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Monosaccharide Profile and Antioxidant Activities of Sulphated Polysaccharide Extracted from Indonesian Brown Seaweed (*Sargassum* sp.)

(Profil Monosakarida dan Aktiviti Antioksida Polisakarida Tersulfat yang Diekstrak daripada Rumpai Laut Perang Indonesia (*Sargassum* sp.))

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ABSTRACT

This research was conducted to determine the monosaccharide profile and antioxidant activities of the sulphated polysaccharide fucoidan, extracted from *Sargassum* sp. harvested in Batam, Indonesia. Crude fucoidan (F_{sar}) was extracted using low pH (pH 2) acid method, obtaining a yield of 5.5% (w/w), and the chemical properties of F_{sar} were subsequently compared to commercial fucoidan (F_{comm}), obtained from Japan. Sulphate contents were determined using spectrophotometric analysis, where F_{sar} (5.21±0.35%) and F_{comm} (8.80±0.19%) differed significantly (p<0.05). Monosaccharide profiling was performed using methanolysis and gas chromatography-flame ionisation detector (GC-FID). F_{sar} contained fucose (138.34 µg/g), 4-*O*-methyl-glucuronic acid (105.39 µg/g), galactose (70.49 µg/g) and glucuronic acid (30.60 µg/g) while F_{comm} contained fucose (520 µg/g) and glucuronic acid (62.93 µg/g) as its main composition. Antioxidant activities were analysed through 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide anion (SOA) and hydroxyl radical (·OH) scavenging activities. DPPH scavenging activity of F_{sar} (40.67±4.0%) was significantly higher (p<0.05) than F_{comm} (10.11±1.0%), while SOA and ·OH scavenging activities showed no significant difference (p>0.05) between F_{sar} and F_{comm}. F_{sar} showed significant differences (p<0.05) in chemical properties and DPPH scavenging activities compared to that of F_{comm}. Fucoidan from Southeast Asia waters has the potential for application as functional ingredients.

Keywords: Brown seaweed; DPPH; fucoidan; monosaccharide profiling; sulphated polysaccharides

ABSTRAK

Penyelidikan ini dijalankan untuk mengenal pasti profil monosakarida dan aktiviti antioksida polisakarida tersulfat fukoidan yang diekstrak daripada *Sargassum* sp., dari Batam, Indonesia. Fukoidan kasar (F_{sar}) diekstrak dengan kaedah pengekstrakan asid pada pH rendah (pH 2) dan telah memperoleh hasil pengekstrakan sebanyak 5.5% (w/w). Seterusnya, ciri kimia F_{sar} telah dibandingkan dengan fukoidan komersial dari Jepun (F_{comm}). Kandungan sulfat dikenal pasti menggunakan kaedah spektrofotometrik dengan F_{sar} (5.21±0.35%) dan F_{comm} (8.80±0.19%) menunjukkan perbezaan yang signifikan (p<0.05). Pemprofilan monosakarida dijalankan menggunakan kaedah metanolisis dan kromatografi gas-pengesan nyala pengionan (GC-FID). F_{sar} didapati mengandungi fukosa (138.34 µg/g), asid 4-*O*-methil-glukuronik (105.39 µg/g), galaktosa (70.49 µg/g) dan asid glukuronik (30.60 µg/g); manakala F_{comm} mengandungi fukosa (520 µg/g) dan asid glukuronik (62.93 µg/g) sebagai monosakarida utama. Aktiviti antioksidan dianalisis menggunakan kaedah pemerangkapan radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH), anion superoksida (SOA) dan radikal hidroksil

(·OH). F_{sar} (40.67±4.0%) menunjukkan aktiviti pemerangkapan radikal bebas DPPH yang lebih tinggi secara signifikan ($p<0.05$) berbanding F_{comm} (10.11±1.0%), manakala aktiviti pemerangkapan SOA dan ·OH tidak menunjukkan perbezaan signifikan ($p>0.05$) antara F_{sar} dan F_{comm}. Secara keseluruhannya, F_{sar} menunjukkan perbezaan yang signifikan ($p<0.05$) dalam ciri kimia dan aktiviti pemerangkapan radikal bebas DPPH berbanding F_{comm}. Ini menunjukkan fukoidan dari perairan Asia Tenggara mempunyai potensi untuk dibangunkan sebagai ingredien kefungsian.

