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ANTIOXIDANT ACTIVITY AND TOXICITY POLYSACCHARIDE EXTRACT FROM RED ALGAE *Eucheuma cottonii* AND *Eucheuma spinosum*

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ABSTRACT

Red algae *Eucheuma cottonii* and *Eucheuma spinosum* which one alternative source of antioxidant and anticancer because contains polysaccharide compound. This research aims to isolate and examine the activity of antioxidant and toxicity polysaccharide extract from red algae *Eucheuma cottonii* and *Eucheuma spinosum* . The study was carried out by isolating the polysaccharide extract using water and methanol-ethanol precipitation. Antioxidant activity of crude extract was examined using 1,1-Difenil-2-pikrilhidrazil (DPPH) method, and toxicity test was carried out using *Brine Shrimp Lethality Test* (BSLT). The research results showed that the crude extract polysaccharide for *Eucheuma cottonii* and *Eucheuma spinosum* have strong antioxidant activity with IC_{50} value of 72,49 ppm and 75,98 ppm. The result of BSLT assay showed that the crude extract polysaccharide has a highest toxicity with LC_{50} value of 165,88 ppm and 337,21 ppm there are classified as toxic. The crude extract polysaccharide *Eucheuma cottonii* and *Eucheuma spinosum* has a potential to be developed as an alternative antioxidant and anticancer agent.

Key words : Red algae, *Eucheuma cottonii*, *Eucheuma spinosum,* polysaccharide, antioxidant, toxicity

1. INTRODUCTION

Indonesia, which is 70% of the archipelago, has a coastline of over 81.000 km with 13. 667 islands have the potential of seaweed that is big enough. Residents who live in coastal areas have always utilize seaweed or algae which is also known by the name of seaweed in various forms, for example, is eaten raw as a salad, vegetables, pickles, cakes or puddings and sweets, as well as ingredients for pharmaceuticals.[12]

From the 555species of algae, there are four tribes algae has been known, such as the blue algae (*Cyanophyceae*), green algae (*Chlorophyceae*), brown algae (*Phaeophyceae*), and red algae (*Rhodophyceae*).[17]

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of a secondary metabolites characterized by a broad spectrum of biological activities with antiviral, antibacterial, and antifungal activities which acts as potential bioactive compounds of interest for pharmateutical applications.[15] Now, the many attention to screening of natural antioxidant because the use of sintetic antioxidant have carsinogen effect.[8]

It can be used as food, beverages, and pharmaceutical, and algae also become important economically because algae contains polysaccharide compounds. Polysaccharides of algae that the most commercial and industrial importance in the field today are the type of carrageenan, alginate, agar, and agarose which are a fraction from agar.[7] Polysaccharides of some marine algae have also been knowned to have biological activity

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associated with pharmacological potential as an anticoagulant, antioxidant, and antitumor.[4]

Preliminary study of the polysaccharide compounds as antitumor and antioxidant of some algae that has been done are purificationantioxidant activity, toxicity, and cytotoxicity from red algae *Rhodymenia palmate*[17]; purification and in vitro antioxidant activity of polysaccharide from green seaweed *Caulerparacemosa*[9]; purification, antitumor and antioxidant activities *in vitro* of polysaccharides from the brown seaweed *Sargassumpallidum* [18].

Eucheuma cottonii and *Eucheuma spinosum* which one type of red algae of interesting to explore for research. It is based on the fact that this red algae have the primary and secondary metabolites of high economic value, such as hydrocolloid compounds used in the food industry, pharmaceutical and cosmetics industries.

Eucheuma cottonii and *Eucheuma spinosum* that can be used as raw material for the manufacture of polysaccharides of carrageenan. Seaweeds *Eucheuma cottonii* and *Eucheuma spinosum* are economically valuable plant because its use is very extensive, as groceries, organic fertilizer industry, cosmetics, textiles, and pharmaceuticals. use of seaweed is caused by the presence of carrageenan which has the properties of carrageenan as stabilizer, thickener, gilling agent, emulsifier, and others. The nature of carrageenan is widely used by many industries as it helps products produced more quality[6].

2. METHOD

Materials Research

The materials of research are the red alga *Eucheuma cottonii*, *Eucheuma spinosum,* aquadest, methanol, ethanol, DPPH, sea water, *Artemia salina* Leach, KBr, NaOCl, ascorbic acid.

Research Tools

The tools of research are hot plate, blender, hammer mill, thermometer, oven, spectrophotometer, incubator, micropipet, filter paper, volumetric flask, etc.

