

Chemical composition antioxidant and antimicrobial activities of fucoidan extracted from two species of brown seaweeds (*Sargassum ilicifolium* and *Sargassum angustifolium*) around Qeshm Island

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Abstract

The chemical compositions, antioxidant and antimicrobial properties of fucoidan extracted from *Sargassum ilicifolium* and *Sargassum angustifolium* were investigated. The results showed that these seaweeds were characterized by an acceptable level of minerals and proteins. The fatty acid profile was dominated by the palmitic acid, which represents about 37.44±0.01% and 23.05±0.01% of the total fatty acids, followed by oleic acid (13.83±0.01% and 45.30±0.01%) for *S. ilicifolium* and *S. angustifolium*, respectively. Swelling capacity, water holding capacity and oil holding capacity were varied in two different temperatures (25°C and 37°C). The antioxidant activities of fucoidan including DPPH, superoxide-hydroxyl radical scavenging, chelating ability and reducing power were considerable. The correlation of sulfate and phenol contents with scavenging superoxide radical ability, chelating ability and reducing power was positive. The antibacterial activity was evaluated by disc diffusion method against *Bacillus subtilis* and *Staphylococcus aureus* (gram positive bacteria) and *Escherichia coli* and *Salmonella enteritidis* (gram negative bacteria). There were no positive results against gram negative bacteria. In the concentration of 5 mg ml⁻¹ small clear inhibition zones was observed against *B. subtilis* and *S. aureus*. On the basis of obtaining results from antioxidant activities, fucoidan is suggested as potential natural and safe antioxidant agents in the nutraceutical industry.

Keywords: Fucoidan, Antioxidant, Antimicrobial, Brown seaweed, *Sargassum*.

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Introduction

Seaweeds are plants with biological active resources in nature. They divided into three major categories “brown algae, red algae and green algae” on the type of pigment and chemical compositions (Pushparajet *al.*, 2014). Among the algae, brown algae are usually widely distributed in tropical and subtropical areas. They usually grow in the intertidal zone on rocky substrates, dead corals, pebbles, shells and other plant materials. The seaweeds contain significant amounts of protein, carbohydrates, minerals, vitamins, fatty acids and antioxidants. The chemical composition of seaweed is variable according to age, environmental factors and geographic distribution (Yaichet *al.*, 2011). The major components of the seaweed can have biological effects due to a variety of sulfated polysaccharides. On the other hand, according to species, the chemical composition of the polysaccharides is different. Sulfated hemi-ester groups as a group of these polysaccharides, attached to the units of sugars in a form called fucoidan that are only found in brown algae and some marine invertebrates (such as sea urchin and sea cucumber) (Li *et al.*, 2008). Numerous studies have been conducted on the biological effects of fucoidan extracted from various species of algae, such as antiviral, antitumor, anti-inflammatory, wound healing, antioxidant and antibacterial (Ale *et al.*, 2011; Kordjazi *et al.*, 2013). Fucoidan is also a sulfated polysaccharide (MW: average 20,000) that designates a group

of certain fucose-containing sulfated polysaccharides with a backbone built of (1→3)-linked α -1-fucopyranosyl or of alternating (1→3)- and (1→4)-linked α -1-fucopyranosyl residues, but also include sulfated galactofucans with backbones built of (1→6)- β -d-galacto- and/or (1→2)- β -d-mannopyranosyl units with fucose or fucoligosaccharide branching, and/or glucuronic acid, xylose or glucose substitutions. They even include acetyl groups and protein (Ponce *et al.*, 2003). In southern shores of the Persian Gulf in Iran, there are numerous species belonging to the family of brown algae such as *Padina*, *Sargassum* and *Cystoseira* with high potential. But little researches (Kurdjaziet *al.*, 2011) have been done on extraction of their bioactive compounds such as fucoidan. Among the brown algae in the genus of *Sargassum*, two species *Sargassum ilicifolium* and *Sargassum angustifolium* are more abundant. They grow in rocky areas of the Persian Gulf. So, it is likely that the antioxidant and antimicrobial effects of fucoidan as a sulfated polysaccharide can be unique in these algae. Therefore, the aim of this study was to determine the chemical composition of *S. ilicifolium* and *S. angustifolium* and extracting the sulfated polysaccharide called "fucoidan" in order to evaluate their antioxidant and antimicrobial activities.

Materials and methods

Sample collection and preparation

S. ilicifolium and *S. angustifolium* were collected from the shallow subtidal zone of Qeshm Island (the Persian Gulf) on rocky substrates by Center of Advanced Study in Marine Biotechnology from February to March 2015. All samples were transferred to laboratory of Gorgan University of Agricultural Sciences and Natural Resources. The samples were washed with tap water to remove mud and sand and then were dried in the oven for 3 days at 60°C to obtain a constant weight and grounded by an electric mill (Industrial Mill, SinaParscenter, 2500M-high power, Iran) at 2700 rpm and then were kept in plastic bags. The powdered samples were stored at -80°C.

Chemical analyses

Proximate compounds

Proximate compounds such as ash, total protein, crude lipids, moisture and total carbohydrate were determined by the method of AOAC (2005).

Water holding capacity (WHC), swelling capacity (SWC) and oil holding capacity (OHC)

Half g of *S. ilicifolium* and *S. angustifolium* powders was centrifuged with 20 ml of deionized water. Then, the samples were incubated in a shaker (IKA KS 4000, Germany) for 24 h at 25°C and 37°C, separately and centrifuged at 14000×g for 30 min (5810R, Eppendorf, Germany). The wet weight of *S. ilicifolium* and *S.*

angustifolium were noted and dehydrated by putting in an oven at 105°C for 2 h. WHC was expressed as g of water held on a dry weight basis. SWC of *S. ilicifolium* and *S. angustifolium* were assessed according to the method described by Kuniakand Marchessault (1972). Half g of *S. ilicifolium* and *S. angustifolium* powders were mixed with 20 ml of deionized water and stirred vigorously. The samples were incubated in a shaker overnight at 25°C and 37°C, separately. SWC was expressed as ml of a swollen sample on a dry weight basis.

