

ANTIOXIDANT ACTIVITY OF INDONESIAN BROWN MARINE ALGAE (*Sargassum turbinarioides*)

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ABSTRACT

Brown marine algae are provided for an excellent source of bioactive compounds especially fucoidan. Fucoidan is primarily composed of L-fucose residues linked by α -1,3 and α -1,4 glycosidic bonds, with sulfate groups as its main chemical components. One of the biological activities of fucoidan is antioxidant. Antioxidant activities of Indonesian brown marine algae were investigated. The phytochemical screening of the crude extract was conducted to identify various phytochemical constituents, including carbohydrates, flavonoids, phenolic compound, terpenoids, and proteins and amino acid. The antioxidant activity of the brown marine algae *Sargassum turbinarioides* was evaluated using free radical scavenging through the DPPH assay and a reducing power assay. The antioxidant capacity of the extracted fucoidan was measured against ascorbic acid, revealing that fucoidan from brown marine algae exhibited lower DPPH radical scavenging activity compared to vitamin C. Meanwhile, the reducing power of the extract increased with higher concentrations, demonstrating a dose-dependent effect. These findings suggest that brown marine algae extract has potential for commercialization as a functional food or dietary supplement owing to its significant antioxidative properties.

Keywords: phytochemical detection, DPPH, reducing power assay, fucoidan.

1. INTRODUCTION

Marine algae represent a promising renewable resource found in marine environments. Approximately 6,000 species of marine algae have been identified and classified into different groups, including brown algae (Phaeophytes), green algae (Chlorophytes), and red algae (Rhodophytes) (Chandini et al., 2008). In recent years, marine algae have attracted significant interest as a source for bioactive compounds used in the development of nutraceuticals and functional foods. They are recognized as excellent sources of various bioactive substances such as dietary fiber, vitamins, carotenoids, proteins, essential fatty acids, and minerals (Fleurence & Levine, 2016). In Asia, Japan is a major consumer of marine algae, with an average annual intake of 1.6 kg (dry weight) per person. In contrast, in India, marine algae are primarily harvested for industrial uses, particularly for extracting phycocolloids like alginate, agar-agar,

and carrageenan, rather than for food consumption or recovery of beneficial biomolecules (Chandini et al., 2008).

The brown alga *Sargassum turbinarioides* contains a diverse array of bioactive compounds and displays a wide range of biological activities. Typically, the cell walls of brown algae and certain marine invertebrates contain fucose-rich sulfated polysaccharides known as fucoidan. While the structure of fucoidan varies across species, its primary components include sulfate and L-fucose, with smaller amounts of other sugars such as D-mannose, D-galactose, D-xylose, L-rhamnose, glucose, arabinose, D-glucuronic acid, and acetyl groups (Bilan & Usov, 2008). Fucoidan composition differs between species but commonly consists of α -1,3- and α -1,4-linked L-fucose residues bound with sulfate groups (Mak et al., 2013). The fucoidan content in dry marine algae ranges from 25% to 30% (Palanisamy et al., 2018).

Fucoidan exhibits multiple biological activities, including anticoagulant, antioxidant, antibacterial (Khalafu et al., 2017), antilipidemic, antiviral, anti-inflammatory, and antitumor effects (Mak et al., 2013; Suresh et al., 2013). However, the quantity of fucoidan can vary among species and to a lesser extent with growth stages and seasonal changes (Kusaykin et al., 2008). Variations in biological activity between different fucoidans are likely influenced by factors such as the degree of sulfation, molecular weight, and sugar composition. Additionally, the structural properties of fucoidan depend on variables like extraction methods, harvest season, seaweed species, geographic location, and maturity of the algae (Mak et al., 2013).

Numerous studies have reported antioxidant activities in various marine algae species. Antioxidants are compounds that delay or prevent cellular damage caused by free radicals by neutralizing and inhibiting the formation of reactive oxygen species (ROS) (Naji et al., 2020). These reactive species, including hydroxyl, peroxy radicals, and superoxide, are generated in human cells through both internal metabolic processes and external factors. Their presence can lead to extensive oxidative damage associated with aging, degenerative diseases, cancer, and other health conditions. Consuming antioxidants from natural sources can help protect the body from such damage (Chandini et al., 2008). However, research on the antioxidant properties of marine algae extracts from Indonesia remains scarce. Therefore, this study aims to evaluate the antioxidant potential of brown marine algae, particularly *Sargassum turbinarioides*, using various in vitro free radical scavenging assays.