Kata kunci: DPPH; fukoidan; pemprofilan monosakarida; polisakarida tersulfat; rumpai laut perang

Introduction

Sargassum sp. is a type of brown seaweeds, which produces fucose-rich sulphated polysaccharides called fucoidan. In South East Asia, Malaysia and Indonesia have abundance source of brown seaweed, especially *Sargassum* sp. The wide availability of *Sargassum* sp. makes it an ideal sample for compositional studies. Fucoidan possess strong biological activities, including anti-oxidant, anti-coagulant and anti-bacterial properties (Ale et al. 2011). Fucoidan has the capacity for free radicals scavenging, thus, can potentially minimising oxidative damage in biological system (Lim et al. 2014). Due to this, fucoidans are commonly applied as a raw ingredients for nutraceuticals and food supplements (Lim et al. 2017).

The fucoidan of brown seaweeds are mainly hetero-polysaccharides, with fucoses with α-1,2-, α-1,3-, or α-1,4-O-glycosidic bonds and sulphated at C2 and/or C4 of the fucoses as the major monosaccharides (Lim et al. 2016). Aside from fucose, fucoidan composed of other monosaccharides, namely mannose, xylose, galactose, glucose, uronic acids, and rhamnose (Lim et al. 2016). The structural characteristics of the fucoidan in terms of monosaccharide linkages and constituents, as well as sulphation patterns are highly correlated to the different bioactivities of fucoidan (Ale et al. 2011).

The modern lifestyle as well as environmental pollution has caused increasing oxidative stress in the human body, which can accelerate the aging rate, cancer and cardiovascular disease (Sharifi-Rad et al. 2020). Fucoidans typically exhibit its antioxidant activities due to the sulphation of the major monomer, fucose, which acts as the electron-withdrawing groups (Wang et al. 2010).

It is clear that structural heterogeneity of fucoidans varies with the seaweed species, and thus seaweeds from different species, geographical locations and seasons need to be studied. The current work was conducted to determine and compare the chemical properties (sulphate, fucose and monosaccharides composition) and antioxidants capacity of fucoidan extracted from Indonesian *Sargassum* sp. (F_{sar}) with the commercially available food-grade fucoidan (F_{comm}). There is not many research conducted on fucoidan from Indonesian *Sargassum* sp., where a search in Scopus database (13th July 2022) with keywords of 'Fucoidan', 'Sargassum' and 'Indonesia' only yielded 5 articles, in which, only 2 articles described on the chemical composition, and none on antioxidant of fucoidan. Thus, this warrants more research on the fucoidan extracted from *Sargassum* sp. harvested in Indonesia.

Materials & Methods

Sample Preparation

Sargassum sp. was provided by Marine Ceuticals Sdn Bhd, Selangor, Malaysia, was harvested from Batam, Indonesia, in May 2015. The seaweed was rinsed with water to remove non-seaweed particles and impurities, such as sand and epiphytes, and subsequently sun-dried. The dried seaweed was then ground and sieved to powdered form (0.25 mm sieve size) as this is the practical and suitable size for handling and extraction. The food-grade fucoidan (92% purity) extracted from *Clasidophon okamuranus* was provided by Yaizu Suisankagaku Industry Co. Ltd, Yaizu City, Japan (F_{comm}).

