Total Phenol and Flavonoid Content and Antioxidant Activity of Extract *Caulerpa racemosa* with Methanol and Ethyl Acetate Solvents from Usaha Jaya Village, Raja Ampat Regency, Southwest Papua Province

ABSTRACT

Caulerpa racemosa is a species of green seaweed that is widely distributed in almost all tropical seas in the world. Those green seaweed contains active compounds that have potential as antioxidants. Differences in phenolic and flavonoid content as an antioxidant can be influenced by post-take handling and solvent polarity. The research aims to study the phenolic and flavonoid content in methanol (polar) and ethyl acetate (semi polar) solvents as well as the antioxidant activity of ABTS (*2,2-azinobis-3-Ethylbenzoathiazoline-6-sulfonic acid*), DPPH (*1,1-diphenyl-2-picrylhydrazyl*), and H_2O_2 (*Hydrogen peroxide*) methods. The highest extract yield was found in the methanol extract which amounted to 6,12%, while the ethyl acetate amounted to 1,80%. The phenolic content of the methanol extract was 40,18 ± 3,06 mgGAE/g, while the ethyl acetate extract had the lowest total phenol content of 17,22 ± 1,37 mgGAE/g. The total flavonoid content of the methanol extract was 84,42 ± 10,20 mgQE/g higher than the ethyl acetate extract was 78,61 ± 5,31 mgQE/g. Antioxidant activity of ABTS method of methanol extract obtained IC50 value of 134,64 ± 19,90 µg/mL with moderate category lower than that of ethyl acetate extract which is 113,50 ± 19,69 µg/mL also with moderate category. Antioxidant activity of DPPH method in methanol extract obtained IC50 reached 96,08 ± 0,45 µg/mL with strong antioxidant category, while IC50 in ethyl acetate extract reached 167,72 ± 1,08 µg/mL with weak category. The antioxidant activity of H_2O_2 method is classified as moderate activity in both solvents with methanol extract of 110,79 ± 2,52 µg/mL and ethyl acetate extract of 123,67 ± 2,08 µg/mL.

Keywords: Antioxidant, ABTS, *Caulerpa racemosa*, DPPH, H_2O_2, total phenolic content, total flavonoid content

1. INTRODUCTION

Indonesia is an archipelagic country with 75% of its territory being water. Therefore, Indonesia has very diverse aquatic biological resources, especially macroalgae [25]. Macroalgae are potential biological resources in intertidal coastal areas and are found in almost all Indonesian waters. Macroalgae germplasm resources spread in Indonesian waters amount to 6,42% of the total world macroalgae biodiversity [36]. Sea grape (*Caulerpa*) is one type of green algae (*Chlorophyceae*) that has edible properties so that it can be utilized by humans for direct consumption or processed into food and non-food products [25].

The nature of *Caulerpa* which is safe for consumption and has been utilized as a food (vegetable) by some coastal communities allows this seaweed to be explored as a source of antioxidants. *Caulerpa* contains constituents as antioxidant ingredients so it is known to be utilized as a source of antioxidants [9]. *Caulerpa* produces secondary metabolites that function as antioxidants. The secondary metabolite compounds produced are phenols, flavonoids, and saponins [11,13,4,43]. The main secondary metabolite content of *Caulerpa* sp is caulerpenin which has anti-inflammatory properties. These bioactive compounds are

Comment [D1]: I suggest to rewrite the title as follows: Total Phenols, Flavonoids and Antioxidant Activity of Methanolic and Ethyl Acetate Extracts of *Caulerpa racemosa* Plants Grown in Usaha Jaya Village, Raja Ampat Regency, Southwest Papua Province

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formed through several biosynthesis pathways with changes from primary metabolites to intermediate metabolites, then to secondary metabolites [3].

Raja Ampat Regency is one of the regions in Southwest Papua Province that is known for its abundant biological resources, especially *Caulerpa racemosa* were found around the coastal areas of Raja Ampat Regency and has been consumed by coastal communities as a substitute for vegetables and has become a daily diet. However, the analysis of chemical composition, total phenolic compounds, and antioxidant activity of *Caulerpa racemosa* found in Raja Ampat waters has not been analyzed. The purpose of this study was to determine the total phenolic and flavonoid content in *Caulerpa racemosa* extracts with methanol (polar) and ethyl acetate (semipolar) solvents and antioxidant activity of ABTS, DPPH and H_2O_2 methods obtained from the waters of Usaha Jaya Village, Raja Ampat Regency, Southwest Papua Province.

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2. MATERIAL AND METHODS

2.1. MATERIAL

Sample Collection : Sea grape (*Caulerpa racemosa*) samples were obtained from Usaha Jaya Village, East Misool district, Raja Ampat Regency, Papua Province (1°59'16"S;130°24'59"E) on January 2024. The samples were washed using fresh water to remove impurities and then dried at room temperature between 28-32°C for 2-3 days. The dried samples were put into plastic seals and packed using sterofoam boxes and then sent to the laboratory for further testing.

Material and Instrumentation : The materials used in the test of the total phenolic content (TPC), total flavonoid content (TFC), dan antioxidant activity assay are; methanol 96%, ethyl acetat 96%, ethanol 96%, Na_2CO_3, reagen Follin-Ciocalteu, gallic acid, reagen quercetin, acetic acid, $AlCl_3$ 4%, distilled water, ascorbic acid, reagen DPPH, reagen ABTS, kalium persulfate, reagen H_2O_2, Buffer fosfate, and filter paper. The tools used in the test are; pestle and mortar, grinder, orbital shaker, rotary evaporator (Buchii rotavapor r-100), centrifuge, spectrofotometri uv-vis single beam (Shimadzu 1280), micropipette, glass ware, pipet, funnel.

