

Aktivitas Antioksidan dan Analisis Proksimat Bubuk Kering Alga Cokelat *Sargassum hystrix*

Antioxidant Activity and Proximate Analysis of Dry Powder from Brown Seaweed *Sargassum hystrix*

Radipta Lailatussifa*, Amir Husni & Alim Isnansetyo

Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada

Jl. Flora Gedung A4 Bulaksumur, Yogyakarta, 55281, Indonesia

*Penulis untuk korespondensi, e-mail: rr.radipta@gmail.com

Abstrak

Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan, kandungan total fenol, kandungan nutrisi, dan kandungan fitokimia dari bubuk kering *Sargassum hystrix*. Ekstrak air dari sampel kering alga cokelat (*S. hystrix*) disiapkan dan diuji kandungan total fenolnya. Jumlah kandungan total fenol ekstrak air yaitu 11,43 g *Gallic Acid Equivalent* (GAE)/100 g berat kering sampel dengan menggunakan metode Folin-Ciocalteu. Aktivitas antioksidan (*Radical Scavenging Activity*/RSA) BHT yang digunakan sebagai standar tergolong tinggi, penghambatan radikal DPPH pada 1 mg sampel kering/ml pelarut yakni 96%. Nilai IC_{50} BHT adalah sebesar $0,227 \pm 0,001$ mg sampel kering/ml pelarut, sedangkan aktivitas penangkapan radikal (RSA) bubuk kering *S. hystrix* sebesar 65,28% pada 1 mg sampel kering/ml pelarut. Nilai IC_{50} bubuk kering *S. hystrix* adalah sebesar $0,616 \pm 0,005$ mg sampel kering/ml pelarut. Kandungan nutrisi dan komponen bioaktif bubuk kering *S. hystrix* seimbang untuk mendukung aktivitas antioksidan sampel. Hasil ini menunjukkan bahwa alga tersebut potensial sebagai sumber antioksidan alami.

Kata kunci: Aktivitas antioksidan, bubuk kering *Sargassum hystrix*, fitokimia, kandungan fenolik, kandungan nutrisi

Abstract

This research aims to know antioxidant activity, total phenolic, nutrient, and screening phytochemistry compounds of *S. hystrix* dry powder. Water extract from the dried sample of brown algae (*S. hystrix*) was prepared and examined for its phenolic compounds. The amount of total phenolic compounds in water extract was about 11.43 g *Gallic Acid Equivalent* (GAE)/100 g of dry basis sample, as measured by using Folin-Ciocalteu method. The antioxidant activity (*Radical Scavenging Activity*/RSA) of BHT as a standard was high about 96% inhibition of radical DPPH with 1 mg dry sample/ml solvent. The IC_{50} of the BHT was 0.227 ± 0.001 mg of dry sample/ml solvent. While the radical activity (RSA) of *S. hystrix* dry powder was about 65.28% at 1 mg dry sample/ml solvent. The IC_{50} of *S. hystrix* dry powder was 0.616 ± 0.005 mg of dry sample/ml solvent. Nutrient contents and bioactive compounds of dry powder *S. hystrix* were balanced to support antioxidant activity of the sample. The results suggest that this alga is a potential source of natural antioxidant.

Keywords: Antioxidant activity, *Sargassum hystrix* dry powder, phytochemistry, the phenolic, compound nutrient contents

Introduction

Free radicals are chemical species like atoms or unstable and very reactive molecules with one or two unpaired electrons in their outermost layer, which can be created in multiple ways. They can be exogenic (e.g. pollution, radiation ultraviolet, infections, tobacco) or endogenic. Free radicals will react with surrounding molecules to get it electron pairs to reach the stability of the atom. That reaction occurs continually in the body, so it can dangerous for the body if the amount of antioxidant in the body less than

reactive oxygen species. It can trigger oxidative stress, so it developed a number of serious illness, such as certain cancers, atherosclerosis, cardiovascular disease, cataract, age-related degenerative disease, and others degenerative disease likes diabetes mellitus, glaucoma, Parkinson's, and Alzheimer's diseases (Barhe & Tchouya, 2016). So it needs the possible therapeutic value of antioxidant from external sources of the body to reduce free radicals in the body and against these illnesses. The provision of antioxidants through diet is a simple means to reduce

the development of illnesses brought on by oxidative stress (Zafra-Stone *et al.*, 2007).

The importance of vegetables as part of a healthy diet is generally accepted. As a consequence of an increasing demand for biodiversity in seeking therapeutic drugs from natural products, there is now a greater interest in marine organisms, especially algae. Seaweeds or marine algae are primitive non-flowering plants without true root stem and leaves (Moubayed *et al.*, 2016). They are kinds of sea vegetables which have a potential of antioxidant sources. They are divided into three major groups, such as Rhodophyta (red algae), Chlorophyta (green algae), and Phaeophyta (brown algae). The prosperity as antioxidant sources is depending on their nutrient and chemical composition.

Antioxidant sources of some foods or diets can come from the presence of vitamins C and D, carotenes, folates, selenium, sulfated polysaccharides likes fucoidan (Chun-Yung *et al.*, 2016; Hifney *et al.*, 2016; Chao *et al.*, 2017), and bioactive compounds including flavonoid, saponins (Tuo *et al.*, 2015), terpenoid, phenols, steroid, alkaloids, and tannins (Zahra *et al.*, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2010; Moubayed *et al.*, 2016). Seaweeds are known to contain a wide variety of bioactive compounds as such offering a rich source of new drugs with potentially lower toxicity.

