Squalen Bull. of Mar. and Fish. Postharvest and Biotech. 11 (2) 2016, 59-67

www.bbp4b.litbang.kkp.go.id/squalen-bulletin

Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology

ISSN: 2089-5690
e-ISSN: 2406-9272

INVESTIGATION ON ANTIOXIDANT COMPOUNDS FROM MARINE ALGAE EXTRACTS COLLECTED FROM BINUANGEUN COAST, BANTEN, INDONESIA

Muhammad Nursid[1*], Endar Marraskuranto[1], Karren Budi Atmojo[2], Tri Mulyo Pandu Hartono[2], Maria Diyah Nur Meinita[2], and Riyanti[2]

[1]Research and Development Center for Marine and Fisheries Product Competitiveness and Biotechnology
Jalan KS Tubun Petamburan VI, Central Jakarta, Indonesia 10260;
[2]Faculty of Marine and Fisheries Science, University of Jenderal Soedirman, Purwokerto, Indonesia

Article history:
Received: 12 April 2016; Revised: 23 June 2016; Accepted: 18 July 2016

Abstract

Marine algae contains bioactive secondary metabolites that have potential to be developed as antioxidant. The aims of this research were to investigate antioxidant activity and total phenolic compound of marine algae collected from Binuangeun Beach, Banten and to characterize antioxidant compounds from selected algae species. Antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay and Ferric Ion Reducing Antioxidant Power (FRAP) Assay, while total phenolic content was estimated by Folin-Ciocalteau method. Isolation of bioactive antioxidant compound was performed using SiO_2 column chromatography and preparative high performance liquid chromatography (HPLC). The antioxidan activity evaluation was conducted to methanol extracts from 20 species (7 Chlorophyta, 9 Phaeophyta and 4 Rhodophyta). Results showed that *Padina australis* extract was found to be the most active. The major bioactive antioxidant compound was identified as fucoxanthin and a polar compound that was suspected as phenolic compound. The extracts of *P. australis* presented the highest phenolic content (58.59 mg GAE/g). A significant correlation between antioxidant capacity and total phenolic content was found, indicating that phenolic compounds are the major contributors to the antioxidant properties of *P. australis*.

Keywords: marine antioxidant, DPPH, FRAP, fucoxanthin, phenolic compounds

1. Introduction

Indonesia has the second longest coastline that is suitable for seaweed growth and development. The seaweed has the potential to be developed as the source for antioxidant compounds. Antioxidant content in seaweed is associated with seaweed's habitat which is generally waters that are continually exposed to UV from sunlight and oxigenated air. Those will generate free radical or reactive oxygen species (ROS). Yet, being exposed continually to ROS, seaweed structural components (e.g. fatty acids) will not experience oxidative damage. This shows a protective system against oxidative stress inside seaweed cells. By donating an electron, antioxidant compound can neutralize the present of free radical or ROS (Kelman et al., 2012; Mallick & Monh, 2000)

There are several kind of ROS e.g. superoxide radical (O_2), hydroxyl radical (OH), peroxyl radical (ROO) and nitric oxide radical (NOO). They attack biomolecules, such as fat, protein, enzyme, DNA, and RNA which could damage the cell or tissue. ROS induces fat oxidation process and produces oxidant such as heptanol and hexanal that contribute to the damage and oxidative process in food material. This is not only causing food damage (quality retardation), but also strongly related to carsinogenesis, mutagenesis, cancer, etc (Apak et al., 2013; Vijayasbakar & Shiyamala, 2012). Various synthetic antioxidants such as butylatedhydroxyltoluene (BHT), butylatedhydroxyanisole (BHA), propyl gallate (PG) and butylatedhydroquinone are made to overcome this. But those synthetics have side effects especially on liver damage and damaging properties such as mutagenic and neurotoxic (Malecke, 2002). So that it is why people would consider choosing a safer natural antioxidant. Seaweed which is rich in phytochemical such as phenols, flavonoids, and carotenoids began

*Corresponding author.
E-mail: mnursid@kkp.go.id

DOI: http://dx.doi.org/10.15578/squalen.v11i2.243

to be explored as a source of natural antioxidant. Antioxidant function as preventing degenerative diseases such as cancer and tumor, blood vessel constriction, aging, osteoporosis, cardivascular, and atherosclerosis had been reported (Tamat, Wikanta & Maulina, 2007).

Phenolic compound is among of compounds that have antioxidant activity. This compound can prevent degenerative disease especially cardiovascular and cancer (Scalbert, Johnson & Saltmarsh, 2005) through neutralizing free radical mechanism. Derivative carotenoid such as fucoxanthin has antioxidant properties (Nomura, Kikuchi, Kubodera & Kawakami, 1997). anticancer (Yoshiko & Hoyoku, 2007) and anti-ultraviolet (Urikura, Sugawara & Hirata, 2011). The relationship between antioxidant activity and polyphenol compound content was revealed by (Dudonne, Vitrac, Coutiere, Woillez & Merillon, 2009) and Vijayabaskar & Shiyamala, (2012) whereas the relationship between antioxidant activity and fucoxanthin content was revealed by (Nursid, Wikanta & Susilowati, 2013). The ability of polyphenol to inhibit free radical activity is caused by its hydroxyl functional group (Wanga, Jonsdottir & Olafsdottir, 2009) while antioxidant activity of fucoxanthin is caused by the presence of allenic bond structure (Peng, Yuan, Wu & Wang, 2012).