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2.2. METHODS

- **Sample Preparation and Extraction**

The dried samples of *Caulerpa racemosa* were cut into small pieces and crushed using a grinder until they were powdered, the samples that were still large were again mashed using a mortar and pestle until the samples became fine powder (simplisia). A total of 25 g of simplisia was dissolved with each solvent (methanol, and ethyl acetate) into Erlenmeyer in a ratio of 1:10 then placed on an orbital shaker at 160 rpm for 72 hours. After that, the sample was filtered using filter paper to produce supernatant. The supernatant obtained was

centrifuged for 10 minutes and then transferred into a tube and evaporated using a rotary evaporator (buchii rotavapor r-100) with a bath temperature of 40-50 ° C at 140 mbar to produce a thick extract. The extract yield was calculated by the formula [5];

$$\text{Yield} = \frac{\textit{final product weight } (g)}{\textit{Initial weight of raw material } (g)} \times 100\%$$

- **Total Phenolic Content (TPC)**

A total of 50 mg of gallic acid was dissolved with 50 mL of 96% ethanol into a dark vial bottle and homogenized. gallic acid parent sample with a concentration gallic acid parent sample with a concentration of 1000 ppm was diluted into 20, 40, 60, 80, 100 ppm concentration series. Each concentration was taken as much as 1 mL placed into a test tube added 4% Follin Ciocalteu reagent as much as 0.4 mL homogenized and incubated for 4-8 minutes then added 5% Na_2CO_3 as much as 4 mL homogenized and diluted to 10 mL using distilled water. The absorbance of the sample was measured in spectrophotmetry with a wavelength of 725 nm.

Each extract sample measured as much as 30 mg was dissolved using 96% ethanol as much as 30 mL so that a stock solution of 1000 ppm was obtained. Taken as much as 1 mL of sample solution added 0,4 mL of follin ciocalteu reagent was homogenized and incubated for 4-8 minutes, after which 5% Na_2CO_3 was added as much as 4 mL homogenized and diluted to 10 mL using distilled water. The absorbance of the sample was measured in spectrophotmetry with a wavelength of 725 nm. Total phenolic was calculated by making a calibration curve of the relationship between the absorbance of gallic acid and the absorbance of the extract sample with the formula [1,39].

$$\text{Total phenol GAE} - \text{c.}\frac{V}{m}$$

- **Total Flavonoid Content (TFC)**

Quercetin standard solution was measured as 50 mg and dissolved using 96% ethanol as much as 50 mL to obtain 1000 ppm stock solution. Dilutions were done at 20, 40, 60, 80, 100 ppm. Each concentration was taken 1 mL and added 1 mL of 4% $AlCl_3$ and 1 mL of 5% acetic acid was homogenized, then diluted to 10 mL using distilled water and incubated for 1 hour. The absorbance of the sample was measured at 420 nm wavelength using uv-vis spectrophotometry.

Each extract sample was measured as much as 30 mg and dissolved with 96% ethanol as much as 30 mL and homogenized. 1 mL of the stock solution was taken and then 1 mL of 4% $AlCl_3$ and 1 mL of 5% acetic acid was added and homogenized, then the sample was diluted to 10 mL using distilled water and incubated for 1 hour. The absorbance of the sample was measured at 420 nm wavelength using uv-vis spectrophotometry. Total flavonoid content was calculated based on quercetin calibration curve [27] with the formula;

$$\text{Total flavonoid} - \frac{C \times V \times Fp}{m} \times 100\%$$

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- **Antioxidant Activity**

ABTS (2,2-azinobis-3-Ethylbenzoathiazoline-6-sulfonic acid) assay

Testing the antioxidant activity of the ABTS method begins with the preparation of ABTS stock solution. ABTS solution was measured 18 mg and dissolved using distilled water as much as 5 mL, then potassium persulfate solution was measured 3 mg and dissolved using distilled water as much as 5 mL. ABTS and potassium persulfate that have been dissolved are put into a measuring flask and the volume is adjusted to 25 mL using ethanol, the solution is then incubated for 12-16 hours at room temperature 22-24 ° C [35].

Samples extract of *Caulerpa racemosa* 1000 ppm were taken as much as 100 µL, 200 µL, 300 µL, 400 µL and 500 µL then added 1 mL of ABTS solution and then sufficient volume up to 5 mL using ethanol so as to obtain a solution with a concentration of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. Furthermore, it was homogenized and incubated for 15 minutes and then measured the absorption at a wavelength of 750 nm. The % inhibition value was calculated using the formula:

$$\%\text{Inhibisi} = \frac{Abs.control\ of\ abts \quad abs\ of\ sample}{Abs.of\ control} \times 100\%$$

DPPH (2,2-difenil-1-pikrilhidrazil) assay

Comment [D30]: Here is the correct name of DPPH (2,2-diphenyl-1-picrylhydrazyl)

The antioxidant activity test of *Caulerpa racemosa* extract samples with DPPH method refers to [19] which has been modified. Each concentration series of extract samples (20, 40, 60, 80, 100 ppm) was taken as much as 3,5 mL and placed into a test tube then added 1,5 mL of 4% DPPH, the solution was homogenized and incubated for 30 minutes at room temperature (37°C) under dark conditions.