Much prior research using the extract of brown seaweed as the source of antioxidant. A variety of brown algae, *Sargassum hystrix* are known to has the highest bioactive compounds than the others (Boonchum *et al.*, 2011). Many research about antioxidant still used extract or fraction to test the value of antioxidant, so it needs complex and expensive tools to get adaptogens for human diets. The present study aimed to know the value of antioxidant compound, total phenolic content, nutrient content and screening phytochemistry of dry powder from *S. hystrix* brown algae as the source of functional food.

Materials and Method

Collection and Preparation of Algae

The brown algae *S. hystrix* J. Agardh was collected from the intertidal zone, Sepanjang coastal, Gunungkidul, Yogyakarta, Indonesia. It was then packed in coolbox and out of the sun after that brought to the laboratory Fisheries Departement of Universitas Gadjah Mada and washing it with flow water. Sample dried at room temperature (± 26 °C) for 5-7 days. The

dried sample was then cut into small pieces of $\pm 0,5$ cm using scissors and blended. While fresh seaweed was identified to Plant Taxonomy Laboratory, Biology Faculty, Universitas Gadjah Mada, Indonesia. Dried seaweed powder was saved in the freezer before used for phytochemistry analysis, total phenolic content, radical scavenging activity (% RSA) and proximate analysis. Phytochemistry analysis was analyzed using the method described by Harborne (1995).

Total Phenolics Content

The total phenolic content in the *S. hystrix* dry powder was determined by the spectrophotometric method based on procedures described by Kang *et al.* (2010). Gallic acid used as a standard solution. Gallic acid concentration 1 mg/ml diluted into several concentration, such as 200; 100; 50; 25; 12.5; and 6.25 $\mu\text{g/ml}$. Then, dry powder of sample 5 mg diluted into aquadest 1 ml and diluted as much as 6x series, ie concentrations of 1,000 $\mu\text{g/ml}$, 500 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$, 125 $\mu\text{g/ml}$, 62.5 $\mu\text{g/ml}$, and 31.25 $\mu\text{g/ml}$. Each concentration of a standard solution and sample extract were taken 10 μl , after that put down in the *microplate 96-well plate* and adding by Folin Ciocalteu reagent 50 μl and incubated for 5 minutes. Furthermore, it added by 7.5% Na_2CO_3 40 μl and incubated for 2 hours in the darkroom at room temperature. After that, read the absorbance using *spectrophotometric microplate* (ELISA reader) on 750 wavelength. Made curve with plotted concentration ($\mu\text{g/ml}$) vs absorbance (nm). The equation of regression standard curve was obtained about $y=ax+b$; $R^2=c$, where x is concentration and y is absorbance. Total phenolic content found in gram GAE (*Gallic Acids Equivalent*) per 100 g sample powder.

Measurement of DPPH (1,1-diphenyl-2-picrylhydrazil) (% RSA)

The scavenging activity of the powder *S. hystrix* on the DPPH based on the procedure described by Khalaf *et al.* (2008) with some modifications. 0.136 mM DPPH 50 μl in the methanol was mixed into powder sample in the methanol solution at concentration of 31.25; 62.5; 125; 250; 500; and 1,000 $\mu\text{g/ml}$. Then it was put in the dark room on the room temperature for 30 minutes. The absorbance was measured with a *spectrophotometric microplate* (ELISA reader) at 517 nm wavelength. BHT used as comparator represent used of commercial or synthetic antioxidant. Control in the absorbance measurement was prepared 100 μl methanol without adding powder sample and set as 0% absorbent. Percentage (%) of free radicals scavenging were measured with the formula:

$$\% \text{RSA} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

Furthermore, graphics made between sample concentration (x) with % inhibition (y). The value of IC₅₀ was measured based on the regression equation.

Proximate Analysis

Sargassum hystrix dry powder has analyzed the compounds of protein, fat, water, and dietary fiber in it. Protein content was analyzed used Micro-Kjeldahl method described by AOAC (1984). Percentage (%) of protein was measured with the formula:

$$\%Protein = \%N \times correction\ factor$$

$$\%N = \frac{(ml\ HCl - ml\ blanko) \times normality \times 14.007 \times 100\%}{mg\ sample}$$

Fat content was analysis used Soxhlet method with the formula:

$$\%Fat = \frac{flask\ fat\ extract\ after\ oven\ at\ 105^{\circ}C - empty\ flask}{5\ gram\ sample} \times 100\%$$

Water content was analysis with method described by AOAC (2005) with the formula:

$$\%Water\ content = \frac{W_0 - W_t}{W_t} \times 100\%$$

While W₀ = weight of sample and cup early; W_t = weight of sample and cup end.

Dietary fiber was analyzed with the method of AOAC 2009.01 dan 2011.25.

Data Analysis

Analytical values represent means of three independent experiments each with duplicate measurements. Duncan's when needed was used to assess the significant difference between dry powder of *S. hystrix* and BHT as a standard sample. Differences among (or between) sample means were reported to be significant when P < 0.05.

Results and Discussions

Screening Phytochemistry

The phytochemical analysis is important in the evaluation of bioactive compounds from medicinal plants (Tuo *et al.*, 2015). Qualitative compounds of *S. hystrix* dry powder are shown in Table 1. Screening phytochemistry was included many types of organic compounds made and saved by the organism, such as chemical structure, biosynthetic, alteration and its metabolism, natural dissemination and biology function, isolation and ratio of chemical composition from plants (Sirait, 2007). It aims to determine the characteristics of bioactive compounds from samples or crude extract which have toxic effect or pharmacology effect that useful if tested with biology system or bioassay (Harborne, 1987). Besides that, these result will be helpful to phytochemists and pharmacologists for identification of new active

Table 1. Screening phytochemistry of *S. hystrix* powder.

