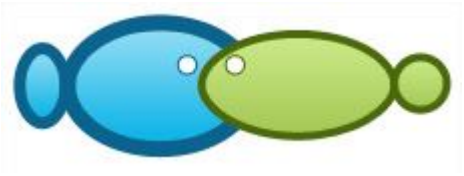

The impact of temperature on the antioxidant activity of fucoidan obtained from brown seaweed *Sargassum hystrix* extracted using EDTA

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Abstract. The harmful side effects, including carcinogenesis and liver toxicity obtained from synthetic antioxidant consumption instigates the need to develop natural forms. In addition, brown seaweed is one of the potential resources for application as an alternative, due to the antioxidant potentials of the fucoidan compounds present. Therefore, this study is aimed at determining the impact of extraction temperature on the antioxidant activity of fucoidan from *Sargassum hystrix* extracted using EDTA. The extraction process was performed using 0.5% EDTA at 50, 70, and 90^{o}C, and the fucoidan characteristics was evaluated for yield, total carbohydrate, sulfate content and also through Fourier-transform infrared (FTIR) analysis. Furthermore, the antioxidant activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA), ferric reducing antioxidant power (FRAP), hydroxyl radical scavenging activity (HRSA), and total antioxidant capacity (TAC). The results showed a *S. hystrix* fucoidan yield of 1.18±0.15 to 2.94±1.06%, with total sugar content at 38.05±15.58 to 52.06±8.11%, and 7.54±1.91 to 27.80±3.59% for sulfate. Moreover, FTIR analysis demonstrated the existence of sulfate and ester functional groups in the molecule, and served as a confirmatory assay for fucoidan. The antioxidant activity of *S. hystrix* fucoidan was evaluated at varied temperatures (50, 70, and 90^{o}C) while using the DPPH method, and IC50 values of 1249.81, 2200.32, and 1818.93 ppm were obtained respectively. Meanwhile, 135.54, 71.38, and 88.04 µM g^{-1} were correspondingly recorded with FRAP, while HRSA showed 1464.55, 2360.00, and 1054.22 ppm, and 232.79, 374.82, and 377.53 mg AAE g^{-1} were respectively obtained with TAC. The antioxidant activity of fucoidan sourced from *S. hystrix* was similar or tended to be better than the commercial variety.
Key Words: antioxidant, DPPH, FRAP, fucoidan, *Sargassum hystrix*.

Introduction. There has been a constant annual increase in the incidence of degenerative diseases, including cancer, premature ageing, as well as diabetes, resulting from free radical exposures (Singh et al 2004). These molecules are known to be highly reactive due to the unpaired electrons present on the outer orbitals. Therefore, reactions with molecules in the surrounding environment are essential to attain stability (Rohmatussolihat 2009). Continuous exposure to free radicals can damage tissues in the body (Kang et al 2010). Furthermore, the potential damages to humans are possibly prevented through natural means, as observed with the ability for body defence systems to produce endogenous antioxidants. The amounts generated are, however, unable to eliminate excessive free radicals (Halliwell 1992). This challenge instigates the need to introduce additional exogenous forms, which possibly encompass both natural and synthetic varieties, depending on the source. Previous studies have shown the tendency for synthetic antioxidants types to initiate some damaging side effects, comprising liver toxicity and carcinogenesis (Kumar et al 2008). Therefore, it is necessary to explore other natural antioxidants sources, including the brown seaweed (El Gamal 2010).

This form of microalgae is known to contain fucoidan, which is characterized by good antioxidant activity (Senthilkumar et al 2013), strong, non-toxic and potentially high antioxidant properties, with applications in pharmaceuticals, cosmeceuticals and functional foods (Barahona et al 2011). In addition, there have also been reports on the bioactivity as an immunostimulant, with the ability to increase macrophage capacity and consequently the body's defence (Sinurat et al 2016). These effects help in cell protection

against damages potentially initiated through free radical exposure. According to Li et al (2008), fucoidan demonstrates a high tendency to inhibit free radicals through the antioxidant effects. Furthermore, disease-mediated cellular damage is prevented, thus instigating the need to develop fucoidan-based antioxidants.