Sample Preparation

The red alga *Eucheuma cottonii* and *Eucheuma spinosum* were washed with fresh water to remove foreign substances. The sample be dried under the sun for 5 days and with oven at 60 ^{o}C for 3 hours. Then the sample milled and sieved with 60-70 mesh.

Extraction of Polysaccharide

The dried seaweed powder of *Eucheuma cottonii* and *Eucheuma spinosum* were extracted with water (1:50 w/v) for 1 hour. The sample was then filtered through whatman paper. The crude extract of polysaccharide can obtained when the supernatant was precipitated with methanol and ethanol (1:1 v/v) for 24 hours. After that, the solution was filtered and the residue then dried, mashed, and be pondered (the crude extract polysaccharide). Then, the crude extract polysaccharide will be determinated of antioxidant activity and toxicity.

Antioxidant assay using DPPH Method (1,1-diphenyl-2-pycrylhydrazyl)

Test of antioxidant activity according to the 1,1-diphenyl-2-pycrylhydrazyl (DPPH) method.[3] The first made the reference solution of a solution of 1 mL of 0.4 mM DPPH and then added with methanol up to 5 mL. Then the test sample

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solution made of coarse polysaccharide extract as much as 5 mg and dissolved in 5 mL of methanol to obtain a solution with a concentration of 1000 ppm (the mother liquor). The mother liquor pipette as much as 100, 200, 300, 400, and 500 mL and then inserted into a test tube to obtain a sample concentration of 20, 40, 60, 80, and 100 ppm. Into each tube is added to a solution of 1 mL of 0.4 mM DPPH, then diluted with methanol up to 5 mL volume and incubated at 37 ^{o}C for 30 min. The absorbance was measured at the maximum wavelength 515 nm with spectrophotometer UV-Vis..

The results of the antioxidant determination were compared to the ascorbic acid as a positive control. The value of the antioxidant activity was calculated using the formula.

$$\% \text{ Antioxidant activity} = \frac{\{(\text{control absorbance}) - (\text{sample absorbance})\}}{(\text{control absorbance})} \text{ x } 100\ \%$$

Toxicity Test using Brine Shrimp Lethality Test (BSLT)

a. Preparation of Shrimp larva

The shrimp eggs were put into container of sea water for hatching, and aerated under 40-60 watt in candescent lamp. The hatcing temperature was maintained in range of 25-30^{o}C for 48 hours. After the eggs hatched, shrimp larvae were taken to be tested.

b. Implementation Test

The toxicity tests done by using Brine Shrimp Lethality Test (BSLT) method. The crude extract polysaccharide were made in concentration 10, 100, dan 1000 ppm and were placed in 3 vials. Ten shrimp larvae were inserted into vial which contain the test compound then the sea water was added to 5mL. As control, using methanol in sea water without extract. The treatment of extract and control have done as much as 3 times restating. Next, all vial incubated under 15 watt in candescent lamp. After incubation,the dead and live of larvaes *Artemia salina* Leach were observed and calculated. The LC_{50} value was determined by using probit analysis. The mortality percentage of shrimp larvae could be determined by formula:

$$\% \text{ Mortality} = \frac{\text{Amount of death test larva} - \text{Amount of death control larva}}{\text{Amount of test larva}} \text{ x } 100\%$$

3. RESULTS AND DISCUSSION

The crude extract polysaccharide from alga *Eucheuma cottonii* and *Eucheuma spinosum* powder were carried out with regular maceration by using aqueous extraction and methanol-ethanol precipitation for 24 hours. The yield of polysaccharide that was obtained from the filter of residu, then be dried, mashed, and be pondered. This study shows the results of extraction of polysaccharides from macroalgae *Eucheuma cottonii* and *Eucheuma spinosum* powder in regular maceration using solvent distilled water and methanol - ethanol precipitation.

