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Antioxidant Activity Test of *Caulerpa racemosa* Extract from Takalar District Using the FRAP (Ferric Reducing Antioxidant Power) Method

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Abstract

Sea grapes or Caulerpa racemosa contain alkaloids, flavonoids, tannins, phenols and steroids. These compositions work as antioxidants. Antioxidants are compounds that can contribute one or more electrons to free radicals, so that the free radicals can be suppressed. The goal of this study was to determine the antioxidant activity of sea grape (Caulerpa racemosa) extract using the FRAP method. Extraction of sea grapes (Caulerpa racemosa) applied maceration and methanol methods. Absorbance of antioxidant activity was measured using a UV-VIS spectrophotometer at a wavelength of 700 nm. The results showed that sea grape extract (Caulerpa racemosa) had an antioxidant activity of 152 mg AAE/g extract. It means that in each gram of extract, it is equivalent to 152 mg of ascorbic acid.

Keywords: *Sea grapes (Caulerpa racemosa), antioxidants, FRAP (Ferric Reducing Antioxidant Power)*

Abstrak

Anggur laut (Caulerpa racemosa) memiliki kandungan alkaloid, flavonoid, tanin, fenol dan steroid yang berfungsi sebagai antioksidan. Antioksidan merupakan senyawa yang dapat menyumbangkan satu atau lebih elektron kepada radikal bebas, sehingga radikal bebas tersebut dapat diredam. Tujuan penelitian ini yaitu untuk mengetahui aktivitas antioksidan pada ekstrak anggur laut (Caulerpa racemosa) menggunakan metode FRAP. Ekstraksi anggur laut (Caulerpa racemosa) menggunakan metode maserasi dan metanol. Absorbansi aktivitas antioksidan diukur menggunakan spektrofotometer UV-VIS pada panjang gelombang 700 nm. Hasil penelitian menunjukkan bahwa ekstrak anggur laut (Caulerpa racemosa) memiliki aktivitas antioksidan sebesar 152 mg AAE/ g ekstrak artinya dalam setiap gram ekstrak setara dengan 152 mg asam askorbat.

Kata kunci*: Anggur laut (Caulerpa racemosa), Antioksidan, FRAP (Ferric Reducing Antioxidant Power*

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INTRODUCTION

Indonesia as an archipelagic country has a very high diversity of seaweed species. Moreover, the experts say that Indonesia is a seaweed barn. In the industrial sector, seaweed is starting to improve, but for the seaweed utilization as an ingredient in the food, cosmetic, pharmaceutical, medical and agricultural industries, Indonesia still necessary to learn from the countries that mastering the processing of seaweed. Therefore, the research on the utilization of seaweed really needs to be developed[1]. One type of algae that has antioxidant activity is a type from the Chlorophyceae group, with tropical climate habitats such as in Indonesia which having the ability to

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defend against ultraviolet (UV) radiation. Then, one of the genera of the Chlorophyceae class that is capable of producing antioxidants is the Caulerpa genus[2].

Caulerpa racemosa has the ability to defend against ultraviolet (UV) radiation which indicates that seaweed is capable of producing antioxidants as a defense system against UV rays and counteracting the free radicals. The capability of *Caulerpa racemosa* to counteract free radicals is due to the presence of folic acid, thiamine and ascorbic acid[1].

Generally, people do not know that sea grapes or *Caulerpa racemosa* have a fantastical economic value. Sea grapes are one of seaweed types that has a fairly high vitamin content, including vitamin A, vitamin C, iron, iodine and calcium[3].

Phytochemical screening was carried out that sea grape extract proved to have chemical compounds in the form of alkaloids, flavonoids, phenols and tannins[4]. Sea grapes (*Caulerpa racemosa*) contain phenolic compounds as non-nutritional components, the components are expected to have a function as antioxidants[3]. A research by Nurlina et al., (2018) stated that sea grapes (*Caulerpa racemosa*) using the DPPH method have antioxidant activity of 1.2466 mg/ml.