There have been numerous reports on fucoidan antioxidant activities after extraction from various species, including *Sargassum muticum* (Balboa et al 2013), *Sargassum wightii* (Prabu et al 2013), *Sargassum hystrix* (Suhaila et al 2019) as well as *Ecklonia maxima* and *Laminaria japonica* (Qu et al 2014). This specific effect is possibly influenced by a number of factors, encompassing the solvent type and also the temperature used during extraction (Rodriguez-Jasso et al 2013; Yuan & Macquarrie 2015). Previous studies have identified some solvents applied in the extraction process, including water (Sinurat & Kusumawati 2017; Suhaila et al 2019; Kurnialahi et al 2020), acid (Sinurat & Maulida 2018), calcium salts (Mak et al 2013), ethanol (Ponce et al 2003) and hot buffers (Hifney et al 2016). However, these five methods have certain weaknesses (Ponce et al 2003; Hifney et al 2016; Sinurat & Kusumawati 2017). The use of ethylenediaminetetraacetic acid (EDTA) at various temperatures (Zhao et al 2017) was an alternative extraction method performed on *L. japonica* seaweed. However, research using this specific technique for *Sargassum hystrix* at varying temperatures has not been widely conducted. The aim of this study, therefore, was to determine the antioxidant activity of fucoidan from *S. hystrix* extracted using EDTA at different temperatures.

Material and Method

Materials. These include the brown seaweed *Sargassum hystrix*, acquired from Teluk Awur, Jepara, Central Java in November 2019. Other ingredients include $CaCl_2$, 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 *Fucus vesiculosus* (Sigma-Aldrich, Germany).

Fucoidan extraction. The fucoidan extraction process was performed with reference to a research by Zhao et al (2017). This involved using dried and mashed seaweed samples weighing approximately 100 grams, followed by extraction using 0.5% (1:30) (w/v) EDTA solvent. Subsequently, the mixture was stirred for 3 hours at varied temperatures of 50, 70, and 90°C using a hot plate stirer. The extracted sample was then cooled at room temperature and filtered using a 500 mesh sieve. Therefore, ethanol was added to the resulting filtrate to attain a concentration of 20%. The extract mixture was further treated with centrifugation at 3000 rpm for 10 minutes. Furthermore, ethanol was again added to the filtrate to achieve 60% concentration before centrifugation for 10 minutes at 3000 rpm. Therefore, only the pellets were collected and dialysed using a dialysis bag as well as distilled water for 24 hours. The outcome of dialysis was then freeze dried using a freeze dryer. This extraction procedure was performed in triplicates.

Determination of yield. The yield was evaluated by computing the ratio of fucoidan extract to dried seaweed weight. Furthermore, the following formula was used in this estimation:

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

Determination of total sugar. The phenol-sulfate test was applied to evaluate the total sugar present in *S. hystrix* fucoidan, using the method by DuBois et al (1956). This involved analysing the fucose and xylose content, after preparing standard fucose (100, 150, 200, 250 and 300 ppm), xylose solutions (5, 10, 15, 20 and 25 ppm) as well as *S. hystrix* sample solution with 500 ppm fucoidan content. Therefore, each solution was treated with 2.5 mL of concentrated H_2SO_4 to evaluate the phenol-sulfate composition, followed by shaking to attain homogeneity and the subsequent incubation in ice water for 20 minutes. In addition, 0.5 mL of phenol (5%) was respectively introduced, before shaking to achieve a stable consistency, and incubating a second time over a 30 minutes period in ice water. The standard fucoidan in this experiment was the commercial variant

extracted from *Macrocystis pyrifera*. Moreover, a Perkin Elmer Lambda 25 UV-VIS spectrophotometer was used to measure the absorbance in each solution prepared, where wavelengths of 490 and 480 nm indicated fucose and xylose standard respectively.

