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INDONESIAN JOURNAL OF MEDICAL CHEMISTRY AND BIOINFORMATICS

Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia

Article

Phytochemical Constituent and Antioxidant Activity Evaluation of Red Seaweed *Eucheuma* sp.

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Abstract: Background: Oxidative stress is a condition in which there is an imbalance between production of free radicals and protective response via antioxidant system. However as age increases, there is a reduction in endogenous antioxidant, hence, there is a need to search for potential exogenous antioxidants that can be derived from natural resources. Indonesia is a megabiodiversity country which has more than 30,000 species of plants and animals. Red seaweed *Eucheuma* sp. is one of marine macroalgae species that exhibit potent biological activities. This study aims to determine the phytochemical constituent and to evaluate the antioxidant activity of red algae *Eucheuma* sp. **Method:** Seaweed *Eucheuma* sp. collected from Lombok, Nusa Tenggara Barat, Indonesia, were extracted by maceration process using three solvents, n-hexane, ethyl acetate, and ethanol, sequentially. Each extract was analyzed for its phytochemical constituents by phytochemistry screening, thin layer chromatography, total phenolic content, total flavonoid content, and total triterpenoid content. Evaluation of antioxidant activity for ethyl acetate extract and ethanol extract were done using DPPH method. **Results:** Phytochemical analysis of *Eucheuma* sp. shows positive result for steroid and triterpenoid. Thin layer chromatography analysis of the *Eucheuma* sp. extracts showed total of 11 phytochemical constituents. Quantitative analysis revealed that the highest value in ethyl acetate extract, with total phenolic content of 29.57 mg gallic acid equivalent/g extract, total flavonoid content of 0.54 mg quercetin equivalent/g extract, and total triterpenoid content of 1.08 mg ursolic acid equivalent/g extract. Moreover, ethylacetate extract of *Eucheuma* sp. showed an active antioxidant activity against DPPH free radical with IC_{50} value of 27.96 μg/mL. **Conclusion:** Thus, ethyl acetate extract of *Eucheuma* sp. collected from Lombok, Indonesia, has a potential to be developed as a natural antioxidant

Keywords: *Eucheuma* sp.; Phytochemistry; Antioxidant; Thin Layer Chromatography; Quantitative analysis

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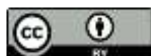

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1. Introduction

Oxidative stress is a condition of imbalance between the production and accumulation of free radicals in cells and tissues against the body's response to detoxify reactive species. If this condition goes continuously, it can trigger cell damage and have an impact on the aging process, chronic obstructive pulmonary disease, neurodegenerative diseases, and cancer [1]. Therefore, the role of antioxidants is important in protecting the body from oxidative stress. There are endogenous and exogenous antioxidants, however as age increases, there is a decreasing in endogenous antioxidant, thus the search for potential exogenous antioxidant which could be derived from natural resources is required. Indonesia is a mega biodiversity country. As a country with a large sea area, Indonesia has a high marine biodiversity which provides the potential to discover various types of chemical

compounds in marine resources that can be developed as medicine [2]. Seaweed algae is one of autotrophic marine species that has bioactive compounds. Seaweed algae are often used as food, cosmetic, and medicinal ingredients. It has attracted the attention of researchers because of its multifunctional bioactive compounds are thought to have a positive effect on health and support the management of diseases such as cancer. Furthermore, this marine species has also reported to have antioxidant and anticancer activities [3,4]. One of Indonesian seaweed species is algae/seaweed *Eucheuma* sp., with its secondary metabolites, shows potential for biological activities.

A previous study by Teo, et al (2020) showed that one of the *Eucheuma* species, namely *Eucheuma cottonii* obtained from Sabah, Malaysia, exhibits antioxidant activity and antibacterial activity which supports the wound healing process [5]. The impressive bioactivities of seaweed algae encouraged us to conduct research on the red seaweed *Eucheuma* sp. collected from Lombok, Nusa Tenggara Barat, Indonesia. In this research, extract of *Eucheuma* sp. (Figure 1) would be analyzed for its phytochemical composition and total content of its secondary metabolites, as well as to conduct an evaluation of its antioxidant activity against DPPH free radicals.