Binuangeun Beach at Lebak District, Banten Province, Indonesia, would be the ideal natural habitat for seaweed growth and development. Preliminary survey result showed that, at least, 22 seaweed species were found in this waters. Exploration on antioxidant and cytotoxic activity as well as fucoxanthin content in brown seaweed from this area was conducted by Nursid et al. (2013). Study conducted by Susilowati et al. (2014) study showed temporal variation of carotenoid compounds from the brown seaweed *Turbinaria decurrens*. Although seaweed has many advantages for food and pharmaceutical industry but research on antioxidant content e.g. phenolic compounds and fucoxanthin in seaweed from Binuangeun Beach has not been conducted yet.

The objective of this research was to investigate antioxidant activity and total phenolic content of seaweed extract from Binuangeun Beach, Banten and also to characterize antioxidant compound from seaweed that has the highest antioxidant activity.

2. Material and Methods

2.1. Collection of the Samples

Seaweeds were collected from Binuangeun Beach, Kabupaten Lebak, Banten at September 2014. Samples were washed thoroughly using freshwater and immediately preserved on ice inside the coolbox. Samples were stored in -20°C shortly after arriving at the laboratory for subsequent analysis. Seaweed identification was conducted in Oseanography Research Center, Indonesian Institute of Sciences, Jakarta.

2.2. Extraction, Fractionation, and Characterization

500 g of seaweed samples were taken out from cold storage and washed thoroughly using freshwater. Samples were macerated for 3 times in 200 mL methanol p.a. Extract was filtered using filter paper and evaporated *in vacuo* until thick extract we obtained. The remaining water and methanol were subsequently dried in vaccuum concentrator until dry extract obtained.

Extraction was conducted using solvent mixture of acetone and methanol (7:3, v/v). The resulting filtrate was partitioned with *n*-hexane, generating two fractions, n-hexane and methanol. The active fraction was fractionated using SiO_2 column chromatography and eluted using solvent mixture of *n*-hexane and acetone with the ratio of 6 : 4 and 1 : 1 and 100% methanol, v/v. Purification of phenolic compound was conducted by preparative HPLC (Shimadzu), characterization of active compound by HPLC (Shimadzu) and LC-qToF-MS (Shimadzu) was used to calculate mass spectra.

2.3. DPPH Assay

Antioxidant evaluation was conducted using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) method according to Sachindra *et al*. (2007) with some modifications. DPPH reagent (Merck) was prepared by diluting 3 mg in 5 mL MeOH (p.a). A methanol diluted seaweed dry extract of 160 µL was poured into 96-well microplate then a 40 µL of DPPH reagent was added (A). Extract control (containing 160 µL of seaweed extract and 40 µL of MeOH) (B), negative control (160 µL MeOH and 40 µL of DPPH reagent) (C) and a blank (200 µL MeOH) (D) were also use in this evaluation. Absorbance of each well was measured using microplate reader (Thermo Scientific) at 517 nm. DPPH free radical inhibition percentage was calculated using the following equation:

$$[(C-D)-(A-B)]/(C-D) \times 100\%$$

Inhibition concentration 50 (IC_{50}) value was calculated using probit analysis. In this evaluation, vitamin C was used as positive control and prepared in the concentration at the range of 1-10 µg/mL.

2.4. FRAP Assay

Antioxidant evaluation using Ferric Ion Reduction Antioxidant Power (FRAP) Assay method was conducted according to Kelman et al. (2012) and Lind et al. (2013). FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ in the ratio 10 : 1 : 1. Upon usage, FRAP reagent was incubated in 37°C for less than one minute. Acetate buffer solution was prepared by diluting 3.1 g of sodium acetate trihidrate in 16 mL acetic acid glacial then aquabidest was added up to the volume of 1 L. TPTZ solution was prepared by diluting TPTZ in 40 mM hydrochloric acid solution. In this analysis, FRAP reagent of 150 µL was poured into 96-well microplate and added with seaweed extract (1 mg/ml dose) for 20 µL. The microplate was incubated in dark room of 27-28 °C for 8 minute, then its absorbance was measured in microplate reader (Thermo Scientific) at the wavelength of 595 nm. Ferrous (Fe^{2+}) standard curve was constructed simultaneously at $FeSO_4 \cdot 7H_2O$ concentration range of 50 - 1000 µM. FRAP value (in µM) was determined using the equation formulated from the standard curve.

2.5. Phenol Content Analysis

Phenol Content Analysis was refered to Anesini et al. (2008). Seaweed methanol extract of 1 mg/ml, 10 % Folin Ciocalteu reagent and 7.5% (w/v) Na_2CO_3, were diluted in aquadest. One mililitre seaweed extract was poured into glass tube then 5.0 ml of Folin Ciocalteu and 4.0 ml of Na_2CO_3 were added. The mixture was incubated at 27-28 °C for 60 min. Absorbance was measured using UV-Vis Spectrometer (Perkin Elmer) at 765 nm. Results were expressed as miligram gallic acid equivalents (GAE)/ g extract. Polyphenol concentration in the extract was determined from gallic acid standard curve for concentration range of 10 - 50 µg/mL.

2.6. Mass Spectrofotometry and Fucoxanthin Analysis

Mass spectrofotometry analysis was conducted by using Liquid Chromatography Mass Spectrophotometer (LC/MS) (Shimadzu) according to Nursid et al. (2016). Fucoxanthin determination was analyzed based on the properties of UV absorbtion and retention time of LC/MS peaks compared to fucoxanthin standard. The analysis of fucoxathin content was calculated base on the standard curve of fucoxanthin.

