International Journal of
Science and Engineering Investigations vol. 2, issue 23, December 2013
ISSN: 2251-8843

Antioxidant Activity of Three-Marine Algae Methanol Extract Collected from North Sulawesi Waters, Indonesia

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Abstract- This study was aimed at studying total phenol content and antioxidant activity of methanol 50, 70, and 95% extracts of marine algae, *Caulerpa sertularoides, Laurencia tronoi*, and *Padina australis* collected from Nain island waters, North Minahasa Regency, North Sulawesi, Indonesia. Total phenol content was measured using Fholin-Chiocalteau method, while the antioxidant activity measurement used *1,1-diphenyl-2-picrihydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP),* and *Ferrous Ion Chelating* (*FIC*) methods. Methanol 50% extract of *Caulerpa sertularoides* gave the highest antioxidant activities of DPPH radical scavenging, FRAP and FIC ($p<0.05$), 38.30±0.44%, 13.07±0.15mg GAE/g and 63.81±4.71%, respectively, while total phenol content of the three methanol concentration extract was not different ($p>0.05$). Methanol 50% extract of *Laurencia tronoi* gave the highest total phenol content, DPPH radical scavenging, FRAP, and FIC ($p<0.05$), 9.48±1.65mg GAE/g extract, 26.54±1.56%, 9.75±0.17mg GAE/g extract and 85.90±3.47%, respectively. Methanol 95% extract of *Padina australis* gave the highest total phenol content and FRAP ($p<0.05$), 8.37±0.60mg GAE/g extract and 7.91±0.29 mg GAE/g extract, respectively, while the highest DPPH radical scavenging and FIC ($p<0.05$) occurred in 50% methanol concentration of the extraction solvent, 21.65±0.50% and 92.29±1.12%, respectively.

Keywords- *Antioxidant Activities , methanol extract, Caulerpa sertularoides, Laurencia tronoi, Padina australis*

I. INTRODUCTION

Human cell electron is usually not in equilibrium due to free radical attack which brings about a variety of diseases as a result of changes in electron chains in the body. Consuming antioxidant-containing plants is one of the solutions, since antioxidant could neutralize the free radicals.

Marine algae are one of antioxidant-sourced food materials which are indicated by the presence of antioxidant activity in the organic solvent extract of the marine algae (Yoshie *et al,* 2004; Santoso *et al,* 2004; Yuan and Walsh, 2006; Wong *et al,* 2009; Cox, *et al,* 2010; Zakaria *et al,* 2011).

The antioxidant activity can be detected by various test methods, such as DPPH radical scavenging (Matsukawa *et al,* 1997; Hwang *et al,* 2010, Andarwulan *et al,* 2010), ferric reducing antioxidant power/FRAP (Chew, *et al,* 2008), and ferrous ion chelating/FIC (Khumar *et al,* 2008).

North Sulawesi waters have the potency as marine algal beds. Gerung (2006) reported that in Manado Bay, there are 4 families, 6 genera and 31 species of green algae, 4 families, 6 genera and 10 species of brown algae, 8 families, 21 genera and 28 species of red algae , including *Caulerpa sertularoides, Laurencia tronoi,* and *Padina australis.*

Indonesian marine waters, including North Sulawesi, is the area exposed to strong ultraviolet radiation, can trigger the development of reactive radical species, so that marine algae growing in this waters alter their metabolisms to produce active compounds as self-defense (Santoso *et al,* 2004), and this makes marine algae be thought to have the antioxidant active compounds in a great number.

Methanol is a good multifunctional solvent for preliminary extraction (Harborne, 2006), so that it has been used for marine algal antioxidant extraction (Santoso *et al,* 2004; Matanjun *et al,* 2008; Khumar *et al,* 2008). Methanol concentration for extraction influences the total phenol content (Chew *et al,* 2008) and the antioxidant activity of the marine algae (Damongilala *et al,* 2013).

Based upon information above, it is important to study about "Antioxidant activities of three-marine algae methanol extract collected from North Sulawesi waters, Indonesia".

II. MATERIALS AND METHODS

A. Samples

C. sertularoides, L. tronoi and *P. australis* were collected from Nain island waters, North Sulawesi Province, in february to march 2012. Marine algae were cleansed, weighed each of 1 kg and packed in the plastic bags. They were transported in crushed ice-contained cool box with a ratio of 1 to 3. The algae directly unused were stored at temperature of -20ºC in a

freezer, and to use them, the algae would be melted at room temperature for 24 hours. Before maceration, samples were previously dried in the room for 3 days and continued with oven-drying at 40ºC for 4-6 hours until 10 times weight reduction, and then fine blended.

