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Activity of Compounds on Seaweed *Eucheuma cottonii* Extract as Antioxidant Candidate to Prevent Effects of Free Radical in Water Pollution

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Abstract Water pollution can make many problems such as, the incidence of disease and poisoning. Pollution in water can produce free radicals and it is can trigger of disease for aquatic organisms. There are some efforts that can be done to provide this problem, such as chemical compound that can reduce the reaction of free radicals. Antioxidants are one of the chemical compounds that can reduce the activity of free radicals. *Eucheuma cottonii* is the one of a seaweed that has many in antioxidant compounds, such as phenol compounds, but it is also rich in iodine fiber and other important minerals. The method used in this research is descriptive explorative and experimental method. This research was conducted with several stages of seaweed extraction. Identification of *Eucheuma cottonii* extract is using FTIR test. The last stage is an antioxidant activity test that includes DPPH test (1,1-diphenyl-2-picrilhidrazil) and Inhibition Concentration 50 (IC 50). The results obtained in this study were based on FTIR test of antioxidant compound in *Eucheuma cottonii* seaweed extract. The one of compounds that have antioxidant activity include galaktosa-4-sulfat. Based on the results of antioxidant activity test using DPPH obtained that seaweed extract *Eucheuma cottonii* active as an antioxidant to ward off free radicals in the waters. The concentration of *Eucheuma cottonii* seaweed extract for preventive 50% concentration of DPPH is 39,926 ppm.

Introduction

Water pollution is a problem that must be addressed immediately. The occurrence of pollution in the body of water can disrupt the normal life of aquatic organisms. Pollution will cause a decrease in water quality and cause disease. This is because in polluted waters it will produce free radicals which spur disease for aquatic organisms (Pratiwi and Lingkungan, 2010). Free radicals are molecules or atoms that have one or more unpaired electrons in their outer orbitals. Free radicals are unstable, very reactive and can take electrons from other molecules in an effort to get an electron pair. These electron-losing molecules can be reactive (Astuti, 2008; Djamil and Anelia, 2009; Selawa, Runtuwene and Citraningtyas, 2013).

In the aquatic environment the process of forming free radicals can be affected by exposure to ultraviolet light and air. The compounds formed are highly reactive and are known as dangerous reactive oxygen (SOR) or reactive oxygen species (ROS) compounds. Free radicals can arise due to various complex chemical processes, free radical formation in the aquatic environment triggered by pollutants, radiation chemicals, poisons and unstable temperatures (Selawa, Runtuwene and Citraningtyas, 2013). In an effort to fulfill its electron peculiarities, free radicals that have unpaired electrons will rapidly attract electrons

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from surrounding biological macromolecules, such as proteins, nucleic acids and deoxyribonucleic acid (DNA). If the oxidized and degraded macromolecules are part of cells or organelles, it can cause damage to these cells (Astuti, 2008).

Some efforts that can be done is to provide active ingredients, that can reduce free radical reactions such as types of antioxidants. It is intended that the waters be stable again and the condition of aquatic organisms back to health. Antioxidants in the chemical sense are electron-giving compounds. Antioxidants work by donating one electron to an oxidant compound, so that the activity of these oxidant compounds can be inhibited. Antioxidants function to stabilize free radicals by supplementing electron deficiencies and inhibiting the occurrence of chain reactions of free radical formation (Astuti, 2008; Malangngi, Sangi and Paendong, 2012).

Antioxidants are divided into two: synthetic antioxidants and natural antioxidants. The use of synthetic antioxidants has high effectiveness, but is not safe because it is carcinogenic, so its use is closely monitored in various countries. These antioxidant products also have quite expensive prices (Djamil and Anelia, 2009; Pramesti, 2013). Natural antioxidants can be found abundantly in nature, both in fruits, vegetables, nuts and sea algae. Generally, chemical compounds produced by marine algae are terpenoid compounds and aromatic compounds that have activities as antioxidants, antimicrobial, antiviral, antimutagen and insecticides. Seaweed is a type of marine algae that contains high antioxidant compounds. The most abundant antioxidant content in seaweed is polyphenolic antioxidant compounds (Ely, Supriya and Naik, 2004; Fayaz *et al.*, 2005; Uy *et al.*, 2005; Suryaningrum, Wikanta and Kristiana, 2006; Suleman Suleman, Sri Andayani, 2018).

