semi-wet seaweed and then stored in polypropylene (PP) sacks. The next treatment is the seaweed is washed with running water and then dried using a sack base by aerating and should not be directly exposed to the sun because it will affect the content of the compounds in it (Yunus, et al, 2009). Furthermore, it is stored in a closed plastic from the drying location to the extraction location at the Chemical and Analysis Laboratory, Faculty of Fisheries and Marine Affairs, Airlangga University, Surabaya

2.2.2 Eucheuma spinosum Extraction

The dried seaweed (simplicia) was cut ± 1 cm with scissors and then ground using a grinder to become simplicia in the form of seaweed flour. The simplicia was weighed as much as 300 grams and put into an Erlenmeyer and the simplicia was soaked (maceration) with 1.5 liters of 99% pro-analysis ethanol solution (1:3) until submerged and stirred with a shaker for 1 hour at a speed of 150 rpm, then allowed to stand for 1 hour. 3x24 hours at room temperature. The filtrate produced from maceration will be filtered with a *Buchner* vacuum and concentrated using a rotary evaporator at a temperature of 40^{o}C in order to obtain a thick extract with a constant weight (Wahyuni, 2016). This process is repeated three times.

2.2.3 Phytochemical Screening

Detection of flavonoid, alkaloid, and terpenoid active compounds in crude extract of *Eucheuma spinosum* using Thin Layer Chromatography (TLC) and precipitation test or color test. The parameter of TLC is the retention factor (Rate of flows), which is the ratio of the distance traveled by the solute to the distance traveled by the mobile phase. The useful Rf value for pure compounds can be compared with the Rf value of standard compounds, therefore the Rf number is always less than 1 (one) (Winirifmawaty, 2011).

Flavonoid Test

Testing by TLC method, the mobile phase used was chloroform: acetone: formic acid (6:6:1) with ammonia vapor staining as visible. Precipitation test for flavonoid test conducted using alkali test method. 50 mg of the extract was put into a test tube, then 3-5 drops of NaOH were added and 3-5 drops of HCl were added. If there is a yellow color deposition, it is identified that the extract contains flavonoids (Septiadi, 2013).

Alkaloid Test

Testing by TLC method, the mobile phase used was chloroform: ethyl acetate (1:1) which was detected with Dragendroff's reagent. A positive reaction indicates the presence of orange spots (Wardhani et al, 2012). The precipitation test for the alkaloid test used the Wagner method. 50 mg of the extract was put into a test tube, then 3-5 drops of Wegner's solution were added. If there is a change in color and a brownish red precipitate, the extract contains alkaloids (Septiadi, 2013).

Terpenoid Test

Testing by TLC method, the mobile phase used was n-hexane: ethyl acetate (4:1) as much as 10 ml with the visible stain of Anisaldehyde-H_2SO_4 reagent accompanied by heating at a temperature of 105^{o}C for 5 minutes on a hot plate. A positive reaction is indicated by the appearance of a red-purple stain (Yuda et al, 2017). The test used the precipitation method, the terpenoid test was carried out using the Salkowki method. 1 ml of chlorofoam was added with 50 mg of extract, then 3-5 drops of H2SO4 were added. If precipitation or color changes to red-brown sample, indicated extract contains terpenoids (Septiadi, 2013).

Saponin Test

The test used is the precipitation test. Saponin test conducted by test methods froth. 50 mg of extract was taken and placed into a test tube then added 6 ml of distilled water and shaken for 30 seconds. If the resulting foam is stable for up to 15 minutes, then the sample contains saponins (Septiadi, 2013).

2.2.4 Determination of Antibacterial Activity

Antibacterial activity test can be done by diffusion method. The working principle of the diffusion method is the diffusion of antibacterial compounds into solid media where the test microbes have been inoculated. Although not all bacteria can be tested accurately by this method, standardization has been made to test certain bacterial pathogens such as streptococci, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*, using specific culture media with various incubation conditions and interpretive criteria. for the zone of inhibition (Balouiri, 2016). This method is carried out by measuring the diameter of the clear zone which is an indication that there is a response from the test microorganism to the object under study (Pratiwi, 2012).

In literature study research, the method used to collect research data is in the form of literature data that has been selected, searched, presented and analyzed. The data sources of this research are looking for data whose substance requires theoretical processing. The data collection carried out in this study was the literature on the antibacterial activity of the acne-causing *Staphylococcus*

epidermidis then selected from the best, reviewed and analyzed and processed.

2.3 Data Analysis

In this study using data analysis in the form of descriptive analysis. and content analysis (*Content analysis*). Descriptive analysis methods provide an overview and information in a clear, objective, systematic and analytical. Data from the literature are presented in tables and pictures later described descriptively.

