Characteristics and antioxidant activity of fucoidan from the brown seaweed *Sargassum hystrix*

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Abstract. The development of natural antioxidants is needed because the consumption of synthetic antioxidants has harmful side effects, such as liver toxicity and carcinogenesis. One natural antioxidant resource is brown seaweed. Brown seaweed contains fucoidan compounds, which have antioxidant potential. The objective of this study was to determine the antioxidant activity of fucoidan extracted from *Sargassum hystrix* from Awur Jepara Bay. Fucoidan extraction was carried out using water-based solvents. The fucoidan characteristics tested included yield, total carbohydrate, sulphate content and FTIR. The methods used to measure antioxidant activity were DPPH RSA (radical scavenging activity) and FRAP. The results showed that *S. hystrix* fucoidan had a yield of 4.67±0.23%, total sugar content of 58.57±1.50%, and sulphate content of 24.96±0.68%. In terms of total sugar and sulphate content the statistical tests indicated that there was no significant difference ($p>0.05$) between S. *hystrix* and commercial fucoidan. The FTIR analysis of *S. hystrix* showed the presence of ester and sulphate functional groups that indicated the presence of fucoidan. The RSA DPPH method resulted in 32.57±1.47; 37.64±0.94; 42.51±0.90; 44.71±1.20; 49.63±2.42 inhibition at the concentrations of 500, 1000, 2000, 3000, and 4000 ppm, respectively. The FRAP method yielded a value of 89.62±5.20 μM g^{-1}. The statistical analysis of the DPPH and FRAP results showed that antioxidant activity of *S. hystrix* fucoidan and commercial fucoidan were not significantly different ($p>0.05$). The antioxidant activity of *S. hystrix* fucoidan was approximately the same as or tended to be better than commercial fucoidan.
Key Words: DPPH, FRAP, FTIR, *Fucus vesiculosus*, sulphate content .

Introduction. Degenerative diseases such as premature ageing, cancer, and diabetes continue to increase every year due to exposure to free radicals (Singh et al 2004). Free radicals are highly reactive molecules because they contain unpaired electrons in their outer orbitals, so they must react with the surrounding molecules to achieve stability (Rohmatussolihat 2009). Continuous exposure to free radicals can damage tissues in the body (Kang et al 2010). The damage caused by free radicals in the human body can be naturally prevented by the production of endogenous antioxidants via the body's defence system. However, the levels of endogenous antioxidants were not able to fight excess free radicals (Halliwell 1992), so that additional exogenous antioxidants are necessary. Depending on the source, exogenous antioxidants may consist of natural antioxidants and synthetic antioxidants. However, the consumption of synthetic antioxidants has damaging side effects, including liver toxicity and carcinogenesis (Kumar et al 2008), and it is necessary to look for sources of natural antioxidants. One source of natural antioxidants is brown seaweed (Ye et al 2009; Gamal 2010).

Brown seaweed contains fucoidan, which has good antioxidant activity (Senthilkumar et al 2013). Fucoidan is reported to have strong, non-toxic and potentially high antioxidant properties with applications in the fields of pharmaceuticals, cosmeceuticals and functional foods (Barahona et al 2011; Li et al 2008). In addition, fucoidan also has bioactivity as an immunostimulant capable of increasing the capacity of macrophages for the body's defence (Sinurat et al 2016). This immunostimulant bioactivity helps antioxidants to protect cells from the damage caused by exposure to

free radicals. Li et al (2008) reported that fucoidan showed high antioxidant activity and has great potential to prevent free radical production and the cell damage mediated by disease; thus, fucoidan-based antioxidants need to be developed.

Research on the antioxidant activity of fucoidan in various species has been reported, including *Sargassum wightii* from Indian waters (Prabu et al 2013), *Sargassum muticum* of the Spanish coast (Balboa et al 2013), *Saccharina japonica* from Chinese waters and *Ecklonia maxima* from South African waters (Guiyan et al 2014). Awur Jepara Bay is an area rich in brown seaweed, including *Sargassum hystrix* (Pramesti et al 2016). Budhiyanti et al (2012) reported that *S. hystrix* had the highest antioxidant activity among species, but studies on the antioxidant activity of *S. hystrix* fucoidan have not been carried out. This study aimed to isolate and determine the antioxidant activity of the fucoidan from the brown seaweed *S. hystrix* from the Awur Jepara Bay, Indonesia.

