

Antioxidant Activity of Metanol Extract of Sea Weeds Obtained from North Sulawesi

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Abstract

In Vitro antioxidant activity of four sea weeds from North Sulawesi –viz., *Gracillaria salicornia*, *Sargassum olygocystum*, *Turbinaria decurens*, *Halimeda macroloba* and *H. durvilae* were evaluated. Total phenol content (TPC) and activity antioxidant of 30%,50%, 70% aqueous were study. TPC using follin Ciocalteu method, while 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, Ferric reducing Antioxidant Power (FRAP) and ferrous ion Chelating (FIC) assay were used to study their AOC(Antioxidant Capacity) . The total phenol content of different metanol concentration of sea weed varied from 3.02±0.68% to 18.83±0.77 g gallic acid equivalent (GAE) per 100 gr dried sample, All sea weed were determined to have highest TPC, DPPH and FRAP in 70 % aqueous metanol . *H durvilae* displayed highest in TPC and DPPH. *G.salicornia* showed highest in FRAP. 42.95±2.24 g GAE/100 g ekstrak. Brown alga *T. decurens* and *S. olygocystum* have FIC activity higher then another seaweeds in 30% metanol concentration.

Keywords: Antioxidant Activity, Metanol Extract , Sea Weeds

1. Introduction

Marine alga, like other photosynthesizing plants, are exposed to a combination of light and oxygen that lead to the formation of free radical and other strong oxidizing agents. However, the absence of oxidative damage in the structural componens of macroalga (i.e., polyunsaturated fatty acids) and their stability to oxidative to oxidation during storage suggest that their cells have protective antioxidative defence systems. In fact algae have protective enzymes (superoxide dismutase, peroxidase, glutathione reductase, catalase) and oxidative molecular (phlorotannins, ascorbate acid, tocopherols, carotenoids, phospholipids, chlorophyllnrelated compounds, bromophenol, catechins, mycosporine-like amino acids, polysaccharides,etc) which are similar to those of vascular plants (Burtin,2003).

Reactive species species (ROS) (e.g., superoxide anion (O_2^-), hydroxylradical (OH.), hydrogen peroxide (H_2O_2), and singlet oxygen (1O_2) are formed as a result of normal metabolic activity and exogenous sources. In pathologic condition, the antioxidant mechanism are often inadequate, as excessive quantities of ROS can be generated. The ROS formed may cause cellular and subcellular damage by peroxidation of membrane lipids, by denaturing cellular protein, by breaking DNA strands, disrupting cellular function. In vivo, cells have their own inherited antioxidative among enzymatic pathway O_2^- are dismutated by superoxide dismutase (SOD) to H_2O_2 , catalase (CAT) reduces H_2O_2 to water and molecular oxygen. Glutation peroxidase (GPX) catalyzes the reduction of H_2O_2 to water and organic peroxide to alcohols at the expense of reduced glutathione (GSH), while glutathione-S-transferase (GST) conjugates xenobiotics with GSH for excretion. Among the nonenzymatic substance, β carotene, GSH, Vitamin –A, vitamin-E and vitamin C scavenge free radical (Patra. *et al.*, 2008) .

Reactive oxygen species (ROS) and oxidative stress have been associated with the onset of chronic disease states in humans, including coronary heart disease (CHD), certain cancer, rheumatoid arthritis, diabetes, retinopathyof prematurity, chronic inflammatory disease of the gastrointestinal tract, as well as diseases associated with cartilage, Alzheimer'disease,other neurology disorder and the aging process.(Matanjun. *et al.* , 2008; Yan. *et al.*). According to Shahidi, (2009), The seaweed's potential as a source of natural antioxidants, contains no contamination of other compounds and has various functions that are safe to use as: medication, supplement, nutraceutical and cosmetics that aim to improve health, reduce the influence of a dangerous disease, and aspects the function of the immune system. Seaweeds have protective effect against liver injury caused by carbon tetrachloride antiproliferatif activity toward HeLa cells, antimicrobial activity and antiviral properties (Chew. *et al.* 2008; Yua., *et al.*, 2005; Kuda and Ikemori, 2009).