Phytochemistry Test	Reagent	Result
Alkaloid	Mayer Wagner Dragendorf	+
Steroid	Glacial acetic acid+H ₂ SO ₄	+
Terpenoid	Glacial acetic acid+H ₂ SO ₄	+
Phenol	FeCl ₃ 5%	+
Tannin	FeCl ₃ 1%	+
Saponine	Water+HCl	+

compounds from plants (Ankanna *et al.*, 2012).

Alkaloid was detected in each solvent, such as Meyer, Wagner, and Dragendorf. Alkaloids are generally crystalline or amorphous powders and generally have a bitter taste and can produce precipitate with iodide form from Hg, Au, and other heavy metals as the basis for the identification of these compounds (Coria-Tellez *et al.*, 2016). Alkaloid which was consisted of *S. hystrix* dry powder has the function to muffle DPPH free radicals so it can trigger decreasing of IC₅₀ values in the samples (Nurjanah *et al.*, 2011). Besides that, alkaloid plays a part in the source of basic substance anti-malaria medicine (Radji, 2005). Steroid and terpenoid were detected in *S. hystrix* dry powder with the solvent of glacial acetic acid and sulfuric acid. The steroid has several functions, such as increasing body stamina effect (aphrodisiac) and anti-inflammation. While triterpenoid compound has functioned as the anti-tumor activity because it can hamper activity of topoisomerase II enzymes by binding with the active side of enzymes and binding DNA, so the enzymes would be locked and couldn't binding DNA (Setzer, 2008). Terpenoids are formed from a sequential assembly of five carbon building blocks (C₅H₈) called isoprene units (Lei *et al.*, 2016). *S. hystrix* dry powder also contains phenolic and tannin content which detected by 5% and 1% FeCl₃ reagent.

Phenolic content and tannin content have been known as a strong antioxidant effect, antidiabetic, antitumor, anticancer, anti-inflammation, antiaging, anti-hepatotoxic, anti-stress, anti-hyperlipidemic, and an anti-hypertension (Balboa *et al.*, 2013; Lailatussifa *et al.*, 2016; Pei *et al.*, 2015). Saponin was detected in *S. hystrix* dry powder with the solvent of water and chloride acid. In algae, saponin has functioned as antioxidant effect via facilitating general neurotransmitter inhibitory effect of GABA system, reduced brain levels of adrenaline and noradrenaline,

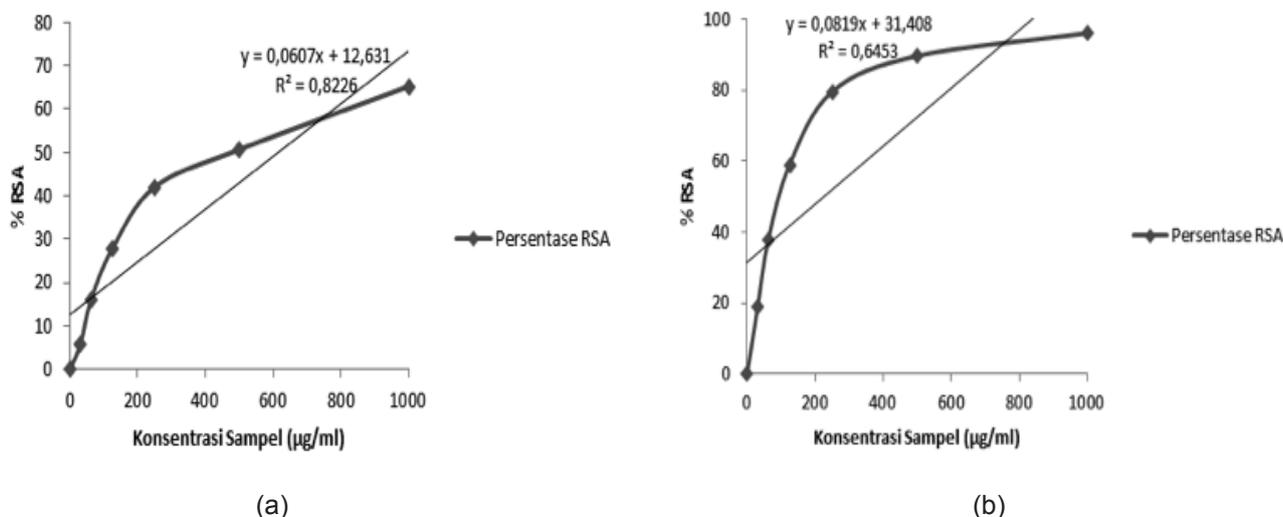


Fig. 1. RSA Percentation of (a) BHT and (b) *S. hystrix* powder.

Table 2. Value of IC_{50} *S. hystrix* dry powder vs BHT.

Sample	IC_{50} Value (mg/ml)	IC_{50} Value (ppm)
BHT	0.227±0.001 ^{a1)}	227.02±1.30 ^a
<i>S. hystrix</i> dry powder	0.616±0.005 ^b	616.22±4.80 ^b

¹⁾ Values with different letters in the same column indicate significant difference ($p < 0.05$)

and reduction in both cerebral metabolic rate for oxygen and cerebral blood flow (Piffare *et al.*, 2015).