Material and Method

Materials. The material used in this study was brown seaweed, *S. hystrix*, obtained from Awur Jepara Bay, Central Java in September 2018. Other ingredients used were $CaCl_2$, ethanol, KBr, H_2SO_4 (Merck, USA), phenols (Merck, USA), L-fucose (Sigma-Aldrich, Germany), xylose (Sigma-Aldrich, Germany), $BaCl_2$ (Merck, USA), gelatine, trichloroacetic acid (Merck, USA), K_2SO_4 (Merck, USA), DPPH (Merck, USA), vitamin C (Merck, USA), $FeCl_3$ (Merck, USA) and commercial fucoidan from *Fucus vesiculosus* (Sigma-Aldrich, Germany).

Fucoidan extraction. Fucoidan extraction was carried out using the method of Sinurat & Kusumawati (2017) with solvent distilled at 85°C. Fucoidan was extracted from the brown seaweed *S. hystrix* harvested from Awur Jepara Bay. The seaweed was washed and dried to dryness. Then, the seaweed was blended and filtered using a sieve with 60 mesh and then weighed to obtain a powder weight of 100 g. Seaweed powder was introduced in a 2 L Erlenmeyer flask and soaked in distilled water (1:20) (b/v). The sample was then stirred for 4 hours at 85°C with a hot plate stirrer. The mixture was filtered using a sieve with 625 mesh, and the filtrate was collected. Crystalline $CaCl_2$ was added to the filtrate to a final concentration of 2% while stirring for 30 minutes at room temperature. The mixture was centrifuged at 3,000 rpm for 40 minutes at 5°C. The filtrate was collected, and the precipitate was removed. Then, ethanol was added to the filtrate (1:2.5), and the mixture was separated into pellets (sediment) and supernatant (filtrate) by centrifuging at 3,000 rpm for 40 minutes. The obtained pellets were dissolved in distilled water (sufficiently) until completely dissolved and dried with a freeze dryer to obtain the fucoidan extract.

Determination of yield. The yield was obtained from the ratio of the weight of the fucoidan extracts to the weight of the dried seaweed. The fucoidan yield was determined using the following formula:

$$\text{Yield (\%)} = (\text{Weight of fucoidan/Weight of dry sample}) \times 100$$

Determination of total sugar. The total sugar content of the *S. hystrix* fucoidan was analysed using the phenol-sulphate test based on the Dubois et al (1956) method. The sugars tested in this study were fucose and xylose. Standard fucose solutions (100, 150, 200, 250 and 300 ppm), standard xylose solutions (5, 10, 15, 20 and 25 ppm) and sample solutions from *S. hystrix* fucoidan (500 ppm) were prepared. Phenol-sulphate testing was carried out by adding 2.5 mL of concentrated H_2SO_4 to each of these solutions, shaking the mixture until homogeneous and then incubated the mixture for 20 minutes in ice water. A total of 0.5 mL of 5% phenol was added to each solution, and the solutions were shaken until became homogeneous and then incubated in ice water again for 30 minutes. Commercial fucoidan from *F. vesiculosus* was used as a standard. The absorbance of all prepared solutions was measured using a Perkin Elmer Lambda 25 UV-

VIS spectrophotometer; the wavelength was 490 nm for the fucose standard and 480 nm for the xylose standard.

Determination of sulphate content. The sulphate content of the fucoidan extract of *S. hystrix* was determined using the $BaCl_2$-gelatine method (Dodgson & Price 1962). The $BaCl_2$-gelatine solution was prepared by weighing 0.5 g of gelatine, which was dissolved in 100 mL of aquabides and then stirred on a hot plate stirrer at 60-70°C until became homogeneous. After obtaining a homogeneous solution, 0.5 g of $BaCl_2$ was added, and the solution was left overnight at 4°C. Sample solutions were prepared by weighing 6 mg of *S. hystrix* fucoidan extract dissolved in 2 mL of 3.5 N HCl until became homogeneous. Then, the samples were hydrolysed for 17-18 hours in an oven at 105°C. After hydrolysis, centrifugation was carried out to ensure that the only sample used was the filtrate. Sample solutions of commercial fucoidan from *F. vesiculosus* were prepared. Standard K_2SO_4 solutions were prepared at the concentrations of 200, 400, 600, 800, and 1,000 ppm. The sample solution was mixed with 3% TCA and $BaCl_2$-gelatine into a 96-well microplate with successive comparisons of 40, 120, and 60 µL. Afterwards, the mixture was mechanically stirred and incubated for 15 minutes, and then the absorbance was measured using a UV-VIS spectrophotometer (Multiply Go) at a wavelength of 360 nm. The standard solutions of K_2SO_4 and commercial fucoidan were treated the same as the sample solution.