Eucheuma cottonii is a type of seaweed that contains high antioxidant compounds, so that it has the potential to counteract the effects of free radicals in the water. *Eucheuma cottonii* is a type of seaweed that contains bioactive compounds that are useful for living things. Seaweed is rich in antioxidant compounds such as phenol compounds, besides it is also rich in iodine fiber and other important minerals (Yusniarti, Lita and Feny, 2009). *Eucheuma cottonii* is included in the type of red seaweed (Rhodophyceae) (Hayashi and Reis, 2012; Damongilala *et al.*, 2013; Mohamed and Abdullah, 2016). Types of seaweed belonging to the class Rhodophyceae or red seaweed contain fikoeritin pigments, carotenoids, chlorophyll a, organic and inorganic compounds. Carotenoids in seaweed are antioxidants that can function to protect various diseases and stress (Suryaningrum, Wikanta and Kristiana, 2006).

Eucheuma cottonii is a type of red algae that can produce polysaccharides such as carrageenan (Estevez, Ciancia and Cerezo, 2004; Hayashi and Reis, 2012; Fathmawati, M.Renardo P and Achmad R, 2014; Manuhara, Praseptiangga and Riyanto, 2016). Carrageenan is a galactose polysaccharide compound and is a hydrocolloid compound consisting of potassium, sodium, magnesium and potassium sulfate esters with galactose 3.6 anhydrogalactose copolymer (Fathmawati, M.Renardo P and Achmad R, 2014). *Eucheuma cottonii* contains polysaccharide. It is an essential component for all organisms and has various biological vital functions, including anti-inflammatory, anticoagulant, antibacterial, has antioxidant activity and is able to inhibit virus attacks (Knutsen *et al.*, 2001; Hayashi and Reis, 2012; Malle, Fransina and Jansen, 2014).

Based on the above problems in this study, we will discuss the activity of the content of the *Eucheuma cottonii* seaweed extract to counteract the effects of free radicals in water through a series of tests. This is done to prove whether the compound in *Eucheuma cottonii* can be used as an antioxidant candidate to

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counteract the effects of free radicals in the waters. The purpose of this study, was to determine the content of antioxidant compounds in seaweed extract *Eucheuma cottonii*. To find out the antioxidant activity of *Eucheuma cottonii* seaweed extract, as an antioxidant candidate to counteract the effects of free radicals in water. To determine the effect of the amount of concentration of *Eucheuma cottonii* seaweed extract in inhibiting free radicals.

Materials and methods

This research was conducted in October to November 2018 at the Laboratory of Fish Health Disease (Faculty of Fisheries and Marine Sciences, University of Brawijaya), Laboratory of Materia Medika Batu, and Organic Chemistry Laboratory (Faculty of Science and Technology, State Islamic University of Malang). The laboratory equipment used in this study, is a set of rotary evaporator, shaker, and glassware which includes glass beakers, measuring cups, measuring flasks, measuring pipettes, micipipets, watch glass, stirrers, porcelain dishes and mortars. The materials used in this study, included red seaweed *Eucheuma cottonii*, aquades, KOH pro analysis, KCl pro analysis and Whatman filter paper no. 41.

Seaweed Extraction Eucheuma cottonii

Eucheuma cottonii is obtained from the location of seaweed cultivation in the Madura strait waters. Handling samples in the field is done by storing samples in the ice box to maintain freshness during the trip from the location of extraction (in the laboratory). Seaweed is dried by drying (for 5-6 days), with the aim that the bioactive compounds contained in it are not lost due to drying. The optimum drying temperature to obtain maximum levels of bioactive compounds is 40°C. Drying is higher than 40°C so the bioactive compounds will be damaged and the levels tend to decrease (Distantina, 2011).