Pre-Treatment

A total of 40 g dried *Sargassum* sp. was pre-treated with ethanol at 1:10 (w:v) for 2 h in a reflux system set at 80 °C for the removal of lipids and coloured pigments. Pre-treated samples were then separated from the solvent through centrifugation (Eppendorf Centrifuge 5810R, 12,000 rpm, 5 min) and subsequently dried in a fume chamber at ambient temperature for 24 h (Khalafu et al. 2017).

Extraction of Fucoidan

Fucoidan extraction was performed by means of low pH acidic solution method, as performed by Khalafu et al. (2017). Extraction was carried out using HCl, where the solution (400 mL) was adjusted to pH 2 with 35 g of the dried pre-treated *Sargassum* sp., heated to 65 °C and stirred for 3 h. After completion of the extraction process, centrifugation was performed (Eppendorf Centrifuge 5810R, 12,000 rpm, 10 min), filtered (Whatmann No. 4 filter paper) and where the fucoidan-containing supernatant was collected. The same extraction procedures and parameters were repeated 5 times for complete fucoidan extraction. The combined extract was then neutralised using 3 M NaOH, and subsequently water was evaporated using a rotary evaporator (Buchi Rotavapor, Switzerland) until approximately 100 mL of extract was obtained. Absolute ethanol (400 mL) was added into the extract to obtain 80% ethanolic solution and was refrigerated (4 °C) for 24 h to allow fucoidan precipitation. Centrifugation was performed at 12,000 rpm for 10 min (Eppendorf Centrifuge 5810R), followed by filtration to recover the precipitates. The precipitates were rinsed with ethanol, and the ethanol was evaporated at 40 °C using a convection oven (Memmert 53L model). Fucoidan extract was kept at 4 °C prior to analyses.

YIELD OF FUCOIDAN

Fucoidan yield (% Fuc), was calculated according to equations (1) (Khalafu et al. 2017)

$$\% \text{ Fuc} = m_{dry} / m_s \times 100 \qquad (1)$$

where m_{dry} is the dry fucoidan mass obtained after extraction; and m_s is the dry seaweed mass used.

FUCOSE CONTENT ANALYSIS

Fucose content was screened through spectrophotometric method (Lim et al. 2014). F_{sar} at concentration of 2 mg/mL (1 mL) was pipetted with 4.5 mL of diluted sulphuric acid (6:1, H_2SO_4:H_2O) and cooled in an ice-water bath for 1 min. Subsequently, the mixtures were heated at boiling temperature (100 °C) for exactly 10 min, and immediately cooled to room temperature by running water. L-cysteine (3%, 0.1 mL) was added into the mixture and incubated for 30 min at room temperature. A series of fucoidan standard using F_{comm} (0.2-0.02%) were prepared for the calibration curve. The reactants (all samples and standards) were measured at 396 nm and 427 nm using a microplate spectrophotometer (BioTek Epoch, USA). Fucose absorbance against other hexoses could be determined by subtracting the absobances ($A_{396} - A_{427}$). Fucose contents of the samples were calculated using equation (2):

$$\text{Fucose (\%)} = R \qquad (2)$$

where *R* is the value obtained from calibration curve; *TV* is total volume; *SV* is sample volume; *DF* is dilution factor; and *m* is the mass of fucoidan.

SULPHATE CONTENT ANALYSIS

Both F_{sar} and F_{comm} (2 mg) were placed into glass tubes and added with 1 M HCl for dissolution. The tubes were covered with screw caps and the mixtures were then heated to 105-110 °C and incubated for 5 h. Samples were then cooled to ambient temperature, after which 0.2 mL were transferred into 2 separate tubes (Tube A and Tube B) for each sample. K_2SO_4 was prepared as standard, with concentration ranging from 0-100 µg/mL, where 0.2 mL were transferred into the tubes, also as Tube A and Tube B. Trichloroacetic acid at 3% (w/v) was pipetted into all Tube A and Tube B. Specifically for Tube A, 1 mL of barium chloride-gelatine reagent (0.5% w/v barium chloride in 0.5% w/v gelatine solution) was added, while for Tube B, 1 mL of gelatine solution (0.5% w/v) was added. All reactants were then mixed and incubated at ambient temperature for 20 min. Subsequently, the absorbance of all samples and standards (Tube A) were determined spectrophotometrically at 360 nm against the corresponding reagent blanks (Tube B) (Lim et al. 2019).

