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Proximate, Phytochemicals, Total Phenolic Content and Antioxidant Activity of Ethanolic Extract of *Eucheuma spinosum* Seaweed

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ABSTRACT

Several health conditions, including aging, joint inflammations, cancer, and the decrease of organ function related to oxidation, have encouraged the investigation of antioxidants' natural sources. Seaweed is one of the marine commodities that has become the focus of researchers, particularly in correlation to health, such as medicines, functional food, and cosmetics. *Euchema spinosum* is a red macroalga naturally thrives in Lemukutan coastal, West Kalimantan. This research measured the proximate composition, phytochemical, total phenol content, and determined the antioxidant activity in vitro from the ethanol extract of *E. spinosum*. Proximate measurements, such as protein was carried out using the micro-Kjeldahl method, fat by the non-polar n-hexane solvent extraction method, and carbohydrate determined using by different calculations. The phytochemicals were identified by reacting to the ethanol extract of *E. spinosum*. The total phenol was determined spectrophotometrically using gallic acid as a positive control. The antioxidant activity was identified by the DPPH free radical scavenging method. The results showed the proximate was composed of 6.00 % carbohydrates, 0.82% fat, and 1.09 % protein. The ethanol extract of *E. spinosum* contained phytochemical components of alkaloids, steroids, tannins, saponins, and flavonoids. The total phenol content of 16.47 ± 0.14 % GEA with the ability to inhibit free radicals (IC50) by 90.10 ppm was classified as a strong antioxidant.

Keywords: Screening, proximate, antioxidant, degenerative, *E. spinosum*,

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INTRODUCTION

Antioxidant activity is the activity of a molecule compound, which in low doses can inhibit or prevent the oxidation of a substrate (Halliwell, 1999). The oxidation process in material for both animal and human needs, as well as cosmetic preparations, will cause the commodity to be damaged. The use of antioxidants aims to prevent oxidation so that it does not cause poisoning or disease due to these foods and cosmetics (Guan et al., 2005; Gupta and Abu-Ghannam, 2011). Exposure to UV rays can cause permanent skin damage, such as skin aging, changes in skin elasticity, and even the worst is to cause skin cancer. This is an important concern in the cosmetic industry (Wijesinghe and Jeon, 2011). The biological activity of several species of seaweeds has been investigated as antioxidants. At the same time, they have been used in the cosmetic, including *S. siliquastrum*, *E. cava*, *S. marginatum*, *T. conoides,* and *P. tetrastomatica*. The compounds like a dieckol, eckol 6.6'-bioeckol, triphlorethol-A, fucodiphloroethol, and several phenolic compounds isolated from seaweed, have been applied in the cosmetic field as anti-aging, body cell protection, whitening, and UV protection (Heo and Jeon, 2009; Wijesinghe and Jeon, 2011). *Euchema spinosum* is one of the seaweeds whose abundance is large in tropical waters such as Indonesia. Apart from naturally occurring in the waters of West Kalimantan, this species has also been cultivated by the coastal communities of Lemukutan Island, West Kalimantan. The use of this species is still minimal as alternative food material, and its use as a cosmetic ingredient is still lacking. For this reason, in this study, phytochemical extraction and testing will be carried out to determine bioactive components accompanied by total phenol and proximate analysis as well as determining the antioxidant activity of *E. spinosum* from West Kalimantan sea.

METHODS AND MATERIALS

Sampling and Identification

The *E. spinosum* was gathered from Lemukutan Island, West Kalimantan, Indonesia. Identification of the sample was carried out at the Laboratory of Marine Science, Faculty of Maths and Natural Sciences, Tanjungpura University. Likewise, the extraction process using ethanol, screening phytochemical and determining of proximate content. Furthermore, the observation of total phenol and antioxidant activity was carried out in the Chemistry Laboratory, Faculty of Maths and Natural Sciences, Tanjungpura University.