2. METHODS

2.1. Tools and materials

Laboratory glassware (Beakers and Erlenmeyer flasks by Duran®), UV-Visible spectrophotometer (Optima® UV-Vis spectrophotometer), Rotary vacuum evaporator (IKA® Rotary Evaporator), Micropipettes (adjustable (10–100 μ L, 100–1000 μ L by Eppendorf®) and filter paper (Whatman®), grinder machine (Retsch®), centrifuge (Eppendorf®), water bath (Mettler®), Vortex mixer (IKA®). *Sargassum turbinarioides* Grunow (dried brown marine algae from Jogjakarta), distilled water laboratory grade. Methanol, ethanol, diethyl ether, acetone, ammonia, hydrochloric acid, sulfuric acid, chloroform and all solvents used were of analytical grade and purchased from Merck Co. (Darmstadt, Germany). Olive oil, Benedict's reagent, Mayer's reagent, Milon's reagent, ascorbic acid, gallic acid, butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol (technical grade), distilled water, phosphate buffer, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other solvents and chemical reagents utilized in this study were of analytical grade quality.

2.2. Preparation and Extraction

Sargassum turbinarioides Grunow were obtained from Drini Beach, Gunung Kidul Regency, Jogjakarta. The samples were sorted, washed, drained, chopped, and dried to obtain *Simplicia*. The *simplicia* was crushed by grinding using a grinder machine (Retsch®); then, *simplicia* was extracted using hot water for 1 H at temperature 80°C. The filtrate was centrifuged (Eppendorf®) for 10 min at 6000 rpm. The supernatant was added Ethanol 96% to supernatant as far as consist precipitation. The precipitate was washed with Ethanol 96%, diethyl ether, and acetone. The yield was air dried for 24 hours at 40 °C and then pulverized (Artemisia et al., 2019).

2.3. Phytochemical detection of crude extract

The phytochemical detection of crude extract was performed for the detection of different phytochemicals like carbohydrates, tannins, saponins, flavonoids, alkaloids, terpenoids and phenols. The phytochemical detection used in the analysis of crude extracts addressed by Dhinakaran et al. (2016) and Singh et al. (2017).

a. Carbohydrate detection

Sample extract (0.5 ml) and Benedict's reagent (0,5 ml) were added and mixed. The mixture was heated and boiled for two minutes in the water bath (Memmert®), then checked for colored precipitate formation (Benedict's test).

b. Flavonoid detection

Ammonia solution (2ml) and aqueous filtrate of each algal extract were mixed and followed by addition of concentrated sulphuric acid forming a yellow coloration. The yellow coloration was disappeared on standing.

c. Alkaloid detection

Filtrate solvent free extract (50 mg) and hydrochloric acid (2 ml) were stirred and filtered. Filtrate (1 ml) and 2 drop of Mayer's reagent were added forming a white creamy precipitate.

d. Phenolic compounds detection

Sample extract (50 mg) and distilled water (5 ml) were dissolved. Few drops of neutral ferric chloride solution were added in the mixture forming a coloration of a dark green.

e. Saponins detection

Sample powder (2 g) and distilled water (20 ml) were boiled in water bath and filtered. Filtrate (10 ml) and distilled water (5 ml) were mixed and shaken vigorously for a stable persistent froth. The frothing was mixed with olive oil (3 drops) and shaken vigorously until emulsification.

f. Steroid detection

Acetic anhydride (2 ml) was added to methanolic extract of each sample (0.5 g) with sulfuric acid (2 ml). The colour will change from violet to blue or green.

a. Terpenoids detection

Sample extract (5 ml) and chloroform (2 ml) were mixed in sulfuric acid (2 ml). The mixture was added to form a layer of red-brown coloration at the interface (Salkowski test).

b. Proteins and amino acids detection

Sample extract (100 mg) and distilled water (10 ml) were dissolved and filtered through Whatman no.1 paper. The filtrate was tested for proteins and amino acids. The Filtrate (2 ml) and Millon's reagent (2 drops) were added and observed for white precipitate (Millon's test).

