Total Content of Phenol and Antioxidant Activity from crude extract Methanol of brown algae (Padina sp) collected from Kayoa Island, North Maluku

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Abstract. Determination of total phenol content and antioxidant activity of brown algae (*Padina sp*) from Kayoa Island and determining the relationship between total phenol content and its antioxidant activity have been carried out. Crude extract of methanol was obtained by maceration of dried algae *Padina sp*. Determination of the extract secondary metabolites was carried out by phytochemical screening which showed the presence of alkaloid, phenolic, steroid, and flavonoid content. The total phenol extract was determined by the Folin-Ciocalteu method and the total phenol content was 4,43 mgGAE/g. Antioxidant activity was analyzed using the DPPH method and the IC50 value was 564,99 µg/mL. The total phenol content of the extract and its IC50 value shows a linear relationship y = 1218x - 4830.7 with a determination value R^2 = 0.9981

1. Introduction

The territorial waters of eastern Indonesia, especially on Kayoa Island, North Maluku, are one of the places that allow for good growth of marine biota. In general, the condition of the aquatic environment in the area is in a fairly good range for marine cultivation activities. The waters around the island show a high level of brightness, this is very supportive for the growth of marine life such as algae, fish and shellfish, but information on other types of algae that live in these waters has not been widely published.

One type of marine biota, namely macroalgae is easily found in coastal areas, generally, macroalgae that live on a mixed substrate of sand and coral fragments have macroalgae that are more diverse than those found on sand substrates [1].

Algae are recognized a b[19]source of polyphenols, enzymes, pigments, polysaccharides and carotenoids and are important source of several vitamins namely A, B1, B12, C, D and E [2]. Macroalgae have been produced to active metabolites including lipids, polysaccharide, polyketides, quinines, cyclic peptide, sterols, glycerol, alkaloids, phlorotannin and diterpenoids that have wide range of biological activities [3]. Algae are used to medical potential due to the bioactive compounds which they have involved to the notice of pharmaceutical industries [4] [5]. They are usually produce biologically active compound and its will grow in the all seas except polar region. Plenty of researchers have been discovered the antibacterial, antiviral, antioxidant, cytotoxic, anti-inflammatory, antidiabetic and antifungal potentials of algae [6] [7].

The macroalgae are present in different types of biological active compound. It will be used to the extract on different solvents with different polarity. The bioactive compound, which is recorded to biological activity were fatty acids, phenolic compounds, ketones, alkanes, acrylic acid, terpenoids, and phlorotannin [8]. there are biological activities and secondary metabolites [9]. They are usually enclosing terpenoids that exhibit biological activities such as vasodilatory effects, larval settlement of hydrozoans, cell toxicity and antioxidant activity [10] [11].

The human body has a limited amount of antioxidants, so if there are many radicals in the body, the body needs antioxidants from outside [12]. Sources of antioxidants can be in the form of natural antioxidants or artificial antioxidants, but currently the use of artificial antioxidants is starting to be limited because it is based on research results that artificial (synthetic) antioxidants such as BHT (Butylated Hydroxy Toluene) can be toxic to experimental animals and are carcinogenic.

One of the natural sources of antioxidants is seaweed or algae. Algae has polyphenol compounds found in some families *alariceae, fucaceae, and sargassaceae* [13]. According to Rice-Evans et al. [14], polyphenols can be antioxidants due to reducing properties, i.e. donor agents or hydrogen contributors. Padina sp. is one of the one reported species of brown seaweed has antioxidant activity [15] – [20] . Chew et al. [21] reported that Padina antillarum has the most antioxidant activity compared *to Caulerpa racemosa* and *Kappaphycus alvarezzi.*

So far, known sources of antioxidants are those from land plants and it is still rare to know the potential of algae as antioxidants. Considering that algae has great potential in Indonesia and has been used as a producer of agar, carrageenan and alginic acid which are used in the fields of food, microbiology, biotechnology, textiles and pharmaceuticals [22]. So it is necessary to have information about the functional potential of algae in the health sector.