Antioxidant Activity

The result of antioxidant activity used DPPH method are shown in Fig. 1. The relative index % RSA only indicated the capacity of the sample, at a given concentration, to reduce the radicals, and in many cases, the increase in the concentration of the antioxidant leads to an increase in the relative indices (Bahre & Tchouya, 2016). Presentation of RSA from *S. hystrix* dry powder at concentration 1000 ppm (1 mg/ml) was 65.28%. Its content was increased when absorbance-concentration were increasing. That value was equal to % RSA from crude extract of fucoidan *S. glaucescens*, with the value of 65% at the same concentration (Chun-Yung *et al.*, 2016), but lower than % RSA from crude extract of *S. latifolium* was about 66% at concentration 0.15 mg/ml and *S. platycarpum* was about 60% at concentration 0.15 mg/ml (Moubayed *et al.*, 2016). Meanwhile, that value was higher than radical scavenging activity (% RSA) of *S. boveanum* extract at concentration 3 mg/ml was about 94% (Zahra *et al.*, 2007), sulfated polysaccharides extract of *S. vulgare* was about 22.2% at concentration 2.5 mg/ml (Dore *et al.*, 2013), crude polysaccharides extract of *S. pallidum* was

about 19.1% at concentration 3.8 mg/ml (Hong *et al.*, 2008), polyphenol extract of *S. polycystum* was about 38.60% at concentration 1 mg/ml (Cahyaningrum *et al.*, 2016), it also higher than DPPH scavenging activity of Ethyl Acetate fraction from *S. marginatum* was about 23.16%, Petroleum Ether fraction of *Turbinaria conoides* was about 19.55%, Petroleum Ether fraction of *Padina tetrastomatica* was about 17.79%, methanolic extract of *T. conoides* was about 17.35%, methanolic extract of *P. tetrastomatica* was about 14.78%, and methanolic extract of *S. marginatum* was about 11.00% at concentration 1000 ppm (Chandini *et al.*, 2008).

However, radical scavenging activity (% RSA) of BHT (*Butylated Hydroxy Toluene*) as standard comparator had the highest value of DPPH radical scavenging activity (% RSA), its value was about 96% at concentration 1 mg/ml. So, DPPH scavenging activity (% RSA) of *S. hystrix* dry powder was higher than several species of brown algae. It caused by the compounds of dry powder *S. hystrix* more complete, it contains primary and secondary metabolites which have the function to reduce free radicals. But, at a different concentration of the same species, % RSA of *S. hystrix* dry powder still lower than the membrane-bound extract of *S. hystrix* in Budhiyanti *et al.* (2012) research, with the value 48.71% at concentration 0.45 mg/ml.

To eliminate the influence of the concentration, the second approach is to estimate the reactivity by determining the coloring intensity IC_{50} of each antioxidant. The IC_{50} is the concentration of DPPH corresponding to the optical change in optical density caused by a change of 50 ppm of the antioxidant (Bahre *et al.*, 2016). The calculation of IC_{50} value requires the determination of the kinetics of the

reaction between DPPH and different concentration of the antioxidant (Dawidowicz *et al.*, 2012). IC₅₀ is similar to EC₅₀ or LD₅₀ in biological measurements. This parameter is defined as the amount of antioxidant necessary to decrease the absorbance of DPPH by 50% of the initial absorbance (Mishra *et al.*, 2012). The smaller amount of antioxidant necessary to decrease 50% radical DPPH, it is more effective and reactive of the antioxidant sample to work. The IC₅₀ value of *S. hystrix* dry powder was about 0.616±0.005 mg/ml or 616,22±4.80 ppm. That value was higher than IC₅₀ value from phlorotannin extract of *S. polycystum* was about 1.20±0.01 mg/ml, polyphenol extract of *S. polycystum* was about 1.27±0,01 mg/ml (Cahyaningrum *et al.*, 2016), water extract of *S. boveanum* was about 3.82 mg/ml (Zahra *et al.*, 2007), sulphated polysaccharides of *S. filipendula* was about 1000 ppm (Costa *et al.*, 2011), sulphated polysaccharides of *Sargassum* sp. was about 800 ppm (Ale *et al.*, 2011), sulphated polysaccharides of *Fucus vesiculosus* was about 800 ppm (Suresh *et al.*, 2013), sulphated polysaccharides of *S. pallidum* was about 1,000 ppm (Ye *et al.*, 2008), and sulphated polysaccharides of *S. plagiophyllum* was about 700 ppm (Suresh *et al.*, 2013). IC₅₀ value of *S. hystrix* dry powder was lower than sulphated polysaccharides of *Ecklonia cava* was about 43.9-100 ppm (Athukorala *et al.*, 2009), sulphated polysaccharides of *Cladosiphon okamuranus* was about 100 ppm (Teruya *et al.*, 2007), and *S. hystrix* extract was about 0.33±0.03 mg/ml (Budhiyanti *et al.*, 2011).

However, the IC₅₀ value of *S. hystrix* dry powder was lower than BHT as standard, that was about 0.227±0.001 mg/ml or 227.02±1.30 ppm. The value of IC₅₀ relatively strong if the value ranges between 50-100 ppm (Khotimah, 2013). However, Suresh *et al.* (2013) mentioned that brown algae with the IC₅₀ value of 1000 ppm were potential to be used as in vitro anticancer. High and low IC₅₀ values are influenced by several factors, such as the solvents used, the amount of dissolved bioactive components (Molyneux, 2004), harvest seasons, harvest sites, and species (Budhiyanti *et al.*, 2012).

