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The Content of Total Phenols and Antioxidant Activity Three Types Sea Algae Taken at the North Sulawesi Waters

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Abstract
This research aims to study the total content of phenolic and antioxidant activity in fractions of hexane, ethyl acetate and water at sea Caulerpa sertularoides sea algae, Laurencia tronoi and Padina australis. The content of total phenols were measured using the method Fholin-Chiocalteau, while the measurement of antioxidant activity using free radical scavengers 1,1-diphenl-2-picryhidrazyl (DPPH), reducing power, and ferrous ion chelating (FIC). Mass fraction of water is highest on Caulerpa sertularoides fraction (12.3 g), and the lowest Padina australis hexane fraction (0.44 g). Caulerpa sertularoides ethyl acetate fraction was found have the highest content of total phenols 123.87 ± 2.67 mg GAE / g extract and reducing the highest power is 36.45 ± 0.27 mg GAE / g, whereas the water fraction of Laurencia tronoi have the lowest total phenol content that is 5,92 mg GAE / g extract and lowest FRAP is 1.33 mg GAE / g extract. Highest activity of free radicals found in the hexane fraction tronoi Laurencia is 82.62 ± 1.54%, the highest FIC at a concentration of 1000 ppm, 1500 ppm and 2000 ppm was found in the water fraction Caulerpa sertularoides respectively 93.76 ± 0.4% ; 94.65 ± 0.74% and 96.50 ± 0.26%. FIC value of three factions in the three types of marine algae increased with increasing concentration of the sample.
Keywords: marine algae; total phenol; antioxidant activity

1. Preliminary

Consuming foods that contain of natural antioxidants increasingly demand a by lot of the consumers because it can inhibit the increase of free radicals in the body which is the source of various diseases. Several health research for the role of antioxidants as inhibitors of the oxidation of Low Density Lipoprotein (LDL) associated with arteroklerosis (Septiana and Rungkat, 2001; Septiana, et al, 2002), as an anti-tumor (Hong et al, 2008 and Mamahit, 2008); as anticancer (Kondoy, Kermer 2008 and 2008), as an inhibitor of hemolysis of red bloodcells (Cheungetal.2002;Dedin,2009).

One food source of antioxidants is that a marine algae, this was confirmed by research Santoso et al (2004), Suryaningrum (2006), Yuan and Walsh (2006), Chew et al (2008), Khumar et al (2008) Tao Wang et al (2009), Santoso et al (2010) and Zakaria et al (2011) who reported that the extracts of marine algae in organic solvents such as methanol, ethanol, butanol, ethyl acetate, hexane, klroform, water and others have antioxidant activity with various test methods.

Method of testing antioxidant activity has been carried out,among others the ability to avoid free radicals 1,1-diphenyl-2-pikrihidrazyl / DPPH (Matsukawa et al, 1997; Ismail and Hong, 2002; Hwang et al, 2010; Banerjee et al, 2012; Sumathi and Krishnaveni, 2012), reducing power (Khumar et al, 2008)

North Sulawesi Sea in addition to having the potential field of fisheries, as well as potential fields of marine algae. In Manado bay there are 4 families, 6 genera and 31 species of green algae; 4 families. 6 genera and 10 families of brown algae, and 8 families, 2 genera and 28 species of red algae (Gerung, 2006), types of marine algae such as Caulerpa sertularoides, tronoi Laurencia and Padina australis.

Marine algae that grows in North Sulawesi is buffeted by tropical strong ultraviolet radiation, which causes increased reactive radical species, to protect marine algae to change its metabolism and stimulate the formation of the active compound (Santoso et al, 2010), so that marine algae that grow in the region North Sulawesi is thought to have antioxidant active compounds in large quantities.

Research on marine algae growing in the waters of North Sulawesi until now many intended as a source of carrageenan and in particular on the type of fiber cotonii Eucheuma spinosum and Eucheuma (Mongi, 2007), while research on marine algae as a source of antioxidants especially on the type of Caukerpa sertularoides, Laurencia tronoi and Padina australis is still lacking done.