The process was followed by the extraction of the polysaccharide *Eucheuma cottonii* with a reference from the modified Distantina and Fahrurrozi, (2011). In the process of this contraction, using an alkaline solution will increase the properties of the carrageenan gel produced. Potassium hydroxide (KOH) was chosen because, the effect of cation on kappa carrageenan produces gel stronger than other alkalis such as NaOH and $Ca(OH)^2$. The use of alkali has two functions, namely to help extract polysaccharides to be more perfect and accelerate the elimination of 6-sulfate from the monomer unit to 3.6-anhydro-D-galactose so that, it can increase gel strength and reactivity of the product to protein. The use of KOH also affects the increase in yield and quality of carrageenan produced (Ramu Ganesan, Shanmugam and Bhat, 2018).

Seaweed is then poured into a sieve, then seaweed is washed with running water while squeezing and boiling with distilled water so that the KOH mixed in the seaweed is lost. This is indicated by the seaweed no longer slippery if held. Next seaweed soaked with KCl. The use of KCl is to remove carrageenan from water and oil. At each process using a high extraction temperature because, high temperatures cause more sulfate content to emerge from seaweed (Perez Recalde *et al.*, 2016; Ramu Ganesan, Shanmugam and Bhat, 2018).

FTIR

Polysaccharide extract *Eucheuma cottonii* 2 mg of was mixed with KBr to form a transparent film. Spectra waves from *Eucheuma cottonii* polysaccharide extract will be seen using the Nicolet Impact 410 FT-IR spectrometer at a wavelength of 400-4000 cm^{-1} (Immanuel *et al.*, 2012).

Antioxidant Activity using DPPH Method

Antioxidant activity using the DPPH method refers to the research of Damongilala *et al.* (2013), 25 mg of dried algae is dissolved with methanol p.a to 25 ml (1000 µl/ml). Then,

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a series of sample concentrations of 100, 200, 400 and 800 µl/mL were then made. In each tube, the sample was added 1 ml of DPPH 1 mMol solution. then added methanol p.a to 5 ml and homogenized. Blank solution and test solution were incubated for 30 minutes, then absorption was read at a wavelength of 517 nm. Percentage of inhibition is calculated by the following formula:

$$\% \text{ inhibition} = \frac{\text{Blanko - sample}}{\text{Blanko}} \times 100\%$$

Next is the Inhibition Concentration 50 (IC 50) test. Inhibition Concentration 50 (IC 50), is an antioxidant concentration that is able to give 50% free radical capture percent compared to control through a line equation. The value of IC 50, is obtained from the intersection of the line between the power resistance and the axis of concentration, then put in the equation y = a + bx, where y = 50 and the value of x indicates IC 50.

Results and discussion

FTIR

Carrageenan extract *Eucheuma cottonii,* identified the contents of its active compound using FTIR in transmittance mode (eight scans, at a resolution of 400-4000 cm^{-1}). The results obtained can be seen in Figure 1.

