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Structure and Biological Activity Analysis of Fucoidan Isolated from *Sargassum siliquosum*

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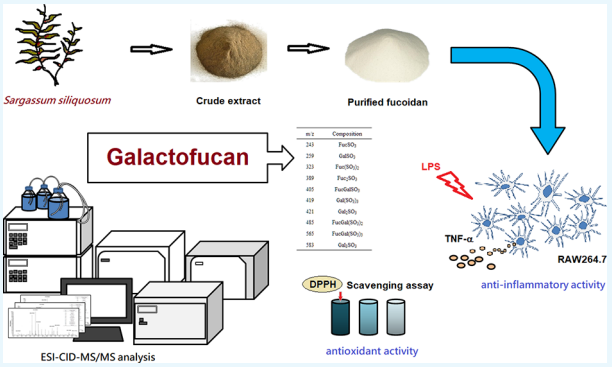
ABSTRACT: Fucoidans are heterologous polysaccharides commonly seen in brown macroalgae and are known for their biological activity including anticancer, antiangiogenic, immunomodulation, and antiviral properties. The brown macroalga *Sargassum siliquosum* was used for the extraction and analysis of fucoidan in this study. The *S. siliquosum* fucoidan was indicated as a galactofucoidan composed of sugars, uronate, and sulfate at a ratio of 12:1:4 and its purity was 85% based on the abovementioned three major components. Structural analysis by electrospray ionization collision-induced dissociation tandem mass spectroscopy revealed that the purified fucoidan consisted of a carbohydrate chain composed of (1→3)-linked or (1→4)-linked L-fucose residues, with sulfate groups at C-2 and C-4 positions. Galactose residues with (1→4)-linkages function as the branch points and they are located at the C-3 or C-4 position of fucose residues. Galactose residues are sulfated mainly at C-4 and C-6, while some sulfation can also be seen at C-2. The fucoidan purified from *S. siliquosum* demonstrated antioxidant, anti-inflammatory, and antilipogenic activities.



INTRODUCTION

Fucoidan is a polysaccharide present in brown macroalgae and members of the echinoderms, such as sea urchins and sea cucumbers,[1] which contains sulfated L-fucose as the major monosaccharide. The other monosaccharides commonly seen in fucoidan include mannose, galactose, glucose, xylose, and glucuronic acid. Brown seaweeds,[2] sea urchins,[3] and sea cucumbers[4] are some of the commercial sources of fucoidan. The structure of fucoidan is usually heterogeneous with branched polysaccharides and varies with algae species. The heterogeneity in the fucoidan structure, attained by variations in sulfate content, constituent monosaccharides, and molecular weight, is highly influenced by both the biological source of fucoidan and the applied extraction methods.[5] Many studies have revealed that fucoidan from brown algae is typically composed of α-(1→3)-L-fucose residues or alternating α-(1→3) and α-(1→4)-linked L-fucose as the backbone with a sulfate group at C-2 of α-(1→3)-linked and C-2 or C-4 of α-(1→4)-linked L-fucose residues. Fucoidan produced from *Fucus evanescens* and *Fucus serratus* was shown to have high levels of α-(1→3) and α-(1→4)-linked L-fucose residues.[6] Fucoidan produced from *Ascophyllum nodosum* also showed a typical structure with a backbone mainly composed of repeating α-(1→3)-linked L-fucose residues with a sulfate group at the position of C-2.[7] In addition, fucoidan produced from *Sargassum stenophyllum* consisted of a backbone composed of β-(1→6)-D-galactose and β-(1→2)-D-mannose residues, which revealed that sulfated galactose could be the other major sugar in fucoidan.[8] Many studies discussed the relationship between the fucoidan structure and its bioactivity.[9] For example, nine species of brown algae were evaluated for the anticoagulant properties of their fucoidans. The common structural feature was the presence of 2-*O*-α-D-glucuronopyranosyl branches in the linear (1→3)-linked poly-α-fucopyranoside chain (present in all except one), and it was suggested that this structure is essential for anticoagulant activity. In contrast, antithrombin activity was only observed in the fucoidan of five strains.[6] It is important to elucidate the structure of fucoidans to understand structure−function relationships. Unfortunately, the structural composition of native fucoidan from brown seaweeds is heterogeneous with fucose and other assorted monosaccharides. In addition, the sulfate groups are positioned at various points. Thus, structural analysis by chemical methods, such as nuclear magnetic resonance spectroscopy, provides limited information on their structure. Mass spectrometry is another

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major technique in carbohydrate structural analysis. Electrospray ionization mass spectrometry (ESI-MS) is a highly sensitive method for the determination of the ionization of sulfated oligosaccharides, with specifics regarding the monosaccharide arrangement and sulfation/branch point positions.[10]

Fucoidan is known to have several biological functions, including antioxidant, anticancer, antiviral, anticoagulant, antithrombotic, and immunomodulatory activities.[9,11,12] In this study, fucoidan from a brown macroalga *Sargassum siliquosum* was isolated and purified. The structure of the fucoidan isolated from *S. siliquosum* was analyzed using ESI-MS. The antioxidant and anti-inflammatory activity was investigated by the di(phenyl)-(2,4,6-trinitrophenyl)-iminoazanium (DPPH) scavenging effect and the inhibition of proinflammatory cytokine tumor necrosis factor (TNF)-α production assays.