A research by Indayani et al., (2019) also claimed that sea grapes (*Caulerpa racemosa*) by using different drying methods would produced different antioxidant content. The antioxidant activity of C1 (sunlight) has a IC_{50} value of 6.882.43 ppm and C2 (temperature 40^0C) has a IC_{50} value of 652.41 ppm.

Based on the background, the researchers would like to propose a title regarding the antioxidant activity test of sea grape extract (*Caulerpa racemosa*) by using other antioxidant testing methods.

METHODOLOGY

The Production of Sea Grape Methanol Extract

The process of sea grape (*Caulerpa racemosa*) methanol extract was carried out by maceration method. Sea grape simplicia powder was weighed as much as 500 grams and then put into the maceration vessel. Add 4 liters of methanol until completely submerged (ratio 1:8) then, the vessel was closed and stored in a dark place protected from light. This process was conducted to avoid compound damage to the sample. Maceration was carried out for 3x24 hours and once a day stirring was carried out to balance the solvent and extractive materials. Remaceration was carried out by adding 4 liters of methanol for 3x24 hours. The filtrate

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was filtered using whatman paper. The results of the filtrate were evaporated by using a rotary evaporator with a temperature of 45 degree celcius.

Phytochemical Screening

a. Alkaloids 0.05 g of sea grape (*Caulerpa racemosa*) sample extract was put into a test tube and then added H_2SO_4 and shaken until mixed. Then, they were filtered and added Wagner reagent by looking at the brown precipitate and dragendorf reagent with orange red precipitate, if there was precipitate, the sample would be positive[5].

b. Flavonoids Sample extract of sea grapes (*Caulerpa racemosa*) as much as 0.05 grams was put into a test tube and added with 0.5 ml of concentrated HCl and 0.1 mg of magnesium, the presence of flavonoids was marked with red, orange or green. Then, the solution contained flavonoids[6]. 0.05 gram of sea grape (*Caulerpa racemosa*) sample extract was added to hot water which had been boiled for 3 minutes, the sample was filtered after that it was dripped with FeCl3 1%, positive test result if the solution was dark blue or blackish green[5].

c. Phenol Sample extract of sea grapes (*Caulerpa racemosa*) as much as 0.05 grams was added hot methanol. The solution was filtered to obtain a filtrate. The filtrate was dripped with 10% NaOH. The positive extract contained phenol if it produced a yellow to red color[6].

d. Saponin Sample extract of sea grapes (*Caulerpa racemosa*) as much as 0.05 gram sample was placed in a test tube. Then hot water was added, and the test tube was shaken. After the tube was shaken, it was left for 30 minutes and 1 drop of 2 N HCL was added. Positive results of the saponin test were indicated by the presence of stable foam[5].

e. Steroids Sample extract of sea grapes (*Caulerpa racemosa*) as much as 0.05 grams of sample was put in a test tube. Then it was added chloroform and then dripped with acetic anhydride about 5 drops. After that it was dripped with $H2SO4$ as much as 3 drops. The steroid test results were positive when the color of the solution turned to be blue[5].

f. Terpenoids Sample extract of sea grapes (*Caulerpa racemosa*) as much as 0.05 grams of sample was placed in a test tube. Then added chloroform and then dripped with acetic anhydride as much as 5 drops. After that it was dripped with $H2SO4$ as much as 3 drops. The terpenoid test results were positive when a brownish red color formed on the surface layer of the sample[5].

Antioxidant activity test using the FRAP (Ferric Reducing Antioxidant Power) method

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a. The Production of reagent solutions 0.2 M phosphate buffer solution pH 6.6. The solution was prepared by weighing 2 grams of NaOH and dissolved with $CO2$ free distilled water to exactly 250 ml in a measuring flask. Then as much as 6.8 grams of $KH2PO4$ dissolved with 250 ml $CO2$ free distilled water in a measuring flask. Then, pipetted 16.4 ml of NaOH into a measuring flask and mixed with 50 ml of $KH2PO4$, then measured to pH 6.6 and made up to 200 ml of free $CO2$ distilled water.