In this research, an investigation will be carried out on the total phenol content and antioxidant activity of brown algae (*Phaeopeceae*) macroalgae which are often found on the coast of Kayoa Island, namely the species *Padina sp*. As well as the correlation between total phenol and its antioxidant activity.

2. Material Method

2.1 Tools and Materials

The tools used in this research include a digital balance, a rotary evaporator, a set of glassware, and a UV-VIS spectrophotometer for the Shimadzu UV-1800 brand. The materials used in this research include *Padina sp* seaweed, methanol pa solution, DPPH, Aquades, cotton, aluminum foil, LB reagent, Meyer reagent, Dragendorf reagent, Folin-Ciocalteu solution, gallic acid, Na_2CO_3, Magnesium band and Aquades.

2.2 Sample Preparation

2.2.1 Sample Collection

The algae were collected from Kayoa Island, North Maluku, Indonesia. After the collection, sample was washed with seawater to remove all the extraneous sand particles, shells and impurities and transported to the laboratory in plastic bags aseptically in the ice box (4°C). Then the samples are washed with tap water followed by distilled water. The samples were identified by their morphological key characters with genus and species level in Productivity and Quality Laboratory Department of Fisheries, Faculty Of Marine And Fisheries Sciences, Hasanuddin University. The Algae samples were blotted in blotting paper then shadow dried in the shade for four days.After that samples grounded into a fine powder.

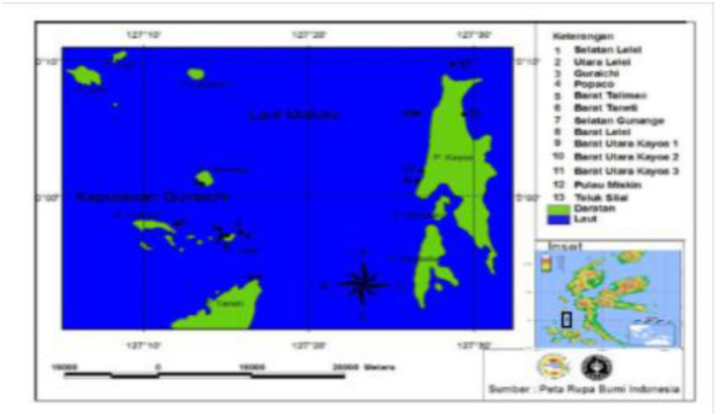

Figure 1. The Map of Kayoa Island, North Maluku

2.2.2 Preparation of algae extraction

The extraction of metabolites was carried out by soaking the dried algae (*Padina sp*) in Methanol (Merck) in an erlenmeyer. Then the sample was kept on Erlenmeyer for three days at room temperature (28°C), and replace the solution every 24 hour. The mixture was filtered through the Whatman No.1 filter paper and the the samples were taken to dryness under reduced pressure at 50°C. Then the crude extract was stored for further studies.

2.2.3 Qualitative phytochemical screening

The phytochemicals such as tannins ($FeCl_3$test), alkaloids, saponins (Frothingtest), steroids (*Lieberman-Buchard*), terpenoids, phenolic content of the brown algae (*Padina sp*) methanol extract was estimated qualitatively by followed the standard protocol [23] [24] [25].

2.2.4 Total Phenolic Determination Analysis

2.2.4.1 Determination of the maximum wavelength of gallic acid

The main solution of 100 ppm gallic acid was prepared by dissolving 0.01 grams of gallic acid in a100 ml volumetric flask, adding 1 ml of methanol then adding distilled water to the limit mark. The 100 ppm main solution was then taken 1 ml and put into a 10 ml measuring flask, added 1 ml of Folin-Ciocalteu reagent, then shaken until homogeneous. Let stand for a few minutes then add 4 ml of 10% Na2CO3, let stand for 15 minutes at room temperature. Furthermore, measurements were made with a visible light spectrophotometer at a wave of 700 to 800 nm to determine the maximum wavelength.