OHC of *S. ilicifolium* and *S. angustifolium* were assessed according to the method described by Caprezet al. (1986). Three g of dried *S. ilicifolium* and *S. angustifolium* powders were mixed with 10.5 g of corn oil and were incubated in a shaker at room temperature for 30 min. The supernatant was collected by centrifuging at 2500×g for 30 min. OHC was expressed as g of oil held on a dry weight basis.

Mineral content and fatty acid composition

The mineral content was evaluated by following the protocol of Ruperez (2002) and measured by atomic absorption spectrophotometer (Perkin Elmer Analyst 800). The results were expressed as ppm and percentage. Fatty acid composition of *S. ilicifolium* and *S. angustifolium* was assessed according to the protocol of Castro et al. (2007).

2.3. Extraction of fucoidan

Twentyg of *S. ilicifolium* and *S. angustifolium* powders were mixed with 1 liter of 85% ethanol. The samples were shaken at room temperature for 12h in order to remove pigments and proteins. Then, samples were washed with acetone, centrifuged at 1800×g for 10 min and dried at room temperature. Five g of dried samples from the above steps were extracted with 100 ml distilled water at 65°C with stirring for 1h. The extraction was conducted twice. The extracts were combined and centrifuged at 18500×g for 10 min. The supernatant was separated and mixed with 1% CaCl₂. Then, the solution was incubated at 4°C overnight in order to precipitate materials such as alginic acid, and centrifuged at 18500×g for 10 min. The supernatant was collected again. Ethanol (96%) was added into the supernatant to obtain the final ethanol concentration of 30%, and was incubated at 4°C for 4 h. The solution was centrifuged at 18500×g for 10 min. 150 ml ethanol was added into the supernatant to obtain the final ethanol concentration of 70%, and was incubated at 4°C overnight. The fucoidan was obtained by the filtration of the solution with a nylon membrane (0.45µm pore size, ALBET-NY-045-47-BL, Spain). The nylon membrane was washed with ethanol (96%) and acetone and dried at room temperature. The yield of the fucoidan was calculated based on the dried samples obtained after the treatment of the powdered sample with 85% ethanol (Yang *et al.*, 2008).

Sulfate content in fucoidan

First, 1.2 ml of 8% TCA was added to 1.1 ml volume of obtained fucoidan. Then, 0.6 ml of the agarose reagent was added to it. The mixture was let stand for 35 min. Samples were shaken and read the optical density at 500 nm in a spectrophotometer (Jackson and Mccandless, 1978).

Antioxidant activities of fucoidan

Total phenolic content

The amount of total phenolic in fucoidan was determined according to the method of Tagaet *al.*(1984). Briefly, the stock solution of Gallic acid (10 mg10 ml⁻¹) was prepared in distilled water. Then, various concentrations ranging from 200-1000 µg ml⁻¹ were prepared. Two ml of 2% sodium carbonate solution was added to 100 µL water extract of sample and incubated at room temperature for 2 min and then 100 µL of 50% Folin-Ciacaltea reagent was added to the reaction mixture and incubated at room temperature in the dark for 30 min. The absorbance of samples was read at 750 nm. The results are expressed as milligram Gallic acid equivalent per gram dry weight of seaweed powder.

DPPH radical scavenging activity

The scavenging activity of the DPPH radical was assayed according to the method of Brand-Williams *et al.* (1995).23.5 mg of DPPH was dissolved in 100 ml of methanol. Then, this solution was diluted with methanol (1:10). Different concentrations of fucoidan extracts (20-150 µg ml⁻¹) were

prepared. 0.1 ml of these solutions and 3.9 ml of DPPH solution was put in tubes to complete the final reaction media. The mixture was shaken vigorously and incubated at room temperature in the dark for 30 min. The absorbance of samples was measured at 517 nm. Methanol was used to adjust zero and DPPH-methanol solution used as a control sample. L-ascorbic acid and Gallic acid were used as two positive controls. DPPH radical scavenging activity was given by the following formula:

$$\text{Scavenging effect (\%)} = [1 - (\text{A Sample } 517) / (\text{A Control } 517)]$$

Hydroxyl radicals scavenging

Hydroxyl radical scavenging activity was measured using a modified method of Smirnoff and Cumbes (1989). Different concentrations of samples (0.11-1.83 mg ml⁻¹) were prepared and incubated with 0.5 ml of EDTA-Fe (2 mM), 1 ml of H₂O₂ (3%) and 360 µg ml⁻¹ crocus in 4.5 ml sodium phosphate buffer (150 mM, pH 7.4) at 37°C for 30 min. Hydroxyl radical was determined by measuring absorbance at 520 nm. For preparing the control, sample was substituted with distilled water, and sodium phosphate buffer replaced H₂O₂. L-ascorbic acid was used as positive control. Hydroxyl radical scavenging activity was given by the following formula:

$$\text{Scavenging effect (\%)} = [1 - (\text{A Sample } 520) / (\text{A Control } 520)]$$

Superoxide radical scavenging

In the system of PMS-NADH, 3 ml of Tris-HCl buffer (16 mM, pH 8.0), 338 µM of NADH, 72 µM of NBT, 30 µM of PMS were prepared. The different concentrations of fucoidan (4.26-153 µg ml⁻¹) were prepared and incubated at room temperature for 5 min. The absorbance was read at 560 nm. In the control, sample was substituted with Tris-HCl buffer. L-ascorbic acid was used as positive control. Superoxide radical scavenging activity was given by the following formula:

$$\text{Scavenging effect (\%)} = [1 - (\text{A Sample } 560) / (\text{A Control } 560)]$$