3. Results and Discussion

3.1. Antioxidant Activity

Approximately 20 seaweed species from Binuangeun Beach of Banten were collected, 7 of which were belonged to Chlorophyta, 9 Phaeophyta and 4 Rhodophyta. FRAP evaluation showed that the highest FRAP value for Chlorophyta was *Chaetomorpha* sp. (11.9 µM/µg), *Padina australis* for Phaeophyta (9.07 µM/µg), and *Hypnea* sp. for Rhodophyta (6.26 µM/µg) (Fig. 1). Ascorbic acid as positive control had FRAP value 76,5 µM/µg. All FRAP values from each seaweed extracts tested were significantly different ($p < 0.01$). In general, Chlorophyta had higher FRAP value compared to Phaeophyta and Rhodophyta.

The antioxidant activity from seaweed extracts also evaluated using DPPH method and was expressed as inhibition percentage. Results showed that *Padina australis* (Phaeophyta) extract of dose 100 µg/mL had the highest antioxidant activity of 53.3% followed by *Sargassum echinocarpum* (Phaeophyta) with 27.97% (Fig 2). Both values were significantly different ($p < 0.01$). *Caulerpa sertularioides* of Chlorophyta showed the highest antioxidant activity (22.9%), while *Hypnea* sp. showed by Rhodophyta (22.79%). In this research, ascorbic acid as a positive control had antioxidant activity of 95% at dose 10 µg/mL.

Different results showed by Kelman et al. (2012) where *Turbinaria ornata* (Phaeophyta) collected from Hawaiian waters had the highest FRAP value compared to *Gayralia oxysperma*, *Chaetomorpha antennina* (Chlorophyta) and *Polysiphonia howei* (Rhodophyta). Boonchum *et al*. (2011) also reported that *T. conoides* collected from Thailand Bay showed highest activity of antioxidant (DPPH and ABTS). In this study, *Chaetomorpha* sp. and *Caulerpa racemosa* had the highest antioxidant activity (FRAP value) for Chlorophyta. It is assumed that the high FRAP value of these species was due to their phenolic compound. Antioxidant activity possesed by tropical seaweed is developed by their ability to build defense system and is reflected from their adaptation to high solar radiation (Zubia, Robeldo, & Pelegri, 2007).

Naturally, seaweed contains antioxidant compound since endogenous antioxidant need is essential for them by the fact that they are intertidal organism. Intertidal organisms build their protection from UV radiation and tidal flux effect. Antioxidant protection of seaweed could be an enzimatic defense such as superoxide dismutase, L-ascorbic acid, glutation, and carotenoids, phlorotannin for Phaeophyta and micosporine for Rhodophyta (Yuan & Wals, 2006).

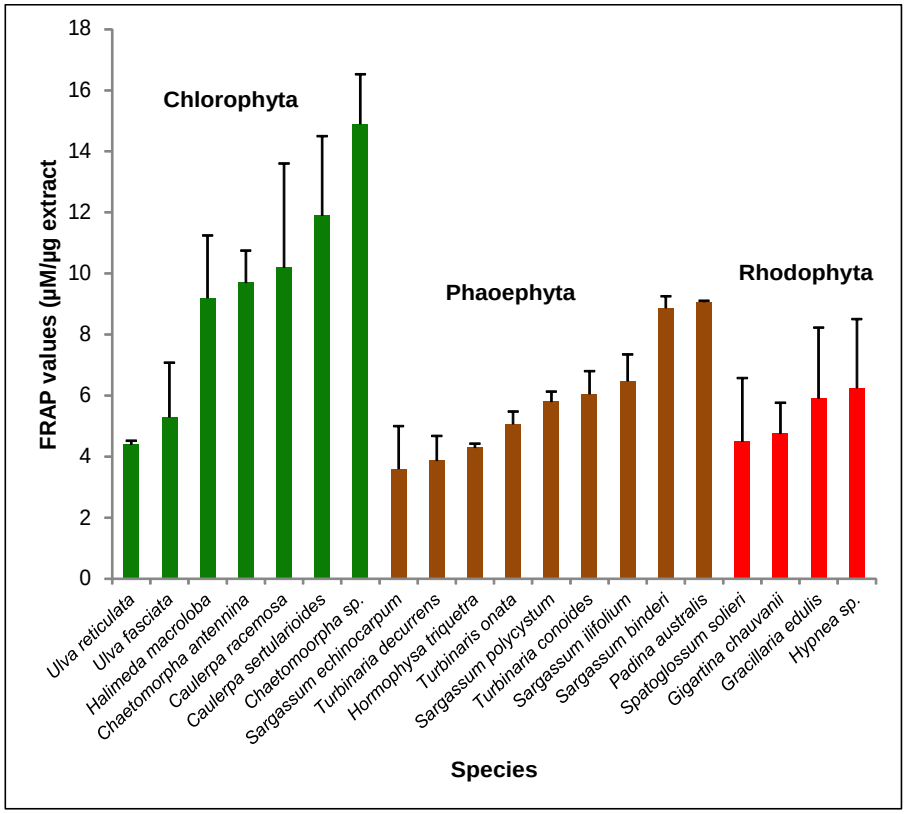

Figure 1. Antioxidant activity of seaweed methanolic extract collected from Binuangeun Beach by FRAP method (error bars ± SD).

