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Ameliorating effects of crude fucoidan extracts from *Sargassum* spp. obtained from Penghu Islands, Taiwan

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Sargassum spp., a predominant seaweed speciesin Taiwan's coastal waters, exhibits a broad spectrum of bioactivities. In the present study, we investigated four *Sargassum* species viz., *S. crassifolium*, *S. siliquosum*, *S. hemiphyllum* and *S. hemiphyllum var. chinense* for various bioactivities. From the four species, we made eight fucoidan extracts, namely SC, SCH, SS, SSH, SH, SHH, SHC, and SHCH with or without a compressional puffing pretreatment. The compressional-puffing process increased the extraction yield of fucoidan and decreased levels of impurities (proteins and polyphenols). Among the eight fucoidans, SS (IC_{50} DPPH 0.46±0.01 mg/mL; IC_{50} $ABTS^{\bullet+}$ 0.09±0.00 mg/mL; FRAP value 67.4±0.9 µmol/g extract, vitamin C equivalent) and SHC (IC_{50} DPPH 0.53±0.17 mg/mL; IC_{50} $ABTS^{\bullet+}$ 0.11±0.00 mg/mL; FRAP value 55.1±0.6 µmol/g extract, vitamin C equivalent) displayed the highest antioxidant activity; SS (36.4±10.1% at 5 mg/mL for α-amylase; 96.3±0.2% at 1 mg/mL for α-glucosidase) and SHC (35.0±2.2% at 5 mg/mL for α-amylase; 96.5±0.2% at 1 mg/mL for α-glucosidase) showed the strongest α-amylase and α-glucosidase inhibitory activities; SH, SHH and SHC had the most potent anti-inflammatory activity; and SHH had a greatest protective effect against damage to pancreatic cells. Hence, SS, SH, SHH, and SHC may be effective health-promoting agents for nutraceutical products.

Keywords: Antidiabetic, Anti-inflammation, Antioxidant, Brown algae, Carbohydrate-digesting enzymes, Pancreatic cells, Reactive oxygen species (ROS), Seaweed

Fucoidans are fucose-enriched sulfated polysaccharides (FCSPs), a polysaccharide category with varying proportions of monosaccharide composition and non-carbohydrate fractions (mainly acetyl and sulfate groups)[1]. Fucoidan exhibits various bio properties, including antioxidant, antibacterial, antiviral, anti-inflammatory, anticancer, anticoagulant, antiadipogenic, antithrombotic, and neuroprotective properties[2]. Recently, studies have found that the efficacy of fucoidan extracts may be related to factors such as seaweed species, growing conditions, location, the physicochemical characteristics of extracts, the extraction method, and analytical techniques, including solvent concentration and extraction time[3]. Gaining a better insight into the fucoidans production using different species of Sargassum and different extraction techniques and determining their bio characteristics becomes exceedingly more important.

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Abbreviation: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FCSPs, fucose-enriched sulfated polysaccharides; SC/SCH; SS/SSH; SH/SHH; and SHC/SHCH (*Sargassum crassifolium*; *S. siliquosum*; *S. hemiphyllum*; and *S. hemiphyllum var. chinense* without/with compressional-puffing pretreatment, respectively).

Pretreatment methods aid in mass transfer by degrading the cell matrix of plant biomass. It has been claimed that several cutting-edge pretreatment methods, such as mechanical disruption (ball milling, high-pressure homogenization, and hydrodynamic cavitation), ultrasound, microwaves, and pulsed electric fields, can also improve the yield and quality of the extracts[4]. Compressional-puffing pretreatment is more effective and simpler than the rotational puffing procedure, which uses high mechanical and explosive pressure on materials and thus increases the extraction yields of fucoidan from *S. glaucescens*[5], phenolic compounds from *Pinus morrisonicola*[6], and bioactive compounds from *Aloe vera*[7].

Reactive oxygen species (ROS) are usually available as superoxide anion ($O2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2), which are often used as biomarkers of inflammation, carcinogenesis, ischemia/reperfusion, and diabetes mellitus[8,9]. Although antioxidant-driven defense techniques can restore oxidative damage, oxidation-induced damage cannot be eliminated, and antioxidants are also necessary. Certain synthetic antioxidants are potentially hazardous to human health[10]. Therefore, there is a need for potent, safe, and low-cost antioxidants derived from naturally occurring compounds.

Diabetes mellitus is a disease that is delineated by abnormalities in insulin levels. Stable blood glucose levels can be regulated by blocking the activity of carbohydrate-digesting enzymes, such as α-amylase, α-glucosidase, and DPP-4 (dipeptidyl peptidase-4)[11]. Antidiabetic medication, also reported as sodium/ glucose co-transporter-2 (SGLT2) inhibitors, has loweredblood sugar levels in diabetics. However, the side effects and cost-effectiveness of synthetic drugs have raised concerns. Pro-inflammatory cytokines, involving tumor necrosis factor alpha (TNF-α) and interleukins (IL-1 & IL-6), are generated by adipose tissue and attached to adipocytes. Elevation of glucose uptake in adipocytes promotes fat accumulation, exacerbating metabolic dysfunction, i.e., insulin resistance, a hallmark of diabetes. Emerging data show a strong relationship between inflammatory responses and the development of type 2 diabetes. Polysaccharides extracted from seaweeds possess anti-inflammatory and antidiabetic properties[12].

The predominant seaweed speciesin Taiwan's coastal waters is Sargassum species, which are widespread brown algae with several varieties. Sargassum species (brown algae) have opted for the current studies because they are an anatomically diverse group compared to other macroalgae (seaweed) and thus exhibited a broad spectrum of bioactivities in previous studies[3,13-15]. The present study hypothesizes that the efficacy of seaweed extracts depends not only on seaweed species but also on their habitat, growth, and extraction conditions. Weexplored four Sargassum species, commonly grown in coastal areas of Taiwan for their fucoidan extracts with differing efficacy to provide diverse bioactivities such as antioxidant, α-glucosidase, and α-amylase inhibitory, anti-inflammation, and pancreatic beta cells protective properties[3,14,16,17]. To the extent of theauthor's knowledge, detailed studies are lacking concerning the inhibition of α-amylase and α-glucosidase applies to desirable bioactivity, anti-inflammation activity, and the protection of pancreatic β cells caused by crude extracts of fucoidan obtained from different Sargassum species and from compressional-puffing-pretreatment. We studied the health-promoting effects of crude extracts of fucoidan. Further, we used compressional puffing treatment for economical extraction of high-quality fucoidan.

Materials and Methods

Chemicals and Materials

Samples of *Sargassum crassifolium*, *S. siliquosum*, *S. hemiphyllum* and *S. hemiphyllum var. chinense*were sampled from Penghu Islands, Taiwan. The samples were water-washed, dried, packed in aluminum foil stacks, and stored until use at 4°C. L-fucose, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), $FeCH_3$, α-amylase, α-glucosidase, streptozotocin (STZ), Bradford reagent, BSA (bovine serum albumin) were obtained from Sigma-Aldrich (St. Louis, USA). DMEM medium, RPMI medium, trypsin/EDTA, penicillin, streptomycin, and fetal bovine serum (FBS) were obtained from Gibco Laboratories (Grand Island, USA). The other reagents, unless otherwise specified, were purchased from Sigma-Aldrich.