B. Chemical compund and reagent

Solvent used was methanol, sodium phosphate, disodium phosphate, trichloroacetic acid (TCA), $FeCl_3$ obtained from Merk. Fholin-Ciocalteau, 1,1-diphenyl-2-picrihidrazyl, ferrozin and gallic acid were gained from Sigma.

C. Sample extract preparation

Each 200 g of dry sample powder was macerated with 2000 ml methanol-water (v/v) solution of 50%, 70% and 95% for 48 hours and filtered. Macerate was separated using a whatman no 1 filter paper, the pulp was then macerated 2 times as before and all macerates were collected and evaporated in a vaccum rotary evaporator at 40ºC to produce semi-solid extracts which was then dried in a vacuum desiccator until the extract weight was constant. The extracts were put into a black-colored glass tube and stored at -20ºC for further analyses.

D. Total phenol content

Total phenol content was measured using Folin-Ciocalteau reagent by modifying methods of Devi *et al* (2008), Ganesan *et al* (2008), and Andarwuan *et al* (2010). As much as 0.1 gram of extract was dissolved in 10 ml methanol in a flask. 0.1 ml extract solution was taken, added 1 ml of 1:2 Folin-Ciocalteau - aquadest and left for 5 min. It was then added 1 ml sodium carbonate 7%, homogenized and incubated at room temperature for 30 minutes in dark condition. The mixture absorbence was measured at 750 nm. Total phenol content was interpreted as mg gallic acid equivalents (GAE)/g extract . The regression equation of gallic acid was y = 0.010 + 0.005x (R^2=0.998).

E. Antioxidant activity

1) 1,1-diphenyl-2-picrihydrazyl (DPPH) radical scavenging

DPPH free-radical scavenging was measured using method modification of Khumar *et al* (2008). 2 ml of DPPH 93 µM was added into 0.5 ml extract (4000 ppm in methanol). The mixture was shaken and incubated at 37^{O}C for 30 minutes; the absorbance was measured at the wavelength of 517 nm. According to Yuan and Wals (2006) and Devi *et al* (2008), DPPH radical scavenging was expressed as % inhibition measured following the equation:

% Inhibtion = {(Control absorbance – sample absorbance)/(control absorbance)} x 100

2) Ferric reducing antioxidant power (FRAP)

FRAP was measured using the method of Khumar *et al* (2008) and Chew *et al* (2008), modified as follows: one-ml of 4000 ppm extract concentration in methanol was mixed with 1 ml phosphate buffer (0,2 M, pH 6,6) and 1 ml potasium ferricyanide, $[K_2Fe(CN)_6]$ 1 %. The mixture was homogenized and incubated at 50^{O}C for 30 minutes (mixture A). 1 ml trichloroacetic acid 10% was added in mixture A (mixture B), and centrifuged (10 minutes, 3000 rpm). Then, 1 ml of mixture B was taken from the upper layer and added 1 ml distilled water and 5 ml $FeCl_3$ 0.1 %. The final mixture was homogenized and the absorbance measured at 700 nm. FRAP value was interpreted as mg equivalent of gallic acid/g extract. The regression equation of gallic acid calibration was y = 0.198 + 0.024x (R^2= 0.991).

3) Ferrous Ion Chelating (FIC)

Ferrous Ion Chelating (FIC) was based on Chew *et al* (2008) method modified as follows: 0.5 ml extract (4000 ppm in methanol) was added 1.8 ml aquadest and 0.5 ml Fe_2SO_4 $7H_2O$ 0.1 mM, then the mixture was homogenized and added 0.5 ml ferrozin (0.25 mM). This final mixture was homogenized, incubated at room temperature for 20 min., and the absorbance was read at 562 nm. Control was made following the procedure above but the sample was replaced with aquadest. FIC value was determined as follows:

FIC (%) ={(Control Absorbance – Sample Absorbance)/ (Control Absorbance} x 100

F. Statistical Analysis

Experiments used 3 treatments, methanol-water (v/v) concentration for extraction: 50, 70 and 95% in each separate marine algal species. The experimental design used Complete Randomized Design with 3 replications. If ANOVA indicates significant difference ($p<0.05$), LSD test was used. Data analysis used the SPSS version 20 software.

III. Results and Discussion

A. Total Phenol Discussion (TPC)

Phenol compounds occur in plants, in which these compounds have aromatic groups strongly absorbing the UV light spectrum. Total phenol content determination uses gallic acid as standard solution that possesses maximum absorption at the wavelength of 750 nm (Devi *et al*, 2008), with Folin Ciocalteau reagents (Harborne, 2006).