RESULTS

Composition Analysis of Fucoidan by ESI-MS. The sugar composition analysis of crude extracts obtained with different extraction strategies from *S. siliquosum* indicated that the average percentage of sugar composition in the crude extract polysaccharides is as follows: fucose, galactose, glucose, xylose, mannose, and rhamnose at 47.13, 24.83, 8.53, 9.07, 6.97, and 3.47%, respectively (Table 1). The *S. siliquosum* fucoidan could be a G-type (the major sugar components are fucose and galactose) or GA-type (the major sugar components are fucose and galactose with uronate) fucoidan. The ratio of sugars, uronate, and sulfate in the purified fucoidan of *S. siliquosum* is 12:1:4 with a composition of 60, 5, and 20%, respectively, per dry weights of the final purified products. This gives an 85% fucoidan purity based on the abovementioned three major components. The major components are fucose, galactose, and sulfate groups, which can be seen from the analysis of the hydrolysis mixture by the ESI-MS data.

Table 1. Sugar Composition Analysis of Crude Extracts from *S. siliquosum*

composition	content (%)
fucose	47.13 ± 0.47
galactose	24.83 ± 0.74
glucose	8.53 ± 4.13
xylose	9.07 ± 0.38
mannose	6.97 ± 2.93
rhamnose	3.47 ± 0.12

Among spectroscopic techniques, ESI-MS is widely recognized as a powerful and highly sensitive analytical method for the determination of the polysaccharide structure. ESI-MS can provide direct information on polysaccharide sequences and substitution positions. The negative ESI-MS spectra of fucoidan are shown in Figure 1. The full spectrum scan contained oligosaccharides with 2 and 3 degrees of polymerization and up to three sulfate groups. The results showed that fucose and galactose with sulfate groups were the main components of the hydrolysis mixture. However, ESI-MS can only reveal the mass-to-charge ratio (m/z, Figure 1). For the purpose of structure analysis, collision-induced dissociation (CID) was carried out for further analysis.

Analysis of the Sulfate Group Position. CID is a mass spectrometry technique used to induce fragmentation of molecular ions in the gas phase using high kinetic energy. In the collision, some of the kinetic energy is converted into internal energy, which results in bond breakage and fragmentation of the molecule into smaller fragments. These fragmented ions can then be analyzed by tandem mass spectrometry. Many studied have indicated that the position of sulfate groups influenced the biological activity of fucoidan. The position of the sulfate group can be determined by analyzing sulfated fucose and galactose fragments using ESI−CID−MS/MS analysis.

The ESI−CID−MS/MS spectra of the sulfated $[FucSO_3]^-$ ion at m/z 243 are shown in Figure 2a. According to Anastyuk et al., tandem mass spectrometry of fucoidan-derived fragments was performed by labeling with heavy oxygen, and the m/z 139 ions may be $^{0,2}X$ or $^{2,4}A$ cleavage.[18] The m/z 183 ions should be an $^{0,2}A$ cleavage. In addition, $^{0,3}A$ and $^{0,2}A$ cleavage (m/z 153 and 183) ions indicated that the sulfate group could be possibly located at C-4 of fucose residues. As indicated in MS data analysis (Figure 3a), the m/z 153 ions of the $^{0,3}A$ cleavage revealed low hydrolysis (or relatively low abundance), and the m/z 139 ions suggested a $^{0,2}X$ cleavage. Consequently, the sulfate group is present at C-2 or C-3 of fucose residues, and the linkage of the sulfate group is located at C-2.

The ESI−CID−MS/MS spectra of the $[GalSO_3]^-$ ion at m/z 259 (Figure 2b) showed intense $^{0,2}A$ cleavage (m/z 199) ions, revealing that the sulfate group is mainly located at C-4 or C-6 of galactose residues. Furthermore, a small amount of $^{0,2}X$ cleavage (m/z 139) ions showed that the sulfate group is also placed at C-2 of galactose residues.

Analysis of the Glycosidic Linkage Type. Many studies have claimed that fucoidan from brown algae is typically composed of α-(1→3)-L-fucose residues or alternating α-(1→3) and α-(1→4)-linked L-fucose residues as the backbone.[19] In this study, the glycosidic linkage type was investigated by analyzing disaccharide residues using ESI−CID−MS/MS analysis.

The ESI−CID−MS/MS spectra of the $[Fuc_2SO_3]^-$ ion at m/z 389 (Figure 3a) resulted in a signal of B_1 and C_1 cleavage ions (m/z 225 and 243), which suggested that the nonreducing end was sulfated fucose residues. As the $^{2,4}A_1$ cleavage ion (m/z 139) content was very low in the MS spectra like in $[FucSO_3]^-$ analysis, the linkage of the sulfate group is located at C-2 rather than C-4, and the presence of $^{2,4}A_2$ and $^{0,3}X_1$ cleavage ions (m/z 285 and 315) indicates that the sulfate group was located at C-2 of the nonreducing fucose residues. In addition, the signal of the $^{0,2}A_2$ cleavage ion at m/z 329 showed that the glycosidic bond of fucose residues was (1→4) linkage. Thus, the ion of m/z 389 was determined to be mainly fucose-(1→4)-fucose or mixed fucose-(1→3)-fucose with a sulfate group located at C-2 of the fucose residues.