MONOSACCHARIDE PROFILING (METHANOLYSIS)

The methanolysis procedure was employed in monosaccharide profiling of F_{sar} and F_{comm}, where 2 mg of the samples were added with 2 mL of 2 M methanolic HCl, and heated to 100 °C for 5 h, with intermittent mixing twice within the 5 h (Lim et al. 2019). After cooling to room temperature, anhydrous pyridine (200 µL) and methanolic sorbitol (internal standard) at 5 - 100 mg/mL was added. Evaporation of solvents from the samples were performed using nitrogen gas and subsequently freeze-dried overnight. After drying, 200 µL anhydrous pyridine was added to dissolve the sample and allowed to stand at room temperature for 1 h. Anhydrous pyridine containing 1.5 mg/mL of 4-dimethylaminopyridine (200 µL) and *N*,*O*-bis(trimethylsilyl) trifluoroacetamide with 10% of chlorotrimethylsilane (400 µL) were pipetted into the sample and incubated at 70 °C for 2 h, allowing the silylation reaction to take place. The reactants were subsequently cooled at -20 °C for 15 min, added with ethyl acetate (400 µL), and then centrifuged.

The supernatants were pipetted into gas chromatography (GC) vials for analysis using gas-chromatography-flame ionisation detector (GC-FID). Separation was conducted on an Agilent Technologies (Mississauga, ON, Canada) model 7890B GC system coupled with a flame ionisation detector (FID), where 1 µL of samples were injected in split ratio of 10:1 with injector temperature of 260 °C. GC column used was HP1 (Agilent 19091Z-413) methylsiloxane column (30 m × 320 µm × 0.25 µm), where analytes were eluted using Helium carrier gas at a flow rate of 2 mL/min. The column temperature was set at 140 °C for 1 min, ramped to 210 °C at 4 °C/min and then increased to 260 °C at a rate of 30 °C/min, and finally held at 260 °C for 5 min. The FID temperature was held at 320 °C. Calibration curves of L-fucose, D-xylose, D-galactose, D-mannose, D-glucose, D-arabinose, D-rhamnose and D-glucuronic acid for quantification were conducted through the same parameters described.

TOTAL PHENOLIC CONTENT (TPC)

Folin-Ciocalteau assay was performed on the F_{sar} and F_{comm} samples to determine the total phenolic contents (Mohd Fauziee et al. 2021). Both F_{sar} and F_{comm} samples were reconstituted with distilled water at 2 mg/mL. The sample solutions (0.3 mL) were added with 1.5 mL of Folin-Ciocalteau reagent and 1.2 mL of 7.5% (w/v) sodium carbonate solution, and subsequently vortexed and kept in the dark condition for 1.5 h to allow the reaction to occur. A calibration curve was prepared using gallic acid (5 - 50 ppm), as well as positive controls (BHA, BHT and ascorbic acid; all at 2 mg/mL) through the procedure above. Absorbance of the reactants (samples, standards and positive controls) at 765 nm were performed using a microplate spectrophotometer. TPC of the samples were then expressed as gallic acid equivalent (GAE %) (Lim et al. 2014).
The total phenolic content was calculated using equation (3):

Total phenolic content (GAE %) =

$$= R \times \frac{TV}{SV} \times DF \times \frac{100}{m} \times \frac{1}{10^6} \tag{3}$$

where R is the absorbance value; TV is total volume; SV is sample volume; DF is dilution factor; and m is the weight of fucoidan.