Sample Preparation

Sample preparation was executed following the procedure of (Fadly et al., 2020; Warsidah et al., 2020). The cleaned *E spinosum* samples were dried at room temperature for 3 days to reduce the moisture content. Furthermore, the sample was roughly chopped and followed by a maceration extraction process using ethanol solvent. The filtrate was then filtered with Whatman paper no 1. The ethanolic extract obtained then concentrated using a rotary evaporator before subjected to the next analysis.

Proximate analysis

Analysis of the ash content is initially by weighing about 2 g sample, then put it in a porcelain dish with a known fixed weight. The sample is charred over the bunsen over low heat until smoking. Then put it in the furnace at a temperature of 500 - 600 ° C until it becomes white ash. The plate containing the ash is cooled in a desiccator and weighed until a fixed weight is obtained.

Analysis of moisture content is based on the ashing thermogravy method. A total of 2 grams of the sample is weighed in a container whose weight is known. Water content was measured using 4 ovens at 105ºC for 3 hours. After that, it is cooled in a desiccator and weighed. The process is repeated so that it gets a constant weight.

Analysis of protein content using the micro-Kjeldahl method. A total of 1 gram of the extract is put into a Kjeldahl flask, digestion using 20 ml of concentrated sulfuric acid by heating until the solution is clear. The solution resulting from the destruction is diluted and distilled with the addition of 10 ml of 10% NaOH. The distillate is collected in 25 ml of a 3% H3BO3 solution. H3BO3 solution was titrated with a standard HCl solution using methyl red as an indicator. From the results of this titration, the total nitrogen can be known. The nitrogen value reflects the protein content (Fadly et al., 2017). The protein content of a sample is calculated by multiplying the total nitrogen and a correction factor.

$$\% \text{ Total Nitrogen} = \frac{\text{ml (HCl) x N (HCl)}}{\text{Sample Weight}} x\ 14.008 \text{ x f}$$

% Protein = % Total Nitrogen x 6.25

Analysis of Fat Content was carried out by the soxhlet method using a diethyl ether solvent. About 2 grams of E. spinosum extract was placed in a Soxhlet device, then filtered with a minimum of 5 hours diethyl ether or until the solvent that had dropped back turned clear. The resulting extract was heated in an oven at 105 ° C, then weighed until a fixed weight was obtained (fat Weight).

$$\% \, Fat\ Content = \frac{Fat\ Weight}{\text{Sample Weight}}\ x\ 100\%$$

The carbohydrate content was determined through different calculations. The calculation is below :

% carbohydrate content = 100% - (A + B + C + D)%

Where: A is an ash content, B is moisture content, C is protein content, D is a fat content

Phytochemical screening

The identification of phytochemicals in the ethanolic extract of *E. spinosum* was carried out using the method reported by (Masriani et al., 2020). It was including testing for alkaloids, steroids, phenols, flavonoids, and saponins. Phytochemical screening is based on a qualitative test between samples with specific reagents for each chemical component, which is characterized by the occurrence of color changes, the formation of deposits, and foam on the surface of the sample. The alkaloid test was carried out by dissolving the *E. spinosum* extract a few drops of 2 N sulfuric acids and then tested with 2 alkaloid reagents, namely dragendorff reagent, and Meyer reagent. A positive test result is obtained when a red to orange precipitate is formed with dragendorff reagent and a yellowish-white precipitate with Meyer reagent. The steroid test was carried out by dissolving the extract in 2 ml of chloroform in a test tube to which 10 drops of acetic anhydride were added and 3 drops of concentrated sulfuric acid. A positive reaction is indicated when a red solution is formed for the first time, then turns blue and green. The phenol test was carried out by extracting *E spinosum* extract in 20 ml 70% ethanol, then the resulting 1 ml extract was added with 2 drops of 5% FeCl3 solution, the formation of a green or blue-green color showed a positive reaction. The flavonoid test was carried out by adding 0.1 mg of magnesium powder and 0.4 ml of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol with the same volume) and 4 ml of alcohol then the mixture was shaken. A positive reaction is indicated by the formation of a red, yellow, or orange color on the amyl alcohol layer. The saponin test was carried out by shaking the *E. spinosum* extract with hot water, the formation of a foam that was stable for 5 minutes, and did not disappear when adding 1 drop of HCl 2 indicated the presence of saponins.