FTIR spectroscopy

The technique of Shih *et al*.[18] was used to do the FTIR analysis. In a nutshell, KBr (w/w, 1:50) and dry

Sargassum powder (less than 100 mesh) were blended and ground uniformly. The translucent pellets of KBr were prepared at 500 kg/cm^2 pressure under vacuumized conditions. A Horiba FT-730 spectrometer (Horiba, Kyoto, Japan) generated the FTIR spectra. The absorbance was measured using 60 scans between 400 and 4000 cm^{-1} with a resolution of 16 cm^{-1} using KBr pellet alone as a background.

Compressional-puffing procedures

The dried macroalgal sample (weight: 2.7 g, moisture content: 12.9%) was puffed by the procedure outlined in our prior work[5]. To put it briefly, the temperature for the compressional puffing conditions was set at 220°C, and the algae sample was automatically fed to the chamber. The detachable cylindrical chamber automatically moved up and down thrice when the chamber attained the desired temperature. This compressed the sample mechanically to a force of about 5 kg/cm^2, and then it immediately opened to cause puffing by releasing the steam pressure fast. Each process required a total reaction time of around 10s. At a temperature of 220°C, the pressure level within the chamber was 18.3 kg/cm^2.

Analytical techniques

According to Huang *et al.*[14], thephenol-sulfuric acid method was employed to quantify thetotal reducing sugar level using galactose (standard). The fucose contentwas estimated corresponding to a previously described method[19] where, as a sugar standard, L-fucose was used. Using galactose as areference, 3,5-dinitrosalicylic acid colorimetry was used to measure the amount of reducing sugar in the algal extracts[20]. The sulfate content of the extracts was estimated by hydrolyzing it with a 1N HCl solution at 105°C for 5 h. The sample's ion chromatographic analysis was performed with a Dionex ICS-1500 utilizing an IonPac AS9-HC column at a flow rate of 1 mL min^{-1} at 30°C with conductometric detection to determine the sample's sulfate content, where K_2SO_4 was used as the standard, and 9 mM Na_2CO_3 was used as the eluent. Bradford's assay calculated the amount of protein in the extracts with BSA (standard). Polyphenols were examined using the Folin-Ciocalteu analytical technique, with gallic acid as the reference[21].

Polysaccharide extraction method

Crude fucoidan extractsfrom *S. crassifolium*, *S. siliquosum*, *S. hemiphyllum*, and *S. hemiphyllum var. chinense* were performed using the following method. Fifteen grams of algal sample was mixed and shaken with 150 mL 95% ethanol to separate pigments, lipids, and other impurities, later, it was centrifuged at 970×g for 10 min. The biomass sample was mixed again with 150 mL distilled water, and the polysaccharide was extracted for 1 h at 85°C and 120 rpm. The mixture was centrifuged for 10 min at 3870 g to separate the supernatant. To precipitate alginate, a 2% $CaCl_2$ (w/v) solution was mixed with the supernatant and shaken at 120 rpm for 1 h. The treated sample was centrifuged @ 3870 ×g for 10 min, and the supernatant was then ultrafiltrated at a speed of 300×g for 10 min using a membrane with an MWCO of 100 kDa to remove calcium and protein. The retentate was collected to further precipitate alginic acid, and 95% ethanol was mixed with the biomass extract, bringing 20% of the final ethanol concentration. Finally, the fucoidan extract was precipitated from the supernatant, which was centrifuged at 9170 g for 30 min by 95% ethanol blending to obtain a 50% final ethanol concentration. The obtained fucoidan extract was collected via centrifugation at 9170g for 30 min and freeze-dried. The extraction yield was derived using the equation given below:

$$\text{Extraction yield (\%)} = \frac{[\text{Extracted solid (dry wt.)}]}{[\text{Sample (dry wt.)}]} \times 100 \quad \ldots(1)$$

DPPH radical-scavenging activity

DPPH radical-scavenging activity was quantified as per protocol[22] where, 50 µL of sample solution was blended with 150 µL of freshly made, 0.1 mM DPPH solution (in methanol). The mixture was briskly shaken for 1 min beforeincubatingat room temperature (25^{o}C)for 30 min in the dark. Later, all the sample's absorbance was read at 517 nm by means of a microplate reader (SPECTROstar Nano; Germany), and the activity of radical scavenging efficiency was determined using the below equation:

$$\text{DPPH radical-scavenger (\%)} = \frac{(1 - \text{A sample})}{(\text{A control})} \times 100 \quad \ldots(2)$$

where, sample A denotes various test sample absorbance, and control A denotes absorbance of blank sample.

ABTS cation radicalscavenging activity

The samples scavenging activity against the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical was assessed using a previously demonstrated technique[23]. The $ABTS^{\bullet+}$ solution combined 88 µL of 140 mM potassium persulfate

with 5 mL of a 7 mM ABTS solution. A 95% ethanol diluted the solution until itreached0.70±0.05 absorbance at 734 nm. For the activity assay, 100 µL of sample solution was blended with a diluted 100 µL $ABTS^{\bullet+}$ solution. After 6 min of incubation at room temperature, the absorbance was determined at 734 nm using an ELISA equipped with a microplate reader (SPECTROstar Nano; Germany). Similarly, the blank was kept adding distilled water replacing thesample. The $ABTS^{\bullet+}$ scavenging activity was determined as follows:

$$\text{ABTS cation radicalscavenger (\%)} = \frac{(1 - \text{A sample})}{(\text{A control})} \times 100 \quad \ldots(3)$$

where, sample A denotes the absorbance of various testing samples, and control A represents theabsorbance of blank.

Frap (ferric reducing antioxidant power) assay

This assay was performed as per Hsiao *et al*.[24] method. To make the FRAP solution, 10 µL of acetate buffer (300 mM) was added until pH 3.6 was attained. The resulting solution was mixed with 1 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and was further blended with 1 mL of 10 mM TPTZ (in 40 mM HCl). Testing samples (50 µL) of varying concentration were prepared and mixed with freshly prepared warm (37°C) FRAP solution (450 µL), and was incubated for 30 min in the dark, where acetate buffer (450 µL) was used as a blank. Using a microplate reader (SPECTROstar Nano; BMG Labtech, Germany), the absorbance was measured at 593 nm. Finally, the FRAP values were assessed using vitamin C (standard) and expressed as µmol of vitamin C/g of dried sample.

Inhibition study of α-amylase

The α-amylase inhibitory activity of polysaccharides was determined using the previously reported method[18]. A 20 µL of α-amylase solution (2 U/mL in 0.02 M pH 6.9 sodium phosphate buffer) were added with 40 µL of negative control sample (distilled water) and incubated for 60 mins at ambient temperature. Later, 20 µL of 1% soluble potato starch solution (in 15 min heated 0.02 M & pH 6.9 of sodium phosphate buffer) was filled in each tube, later 40 µL of DNS (dinitrosalicylic acid) solution was added before incubating them at 100°C for 8 min. Centrifugation of the above mixture at 12,000 ×g for 5 min, 780 µL of distilled water was added, and the absorbance was determined using amicroplate reader at 540 nm. The inhibition rate was estimated with respect to negative control having 100% activity.

Inhibition study of α-glucosidase

The inhibitory activity of α-glucosidase on extracted polysaccharides was measured using the previously described methods[18]. A 72 µL of α-glucosidase (1 U/mL in 0.1 M & pH 6. 9 of maleate buffer) was mixed with an equal amount of sample or negative control (distilled water) in a test tube and later, it was incubated for 30 min at 37°C. Later, 144 µL of maltose (2 mM) or sucrose (20 mM) substrate was added in each tube. The resulting mixture was incubated for 20 min. at 37°C and centrifuged for 5 min at 12,000 ×g, then the reactions were finally halted by adding 576 µL of 1M Na2CO3. Finally, the absorbance was measured using amicroplate reader (SPECTROstar Nano; Germany) at 405 nm. The inhibition ratewas determined with respect to the negative control having 100% activity.