Figure 1. Seaweed *Eucheuma* sp. [6,7]

2. Results

2.1. Phytochemical Composition of Eucheuma sp.

The phytochemical composition of *Eucheuma* sp. extracts can be seen in Table 1. The n-hexane and ethyl acetate extracts of *Eucheuma* sp. are shown to have phytochemical constituent of triterpenoid and steroid, whereas the ethanol extract only has the positive result for steroid.

Table 1: Phytochemical composition of *Eucheuma* sp.

Metabolites	Extract		
	n-Hexane	Ethyl acetate	Ethanol
Saponin	-	-	-
Flavonoid	-	-	-
Tannin	-	-	-
Glycoside	-	-	-
Triterpenoid	+	+	-
Steroid	+	+	+
Alkaloid	-	-	-

2.2. Thin Layer Chromatography Analysis of Eucheuma sp.

The result for TLC analysis is shown in Figure 2, and the retention factor (Rf) of each phytochemical constituent is listed in Table 2. The TLC chromatogram shows that n-hexane extract of *Eucheuma* sp. has the largest number of constituents' spots, with 7 phytochemical constituents, followed by ethyl acetate extract with 6 spots of phytochemical constituents, and the ethanol extract with 2 spots of phytochemical constituents.

Table 2: Retention factor (Rf) of phytochemical constituents in *Eucheuma* sp. extracts.

Extract	Rf Value						
	1	2	3	4	5	6	7
n-Hexane	0.24	0.45	0.58	0.65	0.83	0.93	0.96
Ethyl acetate	0.24	0.34	0.45	0.59	0.69	0.96	-
Ethanol	0.63	0.74	-	-	-	-	-

Rf = Retention factor

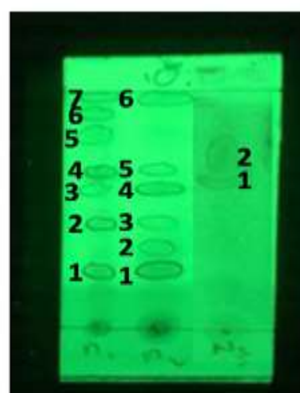

Figure 2. TLC chromatogram of *Eucheuma* sp. (n1: n-hexane extract, n2: ethyl acetate extract, n3: ethanol extract)

2.3. *Quantitative Analysis of Eucheuma sp.*

The quantitative phytochemical analysis of *Eucheuma sp.* extracts can be seen in Table 3. Three extracts of *Eucheuma* sp. were analyzed quantitatively to determine the total content of phenolic, flavonoid, and triterpenoid, respectively. Calibration curve of quercetin as standard of flavonoid and gallic acid as standard of phenol are displayed in Figure 3.

Table 3: Quantitative analysis of Eucheuma sp.

Extracts	Compound Tested		
	Total Phenolic Content (mg gallic acid equivalent/g extract)	Total Flavonoid Content (mg quercetin equivalent/g extract)	Total Triterpenoid Content (mg ursolic acid equivalent/g extract)
n- hexane	4.47	0.47	0.93
Ethyl acetate	29.57	0.54	1.08
Ethanol	10.45	0	1.05

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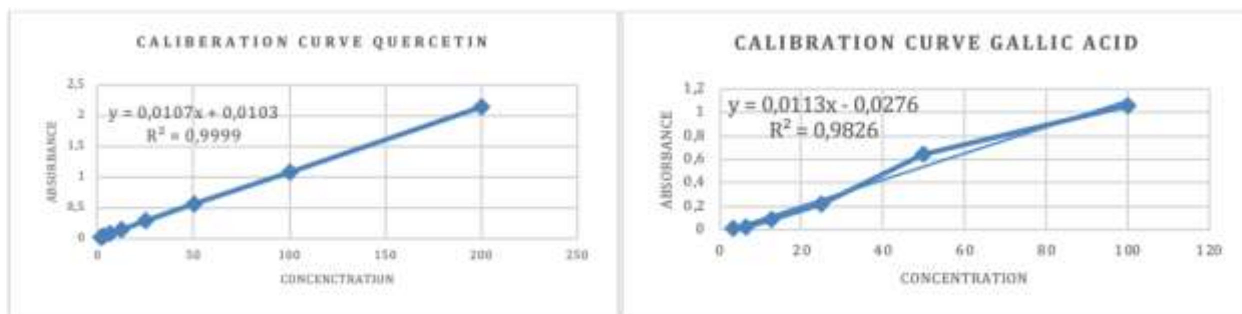