Analysis of Total Phenol Content

The method of determining total phenol refers to (Santoso et al., 2012; Sharma et al., 2011; Yangthong et al., 2009) using the Folin Ciocalteau reagent. About 5 mg of *E. spinosum* extract was dissolved in 2 ml of 96% ethanol, 5 ml of distilled water, and 0.5 ml of Folin-Ciocalteau reagent 50% were added, incubated for 5 minutes, then added 1 ml of 5% Na2CO3. The solution was homogenized and then incubated in the dark for one hour. A UV-Vis spectrophotometer measured the resulting absorption at a wavelength of 725 nm. The absorbance measurement was carried out 3 times. Gallic acid is used as a standard with a concentration series of 0 ppm, 5 ppm, 15 ppm, and 20 ppm. The gallic acid calibration curve is used to determine the levels of phenolic compounds contained in the sample through the regression equation and expressed in units of mg gallic acid equivalent / g extract (mg GAE / g extract) with the calculation formula:

$$C = C1\ x\ \frac{V}{\text{M}}$$

Where :

C is the total phenol (mg GAE / g extract)

M is the weight of extract (g)

C1 is the concentration of gallic acid (mg / l)

V is the volume of extract (l)

Antioxidant Testing

The antioxidant test refers to the method of (Banerjee et al., 2005). Testing begins with the creation of a concentration series, namely: 100, 500, 1000, 2000, 4000, 8000, 16000 ppm. The preparation of 0.1 mM DPPH reagent was done by dissolving 0.002 g of DPPH in 50 ml of 95% ethanol. Each sample was added with 3 ml (1: 3 v / v) DPPH solution. 6 Furthermore, the sample and DPPH were mixed using vortex for 1 minute and incubated for 30 minutes. The absorbance was measured using a Spectrophotometer U-1240 Shimadzu MiniUV at a wavelength of 517 nm, and the blank solution was ethanol. The formula below calculates the percentage of inhibition:

$$\% \text{ Inhibitor} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{Blank absorbance}} x\ 100\%$$

RESULTS AND DISCUSSION

Proximate Analysis

Seaweed is an aquatic abundance organism and one of the marine biological resources that have economic value (Darmawati et al., 2016). Seaweed is a macroscopic creature and multicellular algae that generally reside in coastal areas. The number of seaweed is estimated at around 9,000 species. There are three main groups classification according to their pigments, namely Phaeophyta, Rhodophyta, and Chlorophyta. The global production of seaweed produced from aquaculture exceeds 24 million tonnes (Burtin, 2003).

The results of the proximate analysis of E. spinosum taken from the waters of Lemukutan Island are shown in the following table.

Table 1. Results of the proximate analysis of fresh *E. spinosum*

Proximate analysis	% (Wet Weight)
Ash	6.67
Water	85.43
Protein	1.09
Fat	0.82
carbohydrate	6.00

The nutritional composition of seaweed varies depending on the species, area, season, temperature, climate, geographic area, water, and the conditions of the sea, which may lead to the difference of the nutritional composition of seaweed (Burtin, 2003).

Water is an essential compound in the food due to its impacts on texture, appearance, and taste. Fishery commodities commonly consist of very high water content even it may differ from each other due to the habitat and environmental conditions of the species. The water content in the fresh *Euchema spinosum* from the coastal area of Lemukutan Island was 85.425% calculated from the wet Weight.