Absorbance measurement was performed by uv-vis spectrophotometry with a wavelength of 517 nm. The control used was 3,5 mL of methanol and 1,5 mL of DPPH. The % inhibition value was calculated with the equation [12].

$$\%\text{inhibition} = \frac{Abs\ of\ blank \quad abs\ of\ sample}{abs\ of\ blank} \times 100\%$$

H_2O_2 (Hidrogen peroksida) assay

Hydrogen peroxide scavenging activity is the ability of a compound to scavenge or remove hydrogen peroxide. Testing the antioxidant scavenging activity of the H_2O_2 method refers to the research of [18] which has been modified. Extract samples with various concentration variations were taken as much as 100 µL and 600 µL of 2 mM hydrogen peroxide was added to the test tube and then the volume was sufficient using phosphate buffer (pH 7,4) as much as 4 mL and then homogenized. The sample was incubated for 10 minutes and the absorbance was measured at a wavelength of 230 nm. The percentage of H_2O_2 scavenging activity was calculated based on the equation below:

$$\%Inhibisi = \frac{Abs\ of\ control \quad abs\ of\ sample}{Abs\ control} \times 100\%$$

2.3. Statistical Analysis

All analyses were expressed in triplicate and data were detailed as means ± SD.

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3. RESULTS AND DISCUSSION

Yield extract of *Caulerpa racemosa*

Presentation of extract yield, total phenols and total flavonoids of *Caulerpa racemosa* with methanol and ethyl acetate solvents are presented in Table 1.

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Table 1. Extract yield and phytochemical compound content

Solvent	Yield (%)	TPC ± SD (mgGAE/g)	TFC ± SD (mgQE/g)
Methanol	6,12	40,18 ± 3,06	84,42 ± 10,20
Ethyl acetate	1,80	17,22 ± 1,37	78,61 ± 5,31

Note: TPC (Total Phenolic Content), TFC (Total Flavonoid Content), SD (Standart Deviation)

Table 1 shows that different types of solvents in the extraction process affect the amount of extract produced. Methanol solvent has the highest extract yield (6,12%) than ethyl acetate solvent (1,80) in accordance with the research of [12] methanol solvent (polar) from *Caulerpa lentilifera* extract has the highest average extract yield of 0,42% of ethyl acetate solvent extract (semi-polar) and n-hexane (nonpolar) respectively, which is 0,39% and 0,36%. According to [20] the methanol extract of *Caulerpa raemosa* is the extract with the highest yield of 4,25% while ethyl acetate is 2,08%. Based on research by [19] said that the methanol extract of *Caulerpa filiformis* had extract yields of 8,32% and 15,72%. Based on these results, it shows that *Caulerpa racemosa* contains polar and semi-polar compounds that can dissolve well in methanol solvents.

Extraction aims to obtain bioactive compounds optimally from a material or material. The extraction process is strongly influenced by several conditions such as: extraction method, temperature, time, phytochemical composition and solvent used. If the conditions in the extraction process are the same, then the solvent is an important parameter in the isolation of active compounds [41]. The amount of active compounds that can be extracted is influenced by the polarity of the solvent. Methanol has a fairly wide polarity range so that the number of active compounds that can be extracted is more, both polar, semi-polar to non-polar compounds.

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Total Phenolic Content (TPC)

The analysis of total phenolic content of methanol and ethyl acetate extracts showed phenolic content in methanol extract 40,18 ± 3,06 mgGAE/g and ethyl acetate extract 17,22 ± 1,37 mgGAE/g. The methanol extract of *Caulerpa racemosa* has higher total phenolic content than the ethyl acetate extract.

Table 1 shows that the total phenolic content in this study is higher when compared to the results of research by [19] showing that the methanol extract of *Caulerpa filiformis* has a total phenolic content of 39,31 mgGAE/g from Sechura Bay and 18,78 mgGAE/g from Paracas Bay. However, the results of table 1 are still lower when compared to the research of [12] where the methanol extract of *Caulerpa lentilifera* has a higher phenolic content (154,65 mgGAE/g) than the ethyl acetate extract (141,50 mgGAE/g). these results indicate that differences in the type of solvent in the extraction process will affect the total amount of phenolics produced.

According to [8] most phenolic compounds are polar. The results of research by [9] show that there are differences in the total phenol content produced by *Caulerpa racemosa* extracts from different solvents, namely methanol extract 66,61 mgGAE/g and chloroform extract 123,91 mgGAE/g. [32] stated that the polyphenol content of seaweed has variations from species type, harvest age, season, and geographical location. The process of extraction and drying of samples can affect the total amount of seaweed phenols. The use of solvents in the extraction process affects total phenols [22]. The drying process affects the total phenolics of seaweed [6; 21] Other researchers also said that differences in total phenolics in seaweed are caused by several factors, namely: seaweed type, geographical, seasonal, physiological, and environmental conditions that vary [17,7].

Total Flavonoid Content (TFC)

Table 1 shows the total flavonoid content of methanol and ethyl acetate extracts of *Caulerpa racemosa* from the waters of Usaha Jaya Village has a higher value than total phenolic. The total flavonoid content of *Caulerpa racemosa* of methanol extract was 84,42 ± 10,20 mgQE/g while the ethyl acetate extract was (78,61 ± 5,31). These results are in accordance with the research of [9] with the total flavonoid content of *Caulerpa racemosa* of methanol extract 114,16 ± 0,91 mgQE/g and chloroform extract of 86,33 ± 6,96 mgQE/g. According to research by [12] showed that the flavonoid content of *Caulerpa lentillifera* of methanol extract was 116,82 mgQE/100g. According to [30] showed that the total flavonoids of ethyl acetate extract from *Gracilaria sp*. seaweed had a value of 25,23 ± 0,46 mgQE/g followed by ethanol extract was 21,78 ± 0,32 mgQE/g. While according to [42] showed the total flavonoids of *Eucheuma cottonii* seaweed of ethyl acetate extract was 35,17 ± 1,00 mgQE/g and methanol extract of 17,78 ± 0,31 mgQE/g.