Furthermore, antioxidants from natural sources increase shelf-life of food. Therefore, consumption of antioxidant and or addition of antioxidant in food materials protect the body as well as foods against the oxidative stress. The traditional Japanese diet, seaweeds are commonly used as sushi wrappings, seasonings, condiments, and vegetables and can thus contribute between 10% and 25% of food intake by most Japanese (Skibola, 2004). Antioxidant activity have been attributed to various reactions and mechanism: prevention of chain initiation binding of transition metal ion catalysts, reductive capacity, radical Scavenging, etc. (Zubia. *et al.*, 2006). However, there is little data elucidating the antioxidant activities from north Sulawesi. Hence the present study are aimed to investigate the comparison antioxidant activity of four seaweed.

2. Materials and methods

Sample

Gracillaria salicornia, *Sargasum olygocystum*, *Turbinaria decurens* and *Halimeda macroloba* and *H. durvilae* were collected from Arakan, Manado, Indonesia in the period February-April, 2011 respectively. The sample was thoroughly washed with seawater and fresh water to remove epiphytes and dirt particles. They delivery to laboratory and were stored at -20°C. Until further use. This sample was used for determination of phenolic content, as well as for antioxidant studies. The determination each assay was carried out in triplicate.

Preparation of sample extract

Two-hundred-and-fifty grams freeze sample extracted using 500 ml of 30,50 and 70% v/v methanol overnight for 3 times at room temperature, filtered with filter paper Whatman No. 1 and concentrated down to 30 ml at 40°C by rotary evaporation, (Eyela, Buchi water Bath B-480, Aspirator 13 Iwaki) and storage at -20°C for further analysis.

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich, Ferozin iron reagent, Follin-Ciocalteu's phenol, Sodium carbonate BHT and metanol purchased from Merk. Sodium carbonate, potassium dihydrogen phosphate, iron (III) chloride-6-hydrate, Trichloroacetic acid, Potassium ferricyanide. All other solvent and chemicals were of analytical grade.

Total phenolic content (TPC)

The TPC of the extracts in 30,50, 70% metanol was measured using Follin Ciocalteu method as described by Ganesan. *et al.*, (2008). 50% Follin Ciocalteu's phenol reagent (1 ml) were added to sample extract (0,1 ml) and vortexed, Furthermore added with 7% w/v Na₂CO₃ (1 ml) and the reaction mixture was then measured at 750 nm. TPC was expressed in terms of mg gallic equivalents (GAE)/100 g dried samples. The calibration equation for gallic acid was $y = 0,005x + 0,057$ ($R^2=0.9998$).

Antioxidant activity (DPPH)

DPPH-scavenging potential of different concentration of metanol was measured based on to test the ability of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals by the sea weeds antioxidants. DPPH assay was measured using the method describe by Chew. *et al.*, 2008. 2ml of 93 μM DPPH were added to 1 ml extract solution (containing concentration 10 mg/ml). The mixture was then vortexed vigorously and left for 20 min at room temperature in dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH-scavenging activity relative to the control, using that following equation:

$$\% \text{ Inhibition} = [A_{\text{control}} - A_{\text{sample}}/A_{\text{Control}}] \times 100\%.$$

Ferric-reducing antioxidant Power (FRAP)

Reducing power of crude metanol extract obtained sea weeds was determined by the method prescribed by Chandini. *et al.* 2008. Briefly, 1.0 ml of metanol extract containing 10mg/ml concentration was mixed 1ml of phosphate buffer (0,2 M, pH 6,6) and 1 ml potassium ferricyanide (1%). Reaction mixture was incubate at 50°C for 20 min. after incubation, 1 ml of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. From the upper layer, 1 ml solution was mixed with 1 ml distilled water and 0,5 ml FeCl₃ (0,1%). Absorbance of all the sample solution was mixture at 700 nm. The FRAP Value was expressed in terms of mg gallic equivalents (GAE)/100 g dried samples. The calibration equation for gallic acid was $y = 0.023 x + 0.373$ ($R^2=0.9998$).

Ferrous Ion Chelating (FIC)

Iron chelating of metanol of the seaweeds were used for the present investigation. The-chelating of ferrous ions by the extracts and standards was investigate by the method Kumar. *et al.*, (2008) of 0,1 mM FeSO₄, extract of various dilutions (Concentration range of 10 mg/ml) and 0,25 mM ferrozine were mixed vigorously. The reaction mixtures left standing for 20 minutes. After the mixture had reach equilibrium and the absorbance of solution was measured at 562 nm. The percentage inhibition of ferrozine-Fe²⁺ complex formation was determined using the following formula:

$$\% \text{ Inhibition} = [A_{\text{control}} - A_{\text{sample}}/A_{\text{Control}}] \times 100\%$$

Statistical analysis

All experiments were conducted in triplicate (n=3). The mean of parameter phenol and antioxidant activity were examined for significant by analysis of variance (ANOVA) using Statgraph Centuion XVI. Significant differences between the mean of parameters were determined by using the LSD test (P<0.05).