Total Phenolic Content (TPC)

S. hystrix dry powder was analyzed for total phenolic content to determine the number of phenols contained in the algae. Phenolic activity compound derived from the number of hydroxyl groups on the benzene ring (Dhianawaty & Ruslin, 2015). Total phenolic content analysis performed using the Folin-Ciocalteu reagent and the comparative form of gallic acid. Folin-Ciocalteu method is a method commonly used to measure the antioxidant capacity of natural products. This method is based on the reduction of *phosphomolybdic-tungstic* chromogen by antioxidants

and produces a color change which is measured at 750 nm absorbance (Agbor *et al.*, 2005). The higher amount of phenolic hydroxyl group, the greater the concentration of phenolic component are detected. The principle of Folin-Ciocalteu method is oxidation and reduction colorimetric to measure all of phenolic

Table 3. Total phenolic content and % RSA of *S. hystrix* dry powder.

Concentration of Sample (ppm)	Total Phenol (mg GAE/g dry basis)	% RSA
0	0	0
31.25	21.40±1.97	5.92
62.5	30.21±6.61	16.16
125	40.57±2.70	27.84
250	45.33±5.21	42.08
500	62.12±6.13	50.72
1,000	114.26±10.25	65.28

compounds in the test sample (oxidation of phenolic hydroxyl group) (Khadambi, 2007). Gallic acid used as the standard because it is stable and has strong antioxidant activity because of the hydroxyl group in it (Daneshfar *et al.*, 2008).

The total phenolic content of *S. hystrix* dry powder is shown in Table 3. It showed that the higher concentration of the sample, the total phenolic content would increase. Total phenolic content at concentration 1000 ppm was 114.26 mg GAE/g dry basis=11.43 g GAE/100 g dry basis. The result of total phenolic content of *S. hystrix* powder was higher than several kinds and species of else brown algae, likes *S. muticum* was 230.8±17.1 mg GAE/100 g dry basis (Farvin & Jacobsen, 2013), *S. vulgare* was 7.09 g GAE/100 g extract (Plaza *et al.*, 2010), *E. cava* was 8.30 g GAE/100 g extract (Senevirathne *et al.*, 2006), *P. pavonica* was 1.076±0.87 g GAE/100 g extract (Khaled *et al.*, 2012), *S. binderi* was 0.03 g GAE/100 g extract (Boonchum *et al.*, 2011), *S. horneri* was 2.08 g GAE/100 g extract (Airanthi *et al.*, 2011), and *T. conoides* was 0.11 g GAE/100 g extract (Boonchum *et al.*, 2011). But, total phenolic content of *S. hystrix* powder in this research was lower than total phenolic content of *S. hystrix* extract was about 49.46 g GAE/100 g dry extract (Pratiwi, 2013), *S. polycystum* was 59.30 g GAE/100 g extract (Lailatussifa *et al.*, 2016), *S. kjelmanianum* was 16,3 g GAE/100 g extract and *S. thunbergii* was 11,5 g GAE/100 g extract (Luo *et al.*, 2010).

The high and low total phenolic content were influenced by the internal factor (species, harvest site, and age of sample) also the external factor

(temperature, climate, depth, salinity, tidal zone, and tidal cycle) (Lann *et al.*, 2012). Increasing the value of total phenolic content was directly proportional with increasing of % RSA (Table 3.3). It was indicated that accumulation value of the total phenolic content could trigger free radicals catching ability of a sample. Andayani *et al.* (2008) mentioned that the phenolic compounds contained in plants had antioxidant activity because these compounds could capture peroxide radicals and ferrous metals which catalyzed fat peroxide.

Proximate Analysis

Proximate analysis is an analysis to predict the chemical compounds of the substance. Proximate analysis of *S. hystrix* dry powder is shown in Table 4. Analysis of nutrient content was included water content, protein content, fat content, and fiber content of food. The water content in the sample of food was determined acceptability, freshness, and endurance it substance (Winarno, 2008). Water content obtained in the *S. hystrix* dry powder was 13.43±0.15%. This value was lower than water content of *S. hystrix* in the research of Solarin *et al.* (2014), was 14.33%. However, this value is still eligible for the water content of dried algae by SNI, it was 30% (BSN, 2006). The protein content of *S. hystrix* dry powder was 6.54±0.04%. Its value was equal to the research of Solarin *et al.* (2014), was 6.55%. Makkar *et al.* (2016) mentioned that *Sargassum* sp. brown seaweed has low to moderate amounts of crude protein, it was 6-11%. The protein content of marine algae has varied greatly with species, seasons and nutrients (Stengel *et al.*, 2011). The fat content of *S. hystrix* dry powder was 0.05±0.02. This content was lower than the research of Solarin *et al.* (2014), was 1.9%. But its value still eligible for the standard fat content of *Sargassum* sp., it was 0,5-3,9% (Dawczynski *et al.*, 2007).

Brown seaweeds contain contain few lipids (1-5%), but a majority of those lipids are polyunsaturated n-3 and n-6 fatty acids (Makkar *et al.*, 2016). Brown algae have a balanced $\omega 6/\omega 3$ ratio (0.6-5.1:1), considering that in a healthy human diet the ratio of $\omega 3/\omega 6$ should not exceed 10:1. Differences in the fatty acids composition among species and intraspecies variations in environmental and geographical factors (Balboa *et al.*, 2013). The dietary fiber content of *S. hystrix* dry powder was 31.53±0.18%. This value was higher than the research of Solarin *et al.* (2014), was 17%. The mineral composition and proximate content of brown algae dependent on environmental, geographical, physiological factors and postharvest conditions have been evaluated (Balboa *et al.*, 2013).

