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Antioxidant activity of red algae extract (Rhodophyta) Eucheuma spinosum measured by 2.2-diphenyl-1- picrylhydrazyl method

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Abstract

Objective: To determine the results of the antioxidant concentration of red algae extract (Rhodophyta) Eucheuma spinosum.
Material and Method: The type of research method used in this study is an experimental laboratory.
Results: Antioxidant research results of red algae extract (Rhodophyta) Eucheuma Spinosum dry extract of ethanol fraction using DPPH (2.2-diphenyl-1- picrylhydrazyl) method had IC50 values of 702.387 µg / mL and Vitamin C IC50 of 2,588 µg / mL. Antioxidant levels of Vitamin C are more substantial.
Conclusion: Antioxidants of red algae extract (Rhodophyta) Eucheuma Spinosum dry extract ethanol fraction IC50 value 702,387 µg / mL

Keywords: Antioxidants, DPPH, Eucheuma spinosum, Red Algae, Rhodophyt
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Introduction

Indonesia is known as a country with the second-largest vegetation in the world after Brazil. There are about 30,000 plants in Indonesia, and 9,600 of them include medicinal plants or herbs. In addition, Indonesia is also a maritime country whose earth's surface is dominated by the ocean. Marine natural materials are widely used in agriculture (food), industry, health, and the environment, which are generally sourced from biological organisms.[1]

Indonesian waters as a tropical region have seaweed resources of 6.42% of the world's total seaweed. Seaweed from the red algae class (rhodophyceae) ranks the most from the number of species that grow in Indonesian marine waters, which is about 452 species, after those green algae (chlorophyceae) about 196 species and brown algae (phaeophyceae) about 134 species. Besides its ecological and biological role, seaweed in maintaining the stability of marine ecosystems and as a place to live and protection for other biotas, this group of macroalgae also has economic potential, namely as raw materials in industry and health. Seaweed contains primary and secondary metabolites. The content of primary metabolites such as vitamins, minerals, fiber, alginate, carrageenan, and agar are widely used as cosmetic ingredients for skincare. In addition to its primary content, which has economic value, the secondary metabolite content of seaweed has the potential as a producer of various bioactive metabolites with extensive activities as antibacterial, antiviral, antifungal, cytostatic, and antioxidant. Furthermore, seaweed extract has activity against Escherichia coli and Bacillus cereus.[2-5]

Lately, traditional medicines have experienced significant development as herbal medicines. They are widely consumed again after people tend to use patent medicines or factory-made medicines widely sold over the counter and have many side effects. In addition, traditional medicine is also easy to obtain, and the price is relatively cheaper.[5,6]

Red algae (Rhodophyta) in the class with the species most widely used and has economic value. Red algae live in seawater, from the edge of the sea to the deep sea with a depth of 130 meters. This plant lives as phytobenthos by attaching itself to mud, sand, live coral, dead coral, volcanic rock, and wood substrate. The type of algae in Indonesia that contains the most carrageenan and agar is from the red algae class (rhodophyceae). Red algae containing carrageenan (kara genophyte) are from Eucheuma, Kappaphycus, and Hypnea. While containing agar (agarophyte) from the genera Gracilaria and Gelidium.

Natural antioxidants from algae play an important role in treating various diseases such as anti-inflammatory, antibacterial, antifungal, cytotoxic, antimalarial, antiproliferative, anticancer, and preventing aging. Some marine algae with antioxidant activity include ahnfeltiopsis,

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colpomenia, gracilaria, halymenia, hydroclathrus, laurencia, padina, polysiphonia, and turbinaria. Among the three species of algae, rhodophyta (red algae) produces bioactive compounds that can be used for medicinal purposes in the pharmaceutical field. Euchema sp. from red algae species can reduce blood glucose levels in male Wistar rats given a dose of 4g/kg BW/day, 8g/kg BW/day, and 12gr/kg BW/day.[7]

Using spectrophotometric procedures, various methods are used to determine the antioxidant activity of a plant extract, food, or beverage, including ABTS and DPPH (1.1-diphenyl-2-picrylhydrazyl). DPPH is a free radical compound that is stable so that when used as a reagent in the free radical scavenging test, it is sufficiently dissolved and, when stored in a dry state with good and stable storage conditions for many years. DPPH absorbance values ranged from 515-520 nm.[7]

Based on the description above, the researchers wanted to know the antioxidant activity of the biological wealth, namely red algae extract (rhodophyta), using the DPPH method.