The results showed that the methanol extract was higher when compared to the ethyl acetate extract. In addition, *Caulerpa racemosa* seaweed has a higher total flavonoid content when compared to total phenol. According to [31] flavonoids that bind to sugars tend to dissolve in water (polar), while less polar aglycones such as isoflavones, flavones, flavonon, and flavonols tend to dissolve more easily in semi-polar solvents. Flavonoids are one of the natural antioxidants that have the function of inhibiting the oxidation of low density lipoprotein (LDL) which is a trigger for narrowing of blood vessels. According to [44] suggested that natural flavonoid compounds including kaempferol, miricetin, morin, and quercetin have protective activity by reducing α-tocopherol content in LDL. According to [19] flavonoids are a class of phenolic compounds that form the best antioxidants found in plants.

Antioxidant activity assay

Antioxidant activity is declared very strong if the IC50 value is < 50 µg/mL, strong 50-100 µg/mL, moderate 101-150 µg/mL, and weak 150-200 µg/mL [24]. Antioxidant activity testing of *Caulerpa racemosa* with methanol and ethyl acetate solvents using ABTS, DPPH, and H_2O_2 methods showed strong to moderate antioxidant activity, respectively. The control used in each test was ascorbic acid. The IC50 values in each test are as follows.

ABTS (*2,2-azinobis-3-Ethylbenzoathiazoline-6-sulfonic acid*) assay

The antioxidant activity of *Caulerpa racemosa* ABTS method with methanol and ethyl acetate solvents has free radical scavenging activity shown in Figure 1 methanol extract has an IC50 value of 134,64 ± 19,90 indicating moderate antioxidant activity, while ethyl acetate extract has a better IC50 value of 113,50 ± 19,69 indicating moderate antioxidant activity.

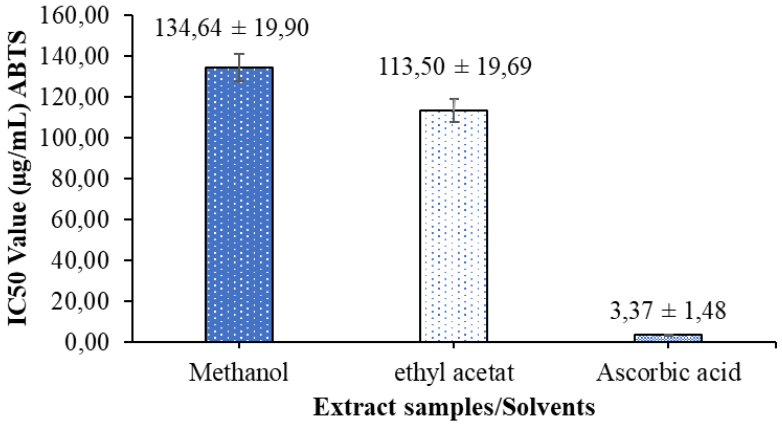

Figure 1. Antioxidant activity of methanol and ethyl acetate extracts of *Caulerpa racemosa* with comparative ascorbic acid by ABTS method

Ethyl acetate extract showed better antioxidant activity than methanol extract. According to [19] in their research showed that the methanol extract of *Caulerpa filiformis* has good antioxidant activity with EC50 values of 2,546 and 4,624. Mamani further explained that the antioxidant capacity of *Caulerpa* has been studied in the form of extracts with antioxidants, which vary in the same or similar species.

The *Padina sp* and *Sargassum sp* seaweed have different antioxidant activities. The IC50 value of *Sargassum sp* extract is higher (64,80-102,48 mg/L) when compared to *Padina sp* by (101,78-126,99) the difference in IC50 results from extracts in the ABTS test can be influenced by the type of sample, solvent, and extraction method [2].

DPPH (*2,2-difenil-1-pikrilhidrazil*) assay

The antioxidant activity of *Caulerpa racemosa* DPPH method with methanol and ethyl acetate solvents has free radical scavenging activity shown in Figure 2. The antioxidant activity of DPPH method of methanol and ethyl acetate extracts of *Caulerpa racemosa* is categorized as strong to weak. The methanol extract has a fairly good IC50 value and is classified as a strong antioxidant at 96,08 ± 0,45 µg/mL, while the ethyl acetate extract has a

much lower IC50 value with a value of 167,72 ± 1,08 µg/mL and is classified as weak antioxidant activity.