1% Oxalic Acid Solution A solution was prepared by dissolving 1 gram of oxalic acid in $CO2$ free water and diluting it in a 100 ml measuring flask.

Solution of Potassium Ferricyanide $K3Fe(CN)$ 6 1 %. The solution was prepared by dissolving 1 gram of potassium ferricyanide in distilled water and diluted in a 100 ml measuring flask.

0.1% $FeCl3$ solution. A solution was prepared by dissolving 0.1 gram of $FeCl3$ in distilled water and diluted in a 100 ml measuring flask.

10% trichloroacetic acid (TCA) solution. The solution was prepared by dissolving 10 grams of TCA in distilled water and diluting it in a 100 ml measuring flask. Preparation of ascorbic acid reference solution.

b. The production of ascorbic acid reference solution

The production of ascorbic acid main liquor concentration (1000 ppm). Main liquor production was carried out by weighing 25 mg of ascorbic acid and then dissolving it with 1% oxalic acid solution to the limit of a 25 ml volumetric flask.

The production of ascorbic acid standard series solutions. The process of the production of standard was conducted by using solution about 1000 ppm main liquor. They were pipetted as much as 1.5 ml, 1.75 ml, 2 ml, 2.25 ml and 2.5 ml, each put in a 25 ml volumetric flask and filled with solution 1% oxalic acid to the limit mark, in order to obtain solutions with concentrations of 60 ppm, 70 ppm, 80 ppm, 90 ppm and 100 ppm.

The production of blank solution. The blank solution was pipetted 1 ml of 1% oxalic acid solution into a blank solution, then mixed it with 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. After incubation, 1 ml of 10% TCA was added and then centrifuged at 3000 rpm for 10 minutes. After centrifuging, the solution was pipetted about 1 ml into a test tube and added 1 ml of distilled water and 0.5 ml of 0.1% $FeCl3$. The solution was allowed to stand for 10-15 minutes and the absorbance was measured at a

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wavelength in the range of 650-730 nm by using a UV-Vis spectrophotometer.

Determination of maximum absorption wavelength. Determination of maximum wavelength was obtained by measuring the absorbance of a standard solution of ascorbic acid at a concentration of 80 ppm. 1 ml of the solution was taken and then mixed with 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide, the mixture was incubated at 50ºC for 20 minutes. After incubation, 1 ml of 10% TCA was added and then centrifuged at 3000 rpm for 10 minutes. After centrifuging, it was pipetted about 1 ml into a test tube and added 1 ml of distilled water and 0.5 ml of 0.1% FeCl3. The solution was allowed to stand for 10-15 minutes and the absorbance was measured at a wavelength in the range of 650-730 nm using a UV-Vis spectrophotometer.

The production of extract solution. The methanol extract was weighed with 3 replications, each of which was 5 mg. Each extract was dissolved with 5 ml of methanol then homogenized.

Antioxidant activity of the FRAP (Ferric Reducing Antioxidant Power)

Ascorbic acid standard antioxidant activity test. Ascorbic acid standard solutions from each concentration of 60, 70, 80, 90 and 100 ppm were pipetted 1 ml each, added 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of $K3Fe(CN)$ 6 1%. After that, incubated for 20 minutes at 50°C. After incubation, 1 ml of 10% TCA was added and then centrifuged at 3000 rpm for 10 minutes. After centrifuging, the solution was pipetted as much as 1 ml into a test tube and added 1 ml of distilled water and 0.5 ml of 0.1% FeCl3. The solution was allowed to stand for 10-15 minutes and the absorbance was measured at 700 nm.