The ESI−CID−MS/MS spectra of the $[FucGalSO_3]^-$ ion at m/z 405 (Figure 3b) showed an intense B_1 cleavage (m/z 241) ion, which suggested that sulfated galactose residues were at the nonreducing end. Moreover, the presence of $^{2,4}A_2$ and $^{0,3}X_1$ cleavage ions (m/z 301 and 315) indicated that the sulfate group was present at C-2 of the nonreducing galactose residues. Because there was no C_1 cleavage (m/z 259) ion signal, the $^{0,2}A_2$ cleavage (m/z 345) ion suggested that the glycosidic bond was (1→4) linkage. Consequently, the m/z 405 ion was galactose-(1→3)-fucose with the sulfate group present at C-2 of galactose residues. Fucose-(1→4)-galactose

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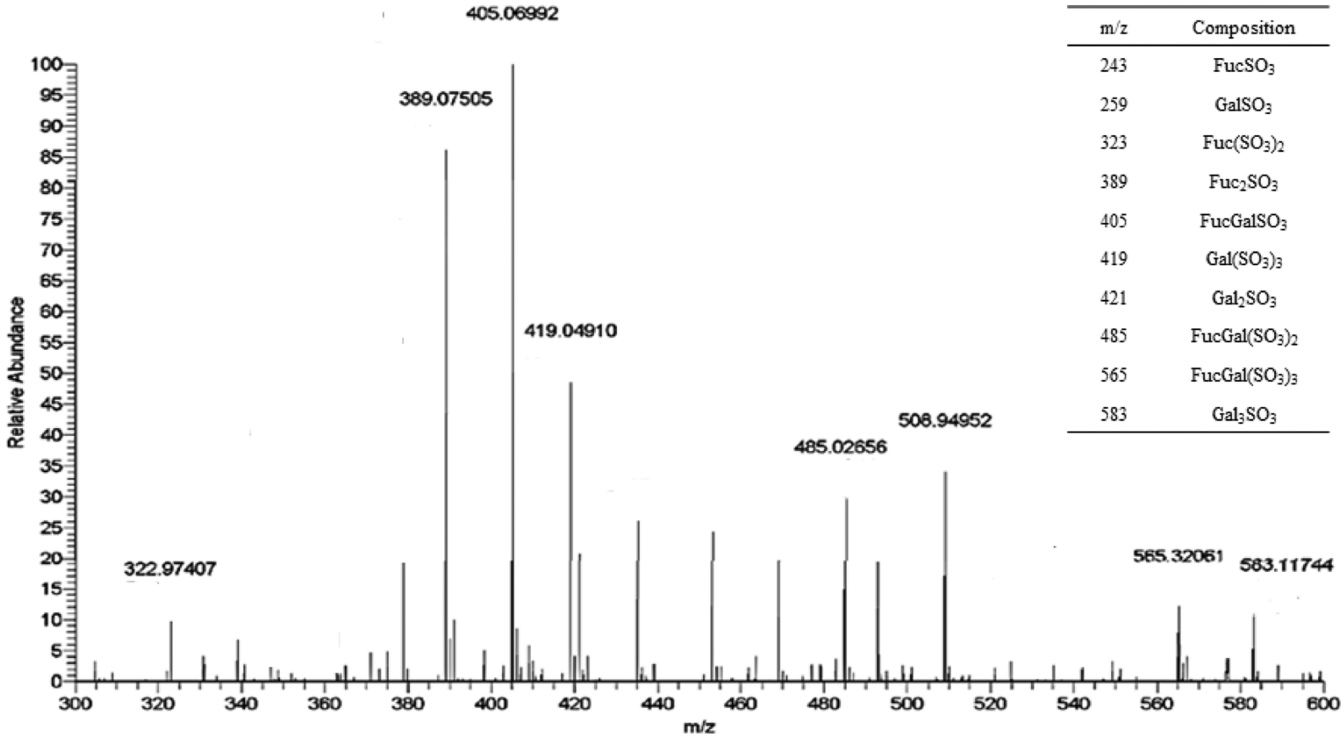

m/z	Composition
243	$FucSO_3$
259	$GalSO_3$
323	$Fuc(SO_3)_2$
389	Fuc_2SO_3
405	$FucGalSO_3$
419	$Gal(SO_3)_3$
421	Gal_2SO_3
485	$FucGal(SO_3)_2$
565	$FucGal(SO_3)_3$
583	Gal_3SO_3

Figure 1. Negative ESI-MS spectra of fucoidan from *S. siliquosum*.

also possibly showed at m/z 405 because the MS spectra have very low presence at $^{2,4}A_2$ cleavage (m/z 285) and $^{0,3}X_1$ cleavage (m/z 331) ions.

The ESI−CID−MS/MS spectra of the $[Gal_2SO_3]^-$ ion at m/z 421 are shown in Figure 3c. The B_1 (m/z 241) ion indicated that sulfated galactose residues were at the nonreducing end. $^{2,4}A_2$ and $^{0,3}X_1$ cleavage ions (m/z 301 and 331) showed that the sulfate group was located at C-2 of the nonreducing galactose residues. The $^{0,2}A_2$ cleavage (m/z 361) ion suggested that the glycosidic bond of galactose residues was (1→4) linkage. Therefore, the m/z 421 ion was found to be galactose-(1→4)-galactose with the sulfate group located at C-2 of galactose residues.