Chelating ability

The chelating ability of the fucoidan was measured using the method of Boyer and McCleary (1987). 4.7 ml of sample in different concentrations (0.1-2 mg ml⁻¹) were mixed with 0.1 ml of FeCl₂ (2 mM) and 0.2 ml of ferrozine (5 mM). Then, the samples were shaken well and stand still for 20 min at room temperature. The absorbance was read at 562 nm. In the control, sample was substituted with distilled water. The Citric acid and EDTA were used as positive control. The chelating activity was given by the following formula:

$$\text{Chelating activity (\%)} = [B - A / B] \times 100$$

Where A is A₅₆₂ of the sample and B is A₅₆₂ of the blank.

Reducing power

Reducing the power of fucoidan was determined by the method of Zhang *et*

al. (2011). One ml of fucoidan solution at different concentrations (10-320 $\mu\text{g ml}^{-1}$) was mixed with 1 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 2 ml of TCA (10%, w/v) was added in order to terminate the reaction. The mixture was mixed with 1.25 ml of distilled water and 0.25 ml of 0.1% (w/v) ferric chloride. After 10 min, the absorbance of samples was measured at 700 nm. The L-ascorbic acid was used as standard.

Antibacterial assays of fucoidan

Test organisms

The test organisms which used in this study were *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 10536) and *Salmonella enteritidis* (ATCC 13076). All microorganisms were obtained from the Persian Type Culture Collection in Iranian Research Organization for Science and Technology (IROST).

Antibacterial test

The agar disc diffusion method was performed for antibacterial susceptibility test (Karting *et al.*, 1991).

All bacteria were grown in Nutrient Broth (Merck, Germany) and incubated at 37°C for 24h. A liquid microorganism suspension corresponding to a 0.5 McFarland scale was applied onto petri dishes containing Mueller–Hinton agar (Merck, Germany), using a sterile swab. The 6 mm discs were impregnated with 30 μL of the fucoidan solution at different concentrations (1-5 mg/ disc) and placed on Muller Hinton agar. The plates were incubated at 37°C for 24-28h and the inhibition zones measured around the discs (mm diameter) using a caliper.

Statistical analysis

Statistical analyses were performed using Analysis of Variance (ANOVA) analysis. The Duncan test ($p = 0.05$) was used to determine any significance of differences between specific means (SPSS, 21 version, 2012, USA). All determinations were performed in triplicate, and the data are expressed as mean \pm standard deviation (SD).

Results

Chemical analyses

The results of chemical composition of *S.ilicifolium* and *S.angustifolium* were presented in Table 1.

Table 1: The chemical analyses of *Sargassum ilicifolium* and *Sargassum angustifolium*.

Compositions	<i>S. ilicifolium</i> ^a	<i>S. angustifolium</i> ^a
Proximate composition		
Ash (% DW)	23.16±0.36	35.01±0.42*
Crude protein (% DW)	10.30±0.25	12.45±1.37
Crude lipid (% DW)	1.28±0.09**	0.50±0.17
Moisture (% DW)	14.80±0.50*	10.55±0.61
Total carbohydrate (%DW)	50.46±7.00*	41.49±4.63
Physicochemical properties		
WHC (g H ₂ Og ⁻¹ DW) at 25 °C	2.46±0.24	2.67±0.20
WHC (g H ₂ Og ⁻¹ DW) at 37 °C	2.74±0.11	2.81±0.19
SWC (ml g ⁻¹ DW) at 25 °C	5.33±0.29**	4.33±0.76
SWC (ml g DW) at 37 °C	3.83±0.29	3.67±0.29
OHC (gg ⁻¹ DW)	3.39±0.17	4.32±0.50*
Mineral composition		
Calcium (%)	3.25±1.23*	2.26±0.34
Magnesium (%)	0.72±0.27	1.01±0.072
Iron (%)	0.07±0.023	0.06±0.01
Manganese (ppm)	24.67±14.43*	13.33±3.5
Copper (ppm)	4.33±1.15	4.33±0.58
Zinc (ppm)	42.33±13.32*	11.67±1.15
Fatty acid composition (%w/w)		
Myristic acid (14:0)	3.63±0.01**	2.54±0.01
Palmitic acid (16:0)	37.44±0.01**	23.05±0.01
Stearic acid (18:0)	0.89±0.01**	2.60±0.01
Arachidic acid (20:0)	7.58±0.01**	3.23±0.01
Palmitoleic acid (16:1)	3.89±0.01**	1.73±0.01
Oleic acid (18:1)	13.83±0.01**	45.30±0.01
α-Linoleic acid (18:2)	6.20±0.01**	8.80±0.10
α-Linolenic acid (18:3)	0.91±0.01**	0.99±0.01
Chemical compositions in fucoidan		
Sulphate (% SO ₄ ⁻²)	9.40±0.09**	3.90±0.48
Total phenolic (mgGAEg ⁻¹)	16.59±0.01**	9.73±0.00

^a Results are expressed as Mean±SD (n = 3).; * p<0.05; ** p<0.01.

DPPH radical scavenging activity

The results of DPPH of *S.ilicifolium* and *S.angustifolium* were showed in Fig 1.