Determination of sulfate content. The fucoidan extract obtained from *S. hystrix* was evaluated for sulfate content based on the method involving $BaCl_2$-gelatine solution (Dodgson & Price 1962). This required the preparation of $BaCl_2$-gelatine by dissolving 0.5 g gelatine in 100 mL distilled water, followed by stirring with a stirrer to attain homogeneity on a hot plate set at 60-70°C. Subsequently, 0.5 g $BaCl_2$ was introduced to the solution before reserving at 4°C overnight. In addition, sample solution preparations involved dissolving 6 mg *S. hystrix* fucoidan extract in 2 mL of 3.5 N HCl to attain homogeneity. This solution was then hydrolysed in an oven at 105°C for 17-18 hours, and was followed by centrifugation to produce the filtrate required for further investigation. The commercial fucoidan sample solutions were prepared from *M. pyrifera*, as well as standard 200, 400, 600, 800, and 1000 ppm solutions of K_2SO_4. Subsequently, the sample solution was combined with $BaCl_2$-gelatine and 3% TCA before introducing in a 96-well microplate. This step was performed with successive comparisons at 40, 120, and 60 µL, followed by the mechanical stirring and incubation over a 15 minutes period. Therefore, UV-VIS spectrophotometer (Multiply Go) was used for absorbance measurements at 360 nm wavelength. The standard commercial fucoidan and K_2SO_4 solutions were then treated similarly as samples.

Analysis of functional groups. The Fourier-transform infrared (FTIR) spectrophotometer was used to evaluate the functional groups present (Perkin-Elmer 577) in fucoidan extracts obtained from *S. hystrix* species. This involved crushing approximately 200 mg potassium bromide (KBr) with 2 mg of sample to attain homogeneity. Therefore, the powder mixtures generated were prepared in a thin and transparent tablet form at 7000 Pa pressure, before placing in a simple pan. This step was required to obtain infrared spectra measurements at wave numbers 500-4000 cm^{-1} (Sinurat & Kusumawati 2017). The commercial fucoidan was then adopted as standard.

DPPH free radical scavenging assay. The scavenging ability against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated to evaluate the antioxidant activity of fucoidan extract using the modified Clarke et al (2013) method. This involved creating homogenized *S. hystrix* fucoidan, *M. pyrifera* fucoidan (500-4000 ppm) and vitamin C (4-10 ppm) sample solutions with distilled water. Therefore, 3 mg DPPH powder was dissolved in 10 mL distilled water to create a DPPH solution (0.76 mM), followed by storage at 4°C prior to use within a maximum of 24 hours. Furthermore, the control and sample solutions were evaluated for absorbance at 517 nm wavelength using the UV-VIS spectrophotometer (Multiply Go). The percent inhibition and IC50 values were used to estimate the antioxidant activity. The following formula was used to evaluate the inhibition percentage:

$$\text{Inhibitoryion activity } (\%) = \frac{(C-D)-(A-B)}{(\mathrm{C}-\mathrm{D})} X\ 100$$

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. This assessment required the method described by Clarke et al (2013), where Fe^{3+} was reduced to Fe^{2+}. The spectrophotometer is possibly used to measure the iron(III) chloride modified into Fe^{2+} complexes at 595 nm wavelength. These changes are potentially observed with a solution color transformation to blue. In addition, acetate buffer solution with pH 3.6 was formulated by adding 0.775 g of sodium acetate trihydrate ($CH_3COON.3H_2O$) to 4 mL of concentrated acetic acid, and followed by dissolution with distilled water to obtain an exact volume of 250 mL. The yield is then stored at 4°C as a stock solution.

Subsequently, 10 mM mL^{-1} 2,4,6-tripyridil-striazine (TPTZ) solution was formulated by dissolving 0.15 g of TPTZ in 40 mM L^{-1} HCl to achieve an exact volume of 50 mL, while the 40 mM L^{-1} HCl solution was prepared through the dissolution of 0.828 mL concentrated HCl in 250 mL distilled water. Therefore, the generated TPTZ solution was reserved at 4°C for use within a 24 hour period. Also, 0.54 g $FeCl_3.6H_2O$ was dissolved in distilled water and made up to approximately 100 mL to produce 20 mM L^{-1} $FeCl_3.6H_2O$ solution, which is possibly stored for up to 24 hours at 4°C. The FRAP reagent was formulated by combining 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3.6H_2O$ solution (10:1:1) before making up to approximately 100 mL with distilled water. Furthermore, the standard $FeSO_4.7H_2O$ (10,000 µM L^{-1}) solutions were produced by dissolving 2.78 g $FeSO_4.7H_2O$ in 1000 mL distilled water, before serially diluting to attain 50, 100, 150, 200, 250 and 300 ppm concentrations. The commercial fucoidan from *M. pyrifera* (1000 ppm), *S. hystrix* fucoidan (1000 ppm), and vitamin C (7 ppm) sample solutions were prepared, and 20 µL were respectively introduced to the 150 µL FRAP reagent in a 96-well microplate. This was followed by absorbance measurements with the UV-VIS spectrophotometer (Multiply Go) at 595 nm wavelength. In addition, the respective standard solutions were also treated to similar conditions.

