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Structural characterisation of algae *Costaria costata* fucoidan and its effects on CCl_4-induced liver injury

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ABSTRACT

Fucoidan is a well-known natural product that is commonly found in brown algae and shows a variety of activities, including immunomodulation, antioxidation, and the combat of carcinogens. The fucoidan fractions of *Costaria costata*, a brown algae introduced from Japan and cultured in northern China, were studied. The fucoidan fractions were extracted, separated, and purified using a combinatorial procedure consisting of enzymolysis, ethanol precipitation, and DEAE and size-exclusion chromatographies. The fundamental characteristics of the four enriched fucoidan fractions (F1–F4), such as their sulphate content and monosaccharide composition, were investigated. FTIR and NMR spectroscopy were employed to further elucidate the structural features of the four fractions. It was found that the F1–F4 fractions all showed oxidative activity against hydroxyl radicals. The bioactive effects of the fucoidan fractions on CCl_4-induced liver injury suggest their potential use as ingredients for functional foods or pharmaceuticals.

1. Introduction

Costaria cantata is an annual plant that is mainly located in Troits Bay in the Sea of Japan and in Primorsky Krai in Russia (Imbs et al., 2009; Imbs, Shevchenko, Semenova, Sukhoverkhov & Zvyagintseva, 2011). It is a cultured species of brown seaweed that was recently introduced into the Dalian coast in northern China. Because it contains a high content of sulphated polysaccharides, increased interest in its aquaculture and sulphated polysaccharides has emerged. It is well known that sulphated polysaccharides in brown algae exhibit a variety of biological activities, which are mainly related to their structural features, including monosaccharide composition, degree of sulphation, and molecular weight (Wijesinghe, Athukorala & Jeon, 2011).

The structural features of polysaccharides vary from species to species, as has been observed in the fraction from *Sargassum tenerrimum* (Sharmistha, Akram, Tuhin, Paul & Bimalendu, 2010), the five fractions from *Hizikia fusiforme* (Li, Wei, Sun & Xu, 2006), and the fractions from *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Saccharina longicruris* (Rioux, Turgeon & Beaulieu, 2007). Imbs et al. (2009) determined the fucoidan content of *C. costata* and its composition of monosaccharides. These researchers found that the fucoidan content increased with growth, and the highest content was obtained in the month of June. The fucoidan content then starts to decrease in the following months. The contents of fucose and SO_3Na also showed a similar trend, and highest yields were obtained from the fucoidan samples collected in June. Anastyuk, Imbs, Shevchenko, Dmitrenok, and Zvyagintseva (2012) further studied the relationship between the structural characteristics of fucoidan and the life stages of *C.costata*. Their findings showed that the seaweed synthesised from a different set of fucoidans and the monosaccharide content and structure of the fucoidans of vegetative algae changed depending on the life stage of the plant. The structural variations of fucoidan are due not only to differences between species but also to the different parts of the seaweed. A specific fucoidan was purified from cultivated *Laminaria japonica* by Ozawa, Yamamoto, Yamagishi, Yamazaki, and Nishizawa (2006).

The structural features of sulphated polysaccharides in various species of brown seaweeds have been studied, and the species-specific differences in monosaccharide composition and structure have been identified. Sulphated polysaccharides extracted from