3. Results and Discussion

3.1 Eucheuma spinosum Seaweed Extract Results

Maceration is a cold extraction method and this method is the simplest where the filter liquid will penetrate the cell wall of the material and will enter the cell cavity containing the active substance, so that the active substance which is the concentrated solution will be pushed out of the cell due to the difference in concentration between the active substance solutions. inside the cell with those outside the cell (Wahyulianingsih et al., 2016). The results of the samples obtained from the filtration, concentrated using a *Rotary evaporator* to obtain a yield of 1.54%. Nurhayati, *et al* (2005) said that the yield value of the maceration results indicated the presence of bioactive components contained in the material. Organoleptically, the *Eucheuma spinosum* seaweed extract that has been obtained is in the form of a thick, yellowish-brown liquid with a distinctive smell of seaweed.

3.2 Phytochemical Screening Results

The results of phytochemical screening that have been carried out, crude extract of *Eucheuma spinosum* contains alkaloids, flavonoids, terpenoids and saponins. These compounds are thought to have the potential to have antibacterial activity. Analysis of compounds in this study using Thin Layer Chromatography (TLC) and Precipitation Test. Chromatographic techniques usually require the solute to be distributed between two phases, namely the stationary phase and the mobile phase. Chemical components move up following the mobile phase because the absorbent absorption of chemical components is not the same so that chemical components can move at different distances based on their level of polarity (Stahl, 2013). This TLC analysis was carried out by spotting the TLC plate extract which was eluted with the mobile phase, if the spotting of the sample was not appropriate it would cause scattered spots (Alen et al, 2017).

Ethanol extract of *Eucheuma spinosum* was analyzed using Thin Layer Chromatography (TLC) to see the color stain pattern and the parameter of TLC is the retention factor (*Rate of flows*), which is the ratio of the distance traveled by the solute to the distance traveled by the mobile phase. Compounds that have a smaller Rf value are polar because they interact strongly with the polar stationary phase of TLC, while compounds with a larger Rf value are less polar because they interact less strongly with the polar stationary phase of the TLC plate (Ratnaningtyas, 2013). Factors that affect the Rf value in TLC are layer activity, thickness, uniformity, elution distance, number of samples applied, solvent, presence of other substances, size, chamber shape and temperature changes (Ratnaningtyas, 2013).

Research conducted by Rarassari (2016) tested *Eucheuma spinosum* extract with the Fourier Transform Infrared (FT-IR) Spectrophotometer test, it was found that the isolates of *Eucheuma spinosum* compounds contained C=O, C=C, C-H and C-O groups. According Rosyidah *et al*. (2010) a compound with the characteristics of aliphatic –OH, C=O, -CH groups and indicated as a flavonoid compound of the flavone group. The antibacterial compounds in the *Eucheuma spinsoum* extract in the research of Rarassari (2016) that have been identified include the compounds 3-octadecane, 9-eicosene, 3-eicosene, 1-hexadecane, 1-nonadecane and 1-octadecane. Also added by Srivastava and Kumar (2011), the active compounds with antibacterial properties are Pentadecane, Heptadecane, 3-Eicosene, 9-Eicosene, 1-Hexadecane, Docosane and 1-Octadecene. Both Eicosene and Heptadecane compounds have strong antimicrobial activity. Eicosene is a compound that has antibacterial properties because it is a fatty acid (Srivastava and Kumar, 2011). Fat activity on antibacterial and fatty acids had the highest concentration in algae. Fatty acids in antibacterials can kill microbes by causing cell membrane disruption because they can penetrate the peptidoglycan in the cell wall without visible changes and damage the bacterial cell membrane (Sanger, 2018). There are phenolic compounds and their derivatives, namely eugenol, which were identified in the extract of Eucheuma spinosum which are also thought to have antibacterial properties. According to Nisar (2017), eugenol

is a derivative of phenolic compounds which has an antiseptic effect and works by damaging cell membranes.

3.2 Antibacterial Activity Test Results

This antibacterial activity test aims to determine the zone of inhibition of the growth of *Staphylococcus epidermidis* bacteria. The results of this antibacterial activity test are based on research data that has been carried out from several related research journals.

Staphylococcus epidermidis is a Gram positive bacterium that is said to be normal flora on human skin that causes swelling (abscess) diseases such as acne, skin infections, urinary tract infections, and kidney infections so that it can be used as test bacteria in the process of antibacterial testing on an active ingredient (Radji,2011). Gram-positive bacteria are more sensitive to antimicrobial compounds because the cell walls of Gram-positive bacteria do not have lipopolysaccharides so that hydrophobic and hydrophilic antimicrobial compounds can pass through the cell walls of Gram-positive bacteria and then interact directly with peptidoglycan in growing bacterial cells and cause cell death.(Manu,2013).

The literature search related to the antibacterial activity test against the acne-causing bacteria *Staphylococcus epidermidis* showed different clear zone diameters. The ability of the inhibition activity of *Staphylococcus epidermidis* bacteria in Bouhlal (2013) study had the largest inhibition zone than the results of other literature studies. The results of the study based on the literature were smaller or the effect was significantly different compared to the positive control of the antibiotic clindamycin in the study from Ermawati (2019). The ability of a material to inhibit or form microbes depends on the high and low concentration of the antimicrobial agent. (Purwanto, 2015). The difference in the diameter of the inhibition zone obtained was due to the concentration of the sample, in this case the ability of the substance thought to be contained in the sample that was able to inhibit bacterial growth (Nurhayati, 2011). In addition to the effect of differences in the type of substance and the concentration of a material, according to WHO, there are technical factors that affect the size of the inhibition in the disc diffusion method, including: inoculum density, disc installation time, incubation temperature, plate size, thickness of agar medium, and spacing. antimicrobial disc, antimicrobial potential of disc, media composition (Darsana, 2012). Alternative natural materials used for research contain secondary metabolites that have potential as antibacterials including phenols, flavonoids, and alkaloids. The active compounds contained in the extract that are antibacterial are increasing with the greater the concentration of the extract so that the ability to inhibit is also greater (Hanapi, *et al*. 2013).