Proper choice of solvent is critical amount of antioxidant chemical compounds that can be extracted is also to determine the high antioxidant activity of the extracts of marine algae. Harborne (2006) stated that in order to obtain the organic matter content of dry plant tissue can be performed by continuous extraction of the powder material using series of solvent by turns alternated from ether (non-polar), then use the more polar ethyl acetate.

The results showed that the antioxidant activity of the extracts of marine algae vary when extracted with different solvent polarity properties of the petroleum ether (PE). Ethyl acetate (EA), diclorometana (DCM) butanol (BuOH) and water (Ganesan et al, 2008; Khumar et al, 2009; Zakaria et al, 2011).

Based on the things that have talked above it is important to conduct research on the antioxidant activity of marine algae Caulerpa sertularoides, tronoi Laurencia and Padina australis in different solvent fraction is the fraction of the level of polarity hexane (non-polar), ethyl acetate (semi-polar) and water (polar).

2. Materials and Methodology

2.1 Sample

Marine algae Sertularoides Caulerpa, Laurencia and Padina australis tronoi taken from marine waters of North Sulawesi Nain Island October-November 2012 period. Marine algae that have been washed clean each weighed 1 kg, put into plastic bags. For long trips (no more than 24 hours) put in a cool box contains crushed ice with a ratio of 1: 3. Marine algae that are not immediately used is stored in a freezer at -20 ° C, when it will be used at room temperature for 24 hours.

Sample before the first macerated dried indoors for 3 days, followed by drying in an oven temperature of 40O C for 4-6 hours until the weight is reduced 10 times, and blend smooth.

2.2 Chemical compounds and reagents

Solvents used are methanol, ethyl acetate, hexane, sodium phosphate, disodium phosphate, potassium ferricianat, trikoloroasetat acid (TCA), FeCl3 obtained from Brand; Folin-Ciocalteau. 1.1-dipenyl-2-pikkrihidrazyl (DPPH), gallic acid Ferrozin and obtained from Sigma.

2.3 Preparation of sample extracts

200 grams of dried powder samples macerated with 2 liters of methanol 50% (v / v) for 48 hours, filtered, maserat separated from the pulp using whatman no 1 filter paper, pulp and then macerated again in the same way as above 2 times, all maserat collected and evaporated with a rotary vacuum evaporator temperature of 40 ° C so that the resulting semi-solid extract (methanol crude extract). Methanol crude extract was further partitioned with each 200 ml of a mixture of n-hexane-methanol (1:1), n-hexane section separated from the water and placed in the evaporation flask, it is repeated until the part n-hexane colorless, subsequent sections water partitioned with 200 ml of ethyl acetate according to the procedure in n-hexane to obtain ethyl acetate part, the remaining part at the end of the partitioning process is part of the water, then the third part is evaporated by vacuum rotary evaporator at 40 ° C. and the fraction n-hexane, ASTAT fractions and fractions ethyl water. The third faction is packaged in dark glass containers and stored at -20 ° C until used for analysis.

2.4 The content of total phenols

The content of total phenols were measured using the Folin-Ciocalteau reagent following the method of Devi et al, (2000) and Ganesan et al (2008) modified. Extract as much as 0.1 gram dissolved in 10 ml of methanol in a test tube, taken from the extract solution was added 0.1 ml 1 ml Folin-Ciocalteau 1:2 in distilled water and allowed to stand 5 minutes. Then added 1 ml of 7% sodium carbonate, homogenized and incubated at room temperature for 30 minutes in the absence of light (dark). Absorbance of the mixture was measured at 750 nm. The content of total phenols interpreted as mg gallic acid equivalents (GAE) / g extract. Galad acid calibration curve was y = 0.002 x - 0.032 (R2 = 0.9936).

2.5 The antioxidant activity

2.5.1 The antidote radical 1,1-diphenyl-2-picrylhydrazyl (DPPH)

DPPH radical scavengers were measured using the method Khumar et al (2008) modified. 2 ml of DPPH 93 lm added to 0.5 ml of extract (2000 ppm in methanol). Mixture shaken and incubated at 37 ° C for 30 min, absorbance was measured at a wavelength of 517 nm. (Yuan and Wals, 2006; Devi et al, 2008): states that the activity of DPPH radical scavengers determined as percent inhibition was calculated by the equation:

% inhibitory = {(Absorbance of control-Absorbance of the sample)/(Absorbance of control)} x 100