FREE RADICAL SCAVENGING ACTIVITY

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the samples were conducted based on Tan et al. (2018). DPPH stock solution was prepared at 0.02% using methanol, and diluted with methanol to achieve 0.7±0.01% absorbance at 516 nm through a 1:10 dilution. Both fucoidan samples (F_{sar} and F_{comm}) was prepared at 2 mg/mL and 200 µL of it was added with 3 mL of methanolic DPPH solution, and then mixed prior to incubation in the dark at room temperature for 30 min. Subsequently, 200 µL of the reactants were transferred to a 96-well microplate and for absorbance measurement at 516 nm. DPPH free radical scavenging activity was calculated using (4):

DPPH free radical scavenging activity (%) =

$$\frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \tag{4}$$

where A_{blank} is the absorbance of the blank; and A_{sample} is the absorbance of the sample.

SUPEROXIDE ANION SCAVENGING ACTIVITY

Superoxide anion (SoA) scavenging activity was analysed based on the procedures conducted by Lim et al. (2014). Both F_{sar} and F_{comm} (0.3 mL at 2 mg/mL) were added with 2.6 mL of 50 mM phosphate buffer (pH 8.24) and 90 µL of 3 mM pyrogallol (dissolved in 10 mM HCl). The capacity of the samples to inhibit the auto-oxidation of pyrogallol were determined based on the absorbance at 325 nm over 10 min, with absorbance measured every 1 min in a 96-well microplate (200 µL) using a BioTek Epoch microplate spectrophotometer. The SoA scavenging activity was calculated based on equation (5):

Superoxide anion scavenging rate (%) =

$$[1 - \left(\frac{A_2 - A_1}{A_0}\right)] \times 100 \tag{5}$$

where A_1 is the absorbance of the sample at 0 min; A_2 is the absorbance of the sample after 10 min; and A_0 is the autoxidation rate of pyrogallol for the blank (the change of absorbance in the blank from 0 min to 10 min).

HYDROXYL RADICAL SCAVENGING ACTIVITY

Both F_{sar} and F_{comm} at 2 mg/mL were used to determine the hydroxyl radical (·OH) scavenging activity. The ·OH were generated via Fenton reaction through reacting $FeSO_4$ with H_2O_2. In order to measure the ·OH scavenging activity, the fucoidan samples prepared were mixed with 1.5 mL of Fenton reaction mixture (1:2 of 9 mM $FeSO_4$:8.8 mM H_2O_2), followed by 0.5 mL of 9 mM salicylic acid (Set A). A second set of reaction was prepared through the same procedure by substituting salicylic acid with distilled water (Set B). Sample blank was prepared by substituting fucoidan sample with distilled water. All mixtures (both Set A and Set B) were incubated for 30 min at 37 °C. Subsequently, all samples and blanks (200 μL) were transferred into 96-well microplate and measured at 510 nm though a microplate spectrophotometer (BioTek Epoch, USA) (Lim et al. 2014). The scavenging activity was calculated using equation (6):

$$\text{Scavenging activity (\%)} = \left[1- \left(\frac{A_2 - A_1}{A_0}\right)\right] \times 100 \qquad (6)$$

where A_1 is the absorbance reading of reaction containing samples and salicylic acid; A_2 is the absorbance reading of reaction containing samples and without salicylic acid; and A_0 is the blank.

STATISTICAL ANALYSIS

Analyses were conducted with three replications (n=3), while sample measurements of each replicate were performed in duplicates. The data generated were reported as mean ± standard deviation. The significance between means (p<0.05) were analysed through ANOVA and post-hoc Duncan's Multiple Range Test using IBM SPSS Statistic Software version 22.