Figure 3. Calibration curve of quercetin and gallic acid

2.4. Antioxidant Activity of Eucheuma sp.

The antioxidant activity of ethyl acetate and ethanol extracts of *Eucheuma* sp. and ascorbic acid (as positive control) is denoted in IC_{50} values on DPPH free radical. The n-hexane extract of *Eucheuma* sp. was not tested due to its non-polarity hence unable to dissolve in polar solvent of ethanol, which serves as solvent for the DPPH experiment. Linear equation of ethyl acetate and ethanol extracts of *Eucheuma* sp. towards DPPH radical is displayed in Figure 4. The line graph between the concentration in logarithm versus percentage of inhibition was used to determine the linear regression equation. The IC_{50} value was obtained from the linear regression equation by substituting "y" with 50% (inhibition) to get x value in log concentration, antilog or inverse log of x value gave IC_{50} value. The IC_{50} value of *Eucheuma* sp. extracts and ascorbic acid on DPPH is summarized in Table 4.

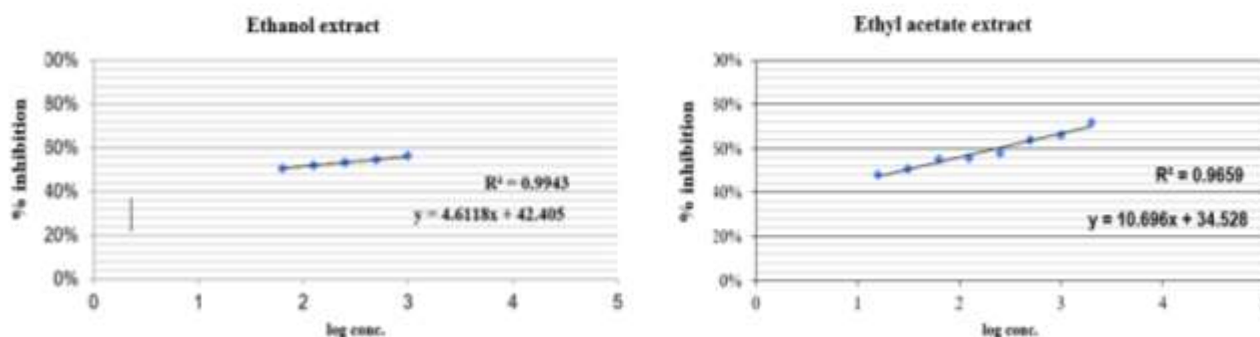

Figure 4 Linear equation of ethyl acetate and ethanol extracts of Eucheuma sp. in various concentrations towards DPPH radicals.

Table 4: Antioxidant Activity of *Eucheuma* sp. extracts and ascorbic acid on DPPH.

Tested Sample	IC_{50} (μg/mL)
Ascorbic acid (positive control)	0.69
Ethyl acetate extract of *Eucheuma* sp.	27.96
Ethanol extract of *Eucheuma* sp.	31.51

3. Discussion

3.1 Phytochemical Composition of Eucheuma sp.

Based on phytochemical analysis, three extracts of *Eucheuma* sp. gave positive result for steroid, meanwhile n-hexane and ethyl acetate extracts have a positive result for triterpenoid. Steroid is a secondary metabolite produced by plants, animals, and microorganism. Biosynthetically, all steroids are derivate of S-squalene-2,3-epoxide. Plant steroid could be classified into seven mayor classes based on biological

function/structure/taxonomic. Phytosterol is one type of plant steroid with integral role in bilayer lipid membrane which control fluidity and membrane permeability, while also showing hypocholesterolemic activity and in vitro anticancer activity. Commonly found phytosterol are β-sitosterol, campesterol, and stigmasterol. Brassinosteroid, another type of phytosterol, which mainly acts as plant's growth regulator, also has shown antioxidant activity and neuroprotective activity in a mammal neuron cell culture model. Previous study by Fasya AG, et al (2019) reported the isolated steroid from *Eucheuma cottonii* extract obtained from Banyuwangi showed the presence of campesterol, stigmasterol, and β-sitosterol [12,13].