Hydroxyl radical scavenging activity (HRSA). The Fenton's reaction was used to determine the hydroxyl radical scavenging activity of fucoidans, based on the methods described by Zhao et al (2017). This involved generating hydroxyl free radicals from $FeSO_4$, and detected by evaluating the hydroxylate activity against salicylate. In addition, 100 µL of each sample (0.25-5 mg mL^{-1}) was added to 1 mL of 9 mM $FeSO_4$ and further mixed with 1 mL of 0.3% H_2O_2 in 9 mM salicylic acid-ethanol solution (0.5 mL). This solution was then incubated for 30 min at 37°C, and the total mixture volume was made up to 5 mL with distilled water. Moreover, using UV–Vis spectrometer (Shanghai Spectrum Instruments co., Ltd.) was used to estimate absorbance at 510 nm. The positive control in this experiment includes vitamin C and BHT, while the hydroxyl radical scavenging activity (HRSA) was evaluated in terms of percentage, using the formula:

$$HRSA\ (\%) = [\{A_0 - (A_1 - A_2)\}/A_0] \times 100$$

where: A_1 represents the sample absorbance;
A_0 is absorbance determined for control experiment;
A_2 denotes the absorbance for blank reagent.

Total antioxidant capacity (TAC). The phosphomolybdenum method was applied during the determination of total anti-oxidative capacity, where ascorbic acid was used as standard (Patel et al 2016). In addition, a mixture comprising fucoidan and 3 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was incubated at 5°C for 90 min. Therefore, absorbance measurements were collected after cooling at 695 nm. The total antioxidant composition was then expressed in terms of mg Ascorbic Acid Equivalent (AAE)/g for each sample, using the following formula:

$$TAC = \frac{(axV)/1000}{G}$$

Where: a = the concentration of vitamin C in the test sample (mg L^{-1});
V = total volume of test solution (mL);
G = weight of sample extract (g);
1000 = conversion factor to total volume of solution (mL).

Data analysis. The data collected were expressed in terms of mean±standard deviation (n = 3), and consequently processed with Statistical Package for Social Sciences (SPSS) and Excel 2013. In addition, Kolmogorov-Smirnov test was used to evaluate normality, and a normally distributed data was continued to parametric testing, comprising LSD and additional assessment with Duncan test. However, abnormally distributed data were separately evaluated with non-parametric tests, including the Kruskal-Wallis and subsequently Mann-Whitney tests.

Results and Discusions

Yield and composition of fucoidan. Table 1 shows a fucoidan yield of 1.18±0.23 to 2.94±1.06% from the *S. hystrix* raw material. Moreover, a similar extraction performed by Suhaila et al (2019) using water showed yields of 4.67±0.23%. Therefore, the higher yield obtained with water in contrast to EDTA was attributed to the greater polarity and the good solvent characteristics in extracting ionic compounds (Sharmila et al 2018). Previous reports have shown the fucoidan output from other species, including *Laminaria japonica* (1.39%) (Qu et al 2014), *Sargassum binderi* (6.16±0.08%), 2.06±0.23% and 3.6±0.07% from *Padina* sp. (Lim et al 2014), and *Sargassum tenerrimum* (Marudhupandi et al 2014), respectively. The yield variations are possibly attributed to the differences in extraction methods, seaweed types, algae age and geographical location (Sinurat et al 2016).