RESULTS & DISCUSSION

YIELD OF FUCOIDAN (F_{sar})

Extraction of brown seaweed is usually performed with hot water, acid or alkali. The yield of fucoidan was reported to be strongly affected by the method of extraction. In the current study, the fucoidan yield obtained from *Sargassum* sp. was 5.5±0.3%. The yield obtained in this study was thus higher as compared to other literatures. This was supported by study of Junaidi (2013), which had found that the maximum yield produced by *Sargassum* sp. extracted using HCl was 3.3% while Sinurat et al. (2015) had reported that their *Sargassum* sp. produced a fucoidan yield of 4.02% when using a combination of HCl and alkali in their extraction method. On contrary, Mak et al. (2013) reported that the yield obtained by alkali extraction ($CaCl_2$) was much higher compared to acid extraction. However, despite the higher yield obtained, alkali extraction usually involves longer extraction durations and higher solution volumes. Another common method for fucoidan extraction is hot water extraction. Using water (60-100 °C) as a solvent, fucoidan's natural bioactivity is maintained without corrupting its structure (Wang & Chen 2016).

CHEMICAL PROPERTIES OF F_{sar} AND F_{Comm}

The fucose content for F_{sar} was 29.21±0.49%, while sulphate contents for F_{sar} and F_{comm} were significantly different (p<0.05) at 5.21±0.35% and 8.80±0.14%, respectively. It has been reported that the yield as well as chemical composition were strongly affected by method of extraction, species of seaweed and geographical factors (Ale et al. 2011; Mak et al. 2013). Wang and Chen (2016) who worked on 6 different species of brown seaweeds, reported that fucoidans have sulphate content at approximately 13%. Sulphate content of F_{sar} and F_{comm} were also lower than those reported by Cumashi et al. (2007) which was in the range of 27.5%-29.6%. The fucose content of F_{sar} was within the range reported by other researchers for fucoidan from *Sargassum* sp. (range of 20-50%) (Junaidi 2013; Sinurat et al. 2015).

In this study, monosaccharide composition was determined by methanolysis, where the methanolic HCl cleaves the glycosidic linkages in the fucoidan samples. When compared with other method such as hydrolysis, methanolysis causes less oxidative damage to the monosaccharides, and is highly effective at cleaving glycosidic linkages (Kamerling & Gerwig 2007). As shown in Table 1, the monosaccharide composition of F_{comm} comprised fucose (520.00 μg/g) and glucuronic acid (62.93 μg/g), while F_{sar} contained fucose (138.34 μg/g), 4-*O*-methyl glucuronic acid (105.39 μg/g), galactose (70.49 μg/g) and glucuronic acid (30.60 μg/g) as their main components. Fucose was the main monosaccharide composition in both F_{sar} and $F_{comm.}$ Overall, F_{comm} had a less complex monosaccharide composition when compared to F_{sar}. The commercially available fucoidan, F_{comm} was derived from *Clasidophon okamuranus* species.

In F_{sar}, the occurrence of 4-*O*-methyl-glucuronic acid is consistent with Ale et al. (2011), where glucuronic acid were found in the fucoidan extracted from *Sargassum henslowianum*. Acidic extraction may produce a higher yield of fucoidan but also are likely to extract other compounds such as alginic acid (Lim et al. 2017). The results showed that especially under acidic conditions the co-extraction other compounds in fucoidan were inevitable.

TABLE 1. Monosaccharide composition of F_{sar} and F_{comm} (n=2)

Monosaccharide composition (µg/g)	F_{sar}	F_{comm}
Arabinose	3.51±0.14^{a}	1.46±0.1^{a}
Rhamnose	6.92±1.18^{a}	1.34±0.04^{b}
Fucose	138.34±0.07^{b}	519.99±0.30^{a}
Xylose	27.24±0.14^{a}	9.26±0.33^{b}
4-*O*-Methyl glucuronic acid	105.39±0.08	ND
Mannose	32.61±0.31^{a}	4.35±0.26^{b}
Galactose	70.49±0.32^{a}	7.31±0.18^{b}
Glucose	10.55±0.26^{a}	2.92±0.31^{b}
Glucuronic acid	30.60±0.02^{b}	62.93±0.47^{a}