Analysis of functional groups. The analysis of the functional groups was conducted using a Fourier transform infrared (FTIR) spectrophotometer (Perkin-Elmer 577); this method was used to identify the types of functional groups in the fucoidan extract of *S. hystrix*. A total of 2 mg of sample was crushed with 200 mg of potassium bromide (KBr) until became homogeneous. Afterwards, the powder mixture was prepared into thin and transparent tablets at a pressure of 7,000 Pa. Then, the tablets were placed into a simple pan to record the infrared spectra at wave numbers 1,000 – 4,000 cm^{-1} (Sinurat & Kusumawati 2017). Commercial fucoidan from *F. vesiculosus* was used as a standard.

DPPH free radical scavenging assay. The fucoidan extracted from *S. hystrix* was analysed for antioxidant activity based on the ability to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical using the method of Clarke et al (2013) with some modifications. Sample solutions of *S. hystrix* fucoidan, *F. vesiculosus* fucoidan (500-4000 ppm) and vitamin C (4-10 ppm) were made using distilled water and homogenized. The DPPH solution (0.76 mM) was made from 3 mg of DPPH powder dissolved in 10 mL of distilled water and then stored at a cool temperature of 4°C before use (maximum 24 hours). The absorbance of the sample and control solutions was then measured at a wavelength of 517 nm using a UV-VIS spectrophotometer (Multiply Go). Antioxidant activity was expressed as the inhibition percent and IC_{50}. The inhibition percent was calculated using the following formula:

$$\text{Inhibitory activity (\%)} = \frac{(C - D) - (A - B)}{C-D} \times 100$$

Where:
A = Sample (160 µL of sample + 40 µL of 0.76 mM DPPH)
B = Sample control (160 µL of sample + 40 µL of distilled water)
C = Negative control (160 µL of distilled water + 40 µL of 0.76 mM DPPH)
D = Blank (200 µL of distilled water)

Ferric reduction antioxidant power (FRAP) assay. The FRAP test was carried out based on the method described by Clarke et al (2013) that involves the reduction of Fe^{3+} to Fe^{2+}. The change from iron (III) chloride to the Fe^{2+} complex can be measured using a spectrophotometer at a wavelength of 595 nm. Changes can be seen as the solution turns blue. An acetate buffer solution at a pH of 3.6 was made from 0.775 g of sodium acetate trihydrate ($CH_3COON.3H_2O$), which was added to 4 mL of concentrated acetic

acid and dissolved in distilled water to exactly 250 mL. Acetate buffers can be stored as stock solutions at 4°C. A solution of 10 mM mL^{-1} 2,4,6-tripyridil-striazine (TPTZ) was prepared from 0.15 g of TPTZ dissolved in 40 mM L^{-1} HCl to exactly 50 mL. A solution of 40 mM L^{-1} HCl was made by dissolving 0.828 mL of concentrated HCl in 250 mL of distilled water. The TPTZ solution was stored for a maximum of 1 day at 4°C before use. A solution of 20 mM L^{-1} $FeCl_3.6H_2O$ was prepared by weighing 0.54 g of $FeCl_3.6H_2O$ and dissolving it in distilled water to exactly 100 mL. The $FeCl_3.6H_2O$ solution can be stored up to 1 day at 4°C before use. The FRAP reagent was made by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3.6H_2O$ solution (10:1:1) and then adding distilled water to exactly 100 mL. The standard solutions of $FeSO_4.7H_2O$ (10,000 µM L^{-1}) were made by weighing 2.78 g of $FeSO_4.7H_2O$ and dissolving the substance in 1,000 mL of distilled water. Then, concentrations of 50, 100, 150, 200, 250 and 300 ppm were made. Sample solutions of *S. hystrix* fucoidan (1,000 ppm), commercial fucoidan from *F. vesiculosus* (1,000 ppm) and vitamin C (7 ppm) were prepared. A sample solution (20 µL) was added to 150 µL of the FRAP reagent in a 96-well microplate, and then the absorbance was measured at a wavelength of 595 nm using a UV-VIS spectrophotometer (Multiply Go). The standard $FeSO_4$ solution, commercial fucoidan and vitamin C were treated in the same manner as the sample.