In general, the main component of algae is a carbohydrate that can reach 40-70 % per dry weight, depending on the type of algae growth and environmental conditions.[1] *Eucheuma cottonii* and *Eucheuma spinosum* which one of a source of carrageenan. *Eucheuma cottonii* to product kappa carrageenan and have contain carrageenan 61,52%.[5] *Eucheuma spinosum* have *c*hemical content is iota carrageenan (65%), protein, fat, crude fiber, water, and ash. Iota Carrageenan is a

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polysaccharide sulfate ester sulfates in which the content is (28-35%).[2]

Eucheuma cottonii and *Eucheuma spinosum* of seaweed were use caused by the presence of carrageenan which has the properties of carrageenan as stabilizer, thickener, gilling agent, emulsifier, and others. The nature of carrageenan is widely used by many industries as it helps products produced more quality.[6]

Antioxidant assay using DPPH method

Test antioxidant activity with DPPH method *Eucheuma cottonii* and *Eucheuma spinosum* polysaccharide extract compared with ascorbic acid as a positive control. The value is used as the percent inhibition data to calculate the IC_{50} value. Value percent inhibition of polysaccharide extract higher with increasing concentration of the sample. Value percent inhibition of polysaccharide extracts against the concentration of the sample shown in Figure 1 and Figure 2.

While the IC_{50} values obtained by regression equation of the relationship between the percent inhibition at concentrations of extracts shown in Table 1. IC_{50} values obtained polysaccharide extract of *Eucheuma cottonii* and *Eucheuma spinosum* antioxidant activity of 72,49 ppm and 75,98 ppm (relatively strong), while the IC_{50} value of 2.283 ppm ascorbic acid (as very strong).

The antioxidant activity from the crude extract and polysaccharides fraction of macroalgae *Eucheuma cottonii and Eucheuma spinosum* carried out by using DPPH method. Excellent DPPH reagent to screen antioxidant compounds that specifically reaction neutralize free radicals by breaking free chain. Protons or hydrogen donor of antioxidant compounds to the free radical DPPH free radical chain will break up to to form a compound that is not radical. It can be written in the following equation:

$$DPPH^{+} + AH \longrightarrow DPPH\text{-}H + A^{+}$$

Free Radical Antioxidant Neutral color free radical
Purple color yellow new

Purple color which reduced the sample mixture is proportional to the antioxidant power of the test .[17]

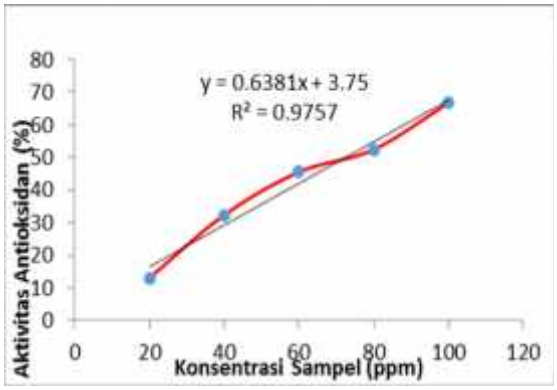

Figure 1. Regresion graph antioxidant activity (%) versus the concentration of the crude extract polysaccharide *Eucheuma cottonii*

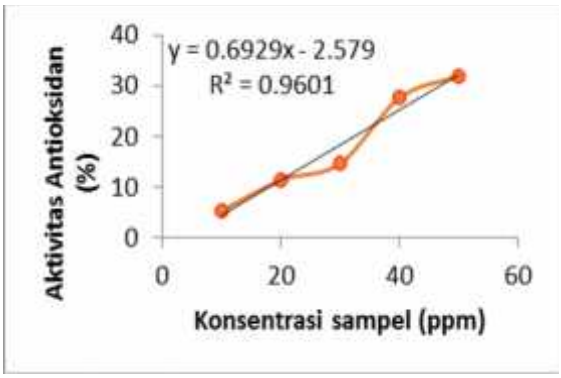

Figure 2. Regresion graph antioxidant activity (%) versus the concentration of the crude extract polysaccharide *Eucheuma spinosum*

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Tabel 1. The value IC_{50} of the crude extract polysaccharide

Sample	IC_{50} (ppm)	Antioxidant activity
Eucheuma cottonii	72,49	Strong
Eucheuma spinosum	75,98	Strong
Ascorbic acid (positif control)	2,283	Very strong

Antioxidant is a compound capable of slowing or preventing the oxidation processes caused by free radicals. DPPH is a free radical compounds are stable at room temperature and is often used for antioxidant activityassay some compounds or extracts of natural materials. DPPH chosen because it is a method that is simple, easy , and using samples in small amounts with a short time.[11]

The antioxidant activity assay of the crude extract compared with ascorbic acid produces a relationship between the concentration of test samples with percent antioxidant activity. Percent antioxidant activity (percent inhibition) was obtained from the difference between the absorbance of DPPH absorbance with the absorbance of the test sample. The ascorbic acid has antioxidant activity percent is much larger than all the the crude extract polysaccharide. This means that ascorbic acid has a damping capability against free radicals are very high, although with lower concentrations.