Antioxidant activity test of sea grape (*Caulerpa racemosa*) extract using the FRAP (Ferric Reducing Antioxidant Power) method. 5 mg of the extract was dissolved in 5 ml of methanol, then pipetted 1 ml, added 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of $K3Fe(CN)$ 6 1%. After that, incubated for 20 minutes at 50°C . After incubation, 1 ml of 10% TCA was added and then centrifuged at 3000 rpm for 10 minutes. After centrifuging, it was pipetted 1 ml into a test tube, and added 1 ml of distilled water and 0.5 ml of 0.1% FeCl3. The solution was allowed to stand for 10-15 minutes and the absorbance was measured at 700 nm. FRAP values were expressed in mg equivalent of ascorbic acid/g extract.

Data Analysis

Data analysis was conducted to specify the antioxidant activity of sea grape extract (*Caulerpa racemosa*), carried out at a wavelength of 650-730

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nm on a UV-Vi spectrophotometer. So that, the absorbance value was obtained, after the absorbance value was obtained, the antioxidant activity of the sample was calculated by entering it into the ascorbic acid standard curve regression equation with a linear equation y = a + bx. Where in every gram of sample was equivalent to every mg of ascorbic acid (ascorbic acid equivalent)[7]. Thereafter, the value of antioxidant activity was calculated using the following formula:

$$\text{antioxidant activity} = \frac{\text{Sample concentration X sample volume (L)}}{\text{extract weight (g)}} X\ fp$$

Information:
y = uptake (A)
a = intercept
b = tilt slope
x = concentration
fp = dilution factor

RESULT AND DISCUSSION

The result can see at the table below.

Table 1. Weight of *Caulerpa racemosa* extract obtained by extraction

Powder *Caulerpa racemosa*	*Caulerpa racemosa* extract	%
500 gram	22.27 gram	4.45%

Table 2. Result phytochemical screening *Caulerpa racemosa* estract

Compound	Reagent	Information	Result
Alkaloids	H_2SO_4 + reagent dragendorf	A brownish red precipitate formed	+
	H_2SO_4 + reagent Wagner	Brown precipitate	+
Flavonoids	HCl + compound magnesium	orange	+
Saponin	Metanol + NaOH 10%	yellow	+
Kloroform	Chloroform + acetic anhydride + H_2SO_4	Blue green	+
Terpenoids	Chloroform + acetic anhydride + H_2SO_4	Blue green	-
Phenol	Methanol + NaOH 10%	yellow	+

Table 3. Maximum wavelenght measurement result

No.	Absorbance	Concentration
1	650	0.321
2	655	0.330

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3	660	0.337
4	665	0.345
5	670	0.352
6	675	0.358
7	680	0.364
8	685	0.366
9	690	0.369
10	695	0.369
11	700	0.372
12	705	0.366
13	710	0.367
14	715	0.363
15	720	0.357
16	725	0.356
17	730	0.352

Note : The maximum wavelenght result obtained the high absorbance value of 0.372 at of 700 nm

Tabel 4. Result of absorbance measurements of ascorbic acid as a comparison solution

No.	Absorbance	Concentration
1	60 ppm	0.162
2	70 ppm	0.189
3	80 ppm	0.218
4	90 ppm	0.260
5	100 ppm	0.281

Tabel 5. Absorbance measurement result and antioxidant activity values of *Caulerpa racemosa* extract

Caulerpa racemosa extract	Absorbance	Antioxidant activity (mgAAE / mg extract)	Antioxidant activity average (mgAAE / mg extract)
Replication 1	0.387	132.8	152
Replication 2	0.456	155.2	
Replication 3	0.496	168	

The researcher used sea grapes (*Caulerpa racemosa*) as plant sample in this study. The obtain sample was cleaned by using running water. After

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that, the sample was dried by airing during 3 days[4]. When the sample already dry, then it was powdered by using a blender. The aim of this process was to increase the surface area of the sample, so that, the distribution of compounds in the solvent during maceration runned optimally. After that, the extraction process was carried out. The solvent that be used in this study was methanol. The refined samples were then extracted by maceration method for 3 × 24 hours using 4 liters of methanol solvent while stirring occasionally[8]. Samples were generally soaked for 3-5 days while stirring occasionally to speed up the process of dissolving the analyte.