2.5.2 Reducing power

Measurements performed using the method of reducing power Khumar et al (2007), Chew et al (2008), Andarwulan et al (2010), modified as follows: extract with a concentration of 2000 ppm in methanol, taken 1 ml was mixed with 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of potassium ferricianida [K2Fe (CN) 6] 1%. Mixture was homogenized and incubated at 50 ° C for 30 minutes (mixture A). 1 ml of trichloroacetic acid (10%) was added to a mixture (mixture B), a mixture of B and centrifuged (10 min, 3000 rpm). Subsequently 1 ml was taken on the top layer of mixture B was added to 1 ml of water and 5 ml of distillate FeCl3 0.1% and the mixture homogenized, mixed absorbance was measured at 700 nm. Reducing power values are interpreted as mg gallic acid equivalent / g extract. Gallic acid calibration curve was y = 0.198 x + 0.024 (R2 = 0.991).

2.5.3. Ferrous Ion Chelating (FIC)

Ferrous ion chelating (FIC) is based on the method of Chew et al (2008) were modified as follows: 0.5 ml of the extract was taken was added 1.8 ml of distilled water and 0.5 ml of 0.1 mM Fe2SO4 7H2O, homogenized mixture and added 0, ferrozin 5 ml (0.25 mM). The final mixture was homogenized and incubated at room temperature for 20 minutes, the absorbance read at 562 nm. Controls were made following the procedure above is only a sample was replaced with distilled water. FIC value is determined by the formula:

FIC (%) ={(Absorbance of control-Absorbance of the sample)/ (Absorbance of control)} x 100

2.6. Statistical analysis

Analysis using descriptive statistics, the data were analyzed using Microsoft Excel as the mean 3 of independently variable with standard deviation (SD).

3. Results and Discussion

3.1 Mass fraction of marine algae

Mass fractions of the three types of marine algae can be seen in Table 1. This table shows that the fractionation with different solvents yield (mass fractions) are different. Three types of marine algae indicates the fraction of the mass of water that has the highest compared with the other 2 fractions (hexane and ethyl acetate). Fraction of water in the past three types of algae are as follows: 12.13 g sertularoides Caulerpa, Laurencia tronoi 10, 32 g, and 7.69 g Padina australis, this means that the chemical components in most marine algae that are polar water soluble solvents polar. Of three marine algae, Caulerpa sertularoides water fraction (green algae) which have the highest masses, while the lowest mass contained in Padina australis hexane fraction (0.44 g). Highest mass fraction of hexane and ethyl acetate fractions are present in each tronoi Laurencia: 2.33 g and 1.84 g. The End (2007) reported that the green alga Ulva retikulata Forskal, water fraction has the highest mass (6.323 g) compared to the hexane fraction (2,715 g) and chloroform (1.413 g).

Table 1

Mass fraction *Caulerpa sertularoides, Laurencia tronoi, Padina australis.*

Types of sea algae	**Mass fraction (g)**		
	Hexane Fraction	Ethylacetate Fraction	Water Fraction
Caulerpa. sertularoides	1,32 ± 0,35	1,29 ± 0,50	12,13 ± 0,88
Laurencia tronoi	2,33 ± 0,32	1,84 ± 0,31	10,32 ± 0,70
Padina australis	0,44 ± 0,15	0,48 ± 0,21	7,89 ± 1,41

3.2 The content of total phenol

Table 2 shows the highest total phenol content was found in the ethyl acetate fraction sertularoides Caulerpa is 123.87 ± 2.67 mg GAE / g extract, followed by n-hexane fraction tronoi Laurencia is 94.29 ± 2.02 mg GAE / g extract, and the lowest content of total phenols found in the water fraction Laurencia tronoi ie 5.92 mg GAE / g extract. This indicates that phenolic compounds in Caulerpa sertularoides more soluble in ethyl acetate (semi-polar solvent), while phenols in Laurencia tronoi (red algae) is more soluble in n-hexane (non-polar solvent). Santoso, et al (2010) reported that total phenol content of the same genus Caulerpa lentilifera with Caulerpa sertularoides encountered higher in the ethyl acetate extract, compared with the methanol extract and n-hexane. Ganesan et al (2008) reported that the 3 types of red algae Kappaphycus Eucheuma ie. Gracilaria edulis and Aconthopora spicifera highest content of total phenols found in fractions of petroleum ether (non-polar fraction) compared with other more polar fractions (ethyl acetate and dichloromethane).