Fucoidan Structure from *S. siliquosum*. Based on ESI−CID−MS/MS analysis, the structure of fucoidan from *S. siliquosum* was determined. As illustrated in Figure 4, fucoidan from *S. siliquosum* has a backbone composed of (1-3)-linked or (1-4)-linked L-fucose residues, and the sulfate groups are located at C-2 and C-4 of fucose residues. Branches are galactose residues with (1→4)-linkages and the branch point is at C-3 or C-4 of fucose residues. The sulfate groups on galactose are mainly present at C-4 or C-6 of galactose residues and some are located at C-2.

Table 2 summarizes various fucoidan structures from different brown algae and their biological activity. It is evident that (1→3)-linked L-fucose seems to exhibit higher antioxidant, anticancer, antiviral, anticoagulant, and immunomodulatory activities. The (1→3)-linked and (1→4)-linked L-fucose residue structure is especially implicated in immunomodification effects.

Biological Activities of Fucoidan from *S. siliquosum*. Antioxidant activity was measured by the DPPH scavenging effect. The DPPH scavenging ability of *S. siliquosum* fucoidan is shown in Figure 5. The EC_{50} of purified fucoidan was 2.58 mg/mL DPPH. The crude extract showed higher antioxidant ability with an EC_{50} of 0.34 mg/mL DPPH. This could be due to the presence of polyphenolic compounds in the crude extract, which has been removed in the further purification steps.[26] However, purified fucoidan from *S. siliquosum* showed higher anti-inflammatory activity compared to the crude extract (Figure 6). The inhibition of TNF-α production reached 14.8% with the 0.25 μg/mL fucoidan-treated group compared to the lipopolysaccharide (LPS)-induced control.

DISCUSSION

The structure and composition of the purified fucoidan are heavily influenced by the exaction methods employed or the macroalgal species.[27] There are major three types of fucoidan with their chemical structure elucidated.[9] F-fucoidan is almost completely composed of sulfated fucose (the official name is sulfated fucan). G-fucoidan consists of sulfated fucose and sulfated galactose as its main components, also called sulfated galactofucan. The third type of fucoidan is GA-fucoidan or U-fucoidan (indicates uronofucoidan), which is composed mainly of fucose, accompanied by other monosaccharides (mostly mannose or galactose but also glucose, xylose, and rhamnose), with significant amounts of uronic acids and sulfate ester. The purified fucoidan from *S. siliquosum* not only shows (1→3)-linked or (1→4)-linked L-fucose residues as the main structure but also has an additional sulfated galactose side chain, and hence, it is classified as galactofucan. This structure predicted that fucoidan from *S. siliquosum* may also exhibit anticoagulant and antithrombotic activities, like the galactan isolated from the red alga *B. occidentalis*.[28]

The fucoidan from *S. siliquosum* possesses (1→3)-linked or (1→4)-linked L-fucose residues as the backbone structure. This is similar to that observed in other reports described in Table 2.[21,22] Moreover, fucoidan from some algae species shows (1→2)-linked and (1→4)-linked L-fucose resi-

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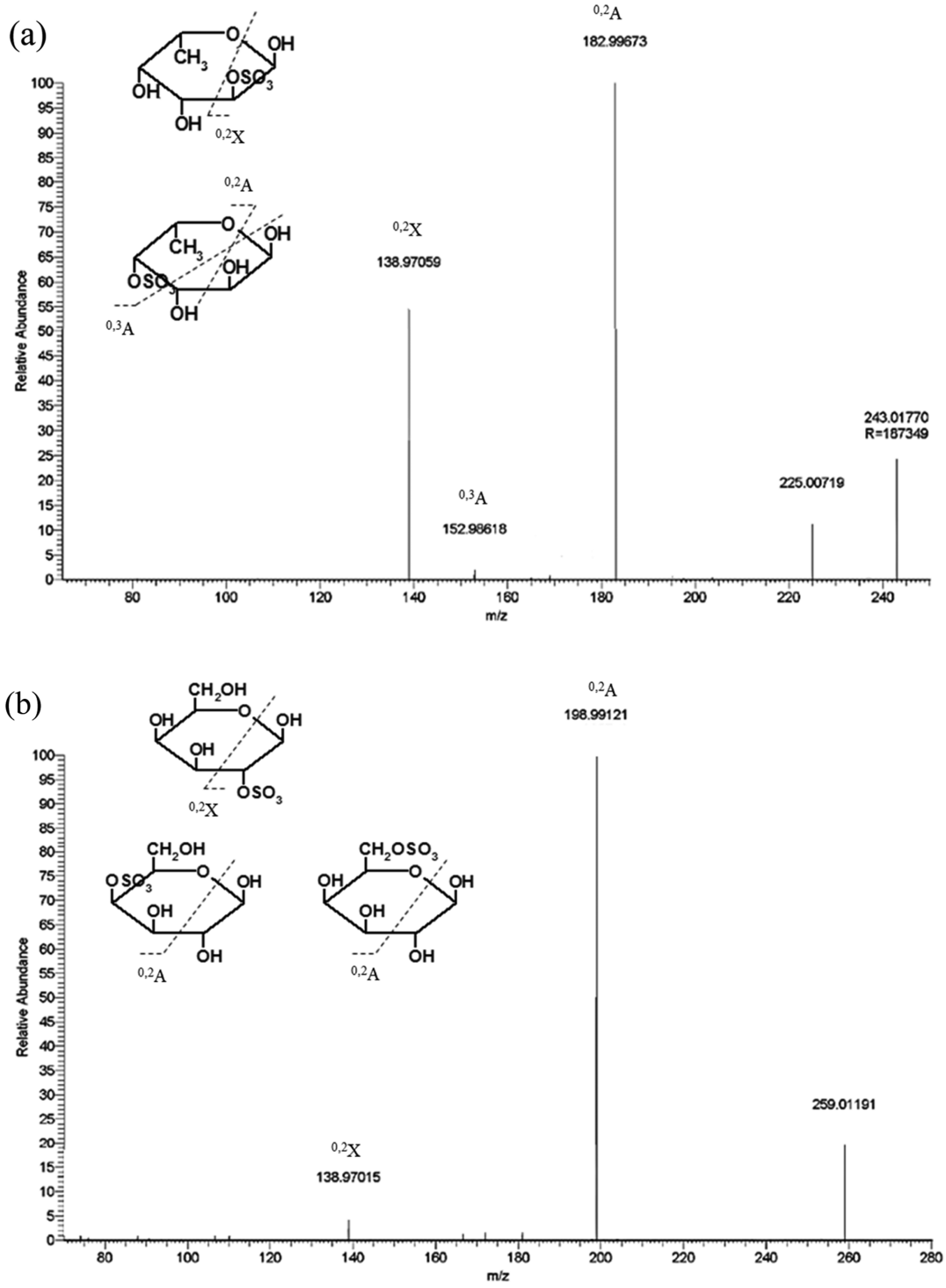