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Sargassum stenophyllum yielded two fractions: one fraction presented a higher percentage of glucuronic acid and a lower sulphate content, and the other fraction exhibited a small amount of α-d-glucuronic acid and a high percentage of sulphate groups concentrated on the fucose residues (Duarte, Cardoso, Noseda & Cerezo, 2001). Bilan, Grachev, Shashkov, Nifantiev, and Usov (2006) determined the structure of fucoidan from the brown seaweed *Fucus serratus* L. using NMR. Further studies examined the composition and structure of the fucoidan from the brown alga *Saccharina latissima* (Bilan et al., 2010). Yang, Chung, and You (2008) used the light scattering technique to determine the physicochemical properties of sulphated fucans from the sporophyll of *Undaria pinnatifida*. Anastyuk, Shevchenko, Nazarenko, Dmitrenok, and Zvyagintseva (2009) and Anastyuk et al. (2010) applied MALDI-TOF and tandem ESI mass spectrometry to analyse the structural characteristics of a fucoidan from *Fucus evanescens*, and their results indicate that the monosaccharide composition can be obtained by MS. Imbs et al. (2009) analysed the ^{13}C NMR spectrum from fractions from *Costaria costata* collected in July and found that the typical structure of fucoidan exhibits a resonance at 16.9 ppm, which was assigned to the CH_3 group of fucopyranose. The biological activities of the fucoidan from *C. costata* and its structural-activity relationship have not been reported.

Because fucoidans are non-toxic and can be naturally extracted, many studies have investigated their biological activities and/or structural–activity relationships (Wijesekara, Pangestuti & Kim, 2011). These activities include immunomodulatory activities (Raghavendran, Srinivasan & Rekha, 2011), anti-coagulative activities (Bruhn, Thomas, Karl-Heinz, Bruhn', & Béress, 1997; Qiu, Amarasekara & Doctor, 2006; Wijesinghe et al., 2011; Zhao et al., 2007), and anti-oxidant activities (Wang et al., 2009). The biological activities of these sulphated polysaccharides were also investigated to determine their anti-cancer functions (Synytsya et al., 2010; Teruya, Konishi, Uechi, Tamaki & Tako, 2007). However, only a few studies have investigates their protective activities against liver injury (Hayashi et al., 2008).

The extracted fucoidans of the newly cultivated *C. costata* from the Dalian coast in northern China were expected to have different structural characteristics and thus different biological activities and medicinal effects because their cultivation and habitat can induce variations in the chemical composition and structure of fucoidans (Rioux, Turgeon, & Beaulieu, 2007). The present study aimed to identify the structural features of the fucoidans extracted and purified from *C. costata* cultivated in the Dalian coast and to examine their protective activities against liver injury.

2. Materials and methods

2.1. Biological materials and reagents

The brown algae *C. costata* was originally introduced from Japan and aqua-cultured in the Dalian coast in Liaoning Province of China. For the animal experiments, Kunming mice weighing between 18 and 22 g were obtained from the Laboratory Animal Centre of Dalian Medical University. The animal experiments were approved by the Ethical Committee (Number: 20120623) and the Laboratory Animal Centre of Dalian Medical University. All of the animal procedures complied with the China National Institutes of Healthy Guidelines for the Care and Use of Laboratory Animals. The mice were housed in a temperature-controlled environment at 25 °C and fed standard laboratory food.

Cellulase and pectase were purchased from the Lanji Science and Technology development Company (Shanghai). The fucoidan and dextran reference standards were obtained from Sigma, and blue dextran (type 2000) was obtained from Boao Biotechnology Company (Shanghai). Bifendate was purchased from Beijing Union Pharmaceutical Company. Commercial assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), malonaldehyde (MDA), and superoxide dismutase (SOD) were purchased from Jiancheng Bioengineering Institute (Nanjing). The ethanol used in the fucoidan extraction was of food grade, and all of the other reagents were of analytical reagent grade.

2.2. Fucoidan extraction and fractionation

Air-dried seaweeds were crushed and resuspended in H_2O. Enzymatic hydrolysis was performed as shown in Fig. 1; during this hydrolysis, the pH value was adjusted to 4.5 using 1 M HCl. The hydrolysate was then subjected to an ethanol precipitation protocol (Fig. 1) to produce crude fucoidan. The crude extracts were re-dissolved, re-precipitated, washed, and lyophilised to obtain a solid fucoidan mixture (F), which was further fractionated using anion column chromatography. As described in Fig. 1, sample F was dissolved and applied onto a 300-mm × 26-mm column packed with DEAE-Sepharose Fast Flow (Pharmacia). The mobile phase parameters were the following: 0–4 h, phosphate buffer (pH 7.4); 4–21 h, NaCl (0–1.3 M). The flow rate was 0.75 mL/min, and an automatic fraction collector was used to collect the elutions (3 mL/tube). The total sugar concentration of the elute was determined using the phenol–H_2SO_4 reaction (DuBois, Gilles, Hamilton, Rebers & Smith,1956). L-Fucose/d-galactose = 3/1 was used as a standard; the relevant fractions were dialysed using ultrafiltration, and the samples were subsequently lyophilised.