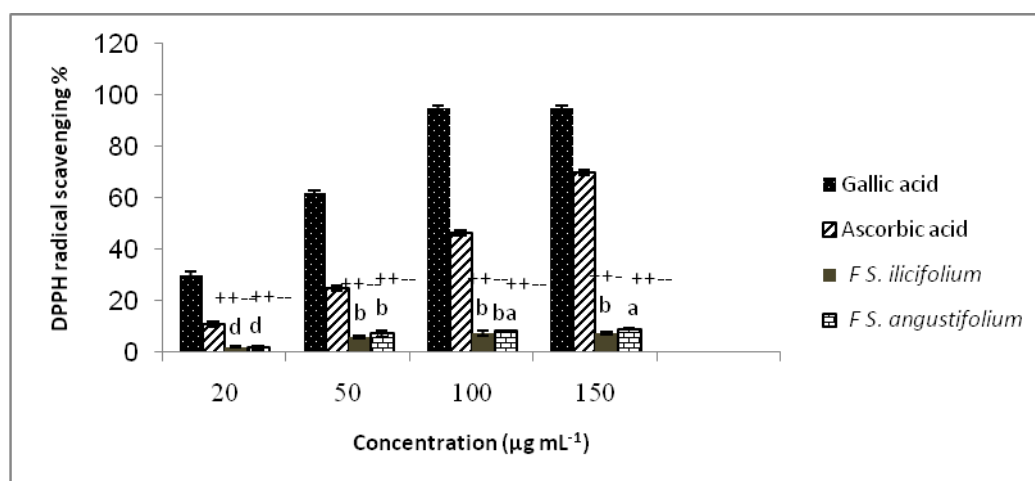


Figure 1 : Scavenging effect of fucoidans on DPPH radicals compared with Gallic acid and L-ascorbic acid. ^(a,b,c,d) Small letters in the graph indicate significant differences ($p < 0.05$) between extracted fucoidans. ++ Significant difference in level of 0.01 between samples and Gallic acid. -- Significant difference in level of 0.01 between samples and ascorbic acid.

Hydroxyl radical scavenging activity

The results of Scavenging effect of fucoidans on Hydroxyl radicals of

S. ilicifolium and *S. angustifolium* were showed in Fig 2.

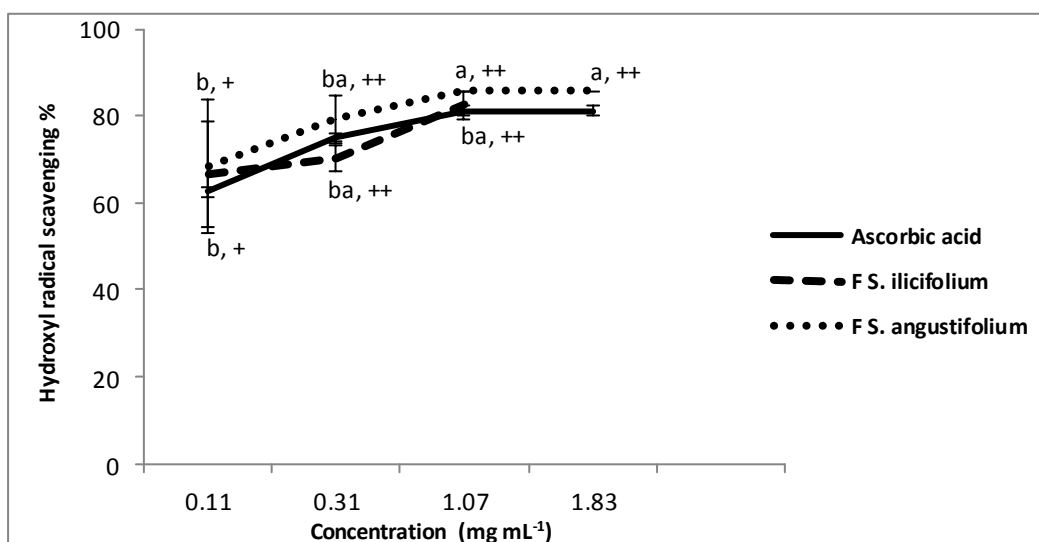


Figure 2: Scavenging effect of fucoidans on Hydroxyl radicals compared with L-ascorbic acid. ^(a,b,c,d) Small letters in the graph indicate significant differences ($p < 0.05$) between extracted fucoidans. ++ Significant difference in level of 0.01 between samples and ascorbic acid.

Superoxide radical scavenging activity

The scavenging activity of fucoidans extracted from *S. ilicifolium* and *S.*

angustifolium on superoxide radicals are shown in Fig. 3.

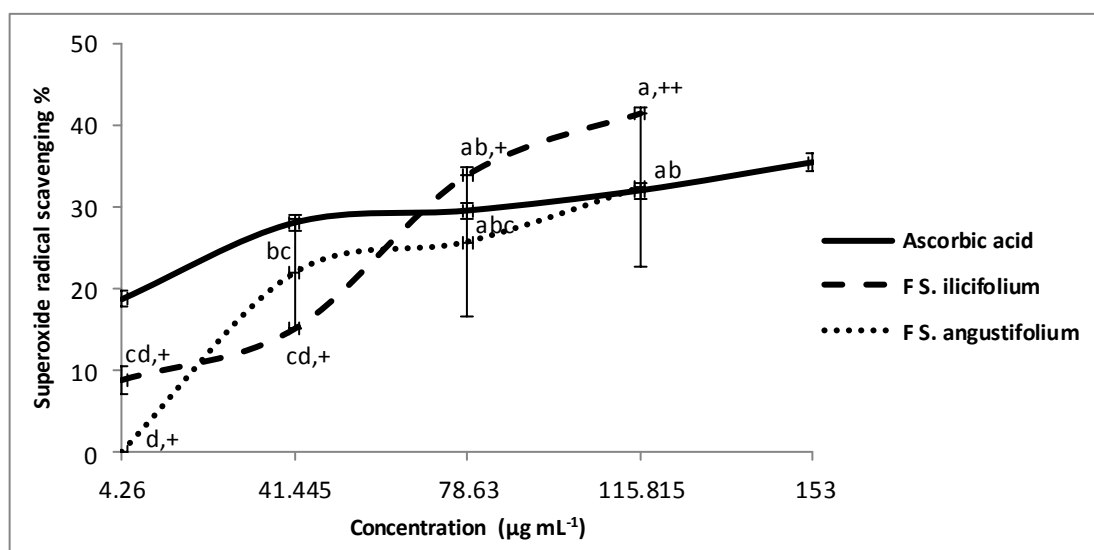


Figure 3: Scavenging effect of fucoidans on Superoxide radicals compared with L-ascorbic acid.
 (a,b,c,d) Small letters in the graph indicate significant differences ($p < 0.05$) between extracted fucoidans. +, + + Significant difference in levels of 0.05 and 0.01 between samples and ascorbic acid.