Cell culture

RAW 264.7 cell lines (BCRC No. 60001) of Murine macrophage and Rat insulinoma cell lines RIN-m5F (BCRC No. 60410) used in this study were procured from the BioSource Collection and Research Center of the Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan). The Murine macrophage cell lines RAW 264.7 were grown at 37°C in a humid environment with 5% CO_2 in DMEM supplemented with 10% FBS and 100 U/mL penicillin-streptomycin solution, whereas Rat insulinoma cell lines RIN-m5F (BCRC No. 60410) were grown at same incubation condition using RPMI added with 100 U/mL solution of penicillin-streptomycin and 10% FBS. These two cells were maintained by changing the media for 2-3 days.

Measurement of nitrite oxide in culture media

Nitrite oxide (NO) in the culture media was measured using a previously described protocol[24]. In a nutshell, RAW 264.7 cells (2 × 105 cells/mL) were incubated for 24 h at 37°C with 5% CO_2 in a 96-well flat-bottom plate. The old culture media was changed with a new medium encompassing LPS (1 µg/mL) or checked samples at varied concentrations. After a 24 h incubation period, the nitrite content in the culture supernatant was assessed by mixing 50 µL of each cultured supernatant with Griess reagent (100 µL) and mixed well before 10 min incubation at room temperature, followed by this the absorbance was measured using a microplate reader (SPECTROstar Nano; BMG Labtech, Germany) at 540 nm, and the quantity of nitrite was standardized using $NaNO_2$ at 0-100 µM concentrations.

Cytotoxicity analysis

According to Hsiao *et al.*[24], MTT assay was used to test the cytotoxicity of extracted polysaccharides. Cells were grown using 5% CO_2 in a wet environment for 24 h at 37°C. Following the medium decantation, the cells were subjected to different concentrations of the test substances in a serum-free medium. Upon 24 h of treatment, the cells were washed using PBS, and MTT reagent (0.1 mg/mL) was applied. After 2-3 h of incubation, DMSO was added and mixed well through pipetting to dissolve the formazan. The absorbance was determined using a microplate reader (SPECTROstar Nano, BMG Labtech, Germany) at 570 nm. The MTT reduction percentage served as a measure of the cell's viability.

Quantification of IL by ELISA

As per the protocol described by Hsiao *et al.*[24], IL-6 was quantified using ELISA. RAW 264.7 cells were cultured in the medium with 5% CO_2 for 24 h at 37°C in a humid atmosphere. After that,the old media was decanted, and various sample concentrations were used to treat cells in a serum-free medium for 24 h. Later, the supernatant was collected, and IL-6 concentration was determined per the manufacturer's protocols in ELISA Max kits (BioLegend, San Diego, USA).

Statistical assessment

Experimental data are shown as the mean and standard deviation of triplicates (n=3). The ANOVA (one-way), the student's t-test, and the Duncan multiple-range test were used to compare data across groups. If the p-value was less than 0.05, statistical findings were deemed significant.

Results and Discussion

FT-IR analyses of *Sargassum* spp.

The FT-IR results of the four Sargassum species viz., *S. crassifolium*, *S. siliquosum*, *S. hemiphyllum* and *S. hemiphyllum var chinense* are shown in Fig. 1.

According to Huang *et al.*[14], the peaks at 3401 and 2940 cm^{-1} specifically correspond to the stretching of O-H and C-H, respectively. The scissoring water vibration and the in-plane ring vibrations of OCH, CCH, and COH are specific for polysaccharides shown by the peaks at 1621 and 1421 cm^{-1}, respectively[14]. The signal observed at 1320 cm^{-1} denotes the C–O stretching vibrations of the saccharide structure (mannuronic acid)[25]. The sulfate group's S=O asymmetric stretching vibration gave rise to the bands approximately 1230 cm^{-1}[14]. The IR bands

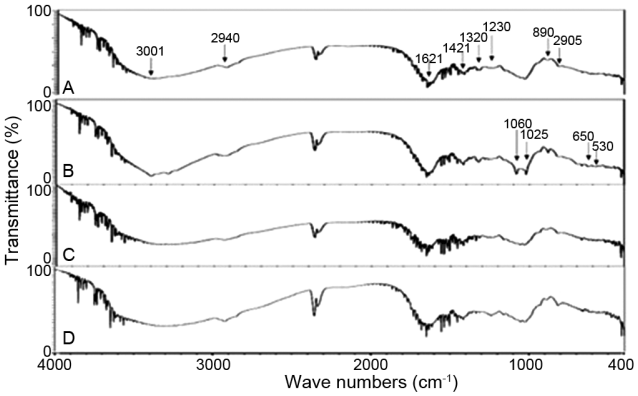

Fig. 1 — FT-IR spectra for (A)*S. crassifolium*;(B)*S. siliquosum*; (C) *S. hemiphyllum*; and (D) *S. hemiphyllum var. chinense*. [Absorption bands at 3401, 2940, 1621, 1421, 1320, 1230, 1080, 1025, 890, 805, 620 and 580 cm^{-1} are indicated]

about 1080 and 1025 cm^{-1} reflect the saccharide structure of guluronic acid[26]. The major peaks at 890 and 805 cm^{-1} corresponded to anomeric CH of β-galactopyranosyl residues and sulfation on C2 of the 3,6-anhydro-L-galactose[27]. Between 620 and 580 cm^{-1}, additional sulfate IR bands are detected. All above peaks are ascribed for symmetric and antisymmetric O=S=O deformations[24]. Given that Sargassum is a significant source of complex carbohydrates, our findings verified that these signals might be primarily ascribed to polysaccharides (alginate and sulfated polysaccharides). In contrast to the other three *Sargassum* spp., *S. siliquosum* displayed clear FT-IR signals for alginate.

Preparation of fucoidan crude extracts from compressional puffing-pretreated Sargassum

Eight crude extracts of fucoidan were used in the study, including SC (*S. crassifolium* without compressional puffing pretreatment), SCH (*S. crassifolium* with compressional puffing pretreatment), SS (*S. siliquosum* without compressional puffing pretreatment), SSH (*S. siliquosum* with compressional puffing pretreatment), SH (*S. hemiphyllum* without compressional puffing pretreatment), SHH (*S. hemiphyllum* with compressional-puffing pretreatment), SHC (*S. hemiphyllum var. chinense* without compressional-puffing pretreatment), and SHCH (*S. hemiphyllum var. chinense* with compressional-puffing pretreatment). The extraction yields of fucoidan extracts for SC, SCH, SS, SSH, SH, SHH, SHC and SHCH were found to be 1.29±0.03, 2.28±0.01, 0.91±0.05, 1.56±0.01, 1.84±0.02, 3.07±0.05, 1.71±0.05, and 3.70±0.08 g/100 g, respectively in dry basis (Table 1), which showed that the compressional-puffing pretreatment enhanced the extraction yield up to 2.16-fold higher (3.70/1.71 =

Table 1 — Chemical compositions for fucoidan extracts from *Sargassum* spp. SC, SCH, SS, SSH, SH, SHH, SHC and SHCH