2.2.4.2 Preparation of Gallic Acid Calibration Curves with Folin-Ciocalteu Reagent.

100 ppm gallic acid main solution was taken 1 ml each; 3 ml; 5 ml; 7 ml. Then diluted with distilled water to a final volume of 10 ml in order to obtain a solution with a concentration of 10 ppm; 30 ppm; 50 ppm; 70 ppm. From each concentration pipette 0.2 ml and then put into a 10 ml volumetric flask, added 1 ml of Folin-Ciocalteu reagent and shaken until homogeneous, let stand for 8 minutes. Added 3 ml of 10% Na_2CO_3 then shaken homogeneously, and then let stand for 60 minutes at room temperature. Measure the maximum absorption wavelength nm, then create a calibration curve with the regression equation y = bx + a.

2.2.4.2 Determination of Total Phenol Content by the Folin-Ciocalteu Method

Pipette 0.2 ml of extract, added 15.8 ml of distilled water and 1 ml of Folin-Ciocalteu reagent and then shaken. Let stand for 8 minutes then add 3 ml of 10% Na_2CO_3 to the mixture. Let the solution sit for 1 hour at room temperature. The absorption was measured using a UV-Vis spectrophotometer at

the maximum wavelength. Three repetitions were performed so that the phenol content obtained was obtained as mg GAE/g of fresh sample.

2.2.5 Determination of Antioxidant Activity

Antioxidant activity was analyzed first by making a DPPH (1,1-diphenyl-2-phikril-hidrazil) solution by dissolving DPPH crystals into methanol at a concentration of 0.01 M and adding mmethanol to a volume of 5 ml, then measuring the absorbance at a wavelength of 517 nm. as the absorbance of the control. The next process is measuring the absorbance of the sample from extraction with n-hexane solvent. The step of measuring the absorbance of the sample by taking 200 mg of the sample and dissolving it in 5 ml of mmethanol while vortexing it for 1 hour. Then, take 1 ml the mixture and add 1 ml of DPPH 0.01 M and methanol until the volume is 5 ml. Then the absorbance of the sample was measured at a wavelength of 517 nm. Repeating the absorbance analysis of the samples extracted with chloroform and ethyl acetate solvents. The absorbance data of the samples obtained were used to determine Inhibition Concentration (IC) (%). Inhibition Concentration (IC) (%) is the concentration of an antioxidant substance that causes 50% DPPH to lose its radical character. Inhibition Concentration (IC50) (%) is calculated by the equation:

$$\% \, Inhibition = \frac{(A_{k\,ontr\,ol} - A_{sampel\,control}}{A} \, x \, 100$$

Explain : $A_{kontrol}$ = Absorbance does not contain samples
A_{sampel} = Absorbance of sample

3. Results and Discussion

3.1 Phytochemical Analysis

The results of the phytochemical test are presented in Table 1.

Table 1. Phytochemical test of crude extrack from brown algae (*Padina sp*)

No.	Phytochemical test	Result test	Difference
1	Alkaloid		
	a. Dragendorff	-	No difference
	b. Mayer c.	+	White sediment
	Wagner	+++	Violet ring
2	Flavonoid		
	a. Timbal asetat	++	White sediment
	b. Mg	-	
3	Steroid	++	Dark Blue
4	Fenolik	+	Dark Green
5	Saponin	-	No Foam

Note: (-): negative; (+): positive but weak; (++): Strong positive; (+++): Positive and very strong

The crude methanol extract of *Padina sp*. Was analyzed for its chemical compounds by color test using several reagents. This test is conducted to determine the presence of flavonoids, phenolics, alkaloids, steroids / terpenoids, and saponins. These compounds determine the characteristics of the active compounds that cause toxic effects or beneficial effects, which is shown by crude plant extracts tested with a biological system [25].