3. Result

3.1. Extract yield

Yield of total methanolic extract of *G. salicornia*, *S. olygocystum*, *T. decurens* and *H. microloba* and *H. durvilae* are given in Table 1. *H. durvilae* extracts showed the highest yield followed with *G. salicornia*, *T. decurens*, *H. microloba* and *S. olygocystum* respectively, The extraction yield of the methanolic extracts range from 0.05% to 2.96 %.

Table 1. Yield of Total Extracts of sea weed of various dilutions of metanol in water on *G. salicornia*, *S. olygocystum*, *T. decurens*, *H. microloba* and *H. durvilae* samples.

Species	Yield of extract (gr (% ,w/w)		
	30% metanol	50% metanol	70% metanol
<i>G. salicornia</i> ,	3.21±0.50(1.28%)	2.42±0.42(0.97%)	2.75±0.46(1.1%)
<i>S. olygocystum</i>	0.67±0.31(0.27%)	0.63±0.38(0.26%)	0.68±0.14(0.27%)
<i>T. decurens</i>	2.04±0.47(0.82%)	1.22±0.04(0.48%)	0.96±0.12(0.38%)
<i>H. macroloba</i>	1.21±0.22 (0.48%)	1.20±0.25((0.48%)	1.55±0.22(0.68%)
<i>H. durvilae</i>	7.40±0.56 (2.96%)	7.20±0.49(2.88%)	2.84±0.52(1.14%)

All the value are means ±SD; SD:Standart deviation.

3.2.Total Phenolic Content

Phenolic compound are commonly found in plants and have been reported to have several biological activity including antioxidant properties. Early reports revealed that marine sea weeds extracts, especially their polyphenols, have antioxidant activity (Ganesan, 2008). The mayor active compound in different sea weed extracts, have been reported to be pholototannins and focoxantin (Ganesan, 2008). Total phenolic content (TPC) of sea weed of various dilutions of metanol in water on *G. salicornia*, *S. olygocystum*, *T. decurens* and *H. microloba*, *H. durvilae* samples (Table 2) showed that the phenolic content in methanolic extracts between soecies and metanol concentration were significant different (P<0,05). The Total phenolic content of the extracts show that there was an increase in yield with increase the concentration of metanol. The highest conten of fenol in 70% methanolic astract was *H. durvilae* (18.83±0.77 g GAE/100 gr samples) and the lowest content of fenol was *G. salicornia*, , 3,2±0.68 g GAE/100 gr samples.

Table 2. Total phenolic content (TPC) of sea weed of various dilutions of metanol in water on *G. salicornia*, *S. olygocystum*, *T. decurens* and *H. microloba*, *H. durvilae* samples.

Seaweeds	Total phenol content (g GAE/100g extract)		
	concentration of methanol		
	30%	50%	70%
<i>G.salicornia</i>	3.02±0.68 ^{ax}	2.95±0.61 ^{ax}	6.33±0.51 ^{ay}
<i>S.olygocystum</i>	3.16±0.81 ^{ax}	5.86±0.66 ^{by}	14.32±0.32 ^{bz}
<i>T. decurens</i>	3.86±0.37 ^{bx}	5.26±0.28 ^{by}	13.71±0.98 ^{cz}
<i>H. macroloba</i>	9.60±0.32 ^{cx}	11.11±1.12 ^{cx}	18.42±0.65 ^{dz}
<i>H.durvilae</i>	2.07±0.33 ^{dx}	2.95±0.43 ^{ay}	18.83±0.77 ^{dz}

All the value are means ±SD; SD:Standart deviation

a,b,c,d Colom wise value with same superscripts of these type indicate no significant (P>0.05)

x,y,z Row wise values with different superscripts of these type indicate significant difference (P<0.05)