The extraction and fractionation procedures were executed multiple times to acquire a sufficient amount of fucoidan fractions for the subsequent studies.

2.3. Size-exclusion chromatography

To estimate the molecular-weight distribution, the four fractions were dissolved with distilled water and subjected to size-exclusion chromatography using columns packed with Sephacryl G-300 (1.6 cm × 80 cm, distilled water at a flow rate 0.3 mL/min). Dextrans (4, 7, and 15 kDa) and dextran blue (>2000 kDa) were used as the standards. The total sugar concentration was assayed using the phenol-sulphuric acid method (DuBois et al., 1956).

2.4. Chemical analyses

The sulphate contents were determined through the gravimetric analysis of HCl hydrolysis and $BaSO_4$ precipitation. The total sugars were assayed using the phenol–sulphuric acid method (DuBois et al., 1956).

2.5. Determination of the monosaccharide composition

The fractions were hydrolysed using 2 M trifluoroacetic acid, and the resulting monosaccharides were converted into their alditol acetate derivatives. The samples were analysed using a GC-2014C gas chromatographer (Shimadzu) equipped with a HP-5 capillary column at 220 °C.

2.6. Fourier-transform infrared spectroscopy (FTIR)

FTIR was performed in KBr pellets (2 mg of polysaccharide in 100 mg of KBr). The spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer from 600 to 4000 cm^{-1}.