Fucoidan extracts	Extraction yield (%)	Total sugar (%)	Reducing sugar (%)	Protein (%)	Total phenolic content (%)	Fucose (%)	SO_4^{2-} (%)
SC	1.29±0.03^{b}	27.5±1.7^{a}	4.67±0.70^{cd}	1.10±0.23^{a}	1.81±0.08^{b}	40.7±1.5^{a}	20.4±1.2^{b}
SCH	2.28±0.01^{f}	42.2±0.5^{bc}	3.53±0.12^{a}	1.30±0.03^{a}	1.24±0.14^{a}	38.7±2.5^{a}	27.4±1.7^{d}
SS	0.91±0.05^{a}	42.8±1.5^{bc}	5.73±0.64^{e}	2.91±0.15^{c}	3.34±0.11^{e}	44.7±2.5^{b}	15.4±1.2^{a}
SSH	1.56±0.01^{c}	48.6±0.8^{d}	3.73±0.31^{bc}	2.07±0.35^{b}	1.63±0.08^{b}	40.7±1.5^{a}	15.0±0.9^{a}
SH	1.84±0.02^{e}	39.6±5.2^{b}	4.33±0.12^{bcd}	1.86±0.46^{b}	2.50±0.19^{c}	51.0±1.7^{c}	23.2±1.3^{c}
SHH	3.07±0.05^{g}	46.3±3.0^{cd}	5.07±0.12^{de}	1.94±0.07^{b}	1.83±0.08^{b}	50.0±1.7^{c}	25.7±1.1^{cd}
SHC	1.71±0.05^{d}	41.8±2.6^{bc}	5.87±0.12^{e}	4.19±0.25^{d}	2.75±0.13^{d}	53.0±2.0^{c}	24.4±0.9^{c}
SHCH	3.70±0.08^{h}	52.2±1.4^{de}	4.27±0.12^{abc}	1.26±0.07^{a}	1.09±0.04^{a}	51.7±1.2^{c}	25.4±2.5^{cd}

[SC, *Sargassum crassifolium* without compressional puffing pretreatment; SCH, *S. crassifolium* with compres-sional puffing pretreatment; SS, *S. siliquosum* without compressional puffing pretreatment; SSH, *S. sili-quosum* with compressional puffing pretreatment; SH, *S. hemiphyllum* without compressional puffing pretreatment; SHH, *S. hemiphyllum* with compressional-puffing pretreatment; SHC, *S. hemiphyllum var. chinense* without compressional-puffing pretreatment; and SHCH, *S. hemiphyllum var. chinense* with compressional-puffing pretreatment)Values are means ± SD (n=3). $^{a-h}$ data in the same column with distinct superscript letters which are significantly different (P <0.05)]

2.16) in the SHC and SHCH group. Total sugar, reducing sugar, protein, polyphenols, fucose, and sulfate contents of SC, SCH, SS, SSH, SH, SHH, SHC, and SHCH are presented in Table 1. In general, the overall sugar content of the extracts rose, while the reduced sugar content decreaseddue tothe compressional-puffing treatments. Proteins and polyphenols in fucoidan extract are considered to be impurities[3]. Our data suggested that compressional-puffing pretreatment decreased the protein content and reduced the polyphenols content of extracts. Fucose and sulfate are recognized as the most important components of fucoidan[24]. Generally, compressional-puffing pretreatment did not affect the fucose and sulfate contents in fucoidan extracts. In addition, these data revealed that fucoidan extracts from *S. hemiphyllum* and *S. hemiphyllum var. chinense* had more fucose content than those from the other two brown algal species. Moreover, fucoidan extracts from *S. siliquosum* had the least sulfate content compared to the other three brown algal species. In summary, compressional-puffing pretreatment can effectively augment the extraction yield of fucoidan. Compressional-puffing pretreatment does not affect the critical components, such as fucose and sulfate, in fucoidan. Compressional-puffing pretreatment seems to decrease impurities, such as proteins and polyphenols. Most importantly, fucoidan extracts from *S. hemiphyllum* and *S. hemiphyllum var. Chinese* had higher fucose and sulfate contents than the other brown algal species, indicating that their biological activities warrant further investigation.

Antioxidant activities of fucoidan crude extracts from compressional puffing pretreated Sargassum

To determine the antioxidant activities of SC, SCH, SS, SSH, SH, SHH, SHC and SHCH, the following methods were used in the current study: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS), and ferric reducing antioxidant power (FRAP) assays. As the data presented in Table 2, in general, compressional puffing pretreatment slightly reduced the antioxidant activities of fucoidan extracts, yet all these fucoidan extracts exhibited antioxidant activities. In addition, biological activity experiments, such as inhibition of carbohydrate-digesting enzymes, anti-inflammation, and protection of pancreatic β cells, were conducted.

Table 2 — Antioxidant activities of fucoidan extracts of *Sargassum* spp.

Fucoidan extracts	DPPH	$ABTS^{\bullet+}$	FRAP
	IC_{50} mg/mL*		Vit. C equivalent (μmol/g extract)
SC	1.39±0.08^{e}	0.15±0.00^{e}	47.6±1.7^{bc}
SCH	1.71±0.04^{f}	0.20±0.00^{h}	42.0±1.5^{a}
SS	0.46±0.01^{b}	0.09±0.00^{b}	67.4±0.9^{e}
SSH	0.94±0.04^{c}	0.16±0.00^{f}	49.9±0.4^{c}
SH	1.13±0.05^{d}	0.10±0.01^{c}	56.3±3.0^{d}
SHH	0.98±0.03^{c}	0.17±0.00^{g}	46.3±0.4^{b}
SHC	0.53±0.17^{b}	0.11±0.00^{d}	55.1±0.6^{d}
SHCH	1.87±0.05^{g}	0.26±0.00^{i}	41.3±0.4^{a}
Vit. C	0.013±0.000^{a}	0.005±0.000^{a}	-

[*Concentrations of crude fucoidans from Sargassum species can scavenge half of ABTS•+ or DPPH free radicals. Values are means ± S.D. (n = 3), and a-i data in the same column with different superscript letters are substantially different (P <0.05)]

Inhibition assays against α-glucosidase and α-amylase of fucoidan crude extracts

The inhibitory activities against carbohydrate-breaking enzymes (α-amylase and α-glucosidase) of SC, SCH, SS, SSH, SH, SHH, SHC and SHCH were estimated, andthe outcomes are provided (Table 3). For α-amylase inhibition, the observed results suggested that SS, SH, SHH and SHC had higher α-amylase inhibitory activity than the other fucoidan extracts. For the inhibition of α-glucosidase, the

observed results suggested that SS, SSH and SHC had higher α-glucosidase inhibitory activity than the other fucoidan extracts. In general, compressional-puffing pretreatment decreased the activity of carbohydrate degrading enzymes (α-amylase and α-glucosidase) inhibitory activities of the fucoidan extracts. In addition, among these extracts, SS and SHC showed the best α-amylase and α-glucosidase inhibition.

Anti-inflammatory activity of fucoidan crude extracts

The anti-inflammatory activities of fucoidan extracts (SC, SCH, SS, SSH, SH, SHH, SHC and SHCH) were tested by assessing their effects in LPS-induced RAW264.7 macrophages via NO production and IL-6 production. At 10-200 µg/mL concentrations, none of these extracts markedly affected the viability of RAW264.7 cells in the presence of 1 µg/mL LPS (Fig. 2A). As illustrated in Figure 2B, when RAW264.7 cells were treated with 1 µg/mL LPS, the NO production was increased from 3.60±0.39 µM to 23.7±2.1 µM. Whereas, LPS-induced NO production was significantly reduced when the RAW264.7 cells were treated with 1 µg/mL LPS in the presence of various concentrations of SC, SCH, SS, SSH, SH, SHH, SHC and SHCH, especially SH and SHH treatments resulted in a marked reduction of LPS-induced NO production. As presented in Fig. 2C, it was observed that when RAW264.7 cells were treated with 1 µg/mL LPS, the IL-6 production increased from 0.00±0.00 ng/mL to 2.07±0.09 ng/mL. Furthermore, when RAW264.7 cells were treated with 1 µg/mL LPS in various concentrations of SC, SCH, SS, SSH, SH, SHH, SHC and SHCH, IL-6 production was significantly decreased. Notably, SH and SHC effectively reduced LPS-induced IL-6 biosynthesis. Taken together, most of the fucoidan extracts investigated herein exhibited anti-inflammatory activity; SH, SHH, and SHC showed greater anti-inflammation activity compared with the other three fucoidan extracts.