3.3. DPPH

DPPH has been used extensively as a free radical to evaluate reducing substance and is useful reagent for

investigating the free radical scavenging activities of compounds (Candini. *et al.*, 2008; Duan. *et al.*, 2006). The radical scavenging activity of, *G. salicornia*, *S. olygocystum*, *T. decurens* and *H. microloba* and *H. durvilae* extracts are shown in fig.1 and expressed as percentage reduction of DPPH absorption the tested compound. Respectively, The highest activity antioxidant was in 70% metanol extract. Activity Antioxidant between concentration of metanol are significant different ($P < 0.05$). The best radical scavenging activity could be obtained in the 70% metanol extract of *H. durvilae* ($64.63 \pm 2.28\%$) followed by *H. microloba* ($57.73 \pm 3.13\%$) *T. decurens* ($48.46 \pm 3.89\%$). These value were lower than those obtained using BHT ($89.35 \pm 4.56\%$ in 200 ppm). All Species chlorophyta and phaeophyta relatively exhibit high DPPH radical scavenging activity i.e H. Tuna, Caulerpa suppressoides and Caulerpa paspaloides (Zubia. *et al.*, 2007) *S.marginatum* and *T.conoides* from India have radical scavenging activity (Chandini. *et al.*, 2008). Matanjun (2008) showed, there was a significant correlation between antioxidant activity and phenolic content of seaweeds. Many algae species contain polyphenol glucinol phenolic (phlorotannins) and these study the activity antioxidant of alga could be due to these compound.

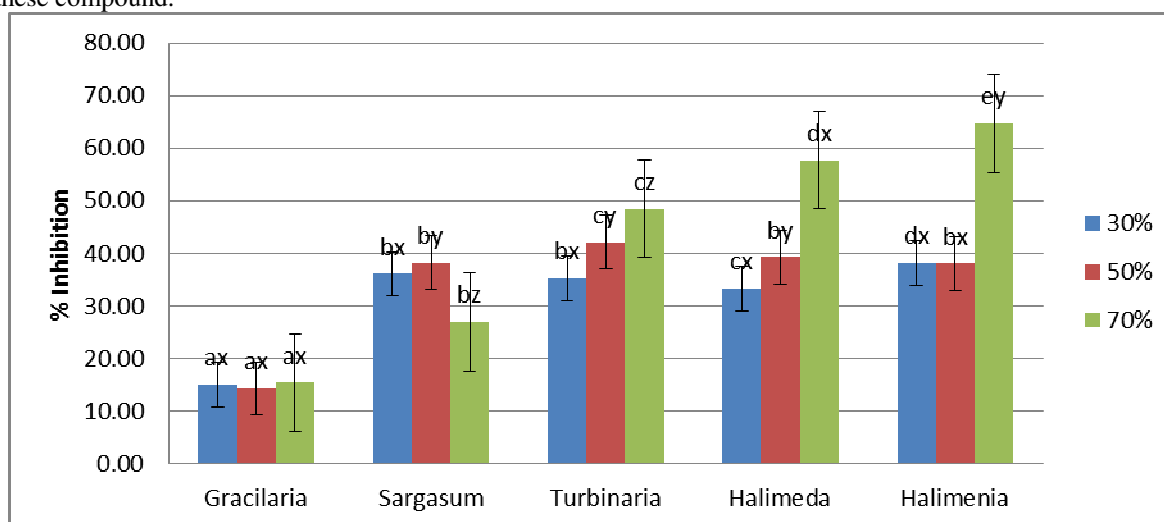


Figure 1 .Free radical scavenging activity of metanolic extract of sea weeds (10 mg/ml ekstrak) Result are mean \pm (n=3).

a,b,c,d,e Indicates significant different ($P < 0.05$) between concentration of extract for a individual seaweeds species; x,y,z indicates a significant ($P < 0.05$) between seaweeds species at each concentration of ekstrak