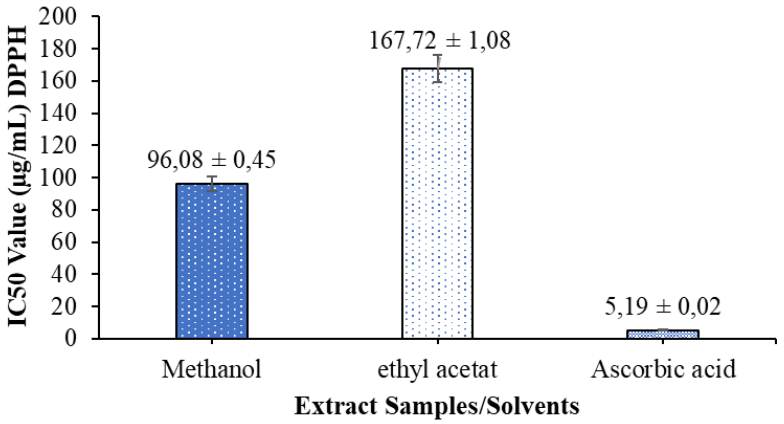

Figure 2. Antioxidant activity of methanol and ethyl acetate extracts with ascorbic acid using DPPH method

The difference in IC50 values in the DPPH method antioxidant test is due to differences in the solvents used. According to [12] explained the effectiveness of antioxidants in methanol extracts in counteracting free radicals is thought to be related to the polar nature of methanol so that many phytochemical components dissolve in it. Bioactive compounds in *Caulerpa lentillifera* that act as antioxidants are alkaloids, flavonoids, phenolics, and steroids/terpenoids. According to [19] mentioned several studies on *Caulerpa* also showed a greater ability of extracts to trap free radicals (DPPH).

Based on the IC50 value of antioxidant activity testing obtained, it shows that methanol extract has the best IC50 value with a strong category while ethyl acetate is weak. These results have better antioxidant activity than the research of [20] which showed the antioxidant activity of *Caulerpa* of methanol extract (IC50 = 132,08 µg/mL) included in the moderate category. Furthermore, [20] explained that the antioxidant activity test in inhibiting free radicals is based on the ability of an ingredient/extract to donate electrons or hydrogen compounds to DPPH free radicals so that more stable free radicals will be formed.

H_2O_2 (*Hydrogen peroxide*) assay

Hydrogen peroxide is a hydroxyl radical that has toxic properties and can cause damage to cells and tissues in the body [26]. This assay measures the reduction in hydrogen peroxide (H_2O_2) concentration after exposure to plant extracts that have antioxidant potential [18]. The hydrogen peroxide scavenging activity of methanol and ethyl acetate extracts of *Caulerpa racemosa* is described in Figure 3.

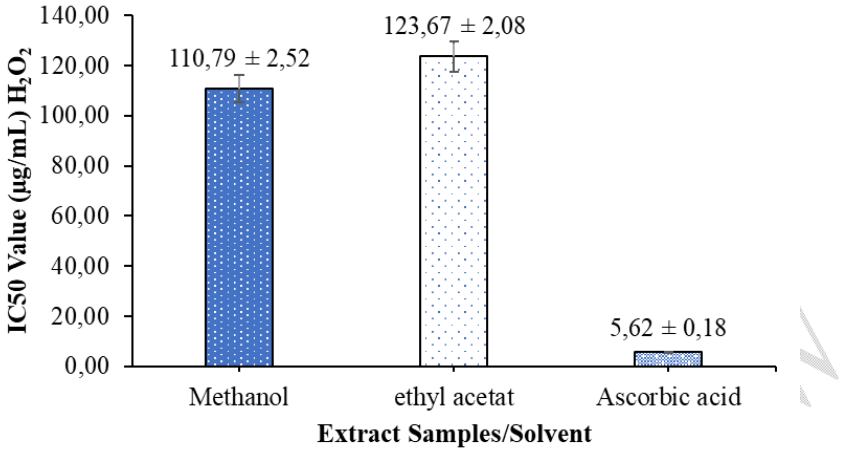

Figure 3. Antioxidant activity of methanol and ethyl acetate extracts of *Caulerpa racemosa* with comparative ascorbic acid H_2O_2 method

H_2O_2 radical scavenging activity in methanol and ethyl acetate extracts showed IC50 values with moderate categories, where methanol extracts (110,79 ± 2,52 µg/mL) had better IC50 values than ethyl acetate extracts (123,67 ± 2,08 µg/mL). Methanol extracts had a percentage of H_2O_2 scavenging of 48,68% at a concentration of 100 ppm, while ethyl acetate extracts amounted to 47,96% at a concentration of 47,96%. These results are still lower when compared to the research of [26], where the scavenging activity of *Caulerpa lentillifera* extract with thermal drying and freeze drying ranges from 50-70%. While in [34] showed lower scavenging activity in methanol extract *Caulerpa racemosa* (20.78%) and *Caulerpa racemosa* var macrophysa (24,91%) where these results are still higher when compared to other green seaweed, namely *Caulerpa scalpelliformis* with a percentage of scavenging (17,64%), *Cladophora vagabunda* (16,71%) and *Ulva Lactuca* (16,32%).

Correlation of Total Phenol and Total Flavonoid Content to Antioxidant Activity

Phenolic compounds in macroalgae have been widely reported to have potential as natural antioxidants, but antioxidants in seaweed are not only caused by phenolic compounds [14]. The role of phenolic compounds as antioxidants has to do with conjugate bonds on the benzene aromatic ring and the number of hydroxyl functional groups [37]. According to [19] the antioxidant activity of macroalgae can be attributed to the phenolic content without ruling out the synergistic action between these compounds. Linear regression analysis between total phenol and total flavonoid content to the antioxidant activity of ABTS, DPPH, and H_2O_2 methods aims to determine the closeness of the relationship between total phenol and total flavonoid content to antioxidant activity.