$^{a-b}$Different alphabets within the same row denotes statistical significance at p<0.05
F_{sar}: fucoidan extracted from *Sargassum* sp. F_{comm}: commercial food-grade fucoidan, ND: not detected

ANTIOXIDANT ACTIVITIES

Previous work has shown that fucoidan exhibits excellent antioxidant activities (Lim et al. 2014). As antioxidants has the ability to inhibit the formation of, or eliminate, free radicals, the incorporation of antioxidants in human diet is strongly encouraged. In this study, F_{sar} and F_{comm} were analysed for their total phenolic content, DPPH, SoA and ·OH scavenging activities. As shown in Table 2, F_{sar} showed no significant difference, (p>0.05) when compared to F_{comm} except for the DPPH free radical scavenging activity where F_{sar} showed higher DPPH value than F_{comm} with 40.67±4.0% and 10.11±1.0%, respectively. The increase in DPPH scavenging activity can also be due to the presence of phenolic compounds which would act as hydrogen donor antioxidants (Husni et al. 2022). It is well-known that fucoidan from different species, extraction method and geographical locations could influence the bioactivities of fucoidan (Ale et al. 2011), which is obvious in the present study.

The antioxidant activities of F_{sar} and F_{comm} were significantly different (p<0.05) when compared to the positive controls of ascorbic acid, BHA, and BHT (synthetic antioxidants). Some of the researchers were reporting about primary and secondary antioxidant capacity of fucoidan. Lim et al. (2014) had showed that fucoidan exhibited higher secondary antioxidant (SoA and ·OH scavenging activities) than primary antioxidant (DPPH free radicals scavenging activity) when compared to those of synthetic antioxidants. Rocha De Souza et al. (2007) who worked on the brown seaweeds *Fucus vesiculosus* and *Padina gymnospora*, showed that the extracted fucoidan have significantly lower IC_{50} values in both the SoA and ·OH scavenging activities. However, in the current study, both F_{sar} and F_{comm} were observed to give a lower value of secondary antioxidant activity compared to ascorbic acid, BHA and BHT.

TABLE 2. Antioxidant analysis of F_{sar} and F_{comm} (n=3)

Antioxidant assays	F_{sar}	F_{comm}	AA	BHA	BHT
TPC (% GAE)	6.40±2.45[b]	3.90±2.80[b]	71.59±3.0[a]	67.78±0.17[a]	68.08±0.3[a]
Free radical (DPPH) (%)	40.67±4.0[b]	10.11±1.0[c]	79.75±0.66[a]	70.67±1.15[a]	65.67±1.15[a]
Superoxide anion (%)	10.72±1.79[c]	10.38±0.96[c]	100[a]	55.47±14[b]	90.61±1.0[a]
Hydroxyl radical (%)	29.49±4.1[c]	28.22±5.1[c]	78.08±2.61[a]	80.82±6.01[a]	49.77±1.0[b]

[a-c]Different alphabets within the same row denotes statistical significance at p<0.05

F_{sar}: fucoidan extracted from *Sargassum* sp., F_{comm}: commercial food-grade fucoidan

AA: ascorbic acid, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, TPC: total phenolic contents, DPPH: 2,2-diphenyl-1-picrylhydrazyl

CONCLUSION

In conclusion, fucoidan extracted from *Sargassum* sp. (F_{sar}) from Indonesia showed different characteristics compared to that of the commercial fucoidan (F_{comm}) from Japan. Fucose was the major monosaccharide, and both are sulphated. Interestingly, F_{sar} showed significantly higher DPPH free radical scavenging activities compared to that of F_{comm}, while no significant differences, (p>0.05) were observed in superoxide anion and hydroxyl radical scavenging activity. This shows the potential of fucoidan from seaweeds harvested from South East Asian waters to be developed further into functional food ingredients.

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