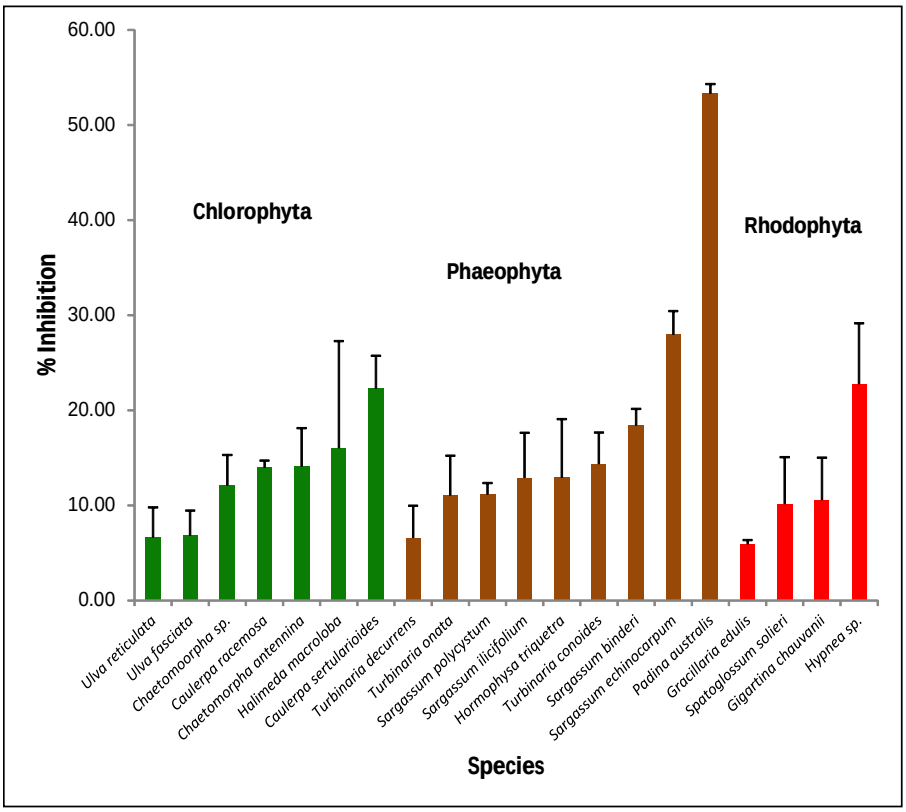

Figure 2. Antioxidant activity of seaweed methanolic extract collected from Binuangeun Beach by DPPH method at dose 100 µg/mL (error bars ± SD).

Active compounds from *Sargasssum* sp. dan *Padina* sp. (Phaeophyta) such as phlorotanins, fucoxanthins, polyphenols and phylopheophylins have a function not only in the metabolism process, but also have a role in controlling free radicals (Foon, Ai, Kuppusamy, Yusoff, & Govindan, 2013).

3.2. Total Phenolic Content

Antioxidant activity correlates to polyphenol content in the extract. *P. australis* had the highest polyphenol content (58.59 mg GAE/g), followed by *C. sertularioides* (28.31 mg GAE/g) and *Hypnea* sp. (22.05 mg GAE/g) (Fig. 3). Total polyphenol content among the extracts were different significantly ($p < 0.01$).

DPPH's antioxidant activity had a significant positive correlation to total polyphenol content ($R=0.865$, $p< 0.01$), also the correlation between FRAP value and total polyphenol content was significant ($R=0.60$, $p< 0.01$). The relationship between total phenolic content and both antioxidant activity of DPPH and FRAP are presented in Fig. 4. Dudonne et al. (2009) showed a strong relationship of them with correlation coefficient value of 0.939 and 0.906, respectively. Stankovic (2011) showed that there was linier correlation between phenolic compound concentration and antioxidant activity using DPPH method ($R = 0.938$). It showed that phenolic compounds in the extract contributed on antioxidant activity. Phenolic compounds have antioxidant properties because of their ability to act as a reducing agent, hydrogen ion donor, and anti radical agent. Phenolic compounds also acts as a metal chelator that protects metal catalytic function in radical initiation process (Wu & Hansen, 2008).

Generally, antioxidant evaluations are classified into two groups based on electron transfer (ET) and hydrogen atom transfer (HAT). DPPH and FRAP antioxidant evaluation belong to ET. In ET-based antioxidant evaluation, antioxidant compound containing extract reacts with fluorescensing compound or another probe compound that causes color reaction. Spectrophotomerically, ET-based evaluation measures antioxidant capacity of an extract to reduce an oxidizing agent indicated by color changes. Therefore, changing color at certain wavelength correlates to antioxidant concentration in the extract (Apak et al., 2013; Huang, Ou, & Prior, 2005).