The antioxidant test of ABTS method showed that ethyl acetate extract was better in inhibiting ABTS free radicals than methanol extract inhibit ABTS free radicals than methanol extract. Based on linear regression analysis the total phenol content of methanol extract (R^2 = 0,98) and ethyl acetate (R^2 = 0,96) has a very strong relationship to the antioxidant activity of ABTS method. Furthermore, the total flavonoid content of extract methanol (R^2 = 0,88) and

ethyl acetate (R^2 = 0,97) also had a very strong relationship to the antioxidant activity of ABTS method. so it is known that the content of total phenols and total flavonoids can act as antioxidant activity in counteracting ABTS radicals. According to [35] the ABTS method is more sensitive in detecting antioxidant concentrations in low levels. low levels. ABTS testing can evaluate antioxidant compounds that do not perform well in the DPPH assay [29].

In testing the antioxidant activity of the DPPH method, the methanol extract was better in inhibiting DPPH free radicals than the ethyl acetate extract. Based on the results of linear regression analysis showed the total phenol content in methanol extract (R^2 =0,97) and ethyl acetate (R^2 = 0,85) has a very strong relationship to the antioxidant activity of DPPH method. At the total flavonoid content of methanol extract (R^2 = 0,68) had a close relationship to the antioxidant activity of the antioxidant activity of DPPH method, on the contrary, the flavonoid content of ethyl acetate extract (R^2 = 0,47) has a relatively weak relationship. It is suspected that the DPPH method has better effectiveness in polar solvents such as methanol than ethyl acetate in analyzing antioxidant activity to assess the content of phenolic compounds and flavonoids [30; 33]. According to [29] the DPPH method is effective in assessing hydroxyl group compounds, while some phenolic compounds such as dihydrochalcones and flavones do not react well with DPPH. In accordance with the results of this study where the methanol solvent has a better advantage in attracting phenolic compounds than ethyl acetate, while the total content of flavonoids has more results than total phenols in both solvents. In accordance with ABTS method antioxidant activity testing which has a better advantage in ethyl acetate solvent.

In testing the antioxidant activity of the H_2O_2 method, methanol extract is better in removing or scavenging hydrogen peroxide than ethyl acetate solvent. Based on the results of linear regression analysis showed the total phenol content of methanol extract (R^2 = 0,84) and ethyl acetate (R^2 = 0,94) had a very close relationship to antioxidant activity of H_2O_2 method. At the total flavonoid content of methanol extract (R^2 = 0,99) and ethyl acetate extract (R^2 = 0,95) also showed a very close relationship to the antioxidant activity of H_2O_2 method. The correlation value shows that the flavonoid compounds of methanol extract have a very strong relationship to the antioxidant activity of the H_2O_2 method. Flavonoid compounds are known to protect in the body caused by H_2O_2. According to [38] flavonoids are significantly able to reduce reactivated oxygen levels in the form of H_2O_2 in the body and increase the activity of antioxidant enzymes. Flavonoids in the form of quercetin and naringin have been shown to protect DNA from oxidative damage caused by H_2O_2, showing their role as effective free radical scavenger [40]. In other studies explain the specific structure of flavonoids with two hydroxyl groups in ring B is essential for inhibiting lipid peroxidation in human erythrocytes exposed to H_2O_2 [10]. According to [16] H_2O_2 is not so reactive, but in certain cases H_2O_2 can be toxic to cells in the body because it can cause hydroxyl radicals of cell nature.

Based on the ABTS and H_2O_2 test methods, the incubation time is faster than the DPPH method so that it can shorten the test time but the reagents needed more, preparation of reagents and samples is quite complicated and requires longer time in the preparation of reagents than the DPPH method. This shows that the DPPH method has the advantage of simplicity and cheaper cost-effectiveness, but the testing of antioxidant activity of ABTS method is more sensitive in evaluating compounds with low levels.

The choice of test method between ABTS, DPPH and H_2O_2 which is more effective depends on the characteristics of the antioxidant compounds to be tested and the nature of the sample matrix. The composition and structure of antioxidants affect the reaction rate significantly. Differences in reaction equilibrium time can affect the wrong estimation. It is important to determine the appropriate test method to evaluate the antioxidant content accurately. Based on the wavelength used, antioxidant results obtained vary based on the absorption characteristics of the antioxidant and the concentration in the measurement system [28].

4. CONCLUSION

The yield of *Caulerpa racemosa* extracts from solvents with different polarities gives an influence on the total phenol and total flavonoid content obtained as well as the antioxidant activity in various methods. Methanol solvent showed a better extract yield (6,12%) in 25 g sample. The total phenol content of the methanol extract was 40,18 ± 3,06 mgGAE/g, while the total flavonoid content of the methanol extract was 84,42 ± 10,20 mgQE/g. IC50 in the ABTS method antioxidant activity of ethyl acetate extract 113,50 ± 19,69 µg/mL. IC50 on antioxidant activity of DPPH method in methanol extract 96,08 ± 0,45 µg/mL. IC50 on the H_2O_2 scavenging activity of methanol extract was obtained 110,79 ± 2,52 µg/mL.