Table 2

The content of total phenols (mg GAE / g extract) on the marine algae Caulerpa sertularoides fraction, tronoi Laurencia, Padina australis

Types of sea algae	**The content of total phenols (mg GAE/g ekstrak)**		
	Hexane Fraction	Ethylacetate Fraction	Water Fraction
Caulerpa. sertularoides	26,5 ± 1,01	123,87 ± 2,67	15,96 ± 0,94
Laurencia tronoi	94,29 ± 2,02	36,92 ± 2,98	5,92 ± 0,19
Padina australis	30,04 ± 0,31	25,21 ± 0,15	8,79 ± 0,26

3.3 Activity to avoid DPPH free radicals

Activity to avoid DPPH free radicals in a solvent fraction of the 3 types of marine algae (Table 3) showed that the hexane fraction (the fraction of non-polar) tronoi Laurencia (red algae) have activity DPPH free radical scavengers highest (82.62%). These results are supported by reports of Ganesan et al (2008) that the activity of free radicals in the 2 types of red marine algae, namely Eucheuma and Gracilaria edulis cappaphycus found to be higher in the fraction of petroleum ether (non-polar) compared to the fraction of ethyl acetate, dichloromethane, butanol and water

Data activity on the DPPH free radicals fraction of marine algae are not positively correlated with total phenol content, the activity of free radicals DPPH on Caulerpa sertularoides ethyl acetate fraction (the fraction which has the highest content of total phenol) is only 1.76%. This is supported by reports Santoso et al, (2010), that the methanol extract of marine algae Caulerpa lentilifera that have activity DPPH free radical scavengers higher than

ethyl acetate extract total phenolic content although ethyl acetate higher than the methanol extract. These results indicate that the relationship between the antioxidant activity of free radicals DPPH and total phenol content does not always correlate potsitif. Lim et al (2002) reported that the marine brown alga Sargassum siliquastrum no positive correlation is due to lignin extracted participating thus affecting the value of total phenol content.
Some factions have activity DPPH free radicals are not detectable fraction tronoi Laurencia water and hexane fraction and ethyl acetate fraction Padina autralis. Ganesan et al, (2008) and Khumar et al, (2008) reported that for DPPH radical scavenging activity in the butanol fraction from Kappaphycus Eucheuma, Acanthophora spicifera, Sargassum marginatum, Padina australis and dichloromethane fractions of Sargassum marginatum showed No. Detection (ND).

Table 3
DPPH free radicals activity in marine algae Caulerpa sertularoides fraction, Laurncia tronoi and Padina australis. Stock samples of 2000 ppm in methanol.

Types of sea algae	**Free Radical Preventive Activity DPPH (%)**		
	Hexane Fraction	Ethylacetate Fraction	Water Fraction
Caulerpa. sertularoides	8,69 ± 0,78	1,76 ± 0,35	5,96 ± 0,78
Laurencia tronoi	82,62 ± 1,54	7,54 ± 0,78	ND
Padina australis	ND	ND	4,20 ± 1,19

Information: ND= No Detection

3.4 Reducing power

Value of reducing power (Table 4) shows the highest value of reducing power was found in the ethyl acetate fraction srtularoides Caulerpa is 36.45 ± 0.27 mg GAE / g means that the chemical components in Caulerpa sertularoides which serves to reduce Fe 3 + ions to Fe 2 + ions are more semi-polar so it can be bound by ethyl acetate. This data is followed by ethyl acetate fraction tronoi Laurencia is 20.05 ± 0.95 mg GAE / g, while reducing the value of the lowest power found in the water fraction Laurencia tronoi 1.33 ± 0.14 mg GAE / g. Value of reducing power three factions on all types of algae indicates that the fraction of water has the lowest value. The low value of reducing power in the water fraction is not possible due to the components in a sample of non-polar, but the polar components bound with other components which are not polar and ambient temperatures require higher to sever ties so that components of polar antioxidants in marine algae samples can be separated and bound water is polar. Hwang et al (2010) reported that the reducing power increased in parallel with increasing concentrations of the marine alga Sargassum hemiphylum samples were extracted with hot water.