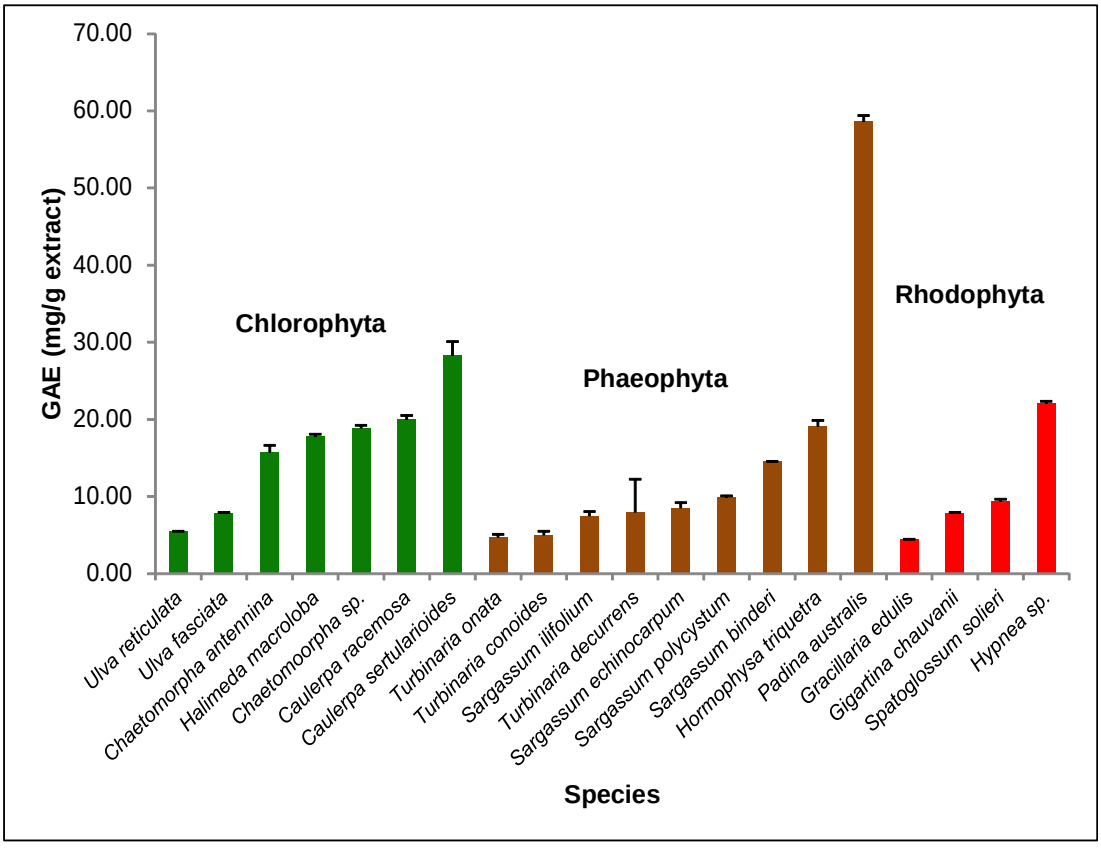

Figure 3. Total phenolic content of seaweed methanolic extract collected from Binuangeun Beach, Banten.

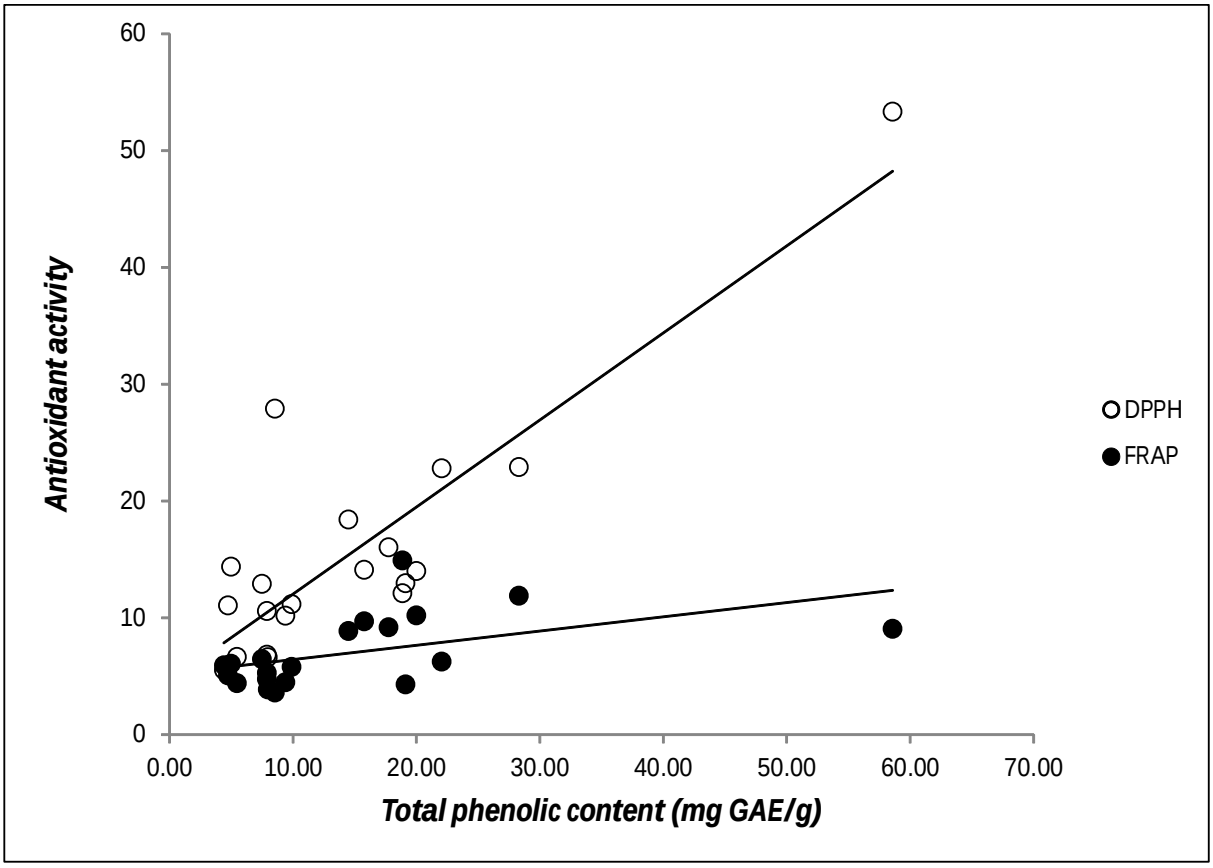

Figure 4. Correlation between antioxidant activity (DPPH and FRAP) and total phenolic content.