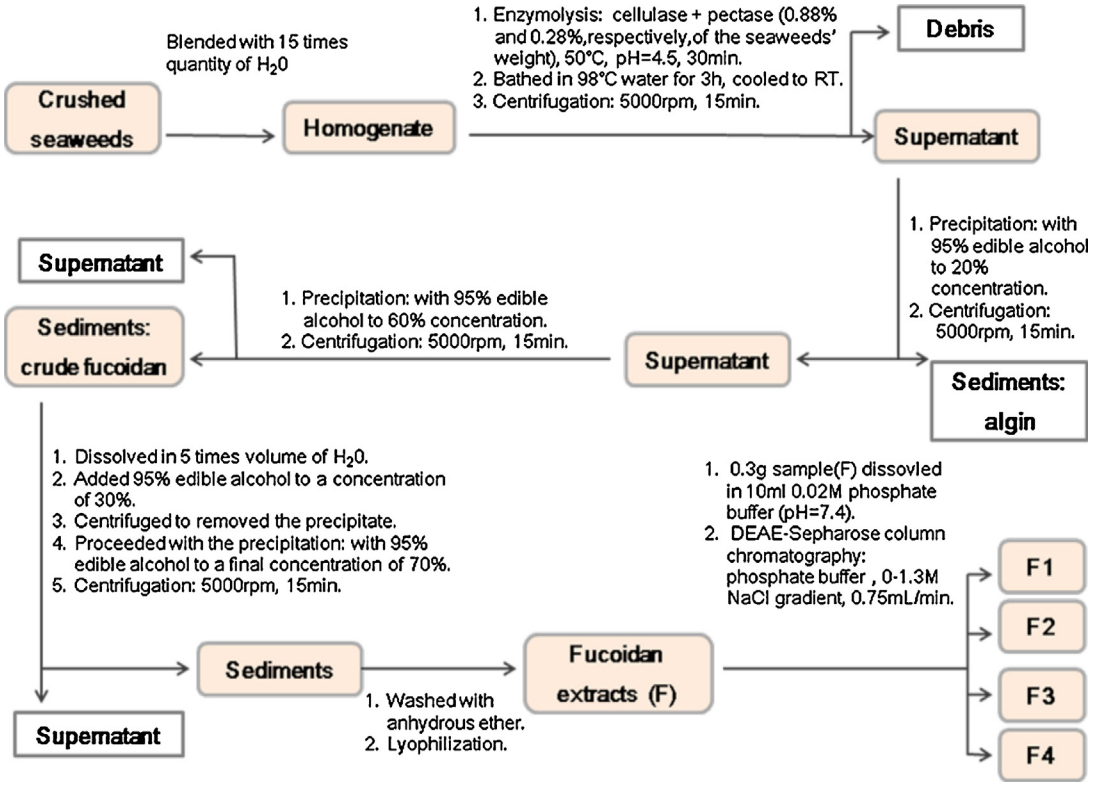

Fig. 1. Diagram of the extraction of fucoidan from dried *C. costata*.

2.7. 2.7 ^{13}C NMR spectroscopy

The NMR experiments were performed using a Bruker AVANCE III 600 MHz NMR spectrometer (Bruker, Berlin, Germany) with a working frequency of 100.6 MHz at 25 °C. The samples were purified using size-exclusion chromatography and then resuspended in dH_2O at a concentration of 20 mg/mL.

2.8. Hydroxyl radical scavenging activity assay

The scavenging activity of F, F1, F2, F3, and F4 against hydroxyl radicals was examined using Fenton's reaction. The initial solution for Fenton's reaction consisted of 0.5 mL of saturated salicylic acid, 3 mL of sodium phosphate buffer (0.2 M, pH 7.4), 0.5 mL of Fe^{2+}–EDTA (3.8 M, mass ratio = 1:1) solution, 1 mL of the sample solution, and 1 mL of H_2O_2 (4 mM). In the control, H_2O_2 was replaced with deionised water. After 90 min of incubation at 25 °C, the reaction was terminated by adding 1 mL of 6 M HCl. An aliquot of 4 mL of cold diethyl ether and 0.5 g of NaCl was then mixed into the solution, and 3 mL of the upper layer of the resulting mixture was subsequently obtained and evaporated to dryness. The residue was redissolved with 0.15 mL of trichloroacetic acid (10%, w/v), 0.25 mL of sodium tungstate (10%, w/v), and 0.25 mL of sodium nitrite (0.5%, w/v), and after 5 min, 0.25 mL of NaOH (1 M) was added. Deionised water was then added to obtain a final volume of 4 mL, and the absorbance at 510 nm was determined. The scavenging activity was calculated as follows: scavenging rate = $[(A_o - A_i)/A_o] \times 100\%$, where A_o is the absorbance of the control, and A_i is the absorbance of the sample.

2.9. CCl_4-induced liver injury studies

One hundred and eighty mice were randomly assigned into 18 groups of ten mice. The mice in these groups were administered physiological saline, physiological saline without CCl_4 injection (CCl_4 negative), bifendate (300 mg/kg·d), of the fucoidan fractions F, F1, F2, F3, or F4 at one of three doses (100, 300, and 500 mg/kg d). The mice received an oral administration of physiological saline, bifendate, or fucoidan daily over a period of one week and were then intraperitoneally injected with 0.4% CCl_4-vegetable oil (10 mL/kg) or vegetable oil (10 mL/kg, CCl_4 negative group only). After 24 h of abrosia, the serum of the mice was sampled and examined using commercial assay kits for ALT and AST. Tissues (approximately 0.3 g per mouse) from the left lobe of the liver were collected from the same position, homogenised in nine volumes of physiological saline, and processed using commercial assay kits for MDA and SOD determination.