Material and Methods

The research method used in this research is exploratory, carried out in May – November 2019, located at the Phytochemical Laboratory and Biopharmaceutical Laboratory, Faculty of Pharmacy, Hasanuddin University. This study used 5 kg of red algae (rhodophyta) extracted by the maceration method using 96% ethanol solvent. Then measured absorbance40 and antioxidant data were compared with vitamin C. Tables and graphs explained the data obtained. Antioxidant data on DPPH radicals (% inhibition) of red algae extract were analyzed, and the IC50 The smaller the IC50, the stronger the antioxidant activity (Blois, 1998). The IC50 was analyzed and calculated using a linear regression equation in this study.

Results

Research on the antioxidant activity test of red algae (Eucheuema Spinosum) and positive control solution (Vitamin C), using DPPH compounds and then measured by spectrophotometry.

The fresh red algae (Rhodophyta) Euchuema Spinosum used in Simplicia manufacture and extracts were taken from Puntondo Beach, Takalar Regency, South Sulawesi. The initial stage is sorting samples or selecting algae according to the type of sample to be used. The red algae were then cleaned using seawater and then cleaned using running water, then dried to obtain a dry sample or Simplicia of that type of algae. Fifty grams of Simplicia from red algae were then dissolved using 90% ethanol for the maceration process. The first stage of washing is seawater, and the second stage of washing uses flowing freshwater. After that, the samples were dried directly under the sun. In order to obtain maximum results, dry red algae (Euchuema Spinosum) were then macerated with 90% ethanol. The maceration method was chosen. After all, it has the advantage of being simple because it does not require complicated equipment and can avoid damage to components that are not heat resistant and are relatively inexpensive. Macerated Simplicia was then put into a rotary evaporator until a light brown dry extract was obtained. The extract obtained from the red algae was then made in six different concentrations, namely 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, and 600 ppm, and made in two replications. Then each sample was reacted with 1000 microliters of DPPH solution as shown in Table 1 below:

After obtaining the % antioxidant activity data, a graph is made between the concentration of the solution (x) and the % antioxidant activity (y), and the linear regression equation is obtained Figure 1 and Figure 2. Therefore, the IC50 value can be determined using a linear regression equation. Microsoft Excel is used to find linear regression equations to facilitate data input. The smaller the IC50, the greater the antioxidant activity. After doing the calculations, the IC50 of red algae extract (eucheuma spinosum) and vitamin C can be seen in Table 2.

In the above study, all data on antioxidant measurements in samples of red algae (eucheuma spinosum) and vitamin C were shown. The Table 3 above shows that the total antioxidant value in the vitamin C sample has a smaller IC50 value of 2.588 ppm, compared to the total antioxidant value in red algae (eucheuma spinosum), which has an IC50 of 702.387 ppm. The smaller the IC50 means, the stronger the antioxidant activity. It can be concluded that the antioxidant activity of the red algae extracts is fragile, while the antioxidant content of vitamin C is solid.

After maceration was carried out, the collected filtrate was evaporated using a rotary evaporator at a temperature of 40°C so that a crude extract was formed. Furthermore, the crude extract is placed on a water bath to produce a thick extract of red algae (eucheuma spinosum).