Table 1

Effect of extraction temperature on the output and chemical composition of commercial and sample fucoidan exracted from *Sargassum hystrix* using EDTA

	Temperature extraction of fucoidan from S. hystrix (%)			*Commercial fucoidan (%)*
	50°C	*70°C*	*90°C*	
Yield	2.92±1.00[a]	1.18±0.15[b]	2.94±1.06[a]	-
Total sugar	38.05±15.58[a]	48.45±10.39[ab]	52.06±8.11[ab]	61.74±11.73[b]
Xylose	32.01±14.35[a]	40.62±8.59[ab]	44.51±7.15[ab]	53.68±10.97[b]
Fucose	6.04±1.23[a]	7.83±1.82[a]	7.57±0.97[a]	8.07±0.93[a]
Sulfate	7.54±1.91[a]	27.80±3.59[b]	26.06±5.16[b]	44.36±4.47[c]

Note: The same letter in the same line indicates no significant difference ($p > 0.05$).

Table 1 shows the total carbohydrate contained in *S. hystrix*, which was within the range 38.05±15.58 to 52.06±8.11%, and the value was relatively lower than the output recorded for the commercial variant obtained from *M. pyrifera* (61.74±11.73%). However, statistical analysis demonstrated the absence of any significant differences ($p > 0.05$). The total sugar content in the tested sample was relatively lower than the result of fucoidan extracted *Lessonia nigrescens* with ethanol (57.46±1.23%) (Qu et al 2014) and *S. binderi* (76.25%) (Sinurat & Kusumawati 2017). These variations were possibly attributed to the method of extraction adopted (Ponce et al 2003). Also, this phenomenon is more significant, due to the high solubility of polysaccharides in aquadest (Sinurat & Kusumawati 2017). The factors of geographical location seaweed species used have also been implicated as influences on the total sugar content (Anastyuk et al 2012).

Table 1 shows the sulfate content of *S. hystrix* fucoidan and the commercial variety, at 7.54±1.91 to 27.80±3.59%, and 44.36±4.47%, respectively. Wang & Chen (2016) reported on the quantity generated from *S. glaucescens* (15.28%), *S. horneri* (14.08%), and *L. japonica* species (18.36%) using ethanol solvents. These values were generally lower than the sulfate content observed with *S. hystrix* in the current study. However, Men'shova et al (2013) reported a comparable value of 28.8% with *Sargassum polycystum* fucoidan exracted using ethanol. The variations in sulfate content are possibly attributed to differences in species, extraction methods and geographical locations (Skriptsova et al 2010).

Analysis of functional groups. The Fourier-transform infrared spectroscopy (FTIR) analysis was used to determine the functional group patterns in fucoidan extracted from *S. hystrix*. Table 2 shows the results of both the commercial variant and the samples treated 50, 70 and 90°C. The spectra demonstrated fucoidan formation at a wavelength of 1000-1500 cm^{-1}. Moreover, the peak value for commercial and *S. hystrix* yields was observed at 1010-1090 cm^{-1}, representing the existence of glycosides (C-O-C) (Pereira et al 2013). The alginic acid residues or sulfate (S-O) groups were indicated by the presence of a broad peak at 1120-1270 cm^{-1} (Fernando et al 2017). Meanwhile, absorption peaks at 1253-1220 cm^{-1} were primary and secondary to the O-sulfate group, therefore indicating the polysaccharide and fucoidan composition of seaweed (Pereira et al 2009).

The absorption bands for commercial and *S. hystrix* sourced variants were observed at 1645, 1614, 1651 and 1615 cm^{-1}, indicating the presence of C=O uluronic bands from carbonyl groups (Na et al 2010). Moreover, absorption at about 1600 cm^{-1} shows the uronic acid present (Hifney et al 2016).

Table 2

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

S. hystrix fucoidan (cm^{-1})			*Commercial fucoidan (cm^{-1})*	*Reference wavelength (cm^{-1})*	*Interpretation of functional groups**
50°C	*70°C*	*90°C*			
3436	3413	3447	3453	3230-3550	O-H (Hydroxile)
1614	1651	1615	1645	1350-1800	C-O (Aldehide, ketone,
1421	1415	1420			ester)
1256	1248	1255	1166	1000-1300	S=O (Sulfate, sulfonate)
1035	1035	1035			
619	532	614	683	350-700	COOH (Carboxylic acid)
	469		580		

* Pretsch et al (2009).