Table 4
Reducing the value of the fraction of power in the marine algae Caulerpa sertularoides, tronoi Laurencia, Padina australis

Types of sea algae	**Reducing power (mg GAE/g)**		
	Hexane Fraction	Ethylacetate Fraction	Water Fraction
Caulerpa. sertularoides	12,65 ± 0,18	36,45 ± 0,27	7,36 ± 0,01
Laurencia tronoi	10,97 ± 0,27	20,05 ± 0,95	1,33 ± 0,14
Padina australis	11,04 ± 0,21	7,56 ± 0,12	2,71 ± 0,31

3.5 Ferrous ion chelathing (FIC)

Metal ion binding ability of antioxidant components are usually evaluated by using the test parameters Ferrous ion chelating (FIC). Class of phenolic antioxidant components such as flavonoids can function as chelating ion (Mamahit, 2008). Other components that can function as chelating metal ions are acidic tanat as other sites of the galloyl group (Santoso, et al, 2004).
Data activity on the marine algae stuck ion fraction hexane, ethyl acetate and water can be seen in Figure 1, 2 and 3. Stuck ion activity increased with increasing concentration of the sample, where the higher concentration samples, stuck ion activity increases. Chew, et al, (2008) and Khumar, et al, (2008) reported that the stuck ion activity increased with increasing concentrations of the samples on marine algae Padina antilarum, Caulerpa racemosa, and Kappaphycus alvarezii.
In the hexane fraction was found stuck ion activity is highest in the marine alga Caulerpa sertularoides, while the activity of stuck ion lowest was on Padina australis (Figure 1).

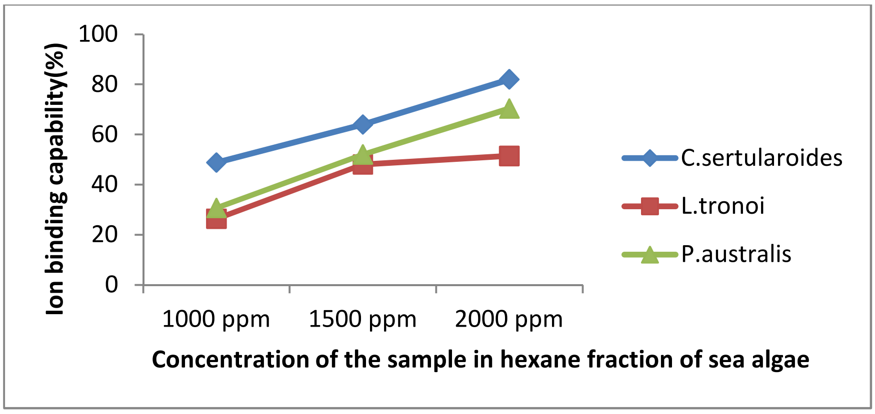

Figure 1. Relationship with the concentration of sample ions in stuck activity hexane fraction 3 types of marine algae

Stuck ion activity in the ethyl acetate fraction (Figure 2) shows the highest yield at the lowest and Padina australis found in Caulerpa sertularoides

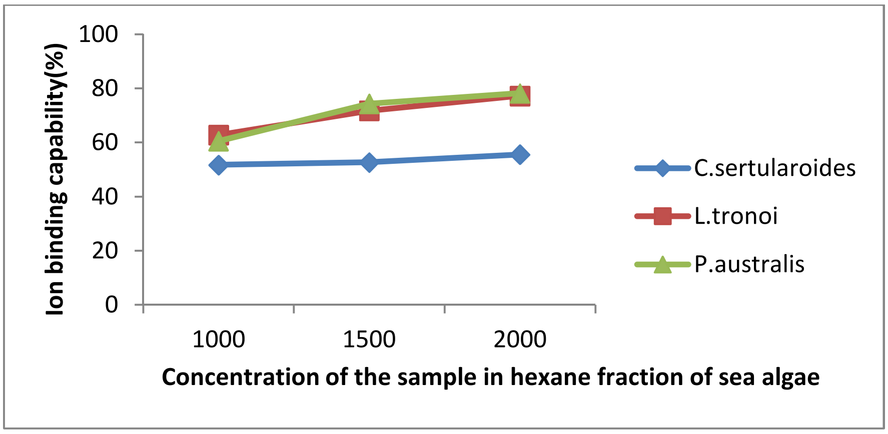

Figure 2. Graph showing the relationship stuck sample concentration ion activity in the ethyl acetate fraction 3 types of marine algae