2.4. DPPH radical scavenging activity

This method were determined by the method of (Chandini et al., 2008). Briefly, 2.0 ml of 0.16 mM DPPH solution (in methanol) were added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed by vortex mixer (IKA®) for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at

517 nm. Sample blank and control samples were performed according to the method. Ascorbic acid was used as a positive control. The % inhibition was calculated by using the following equation:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of sample} - \text{absorbance of blank})}{(\text{Absorbance of control})} \times 100$$

2.5. Reducing Power Assay

The reducing power assay was performed according to [Lim et al., \(2014\)](#) and [Zhou et al., \(2020\)](#) with slight modification. Briefly, 2 mg/ml of extract (in aquades), were mixed with phosphate buffer (2.5 ml, 0.1 mol/L, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1% w/v). The solutions incubated for 20 min at 50°C. The solutions added Trichloroacetic acid (2.5 ml, 10%). The mixtures were centrifuged at 2000 g for 10 min. The filtrate of the sample (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml $FeCl_3$ 0.1% (w/v). The solution was left at room temperature for 30 min before measuring the absorbance at 700 nm in a 96-well plate (200 μ l) using a microplate spectrophotometer reader. The reducing power assay was performed in triplicate. The results were expressed as gallic acid equivalents (mg GAE/100 g) and compared with BHA, BHT and ascorbic acid (2 mg/ml)

3. RESULTS AND DISCUSSION

3.1. Extract

The simplicia of *Sargassum turbinarioides* Grunow was extracted using hot water at temperature 80°C for 1 hours (Artemisia et al., 2019). The yield of each extract is presented in [Table 1](#).

Table 1. Yield of *Sargassum turbinarioides* Grunow extract

Extract	Mass of samples	Extract Mass	Yield
	(g)	(g)	(%)
<i>Sargassum turbinarioides</i>	10.00	0.74	7.36

The extract of *Sargassum turbinarioides* had a low % yield value because extract containing secondary metabolites especially fucoidan sulfate and the primary metabolites is carbohydrates. The hot water extraction method may not effectively solubilize all bioactive compounds present in *Sargassum turbinarioides*, leading to a lower yield. The structural components of the algae, such as cellulose or lignin, may hinder the extraction process and trap bioactive compounds, leading to a lower yield.

The fucoidan sulfate content depend on the time of harvesting samples. The yield of fucoidan sulfate showed a significant increase from July to September, followed by a decline in October. These fluctuations in fucoidan sulfate content reflect the maturity stage of the algae sample. September marks the beginning of spring, with rising water temperatures. During June and July, the algae began to mature, with sporophylls observed to be larger in size. The production of secondary metabolites, particularly fucoidan sulfate, may be associated with various chemical and physiological changes, such as the degradation of the algal blade, changes in photosynthetic pigments, and sporophyll maturation. Throughout the growing season, the fucoidan sulfate content remained relatively stable ([Mak et al., 2013](#)).

3.2. Phytochemical detection of crude extract

Phytochemical screening of the *Sargassum turbinarioides* extract was performed to detect the presence of carbohydrates, flavonoids, phenolic compounds, terpenoids, proteins and amino acids. Phenolic compounds and flavonoids have been reported as antioxidant compounds. The

presence of sulphate polysaccharide in brown marine algae have been reported as the antioxidant (Lim et al., 2014). The results of the phytochemical screening of the *Sargassum turbinarioides* extract are summarized in Table 2.

Phytochemical screening of the *Sargassum turbinarioides* extract was performed to detect the presence of carbohydrates, flavonoids, phenolic compound, terpenoids, and proteins and amino acid. Phenolic compounds and flavonoids have been reported as antioxidant compounds

Table 2. Phytochemical Analysis Result

No	Phytochemicals	Result
1	Carbohydrate	+
2	Flavonoid	+
3	Alkaloid	-
4	Phenolic compound	+
5	Saponin	-
6	Steroid	-
7	Terpenoid	+
8	Proteins and amino acid	+

3.3. DPPH radical scavenging activity

DPPH has been widely used as a stable free radical to evaluate the reducing potential of substances and to study free radical scavenging activities. The DPPH radical scavenging activity (%) of the extract is presented in a table, where the *Sargassum turbinarioides* extract exhibited significantly higher scavenging activity. This activity is attributed to the capability of the extract to donate electrons to pair with the unpaired electron of the DPPH radical (Chandini et al., 2008). Table 3 compares the percentage of DPPH radical scavenging or inhibition between the extract and the standard antioxidant, vitamin C.