DPPH radical scavenging activity. Table 3 shows the result of antioxidant activity evaluation, which was based on the IC_{50} value obtained using DPPH method. Particularly, the commercial variant showed a value of 1634.55 ppm, while the outcome for fucoidan sourced from *S. hystrix* at extraction temperature treatments of 50, 70 and 90°C were 1249.81, 2200.32 and 1818.93 ppm, respectively. The temperature values were estimated to only influence yield (Zhao et al 2017). However, differences in IC_{50} are possibly attributed to decreased viscosity observed during higher heat treatments (Saravana et al 2016). Schrieber & Gareis (2007) acknowledged the potential impact of hydrolyzate molecular weight on solution viscosity, as the heavier molecules facilitate the production of solutions with reduced viscosity.

Table 3

DPPH IC_{50} value of vitamin C, BHT, commercial fucoidan and fucoidan from *Sargassum hystrix* at different temperature extraction

Sample	*IC_{50} (ppm)*
Vitamin C	11.03 ± 1.95^{d}
BHT	13.48 ± 1.29^{d}
Commercial fucoidan	1634.00 ± 409.72^{ab}
S. hystrix fucoidan (50°C)	1249.81 ± 207.81^{a}
S. hystrix fucoidan (70°C)	2200.32 ± 141.15^{c}
S. hystrix fucoidan (90°C)	1818.93 ± 208.73^{bc}

Note: The same letter in the same column shows no significant differences ($p > 0.05$).

The results showed significantly lower inhibition capacity with both fucoidan samples evaluated, in contrast with vitamin C (IC_{50} = 11.03 ppm) and BHT (IC_{50} = 13.48 ppm). Lim et al (2014) reported a similar outcome, where *S. binderi* fucoidan demonstrated lower inhibition activity at an IC_{50} of 2010±0.29 ppm, compared to vitamin C (IC_{50} = 3.49±0.19 ppm). This outcome was also lesser than the value for *S. hytrix* obtained in this current investigation at 1249-2200 ppm, Moreover, higher values were recorded for *Sargassum glaucescens*, purified into 4 fractions at 4300 ppm, 4270 ppm, 4570 ppm and 5150 ppm, respectively (Huang et al 2016).

Ferric reduction antioxidant power (FRAP). The FRAP method was based on the sample's ability to reduce Fe^{3+} to Fe^{2+} (Shah & Modi 2015). Table 4 shows the results, and vitamin C, BHT as well as the commercial fucoidan served as a comparison. These respectively generated 131.375 µM g^{-1} at 14 ppm concentration, 133.181 µM g^{-1} at 20 ppm, and 60.403 µM g^{-1} at 1000 ppm. Meanwhile, the FRAP result for *S. hystrix* treated with temperatures of 50, 70 and 90°C and 1000 ppm concentrations were 135.54, 71.38, and 88.04 µM g^{-1}, respectively.

Table 4

FRAP value of vitamin C, BHT, commercial fucoidan dan fucoidan from *S. hystrix* at different temperature extraction

Sample	*Concentration (ppm)*	*FRAP value (μM g^{-1})*
Vitamin C	14	131.38±3.82[a]
BHT	20	133.18±16.53[a]
Commercial fucoidan	1000	60.40±6.14[b]
S. hystrix fucoidan (50°C)	1000	135.54±6.29[a]
S. hystrix fucoidan (70°C)	1000	71.38±6.14[c]
S. hystrix fucoidan (90°C)	1000	88.04±4.23[d]

Note: The same letter in similar column indicates the absence of any significant differences ($p>0.05$).

The results show a significantly higher FRAP value in *S. hystrix* compared to the commercial variety. However, a contrast between treatments at different temperatures show the greatest value at 50°C, followed by 90°C and 70°C, respectively. The sulfate content was also determined to influence antioxidant activity, as the test showed greater amounts in the commercial fucoidan compared to the sample investigated. However, a contrast between various temperature treatments indicates higher composition at 70°C, while 50°C produced the least.