Table 4. Proximate analysis of *Sargassum hystrix* dry powder.

Test Type	Content (%)
Water Content	13.43±0.15
Protein Content	6.54±0.04
Fat Content	0.05±0.02
Dietary Fiber Content	31.53±0.18

Conclusion and Recommendation

Conclusion

S. hystrix dry powder had antioxidant activity with the IC_{50} value was 0.616±0,005 mg/ml. Antioxidant activity of the sample supported by a variety of bioactive compounds, such as alkaloids, steroids, terpenoids, phenols, tannins, and saponins for chelating free radicals and balanced nutritional composition that has potential as a functional food. The total phenolic content of the algae powder was directly proportional to the antioxidant activity (%RSA) in the samples with increasing of sample absorbance. It means the amount of total phenol content in the samples affect the antioxidant activity of the sample.

Recommendation

It's necessary to do a study of the potential antioxidant activity (% RSA, IC_{50}) correlated with total phenol and nutrient content from the dry powder of other algae species.

References

- Agbor, G.A., J.E. Oben., J.Y. Ngogang., C. Xinxing & J.A. Vinson. 2005. Antioxidant capacity of some herbs/spices from cameroon: a comparative study of two methods. *J. Agr. Food Chem.* 53: 6819-6824.
- Airanthi, M.W., M. Hosokawa & K. Miyashita. 2011. Comparative antioxidant activity of edible Japanese brown seaweeds. *J. Food Sci.* 76: 104-111.
- Ale, M.T., H. Maruyama., H. Tamauchi & J.D. Mikkelsen. 2011. Fucose-containing sulfated polysaccharides from brown seaweeds inhibit proliferation of melanoma cells and induce apoptosis by activation of caspase-3 in vitro. *Mar. Drugs* 9: 2605-2621.
- Andayani, R., L. Yovita & Maimunah. 2008. Penentuan aktivitas antioksidan, kadar fenolat total dan likopen pada buah Tomat (*Solanum lycopersicum* L). *J. Sains dan Teknologi Farmasi* 13: 31-37.

- Ankanna, S., D. Suhurulatha & N. Savithramma. 2012. Chemotaxonomical studies of some important monocotyledons. *Bot. Res. Int.* 5: 90-96.
- AOAC. 1984. Official Methods of Analysis of The Association Analytical Chemist. Inc. Washington D. C.
- AOAC. 2005. Official Method of Analysis of the Association of Official Analytical Chemist. Benyamin Franklin Station. Washington D. C.
- AOAC. 2009. Integrated Total Dietary Fiber, Assay Procedure Including Resistant Starch and Non Digestible Oligosaccharides. Megazyme International Ireland.
- AOAC. 2011. Integrated Total Dietary Fiber, Assay Procedure Including Resistant Starch and Non Digestible Oligosaccharides. Megazyme International Ireland.
- Athukorala, Y., G.N. Ahn., Y.H. Jee., G.Y. Kim., S.H. Kim & J.H. Ha. 2009. Antiproliferative activity of sulfated polysaccharide isolated from an enzymatic digest of *Ecklonia cava* on the U-937 cell line. *J. Appl. Phycol.* 21: 307-314.
- Badan Standarisasi Nasional. 2006. Cara Uji Kimia-Bagian 2: Penentuan Kadar Air pada Produk Perikanan, SNI-01-2354.2-2006. Jakarta.
- Bahre, T.A & G.R.F. Tchouya. 2016. Comparative study of the anti-oxidant activity of the total polyphenols extracted from *Hibiscus Sabdariffa* L., *Glycine max* L., *Merr.*, yellow tea and red wine through reaction with DPPH free radicals. *Arab. J. Chem.* 1-8.
- Balboa, E.M., E. Conde., A. Moure., E. Falque & H. Dominguez. 2013. In vitro antioxidant properties of crude extracts and compounds from brown algae. *Food Chem.* 138: 1764-1785.
- Boonchum, W., Y. Peerapornpisal., D. Kanjanapothi., J. Pekkoh., C. Pumas & U. Jamjai *et al.* 2011. Antioxidant activity of some seaweed from the gulf of Thailand. *Int. J. Agr. Biol.* 13: 95-99.
- Budhiyanti, S.A., S. Raharjo., D.W. Marseno & I.Y.B. Lelana. 2011. Free radical scavenging, metal chelating, and singlet oxygen quenching activity of fractionated brown seaweed *Sargassum hystrix* extract. *J. Biol. Sci.* 11: 288-298.
- Budhiyanti, S.A., S. Raharjo., D.W. Marseno & I.Y.B. Lelana. 2012. Antioxidant activity of brown algae *Sargassum* species extract from the coastline of Java Island. *American J. Agr. Biol. Sci.* 7: 337-346.
- Cahyaningrum, K., A. Husni & S.A. Budhiyanti. 2016. Aktivitas antioksidan ekstrak rumput laut cokelat (*Sargassum polycystum*). *AGRITECH* 36: 2-9.
- Chandini, S.K., P. Ganesan & N. Bhaskar. 2008. In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chem.* 107: 707-713.
- Chao Li, X. Li., L. You., X. Fu & R.H. Liu. 2017. Fractination, preliminary structural characterization and bioactivities of polysaccharides from *Sargassum pallidum*. *Carbohydr. Polym.* 155: 261-270.
- Chun-Yung Huang., S.J. Wu., W.N. Yang., A.W. Kuan & C.Y. Chen. 2016. Antioxidant activities of crude extracts of fucoidan extracted from *Sargassum glaucescens* by a compressional-puffing-hydrothermal extraction process. *Food Chem.* 197: 1121-1129.
- Coria-Tellez, A.V., E. Montalvo-Gonzalez., E.M. Yahia & E.N. Obledo-Vazquez. 2016. *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian J. Chem.* xxx: xxx-xxx.
- Costa, L.S., G.P. Fidelis., C.B.S. Telles., N. Dantas-Santos., R.B.G. Camara & S.L. Cordeiro *et al.* 2011. Antioxidant and antiproliferative activities of heterofucans *Sargassum filipendula*. *Mar. Drugs.* 9: 952-966.
- Daneshfar, A., H.S. Ghaziaskar & N. Homayoun. 2008. Solubility of gallic acid in methanol, ethanol, water, and ethyl acetate. *J. Chem. Eng.* 53: 776-778.
- Dawczynski, C., R. Schubert & G. Jahreis. 2007. Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chem.* 103:891-899.
- Dawidowicz, A.L., D. Wianowska & M. Olszowy. 2012. On practical problems in estimation of antioxidant activity of compounds by DPPH method (Problems in estimation of antioxidant activity). *Food Chem.* 131: 1037-1043.
- Dhianawaty, D & Ruslin. 2015. Kandungan total polifenol dan aktivitas antioksidan dari ekstrak metanol akar *Imperata cylindrica* (L) Beauv. (Alang-alang). *MKB* 47: 60-64.
- Dore, C.M.P., M.G.D.C.F. Alves., L.S.E.P. Will., T.G. Costa., D.A. Sabry., L.A.R.S. Rego., C.M. Accardo., H.A.O. Rocha., L.G.A. Filgueira & E.L. Leite. 2013. A sulfated polysaccharide, fucans,