3.1.1 Alkaloid Test

Analysis of the sample of *Padina sp* extracted with methanol solvent showed that the extract identified alkaloids by testing using Meyer and Wagner reagents. The principle of this analytical method is the precipitation reaction and color changes that occur due to ligand replacement. The

nitrogen atom which has a lone pair in the alkaloid compound can replace the Bi ion in the Mayer reagent. Nitrogen as part of its cyclic system in alkaloid compounds has various substituents such as amine, amide, phenol and methoxy groups so that it is semipolar [24]. Meanwhile, Dragendorf reagent did not show any changes. In the alkaloid test with Wagner's reagent there will be a reaction between nitrogen and potassium ions (K +) to form a precipitated alkaloid potassium complex [26]. Alkaloid compounds have the effect of triggering the nervous system, raising blood pressure, reducing pain, and antimicrobials.

3.1.2. Flavonoid Test

Analysis of the *Padina sp* sample extracted with methanol solvent showed that the tested extract identified flavonoids. Flavonoid compounds are polar compounds because they have an unsubstituted hydroxyl (-OH) group so that hydrogen bonds can be formed. The presence of flavonoids in the results of this test is because there are several terpenoid compounds that have a cyclic structure in the form of alcohol which causes these compounds to tend to be semipolar so that their bonds with methanol solvent which are polar are very weak [24]. This is indicated by two different tests, only one identified to contain flavonoids is shown in Table

3.1.3 Steroid Test

Analysis of the *Padina sp* sample extracted with methanol solvent showed that the extract tested showed the presence of steroids. This analysis is based on the ability of terpenoid and steroid compounds to form color by concentrated H2SO4 in hydrochloric acid solvent. Positive results are given to samples forming red orange for triterpenoid analysis and blue for steroid analysis [27]. The analysis results showed the formation of a blue color indicating the presence of a steroid compound in the extract.

3.1.4 Phenolic Test

Analysis of the *Padina sp* sample extracted with methanol solvent showed that the extract tested was identified to have a phenolic compound in it, this is an indication to further explore how much total phenolic content is possessed by this *Padina sp* extract. Phenolics have aromatic rings with one or more hydroxy (-OH) groups and other accompanying groups. The largest group of phenolic compounds are flavonoids, this was confirmed in the phytochemical test of the flavanoid compound in the extract. Phenolic compounds that have the possibility of phenolic compounds such as monocyclic phenols, phenyl propanoids, polyphenols (lignins, melanins, tannins), and phenolic quinones [28].

3.1.5 Saponin Test

Analysis of the *Padina sp* sample extracted with methanol solvent showed that the samples tested did not form foam.

4. Determination of Total Phenol Levels

4.1 Results of Determination of Total Phenol Levels

Table 2. Data on the measurement results of standard absorbance of gallic acid

[Gallic Acid] (mg/mL)	Absorbance
0,001	0,106
0,002	0,223
0,004	0,436
0,008	0,825
0,016	1,667

The following is the correlation between concentration of gallic acid and the absorbance to determine the linear regression:

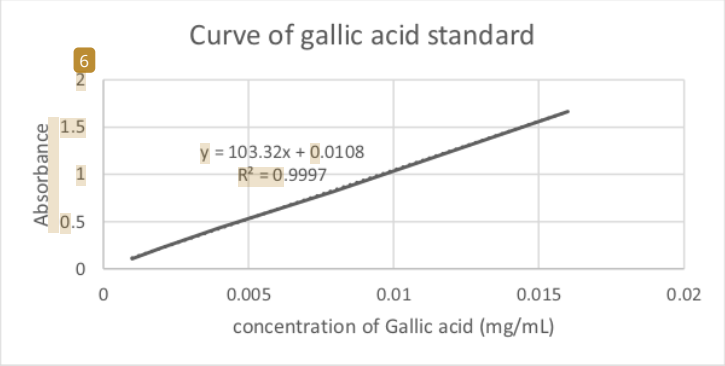

Figure 2. Correlation between Gallic acid standard and Absorbance

The determination of the total phenol content compound in the crude extract of Methanol of *Padina sp* from Kayoa Island was conducted by Folin-Ciocalteu method. The principle of the Folin-Ciocalteu method is oxidation of hydroxyl phenolic groups. This react oxidizes phenolate