Chelating effect

Ferrous-ion chelating effect in the crude extracts of the fucoidan is shown in Fig.

4.

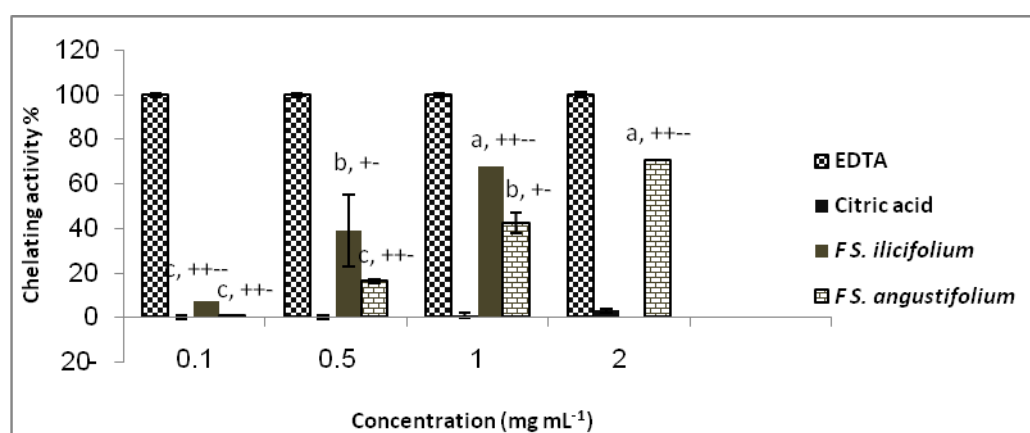


Figure 4: Chelating effect of fucoidans on ferrous-ion compared with EDTA and citric acid.
 (a,b,c,d) Small letters in the graph indicate significant differences ($p < 0.05$) between extracted fucoidans. +, + + Significant difference in levels of 0.01 and 0.05 between samples and EDTA. +, + - Significant difference in levels of 0.01 and 0.05 between samples and citric acid.

Reducing power effect

The effect of reducing power in the fucoidan extracted from *S. ilicifolium*

and *S. angustifolium* are showed in Fig

5.

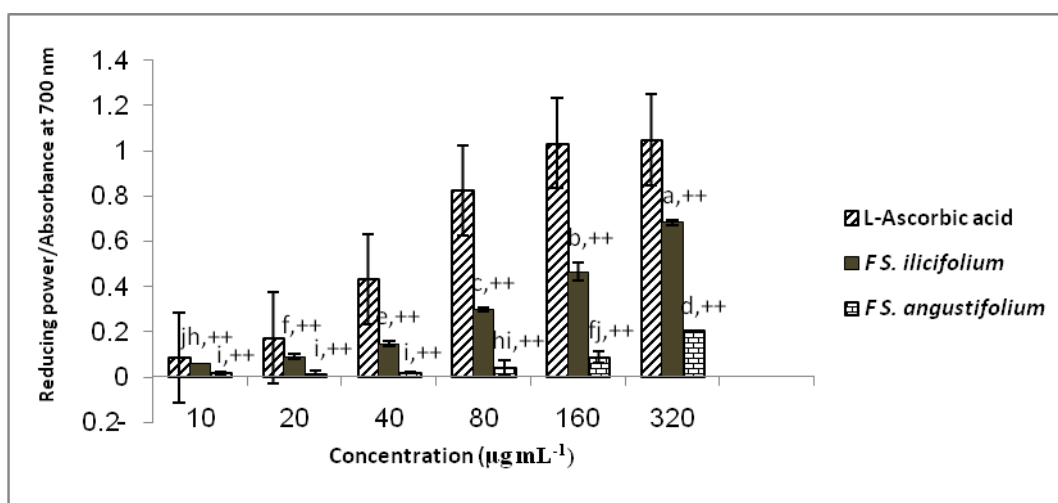


Figure 5: Reducing power of fucoidans compared with L-ascorbic acid. ^(a,b,c,d,e,f,j,h,i) Small letters in the graph indicate significant differences ($p < 0.05$) between extracted fucoidans. ++ Significant difference in level of 0.05 between samples and L-ascorbic acid.

Discussion

The ash content is the total mineral content of the seaweeds after ignition or complete oxidation. Ash contents of *S. ilicifolium* and *S. angustifolium* were found as $23.16 \pm 0.36\%$ and $35.01 \pm 0.42\%$ of dry weight (DW), respectively. Proteins are a dominant component in all plant cells, and mostly important for cellular structures and biological functions.