3.3. Ferric Reducing antioxidant Power

The antioxidant power was measured by FRAP method at λ 700 nm which is based on comparison of the total of antioxidant with the reducing capacity of the samples. In FRAP, The AOC was determined based on the ability of the antioxidant components in the samples to reduce ferric (III) to ferrous (II) in a redox-linked colourimetric reaction (Li. *et al.*, 2006) that involves single electron. The reducing power of *G. salicornia*, *S. olygocystum*, *T. decurens* and *H. microloba* extracts was concentration of metanol-dependent as the concentration increase from 30 to 70%, there was an increase the % inhibition except *S.olygocystum*. Figure 2. Illustrates the total antioxidant power of the sea weed extracts (10 mg/ml). The result show that *G. salicornia*, exhibits highest, 42.95 ± 2.24 g GAE/100 g ekstrak .When compare with BHT (80.37 ± 3.14 gr/100 gr ekstrak) that antioxidant power was significantly less. The AOC among *S.olygocystum*, *H. durvilae* and *T.decurens* are non-significant different ($P > 0.05$).Then reducing ability of a compound greatly depends on the presence of reductones, which are exhibit oxidative potential by breaking the freeradical chain by donating a hidrogen atom.

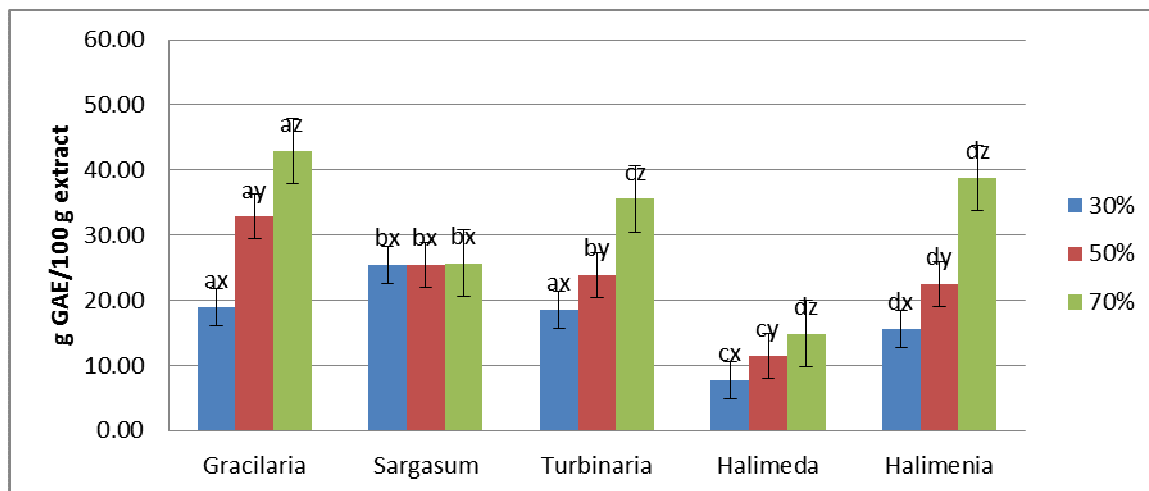


Figure 2. Comparison of persen of Reducing power activity of metanolic extract of sea weeds (g GAE/100 g ekstrak). Consenration of sampel 10 mg/g Result are mean±(n=3).

a,b,c,d, Indicates significant different ($P<0.05$) between consenration of extract for aindividual seaweeds species; x,y,z indicates a significant ($P<0.05$) between seaweeds species at each consenration of ekstrak

3.4.Ferrous ion chelating (FIC).

All the sea weeds extracts show have metal ion chelating activity in concentrasi metanol and species significantly ($p<0.05$). The ability of *G. salicornia*, , was an increase with increase the concentration of metanol, in contrary with *S. Olygocystum*, *T. decurens* and *H. macroloba*.The highest chelating ability in 70% Of metanol was *G. salicornia*, , ($54.07\pm3.01\%$) , Otherwise *S. olygocystum* ($76,14\pm3.36\%$), *T. decurens* ($86,18\%$) and *H.macroloba* $72.24\pm0.50\%$ have highest in metanol 30%. These value were still lower than those obtained using BHT ($40.59\pm5.16\%$ In 200 ppm). Prolotannins which are usually present in brown sea weeds are strong chelators of heavy metals. Iron is known to generate free radicals through the Fenton & Haber-weiss reaction. Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent axidative damage. Metal ion chelating capacity plays a significant role in the antioxidant mechanism since it reduces the concentration of the catalyzing transition metal LPO. It is reported that chelating agents that form σ bonds with a metal, are effective a secondary antioxidants since they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion. Metal binding capacities of dietary fibers are well known, e.g. the inhibitory effects on ferrous absorbtion of algal dietary fibers such as carrageenan might have caused the decrease of ferrous ion in the assay sysem. (Kumar. *et.al.*, 2008 and chew. *et al.*, 2008)