REFERENCES

1. Arifin, M. H., A. M. Jacoeb, A. Abdullah, P. H. Riyadi. 2023. *Drying Method Comparison of Black Mangrove Leaves (Rhizopora mucronata) for an Antioxidant Activity Assay*. Food Research, 7 (3) : 67-75.
2. Abdullah A, Nurjanah, A. I. S. Nasution. 2021. *Characterization of active fraction of brown seaweed fucosanthin biopigment as antioxidant and UV-Protector*. Jurnal Pengolahan Hasil Perikanan Indonesia, 24 (1): 131-147.
3. Anulika, N.P., E.O. Ignatius, E.S. Raymond, O.I. Osasere, dan A.H. Abiola. 2016. *The Chemistry of Natural Product: Plant Secondary Metabolites. International Journal of Technology Enhancements and Emerging Engineering*, 4 (8): 1-8.
4. Anwar L.O., R. L. Bubun., Rosmawati. 2019. *Potential Antioxidant Bioactive Compounds and Chemical Composition of Fresh, Semi-dried, and Dried Caulerpa racemosa Green Seaweed*. SENASPRO.
5. AOAC. Association of Official Analytical Chemist. 2005. Official Method of Analysis of The Association of Official Analytical of Chemist. Arlington: The Association of Official Analytical Chemist, Inc.
6. Badmus U.O, M. A. Taggart, K. G. Boyd. 2016. The effect of different drying methods on certain nutritionally important chemical constituents in edible brown seaweeds. Journal of Applied Phycology, 31 (6): 3883-3897.
7. Balboa E. M, Gallego-Fabrega. C, A. Moure, H. Dominguez. 2016. Study of the seasonal variation on proximate compositian of oven-dried *Sargassum muticum* biomass collected in Vigo Ria, Spain. Journal of Applied Phycology, 28 (3): 1943-1953.
8. Bangol E, L.I. Momuat, J. Abidjuju. 2014. *Antioxidant activity of ethanol and n-hexane extracts of santa maria grass* (*Artemisia vulgaris L.*) *leaves on fish oil.* Jurnal Ilmiah Sains, 14 (2): 129-135.

Comment [D34]: From the reference no. 1 to 13, the year is written after authors names while from 14 to 44 is written after the journal name.

Comment [D35]: I didn't find any published research or book chapter with this title

9. Belkacemi L., Mahmoud B., Ali C. D., Youcef B. 2020. Antioxidant and antibacterial activities and identification of bioactive compounds of various extracts of Caulerpa racemosa from Algerian coast. Asian Pacific Journal of Tropical Biomedicine; 10 (2): 87-94.
10. Bilto. Y. Y, S. Suboh, T. Aburjai, S. Abdalla. 2012. Structure-activity relationships regarding the antioxidant effedts of the flavonoids on human erythrocytes. Natural Science, 4 : 740-747.
11. Chew YL, Lima YY, Omar M, Khoo KS. 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT41:1067-72.
12. Diharmi. A, Edison, M. Ilza, Dahlia, R. Saputra. 2024. *Antioxidant activity, total phenolics, flavonoids and saponins of sea grape (Caulerpa lentillifera) extracted with different polarity solvents*, Jurnal Teknologi Industri Pertanian. AGROINTEK; 18 (3): 761-768.
13. Djapailala F.Y., Lita A.D.Y Montolalu., Feny M. 2013. Total Phenol Content in Seaweed (Caulerpa racemosa) with Potential as Antioxidant, Media Teknologi Hasil Perikanan; 1 (2): 1-5.
14. Erniati, Syahrial, Erlangga, Imanullah, Y. Andika. Aktivitas Antioksidan dan Total Fenol Rumput Laut *Sargassum sp*. Dari Perairan Simeulue, Aceh. JPHPI. 2024;27(3): 186-196.
15. Handayani S, A. Najib, Wisdawati, A. Khoiriyah. 2020. Antioxidant activity of *Caulerpa lentillifera J. Agardh* by *1,1-diphenyl-2picrylhydrazyl* free radical scavenging method, jurnal kesehatan, 13 (1): 61-70.
16. Lakhsmi. T. M., R. Radha, N. Jayshree. Invitro Antioxidant Activity, Total Phenolic, and Total Flavonoid Content in Extracts from the Bark of *Dalbergia sisso* Roxb. 2014;5(5): 226-231.
17. Machu L, L. Misurcova, J.V Ambrozova, J. Orsavova, J. MLcek, J. Sochor, T. Jurikova. Phenolic content and antioxidant capacity in alga food product. Molecules. 2015;20(1): 1118-1133.
18. Madhuranga. H. D. T., D. N. A. W. Samarakoon. Advancing In vitro Antioxidant Activity Assessment: A Comprehensive Methodological Review and Improved Approaches for DPPH, FRAP, and H_2O_2 Assays. Journal of Natural & Ayuverdic Medicine. 2023;7(4):000431
19. Mamani. J, J. Chavez, E. Apumayta, P.G-Kodaka. Antioxidant activity and total phenolic content in *Caulerpa filiformis* (Chlorophyta) from Sechura Bay and Paracas Bay, Peru. Revista peruana de biologia. 2020;27(1): 061-066.
20. Marraskuranto E, M. Nursid, S. Utami, I. Setyaningsih, K. Tarman. *Phytochemical content, antibacterial and antioxidant potential of Caulerpa racemosa extracted with different solvents*. JPB Kelautan dan Perikanan. 2021;16(1): 1-10.
21. Masyrikhiyah R. *Antioxidant activity and total phenolics of Gracilaria sp seaweed*. Kabupaten Brebes. Jurnal Pengolahan Hasil Perikanan Indonesia. 2021;24(2): 236-242.
22. Mazumder A, S.L. Holdt, De Francisci D, M.M. Alvorado, H. N. Mishra, I. Angelidaki. Extraction of alginate from *Sargassum muticum*: Process optimization and study of it functional activity. Journal of Applied Phycology. 2016;28(6): 3625-3634.
23. Mokoginta T.A, A. Yudhistira, Deby A. Mpila. *Antioxidant activity of ethanol of seaweed Caulerpa racemosa from Mantehage island North Sulawesi*. PHARMACON. 2021;10(3)
24. Molyneux P. The use of stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioksidan activity. Songklanakarin Journal Science Technology. 2004;26(2): 211-219.