Table 3. Results of DPPH scavenging activity

Sample	Concentration (ppm)	Inhibition (%)	IC ₅₀ (ppm)
<i>Sargassum turbinarioides</i> Extract	2000	25	7627
	4000	39	
	6000	39	
	8000	55	
Vitamin C	4	31	6.01
	6	49	
	8	66	
	10	73	

However, the extract showed lower DPPH scavenging activity compared to vitamin C. Fucoidan derived from various marine algae sources generally exhibits strong hydroxyl radical scavenging but relatively lower activity against DPPH radicals (Lim et al., 2014). Additionally, when *Sargassum turbinarioides* extract was precipitated with methanol, this may have contributed to the reduced inhibition activity observed. The method used for extracting the compounds from *Sargassum turbinarioides* could impact the yield and potency of antioxidant compounds, potentially leading to lower activity.

2.6. Reducing Power Assay

Many studies have shown that the reducing power of extracts correlates with their antioxidant activity. Antioxidants achieve this by donating electrons to reduce the Fe³⁺/ferricyanide complex to its ferrous (Fe²⁺) form, thereby helping to neutralize oxidized intermediates involved in lipid peroxidation (Irshad et al., 2012; Mukherjee et al., 2011; Zhou et al., 2020).

Table 4. Results of Reducing Power Assay

Sample	Concentration (ppm)	Activity (μM)
<i>Sargassum</i>	5000	2.13

<i>turbinarioides</i>	10000	4.99
Extract	15000	7.88
	20000	9.03

Antioxidants assessed by the reducing power assay are capable of donating electrons, which enables them to reduce oxidized intermediates formed during lipid peroxidation. This electron donation allows antioxidants to function as both primary and secondary defenders against oxidative damage. In the assay, antioxidants reduce potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}). Subsequently, potassium ferrocyanide reacts with ferric chloride (Fe^{3+}) to produce ferrous (Fe^{2+}) complexes that exhibit maximum absorbance at 700 nm (Lim et al., 2014; Zhou et al., 2020). The intensity of the blue color, known as Perl's Prussian blue, measured at 700 nm reflects the quantity of Fe^{2+} complexes formed. Thus, an increase in absorbance at this wavelength indicates a greater reducing (electron-donating) ability of the antioxidant compounds in the sample (Mukherjee et al., 2011).

The dose dependency of antioxidant activity was examined by measuring the reducing power, providing an overall indication of the reductive compounds present in the extract. The reducing power increased proportionally with higher extract concentrations, demonstrating that the scavenging activity of seaweed extracts depends on concentration. In contrast, the positive control, vitamin C, did not exhibit dose-dependent behavior. The percentage of scavenging activity rose as the extract concentration increased (Lim et al., 2014). Among all tested samples, the reference compound, ascorbic acid, showed the highest reducing power. This reducing ability is likely attributed to the substances' capacity to donate hydrogen atoms.

4. CONCLUSION

The results demonstrate that brown marine algae can serve as a valuable source of natural antioxidant compounds, as their extracts exhibit notable antioxidant activity. These findings provide a useful foundation for further research aimed at isolating, identifying, and characterizing the specific compounds responsible for the enhanced antioxidant effect. Bioactive compounds derived from brown marine algae hold considerable promise for significant advancements in various food and medical applications. The extracts containing these compounds have potential uses as natural antioxidants in an array of food and pharmaceutical products. They also possess commercial potential to be developed and marketed as natural antioxidant bioingredients.

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6. CONFLICT OF INTEREST

In this study the authors declare free from conflicts of interest.