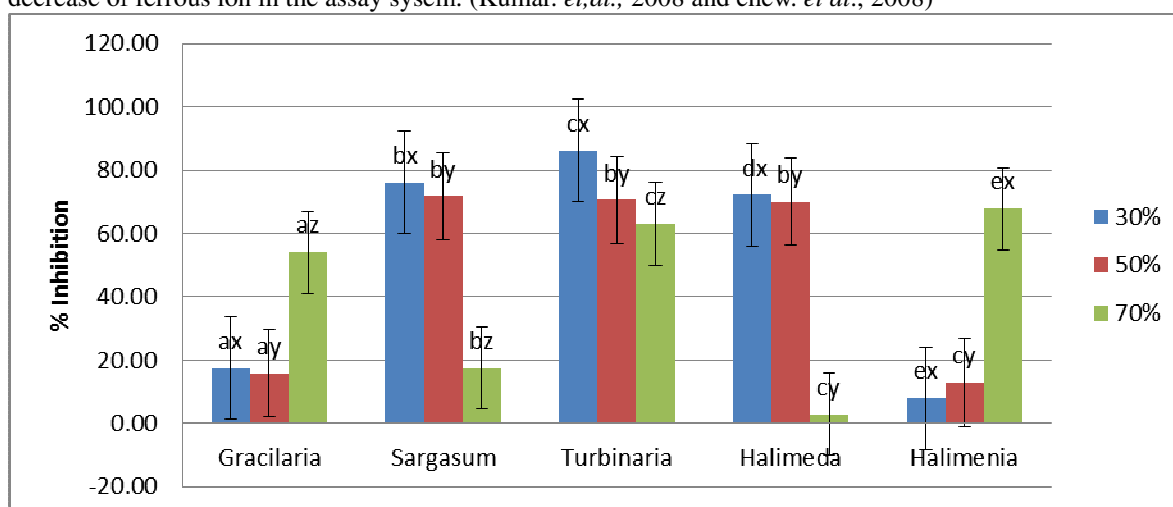


Figure 3. Comparison of persen of Ferrous Ion Chelating activity of metanolic extract of sea weeds (10 mg/ml) Result are mean±(n=3).

a,b,c,d,e Indicates significant different ($P<0.05$) between consenration of extract for aindividual seaweeds species; x,y,z indicates a significant ($P<0.05$) between seaweeds species at each consenration of ekstrak

Discussion

The results of this study showed that the concentration of 30% , 50% and 70% methanol gave significant effect on total phenolic content and antioxidant activity. Phenol content and DPPH and FRAP antioxidant activity increased with increasing concentrations of methanol. Phenol is a very important component in seaweed because of its ability to reduce free radicals, since hydroxyl groups of phenolic compounds is linked with antioxidant activity and plays an important role in stabilizing lipid peroxidation. (Vinayak and Shiyamala, 2012).The important product of Lipid peroxidation is malonaldehyde that reflects the level of lipid peroxidation in clinical diagnosis (Fukunakz. *et al.*, 1995) Algae contains phenolphloroglucinol (phlorotannin) which serves as a reducer of reactive oxygen species, chelating metals, enzyme modulators and prevent lipid peroxidation. (Matanjun. *et al.*, 2007). Antioxidant activity of the sample has a strong correlation to the value (FRAP). Reducing power characteristic indicate that antioxidant compound is the electron donor and can reduce intermediate oxidation of lipid peroxidation process, so it can function as primary and secondary antioxidant .There is also a strong relationship between the antioxidant phenol content activity.

At a concentration of 70% methanol *H. microloba* has phenol content, DPPH and FRAP values are quite high. Boonchumi. *et al.*, (2011), found that *H macroloba* have antioxidant activity DPPH, reducing power, superoxide radicals and anti-lipid peroxidation. By Yoshie. *et al.*, (2002) Composition of phenolic compounds and other phenolic compounds will differ even between the same species, as in *Halimeda spp.* A very large number on *H.macroloba* is epigallocatechin for 20.000µg / g dry weight acid cafeat and hespiridin only on *H. macroloba*. Catechol on *H.macroloba* 5 times from *H.opuntia. myricetin* and morin on *H.macroloba* average 2 times larger than *H.opuntia*.