Reducing power assay. The reducing power of fucoidan was assayed according to the method developed by Kumar et al (2008). The sample was dissolved in distilled water at different concentrations. The sample solution (1 mL) was mixed with sodium phosphate buffer (2.5 mL, 0.2 mol L^{-1}, pH 6.6) and potassium ferricyanide aqueous solution ($K_3Fe(CN)_6$, 2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes, followed by the addition of trichloroacetic acid solution (2.5 mL, 10%) and aqueous ferric chloride ($FeCl_3$, 0.5 mL, 1%). The absorbance of the reaction mixture was measured at 700 nm on a UV-2000 spectrophotometer (Unico (Shanghai) Instrument Co., Ltd., China). The reducing power was proportional to the absorbance of the reaction mixture.

Data analysis. Data analysis was performed using the Minitab program. The normality test was performed using the Kolmogorov-Smirnov test because this test can be used for quantitative data, single data, and large or small n. The research data were normally distributed with terms $p>0.05$. Two independent parametric T-tests were used.

Statistical analysis. All data were tested for assumptions of normality and homoscedasticity by the Bartlett's test and were arcsine-transformed when required. Comparisons between the nursery site at Tekek and natural reef at Renggis were conducted using t-test. Variations between sampling months were compared using one-way ANOVA, followed by Tukey's post hoc test. Significance of differences was defined at $p<0.05$. Statistical analyses were performed using MINITAB® software.

Results and Discussion

Yield and composition of fucoidan. Table 1 shows that the fucoidan yield of *S. hystrix* was 4.67±0.23%. Balboa et al (2013) reported that the yield of fucoidan from *S. muticum* (Yendo) was also 4%. The yields of fucoidan from other species that have been reported include 1.39% of *S. japonica* (Guiyan et al 2014), 6.16±0.08% of *Sargassum aquifolium* 2.06±0.23% of *Padina* sp. (Lim et al 2014), and 3.6±0.07% of *S. tenerrimum* (Marudhupandi et al 2014). Differences in yield can be caused by differences in extraction methods, seaweed types, algae age and geographical location (Sinurat et al 2016).

The total carbohydrate content of *S. hystrix* was 58.57±1.50% (Table 1), lower than that of commercial fucoidan *F. vesiculosus* (63.00±0.74%). Based on the statistical analysis, the total carbohydrate contents of *S. hystrix* fucoidan and commercial fucoidan were not significantly different ($p>0.05$). The total carbohydrate content of *S. hystrix* was similar to that of *Sargassum glaucescens* (59.52%) obtained with a compressional-Puffing-hydrothermal extraction process, but it was still lower than that of *Sargassum horneri* (61.82%) and *Sargassum cristaefolium* (69.87%) (Huang et al 2015).

Table 1
Yield and chemical composition of *Sargassum hystrix* fucoidan and commercial fucoidan

	Fucoidan from S. hystrix (%)	*Commercial fucoidan (%)*
Yield	4.67±0.23	-
Total sugar	58.57±1.50[a]	63.00±0.74[a]
Fructose	54.29±1.36[b]	58.47±0.92[b]
Xylose	4.27±0.14[c]	4.53±0.18[c]
Sulphate	24.96±0.68[d]	30.94±1.26[d]

The same letter in the same row indicates no significant difference (p>0.05).

The sulphate content of *S. hystrix* fucoidan, as shown in Table 1, was 24.96±0.68%, while the sulphate content of commercial fucoidan was 30.94±1.26%. Although the sulphate content of *S. hystrix* fucoidan was higher than that of commercial fucoidan, the difference was not significant (p>0.05). Wang & Chen (2016) reported sulphate contents of 15.28% in *S. glaucescens*, 14.08% in *S. horneri*, and 18.36% in *S. japonica* using ethanol solvents. The sulphate contents of these species were lower than the sulphate content of *S. hystrix* obtained in the present study. However, Men'shova et al (2013), using *S. polycystum*, reported a sulphate content of 28.8%, a value higher than the sulphate content of *S. hystrix*, using ethanol solvents. Differences in sulphate content can be caused by different species, extraction methods and geographical locations (Skriptsova et al 2009).