ANOVA shows that total phenol content of *C. sertularoides* in methanol concentration treatment was not significant ($p>0.05$), despite higher content in methanol 50% extract (18.87 mg GAE/g extract) than two other methanol concentrations. These data were in agreement with Chew *et al* (2008) that the highest total phenol content was recorded in *C. rasemosa* in the extract of 50% methanol (144 mg GAE/100g dry sample) followed by 20% methanol (114 mg GAE/100g dry sample) dan then 100% methanol (96.5mg GAE/100g dry sample), respectively.

ANOVA for the effect of methanol extraction concentration on the total phenol content of *L. tronoi* shows significant difference ($p<0.05$). The highest total phenol content was recorded in methanol 50% extract (9,48mg GAE/g extract), which supported the finding of Chew *et al* (2008) that the highest total phenol content in red alga, *Kappapychus alvarezii*, was found in methanol 50% extract. Damongilala *et al* (2013) also reported that total phenol content of methanol 60% extract in red algae, *Eicheuma spinosum*, was higher than those in methanol 70% and 80% extracts. Demirel *et al* (2011) found

that total phenol content in the methanol extract of *Laurencia obstusa* was 32.2 ± 1.9 mg GAE/g.

ANOVA for the effect of methanol concentration extraction treatment on total phenol content in *P. australis* indicated significant difference ($p<0.05$), in which the higher the methanol concentration for extraction, the higher the total phenol content. Total phenol content in the extract of 50, 70, and 95% methanol concentration were 3.55mg GAE/g extract, 6.11mg GAE/g extract and 8.7mg GAE/g extract, respectively. Table 1 exhibits total phenol content values of three methanol extract concentrations in 3 algae species.

TABLE I. TOTAL PHENOL CONTENT OF THREE METHANOL EXTRACTION CONCENTRATIONS IN *C. SERTULAROIDES, L.TRONOI* AND *P. AUSTRALIS.*

Alga Species	Total phenol content (mg GAE/g extract)		
	Methanol Extract 50%	Methanol Extract 70%	Methanol Extract 95%
C.sertularoides	18.87±1.39 (a)	18.53±2.00 (a)	15.63±1.19 (a)
L.tronoi	9.48±1.65 (a)	6.00±0.57 (b)	5.97±0.53 (b)
P.australis	3.55±0.12 (c)	6.11±0.59 (b)	8.37±0.60 (a)

Notes: Similar alphabets on the same row indicates no difference ($p>0.05$) among methanol extraction concentration treatments in each marine algae used.

B. Antioxidant activity (AOA)

1) 1,1-diphenyl-2-picryhyhydrayl (DPPH) radical scavenging

DPPH radicals are compounds possessing free radical nitrogen and easily damaged by the free radical destroyers. This test was utilized to examine the antioxidant compounds functioning as hydrogen donor (Chew *et al*, 2008). DPPH was extensively employed as free radicals to evaluate the "reducing substance*"* and functions to study the damaging activity of the free radical compounds (Khumar *et al*, 2008).

DPPH free radical scavenging of *C. sertularoides* and *L. tronoi* in methanol extraction concentration treatments showed significant difference ($p<0.05$), in which methanol 50% extract had the highest value (Table 2). Higher methanol extraction concentration causes decreased percentage of DPPH free radical scavenging, and it is correlated with total phenol content. Chew *et al* (2008) reported that the methanol extract of *C. rasemosa* and *K. alvarezi* possessing high total phenol content also had a high DPPH radical scavenging indicated with low IC_{50} value. Zubia *et al* (2007) further reported that *Laurencia obtusa* possessing total phenol content of 4.17% dry weight had the EC_{50} DPPH of 37.50 mg/ml, while *L. intricata* possessing total phenol content of 2.51% dry weight had the EC_{50} of 52.56 mg/ml.

The highest free radical scavenging of *P. australis* occurred in methanol 50% extract ($p<0/05$) and exhibits no correlation with total phenol content with the highest in methanol 95% extract ($p<0.05$). Widjaja *et al* (2011) found that high total phenol content in marine algae did not always have positive correlation with the free DPPH radical scavenging.

The values of free DPPH radical scavenging in methanol 50% extract of *C. sertularoides*, *L.tronoi*, and *P.australis* was 38.23%, 26.54% and 21.50%, respectively, but these values were still below the value of free radical scavenging BHT 200 ppm. Several findings indicated that free DPPH radical scavenging in 2000 ppm methanol concentration of *Laurencia obstusa* was 3.62 ± 0.2% (Demirel *et al*, 2011), in ethanol ekstract of *entereromorpha compressa* 49.0% (Shanap *et al*, 2011), in methanol extract of red snail (*Carithidea obtusa*) with IC_{50} 58.11 ppm (Purwaningsih, 2012), respectively.