The process of extracting sea grapes (*Caulerpa racemosa*) used the maceration method with a remaceration system or repeated maceration by applying the same volume of solvent 2 times[9]. The extraction process lasted for 3 days and occasional stirring was carried out. After 3 days of filtering, then re-maceration was carried out again for 3 days. Furthermore, the sample was then concentrated using a rotary evaporator to obtain a thick extract.

The results of the phytochemical screening indicated that sea grape extract (*Caulerpa racemosa*) showed positive results for secondary metabolites of alkaloids, flavonoids, phenols, tannins and steroids. While the saponins and terpenoids were negative. After adding sulfuric acid, the alkaloid compounds in the extract of sea grapes (*Caulerpa racemosa*) were added to the dragendorf reagent and 3 drops of wagner reagent each other, so that the dragendorf reagent formed a reddish-brown precipitate and the wagner reagent formed a brown precipitate[5].

Flavonoid compounds after adding 0.5 ml of concentrated HCL and magnesium powder[6]. The results obtained from the flavonoid test were that there was a change in the color of the filtrate from orange to red and a little foam appeared. The presence of phenol groups was shown in green-black or dark blue after adding FeCl3, FeCl3 showed positive results. It was possible that the sample contained phenol compounds and it was possible that one of them was tannins because tannins were polyphenolic compounds[10]. Phenol compounds after the addition of methanol and NaOH and a yellow color was formed[5]. Steroid compounds after the addition of chloroform, acetic acid anhydride and $H2SO4$ and a green-blue color was formed[6].

The analysis in this research was conducted by using a single beam type UV-VIS spectrophotometry which measured at a wavelength of 650-730 nm. Then, the maximum wavelength of 700 nm was obtained. From each

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standard solution concentration of 60, 70, 80, 90 and 100 ppm, the absorbance was measured at a wavelength of 700 nm, then from the absorbance data obtained a linear line equation was created. During the work process, TCA was added to precipitate the potassium ferricyanide complex.

The regression results from the concentration (x) with the absorbance value (y) of standard ascorbic acid obtained an equation that was y = 0.0031x - 0.0252 with a value of *R* 2 = 0.9915 and the absorbance was included into the equation. Based on the results of the absorbance value of the samples on sea grape extract (*Caulerpa racemosa*), in replication 1 was 0.387, in replication 2 was 0.456 and in replication 3 was 0.496. Whereas for ascorbic acid or vitamin C as a comparison with different concentrations of ppm, the absorbance value was obtained at a concentration of 60 ppm which was 0.162, at a concentration of 70 ppm which was 0.189, at a concentration of 80 ppm which was 0.218, at a concentration of 90 ppm which was 0.260, at a concentration of 100 ppm that was equal to 0.281. Based on the results of the antioxidant activity value of the sea grape (*Caulerpa racemosa*) extract sample, the antioxidant activity value was obtained in replication 1, which was 132.8 mg AAE/g extract, in replication 2, it was 155.2 mg AAE/g extract, in replication 3, it was of 168 mg AAE/g extract. With the result that from the three replications of sea grape (*Caulerpa racemosa*) samples, the average value of sea grape (*Caulerpa racemosa*) extract samples was 152 mgAAE/g extract, so that, every gram of extract was equivalent to 152 mg of ascorbic acid.

CONCLUSION

Based on the result of this study, it can be concluded that there is antioxidant activity in sea grape extract (*Caulerpa racemosa*) and the average value of the methanol extract sample of sea grape (*Caulerpa racemosa*) is 152 mgAAE/g extract. It means that in every gram of extract it is equivalent to 152 mg of ascorbic acid.

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