The third type of solvent fractions (n-hexane, ethyl acetate, water), the water fraction showed the highest stuck ion activity on the three types of marine algae (Figure 3), it is clear that the chemical components that function to stuck ions on the three types of algae are polar. These data are supported by reports Khumar, et al, (2008), that of the three types of solvent extractors are used as ingredients in marine algae Kappaphycus alvarezii with different levels of polarity, namely methanol, ethanol and ethyl acetate have mengkelat ion activity (IC50) of each 3.08 respectively; 3.83, and 4.38 mg / ml. These data show that the more polar methanol has a higher stuck ion activity.

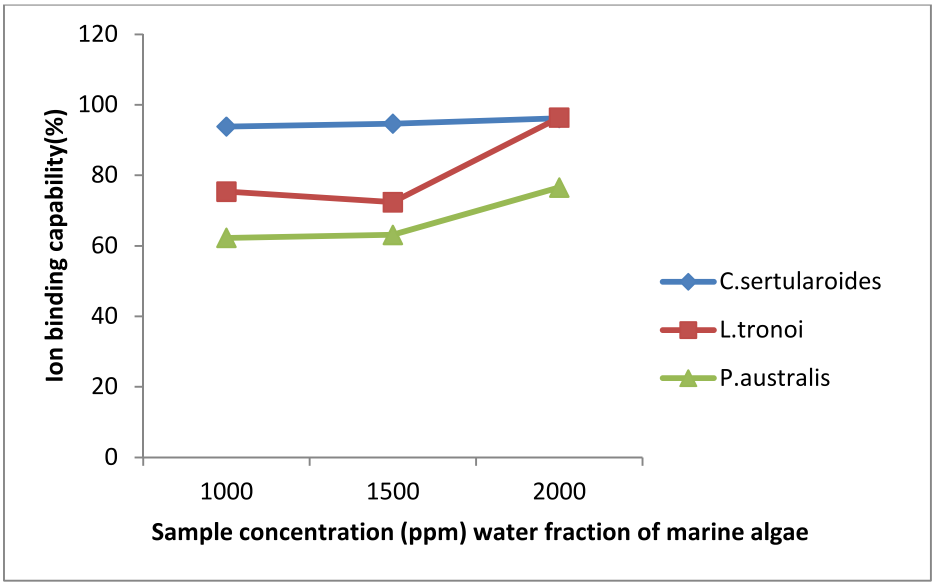

Figure 3. Graph showing the relationship with the concentration of sample ion binding activity on the water fraction 3 types of sea algae

4. Conclusion

Fraction of water generally has a larger mass than the mass of the other two fractions, fractions of water has a mass Caulerpa sertularoides Highest (12.13 g), while the lowest mass (0.44 g) contained in the hexane fraction Padina australis. The highest content of total phenols present in Caulerpa sertularoides ethyl acetate fraction (123.87 mg GAE / g extract), while the lowest content of total phenols present in water Laurencia tronoi fraction (5.92 mg GAE / g extract). Activities highest DPPH free radicals found in Laurencia tronoi hexane fraction (82.68%), other factions have activity DPPH free radicals is very low (<10%) even at a fraction (the fraction of water tronoi Laurencia, the fraction of ethyl acetate and hexane fraction Padina australis) activity of DPPH free radicals was not detected (ND). Reducing power is highest in Caulerpa sertularoides ethyl acetate fraction (36.45 mg GAE / g) and the lowest in Laurencia tronoi water fraction (1.33 mg GAE / g). Fraction of water generally has the highest ion mengkelat activity compared with the other two fractions (hexane and ethyl acetate), the highest ion mengkelat activity found in the water fraction sertularoides Caulerpa. On all fractions, the higher the concentration of the sample, the higher the activity of stuck ion.

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