The value of the percent inhibition is used as the data to calculate the IC_{50} value, which is obtained from the linear regression equation that stated the relation between the concentrationof the test solution (ppm) versus antioxidant activity (%). The parameters used to determine the antioxidant activity is Inhibition Concentration (IC_{50}) is the concentration of a substance that can cause a reduction in antioxidant activity of DPPH by 50 %. The smaller the IC_{50} values,the greater the antioxidant activities.[11]

From the results of this study indicate that extracts polysaccharides of macroalgae *Eucheuma cottonii and Eucheuma spinosum* has IC_{50} value of 72,49 ppm and 75,98 ppm, while for ascorbic acid have a IC_{50} value of 2.283 ppm. Based on the IC_{50} value indicates that the polysaccharide extract has antioxidant activity which is much smaller than ascorbic acid. If IC_{50} <50 ppm, the antioxidant power is very strong, IC_{50} potent antioxidant power strong of 50-100 ppm, 101-150 ppm IC_{50} moderate antioxidant power, and IC_{50}> 150 ppm antioxidant power is weak.[13] Based on these results polysaccharide extract has a powerful antioxidant, while ascorbic acid has a very strong antioxidant. Because of its powerful antioxidant, meaning crude extract polysaccharides of macroalgae *Eucheuma cottonii and Eucheuma spinosum* has the compounds that are antioxidants. A compound containing hydroxyl groups, polyhydroxyl, or carbonyl have the antioxidant activity because the compound will react with free radicals through a mechanism of proton donor of the hydroxyl group contained in the active compound or compounds are antioxidants.[14]

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In addition to hydroxyl groups, sulfate groups of the polysaccharide sulfate also acts as an antioxidant.[16]

Toxicity assay with BSLT (Brine Shrimp Lethality Test) method

Toxicity from the crude extract of *Eucheuma cottonii and Eucheuma spinosum* can be know with determined value of LC_{50}. The observations were made after 24 hours by counting the number of shrimp larvae mortality, furthermore LC_{50} values determined using probit –log concentration graph. The results of calculation of value LC_{50} from the crude extract and polysaccharide fraction showed on Table 2.

The obtained LC_{50} values (Table 2) showed that the crude extract *Eucheuma cottonii and Eucheuma spinosum* have the LC_{50} values of 165,88 ppm and 337,21 ppm and it can be classified to be toxic.

Tabel 2. The value LC_{50} of the crude extract polysaccharide

Sampel	LC_{50} (ppm)	Toxicity
Eucheuma cottonii	165,88	Toxic
Eucheuma spinosum	337,21	Toxic

Toxicity assay with use BSLT method (*Brine Shrimp Lethality Test*) is one of the methods of screening using shrimp larvae Artemia salina Leach as test animals to determine the toxicity of an extract or a new compound derived from plants. The toxicity test with this method has been shown to have a correlation with the power cytotoxicity of anticancer compounds. Moreover this method is easy to do, inexpensive, fast, and accurate enough.[10]

Polysaccharide extract samples can be determined based on the value of its toxicity effects of the calculation of Artemia salina Leach mortality data by using charts log concentration of the sample against probit value. Drugs given as the median lethal concentration termed the concentration or LC_{50}.According to[10], the death of *Artemia salina* Leach becomes a parameter to indicate the presence of active substances that are cytotoxic , the level of toxicity of a test compound can be seen from the graph the value of LC_{50} values using probit versus log concentration of the sample. An extract is considered highly toxic if the LC_{50} value < 30 ppm, toxic when LC_{50} values 30-1000 ppm, and is not toxic when LC_{50}> 1000 ppm. The smaller the value, the more toxic LC_{50} test compound. Based on the results of research showed that the crude extract from the red alga *Eucheuma cottonii and Eucheuma spinosum*, is toxic because it has a LC_{50} values 30-1000 ppm shows the high toxicity.[10]

4. CONCLUSION

Based on the research that has been done about antioxidant activity and toxicity assay from the crude extract polysaccharide of red algae *eucheuma cottonii* and *eucheuma spinosum* that shows the IC_{50} values of strong and the LC_{50} values of classificated toxic. This shows that indicate the content of primary metabolites from *eucheuma cottonii* and *eucheuma spinosum* have antioxidant activity of strong and toxic polysaccharide (positive correlation as the initial screening for anticancer).