FRAP antioxidant evaluation involves electron transfer reaction of which ferric salt, $Fe(III)(TPTZ)_2Cl_3$ acts as an oxidizing agent. The higher the capacity of an extract in reducing Fe^{3+} to Fe^{2+}, the more active the extract. Meanwhile, DPPH antioxidant evaluation is based on the principle that radical DPPH• recives hydrogen atom from antioxidant molecule (hydrogen atom donation from antioxidant molecule). The changing color from violet to yellow means the formation of DPPH during the hydrogen donation. In this case, antioxidant compound containing seaweed extract reduces DPPH color due to its ability to donate hydrogen atom.

3.3. Isolation and Characterization of Antioxidant Compound

Antioxidant screening results showed that *P. australis* had the highest activity among collected seaweeds. In the subsequent study, exploring the main antioxidant compounds was focused on *P.australis*. Antioxidant compound isolation was conducted using DPPH assay guided isolation.

Results from DPPH evaluation of *n*-hexane and methanol fractions showed that *n*-hexane fraction had IC_{50} value higher than 1,000 µg/mL, while methanol fraction had IC_{50} value of 84.7 µg/mL. Subsequently, MeOH fraction was fractionated using SiO_2 column chromatography and was eluted using solvent mixture of *n*-hexane and acetone with the ratio of 6 : 4 and 1 : 1 and finally with 100% methanol. This process resulted four fractions which were then evaluated for its DPPH assay. The result showed that IC_{50} value of fraction F1,F2, F3,and F4 were > 1000 µg/mL, 126.0, 20.5 µg/mL and 72.5 µg/mL respectively. Based on HPLC analysis, F2 was identified as fucoxanthin with UV maximum absorption at 260 nm and 450 nm (Fig. 5) .

Fraction containing fucoxanthin produced yellow color and can be differentiated from the more polar fraction which was showed by green color (Fig. 6). It is assumed that it was another antioxidant active compound of phenolics.

The most active fraction, F5, (IC_{50} = 20.5 µg/mL) was classified as polar compound since it was eluted by polar mixture solvent of acetone : methanol (1 : 1). This fraction was purified using preparative HPLC and resulted five fractions. The DPPH assay results showed that fraction 3 and 4 were active with the IC_{50}

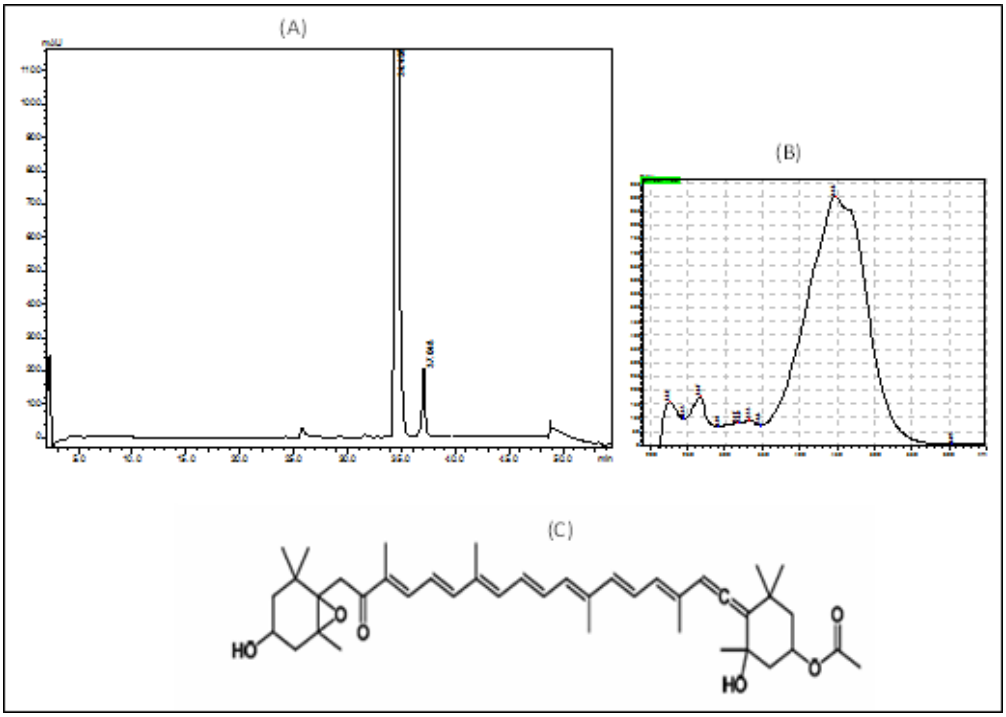

Figure 5. Chromatogram of F2 that identified as fucoxanthin (A), UV absorbance of fucoxanthin (B) and chemical structure of fucoxanthin (C).

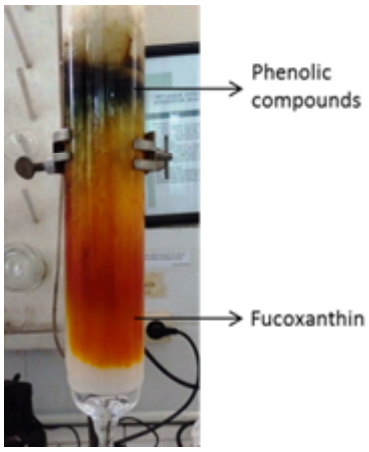

Figure 6. Fucoxanthin and suspected phenolic compound that separated by SiO_2 open column chromatography.

value of 13.4 and 53.2 µg/mL, respectively. Preliminary analysis showed that fraction 5.3 had ion peak at m/z 274.1831 (Fig. 7).