Table 3 — Inhibitory activities against α-amylase and α-glucosidase for SC, SCH, SS, SSH, SH, SHH, SHC and SHCH

Fucoidan extracts	α-amylase inhibition[1] (%)	α-glucosidase inhibition[2] (%)
SC	15.1±3.1^{3,ab}	47.3±3.8^{b}
SCH	22.1±7.3^{b}	29.3±0.6^{a}
SS	36.4±10.1^{d}	96.3±0.2^{e}
SSH	23.7±2.5^{bc}	92.1±1.2^{d}
SH	41.4±5.9^{d}	58.7±0.7^{c}
SHH	34.6±10.9^{cd}	58.5±1.6^{c}
SHC	35.0±2.2^{cd}	96.5±0.2^{e}
SHCH	11.8±1.2^{a}	29.1±3.4^{a}

[[1]For the α-amylase inhibition, 5 mg/mL concentration was used for SC, SCH, SS, SSH, SH, SHH, SHC and SHCH, whereas [2]for α-glucosidase inhibition, 1 mg/mL concentration was used. Values are mean ± SD (n = 3); [a-e] Data in the same column with different superscript letters are substantially different (P <0.05)]

Attenuation of STZ-induced cytotoxicity in RIN-m5F cells by crude extract

The MTT assay was adopted for vitality determination of RIN-m5F cells after the cell's treatment with various STZ doses for 24 h to determine the cytotoxic effect of STZ on RIN-m5F cells. As illustrated in Fig. 3A, at 5 mM concentration, STZ reduced the RIN-m5F cells' vitality to approximately 42.1%±2.9% to 44.3%±1.2%. Thus, the concentration of 5 mM for STZ was used in further cellular experiments. Pretreatment of RIN-m5F cells with different concentrations (0-200 µg/mL) of SC, SCH, SS, SSH, SH, SHH, SHC and SHCH, after the cell treatment with 5 mM STZ for 24 h, revealed that SHH could significantly mitigate STZ-induced cell cytotoxicity and vitality of cells, were reversed from 54.3%±4.4% to 68.1%±2.0% and 69.0%±2.3%

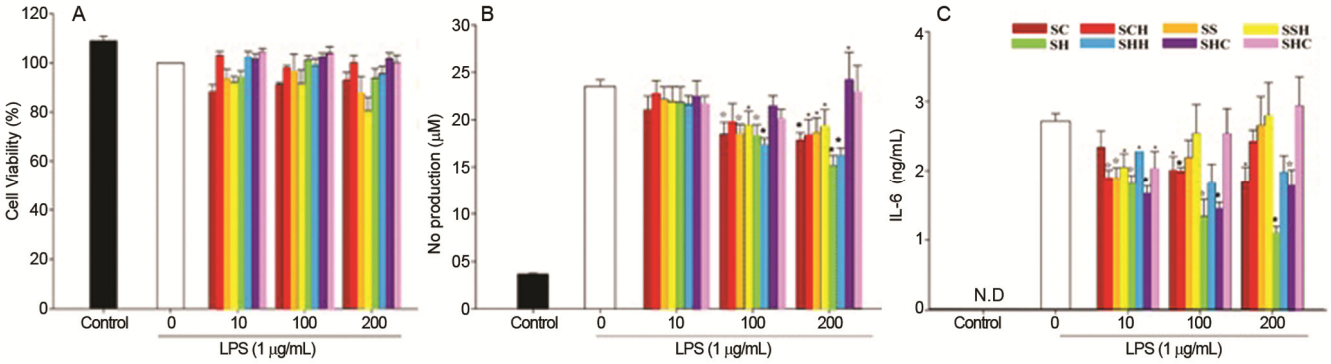

Fig. 2 — Effects of various concentrations of SC, SCH, SS, SSH, SH, SHH, SHC and SHCH on cell viability, LPS-induced NO production, and IL-6 production in RAW 264.7 macrophages. (A) cell viability; (B) NO production; and (C) IL-6 production. [Values and mean ± SD (n = 3). Bars having symbols $^{*}P$ <0.05, $^{\star}P$ <0.005, and $^{\star}P$ <0.001 are substantially different compared with the vehicle (only LPS)]

at SHH concentrations of 12.5 and 25 µg/mL, respectively (Fig. 3B). As a result, SHH may help protect pancreatic cells from oxidative damage.

The compressionalpuffing process has been investigated in numerous studies, and the findings have demonstrated that the compressional puffing process aids the extraction of bioactive components from plant tissues. Huang *et al*.[5] applied the compressionalpuffing process to extract bioactive compounds from seaweed. Compressional puffing was able to break down the cellular structure of the seaweed, which facilitated the subsequent extraction of various compounds. Furthermore, compared with other pretreatments, such as ion-liquid, ultrasonic-, and microwave-assisted pretreatments, compressional-puffing provides several advantages such as a simple operating procedure, reactant-saving, less pollution, and the ability for continuous production, as well as enhancing the extraction yield. Chiang *et al*.[6,28] successfully utilized the compressional-puffing process to pretreat *P. morrisconicola* and extract phenolic compounds. Similarly, Jhang *et al*.[7] pretreated *A. vera* with compressional puffing, which facilitated the extraction of antioxidant compounds from *A. vera*. In the present study, eight fucoidan extracts were obtained, namely SC, SCH, SS, SSH, SH, SHH, SHC and SHCH, extracted from four different species of *Sargassum* species both with and without compressional-puffing pretreatment. The results showed that the compression-puffing process effectively increased the extraction yield of fucoidan, increased the total sugar content, and decreased the protein and polyphenols contents (as impurities) (Table 1). These results are found to be in line with those of earlier investigations[3,5].