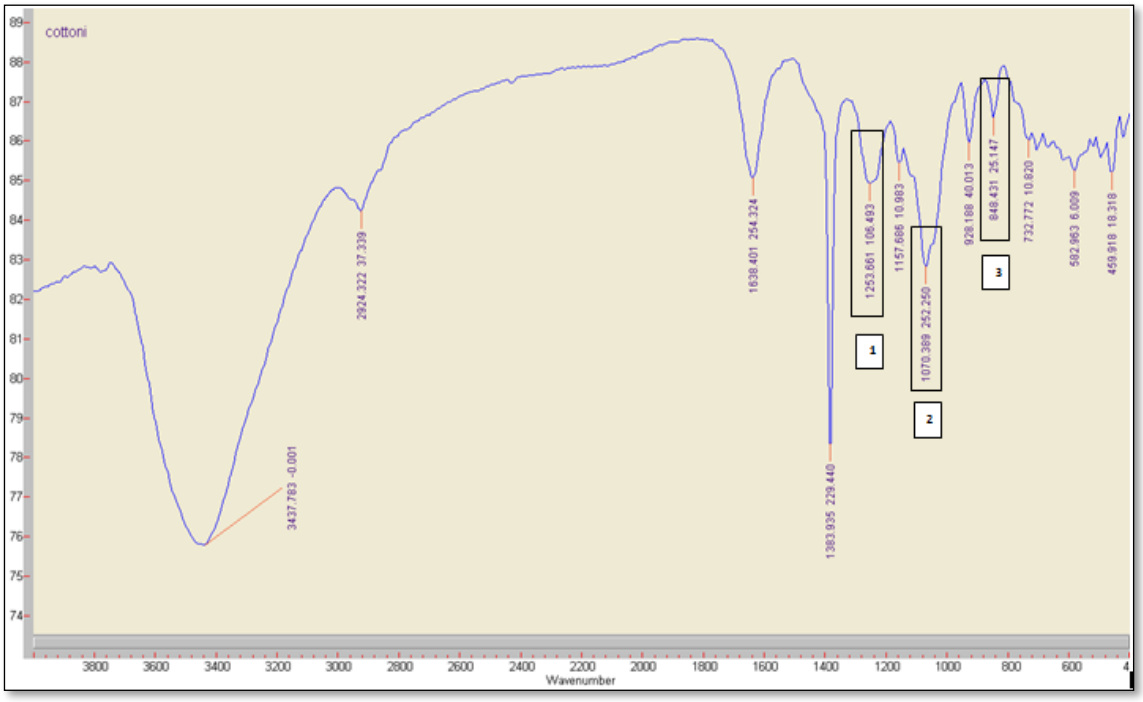

Figure 1. FTIR Test Results *Eucheuma cottonii* Extract.

Based on the above results, it can be seen that the *Eucheuma cottonii* extract contains compounds in absorbance of 1253,661 cm^{-1} at peak 5; 1070,389 cm^{-1} in peak 7; 848,431 cm^{-1} in the 9 peak, according to Uy *et al.*, (2005) :

1. The absorbance file in the area of 1210-1260 cm^{-1} is a bond of sulphate esters which are owned by all types of carrageenan.
2. The absorbance file in the area of 1010-1080 cm^{-1} is a glycoticic bond that belongs to all types of carrageenan.
3. The absorbance at 840-850 cm^{-1} is a galactose-4-sulfate bond that is owned by carrageenan type kappa.

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Based on the literature, the content of *Eucheuma cottonii* extract compound contains galactose-4-sulfate bonds in the 9 peak, which is included in the sulfate galactose group (polysaccharide sulfate). Polysaccharides belong to a very diverse class of macromolecules. Seaweed contains a variety of different types of polysaccharides, where the chemical structure is related to the taxonomic classification of algae and their cell structure. Polysaccharide sulfates, can inhibit the activity of bacteria and also viruses and act as antioxidant compounds. Total polysaccharide compounds contained in seaweed are about 76% of dry weight. Among the many different algae polysaccharides, the most important are galactan, fucoidan, laminarin and alginate (Castro, Zarra and Lamas, 2004; Abidin *et al.*, 2011; Chojnacka, 2012).

Carrageenan is a generic name for the family of polysaccharides, obtained by extraction of certain red seaweed species (Rhodophyta) (Chen *et al.*, 2014). Carrageenan is included in hydrophilic linear sulfate galactans. This compound consists of 3.6-anhydro-D-galactose, forming a repetition unit disaccharide from carrageenan. Galactant sulfate is found in seaweed cell walls. Galactan is a macromolecule containing a disaccharide based repetition unit. Depending on the optical configuration the second unit is distinguished on the distribution (D) and karaginana (L). The subtituents of the main galactant chain are sulfate groups, methoxyl groups, pyruvic acid acetate and glucosyl side chains. These groups, can be irregularly distributed through macromolecules (Campo *et al.*, 2009).

Carrageenan can function as an antioxidant. Antioxidants in the chemical sense are electron-giving compounds. Antioxidants work by donating one electron to an oxidant compound, so that the activity of these oxidant compounds can be inhibited. Antioxidants function to stabilize free radicals, by supplementing electron deficiencies and inhibiting the occurrence of chain reactions of free radical formation (Astuti, 2008; Malangngi, Sangi and Paendong, 2012).