Figure 2. Negative ESI−CID−MS/MS spectra of (a) $[FucSO_3]^-$ at m/z 243 and (b) $[GalSO_3]^-$ at m/z 259.

dues.[21,24,29] Substantial biological activities of fucoidan from different brown algae have recently been defined.[30−32]

Higher sulfate groups containing fucoidan manifested stronger antioxidant, antiviral, anticoagulant, and immunomodulatory activities. Including that the higher sulfate revealed the antioxidant activity and the antiviral and anticoagulant effects under fucoidan may due to the structural similarity to heparin sulfate found in mammalian mucosa.[1]

In addition to antiviral activity,[29] fucoidan also demonstrates anti-inflammation activity effects in some studies. Yang et al. evaluated the effect of fucoidan on the expression of inducible nitric oxide synthase in a macrophage and it was determined that fucoidan might be associated with its NO blocking and anti-inflammatory effects.[33] The fucoidan isolated from *S. siliquosum* has a (1→3)-linked or (1→4)-linked L-fucose residue backbone and exhibits anti-inflammation activity. A low-dose *S. siliquosum* fucoidan (0.25−0.5 μg/mL) treatment can inhibit the TNF-α production of the LPS-induced RAW264.7 macrophages. Low-dose fucoidan showed anti-inflammatory activity, but an increase in fucoidan concen-

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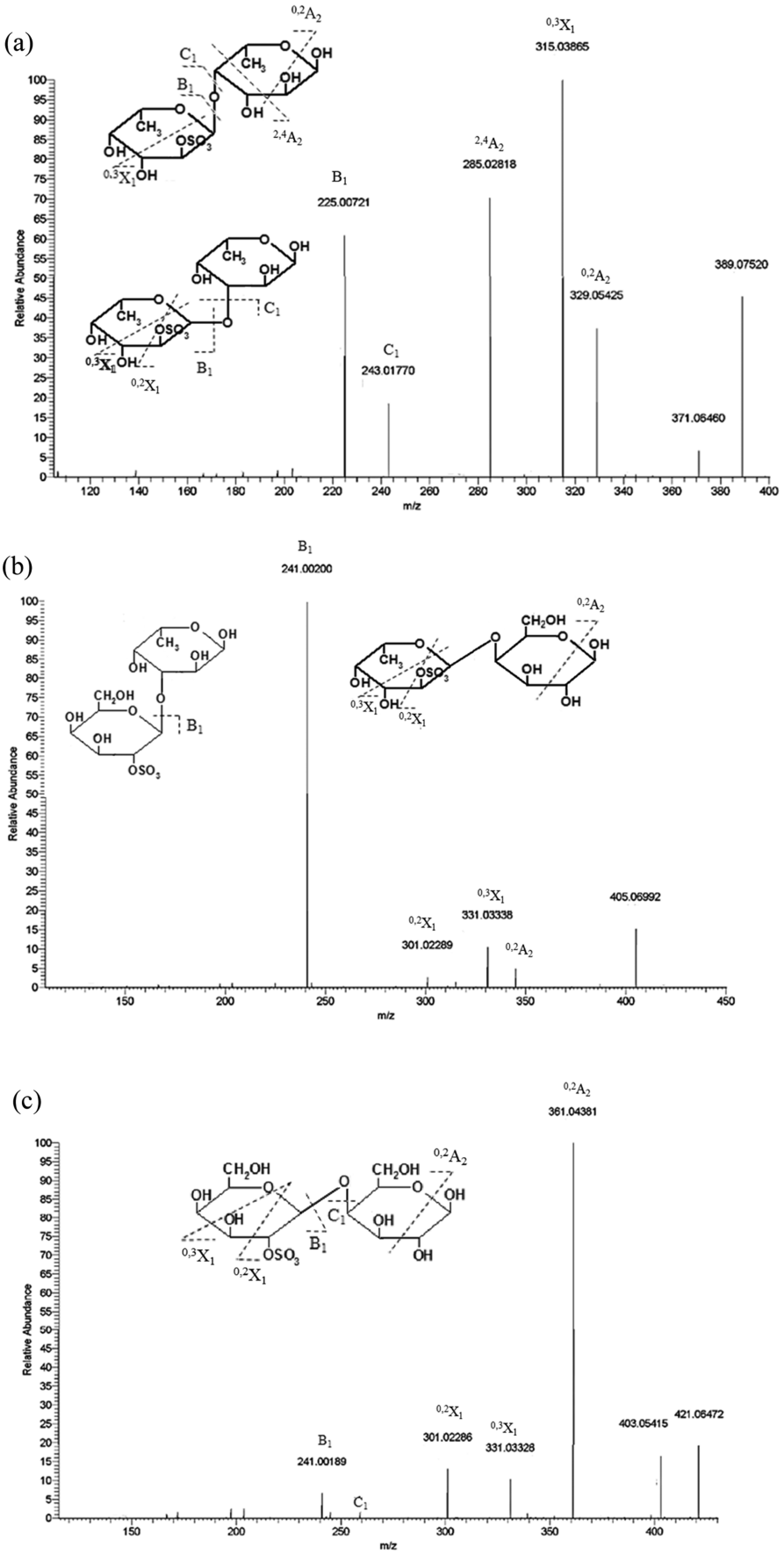