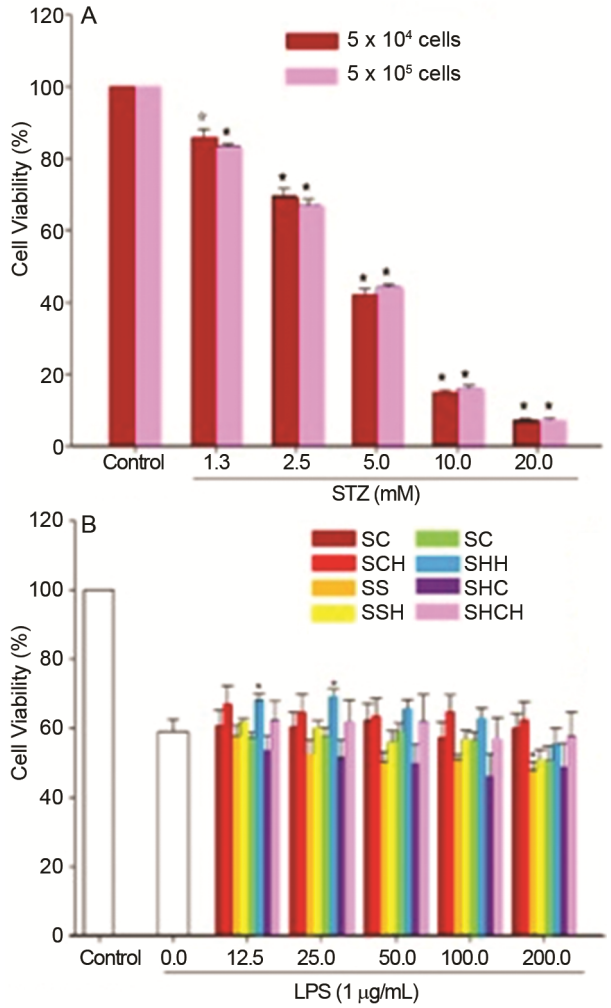

Fig. 3 — Effects of SC, SCH, SS, SSH, SH, SHH, SHC, SHCH, and streptozotocin (STZ) on the cell viability of RIN-m5F cells: (A) RIN-m5F cells were treated with different concentrations (0-20 mM) of STZ for 24 h, and measured the cell viability. Results have been given as mean ± SD of three individual tests. [Bars with symbols $^{\star}P$ <0.005 and $^{\star}P$ <0.001 significantly differ from the control]. (B) RIN-m5F cells were pretreated with various concentrations (0-200 µg/mL) of SC, SCH, SS, SSH, SH, SHH, SHC, and SHCH, then treatment with 5 mM STZ for 24 h, and measured the cell viability. [Bars with the symbol $^{*}P$ <0.05 are significantly different compared with the vesicle (only STZ)]

The principal seaweed colloids among red seaweed (*Osmundea pinnatifida*, *Grateloupia turuturu* and *Gracilaria gracilis*), brown seaweed (*Sargassum muticum* and *Saccorhiza polyschides*), and green seaweed (*Codium tomentosum*) present in Buarcos Bay (Figueira da Foz, Portugal) were identified by Rodrigues *et al*.[26] using FT-IR methods. The researchers discovered that *Gracilariagracilis* and *O. pinnatifida* mostly produced agar, but *G. turuturu* was connected to carrageenan seaweed producers. Moreover, the primary polysaccharides in *Saccorhiza polyschides* and *S. muticum* were alginates and fucoidans. Similar to the findings of Rodrigues *et al*.[26], alginate and sulfated polysaccharides were found to be the most prevalent colloids in *S. crassifolium*, *S. siliquosum*, *S. hemiphyllum* and *S. hemiphyllum var. chinense* (Fig. 1). Interestingly, *S. siliquosum* exhibited obvious alginate FTIR signals compared to the other three *Sargassum* spp., which is a novel discovery.

In brown seaweed species, the fucose and sulfate contents are considered the most significant elements that influence the bioactivities of fucoidan[29]. Herein, we obtained fucoidan extracts from four different species of Sargassum and found that their fucose and sulfate contents varied (Table 1). Therefore, the biological activities of fucoidans extracted

from different Sargassum species require in-depth examination. The development of cancer, atherosclerosis, respiratory illnesses, inflammation, and ischemia/reperfusion are all believed to be significantly influenced by oxidative stress[8,30]. Thus, it may be highly advantageous to develop antioxidant agents from naturally occurring sources. Table 2 shows the DPPH, ABTS and FRAP antioxidant activities of SC, SCH, SS, SSH, SH, SHH, SHC and SHCH. The analyses revealed that compression-puffed samples (SCH, SSH, SHH and SHCH) had lower antioxidant activities than non-puffed samples (SC, SS, SH and SHC). Although fucoidan's antioxidant activity has been established, the connection between its chemical structure and antioxidant capacity has not yet been conclusively demonstrated. Several reasons restrict the activity of fucoidans. The primary reason is the large structural diversity of these polysaccharides, which has prevented the establishment of a structure-activity relationship. The secondary reason is that many sulfated polysaccharides contain several other molecules (sometimes impurities). These latter molecules may have their own activities[31]. Moreover, previous research demonstrated that it is difficult to entirely remove the impurities such as proteins, phenolic chemicals, and alginic acids that may be present in crude fucoidan extracts[1,3]. In the present study, we also found that polyphenols were coextracted using our extraction method (Table 1). Although the amount of coextracted polyphenols was tiny, the possibility that these compounds may have contributed to the observed antioxidant activity cannot be excluded. These results were similar to previous findings[1,31]. Among these fucoidan extracts, SS (fucoidan extract from *S. siliquosum*) and SHC (fucoidan extract from *S. hemiphyllum var. chinense*) showed the most potent DPPH, ABTS, and FRAP activities, suggesting that these two species (*S. siliquosum* and *S. hemiphyllum var. chinense*) maybe a viable resource for extracting fucoidan with a superior antioxidant capacity.

Dietary starch is the primary source of glucose in the blood, which is broken down via hydrolysis. In general, intestinal absorption and starch breakdown are primarily mediated by the enzymes α-amylase and α-glucosidase, respectively. Limiting the activity of these enzymes has been proposed to dramatically reduce gastrointestinal rises in blood glucose after a mixed carbohydrate diet and may therefore represent a crucial technique in managing hyperglycemia, particularly in individuals with type II diabetes[32]. Gunathilaka *et al*.[33] recommended that there is tremendous potential for developing novel antidiabetic medication and nutraceuticals using marine brown algae extracts. Cho *et al*.[34] showed that sulfate-rich fucoidan obtained from *Undaria pinnatifida* hindered α-amylase. The authors anticipated that the inhibitory capacity of sulfate-rich fucoidan was due to better mobility and higher diffusion offered by their lower viscosity. In the current study, SS and SHC had the greatest α-amylase and α-glucosidase inhibitory activities compared with the other fucoidan extracts (Table 3). In SS and SHC, no significant correlations were found due to variations in the sulfate content. Therefore, further examination of physicochemical properties such as viscosity in SS and SHC is needed. However, it can be found that the IC_{50} of α-glucosidase inhibitory activities for SS and SHC were 0.52 ± 0.00 and 0.52 ± 0.00 (mg/mL), respectively. This data is superior to a hydroethanolic extract from *Ecklonia arborea* (0.65 ± 0.00 mg/mL) reported previously[35]. Previously, we also found that SS and SHC had the highest antioxidant activities (Table 2). Hence, fucoidan extracts with a high antioxidant activity could also possess high α-amylase and α-glucosidase inhibitory activities. The generation of ROS may harm cells and tissues when they interact with cellular components such asDNA, proteins, and lipids. Consequently, there may be a reduction in insulin sensitivity, an increase in insulin resistance, and an impairment of insulin signaling, all of which are characteristics of diabetes. In addition to its role in the pathophysiology of diabetes, oxidative stress also aids in the growth of diabetic complications such as neuropathy, nephropathy, and retinopathy. The prevention and treatment of diabetes and its accompanying consequences may thus benefit from lowering oxidative stress, which may be a key therapeutic approach. Furthermore, it is reasonable to suppose that *S. siliquosum* and *S. hemiphyllum var. chinense* could be good resources for the extraction of fucoidan with promising antihyperglycemic properties.