(alkaline salts), reducing heteropoli acid into a molybdenum-tungsten complex (Mo-W). Fenolate is only found in alkaline solutions, but folin-ciocalteu and its products are unstable in alkaline conditions. During a live reaction, the phenolic-hydroxyl group reacts with folin-ciocalteu reaction, forming a blue phosphotungstat-phospfomodat complex with an unknown structure and can be detected with a spectrophotometer. The blue color that is formed will be more concentrated equivalent to the concentration of phenolate ions formed, this means that the greater the concentration of phenolic compounds, the more phenolate ions will reduce heteropoly acid so that the resulting blue color gets thicker[29] .

Based on the linear equation obtained i.e. y = 103.32x + 0.0108, with R2= 0.9997 approaching linearity where R2= 1, can be calculated the total phenol owned by the *crude* extract *Padina sp* with the resulting sample absorbance value of 0.244. This indicates that the crude extract of methanol from *Padina sp* has a total phenol content is 4.43 mg GAE/g of powder.

5. Antioxidant Activity Test

Table 3. Percentage of DPPH on Crude extract of *Padina sp*

No	Konsentrasi (µg/mL)	Aktivitas Antioksidan (%)	Nilai IC-50 (µg/mL)
1	10	2,99	
2	20	4,05	
3	40	6,29	564,996*
4	80	9,47	
5	160	15,76	

*three repetitions are performed

Tests of antioxidant activity on table 3 showed that the *crude* extract of Methanol *Padina sp* showed antioxidant activity. *Padina sp* extract has an IC_{50} value of 564.99 µg/mL higher than reported in Kepulauan Seribu, Jakarta was 267.1 ppm, and Husni et al on Drini Beach, Gunung Kidul where the reported of IC_{50} is 37.68 µg/mL[30].

Where the lower the IC_{50} value of a sample shows the ability of its antioxidant activity to be high. This is also reinforced by the determination of the total phenol content owned by the extract.

6. Correlation of Total Phenol Content and Antioxidant Activity

Antioxidant activity is closely related to the content of secondary metabolites that serve as antioxidants such as phenol compounds [17]. The correlation curve of the total levels of phenol compounds and antioxidant IC_{50} results of this study can be seen in Figure 3.

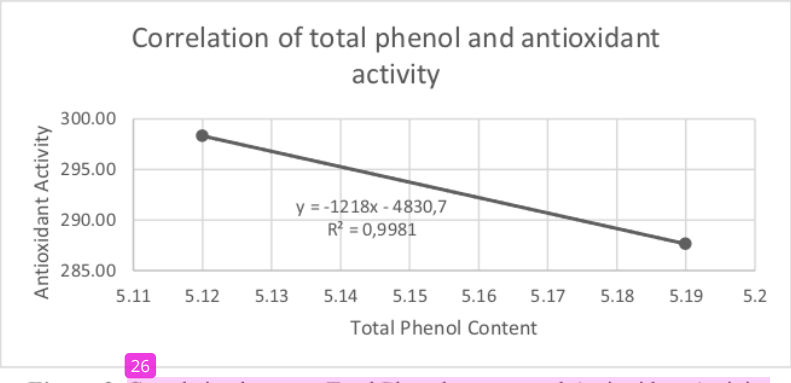

Figure 3. Correlation between Total Phenol content and Antioxidant Activity

Based on Figure 3 it can be seen that the total levels of phenol content are negatively correlated with the antioxidant IC_{50}. Based on the determination coefficient can be interpreted that 99% IC50 antioxidant *Padina sp.* is affected by the total level of phenol content. The lower value of antioxidant IC50 indicates higher antioxidant activity. Based on Figure 3 it can be stated that the antioxidant activity of *Padina sp*. increase as the total level of phenol compounds increases.

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