After getting the dry extract, dilution of the extract was obtained from red algae using methanol PA and DPPH solution. Then, red algae

Table 1. **Inhibitory activity of red algae extract**

Sample	% Antioxidant activity					
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm
Red algae (Eucheuma spinosum)	3.413	16.780	17.861	23.549	32.594	45.791

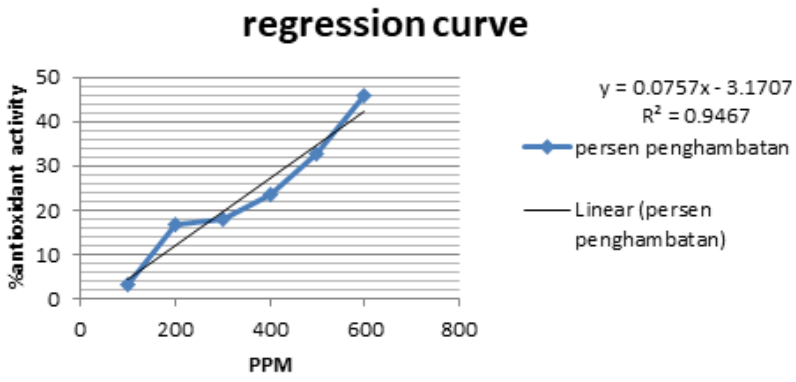

Figure 1. **Linear regression curve of red algae extract**

extract was made in six concentrations, namely 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm, and each. Each replication is made in two replications. Then measurements were made with a spectrophotometric device at the maximum wavelength.

Discussion

Red alga is a type of algae with more biological activity than other types of algae. The Rhodomelaceae dominates the chemical compounds present in red algae. Red algae are the primary source of halogenated compounds such as laurenterol, halomon, callicladol, and other compounds that-

Table 2. **Inhibitory activity of vitamin C concentration**

Sample	% Antioxidant activity				
	0.5 ppm	1 ppm	1.5 ppm	2 ppm	2.5 ppm
Vitamin C	9.946	20.924	34.239	35.924	48.587

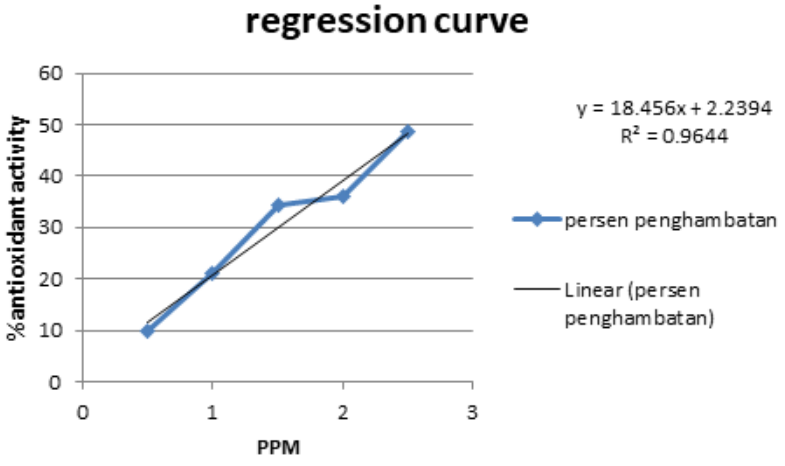

Figure 2. **Linear regression curve of activity antioxidant vitamin C**

Table 3. **IC_{50} red algae and vitamin C**

Sample	IC_{50} score (PPM)	Interpretation
Red Algae (Eucheuma spinosum)	702.387	Very Weak
Vitamin C	2.588	Very Strong

belong to Eucheuma spinosum to the class of red algae (Rhodophyceae) in the form of a cylindrical thallus, smooth surface, dark brown green-brown color, green-yellow or red-purple. Morphologically, this species has spines that grow in a circular row. Eucheuma Spinosum contains carrageenan, a polysaccharide, a hydrocolloid compound consisting of esters of potassium, sodium, and magnesium or calcium sulfate with galactose and a copolymer of 3, 6-anhydrogalactose. Utilization of carrageenan, among others, as a stabilizer, thickener, and emulsifier.[8]

The purpose of this study was to determine the antioxidant levels of red algae extract (eucheuma spinosum) by comparing antioxidant levels of vitamin C using DPPH (1.1-diphenyl-2-picrylhydrazyl) compounds. As measured by a spectrophotometer.