The amount of crude protein was $10.30 \pm 0.25\%$ and $12.45 \pm 1.37\%$ of DW for *S. ilicifolium* and *S. angustifolium*, respectively. Lipids were the minor components of seaweeds. The content of total lipid was observed as $1.28 \pm 0.09\%$ and $0.50 \pm 0.17\%$ of DW, respectively, for *S. ilicifolium* and *S. angustifolium*. One of the fundamental and analytical procedures which need to be performed is the moisture content. Moisture assays can be one of the most important analyses on a food product

and affects the stability of food materials. The moisture content of *S. ilicifolium* and *S. angustifolium* was observed as $14.80 \pm 0.50\%$ and $10.55 \pm 0.61\%$, respectively. Total carbohydrate content of *S. ilicifolium* and *S. angustifolium* was $50.46 \pm 7.00\%$ and $41.49 \pm 4.63\%$ of DW, respectively (Table 1). The centrifugation method was employed to determine the physicochemical properties like WHC, SHC and OHC (Table 1). The effect of temperature on WHC and SWC was investigated in our study. At 25°C , WHC of *S. ilicifolium* was $2.46 \pm 0.24 \text{ g g}^{-1}$ of DW and a slight increase ($2.74 \pm 0.11 \text{ g g}^{-1}$ DW) was observed after incubating at 37°C . Interestingly, upon incubation at 37°C , the SWC of the seaweed decreased significantly ($p < 0.05$) from 5.33 ± 0.29 (at 25°C) to 3.83 ± 0.29 . In case of *S. angustifolium*, the WHC and SWC were $2.67 \pm 0.20 \text{ g/g}$ DW and $4.33 \pm 0.76 \text{ ml g}^{-1}$ DW,

respectively at 25°C and 37°C. The values of WHC increased slightly to $2.81 \pm 0.19 \text{ gg}^{-1} \text{ DW}$, but the values of SWC decreased to $3.67 \pm 0.29 \text{ ml g}^{-1} \text{ DW}$. This increase in WHC can be attributed to increase in the solubility of fibers and proteins. Oil holding capacity (OHC) is an important and dominant property of food ingredients. The entrapment of oil by capillary action is generally represented as OHC of the seaweeds. OHC is related to how staying the polar side chains of amino acids on the surface of protein molecules. The OHC of *S. ilicifolium* and *S. angustifolium* was found to be $3.39 \pm 0.17 \text{ gg}^{-1}$ and $4.32 \pm 0.50 \text{ gg}^{-1} \text{ DW}$, respectively (Table 1).

The seaweeds are very useful for the values of nutritional ingredients such as fiber, minerals and amino acids. Therefore, these positive features make seaweed as a nutritive and low-energy food. Overall, minerals and trace elements are required for human nutrition. Based on these requirements, the major constituents of seaweeds are ranging from 8–40% (Ruperez, 2002). So, seaweeds in compared with ground plants are a rich source of minerals. They can be used as a food supplement to help daily intake of some essential minerals and trace elements, especially for pregnant women and the elderly. The mineral content (Table 1) of *S. ilicifolium* and *S. angustifolium* were indicated that the brown seaweed of *S. ilicifolium* contain high amount of calcium ($3.25 \pm 1.23\%$), whereas *S. angustifolium* contains high amount of

magnesium ($1.01 \pm 0.072\%$). Both calcium and magnesium play an indispensable role in prevention of osteoporosis. Also, recent reports suggest that low magnesium levels lead to the deposition of heavy metals in the brain and cause progress in Parkinson's and Alzheimer's diseases (Syadet *al.*, 2013). Thus the presence of magnesium in food is essential in regulating central nervous system excitability and normal functions (Murck, 2002). Similar to calcium and magnesium, iron was also found to be an abundant mineral in both seaweeds ($0.07 \pm 0.023\%$ in *S. ilicifolium* and $0.06 \pm 0.01\%$ in *S. angustifolium*). Whereas the amount of manganese was $24.67 \pm 14.43 \text{ ppm}$ and $13.33 \pm 3.51 \text{ ppm}$ in *S. ilicifolium* and *S. angustifolium*, respectively. Apart from these major elements, *S. ilicifolium* and *S. angustifolium* have trace elements like copper and zinc in the concentrations of $4.33 \pm 1.15 \text{ ppm}$ (Copper); $42.33 \pm 13.32 \text{ ppm}$ (Zinc) and $4.33 \pm 0.58 \text{ ppm}$ (Copper), $11.67 \pm 1.15 \text{ ppm}$ (Zinc), respectively.

Fatty acids are an important nutrient for the human heart. It has been found that seaweeds in comparing with terrestrial vegetables contain more amounts of polyunsaturated fatty acids (PUFA). For example, the fatty acids have useful functions such as cardio-protective, cytotoxic, anti-mitotic, anti-cancer, anti-viral and anti-mutagenic activities (Ortiz *et al.*, 2006). The data obtained from the fatty acid composition of *S. ilicifolium* and *S. angustifolium* are given in Table 1.

There was a mixture of both saturated and unsaturated fatty acids. The saturated fatty acids present in two brown seaweeds were myristic acid (3.63 ± 0.01 , $2.54 \pm 0.01\%$ w/w), palmitic acid (37.44 ± 0.01 , $23.05 \pm 0.01\%$ w/w), stearic acid (0.89 ± 0.01 , $2.60 \pm 0.01\%$ w/w) and arachidic acid (7.58 ± 0.01 , $3.23 \pm 0.01\%$ w/w). The unsaturated fatty acids include palmitoleic acid (3.89 ± 0.01 , $1.73 \pm 0.01\%$ w/w), oleic acid (13.83 ± 0.01 , 45.30% w/w), α -linoleic acid (6.20 ± 0.01 , $8.80 \pm 0.10\%$ w/w), and α -linolenic acid (0.91 ± 0.01 , $0.99 \pm 0.01\%$ w/w). *S. ilicifolium* and *S. angustifolium* contained a total of 74.37% and 88.24% of fatty acids, of which $49.54 \pm 0.01\%$ (w/w) and $31.42 \pm 0.01\%$ (w/w) were saturated fatty acids; $24.83 \pm 0.01\%$ (w/w) and $56.82 \pm 0.01\%$ (w/w) were unsaturated fatty acids, respectively. So, the results of fatty acid analysis revealed that both *S. ilicifolium* and *S. angustifolium* are rich in MUFA and PUFA, which possess important health benefits. In particular, oleic acid (MUFA) and α -linolenic acid (PUFA) present in both the seaweeds which might help in lowering the blood cholesterol, act as excellent antioxidants, strengthen the cell membrane, repair the damaged cells and tissues, improve the functioning of heart and fight against cancer.