- isolated from brown algae *Sargassum vulgare* with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. *Carbohydr. Polym.* 91: 467-475.
- Farvin, K.H.S & C. Jacobsen. 2013. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish Coast. *J. Food Chem.* 138: 1670-1681.
- Harborne, J.B. 1987. *Metode Fitokimia*. Second Edition. Padmawinata K., Soediro I., penerjemah. *Phytochemical Methods*. ITB. Bandung.
- Harborne, J.B. 1995. *Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan*. ITB Publisher. Bandung.
- Hifney, A.F., M.A. Fawzy., K.M. Abdel-Gawad & M. Gomaa. 2016. Industrial optimization of fucoidan extraction from *Sargassum* sp. and its potential antioxidant and emulsifying activities. *Food Hydrocolloid.* 54: 77-88.
- Hong, Ye., K. Wang., C. Zhou., J. Liu & X. Zeng. 2008. Purification, antitumor and antioxidant activities in vitro of polysaccharides from the brown seaweed *Sargassum pallidum*. *Food Chem.* 111: 428-432.
- Kang, C., Y.B. Jin., H. Lee., M. Cha., E. Sohn., J. Moon., C. Park., S. Chun., E. Jung., J. Hong., S.B. Kim., J. Kim & E. Kim. 2010. Brown alga *Ecklonia cava* attenuates type 1 diabetes by activating AMPK and Akt signaling pathways. *J. Food Chem. Technol.* 48: 509-516.
- Khadambi, T.N. 2007. Extraction of Phenolic Compounds and Quantification of the Total Phenol and Condensed Tannin Content of Bran Fraction of Condensed Tannin and Condensed Tannin Free Sorghum Varieties. Thesis. Department of Food Science, Faculty of Natural and Agricultural Sciences, University of Pretoria.
- Khalaf, N.A., A. Shakiya., A. L-Othman., E.L.-Agbar & Z.H. Farah. 2008. Antioxidant activity of some common plants. *Turkish J. Biol.* 32: 51-55.
- Khaled, N., M. Hiba & C. Asma. 2012. Antioxidant and antifungal activities of *Padina pavonica* and *Sargassum vulgare* from the Lebanese Mediterranean Coast. *Adv. Environ. Biol.* 6: 42-48.
- Khotimah., K., Darius & B.B. Sasmito. 2013. Aktivitas senyawa aktif alga cokelat (*Sargassum filipendula*) sebagai antioksidan pada minyak ikan Lemuru (*Sardinella longiceps*). *Jurnal THPi*, 1: 10-20.
- Lailatussifa, R., A. Husni & A.E. Nugroho. 2016. Anti-stress activity of *Sargassum polycystum* extracts using a cold restraint stress model. *Food Sci. Biotechnol.* 25: 1-6.
- Lann, K.L., C. Ferret., E. VanMee., C. Spagnol., M. Lhuillery & C. Payri. 2012. Total phenolic, size-fractionated phenolics and fucoxanthin content of tropical Sargassaceae (fuciales, phaeophyceae) from the South Pacific Ocean: spatial and specific variability. *Phycol. Research.* 60: 37-50.
- Lei, Y., C. Yang., C. Li., Q. Zhao., L. Liu., X. Fang & X.Y. Chen. 2016. Recent advances in biosynthesis of bioactive compounds in traditional Chinese medicinal plants. *Sci. Bull.* 61: 3-17.
- Luo, H.Y., B. Wang., C.G. Yu., Y.I. Qu & G.I. Su. 2010. Evaluation of antioxidant activities of five selected brown seaweeds from China. *J. Med. Plants Research.* 4: 2557-2565.
- Makkar, H.P.S., G. Tran., V. Heuze., S. Giger-Reverdin., M. Lessire., F. Lebas & P. Ankers. 2016. Seaweeds for livestock diets: A review. *Animal Feed Sci. Technol.* 212: 1-17.
- Mishra, K., H. Ojha & N.K. Chaudhury. 2012. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chem.* 130: 1036-1043.
- Molyneux, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarinn J. Sci. Technol.* 26: 212.
- Moubayed, N.M.S., H.J. Al Hourii., M.M. Al Khulaifi & D.A. Al Farraj. 2016. Antimicrobial, antioxidant properties and chemical composition of seaweeds collected from Saudi Arabia (Red Sea and Arabian Gulf). *Saudi J. Biol. Sci.* xxx: xxx-xxx.
- Nurjanah., A., Abdulla & A. Apriand. 2011. Aktivitas antioksidan dan komponen bioaktif pada keong ipong-ipong (*Fasciolaria salmo*). *J. Pengolahan Hasil Perikanan Indonesia*, XIV: 22-29.
- Pei Teng Chan., P. Matanjun., S.M. Yasir & T.S. Tan. 2015. Histopathological studies on liver, kidney, and heart of normal and dietary induced hyperlipidaemic rats fed with tropical red seaweed *Gracilaria changii*. *J. Func. Foods* 17: 202-213.
- Pifarre, P., M. Simo., J.D. Gispert., P. Plaza., A. Fernandez & J. Pujol. 2015. Diazepam and Jacobson's progressive relaxation show similar attenuating short-term effects on stress-related