7. REFERENCES

- Artemisia, R., Nugroho, A. K., Setyowati, E. P., & Martien, R. (2019). The Properties of Brown Marine Algae *Sargassum turbinarioides* and *Sargassum ilicifolium* Collected from Yogyakarta, Indonesia. *Indonesian Journal of Pharmacy*, 30(1), 43.
- Bilan, M. I., & Usov, A. I. (2008). Structural Analysis of Fucoidans. *Natural Product Communications*, 3(10), 1934578X0800301.
- Chandini, S. K., Ganesan, P., & Bhaskar, N. (2008). In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chemistry*, 107(2), 707–713.

- Dhinakaran, D. I., Rajalakshmi, R., Sivakumar, T., & Jeeva, S. (2016). Antimicrobial Activities and Bioactive Metabolites from Marine Algae *Valoniopsis pachynema* and *Sargassum swartzii*. *Journal of Pharmacognosy and Phytochemistry*, 4(1).
- Fleurence, J., & Levine, I. (2016). *Seaweed in health and disease prevention*. Elsevier.
- Irshad, Md., Zafaryab, Md., Singh, M., & Rizvi, M. M. A. (2012). Comparative Analysis of the Antioxidant Activity of *Cassia fistula* Extracts. *International Journal of Medicinal Chemistry*, 2012, 1–6.
- Khalafu, S. H. S., Wan Aida, W. M., Lim, S. J., & Maskat, M. Y. (2017). Effects of deodorisation methods on volatile compounds, chemical properties and antioxidant activities of fucoidan isolated from brown seaweed (*Sargassum* sp.). *Algal Research*, 25, 507–515.
- Kusaykin, M., Bakunina, I., Sova, V., Ermakova, S., Kuznetsova, T., Besednova, N., Zaporozhets, T., & Zvyagintseva, T. (2008). Structure, biological activity, and enzymatic transformation of fucoidans from the brown seaweeds. *Biotechnology Journal*, 3(7), 904–915.
- Lim, S. J., Wan Aida, W. M., Maskat, M. Y., Mamot, S., Ropien, J., & Mazita Mohd, D. (2014). Isolation and antioxidant capacity of fucoidan from selected Malaysian seaweeds. *Food Hydrocolloids*, 42, Part 2, 280–288.
- Mak, W., Hamid, N., Liu, T., Lu, J., & White, W. L. (2013). Fucoidan from New Zealand *Undaria pinnatifida*: Monthly variations and determination of antioxidant activities. *Carbohydrate Polymers*, 95(1), 606–614.
- Mukherjee, S., Pawar, N., Kulkarni, O., Nagarkar, B., Thopte, S., Bhujbal, A., & Pawar, P. (2011). Evaluation of free-radical quenching properties of standard Ayurvedic formulation Vayasthapana Rasayana. *BMC Complementary and Alternative Medicine*, 11(1), 38.
- Naji, K. M., Thamer, F. H., Numan, A. A., Dauqan, E. M., Alshaibi, Y. M., & D'souza, M. R. (2020). Ferric-bipyridine assay: A novel spectrophotometric method for measurement of antioxidant capacity. *Heliyon*, 6(1), e03162.
- Palanisamy, S., Vinosha, M., Manikandakrishnan, M., Anjali, R., Rajasekar, P., Marudhupandi, T., Manikandan, R., Vaseeharan, B., & Prabhu, N. M. (2018). Investigation of antioxidant and anticancer potential of fucoidan from *Sargassum polycystum*. *International Journal of Biological Macromolecules*, 116, 151–161.
- Singh, S., Ramaiah, M., Devgan, M., & Sarkar, B. K. (2017). Phytochemical and comparative antioxidant evaluation by DPPH and reducing power assay of *Hybanthus enneaspermus*. *Acta Sci Pharm Sci*, 1(1), 01–4.
- Suresh, V., Senthilkumar, N., Thangam, R., Rajkumar, M., Anbazhagan, C., Rengasamy, R., Gunasekaran, P., Kannan, S., & Palani, P. (2013). Separation, purification and preliminary characterization of sulfated polysaccharides from *Sargassum plagiophyllum* and its in vitro anticancer and antioxidant activity. *Process Biochemistry*, 48(2), 364–373.
- Zhou, J., Yang, Q., Zhu, X., Lin, T., Hao, D., & Xu, J. (2020). Antioxidant activities of *Clerodendrum cyrtophyllum* Turcz leaf extracts and their major components. *PLOS ONE*, 15(6), e0234435.