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

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

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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<sub>50</sub> BHT adalah sebesar 0,227 ± 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<sub>50</sub> bubuk kering *S. hystrix* adalah sebesar 0,616 ± 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<sub>50</sub> 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<sub>50</sub> 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 of seaweed as the source of antioxidant 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, carotenoids, folates, selenium, sulfated polysaccharides like 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, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007). Seaweed is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Yan & Asmah, 2007).

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 ºC) for 5-7 days. The dried sample was then cut into small pieces of ± 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 was 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 μg/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 μg/ml, 500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5 μg/ml, and 31.25 μg/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 Ciocalteau reagent 50 μl and incubated for 5 minutes. Furthermore, it added by 7.5% Na2CO3 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 (μg/ml) vs absorbance (nm). The equation of regression standard curve was obtained about y=ax+b; R2=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-picrylhidrazil) (% 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 μg/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) of free radicals scavenging were measured with the formula:

%RSA = (control absorbance - sample absorbance) / control absorbance x 100%
Furthermore, graphics made between sample concentration (x) with % inhibition (y). The value of IC$_{50}$ 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:

$$
\% Protein = \frac{N \times \text{correction factor}}{mg \ sample}
$$

Fat content was analysis used Soxhlet method with the formula:

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

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

$$
\% Water \ content = \frac{W_2 - W_1}{W_1} \times 100\%
$$

While $W_2$ = weight of sample and cup early; $W_1$ = 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 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 IC$_{50}$ 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 ($C_5H_8$) called isoprene units (Lei _et al._, 2016). _S. hystrix_ dry powder also contains phenolic and tannin content which detected by 5% and 1% FeCl$_3$ 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,

| 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% | + |        |
| Saponin Water+HCl | + |        |
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and reduction in both cerebral metabolic rate for oxygen and cerebral blood flow (Piffareti 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

![Fig. 1. RSA Perccentage of (a) BHT and (b) S. hystrix powder.](image)

| Sample                  | IC$_{50}$ Value (mg/ml) | IC$_{50}$ Value (ppm) |
|-------------------------|-------------------------|-----------------------|
| BHT                     | 0.227±0.001$^{a}$       | 227.02±1.30$^{a}$     |
| S. hystrix dry powder   | 0.616±0.005$^{b}$       | 616.22±4.80$^{b}$     |

$^{a}$ Values with different letters in the same column indicate significant difference ($p<0.05$)

![Table 2. Value of IC$_{50}$ S. hystrix dry powder vs BHT.](image)
reaction between DPPH and different concentration of the antioxidant (Dawidowicz et al., 2012). IC$_{50}$ is similar to EC$_{50}$ or LD$_{50}$ 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 the amount of antioxidant necessary to decrease 50% radical DPPH, it is more effective and reactive of the antioxidant sample to work. The IC$_{50}$ 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 IC$_{50}$ 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). IC$_{50}$ 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 IC$_{50}$ 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 IC$_{50}$ relatively strong if the value ranges between 50-100 ppm (Khotimah, 2013). However, Suresh et al. (2013) mentioned that brown algae with the IC$_{50}$ value of 1000 ppm were potential to be used as in vitro anticancer. High and low IC$_{50}$ 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 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. vulgar 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. homeri 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 (Boonchum et al., 2011), 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%.
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