This study resulted two antioxidant compounds isolated from *P. australis* which were fucoxanthin (carotenoid) and unidentified polar compound from fraction 5.3 and 5.4. The aforementioned discussion showed that fucoxanthin had IC_{50} value of 126.0 µg/mL, while fraction 5.3 has more active antioxidant capacity than fucoxanthin (IC_{50} = 13.4 µg/mL). The antioxidant activity of the fraction 5.3 was almost ten times powerful than that of fucoxanthin and its activity almost the same with ascorbic acid (7.58 ±0.74 µg/mL).

The previous study revealed that *P. australis* had a quite high fucoxanthin content with 86.9 mg/gram ethyl acetate fraction (Nursid et al., 2013). This study found that fucoxanthin content was 77.1 mg/g extract. Isolation of fucoxanthin from *P. australis* collected from Malaysian waters found that fucoxanthin had cytotoxic activity against human lung cancer H1299 with IC_{50} value of 2.45 mM (Jaswir, Noviendi, Salleh, Taher, & Miyashita, 2011).

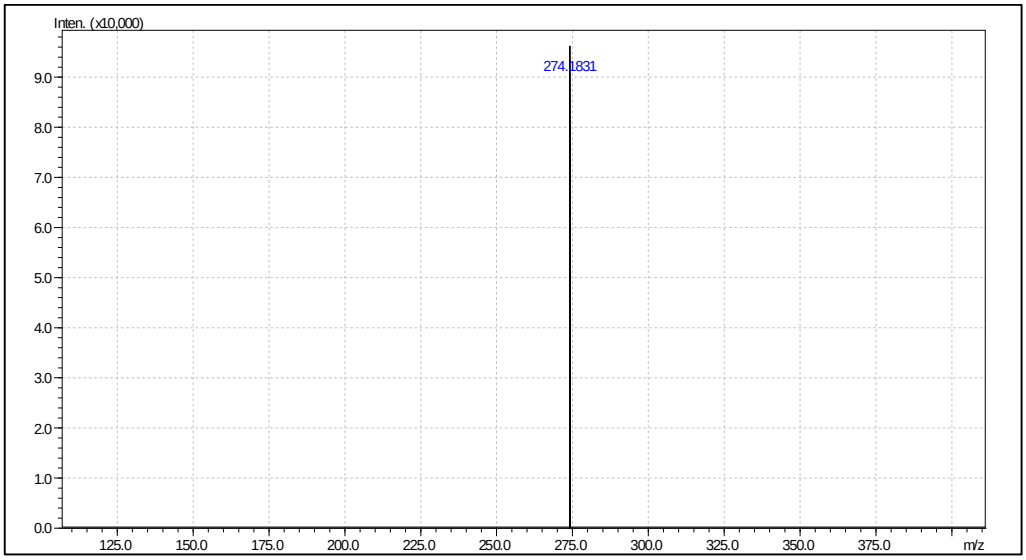

Figure 7. Molecular weight m/z of active isolate that resulted by LC-qToF-MS.

In this study, the high antioxidant activity of *P. australis* most probably was caused by fucoxanthin content and the high concentration of phenolic compound. Combination between these two active compounds caused *P. australis* extract had a strong antioxidant properties. Based on results from this study, fucoxanthin and phenolic compound were selected as standard for determining the quality of *P. australis* raw material for the purpose of, such as, commodity utilization as pharmaceutical or nutraceutical resource material (as standardized herbal medicine or even phytopharmacy). It is also known that *P.australis* has the potential to be developd as natural antioxidant.

4. Conclussion

Among seaweed tested for FRAP evaluation, *Chaetomorpha* sp. (Chlorophyta) had the highest activity (11.9 µM/µg), followed by *P. australis* (Phaeophyta; 9.07 µM/µg), and *Hypnea* sp. of Rhodophyta (6.26 µM/µg). DPPH results at 100 µg/mL dose showed that *P. australis* had the highest antioxidant activity of 53.3% and followed by *S. echinocarpum* (Phaeophyta) 27.97%. For Chlorophyta, *C. sertularioides* (22.9%) had the highest activity, while *Hypnea* sp. activity for of Rhodophyta possesed the highest (22.79%). *P.australis* had the highest total phenolic content (58.59 mg GAE/g). It was evident that antioxidant activity correlated to total phenolic content. Antioxidant major compound in *P. australis* was identified as fucoxanthin (carotenoid) which was suspected caused by polar compound of phenolic group. It was also evident that total phenolic content correlated to antioxidant activity and it also showed that this compound contributed to the antioxidant activity of seaweed.

5. Acknowledgment

This research was funded by the Ministry of Marine Affairs and Fisheries of Indonesia in 2014. We thank to Ms. Sri Iswani for her support on LC/MS analysis.

References

Anesini, C., Ferraro, G.E., & Filip, R. (2008). Total polifenol content and antioxidant capacity of commercially available tea (*Camelia sinensis*) in Argentina. *J. Agric. Food Chem. 56*, 9225–9229.

Apak, R., Gorinstein, S., Böhm, V., Schaich, K,M., Özyürek, M., & Güçlü, K. (2013). Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). *Pure Appl. Chem. 85*(5), 957–998.