In general, the yield of fucoidan extracted from brown seaweeds is very variable due to different factors such as environmental conditions, habitat and the season of brown seaweed collection,

physiological factors and extraction methods.

The yield of fucoidan extracted from *S. ilicifolium* and *S. angustifolium*, was 0.75% and 2.5%, respectively. These yields obtained from these species were lower than the reported values (1.1–8.8%) from other brown seaweeds (Ruperez *et al.*, 2002; Yang *et al.*, 2008). Also, sulfate content in fucoidan extracted from *S. ilicifolium* and *S. angustifolium* was $9.40 \pm 0.09\%$ and $3.90 \pm 0.48\%$ (Table 1). Some of the reasons for such differences in the chemical composition of fucoidan polymers are species type, growing conditions, harvest season, extraction procedures and analytical methods (Ponce *et al.*, 2003; Yang *et al.*, 2008).

The presence of phenolic compounds can affect the antioxidant capacity. Phenol amounts in the fucoidan were observed as $9.73 \text{ mg Gallic acidg}^{-1} \text{ DW}$ in *S. angustifolium* and $16.59 \text{ mg Gallic acidg}^{-1} \text{ DW}$ in *S. ilicifolium* (Table 1). Study of Chandini *et al.* (2008) on the aqueous fractions of *Sargassum arginatum* and *Turbinaria conoides* indicated that phenol content was low (0.29 and $0.86 \text{ mg Gallic acidg}^{-1} \text{ DW}$, respectively). Chew *et al.*, (2008) with study on *Kappa phycusalvarezzi* and *Padina antillarum* noted that the difference in phenol content depended on the variety of seaweed. Also, they reported $1.15 \text{ mg Gallic acid/ g DW}$ in *K. alvarezzi* and $24.30 \text{ mg Gallic acidg}^{-1} \text{ DW}$ in *P. antillarum*. In general, it is demonstrated that the amount of phenol is influenced directly by sunlight and weather condition. Even, phenol level

of the same species in the two areas or in two different countries is quite different. For example, *Fucus serratus* samples harvested in tropical area such as the Canary Islands had higher phenol levels than the same species harvested in Ireland (Flodinet *et al.*, 1999). Also, the phenol levels in seaweed are related to growth location on the beach. Since, the presence of seaweeds in the high intertidal zone leading to absorb higher levels of ultraviolet radiation. In result, the seaweed produces more phenols against stress caused by ultraviolet radiation. In contrast, seaweeds located in the lower intertidal zone have the lower phenol levels due to submerge location.

DPPH radical scavenging power used for evaluating the antioxidant activities of samples in a short time. A color change from purple to yellow was observed in this study. This color change represents antioxidant activities by the donation of hydrogen to the DPPH molecule. Park *et al.* (2004) noted that DPPH radical scavenging activity is in relation to bond formation with unpaired electrons of the radical. The scavenging effect of the DPPH free radical of the fucoidan was depicted in Fig. 1. The absorbance was decreased with increasing the concentration of the samples. Decrease in the absorption is equal to an increase in antioxidant activity, but in general, the scavenging effect of the DPPH free radical in the extracted fucoidan from two brown seaweeds *S. ilicifolium* and *S. angustifolium* were low, which may be

because of weak hydrogen donating ability of the samples. Also, in this study the scavenging effect of the DPPH free radical of L-ascorbic acid and Gallic acid increased with increasing concentrations (20-150 $\mu\text{g ml}^{-1}$). The scavenging activity of the DPPH free radical in Gallic acid was shown >90 % at the concentrations of 100 and 150 $\mu\text{g ml}^{-1}$, while, the scavenging activity of the DPPH free radical in *S. ilicifolium* and *S. angustifolium* samples increased from 2.22% and 1.87% to 7.53% and 9.20% at the concentrations of 20 $\mu\text{g ml}^{-1}$ and 150 $\mu\text{g ml}^{-1}$, respectively.

Devi *et al.* (2008) reported that this scavenging ability is different between the seaweed species. On the other hand, Siriwardhana *et al.* (2003) noted to a correlation between the total phenol content of seaweeds and the scavenging effect of the DPPH free radical. In the present study, except in higher concentration (150 $\mu\text{g ml}^{-1}$), there was no significant difference between *S. ilicifolium* and *S. angustifolium*. Therefore, these results showed the species that have a high amount of total phenol; them necessarily not showing a goodness DPPH scavenging effect.

Hydroxyl radical scavenging activity

As it is shown in Fig. 2, all samples, especially extracted fucoidan from *S. angustifolium* represented the detectable scavenging activity on hydroxyl radicals by increasing the concentration. At a concentration of 1.07 mg ml^{-1} , the extracted fucoidan from *S. ilicifolium*

and *S. angustifolium* showed an excellent scavenging activity of the hydroxyl radical (82-85%). The result showed that although fucoidan of *S. ilicifolium* had more sulfate and phenol contents than the *S. angustifolium*, but no significant difference ($p>0.05$) was observed between species. Overall, the scavenging ability of two fucoidans extracted from these brown seaweeds was better than L-ascorbic acid. The study by Shon *et al.* (2003) noted that there are two antioxidant mechanisms, one is preventing the hydroxyl free radical production and the other is removing the hydroxyl free radicals produced. The hydroxyl free radicals can in the presence of metal ions (such as iron and copper) can produce hydrogen peroxide.