Antioxidant Activity of Eucheuma cottonii Extract based on Color Change

1.1-diphenyl-2-picrylhydrazyl (DPPH) is a test method that is usually used to test the activity of antioxidant compounds in test samples. 1.1-diphenyl-2-picrylhydrazyl (DPPH) is an unstable nitrogen-containing organic compound, with a strong absorbance of a maximum wavelength of 517 nm. In principle, the free radical deterrent method is a measure of the deterrence of synthetic free radicals in polar organic solvents, such as methanol and water at room temperature by a compound that has antioxidant activity (Daud, 2002; Tristantini et al., 2016).

The process of deterring free radicals is through the mechanism of taking hydrogen atoms from antioxidant compounds by free radicals, so that free radicals capture one electron from antioxidants. DPPH synthetic free radical compounds react with antioxidant compounds, by taking hydrogen atoms from antioxidant compounds to obtain electron pairs (Daud, 2002; Stevi G.Dungir, Dewa G. Katja, 2012). DPPH solution, which is initially purple after reacting with natural antioxidants will form a yellow color. The higher antioxidant content, the purple color in the DPPH solution will decrease and form a yellow color (Daud, 2002; Malangngi, Sangi and Paendong, 2012).

The antioxidant activity of the *Eucheuma cottonii* extract was tested by adding DPPH solution to the sample solution with several concentrations. Testing of antioxidant activity was carried out by distinguishing colors between blank solutions. Sample solutions of *Eucheuma cottonii* extract which had been incubated at 37°C for 30 minutes. The results of sample color changes can be seen in table 1.

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Table 1. Results of Color Changes in Antioxidant Activity Tests

Concentration	Discoloration	Indicator of Reactions to Free Radicals
Eucheuma cottonii		
0 ppm	Purple	not active
100 ppm	Violet	not active
200 ppm	Yellow	active
400 ppm	Yellow	active
800 ppm	Yellow	active

Based on the results obtained (Table 1), it can be concluded that qualitatively the *Eucheuma cottonii* extract is active as an antioxidant. This can be seen from the color changes that occur. At a concentration of 100 ppm, the extract has shown light purple and then for a concentration of 200-800 ppm it shows a light-yellow color. This can strengthen the results, that DPPH solution homogenized with a solution containing antioxidant compounds will turn yellow.

DPPH is a free radical molecule in purple. Based on the results above, it can be explained a solution, that has a purple color indicates the content of free radicals is still contained in the solution. The yellow solution, shows an antioxidant reaction that can reduce free radicals in the sample solution. Yellow color changes can occur, because antioxidant compounds give one electron to DPPH so that damping occurs on DPPH free radicals. When the purple DPPH solution meets the electron donor material, DPPH will be reduced, causing the purple color to fade and be replaced by the yellow color from the picril group. The antioxidant testing of the DPPH method, was carried out by looking at the color changes of each sample after incubation with DPPH. If all DPPH electrons are paired with electrons in the extract sample, there will be a change in color of the sample starting from dark purple to bright yellow. Changing the color of the solution from purple to yellow, shows the efficiency of antidote to free radicals (Daud, 2002; Malangngi, Sangi and Paendong, 2012; Stevi G. Dungir, Dewa G. Katja, 2012).

Antioxidant Activity of Eucheuma cottonii Extract

The next antioxidant activity test, was measuring the absorbance value using a UV-Vis spectrophotometer at a wavelength of 517 nm. The results of the measurement absorbance values of blank solutions and solution samples of *Eucheuma cottonii* extract, can be seen in Table 2.

Table 2. Results of Inhibiting (%) *Eucheuma cottonii* Extract

Concentration (ppm) *Eucheuma cottonii*	Inhibition (%)			Average (%) inhibition
	1	2	3	
0				
100	5.254	4.181	5.480	4.972
200	8.362	5.650	6.723	6.911
400	8.701	8.305	8.249	8.418
800	6.780	9.040	9.040	8.286