Analysis of functional groups. Patterns of the functional groups in *S. hystrix* fucoidan were determined using FTIR analysis (Fourier transform infrared spectroscopy). Figure 1 shows the functional group detected at the *S. hystrix* fucoidan and commercial fucoidan.

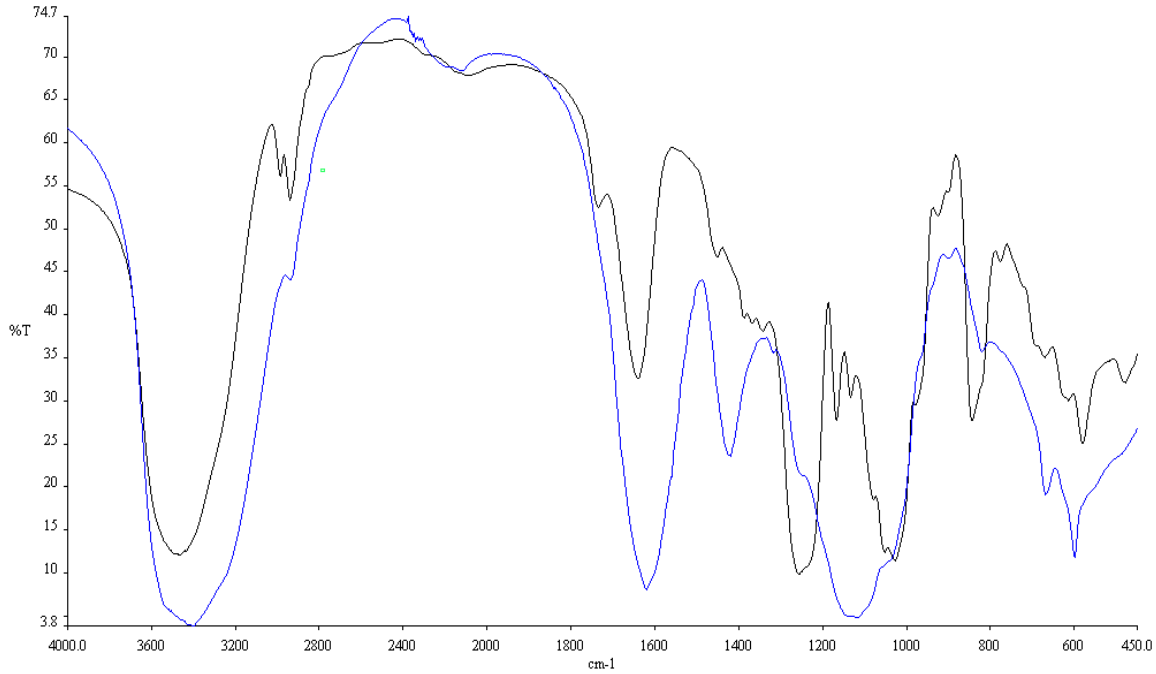

Figure 1. Infrared spectrum of *S*argassum *hystrix* fucoidan and commercial fucoidan.
: *S. hystrix* fucoidan
: Commercial fucoidan

Based on Table 2, *S. hystrix* fucoidan and commercial fucoidan from *F. vesiculosus* had similar wave numbers. According to Na et al (2010), absorption at an area of approximately 3,400 cm^{-1} shows an O-H stretch band originating from a hydroxyl group, whereas absorption in the area of 1,600 cm^{-1} indicates the presence of a C = O stretching band of the carbonyl group. According to Sinurat et al (2016), the absorption area of approximately 1,600 cm^{-1} indicated the presence of uronic acid, and *S. hystrix* fucoidan

and commercial fucoidan showed peaks at 1,631-1,637 and 1,639 cm^{-1}, respectively. Uronic acid is a component that is sometimes found at different concentrations in fucoidan extracts from different species.