In this study, the red algae (eucheuma spinosum) used came from Putondo Beach, Laikang Village, Mangara' Bombang District, Takalar Regency.

The DPPH radical absorption method is used to test antioxidant activity because it's a simple, easy method and uses a small number of samples

quickly.35 Measurement of the antioxidant activity of the sample was carried out at a wavelength of 517 nm, which is the maximum wavelength of DPPH, with a DPPH concentration of 50 mM. The presence of antioxidant activity in the sample resulted in a change in the color of the DPPH solution in methanol, which was originally dark violet to pale yellow. The antioxidant activity of this red algae extract (eucheuma spinosum) was expressed in the percentage of its inhibition against DPPH radicals. The percentage of inhibition was obtained from the difference in absorption between the absorbance of DPPH and the absorbance of the sample as measured by a UV-Vis spectrophotometer.[9]

Furthermore, measurements were made using a spectrophotometer. The spectrophotometer used is a UV-Vis spectrophotometer which is useful for determining the concentration of compounds that can absorb radiation in the ultraviolet (200-400 nm) or visible (400-800 nm) region. This analysis is used to determine the absorbance of the sample solution being measured.[6]

Based on the research results, red algae extract (eucheuema spinosum) has antioxidant activity. The color change of DPPH indicated antioxidant activity from violet to yellow, which causes no opportunity for these electrons to resonate where this change can be measured and recorded with a spectrophotometer. Crude methanol extract is an extract that has more potent antioxidant activity than the extract—chloroform and ethyl acetate.[7]

Red algae contain polyphenols and carotenoids. Polyphenols and carotenoids the mechanism of polyphenolic compounds as antioxidants are by donating hydrogen from their hydroxyl groups. Polyphenols are components that play a role in antioxidant activity in fruits and vegetables. The mechanism of carotenoid compounds as secondary antioxidants works by binding to singlet oxygen and converting it to triplet oxygen. From the mechanism of action of carotenoid antioxidants above, bixin carotenoids can be classified as secondary antioxidants.[7]

The classification of antioxidant strength criteria can be divided into 5, namely: <50 ppm very strong, 50-100 ppm strong, 100-150 ppm moderate, 150-200 ppm weak, >200 ppm very weak.

Based on this category, the antioxidant levels in this study were categorized as very weak (702.387 ppm) because the extract used was too dry. There was salt in the sample so that the polyphenol content of the red algae was reduced so that the antioxidant content of the red algae extract eucheuema spinosum became very weak.[8]

Red algae (eucheuma spinosum) by comparing three extracts of red algae, namely processed red algae, washing them until they are white to remove epiphytes, marine organisms, and marine rocks. Wet and dry Simplicia. This study showed that the most potent antioxidant activity was found in processed algae extracts with an IC value of 333.66 g/mL, fresh algae 418.32 g/mL, and dried algae 472.14 g/mL.[9]

Meanwhile, as a control solution for comparison, vitamin C obtained solid results (2.588 ppm). Because vitamin C is known as the most powerful water-soluble antioxidant, vitamin C also effectively scavenges the formation of free radicals. Vitamin C can directly react with superoxide anions, hydroxyl radicals, singlet oxygen, and lipid peroxides. As a reducing agent, ascorbic acid will donate one electron to form unreactive emi dehydroascorbate and then undergo a disproportionation reaction to form unstable dehydroascorbate.[10]

Similar study by comparing three red algae extracts, namely processed, wet, and dry, this study showed that the weakest antioxidant activity was found in dry algae extracts with an IC value of 472.14 ppm, it can be concluded that the criteria for red algae extract in this study has very weak criteria.[10] Using 55% ethanol, 75% ethanol, and 95% as solvents, where the IC50 of the ethanol solvent is ethanol 55% IC50 1.179.245ppm, 75% IC50 1,190,476 ppm, and 95% IC50 ethanol 4,032,256 ppm where the three extracts with ethanol solvent criteria are very weak.[11]