Triterpenoid is a phytochemical compound built of three terpene unit with molecular formula of $C_{30}H_{48}$. This compound mainly found on seaweed, nonetheless it could also be found in wax-like coating of various fruits and plants, such as thyme, oregano, olive, and apple. Triterpenoid has various biological activity, such as an antioxidant, antimicrobial, antiangiogenic, and antiallergic [14]. Previous study by Putri T, et al (2019) reported that ethyl acetate and ethanol extract of seaweed *Eucheuma cottonii* contain triterpenoid [8].

Quantitative analysis of total phenolic, total flavonoid, and total triterpenoid content for *Eucheuma* sp. extracts shown in Table 3. Total phenolic content was determined by substituting 'y' value on linear regression equation of calibration curve of gallic acid as standard compound ($y = 0.0113x - 0.0276$ and $R^2 = 0.9826$) with absorbance value of tested extract to get x value which is equal to total phenolic content in mg gallic acid equivalent/g sample. Ethyl acetate extract of *Eucheuma* sp. shows the highest total phenolic content which contains 29.57 mg gallic acid equivalent/g extract, followed by ethanol extract which contains 10.45 mg gallic acid equivalent/g extract, and n-hexane extract which contains 4.47 mg gallic acid equivalent/g extract. Phenol is a compound with aromatic rings and hydroxyl group. Phenolic group could be classified into flavonoid and non-flavonoid. Phenol has a wide spectrum of biological activities that can act as an antioxidant, antimutagenic, and gene expression modification [15,16].

Total flavonoid content was also determined by substituting 'y' value on linear regression equation of calibration curve of quercetin as standard compound ($y = 0.0107x + 0.0103$; $R^2 = 0.9999$) with absorbance value of sample extract, total flavonoid content is expressed in mg quercetin equivalent/g sample. Ethyl acetate extract of *Eucheuma* sp. shows the highest flavonoid content with 0.54 mg quercetin equivalent/g extract, followed by n-hexane extract with 0.47 mg gallic acid equivalent/g extract, whereas ethanol extract does not contain flavonoid of quercetin. All three extracts of *Eucheuma* sp. are showing a near zero value of flavonoid content, in which it correlated with the qualitative phytochemical analysis, whereby all three extracts showed negative results for flavonoid. Thus, based on the total phenol and flavonoid content, it could be summarized that most of phenolic compound in *Eucheuma* sp. are non-flavonoid.

Total triterpenoid content was determined by linear regression equation of calibration curve of standard compound of ursolic acid obtained from previous study by Wei L (2015) [10]. Substituting 'y' value on linear regression equation of $y = 0.0605x – 0.0122$ ($R^2 = 0.9991$) with absorbance value of sample extract afforded x value which is equal to total triterpenoid content in mg ursolic acid equivalent/g sample. Ethyl acetate extract of *Eucheuma* sp. shows the highest triterpenoid content with 1.08 mg ursolic acid equivalent/g extract, followed by ethanol extract and n-hexane extract which contain 1.05 and 0.93 mg gallic acid equivalent/g extract, respectively.

Based on qualitative phytochemical analysis, triterpenoid was not detected on ethanol extract of *Eucheuma* sp., whereby it was detected on both n-hexane and ethyl acetate extracts. Meanwhile, total triterpenoid content analysis shows ethanol extract of *Eucheuma* sp. has a higher triterpenoid content than n-hexane extract. This is caused by during qualitative phytochemical analysis, non-polar terpenoid such as diterpenoid and triterpenoid were detected, whereas polar terpene (monoterpene) containing in ethanol extract cannot be detected, hence giving a negative result for ethanol extract. In contrast, determination of total triterpenoid content in *Eucheuma* sp. extract using a visible spectrophotometer

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gave a higher accuracy and sensitivity which could detect terpenoid (monoterpene) in ethanol extract of *Eucheuma* sp.