The NO is an inflammatory mediator triggered by inflammatory cytokines or bacterial LPS in various cell types, including macrophages[36]. According to Wang *et al*.[16], fucoidan was isolated from *S. siliquosum* and used by the authors as an anti-inflammatory agent. It contains a (1→3)- or (1→4)-linked L-fucose residue

backbone. The LPS-induced RAW264.7 macrophages' ability to produce TNF-α was inhibited by a low dosage of *S. siliquosum* fucoidan (0.25-0.5 μg/mL). However, an increase in fucoidan concentration can enhance the inflammatory response. It has been found that the molecular weight of fucoidan also impacts its anti-inflammatory properties. For instance, murine cytotoxic T cells are more prevalent when high-molecular-weight fucoidan produced from *Cladosiphon okamuranus* is used[37]. Whereas the concentration of fucoidan in the present study ranged from 10-200 μg/mL, likely due to the different extraction methods, the anti-inflammatory activity of fucoidan was also observed (Fig 2 B and C). In addition, Fig. 2B denotes that compared to the control, LPS significantly induced NO production in RAW 264.7 macrophages. Among the tested fucoidan extracts, LPS-induced NO production was shown to be significantly attenuated following treatments with SC, SCH, SS, SSH, SH, and SHH. SH and SHH markedly reduced LPS-induced NO production. By reducing their gene expression, fucoidan can dramatically reduce inflammatory intermediaries and cytokines in LPS-stimulated RAW 264.7 macrophages[38]. In Fig. 2C, it is evident that compared to the control, LPS was shown to dramatically increase IL-6 synthesis in RAW 264.7 macrophages. Among the fucoidan extracts examined in this study, LPS-induced IL-6 production was significantly mitigated by SC, SCH, SS, SSH, SH, SHH, SHC, and SHCH. SH and SHC were both shown to have a notable influence on the reduction of LPS-induced IL-6 production. Therefore, it can be inferred that SH, SHH and SHC (fucoidans extracted from *S. hemiphyllum*, compressional-puffed *S. hemiphyllum*, and *S. hemiphyllum var. chinense*, respectively) had more potent anti-inflammation activity, suggesting that the Sargassum species (*S. hemiphyllum* and *S. hemiphyllum var. chinense*) and the high fucose contents in SH, SHH and SHC (Table 1) may play an important role in extracting fucoidan with a capacity of promoting human health by modulating the immune system.

STZ is a D-glucopyranose derivative of N-methyl-N-nitrosourea (MNU), which causes diabetes. Due to its toxic impact on pancreatic cells, STZ is frequently employed in experimental animal models to cause diabetes mellitus. In addition, it is also known to have broad-spectrum antibacterial activity. Increased ROS and/or reactive nitrogen species generation, which causes oxidative stress, significantly mediates this pathogenic impact[29]. Therefore, agents with antioxidant activity could confer a protective effect in cells against STZ-induced pancreatic β cell damage. Previous studies used RIN-m5F pancreatic β cells and explored the effects of rice husk silica liquid (RHSL) after STZ stimulation. As a result, the authors found that RHSL has reduced STZ-induced ROS-mediated apoptosis in RIN-m5F cells[39]. However, only a few studies have been conducted on the alleviating ability of fucoidan in STZ-induced damage in RIN-m5F cells. All fucoidan extracts investigated herein exhibited antioxidant activities (Table 2), hence their ability to protect pancreatic β cells was measured. Figure 3B shows that SHH significantly mitigated STZ-induced cytotoxicity in RIN-m5F cells. In Table 1, it can be seen that SHH has a relatively high fucose content and a high sulfate content, which indicates that high fucose and sulfate contents in SHH could contribute, at least in part, to the protective effect against STZ-induced pancreatic β cell damage. However, further study is necessary for the exploration of the precise underlying mechanisms that are involved.

In summary, our findings indicate SHH (fucoidan extracted from compressional-puffed *S. hemiphyllum*) could find various commercial applications as a naturally occurring and safe agent capable of protecting pancreatic β cells. Overall, no correlation was found between the structure data and biological activities in the present study. Since they are extracts and not pure molecules, it is impossible to attribute the observed biological action to a specific structure. Each extract contains several fragments that might interact with other compounds or operate together synergistically. Future work is warranted to evaluate the biological functions in different extract fractions of fucoidan and to give complete insight into the relationship between structure and biological activities.

Conclusions

In the present investigation, four species of Sargassum were pretreated with or without compressional puffing, and eight fucoidan extracts were obtained. Comparisons of the puffing-pretreated samples with the non-puffed samples revealed the former type had a higher extraction yield of fucoidan, higher total sugar content, and lower impurities (proteins and polyphenols). Further analyses of biological activity showed that, in general, fucoidan

extracts obtained from *Sargassum siliquosum*, *S. hemiphyllum* and *S. hemiphyllum var. chinense* exhibited various promising biological activities. Among the eight fucoidan extracts tested, SS and SHC showed the best antioxidant activity; SS and SHC had the most potent α-amylase and α-glucosidase inhibitory activities; SH, SHH, and SHC possessed the strongest anti-inflammation activity; and SHH had a superior protective effect in pancreatic β cells. Hence, SS, SH, SHH, and SHC could have the potential as health-promoting agents in several different industries.

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References

1 Imbs TI, Skriptsova AV &Zvyagintseva TN, Antioxidant activity of fucose-containing sulfated polysaccharides obtained from *Fucus evanescens*by different extraction methods. *J Appl Phycol*, 27 (2015) 545.

2 Shiao WC, Kuo CH, Tsai YH, Hsieh SL, Kuan AW, Hong YH & Huang CY, *In vitro* evaluation of anti-colon cancer potential of crude extracts of fucoidan obtained from *Sargassum glaucescens*pretreated by compressional-puffing. *Appl Sci*, 10 (2020) 3058.

3 Yang WN, Chen PW & Huang CY, Compositional characteristics and *in vitro* evaluations of antioxidant and neuroprotective properties of crude extracts of fucoidan prepared from compressional puffing-pretreated *Sargassum crassifolium*. *Mar Drugs*, 15 (2017) 183.

4 Ummat V, Sivagnanam SP, Rajauria G, O'Donnell C & Tiwari BK, Advances in pre-treatment techniques and green extraction technologies for bioactives from seaweeds. *Trends Food Sci Technol*, 110 (2021) 90.

5 Huang CY, Wu SJ, Yang WN, Kuan AW & Chen CY, Antioxidant activities of crude extracts of fucoidan extracted from *Sargassum glaucescens* by a compressional-puffing-hydrothermal extraction process. *Food Chem*, 197 (2016) 1121.

6 Chiang PS, Lee DJ, Whiteley CG & Huang CY, Antioxidant phenolic compounds from *Pinus morrisconicola* using compressional-puffing pretreatment and water–ethanol extraction: Optimization of extraction parameters. *J Taiwan Inst Chem Eng*, 70 (2017) 7.

7 Jhang LJ & Lee DJ, Compressional-puffing pretreatment for enhanced antioxidant compounds extraction from *Aloe vera*. *J Taiwan Inst Chem Eng*, 81 (2017) 170.

8 Wang J, Zhang QB, Zhang ZS & Li Z, Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. *Int J Biol Macromol*, 42 (2008) 127.

9 Asmat U, Abad K & Ismail K, Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharm J*, 24 (2016) 547.

10 Lorenzo JM, González-Rodríguez RM, Sánchez M, Amado IR & Franco D, Effects of natural (grape seed and chestnut extract) and synthetic antioxidants (buthylatedhydroxytoluene, BHT) on the physical, chemical, microbiological and sensory characteristics of dry cured sausage "chorizo". *Food Res Int*, 54 (2013) 611.

11 Shirosaki M & Koyama T, *Laminaria japonica* as a food for the prevention of obesity and diabetes. Adv. *Food Nutr Res*, 64 (2011) 199.