Boonchum,W., Peerapornpisal,Y., Kanjanapothi,D., Pekkoh,J., Pumas,C., Jamjai,U., Amornlerdpison,D., Noiraksar,T., & Vacharapiyasophon, P. (2011). Antioxidant activity of some seaweed from the gulf of Thailand. *Int. J. Agric. Biol. 13*, 95–99.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., & Merillon, J,M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agric. Food Chem. 57*, 1768–1774.

Fung, A.Y.C. (2012). The fucoxanthin content and antioxidant properties of *Undaria pinnatifida* from Marlborough Sound, New Zealand. Thesis. Auckland University of Technology University. 78 pp.

Foon, T,S., Ai, L,A., Kuppusamy, P., Yusoff, M,M., & Govindan, N. (2013). Studies on in-vitroantioxidant activity of marine edible seaweeds from the east coastal region of Peninsular Malaysia using different extraction methods. *Journal of Coastal Life Medicine 1*(3), 193–198

Huang, D., Ou, B., & Prior, R,L. (2005). The Chemistry behind antioxidant capacity assay. *J. Agric. Food Chem*, *53*, 1841–1856.

Jaswir, I., Noviendi, D., Salleh, H,M., Taher, M., & Miyashita. (2011) Isolation of fucoxanthin and fatty acids analysis of *Padina australis* and cytotoxic effect of fucoxanthin on human lung cancer (H1299) cell lines. *Afr. J. Biotechnol. 10*(81),18855–18862.

Kelman, D., Posner, E,K., McDermid, K,J., Tabandera, N,K., Wright, P,R., & Wright, A.D. (2012) Antioxidant activity of Hawaiian marine algae. *Mar. Drugs*, *10*, 403–416.

Lind, K,F., Hansen, E., Østerud, B., Eilertsen, K,E., Bayer, A., Engqvist, M., Leszczak, K., Jørgensen, T,O., & Andersen,J,H. (2013) Antioxidant and anti-inflammatory activities of barettin. *Mar. Drugs 11*, 2655–2666.

Mallick, N & Mohn, F.H. (2000). Reactive oxygen species: Response of algal cells. *J. Plant. Physiol. 157*, 183–193.

Malecke, M. (2002). Antioxdant properties of the unsaponifiable matter isolated from tomato seeds, oat grains and wheat germ oil. *Food Chemistry*, *79*: 327-330.

Nomura, T., Kikuchi, M., Kubodera, A., & Kawakami, Y. (1997). Proton-donative antioxidant activity of fucoxanthin with 1,1-diphenyl-2-picrylhydrazyl (DPPH). *Biochem. Mol. Biol. Int. 42*(2), 361–70.

Nursid, M., Wikanta, T., & Susilowati,R. (2013). Antioxidant activity, cytotoxicity and fucoxanthine content of macrolagae collected from Binuangeun Beach, Banten Indonesia. *J . Mar. Fish Post Harvest Biotechnol. 8*(1),73–83.

Nursid,.M., Maharani, A.P., Riyanti., & Marraskuranto, E. (2016). Cytotoxic activity and secondary metabolite characteristics of sea cucumber *Actinopyga* Sp. methanolic extract. *Squalen Bull of Mar and Fish Postharvest and Biotech. 11* (1) 23–30.

Peng, J., Yuan, J,P., Wu, C,F., & Wang, J,H. (2011). Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. *Mar. Drugs 9*, 1806–1828.

Sachindra, N,M., Sato, E., Maeda, H., Hosokawa, M., Niwano, Y., Kohno., & Miyashita, K. (2007). Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *J. Agric. Food Chem. 55*, 8516–8522.

Scalbert, A., Johnson, I,T., & Saltmarsh,M. (2005). Polyphenol: antioxidantand beyond. *Am. J. Clin. Nutr. 81*, 2155–217S.

Stankovic, M,S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extract. *Kragujevac J Sci* 33, 63–72.

Susilowati, R. & Januar, H.I. (2014). Temporal variation physical and chemical stability of carotenoids bioactive compounds from the brown alga *Turbinaria decurrens*. *J. Mar. Fish. Post Harvest Biotechnol. 9* (1), 21–28.

Tamat, S,R., Wikanta, T., & Maulina, L,S. (2007). Antioxidant activity and toxicity of bioactive compound of *Ulva reticulata* green macroalgae. *Indones. J. Pharm. 5*, 31–36.

Urikura,I., Sugawara,T., & Hirata,W. (2011). Protective effect of fucoxanthin against UVB-induced skin photoaging in hairless mice. *Biosci. Biotechnol. Biochem*, *75* (4),757–760.

Vijayabaskar,P., Shiyamala,V. (2012) . Antioxidant properties of seaweed polyphenol from *Turbinaria ornata* (Turner) J. Agardh, 1848. *Asian Pac J Tropl Med*, S90–S98.

Wanga, T., Jonsdottir, R., & Olafsdottir, G. (2009). Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem*, *116*, 240–248.

Wu, X, J. & Hansen, C. (2008). Antioxidant capacity, phenolic content, polysaccharide content of *Lentinus edodes* grown in Whey permeate based submerged culture. *J. Food Sci.* 73, 434–438.

Yoshiko, S., & Hoyoku, N. (2007). Fucoxanthin, a natural carotenoid, induces G1 arrest and GADD45 gene expression in human cancer cells. *In Vivo 21*, 305–310.

Yuan, Y,V. & Wals, N,A. (2006). Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food Chem. Toxicol*, *44*, 1144–1150.

Zubia, M., Robledo, D., & Pelegri,Y,F. (2007). Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. *J. Appl. Phycol. 19*, 449–458.