Thin layer chromatography (TLC) analysis of *Eucheuma* sp. shows that n-hexane extract contains the most constituents with a total of seven phytochemical compounds, followed by ethyl acetate extract with a total of six phytochemical compounds, and ethanol extract with a total of two phytochemical compound. This TLC analysis is in concordance with qualitative phytochemical analysis, in which, both n-hexane and ethyl acetate extracts have more positive results for phytochemical constituents than that of ethanol extract. There are four phytochemical constituents with the same Rf value of 0.24; 0.45; 0.58 and 0.96, in both n-hexane and ethyl acetate extracts. This result shows that there is similarity in four phytochemical compounds in both extracts, however, further analysis is needed to identify those four compounds by using the standard compounds, such as gallic acid for phenolic compound, ursolic acid for triterpenoid compound, and quercetin for flavonoid compound. TLC chromatogram shows significant Rf difference between two extracts (n-hexane and ethyl acetate extracts) and ethanol extract, causing by the used mobile phase were different. Ethyl acetate and n-hexane extracts use mobile phase of the mixture of ethyl acetate and n-hexane in 10:1 ratio, whereas ethanol extract uses mobile phase of the mixture of chloroform and methanol in 1:10 ratio.

3.2 Antioxidant Activity of Eucheuma sp.

DPPH is a free radical with unpaired electron on nitrogen atom which could receive hydrogen atom from antioxidative compound. Mixing of DPPH solution with antioxidative compound would produce a reduction reaction which lead to the decolorization process of DPPH from violet to yellow. The amount of DPPH decolorization is proportional to the amount of antioxidant properties in tested sample [11]. Reduction of DPPH radical can be observed through visible spectrophotometry at 517 nm of wavelength. Evaluation of antioxidant activities for *Eucheuma* sp. extract against DPPH provides IC_{50} value of 27.96 μg/mL for ethyl acetate extract, 31.51 μg/mL for ethanol extract, and 0.69 μg/mL for ascorbic acid as a positive control. Classification of antioxidant activity based on IC_{50} value provided by Phongpaichit (2007) is displayed in Table 5 [17].

Table 5: Classification of antioxidant activity based on IC_{50} value

IC50 (μG/ML)	Antioxidant Activity
>250	Inactive
>100-250	Weak
>50-100	Moderate
10-50	Active
<10	Very Active

Based on Table 5, ascorbic acid as positive control has the greatest antioxidant activity against DPPH with IC_{50} value of 0.69 μg/mL. Ethyl acetate and ethanol extracts of *Eucheuma* sp. exhibited an active antioxidant activity against DPPH with IC_{50} value of 27.96 μg/mL and 31.51 μg/mL, respectively.

Referring to the prior phytochemical analysis, triterpenoid compound was found in ethyl acetate extract, but absent in ethanol extract. Thin layer chromatography analysis also shown more phytochemical components in ethyl acetate extract (6 components) compared to ethanol extract (2 components). Moreover, the quantitative analysis of total phenolic, total flavonoid, and total triterpenoid content has confirmed that ethyl acetate extract of *Eucheuma* sp. has the highest content of those total phenolic, total flavonoid and total triterpenoid compared to both of ethanol and n-hexane extracts. All these results would be able to contribute to the higher antioxidant activity of ethyl acetate extract than that of ethanol extract and n-hexane extract. Previous study by Gülçin, et al (2006) found

that triterpenoid in form of glycoside triterpenoid (OGH), shows a strong antioxidant activity when it is tested using ferric thiocyanate method. Approximately 30 μg/mL of OGH to exhibit 95.3% of antioxidant activity in total [18]. Other than triterpenoid, phenolic compounds are also able to act as an antioxidant through various mechanisms. Hydroxyl group of phenol could act as hydrogen donor and interact with free radical in a termination reaction to split up or interrupt the free radical chain reaction. Furthermore, hydroxyl group is able to interact with various protein, acting as antioxidant by inhibiting enzymatic process related to free radical production such as P450, lipoxygenase, and xanthine oxidase [19].