Table 2

Functional group analysis of *Sargassum hystrix* fucoidan and commercial fucoidan

Wavelength (cm^{-1})		*Reference wavelength (cm^{-1})*	*Interpretation of functional groups (Pretsch et al 2019)*
S. hystrix	Commercial		
3,410-3,411	3,467	3,200-3,500	O-H (Alcohol)
2,939-2,940	2,941	2,700-3,000	C-H (Alkane)
1,619-1,621	1,639	1,350-1,800	C=O (Aldehyde, ketone, carboxylate acid, ester)
1,118-1,119	1,256	1,000-1,300	S=O (Sulfonate, sulphate)
3,410-3,411	3,467	3,200-3,500	O-H (Alcohol)

The results obtained from the infrared spectrum help to determine the specific types of functional groups that exist in a molecule. The sulphate group is a specific marker of fucoids that affects bioactivity (Li et al 2008). In thepresent study, the infrared spectrum data determined the presence of sulphate esters, a characteristic of fucoidans that indicates the presence of sulphate polysaccharides. In addition to sulphate ester groups, fucoidan characteristics are also reinforced at the wave lengths of 800 cm^{-1} (C-O-S), which represent equatorial or axial sulphate positions (Sinurat et al 2011).

At 2,900 cm^{-1}, a C-H stretch of a pyranose ring was observed (Marudhupandi et al 2014). In the area of 2,900 cm^{-1}, *S. hystrix* showed absorption, but the absorption was not as strong as that of commercial fucoidan. Absorbance in the area of 1,400-1,600 cm^{-1} indicates the presence of carbonyl groups (Na et al 2010). Sinurat et al (2016) reported that absorbance in the area of 1,600 cm^{-1} potentially indicated uronic acid. In fucoidan, there was a sulphate ester group observed in the area of 1,255-1,258 cm^{-1}. In commercial fucoidan, there is an absorption band at 1,256 cm^{-1}, but in *S. hystrix*, the absorption at this point was very weak. This difference could occur due to the influence of extraction methods and fucoidans that were not been purified. The absorption bands in the regions 1,100 and 1,027 cm^{-1} show the C-O and C-C bonds from the pyranose ring and the C-O-C bonds from glycosides, respectively (Marudhupandi et al 2014). Maciel et al (2008) reported that the 840-820 cm^{-1} (C-O-S) absorption band indicated the substitution of 4-sulphate and 6-sulphate in the monosaccharide unit. It has also been reported that the polysaccharide fraction of *Sargassum stenophyllum* showed an absorption band at 837 cm^{-1}, indicating a sulphate group in the C-4 position of the monomer structure (Pereira et al 2002).

DPPH radical scavenging activity. The DPPH test results are shown in Figures 2 and 3. DPPH inhibitory activity shows the ability of the sample to reduce free radicals. At the concentrations of 500-4,000 ppm, the inhibitory activity of *S. hystrix* fucoidan was 32.57±1.47 to 49.63±2.42%. At the same concentrations, the inhibition activity of commercial fucoidan was 37.81±4.29 to 40.03±1.38%. The curves of the commercial fucoidan from *F. vesiculosus* showed decreased inhibitory activity at a concentration of 2,000 ppm, and the activity then subsequently increased in a concentration-dependent manner. Based on the statistical tests conducted, it can be concluded that the DPPH antioxidant activities of *S. hystrix* and *F. vesiculosus* were not significantly different ($p>0.05$). The curve shows that the percent inhibition of the *S. hytrix* and *F. vesiculosus* compounds were lower than that of vitamin C. Vitamin C at the concentrations of 4 to 10 ppm showed inhibitory activities of 28.07±0.72 to 50.37±0.23%, respectively. The vitamin C curve did not overlap with the curves of *S. hystrix* and *F. vesiculosus* because the concentration was too low.