- brain glucose consumption. *Eur. Psychiat.* 30: 187-192.
- Plaza, M., M. Amigo-Benavent., M.D. del Castillo., E. Ibanez & M. Herrero. 2010. Facts about the formation of new antioxidants in natural samples after subcritical water extraction. *Food Res. Int.* 43: 2341-2348.
- Pratiwi, T. 2013. Uji Aktivitas Ekstrak Metanolik *Sargassum hystrix* dan *Eucheuma denticulatum* dalam Menghambat α -Amilase dan α -Glukosidase. Universitas Gadjah Mada. Yogyakarta.
- Radji, M. 2005. Peranan bioteknologi dan mikrobia endofit dalam pengembangan obat herbal. *Majalah Ilmu Kefarmasian* 2: 113-126.
- Senevirathne, M., S.H. Kim., N. Siriwardhana., J.H. Ha., K.W. Lee & Y.J. Jeon. 2006. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci. Technol. Int.* 12: 27-38.
- Setzer, W.N. 2008. Non-intercalative triterpenoid inhibitors of topoisomerase ii: a molecular docking study. *Compounds J.* 1: 13-17.
- Sirait, M. 2007. Penuntun Fitokimia dalam Farmasi. Institut Teknologi Bandung. Bandung.
- Solarin, B.B., D. A. Bolaji., O. S. Fakayode & R.O. Akinnigbagbe. 2014. Impacts of an invasive seaweed *Sargassum hystrix* var. *fluitans* (Børgesen 1914) on the fisheries and other economic implications for the Nigerian coastal waters. *IOSR J. Agr. Vet. Sci.* 7.
- Stengel, D.B., S. Connan & Z.A. Popper. 2011. Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. *Biotechnol. Adv.* 29: 483-501.
- Suresh, V., N. Senthilkumar., R. Thangam., M. Rajkumar., C. Anbazhagan., R. Rengasamy., P. Gunasekaran., S. Kannan & P. Palani. 2013. Separation, purification, and preliminary characterization of sulfated polysaccharides from *Sargassum plagiophyllum* and its in vitro anticancer and antioxidant activity. *Process Biochem.* 48: 364-373.
- Teruya, T., T. Konishi., S. Uechi., H. Tamaki & M. Tako. 2007. Anti-proliferative activity of oversulfated fucoidan from commercially cultured *Cladosiphon okmuranus* TOKIDA in U937 cells. *Int. J. Biol. Macromol.* 41: 221-226.
- Tuo, K., S. Beourou., A.O. Toure., K. Ouattara., S. Meite., A.A.B. Ako., S.S. Yao., D. Koffi., B. Coulibay., A. Coulibaly & A.J. Djaman. 2015. Antioxidant activities and estimation of the phenols and flavonoids content in the extracts of medicinal plants used to treat malaria in Ivory Coast. *Int. J. Curr. Microbiol. App. Sci.* 4: 862-874.
- Winarno, F.G. 2008. Kimia Pangan dan Gizi: Edisi Terbaru. Gramedia Pustaka Utama. Jakarta.
- Yan, S & R. Asmah. 2010. Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf, and torch ginger flower. *Int. Food Res. J.* 17: 417-423.
- Ye, H., K. Wang., C. Zhou., J. Liu & X. Zeng. 2008. Purification, antitumor and antioxidant activities in vitro of polysaccharides from the brown seaweed *Sargassum pallidum*. *Food Chem.* 111: 428-432.
- Zafra-Stone, S., T. Yasmin., M. Bagchi., A. Chatterjee., J.A. Vinson & D. Bagchi. 2007. Berry anthocyanins as novel antioxidants in human health and disease prevention. *Mol. Nutr. Food Res.* 51: 675-683.
- Zahra, R., M. Mehrnaz., V. Farzaneh & S. Kohzad. 2007. Antioxidant activity of extract from a brown alga, *Sargassum boveanum*. *Afr. J. Biotechnol.* 6: 2740-2745.