Previous in vitro study by Putri T, et al (2019) which analyzed the antioxidant activity of *Eucheuma* sp. collected from Labuan Aji, Nusa Tenggara Barat, showed a similar result with this study, in which, ethyl acetate extract of *Eucheuma* sp. with IC_{50} value of 430.50 ppm on DPPH, gave a stronger antioxidant activity compared to ethanol extract with IC_{50} value of 1386.79 ppm on DPPH [8]. Another study on antioxidant activity of seaweed *Eucheuma* sp. was done by Wulandari (2018), by using seaweed *Eucheuma cottonii* collected from Madura, Indonesia. Potassium hydroxide is used in the extraction process of this seaweed. The use of potassium hydroxide was correlated to its function as polysaccharide extractor, thus giving more carrageenan, a family of polysaccharide that is able to stabilize free radicals by electron supplementation and free radical chain interruption, thus able to act as antioxidant. Wulandari et al. reported that *Eucheuma cottonii* extracted by potassium hydroxide showed an active antioxidant activity with IC_{50} value of 39.94 ppm against DPPH free radical [20].

4. Materials and Methods

4.1 Extraction of Seaweed Samples

Eucheuma sp. collected from Lombok, Nusa Tenggara Barat, Indonesia. The fresh seaweed of *Eucheuma* sp. were washed to clean it by mixing of sand, mud, shell fragments, and also coral. The cleaned *Eucheuma* sp. were dried and grinded become powder. The dried powder of *Eucheuma* sp. were macerated using batch maceration process with three different solvents, n-hexane, ethyl acetate, and ethanol sequentially. Each filtrate was then concentrated into extract using rotary evaporator.

4.2 Procedure of Phytochemical Analysis [8]

4.2.1 Test for Saponin

To test for saponin, flavonoid, tannin and glycoside, the extracts were diluted with 10 ml of hot water and filtered. Two milliliters of the filtrate would then be used to test the presence of each compound. The test for saponin was done by shaking the extract for 10 seconds and observing it for 10 minutes for the formation of foam.

4.2.2 Test for Flavonoid

The test for flavonoid was done by adding 0,5 ml of concentrated HCl and 4 cm of magnesium ribbon to the filtrate. The presence of flavonoid compounds will give red, orange, or green colour to the reacting substances.

4.2.3 Test for Tannin

The test for tannin was done by adding 1 ml of 10% $FeCl_3$ solution to the filtrate and observing the formation of dark blue or blackish green color.

4.2.4 Test for Glycoside

The test for glycoside is done by evaporating the filtrate and adding 1 ml of anhydrous acetic acid and 2 ml of concentrated H_2SO_4 to form blue or green color in its presence.

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4.2.5 Test for Triterpenoid and Steroid

Detecting triterpenoid and steroid was done by adding 5 ml of heated ethanol to the sample, 2 ml of which will be added with 0.5 ml of chloroform, 0.5 ml of glacial acetic acid, and 2 ml of concentrated H_2SO_4, which will be run through the test tube wall. The presence of triterpenoid compound will form a brown or violet ring, while the formation of a greenish blue color indicates the presence of steroid compound.

4.2.6 Test for Alkaloid

Test for alkaloid is done by adding the extract with 5 ml of $CHCl_3$ and 2 drops of NH_4OH. This mixture is filtered, then 2 ml of the filtrate is evaporated. Subsequently, 5 ml of HCl 2N was added to the evaporated filtrate and the mixture was divided into 3 separate test tubes. The first test tube was used as a blank, the second was added with 3 drops of Dragendroff reagent, and the third was added with 3 drops of Meyer reagent.

4.2.7 Procedure of Thin Layer Chromatography Analysis [9]

Thin layer chromatography was done using TLC plate as a stationary phase, and mixture of solvents (chloroform and methanol in ratio of 1:10 for ethanol extract, ethyl acetate and n-hexane in ratio of 10:1 for n-hexane and ethyl acetate extract). Each extract was dripped into the plate then placed into a glass filled with its corresponding solvent. Different compound in each sample will move at different rates depending on its polarity against the stationary phase and solubility towards the mobile phase. This procedure will separate each sample's metabolite contents and data are obtained in Rf, defined as the distance taken by sample compared to solvent.