The latest breakthrough is necessary to herbal therapy to treat a disease caused by the bacterium *Staphylococcus epidermidis* one of them by way of natural herbal treatments to reduce bacterial resistance to antibiotics. Treatment using antibiotics such as clindamycin in the long term can cause bacteria to become resistant to antibiotics (Narulita, 2019). Clindamycin can work as either bacteriostatic or bactericidal depending on the concentration of the drug, the site of infection and the organism causing the infection. Clindamycin is a narrow-spectrum group or has a narrow activity that works only against gram-positive bacteria (Mulyani et al, 2017).

4. Conclusion

Based on the results of research and literature searches that have been carried out, it can be concluded that the crude extract of *Eucheuma spinsoum* contains alkaloids, terpenoids, flavonoids and saponins and has the potential to have inhibitory activity against the growth of *Staphylococcus epidermidis* bacteria. After doing this research, it is recommended for further research that is necessary to do further testing with different methods so that the most optimal concentration of crude extract of *Eucheuma spinosum* can be known.

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Table 1. Phytochemical Test Results of *Eucheuma spinosum* Crude Extract with Precipitation Test at the Chemical and Analysis Laboratory, Faculty of Fisheries and Marine Affairs, Airlangga University, Surabaya

No.	Test sample	*Eucheuma spinosum* Extract	Color	Color standard
1.	Alkaloids	+	Red-brown sediment	Red-brown
2.	Flavonoids	+	Yellow sediment	Yellow sediment
3.	Terpenoids	+	Red-brown sediment	Red-brown
4.	Saponins	+	Foamy	Foamy

Description: Sign (+) = Contains compound
Sign (-) = No compound

Table 2. Phytochemical Test Results of Crude Extract of *Eucheuma spinosum* using Thin Layer Chromatography (TLC)

Secondary Metabolites	TLC system	Reagent	Results	Description	Rf value
Flavonoids	Stationary phase: TLC plate Mobile phase: chloroform: acetone: formic acid (6:6:1)	Ammonia Vapor	Brownish yellow spot	(+)	0,3
Alkaloids	Stationary phase: TLC plate Mobile phase: chloroform: ethyl acetate (1:1)	Dragendorff	Orange spot	(+)	0,3
Terpenoids	Stationary phase: TLC plate Mobile phase: n-hexane: ethyl acetate (4:1)	Anisaldehyde-H_2SO_4	Brownish red spot	(+)	0,45

Description: Sign (+) = Contains compound
Sign (-) = No compound

Table 3. Antibacterial Activity Test Results on *Staphylococcus epidermidis*

No	References	Inhibition Zone Average (mm)	Description	Inhibition Zone Category
1.	Siregar, 2012	4,00 (200µg = 0,2 µl)	*Eucheuma* sp. extract	Weak
2.	Bouhlal, 2013	26,5 (30µl)	*Pterosiphonia complanata* extract	Very strong
3.	Susilowati, 2015	4,7 (30 µl)	*Sargassum* spp. extract	Weak
4.	Anggraeni, *et al*.(2019)	8,6 (80 µl)	*Thalassiosira* sp.	Moderate

			extract	
5.	Ermawati (2019)	32,15	Clindamycin 10µl (Control)	Very strong

Table 4. Bacteria Inhibition Zone Category

Bacteria Inhibition Zone	Category
≥ 20 mm	Very Strong
10-20 mm	Strong
5-10 mm	Moderate
≤ 5 mm	Weak

Figure 1. *Eucheuma spinosum* Seaweed Crude Extract

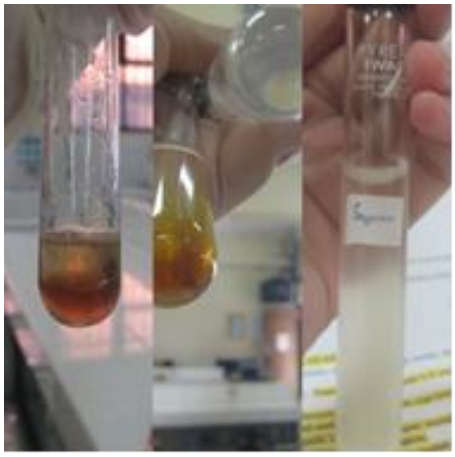

Figure 2. *Eucheuma spinosum* Phytochemical Precipitation Test Result

Figure 3. Phytochemical screening test results (Thin Layer Chromatography Test) crude extract of *Eucheuma spinosumw*