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REFERENCES

[1] Angka S.L. &Suhartono M.T. (2000). Marine Biotechnology results. Center for Coastal and Marine Resource. Bogor: IPB. Edition I. 149p.

[2] Atmadja, W.S., Sulistijo., Kadi, A., Sahari., 1996. Intro Type Seaweed in Indonesia. 30p. LIPI, *Jakarta*.

[3] Chow S.T., Chao W.W. & Chung Y.C. (2003). Antioxidative Activity and Safety of 50% Ethanolic Red Bean Extract (*Phaseolusraditus L. VarAurea).* J. Food Science, 68(1):21-25.

[4] Cristiane M.R., De Souza., C.T. Marques., D.C.M. Guerra., F.R.F. Da Silva., Rocha., dkk. (2006). Antioxidant Activities OfSulphated Polysaccharides From Brown And Red Seaweed. Springer Science and Business Media B.

[5] Hambali E., Ani, S., Wadli (2004). Make a variety of processed seaweed. Publisher Swadaya. Jakarta.

[6] Hidayat A. 2001. Seaweed Cultivation. Publisher Usaha Nasimal. Surabaya.

[7] Hijaz M.N. (2009). Antioxidant Activity Test Red Algae type of carrageenan in Eucheuma spinosum and Gracilaria verrucosa (Tesis). Universitas Islam Negeri Malang. Malang

[8] Kong, F., Zhang, M., Liao, S., Yu, S., Chi, J., Wei, Z., 2010. Antioxidant Activity of Polysaccharide-enriched Fractions Extracted from Pulp Tissue of Litchi Chinensis Sonn*, Molecules*, **15:** 2152-2165.

[9] Mahendran S. &Saravanan S. (2013).Purification And In Vitro antioxidant Activity Of Polysaccharide Isolated From Green Seaweed *Caulerpa racemosa*. International Journal of Pharma and Bio Sciences, 4(4):(B) 1214-1227.

[10] Meyer B. N., Ferrigni N. R., Putman J. E., Jacbsen L.B., Nicols D. E., &Mc Laughlin J. L. (1982). Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. Plant Medica.

[11] Molyneux P. (2004). The Use of The Stable Free Radical Diphenyl picyrylhydrazyl (DPPH) for Estimating Antioxidant Activity. Songklanakarin. J. Sci. Technol, 26(2):211-219.

[12] Murdinah., Nurbaity., Apriani., Nurhayati., dan Subaryono SP. 2012 *To Make of jelly from red algae Gracilaria sp*. Publisher Swadaya. Jakarta.

[13] Nihiati I. A., Hertiani T. &Rohman T.(2008). Rhizome Extract Antioxidant Power Extract Ethanol From *Rhizoma* (*Boesenbergia Pandurata*) with Free Radical arrest Method of DPPH (1,1-difenil-2-pikrilhidrazil) Method. Publisher *Majalah Obat Tradisional,*13 (45)**:** 101– 108.

[14] Oke J.M. & Hamburger M.O. (2002). Screening of Some Nigerian Medicinal Plants for Antioxidant Activity Using DPPH Radical. Afr. J. Biomed Res, 5: 77-79.

[15] Solomon RDJ., Santhi VS. (2008). Purification of bioactive natural product against human microbial pathogens from marine seaweed Dictyota acutiloba. J. Ag. World. J

ISSN 1411-2132
Volume 17 No. 2

Microbiol. Biotechnol. 24:1747-1752.

[16] Souza B.W.S., Cerqueira M.A., Bourbon A.I., Pinheiro A.C., Martins J.T., Teixeira J.A., dkk. (2012). Chemical Characterization, Antioxidant Activity of Sulfated Polysaccharide from The Red Seaweed *Gracilariabirdiae*. Food Hydrocolloids,27**:** 287-292.

[17] Wikanta T., Januar H.I. &Nursid M. (2005). Uji Aktivitas Antioksidan, Toksisitas dan Sitotoksisitas Ekstrak Alga Merah *Rhodymenia palmata*. Jurnal Penelitian Perikanan Indonesia, 11 (4):41-49.

[18] Ye, H., Wang, K., Zhou, C., Liu, J., Zeng, X., 2008. Purification, Antitumor and Antioxidant activities in Vitro of Polysaccharides from The Brown Seaweed *Sargassum pallidum. Food Chemistry.* 111**:** 428-432.