Comment [D36]: This reference is not mentioned in the text

Comment [D37]: This reference is not mentioned in the text

25. Muliani S., Rosmaiti., Muhammad F. I. *Analysis of Kualalangsa Waters Quality Suitability for Sea Grape Cultivation (Caulerpa Recemosa) Viewed with GIS*. Jurnal Ilmiah Samudra Akuatika. 2021;5(2):66-75.
26. Nguyen, V. T., J.P. Ueng, G.J. Tsai. Proximat composition total phenolic content, and antioxidant activity of seagrape (*Caulerpa lentillifera*). J Food Sci.2011;76:C950-8.
27. Oktaria. D., M. P. Marpaung. Penetapan Kadar Flavonoid Total dan Aktivitas Antioksidan Ekstrak Akar Nipah (*Nypa fruticans Wurmb*) Dengan Metode Spektrofotometri UV-Vis. Lantanida Journal. 2023; 11(1):36-50.
28. Olszowy M, Dawidowicz A.L. Is it possible to use the DPPH and ABTS methods for reliable estimation of antioxidant power of colored compounds. Chemical Papers. 2018;72(2):393-400. doi: 10.1007/S11696-017-0288-3
29. Platzer M, Kiese S, Herfellner T, Schweiggert-Weisz U, Miesbauer O, Eisner P. Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. Molecules. 2021;26: 1244
30. Purwaningsih. I., R. Sapriani, R. Indrawati. Antioxidant Activity of Methanol Extract of Kesum Leaves (*Polygonum minus* Huds.) DPPH Method. Jurnal Laboratorium Khatulistiwa. 2018;1(2):161-165
31. Purwaningsih S, E. Deskawati. *Characteristics and antioxidant activity of Glacilaria sp seaweed*. asal Banten. Jurnal Pengolahan Hasil Perikanan Indonesia. 2020;23(3):503-512.
32. Rajauria G, B. Foley, N. Abu-Ghannam. Identification and characterization of phenolic antioxidant compounds from brown irish seaweed *Himanthalia elongata* using LC-DAD-ESI-MS/MS. Journal Innovative Food Science and Emerging Technologies. 2016;37(1): 1-7.
33. Ridwanto. R, A.Trizaldi, Z. Rani, A. S. Daulay, H. M. Nasution, D. Miswanda. Antioxidan Activity Test of Methanol Extract of Gaharu (*Aquilaria Malaccensis* Lam.) Bark with DPPH (1,1 *Diphenyl-2-Picrylhydrazyl*) Method. 2023;3(2): 232-240.
34. Roy S. Screening and partial characterization of natural antioxidants from seaweeds collected from Rameshwaram Southeast Coast of India. Journal of Marine Science Research an Oceanography. 2020;3(1): 1-12.
35. Sari. A. E. N. Determination of Total Flavonoid Levels and Antioxidant Activity Test of Ethanol Extract and Fraction of Waru Bark (Hibiscus toliaceus L.) using the ABTS Method. Jurnal Jamu Kusuma. 2022;2(2): 96-106.
36. Sarita, I Dewa, Ayu, Anix. D., I Made, Subrata., N. Putri, Sumaryani. Identification of Seaweed Species Found in the Natural Ecosystem of Nusa Penida Waters; Jurnal Edukasi Matematika dan Sains. 2021;10(1): 141-154.
37. . Sedjati. S., E. Supriyantini, A. Ridlo, N. Soenardjo, V.Y. Santi. Pigment Content, Total Phenolic and Antioxidant Activity of Sargassum sp. Jurnal Kelautan Tropical. 2018;21(2) : 137-144.
38. Su. J, X. Zhang, Q. Kan, X. Chu. Antioxidant activity of *Acanthopanax senticosus* flavonoids in H2O2-induced colitis in mice. Molecules. 2022;27: 1-17
39. Syafitri, N.E., M. Bintang, S. Falah. Kandungan Fitokimia, Total Fenol, dan Total Flavonoid Ekstrak Buah Harendong (*Melastoma affine* D. *Don*). J.Current Biochemistry. 2014;1(3): 105-115.
40. Thangavel. M, J. Gopi, D. Balakrishnan. Protection of hydrogen peroxide and metal induced DNA damage by flvonoids. Internasional Journal of Experimental Research and Review. 2023;32: 408-421.
41. Truong, D. H., D. H. Nguyen, N. T. A. Ta, A. V. Bui, T. H. Do, H. C. Nguyen. Evaluation of the Use of Different Solvents for Phytochemical Constituen, Antioxidant,

and *In Vitro* Anti-Inflammatory Activities of *Severinia buxifolia*. Journal of Food Quality. 2019;vol. 2019, Artitcle ID 8178294, 9 page.
42. Yanuarti R, Nurjanah, A. Effionora, T. Hidayat. *Antioxidant activity of Turbinaria conoides and Eucheuma cottoni seaweed extracts*. Jurnal Pengolahan Hasil Perikanan Indonesia. 2017;20(2): 230-237.
43. Yoga W. K., Komalasari H. *Potential of Green Algae (Caulerpa racemosa) as a Source of Natural Antioxidants*. Jurnal Teknologi dan Mutu Pangan. 2022;1(1): 15-18.
44. Zhu, Yan Q, Huan Y, Chen Z.Y. Insteractions between flavonoids and α-tocopherol in human lowdensity lipoprotein. The Journal of Nutritional Biochemistry. 2000;11(1): 14-21.