The antioxidant activity of crude extract of green algae H. gracilis using the DPPH radical. The absorbance value was used to calculate the inhibition percentage that indicated the active compound's ability in the extract to scavenge DPPH free radicals. Antioxidant activity of crude extract of H. gracilis with methanol as solvent and ethyl acetate fraction. Categorized as very strong if the IC50 value is <50 ppm, strong is 50-100 ppm, moderate is 101-150 ppm, and weak is >150 ppm. Extract H. gracilis in methanol and ethyl acetate fraction had IC50 values of 290.49 ppm and 375.50 ppm, respectively, so that based on that category the antioxidant activity of green algae extract H. gracilis was very weak.[12]

The antioxidant activity on the crude extract of green algae H. opuntia using the DPPH radical. Ethanol H. opuntia extract and ethyl acetate extract are presented. A compound is categorized as very strong if the IC value is50 ppm, strong is 50-100 ppm, moderate is 101-150 ppm, and weak is >150 ppm. The H. opuntia extract in methanol and the ethyl acetate fraction had IC values of 143.63 mg/L and 75.51 mg/L, respectively, so that based on that category, the antioxidant activity of the ethyl acetate extract of the green algae H. Opuntia was included in the strong category. Meanwhile, the ethanol extract of green algae H. opuntia had moderate

antioxidant activity.

The antioxidant activity of the crude extract of the green silpau algae Dictyosphaeria versulysii using the DPPH radical using methanol as a solvent with concentrations of 50ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm and replicated three times. From the research conducted, the IC50 value of silpau methanol extract (547.97 mg/L) shows that the silpau methanol extract is categorized as a weak antioxidant. This may be because the methanol extract from silpau is not a pure compound, but it still contains other compounds that may not have antioxidant activity.[13]

Antioxidant testing of freshwater microalgae navicula sp, oscillatoria sp, carteria sp and compared it with vitamin C, using DPPH solution with concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm for microalgae while for vitamin C with a concentration of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm. After measuring IC50, it was found that IC50 differed in navicula sp IC50 41.304 (powerful), oscillatoria sp. IC50 23.401 (very strong), Carteria sp IC50 51.433 (strong) while vitamin C (comparison) 3.079 (very strong). In this study, it can be seen that of the three freshwater microalgae, oscillatoria sp which has a powerful antioxidant compared to the other two freshwater microalgae.[14]

To determine the antioxidants from methanol extract, ethyl acetate, and N-hexane from brown algae sargassum polycystum and turbinaria decurrens. The antioxidant activity was tested using the DPPH method, measured using UV-Vis, and compared with vitamin C using the maceration method. The results showed that methanol, ethyl acetate, and n-hexane extracts from Sargassum polycystum had a low antioxidant activity with an IC50 value of 340.06, 180.54, and 502.25. Methanol extract, ethyl acetate, n-hexane from turbinaria decurrens had a low antioxidant activity with IC50 values of 491.02, 411.80, 502.7. This potential is lower than the IC50 of 1.72.[15]

Conclusion

Based on the results obtained from antioxidant measurements from red algae extract (rhodophyta) eucheuema spinosum and vitamin C as positive control solution, conclusions can be drawn that extract of red algae (rhodophyta) eucheuema spinosum ethanol fraction dry extract has an IC50 value of 702.387 g/mL. Moreover, vitamin C extract has an IC50 of 2.588 g/mL, which is very strong. Further research is needed to examine antioxidants from red algae (eucheuma spinosum) from other waters to compare the differences in red algae from the waters of Takalar Regency. In addition, it is necessary to do more research related to the antioxidant activity of other algae with different fractions to compare the antioxidants of red algae extract.

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Conflict of Interest

The authors report no conflict of interest.

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