All living organisms contain a complex system of enzymes antioxidan. Such as thioredoxin system required for life. Antioxidants in biological systems have multiple functions, including defense against the damage of oxidative and play as major signaling pathway in the cell. The main role of antioxidants in cells is to prevent damage by reactive oxygen species (Devi. *et al.*, 2011). ROS hydroxyl, superoxide and peroksidradikal formed in human cells through caused by the damage of intensive oxidative which lead to geriatric degenerative conditions of human carotenoid disease. Carotenoids are natural pigments from plants to react quickly and free radicals and slow down and reduce the progression of damage oksidatif (Shou. *et al.*, 2003).

G. salicornia, , showed antioxidant activity the highest reducing power (FRAP).According Nazir. *et al.*, (2011), the results compound structure elucidation *G.salicornia* ethyl acetate fraction was identified as 22-dehydrocholesterol, cholesterol, oleic acid and stigmasterol. Red algae contain mainly kolesterosterol, demosterol and 22-dehydrocholesterol. Oleic acid degrading the risk of coronary heart disease. Oleic acid also has a protective effect against cardiovascular complications of diabetes because the levels of glutathione, total lipids and triglycerides profitable affected, lowering the activity of tissue factor in diabetes-hyperglycemik can protect the network from the risk of thrombosis.

Ion chelating activity has the highest activity at a concentration of 30% methanol. *T.decurens* has the highest ion chelating activity, it means that it has the highest ability to reduce Fe +3 ions. Brown algae are phlorotannin which is a strong chelator of heavy metals. (Nwosu. *et al.*, 2011). Phlorotannin can serve as antiproliferasi, bactericidal, inhibiting H₂O₂., prevent DNA damage, and treat hypertension (Gornish & Garbary. 2010). *Turbinaria* sp. were found to have antioxidant and anti-inflammatory activity. Species recorded have compounds the essential nutrients that the mineral salts (K, Ca and Fe), soluble fiber, protein can be digested and slightly PUFA 10 (Chakraborty, 2013). Seaweeds are also a rich source of PUFA and fatty acids inhibit the growth of systemic spread function of breast cancer through a mechanism independent of the host immune system, except through intracellular lipid peroxidation (deveri. *et al.*, 2001). seaweed is considered as the metabolic bioactive through a broad spectrum of biological activities, which *Gracilaria salicornia* and *Sargasum oligocystum* in vitro activity against *Leishmania* major promastigote evaluated by MTT assay test. Where treatment with pentavalent antimonial therapy showed failure. (Fouladvand. *et al.*, 2011).

In this research found the highest antioxidant activity present in *H. durvilae* in line with the highest content of phenols at a concentration of 70% methanol extract. *H. durvilae* is a type of red algae that contain high pigment and has a high content of polysakarida polysakarida components such as low molecular weight, pigment, protein or polypeptide also affect antioxidant activity (Kumar. *et al.*, 2008). . *G.Salicornia* red seaweed and *H. durvilae* methanol at a concentration of 70% based on this research has antioxidant activity and reducing power ion chelating the highest compared with another else. Bioactive compound contained in seaweed is beneficial to cure degenerative diseases. Polysaccharide sulfate can prevent the damage of oxidative living organism. Activities range depending on the amount of sulfate, molecular weight, and posisisulfat on sugar glycosides. Fosfat and

sulfate content in polysakarida make different on their activity biology. (Yuan. *et al.*, 2005). Based on these research we conclude that Seaweed *gracillaria salicornia*, *Sargasum olygocystum*, *Turbinaria decurens*, Halimeda *H. durvilae* macroloba and can be used as a source of natural antioxidant products for pharmacolog purposes. Of the five species of seaweed is the most *H.durvilae's* potential is low because although the antioxidant activity when compared with control BHT, but it is edible, often consumed fresh as a vegetable or used as a salad.

Conclusion

In this study showed that *H. durvilae* and *G.salicornia* extract showed highest extraction efficiency respectively. In 70% metanol extracts have highest value in Total fenol and antioxidant ctivity DPPH, FRAP. *H. durvilae*. showed significantly higher TPC and DPPH than *G. salicornia*, *S. polycystum* and *T. macroloba*. Frap ability of *G. salicornia* showed better than the other sea weeds. Chelating ability Of *S. polycystum*, *T. decurens* and *H. macroloba* performed that highest in 30%.

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