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Based on the results obtained (Table 2), it can be concluded that the higher the concentration of the sample the higher the level of inhibition. Obtained the highest results at a concentration of 800 ppm the inhibition percentage value was 8.286%. This is in accordance with the statement of (Pramesti, 2013), that the value of inhibition level increases with increasing sample concentration, because more antioxidant compounds in the sample inhibit DPPH free radicals. Blank absorbance and absorbance of the samples obtained were used to determine the percent (%) of antioxidant activity. Percent (%) of antioxidant activity, is one parameter that shows the ability of an antioxidant to inhibit free radicals. The higher percent (%) of antioxidant activity, shows the number of hydrogen atoms given active compounds to DPPH radicals so that it is reduced to DPPH-H (1.1-diphenyl-2-picrylhydrazine). The percentage of inhibition, showed that DPPH activity was lost by the percentage of inhibition in the test sample. This shows, that if the higher the percentage inhibition of a sample, he is able to reduce the radical DPPH. Antioxidant testing on *E. cottonii* methanol extract using a comparative material with a type of synthetic antioxidant namely ascorbic acid. The use of comparators to find out how strong the antioxidant potential is in the extract, when compared to synthetic antioxidants that have often been used such as ascorbic acid (Hanapi and Fasya, 2013; Mardiyah, Fasya and Amalia, 2014).

To determine the strength of antioxidant activity, inhibition concentration 50 (IC 50) is needed. Based on the results of the average (%) inhibition, a linear regression equation was obtained from *Eucheuma cottonii* and ascorbic acid extract (Tristantini et al., 2016). The linear regression equation *Eucheuma cottonii* is y = 1.145x + 4.2844, so that IC 50 is obtained at 39,926 ppm. This shows, that at a concentration of 39,926 ppm the extract can inhibit 50% of DPPH free radicals. A compound is said to be an antioxidant very strong, if the IC 50 value is less than 50, strong (50-100), moderate (100-150), and weak (151-200). The smaller IC 50 value, the higher the antioxidant activity (Djamil and Anelia, 2009; Tristantini et al., 2016). Based on the statement above, it can be said that *Eucheuma cottonii* extract has strong antioxidant activity. Inhibition concentration (IC 50) is a parameter that shows the concentration of the test extract which can capture radicals as much as 50%. According to Dimara and Yenusi (2011), the smaller the IC 50 value of a test compound, the more active the compound as a free radical or antioxidant catcher. IC 50 is the concentration needed to reduce 50% of the substrate concentration (DPPH radical). The value of IC 50 is considered a good measure of the antioxidant efficiency of pure compounds or extracts.

Eucheuma cottonii extract as a Prevent Effects of Free Radical in Water Pollution

Eucheuma cottonii extract can be used as a as a prevent effects of free radical in water pollution. This is due to the content of sulfate galactose as a natural antioxidant compound, which is very useful for stabilizing the aquatic environment. The existence of this research will be a reference on how to counteract free radicals in the waters, so as not to cause pollution and danger to aquatic organisms such as diseases. The existence of this study, can be used as a reference for further research to find out more details. It is about the use of sulfur galactose compounds from *Eucheuma cottonii* and the right concentration for public water pollution problems. In this study using the FTIR method to determine the compounds contained in *Eucheuma cottonii* extract. This method is caused by knowing the functional groups and bonds contained in a compound from bioactive extract, on the infrared spectrophotometer, also equipped with fourier transformation, which is useful for analysis of spectrum results (Distantina and Fahrurrozi, 2011). According to

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Anam, Sirojudin and Firdausi (2007), the analysis of infrared spectrophotometers was carried out by comparing the functional groups of samples, absorbance bands formed in the infrared spectrum using correlation tables and using a spectrum of known comparative compounds. So, using this method can be clearly illustrated what compounds are contained.

Conclusions and suggestion

Based on the results obtained, it was found that carrageenan extract *Eucheuma cottonii* contained compounds classified as sulfate galactose. Based on the results of the antioxidant activity test using DPPH, it was found that *Eucheuma cottonii* seaweed extract was active as an antioxidant to counteract free radicals in the waters. The amount of concentration of *Eucheuma cottonii* seaweed extract in counteracting 50% of DPPH concentration was 39.926 ppm. Based on the research that has been done, it can be suggested that further research needs to be conducted on a wider range of concentrations.

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