12 Akbarzadeh S, Gholampour H, Farzadinia P, Daneshi A, Ramavandi B, Moazzeni A, Keshavarz M &Bargahi A, Anti-diabetic effects of *Sargassum oligocystum* on Streptozotocin-induced diabetic rat. Iran. *J Basic Med Sci*, 21 (2018) 342.

13 Wang SH, Huang CY, Chen CY, Chang CC, Huang CY, Dong CD & Chang JS, Isolation and purification of brown algae fucoidan from *Sargassum siliquosum* and the analysis of anti-lipogenesis activity. *Biochem Eng J*, 165 (2021) 107798.

14 Huang CY, Kuo CH & Chen PW, Compressional-puffing pretreatment enhances neuroprotective effects of fucoidans from the brown seaweed *Sargassum hemiphyllum* on 6-hydroxydopamine-induced apoptosis in SH-SY5Y cells. *Molecules*, 23 (2018) 78.

15 Lin HC, Tsai WS & Chiu TH, Antioxidant properties of seven cultivated and natural edible seaweed extracts from Taiwan. *J Aquat Food Prod Technol*, 21 (2012) 248.

16 Wang SH, Huang CY, Chen CY, Chang CC, Huang CY, Dong CD & Chang JS, Structure and biological activity analysis of fucoidan isolated from *Sargassum siliquosum*. *ACS Omega*, 5 (2020) 32447.

17 Huynh TG, Yeh ST, Lin YC, Shyu JF, Chen LL & Chen JC, White shrimp *Litopenaeusvannamei* immersed in seawater containing *Sargassum hemiphyllum var. chinense* powder and its extract showed increased immunity and resistance against Vibrio alginolyticus and white spot syndrome virus. *Fish Shellfish Immunol*, 31 (2011) 286.

18 Shih MK, Hou CY, Dong CD, Patel AK, Tsai YH, Lin MC, Xu ZY, Perumal PK, Kuo CH & Huang CY, Production and Characterization of *Durvillaea antarctica* Enzyme Extract for Antioxidant and Anti-Metabolic Syndrome Effects. *Catalysts*, 12 (2022) 1284.

19 Wang CY, Wu TC, Hsieh SL, Tsai YH, Yeh CW & Huang CY, Antioxidant activity and growth inhibition of human colon cancer cells by crude and purified fucoidan preparations extracted from *Sargassum cristaefolium*. *J Food Drug Anal*, 23 (2015) 766.

20 Chien HI, Tsai YH, Wang HMD, Dong CD, Huang CY & Kuo CH, Extrusion puffing pretreated cereals for rapid

production of high-maltose syrup. *Food Chem*, 15 (2022) 100445.

21 Margraf T, Karnopp AR, Rosso ND & Granato D, Comparison between Folin-Ciocalteu and Prussian Blue assays to estimate the total phenolic content of juices and teas using 96-well microplates. *J Food Sci*, 80 (2015) C2397.

22 Wang CY, Wu TC, Wu CH, Tsai YH, Chung SM, Hong YH & Huang CY, Antioxidant, anti-inflammatory, and HEP G2 cell growth-inhibitory effects of aqueous-ethanol extracts obtained from non-puffed and compressional-puffed *Sargassum crassifolium*. *J Mar Sci Technol*, 28 (2020) 200.

23 Huang CY, Tsai YH, Hong YH, Hsieh SL & Huang RH, Characterization and antioxidant and angiotensin I-converting enzyme (ACE)-inhibitory activities of gelatin hydrolysates prepared from extrusion-pretreated milkfish (*Chanoschanos*) scale. *Mar Drugs*, 16 (2018) 346.

24 Hsiao WC, Hong YH, Tsai YH, Lee YC, Patel AK, Guo HR, Kuo CH & Huang CY, Extraction, Biochemical Characterization, and Health Effects of Native and Degraded Fucoidans from *Sargassum crispifolium*. *Polymers*, 14 (2022) 1812.

25 Smitha B, Sridhar S & Khan A, Chitosan–sodium alginate polyion complexes as fuel cell membranes. *EurPolym J*, 41 (2005) 1859.

26 Rodrigues D, Freitas AC, Pereira L, Rocha-Santos TA, Vasconcelos M.W, Roriz M, Rodríguez-Alcalá LM, Gomes AM & Duarte AC, Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal. *Food Chem*, 183 (2015) 197.

27 Fernando IP, Sanjeewa KKA, Samarakoon W, Lee WW, Kim HS, Kim EA, Gunasekara UKDSS, Abeytunga DTU, Nanayakkara CM & De Silva ED, FTIR characterization and antioxidant activity of water soluble crude polysaccharides of Sri Lankan marine algae. *Algae*, 32 (2017) 75.

28 Chiang PS, Lee DJ, Whiteley CG & Huang CY, Extracting antioxidant phenolic compounds from compressional-puffing pretreated *Pinus morrisonicola*: Effects of operational parameters, kinetics and characterization. *J Taiwan Inst Chem Eng*, 75 (2017) 70.

29 García-Vaquero M, Rajauria G, O'Doherty J.V & Sweeney T, Polysaccharides from macroalgae: Recent advances, innovative technologies and challenges in extraction and purification. *Food Res Int*, 99 (2017) 1011.

30 Vijayabaskar P, Vaseela N & Thirumaran G, Potential antibacterial and antioxidant properties of a sulfated polysaccharide from the brown marine algae *Sargassum swartzii*. *Chin J Nat Med*, 10 (2012) 421.

31 Hifney AF, Fawzy MA, Abdel-Gawad KM & Gomaa M, Industrial optimization of fucoidan extraction from *Sargassum* sp. and its potential antioxidant and emulsifying activities. *Food Hydrocoll*, 54 (2016) 77.

32 Kwon YI, Apostolidis E & Shetty KJBT, *In vitro* studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresour Technol*, 99 (2008) 2981.

33 Gunathilaka TL, Samarakoon K, Ranasinghe P & Peiris LDC, Antidiabetic potential of marine brown algae—a mini review. *J Diabetes Res*, 2020 (2020) 1230218.

34 Cho ML, Lee BY & You SG, Relationship between oversulfation and conformation of low and high molecular weight fucoidans and evaluation of their *in vitro* anticancer activity. *Molecules*, 16 (2010) 291.

35 Múzquiz de la Garza AR, Tapia-Salazar M, Maldonado-Muñiz M, de la Rosa-Millán J, Gutiérrez-Uribe JA, Santos-Zea L, Barba-Dávila BA, Ricque-Marie D & Cruz-Suárez LE, Nutraceutical potential of five Mexican brown seaweeds. *Biomed Res Int*, 2019 (2019) 3795160.

36 Swindle EJ & Metcalfe DD,The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. *Immunol Rev*, 217 (2007) 186.

37 Shimizu J, Wada-Funada U, Mano H, Matahira Y, Kawaguchi M & Wada M, Proportion of murine cytotoxic T cells is increased by high molecular-weight fucoidan extracted from Okinawa mozuku (*Cladosiphonokamuranus*). *J Health Sci*, 51 (2005) 394.

38 Jeong JW, Hwang SJ, Han MH, Lee DS, Yoo JS, Choi IW, Cha HJ, Kim S, Kim HS & Kim GY, Fucoidan inhibits lipopolysaccharide-induced inflammatory responses in RAW 264.7 macrophages and zebrafish larvae. *Mol Cell Toxicol*. 13 (2017) 405.

39 Chen HY, Chiang YF, Wang KL, Huang TC, Ali M, Shieh TM, Chang HY, Hong YH & Hsia SM, Rice husk silica liquid protects pancreatic β cells from streptozotocin-induced oxidative damage *Antioxidants*, 10 (2021) 1080.