TABLE II. FREE DPPH RADICAL SCAVENGING ACTIVITY OF 3 METHANOL EXTRACTION CONCENTRATIONS IN *C. SERTULAROIDES, L. TRONOI,* AND *P. AUSTRALIS.* SAMPLE STOCK WAS 4000 MG/L.

Alga Species	Free DPPH radical scavenging (%)		
	Methanol Extract 50%	Methanol Extract 70%	Methanol Extract 95%
C.sertularoides	38.30±0.44 (c)	33.63±1.44 (b)	27.84±0.99 (a)
L.tronoi	26.54±1.56 (b)	21.91±0.55 (a)	21.64±2.44 (a)
P.australis	21.65±0.50 (c)	17.55±0.36 (b)	15.74±0.33 (a)

% Free DPPH radical scavenging Butilated Hydroxy Toluena/BHT 200 ppm=89.49%

Notes: Different alphabets on the same row indicate significant difference ($p<0.05$) among methanol extract concentrations in each species of marine algae.

2) Ferric reducing Antioxidant power (FRAP)

Halverson *et al* (2002) claimed that the FRAP method based on Fe^{3+} reduction to Fe^{2+} by the antioxidant, depending on the complex reaction of Fe (III)TPTZ to Fe (II) TPTZ, was indicated by blue color with maximum absorption at the wavelength of 593 nm.

The FRAP value of methanol extraction concentration treatment in those three alga species (Table 3) shows significant difference ($p<0.05$). The methanol 50% extract in *C. sertularoids* and *L. tronoi* possesses the highest FRAP value, which is correlated with total phenol content of the algae. Matanjun *et al* (2008) reported that *C. lentilifera* and *C. rasemosa* has high total phenol content and FRAP value, while the present study showed that in *P. australis,* the highest FRAP value occurred in the extract of 95% methanol concentration. Matanjun *et al* (2008) found that brown algae, *S. polysistum,* possessing high total phenol content also had a high FRAP value.

TABLE III. FRAP VALUE OF METHANOL EXTRACTION CONCENTRATIONS IN *C. SERTULAROIDES*, *L. TRONOI*, AND *P.AUSTRALIS*

Marine alga species	FRAP value (mg GAE/g extract)		
	Methanol Extract 50%	Methanol Extract 70%	Methanol Extract 95%
C.sertularoides	13.07±0.15 (c)	11.74±0.55 (b)	10.63±0.28 (a)
L.tronoi	9.75±0.17 (c)	7.88±0.22 (b)	6.90±0.21 (a)
P.australis	0.98±0.06 (a)	3.48±0.44 (b)	7.91±0.29 (c)

Notes: Similar alphabets on the same row indicates no difference (p>0.05) among methanol extraction concentration treatments in each marine algae used.

3) Ferrous ion chelating (FIC)

Ferrous ion chelating (FIC) is usually utilized to evaluate the ability of antioxidant to bind metal ions. One of chemical compounds capable of functioning as metal ion chelating antioxidant is flavonoid belonging to phenolic group (Mamahit, 2008).

Methanol 50% extract of *C. sertularoides*, *L. tronoi*, and *P. australis* (Table 4) showed the highest FIC value (p<0.05), and this value was correlated with high total phenol content of *C. sertularoides* and *L. tronoi* methanol 50% extract. Santoso *et al* (2004) reported that polyphenols contained in the methanol extract of marine algae exhibits very good capability of chelating the ion.

TABLE IV. FIC VALUES OF METHANOL EXTRACTION CONCENTRATIONS IN *C. SERTULAROIDES*, *L. TRONOI*, AND *P. AUSTRALIS.* SAMPLE STOCK WAS 4000 MG/L

Marine algal species	FIC Values (%)		
	Methanol Extract 50%	Methanol Extract 70%	Methanol Extract 95%
C.sertularoides	63.81±4.71 (c)	51.42±2.58 (b)	4.56±1.50 (a)
L.tronoi	85.90±3.47 (b)	61.68±6.17 (a)	58.69±4.99 (a)
P.australis	92.29±1.12 (c)	80.99±1.07 (b)	22.93±6.87 (a)

Notes: Similar alphabets on the same row indicates no difference (p>0.05) among methanol extraction concentration treatments in each marine algae used.

IV. CONCLUSION

Metanol 50% extract of *Caulerpa sertularoides* gave the highest antioxidant activities of DPPH free radical scavenging, FRAP and FIC (p<0.05), while total phenol content of the three methanol concentration was not significant (p>0.05). The methanol 50% extract of *Laurencia tronoi* gave the highest total phenol content, DPPH free radical scavenging, FRAP, and FIC (p<0,05). *Padina australis* gave the highest total phenol content and FRAP (p<0.05) which occur in methanol 95% extract, while the highest DPPH free radical scavenging and FIC (p<0.05) occurred in methanol 50% extract.

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