4.2.8 Procedure for Determination of Total Phenolic Content [9]

10 mg of sample extract is dissolved in 10 mL 75% ethanol and 0.5 mL of solution was then placed into reaction tubes. Following this, 2% Na_2CO_3 and 0.5 mL of phenol folin-ciocalteu reagent (dissolved in distilled water with a 1:100 ratio) were added into the solution in which the mixture would subsequently be incubated in a dark room for 30 minutes. The absorbance was measured using spectrophotometry at 765 nm wavelength. Standard solution was made by diluting 1000 μg/mL gallic acid with 75% ethanol and diluted into a dilution series of 0, 6.25, 12.5, 25, 50, 100 μg/mL. Data obtained from absorbance of gallic acid solution measured using spectrophotometry at 765 nm wavelength was then processed into a linear regression equation. Average of sample extract absorbance was thereafter interpolated into the equation to obtain total phenolic content.

4.2.9 Procedure for Determination of Total Flavonoid Content [9]

10 mg of sample extract dissolved into 10 mL 75% ethanol was placed into reaction tubes, each tube containing 600 μL of solution. 30 μL of 20% $AlCl_3$ and 30 μL of 2M CH_3COOK were added into the reaction tubes. Afterwards, mixture was added distilled water to reach a volume of 1.5 mL, and homogenization was done with vortex, prior to a 30-minute incubation in a dark room with room temperature. Absorbance was measured using spectrophotometer at 440 nm wavelength. Standard solution was made by diluting 1000 μg/mL quercetin with 75% ethanol to create a dilution series of 0, 6.25, 12.5, 25, 50, 100 μg/mL. Data obtained from absorbance of quercetin solution which was measured using spectrophotometer at 440 nm was then plotted into a linear regression equation. Average of sample extract absorbance was interpolated into the equation to obtain total phenolic content.

4.2.10 Procedure for Determination of Total Triterpenoid Content [10]

200 μL of extract solution was added into a 10 mL volumetric flask and heated to evaporation with a water-bath. Add 1 mL of 5% vanillin acetic solution and 1.8 mL of Sulphur acid into the flask, followed by incubation with a temperature of 70 degrees Celsius for 30 minutes. Mixture was then cooled and diluted to 10 mL with acetic acid

solution. The absorbance was measured using spectrophotometer with wavelength of 573 nm. Total triterpenoid content was determined using standard ursolic acid calibration curve from previous study by Wei L, et al (2015), Y = 0,0605X – 0,0122 (R^2 = 0,9991).

4.2.11 Procedure of Antioxidant Analysis by DPPH Method [11]

Antioxidant activity evaluation was done using DPPH method. 10 mg of extract is dissolved with 10 mL 75% ethanol, obtaining a solution with concentration of 1000 μg/mL, which would undergo a dilution series to obtain tubes containing extract concentration of 1000, 500, 250, 125, 62.5, 31.25, and 15.625 μg/mL respectively. DPPH solution was made by dissolving 1.25 mg of DPPH in 25 mL 75% ethanol, giving a DPPH solution with 50 μg/mL concentration. Each reaction tube was added 750 μL of DPPH reagent solution then incubated in a dark, room temperature environment for 30 minutes. Each sample and a control solution of DPPH reagent 50 μg/mL was then measured for its absorbance using spectrophotometry at 517 nm wavelength. Percentage of inhibition for each sample towards DPPH was calculated using the following formula:

$$\text{DPPH Inhibition (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

A graph was then plotted with log concentration as the 'x' axis and inhibition percentage as the 'y' axis to obtain linear regression equation. If the coefficient of determination comes near to the value of 1, the equation could be used to calculate IC_{50} value ('X' value) by substituting 'Y' value to 50%.

5. Conclusions

Phytochemical compound found in *Eucheuma* sp. originated from Lombok, Nusa Tenggara Barat, Indonesia, are triterpenoid and steroid. Thin layer chromatography analysis showed a total of 11 phytochemical constituents in *Eucheuma* sp. extracts. Ethyl acetate extract shows the highest total phenolic, total flavonoid and total triterpenoid content. Antioxidant activity evaluation revealed that ethanol extract and ethyl acetate extract of *Eucheuma* sp. had an active antioxidant activity against DPPH.