Figure 3. Negative ESI−CID−MS/MS spectra of (a) $[Fuc_2SO_3]^-$ at m/z 389; (b) $[FucGalSO_3]^-$ at m/z 405; and (c) $[Gal_2SO_3]^-$ at m/z 421.

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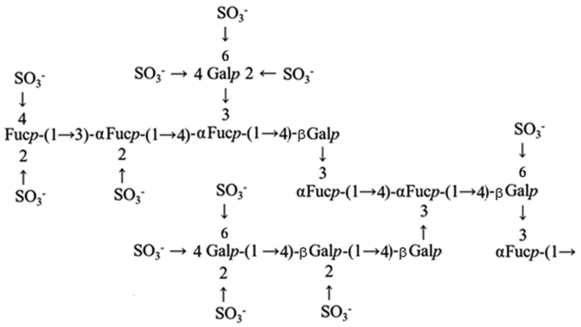

Figure 4. Summary of main structure prediction of fucoidan from *S. siliquosum*.

tration might promote the effect of inflammatory response. The anti-inflammatory activity of fucoidan is also influenced by the molecular weight of fucoidan. For example, high-molecular weight fucoidan prepared from *Cladosiphon okamuranus* promotes an increase in the proportion of murine cytotoxic T cells.[34]

Macroalgal or seaweed-based sulfated polysaccharides have been shown to have immunomodulatory activities. Fucoidan could induce the production of interleukin-1 (IL-1) and interferon-γ (IFN-γ) in vitro, enhance the functions of T lymphocytes, B cells, macrophages, and natural killer cells, and promote the primary antibody response to sheep red blood cells in vivo.[35] Research also showed that arabinogalactan and fucoidan are activators of lymphocytes and macrophages, mainly mediated by the production of free radicals (NO and H_2O_2) and cytokines (TNF-α and IL-6). Therefore, fucoidan could act as an immunomodulating agent. The results obtained in this study showed that an increase in fucoidan concentration will increase the production of TNF-α, even if the production is lower than in LPS-induced RAW264.7 macrophage control.

The preliminary data in this study reveal that *S. siliquosum* fucoidan is capable of several beneficial biological activities, including antioxidant and anti-inflammation activities (Table 2). Also, a previous study from our research group demonstrated antilipogenic activity by purified *S. siliquosum* fucoidan[36] (Supporting Information Figure S1).

CONCLUSIONS

The *S. siliquosum* fucoidan has a major sugar chain of fucose and galactose and is thus classified as a galactofucoidan with a backbone composed of (1→3)-linked or (1→4)-linked L-fucose residues, and the sulfate groups are located at C-2 and C-4 of fucose residues. Branches consist of galactose residues with (1→4)-linkages and the branching point is at C-3 or C-4 of fucose residues. The relationship between the structure of *S. siliquosum* fucoidan and physiological activities (e.g., antioxidant, anti-inflammation, and antilipogenesis activities) was identified. *S. siliquosum* fucoidan also possesses immunomodulatory activities under different treated concentrations. This work demonstrated that fucoidan produced from *S. siliquosum*

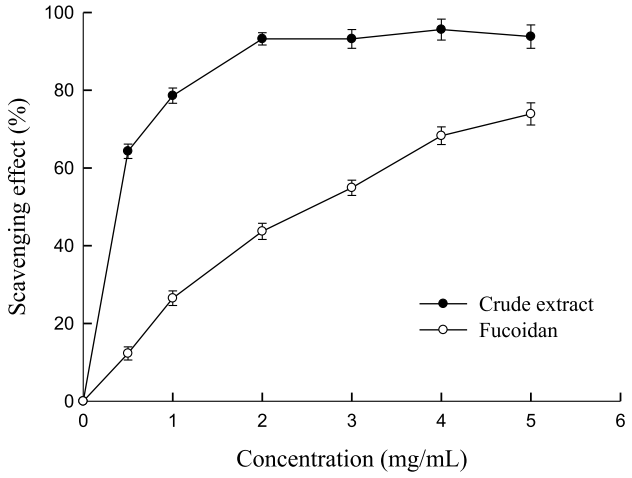

Figure 5. Free radical scavenging effect of the crude extract and fucoidan from *S. siliquosum* by DPPH assay.