Also, in other reports, Qi *et al.* (2005) and Xing *et al.* (2005) found that both mechanisms are effective in the ability of hydroxyl radical scavenging. Since, the molecules such as sulfated polysaccharides (fucoidan) are able to chelate iron and scavenge the hydroxyl free radicals.

In this study, the scavenging effect of fucoidan extracted from *S. ilicifolium* and *S. angustifolium* on superoxide radical had no significant difference, but, they indicated a significant difference in comparing with positive control like L-ascorbic acid. The superoxide radical scavenging ability of fucoidans was less than 50% in selected concentrations. The extracted fucoidan from *S. ilicifolium* indicated more scavenging activity on superoxide free radicals compared with L-ascorbic acid.

Also, *S. ilicifolium* fucoidan showed more scavenging ability in high concentration. Sulfate and phenol contents in the fucoidan samples were 9.40%SO₄⁻², 16.59 mg GAE/ g sample and 3.90%SO₄⁻², 9.73 mg GAE/ g sample, respectively. Presumably, sulfate and phenol amounts can affect on the antioxidant activity of fucoidans. These results were in accordance with Zhang *et al.* (2003) and Qi *et al.* (2005). In most organisms, superoxide is a weak oxidant which can break down and become another form.

According to the result, it can stimulate pathogenic agents such as arthritis and Alzheimer's disease. In general, these results showed that the antioxidant activities of fucoidans extracted from *S. ilicifolium* and *S. angustifolium* were dependent on the superoxide radical scavenging ability.

Ferrous ions stimulate lipid peroxidation and also, they are an effective peroxidant in food systems (Yamauchi *et al.*, 1988). Ferrous ion-chelating effects in fucoidans extracted from *S. ilicifolium* and *S. angustifolium* in the concentration of 1mg ml⁻¹ were 67.54% and 42.05%, respectively. Interestingly, the fucoidans extracted from *S. ilicifolium* could not chelate ferrous-ion in higher concentration and stayed constant, but chelating effect of the Fucoidan extracted from *S. angustifolium* on ferrous-ion increased to 70.70% with increasing concentration. In fact, these results indicated the fucoidan extracted from *S. ilicifolium* had the high metal ion-binding ability at concentrations of less

than 2 mg ml⁻¹. Also, the fucoidan extracted from *S. ilicifolium* had the highest ratio of sulfate and phenol contents resulting in the highest ferrous ion-chelating activity at concentrations between 0.1 and 1 mg ml⁻¹. In total, the relationship between concentration and chelating effect is complicated.

The substitution position of sulfate group may be effective on chelating ability, but this mechanism is not completely understood.

The value of higher absorbance indicated higher reducing power effect in the samples. As a result, in this study, the reducing power of fucoidan samples was shown suitable in the tested concentrations. The reducing power of *S. ilicifolium* and *S. angustifolium* at the concentration of 10 µg ml⁻¹ were 0.06 and 0.02, and at the concentration of 320 µg ml⁻¹, and were 0.683 and 0.205, respectively, which were less than of L-ascorbic acid. In addition, the relationship between reducing power and the amount of sulfate and phenol were significant. The reduction agents such as polysaccharides are responsible for reducing effect and shows antioxidant action with breaking the radical free chains and donating a hydrogen atom. Also, Zhu *et al.* (2002) noted that in most cases, in addition to the antioxidant action, most nonenzymatic and antioxidative activities such as peroxide inhibition and scavenging of free radicals contribute in the redox reactions. The results of this study indicated that the effect of reducing power in the fucoidan

samples may be due to their role in the anti-oxidation activities.

Variation in antibacterial activity of fucoidan extracted from seaweeds may be because of using different extraction methods and the type of selective solvent. In this study, the disc diffusion method was used for antimicrobial activity.

This method is simple and economically justified. Also, the disc diffusion method is used to identify a resistant organism or a specific antibiotic that is susceptible. Salvador *et al.* (2007) reported that the National Committee of Clinical Laboratory Standards (NCCLS) has accepted this standardized procedures for determining antimicrobial susceptibility. In this examination, the filter paper disc impregnated with extracted fucoidan from *S. ilicifolium* and *S. angustifolium*, and after incubating it was observed that only at the concentration of 5 mg ml there were small clear inhibition zones against *B. subtilis* and *S. aureus*. The low activity of the fucoidan against the gram negative bacteria may be due to the presence of outer membrane and a protoplasmic space. In fact that it is a barrier to antibiotic molecules penetration. Because, there are enzymes in the protoplasmic space that are able to destroy alien molecules. The inhibitory zones of the extracted fucoidan from brown seaweeds against *B. subtilis* were much less than the positive control such as Amoxicillin. These results indicated that the

bioactive compounds for playing as antibacterial agent have different characteristics. Furthermore, it may be due to their concentration level that was used. In this study the same concentration was used for measurement of antioxidants and microbial activities. Previous reports have noted that the gram positive bacteria were effectively controlled by seaweed extracts as compared with gram negative bacteria (Tuneyet *al.*, 2006; Pushparajet *al.*, 2014). In total, the separation and purification of the biologically active compounds for developing new drugs to control the deadly diseases is needed. Also, further researches are needed about the fucoidan presence in brown seaweeds in order to combat with various diseases. In this study, chemical composition of *S. ilicifolium* and *S. angustifolium* seaweeds and functional properties of fucoidan were evaluated. As a result, it is suggested that the fucoidan of both brown algae can be a promising ingredient with antioxidant activities for food and medical industries and even used as nutritional supplements or medication in some specific diseases. Therefore, those seaweeds are a good source of pharmaceutical raw materials. Hence, their commercial value can be enhanced by improving the quality.

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