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References

1. Liguori I, et al. Oxidative stress, aging, and disease. Clin Interv Aging. 2018; 13: 757-72
2. Munifah I. Prospek pemanfaatan alga laut untuk industri. Squalen. 2008; 3(2): 58-9
3. Pooja S. Algae used as medicine and food-a short review. Journal of Pharmaceutical Sciences and Research. 2014: 6(1); 33-5.
4. Gamal AAE. Biological importance of marine algae. Saudi Pharmaceutical Journal. 2010; 18: 1-25
5. Teo BSX, Gan RY, Aziz SA, Sirirak T, Asmani MFM, Yusuf E. In vitro evaluation of antioxidant and antibacterial activities of Eucheuma cottonii extract and its in vivo evaluation of the wound healing activity in mice. Journal of Cosmetic Dermatology. 2020.
6. Sabaani NJ, Sepe M, Penaredondo MAE. Antibacterial activity of liquid soap with combined sargassum sp. and eucheuma sp. seaweed extracts. AACL Bioflux. 2019; 12(5): 1514-23
7. Rumput laut, solusi bengkak akar krisan [Internet]. Indonesia: Pusat Penelitian dan Pengembangan Hortikultura; 2017 Jun [cited on 2020 Feb]. Available from: http://hortikultura.litbang.pertanian.go.id/berita-859-rumput-laut-solusi-bengkak-akar-krisan.html
8. Putri T, Arsianti A, Subroto PAM, Lesmana E. Phytochemical analysis and antioxidant activity of marine algae Eucheuma sp. In: Wulan PPDK, Gozan M, Astutiningsih S, Ramahdita G, Dhelika R, Kreshanti P. 3rd International Symposium of Biomedical Engineering''s Recent Progress in Biomaterials, Drugs Development, and Medical Devices; 2019 April 9; Indonesia. AIP; 2019.

9. Arsianti A, et al. Phytochemical composition and evaluation of marine algal sargassum polycystum for antioxidant activity and in vitro cytotoxicity on hela cells. Pharmacogn J. 2020; 12(1): 88-94
10. Wei L, Zhang W, Yin L, Yan F, Xu Y, Chen F. Extraction optimization of total triterpenoids from jatropha curcas leaves using response surface methodology and evaluations of their antimicrobial and antioxidant capacities. Electronic Journal of Biotechnology. 2015; 88-95
11. Kedare SB, Singh RP. Genesis and development of dpph method of antioxidant assay. J Food Sci Technol. 2011; 48(4): 412-22
12. Gunaherath GMKB, Gunatilaka AAL. Plant steroids: occurrence, biological significance, and their analysis. In: Encyclopaedia of Analytical Chemistry. USA: Wiley; 2014
13. Fasya AG, Baderos A, Madjid ADR, Amalia S, Megawati DS. Isolation, identification and bioactivity of steroids compounds from red algae eucheuma cottonii petroleum ether fraction. In: Romaidi, Wahyudi D, Daryono RNH, Yusnawan E, Kikuchi A. International Conference on Biology and Applied Science; 2019 Mar 13-14; Indonesia. AIP; 2019
14. Alihosseini F. Plant-based compounds for antimicrobial textiles. In: Sun G. Antimicrobial textiles. Cambridge: Woodhead Publishing; 2016
15. Sulaiman CT, Balachandran I. Total phenolics and total flavonoids in selected Indian medicinal plants. Indian J Pharm Sci. 2012; 74(3): 258-60
16. Maqsood S, Benjakul S, Abushelaibi A, Alam A. Phenolic compounds and plant phenolic extracts as natural antioxidants in prevention of lipid oxidation in seafood: a detailed review. Comprehensive Reviews in Food Science and Food Safety. 2014; 13: 1125-9
17. Phongpaichit S, et al. Biological activities of extracts from endophytic fungi isolated from garcinia plants. FEMS Immunol Med Microbiol. 2007; 51(3): 517-25
18. Gülçin I, Mshvildadze V, Gepdiremen A, Elias R. The antioxidant activity of a triterpenoid glycoside isolated from the berries of hedera colchica: 3-o-(β-D-glucopyranosyl)-hederagenin. Phytotherapy Research. 2006; 20: 130-4
19. Pereira DM, Valentão P, Pereira JA, Andrare PB. Phenolics: from chemistry to biology. Molecules. 2009; 14: 2202-2211
20. Wulandari D, Kilawati Y, Fadjar M. Activity of compounds on seaweed eucheuma cottonii extract as antioxidant candidate to prevent effects of free radical in water pollution. Research Journal of Life Science. 2018; 5(3): 173-82