Hydroxyl radical scavenging activity (HRSA). The antioxidant ability is evaluated based on the HRSA principles. This was focused on the capacity to capture the hydroxyl free radicals produced by $FeSO_4$ generated through the fenton reaction and their propensity to hydroxylate salicylates (Zhao et al 2017). Furthermore, these analysis procedures were performed on vitamin C, BHT, and commercial fucoidan. Table 5 shows the results as expressed in IC_{50}, at 278.45, 341.35, and 1906.39 ppm, respectively, while the outcome for *S. hystrix* were 1464.55, 2360.00, and 1054.22 ppm for the temperature treatments at 50, 70 and 90°C, respectively. These findings show higher IC_{50} values in the fucoidan samples, therefore indicating better antioxidant activity in vitamin C and BHT.

Table 5

HRSA IC_{50} value of vitamin C, BHT, commercial fucoidan and fucoidan from *S. hystrix* at different temperature extraction

Sample	*IC_{50} (ppm)*
Vitamin C	278.45±0.98[d]
BHT	341.35±10.19[d]
Commercial fucoidan	1906.39±537.58[bc]
S. hystrix fucoidan (50°C)	1464.55±140.07[ab]
S. hystrix fucoidan (70°C)	2360.00±536.93[c]
S. hystrix fucoidan (90°C)	1054.22±125.19[a]

Note: The same letter in the same column shows no significant differences ($p>0.05$).

Table 5 shows the lowest IC_{50} in treatments at 90°C, and was further considered to produce the most significant antioxidant activity amongst other *S. hystrix* fucoidan samples. Moreover, additional factors assumed to have an impact on the antioxidant effects include seaweed maturation, salinity, molecular weight, as well as sulfate (Qi et al 2005) and uronic acid composition (Ruperez et al 2002). In addition, higher activities observed with the 90°C treatments were attributed to the hydrolysis of alginic to uronic acid (Yuan & Macquarrie 2015). This finding was congruent with the report by Zhou et al (2008), and is also supported with the research by Ruperez et al (2002), where fucoidan extracted from *Fucus vesiculosus* demonstrated greater antioxidant effects resulting from the significantly high uronic acid content.

Total antioxidant capacity (TAC). This assessment was performed using ascorbic acid as a standard, according to the phosphomolybdenum method (Patel et al 2016). Table 6 shows the TAC value recorded for each *S. hystrix* fucoidan sample, and the highest value was recorded with the 90°C temperature treatment, while the 50°C had the least outcome. This variation was attributed to the impact of temperature used in the extraction process on the componds present in fucoidan. Yuan & Macquarrie (2015) reported on the ability for samples extracted with 90°C to cause alginate hydrolysis into uronic acid, and the consequent development of high antioxidant activity at greater levels (Zhou et al 2008).

Table 6

TAC value of commercial fucoidan dan fucoidan from *S. hystrix* at different temperature extraction

Sample	*TAC value (mg AAE g^{-1})*
Commercial fucoidan	341.01±9.37[a]
S. hystrix fucoidan (50°C)	232.79±22.35[a]
S. hystrix fucoidan (70°C)	374.82±9.74[a]
S. hystrix fucoidan (90°C)	377.53±15.36[a]

Note: The same alphabet in the same column shows no significant differences ($p > 0.05$).

Conclusions. The extraction temperature was estimated to influence the antioxidant activity fucoidan extracted from *Sargassum hystrix* using EDTA. Furthermore, the antioxidant activity after treatments with 50, 70, and 90°C yielded respective IC_{50} values of 1249.81, 2200.32, and 1818.93 ppm, using the DPPH method, while 135.54, 71.38, and 88.04 µM g^{-1} was respectively produced with FRAP, the HRSA technique generated 1464.55, 2360.00, and 1054.22 ppm, correspondingly, and 232.79, 374.82, as well as 377.53 mg AAE g^{-1} were recorded with TAC. The antioxidant properties of *S. hystrix* fucoidan extracted using EDTA were similar or tended to be better than those of the commercial fucoidan.

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