Ash content describes the number of minerals that do not burn into volatile substances. Most of the food is 96% composed of water and organic matter, while the rest are inorganic materials known as mineral elements, which can be identified by the ash content (Apriyantono et al., 1989). The ash content of from the coastal area of Lemukutan Island was 6.67% from the wet Weight. Protein content in the *Eucheuma spinosum* taken from Lemukutan waters was 1.09% wet Weight. Anyhow, the results of research conducted by (Diharmi et al., 2012) showing the protein content of red seaweed (Rhodophyceae) *Eucheuma spinosum* in Nusa Penida waters, which ranges from 4.85 – 5.95%, while the results of (Yulianingsih and Tamzil, 2017) study found that the protein content of *E. cottonii* growing on Karimun Jawa Island ranged from (1.87 to 2.09%). Proximate content, especially protein, acts as a building substance, regulatory agent, and combustion agent. As a building block for protein, it may form new tissues that contribute to the growth, repair the damaged tissue, and reproduce, so its existence as a source of human food is significant.

The results of the analysis of fat in *E. spinosum* were minimal, amounting to 0.82%, as was reported by Wong and Cheung (2000), namely the fat content in *S. filamentosa* species 1.10% and *Hypnea floresii* species 2.46% dry weight. The low-fat content in seaweed supports this food becomes a healthy diet food.

The carbohydrate content calculated using the system by difference is 6.00% wet weight. According to the research of (Diharmi et al., 2012), the red seaweed (Rhodophyceae) shows that carbohydrate content ranges from 53.44 to 56.80%. (Reskika, 2011) research results showed the carbohydrate content of Ulva sp., about 46-51%. Research by (Yulianingsih and Tamzil, 2017) regarding the proximate composition of seaweed from several regions in Eastern Indonesia shows that carbohydrate content ranges from 71.22% to 73.81% of dry weight. This percentage is higher than the carbohydrate content in seaweed that grows in Karimunjawa waters, 58.29%. The difference in the value of carbohydrates in the two seaweeds shows that the chemical composition of seaweed is not only influenced by the type of seaweed but also by habitat conditions. (Vonne, 2009) states that as an organism capable of photosynthesis, the chemical composition of seaweed is not only influenced by nutrient concentrations in the waters but also by temperature, depth, seasonal variations, and geographic location.

Phytochemical Screening

Phytochemical testing on the ethanol extract of seaweed *Eucheuma spinosum* showed the presence of compounds including alkaloids, phenols, flavonoids, steroids, and saponins. The detection of phytochemicals is following the colors generated by the chemical reaction against the particular reagent (Masriani et al., 2020). The screening of those samples are an indication of the dominant component contained in these plants as well as being the starting point in carrying out further biological activity tests.

Table 2. Phytochemical test results of the ethanol extract of *E spinosum*

Phytochemical screening	Reagents / Chemcial	Ethanol extract
Alkaloids	Dragendorff Mayer	++ ++
Steroids	Liebermann-Burchard	++
Flavonoids	Serbuk magnesium dan amil alcohol	+++
Phenolics	FeCl3	+++
Saponins	Forth test	++

Total Phenol Content

Seaweed extract allows it to be used as a source of antioxidants because it has the ability to inhibit fat peroxidation and can reduce some of the effects of free radicals. Phenolic is a compound that is widely found in almost all types of seaweed and has the potential as an antioxidant (Kurniawati et al., 2016). (Bangol et al., 2014) stated that most phenolic compounds are polar. (Chakraborty et al., 2013b) noted that brown seaweed contains phenolic compounds with different levels between species. Our observation found the total phenol content in the ethanol extract of *E.spinosum* was 16.47 ± 0.14 mg GAE/g. The phenolic compound in the ethanol extract of *E. spinosum* is relatively low compared to the total phenolic in several species of red and brown seaweed. *Turbinaria conoides* extracted with ethyl acetate was higher (0.211 g GAE / g) than methanol (0.195 g GAE / g) because ethyl acetate was able to extract phenolic

better (Chakraborty et al., 2013a). The absorbance of gallic acid as standard in our study is displayed in Picture 1.
E. cottonii from Lontar Beach, Tirtayasa District, Serang Regency, Banten, which was extracted with methanol, was higher (0.141 g GAE / g) than ethyl acetate (0.134 g GAE / g) (Yanuarti et al., 2017). The phenolic content will increase in the extract following the increase of the solvent polarity (Ganesan et al., 2008).