3. Results and discussion

3.1. Separation and chemical analysis of the fucoidan fractions

The fucoidan extracts (F) were obtained after a series of precipitation processes (as described in Fig. 1), and four fractions (F1–F4) were obtained using anion-exchange chromatography on DEAE-Sepharose (Fig. 2). The yield of F was 1.87% of dried seaweed weight, and the yields of F1–F4 are displayed in Table 1. All of the F1–F4 fractions demonstrated a single peak on the Sephacryl G-300 chromatograph and exhibited considerable variation in their molecular weights, which ranged from 7.6 to 135.6 kD (Table 1). Table 1 also shows the carbohydrate and sulphate contents of the individual fractions. Furthermore, the monosaccharide composition of the fucoidan extracts and the four fractions are shown in the table. Notable differences can be observed in the composition. F1 mainly consists of galactose and fucose, and mannose is dominant in F2. In F3 and F4, fucose is the major constituent. It appears that the composition of the fucoidan extracts is different from that of the fractions with a relatively high content of glucose. This finding may

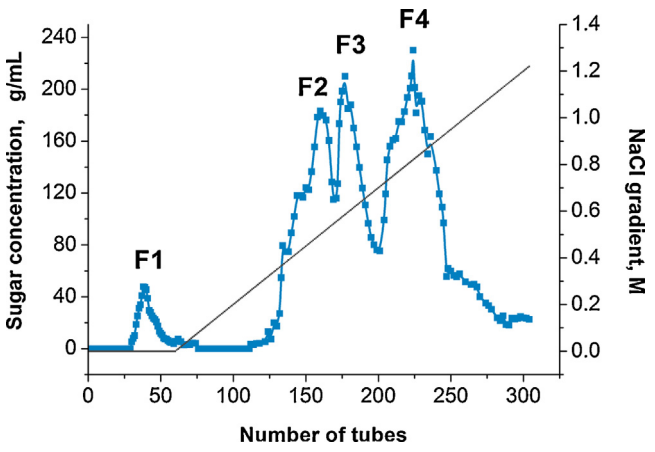

Fig. 2. DEAE-Sepharose chromatography of the fucoidan extracts.

indicate that the F fraction contains some impurities. Because F2 demonstrated a very low sulphate content and a large amount of mannose, we propose that F2 is a type of mannose polysaccharide. Among the other three fractions, F1 has a relatively low fucose content, whereas F3 and F4 are typical fucoidan fractions with fucose molar ratios of 44.7% and 46.6%, respectively.

3.2. IR spectroscopy of F1–F4

The IR spectra of the four fractions showed the typical absorption bands of polysaccharides (Fig. 3). The intensity of the bands at 3600–3200 cm^{-1} was assigned to the deformation of O—H. The bands between 3000 and 2900 cm^{-1} were attributed to the C—H stretching frequency, and the 1430–1400 cm^{-1} bands were attributed to the angular deformations of C—H bonds. The strong absorption at approximately 1050 cm^{-1} corresponded to the C—O—C/C—OH stretching frequency. The four fractions all showed absorption at 1650–1620 cm^{-1}, indicating the presence of acylamino. With the exception of F2, the other three fractions exhibited strong absorption bands at 1260–1240 cm^{-1}, which were consistent with their sulphate contents. The sulphate contents were attributed to the asymmetric O=S=O stretching vibration of sulphate esters. F1 exhibited a distinct band at 821 cm^{-1}, which may indicate the presence of equatorial sulphate groups on the C-2 and C-3 positions, although C-6 may be the primary position for the linkage of sulphate groups (Duarte, Cardoso, Noseda, & Cerezo, 2001). The peaks at 881 and 819 cm^{-1} found for F2 are consistent with the characteristic absorption of mannopyranose. In the F3 and F4 spectra, the bands at approximately 817 and 850 cm^{-1} were attributed to the C—O—S bending vibration of the sulphate substituent, both at the axial C2 and the equatorial C4 positions.