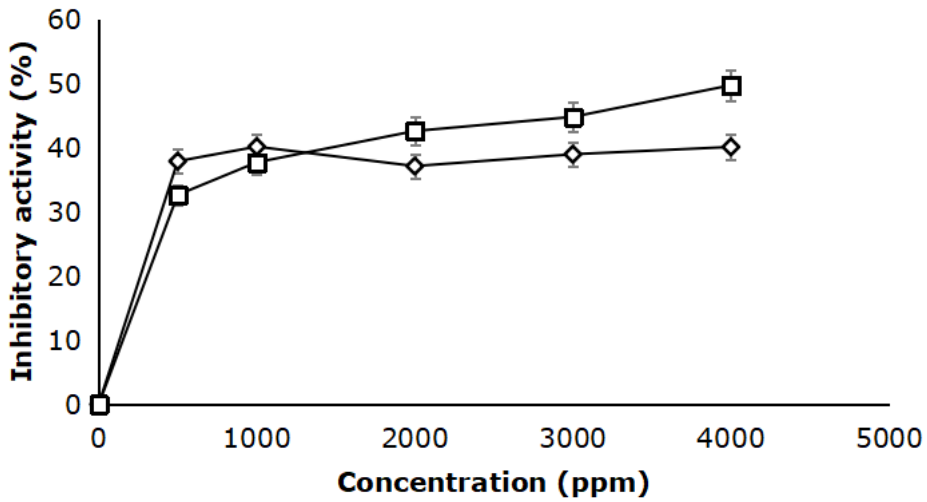

Figure 2. Effect of the concentration of *Sargassum hystrix* fucoidan (◇) and commercial fucoidan (□) on the inhibition of DPPH radicals.

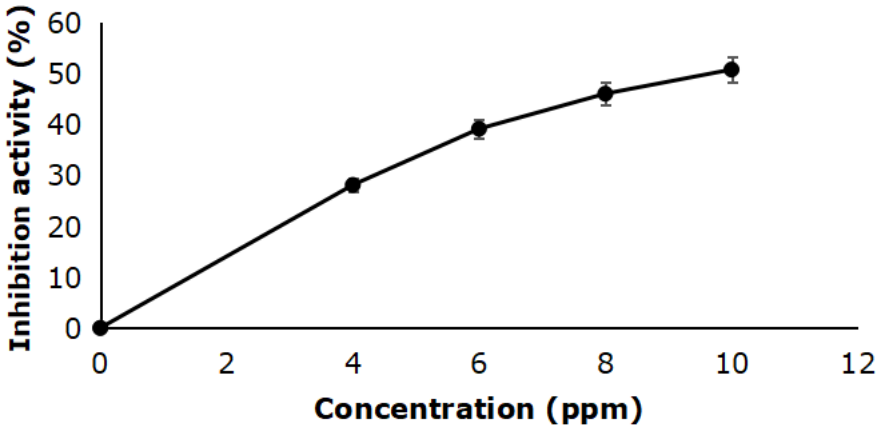

Figure 3. Effect of vitamin C concentration on the inhibition of DPPH radicals.

The inhibitory activity of *S. hystrix* fucoidan and commercial fucoidan was much lower than that of vitamin C (IC_{50} = 9.4 ppm). Similar results were reported by Lim et al (2014), who found that the inhibitory activity of *S. aquifolium* fucoidan was much lower (IC_{50} 2,010±0.29 ppm) than that of vitamin C (IC_{50} = 3.49±0.19 ppm). The IC_{50} of *S. aquifolium* was lower than of *S. hytrix* obtained in the present study. *S. hystrix* has an IC_{50} value of 4,775 ppm, almost the same as that of *S. glaucescens,* which has been purified into 4 fractions with IC_{50} values of 4,300 ppm, 4,270 ppm, 4,570 ppm and 5,150 ppm (Huang et al 2015).

Ferric reduction antioxidant power (FRAP). The FRAP results can be seen in Table 3. The FRAP value of *S. hystrix* fucoidan (89.62±5.20 μM g^{-1}) at a concentration of 1,000 ppm was lower than that of commercial fucoidan (53.95±8.84 μM g^{-1}) at the same concentration. This finding indicates that the antioxidant activity of fucoidan standards is better than that of *S. hystrix*. This result is consistent with the results reported by Lim et al (2014); the higher the sulphate content is, the higher the bioactivity. However, based on the statistical tests conducted, the FRAP results of the fucoidan from *S. hystrix* and *F. vesiculosus* were not significantly different ($p>0.05$). Vitamin C has a much better activity; at a concentration of 7 ppm, the activity of vitamin C reached 64 μM g^{-1}, while the activities of the other samples at a concentration of 1,000 ppm were still higher than that of vitamin C. Vitamin C has a higher inhibitory activity because it has been purified; therefore, *S. hystrix* needs to be purified so that its bioactivity can increase.