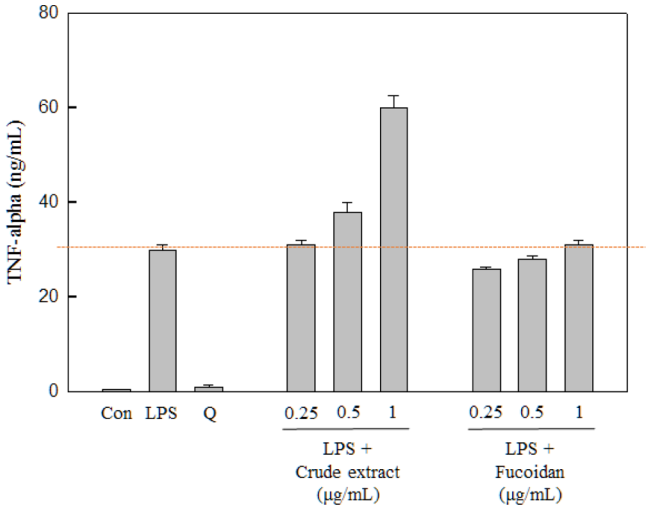

Figure 6. Anti-inflammatory activity of the crude extract and fucoidan from *S. siliquosum*.

Table 2. Fucoidan Structure Obtained from Different Brown Algae and Their Biological Activity

species	composition	biological activity	reference
F. evanescens	[3)-α-L-Fucp-(2SO_3^-)-(1→4)-α-L-Fucp-(2,3SO_3^-)-(1→]	anticancer activity	20
Sargassum crassifolium	[3)-α-L-Fucp-(1→3)-α-L-Fucp (SO_3^-)-(1→4)-α-L-Fucp-(SO_3^-)-(1→]	immunomodulatory activity	21
Stoechospermum marginatum	[3)-α-L-Fucp-(1→3)-α-L-Fucp (SO_3^-)-(1→4)-α-L-Fucp-(SO_3^-)-(1→]	antiviral activity	22
Undaria pinnatifida	[3)-α-L-Fucp-(2,3SO_3^-)-(1→3)-α-L-Fucp-(1→]	antitumor activity	23
Ecklonia kurome	[3)-α-L-Fucp-(1→3)-α-L-Fucp (4SO_3^-)-(1→2)-α-L-Fucp-(4SO_3^-)-(1→]	anticoagulant activity	24
A. nodosum	[3)-α-L-Fucp-(1→3)-α-L-Fucp-(1→3)-α-L-Fucp-(1→3)-α-L-Fucp-(1→]	anticoagulant activity	2
Sargassum polycystum	[3)-α-L-Fucp-(1→3)-α-L-Fucp-(1→]	antioxidant activity, anticancer activity	25
S. siliquosum	[3)-α-L-Fucp-(2SO_3^-)-(1→4)-α-L-Fucp-(1→]	antioxidant activity, anti-inflammatory activity, anti-lipogenesis activity	this study

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could be a potential functional food ingredient for the future development of physiologically active products.

MATERIALS AND METHODS

Preparation of the Crude Extract from *S. siliquosum*. *Sargassum* species were collected from the Penghu coastal area, Taiwan, in December. Freshly picked brown seaweed was washed thoroughly with fresh water to remove epiphytes and salt, followed by oven drying using hot air at 60 °C for 48 h. Then, the dry seaweed was powdered using a grinder. Cell disruption was done to break down the cell wall by an extrusion−hydroextraction process to enhance extraction performance.[13] The *Sargassum* powder was subjected to cell disruption with a single-shaft extruder (Tsung Hsing Co. Ltd., Kaohsiung, Taiwan). The operating temperature was 115 °C, the feeding rate was 10 kg/h, and the screw speed was 360 rpm, and *Sargassum* powder with 35% water content was used, followed by oven drying using hot air at 60 °C for 48 h. Then, the dry seaweed extrudate was powdered using the grinder and stored at 4 °C.

Next, 1 g of dried seaweed powder was washed with 95% ethanol with a liquid/solid ratio of 10 mL/g at room temperature for 4 h to remove lipids, mannitol, pigments, and some salts. The residue was recovered by centrifugation and extracted with distilled water. Microwave-assisted extraction with a microwave power of 750 W, 10 min of extraction time, and 15 mL/g liquid/solid ratio was performed. The supernatant was then collected and dialyzed against distilled water for 24 h using 14 kDa cutoff dialysis membranes. Finally, the supernatant was filtrated through a 0.22 μm filter to obtain the crude extract.