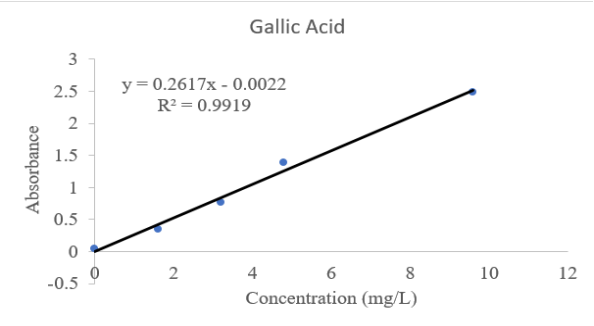

Figure 1. Chart of gallic acid absorbance

Antioxidant Activity
Antioxidant activity is the ability of a compound to inhibit oxidation reactions, which can be expressed by the percentage of inhibition. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical that can react by delocalizing free electrons in a molecule, so that the molecule becomes relatively stable (Dewi et al., 2020; Fadly et al., 2020). The chart of absorbance of DPPH used in our investigation is can be seen in Picture 2. The substance effectiveness against free radicals is commonly expressed in IC50, and the concentration effectively reduces 50% free radicals (Chakraborty and Joseph, 2016). The lower the IC50 value, the more potent the antioxidant activity. Our finding showed the ethanol extract of *E. cotoni* obtained an IC50 value of 90.10 ppm, while the IC50 value of ascorbic acid as a comparison was 4.83 ppm. The chart of percent inhibition of the ethanol extract from *E. cotoni* showed in Picture 3.
Ascorbic acid, which was used as a positive control, had very strong antioxidant activity. This value is considered appropriate because vitamin C is one of the antioxidants commonly consumed by the public, which is found in food ingredients (Rodwell et al., 2018).

CONCLUSION

Euchema spinosum contains proximate carbohydrates of 6.00%, 0.82% fat, and 1.09% protein calculated from wet Weight. The ethanol extract was positive for alkaloid, flavonoid, phenol, steroid, and saponin compounds. The total phenol content was 16.47 ± 0.14 mg GAE / g, and antioxidant determination showed an IC50 of 90.10 ppm.

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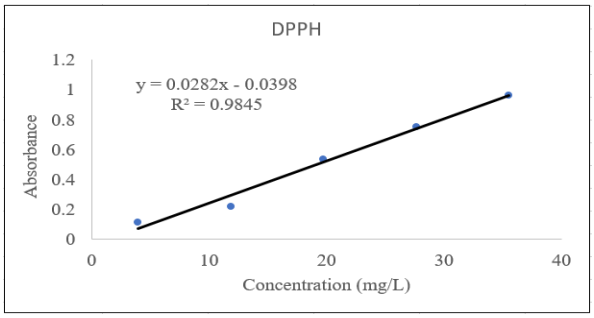

Figure 2. Chart of DPPH absorbance

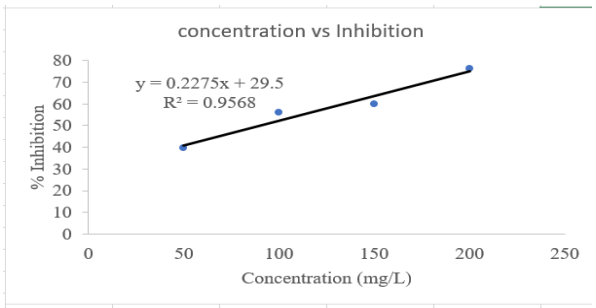

Figure 3. Chart of % inhibition of *E.spinosum* against free radical DPPH

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