Table 3

FRAP values of *Sargassum hystrix* fucoidan, commercial fucoidan, and vitamin C

Sample	*Concentration (ppm)*	*FRAP value (μM g^{-1})*
Vitamin C	7	64.35±2.05
Commercial fucoidan	1,000	53.95±8.84[a]
S. hystrix fucoidan	1,000	89.62±5.20[a]

The same letter in the same column indicate no significant differences (p>0.05).

Prabu et al (2013) reported that fucoidan from *S. wightii* from Indian waters had a FRAP value of 87.96±3.61 μM g^{-1} at a concentration of 8,250 ppm with the antioxidant activity of *S. hystrix*, which had a FRAP value of 89.62±5.2 μM g^{-1} at a concentration of 1,000 ppm, was much higher because a smaller FRAP value indicates better antioxidant activity.

Reducing power. The principle of reducing power is that antioxidants react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with iron chloride (Fe^{3+}) to form iron (Fe^{2+}) complexes that have a maximum uptake at 700 nm (Wang et al 2008). Figure 4 shows the reducing power *S. hystrix*, *F. vesiculosus* and vitamin C. The absorbance values of *S. hystrix* fucoidan at the concentrations of 2,000, 2,500, 3,000, 3,500, and 4,000 ppm were 0.25, 0.28, 0.33, 0.41, and 0.48, respectively. Moreover, at 2,000, 2,500, 3,000, 3,500, and 4,000 ppm, commercial fucoidan had absorbance values of 0.26, 0.33, 0.37, 0.40, and 0.44, respectively. In general, the absorbance values of *F. vesiculosus* were higher than those of *S. hystrix*. Therefore, the antioxidant reducing power of *F. vesiculosus* was higher than that of *S. hystrix*. This result was in accordance with the results reported by Guiyan et al (2014) that showed the correlation of fucoidan reducing power with sulphate content. The reducing power of vitamin C is higher than that of fucoidan. Wang et al (2008) reported that the absorbance of *S. japonica* fucoidan at a concentration of 2,000 ppm was 0.15. Therefore, the reducing power of *S. hystrix* fucoidan was higher than that of *S. japonica* fucoidan. Differences in results can be caused by the seaweed species used, thus affecting the antioxidant bioactivity of fucoidan. In addition, antioxidant activity is also impacted by extraction methods, environmental exposure and seasons (Stanislov et al 2012).

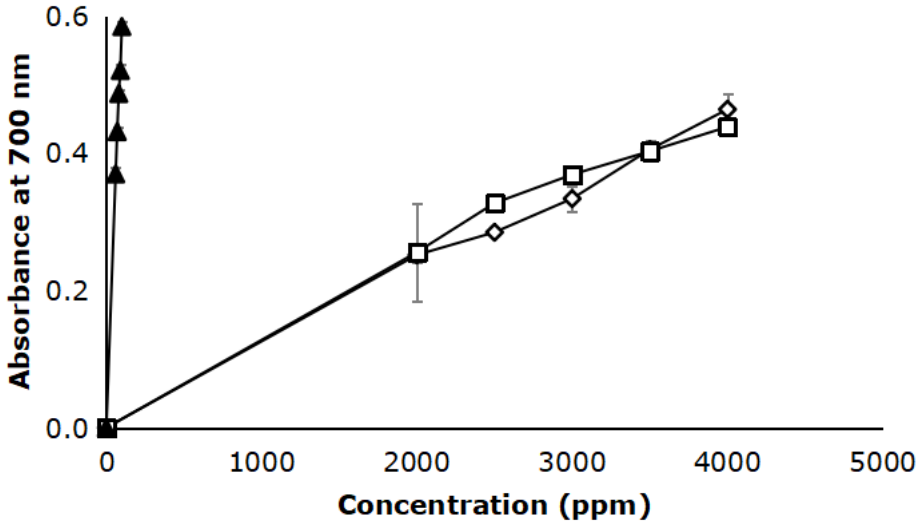

Figure 4. Effect of the concentration of *Sargassum hystrix* fucoidan (◇), commercial fucoidan (□), and vitamin C (Δ) on reducing power.

Conclusions. The antioxidant activity of *S. hystrix* fucoidan was lower than that of vitamin C, with an IC_{50} DPPH value of 4,775 ppm and an FRAP value of 89.62±5.20 μM g^{-1}.

The antioxidant activity of *S. hystrix* fucoidan was similar to or tended to be higher than that of commercial fucoidan from *F. vesiculosus.*

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