Purification of Fucoidan from the Crude Extract. The crude extract from *S. siliquosum* is not homogeneous and it has other contaminating compounds such as protein, alginate, and phenolic compounds.[14] Therefore, to obtain high purity of fucoidan products, crude fucoidan was isolated by precipitation with ethanol. First, the crude extract was mixed with ethanol at a final concentration of 20% to precipitate alginate and pigments.[15] The mixture was centrifuged at 9000*g* for 30 min, the supernatant was collected, and 95% ethanol was added to attain a final ethanol concentration of 50% to obtain crude fucoidan.[15] After centrifugation, the supernatant was removed, and the pellet was dried for further analysis. The pellet obtained was dissolved in deionized water and was used as crude fucoidan for further purification.

The protein content of the crude fucoidan extract was removed by isoelectric precipitation. Proteins were precipitated by continuously adding acid from pH 6 to 3 until reaching the isoelectric point. The final solution pH was controlled as 3 and incubated for 4 h at room temperature. The sample was then centrifuged at 6000 rpm for 3 min to remove the precipitate. The supernatant was then dialyzed with 14 kDa cutoff membranes against distilled water to eliminate low molecular compounds. Uronic acid was removed by adding 2% calcium chloride and incubating at 4 °C for 4 h. The sample was then centrifuged at 6000 rpm for 3 min to remove the precipitated uronic acid. The supernatant was then dialyzed, filtered, and lyophilized for further experiments. A total of 300 mg of fucoidan after protein and uronic acid removal was dissolved in distilled water at a concentration of 10 mg/mL and applied to a DEAE Sephadex A-25 (XK16 mm/40 cm, GE Healthcare, USA) column equilibrated with distilled water. After loading with fucoidan, the column was eluted with 2 M NaCl concentration at a flow rate of 1 mL/min. Samples for analysis were collected in test tubes using an automated step-by-step fraction collector. Fractions containing fucoidan were collected, dialyzed, filtered, and lyophilized to obtain purified fucoidan.

Preparation of Purified Fucoidan Hydrolysate. The dry purified fucoidan biomass was mixed with dilute sulfuric acid to reach a final concentration of 4% (w/v). The mixture was autoclaved at 121 °C for 20 min for acid hydrolysis.[16] After slowly cooling to room temperature, the fucoidan hydrolysate was neutralized with $CaCO_3$ and the solid precipitate was removed by centrifuging at 13500 rpm for 5 min. The supernatant was further filtered and analyzed by ESI-MS experiments.

Mass Spectrometry Analysis. ESI-MS experiments were performed with an LTQ Orbitrap XL mass spectrometer adjusted to the negative mode. The sample was dissolved in a 1:1 methanol−water solution at a concentration of 10 mg/L and introduced into the mass spectrometer. The mobile phase ($MeOH/H_2O$, 1:1 v/v) was delivered using a syringe pump at a flow rate of 100 μL/min. The capillary voltage was maintained at 3 kV, while the cone voltage was 50 V. For CID-MS/MS ion scanning, the collision energy was adjusted between 10 and 50 eV for optimal sequence information.

Antioxidant Activity Tests. Antioxidant activity was measured by the DPPH scavenging effect according to the method of Shimada et al.[17] DPPH is a stable free radical that has been widely used to determine the free radical-scavenging activity of antioxidants. A total of 0.1 mM DPPH in 50% methanol was prepared, and a total of 1 mL of the solution was added to sample solution (1 mL) at different concentrations (0.5−5 mg/mL). The mixture was shaken at room temperature for 15 min in the dark. Then, the absorbance was measured at 517 nm using a spectrophotometer. The absorbance of the start point when 0.1 mM DPPH was added into the sample solution is 100% of oxidant amounts. The absorbance of 50% methanol solution mixed with the sample solution was considered as the blank (0% of oxidant amounts) against which the antioxidant activity was measured. The antioxidant activity was represented as the oxidant-scavenging effect, and the calculation equation is listed as follows

$$\text{Scavenging effect (\%)} = (1 - \text{percentage of remain oxidants}/100\%\ \text{oxidants}) \times 100$$

Anti-inflammatory Activity Tests. Anti-inflammatory activity was evaluated in the RAW264.7 cell line. The estimated TNF-α content level after LPS exposure reflected the anti-inflammatory activity. The TNF-α concentration of the cell culture supernatants was determined using the TNF-α ELISA kit (BioVision, US). The experiment was started after overnight culture in a 24-well plate (1×10^5 cells/well, 500 μL medium/well), the cells were pretreated with the fucoidan extract (0.25−1 μg/mL) for 1 h and 1 μg/mL LPS for additional 24 h, and the culture supernatant from each well was collected at the end of scheduled experiments and used to measure TNF-α concentration. Quercetin, which is a strong inhibitor for proinflammatory cytokine production, was used as the positive control and a concentration of 50 μM was used for the pretreatment before the LPS was treated on cells.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04591.

HepG2 cells stained with oil red treatment after free fatty acid (FFA)-induced lipid accumulations. Antilipogenesis activity of the crude extract (a) and fucoidan (b) from *S. siliquosum* tested by treating HepG2 cells (PDF)

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Notes

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■ ABBREVIATIONS

ESI-MS,	electrospray ionization mass spectrometry
CID,	collision-induced dissociation
DPPH,	di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium
TNF,	tumor necrosis factor

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