3.3. ^{13}C NMR analysis of F1–F4

Similar to many other polysaccharides, the ^{13}C NMR spectra of the fucoidan fractions from *C. costata* exhibited a specific degree of multiplicity in the diversity of the positions of the inter-glycosidic linkages and/or the multiplicity of the patterns of sulphation of the fucosyl residues (Fig. 4). The F1, F3, and F4 fractions contained intense signals in the anomeric C1 region (100–106 ppm), and the major signal was centred at 100.4 ppm and was assigned to sulphated → 3) – α-L-Fuc*p*(1 → units (Bilan, Grachev, Shashkov, Nifantiev, & Usov, 2004; Bilan et al., 2006). The signals that appeared in high-field (16.5–18.5 ppm) regions are also typical of α-l-fucopyranose residues (Vishchuk, Ermakova, & Zvyagintseva, 2011; Imbs et al., 2009), and the region at 65–83 ppm showed complex signals assigned to pyranoid ring carbons C2—C5. The intense signal at 100.4 ppm in the anomeric region observed on the spectrum of F2 should correspond to mannopyranose because the spectrum exhibited no C6 in the fucose residue (Bilan et al., 2006; Zhang, 1999). These findings match the features of the monosaccharide composition of F2, which contains a high proportion of mannose. Although F2 was found to contain fucose, it showed a lack of fucose signals in its spectrum, which is reasonable because the total carbohydrate content was less than 60% and the proportion of fucose was much less than that found for the other fractions. The ^{1}H NMR spectrum of F2 (not shown) contained no signals in the high-field (1–1.5 ppm) regions, which also suggests the failure to detect fucose units. Therefore, the strong signal at 68.2 ppm may be attributed to (1 → 6)-α-mannose residues (Zhang, 1999).

3.4. Hydroxyl radical scavenging activity assay

The capacities of the fucoidan extracts (F) and F1–F4 to scavenge hydroxyl free radicals are illustrated in Fig. 5. All of the fractions showed profound antioxidant activities against ·OH, and an increase in the scavenging rate was obtained with an increase in the concentration. Of the five samples, the crude extract exhibited the most efficient anti-oxidative capacity at 10 mg/mL with a scavenging rate of 67.4%. The scavenging rats of the purified F1–F4 fractions at 10 mg/mL were 63.3%, 50.2%, 53.9%, and 59.1%, respectively.

3.5. Protective CCl_4-induced liver injury studies

Serum ALT and AST are important indicators for the determination of liver damage. ALT is mainly distributed in the cytosol. The ALT level in the liver is 100-fold higher than the level observed in the serum. However, after liver injury, ALT diffuses into the blood. Thus, the serum ALT content is an important indicator of liver injury. In contrast, 20% of the AST content is distributed in the cytosol, and the remaining 80% is present in mitochondria. Moreover, elevated serum AST activity is closely related with mitochondrial damage.

As shown in Fig. 6, an intraperitoneal injection of CCl_4 induced a notable increase in ALT/AST activities, suggesting severe damage to the liver cells. The analysis of the mice that were intragastrically administered one of the five fractions revealed that the serum ALT levels of the mice that received the highest dose was significantly different ($P < 0.01$) compared with those obtained for the control group. F3 and F4 at a dose of 300–500 mg/kg·d significantly

Table 1
Yields and characteristics of the fucoidan mixture (F) and its four fractions (F1–F4) derived from *C. costata*.

Fractions	[a]Yields (%)	Carbohydrate content (%)	Sulphate content (%)	Molecular weight (kDa)	Monosaccharide composition (mol%)				
					Fuc	Gal	Man	Xyl	Glc
F	1.87	79.6	13.2		18.3	25.6	25.1	3.6	27.4
F1	2.8	53.9	11.4	19.8	30.8	52.3	4.2	8.3	4.5
F2	27.6	56.9	1.0	7.6	17.4	7.6	60.6	6.8	7.6
F3	11.4	46.1	17.6	135.6	44.7	15.9	13.9	21.1	4.3
F4	23.5	54.5	23.5	80.3	46.6	17.0	11.6	17.2	7.6

[a] Weight of the fraction/weight of all of the fractions.

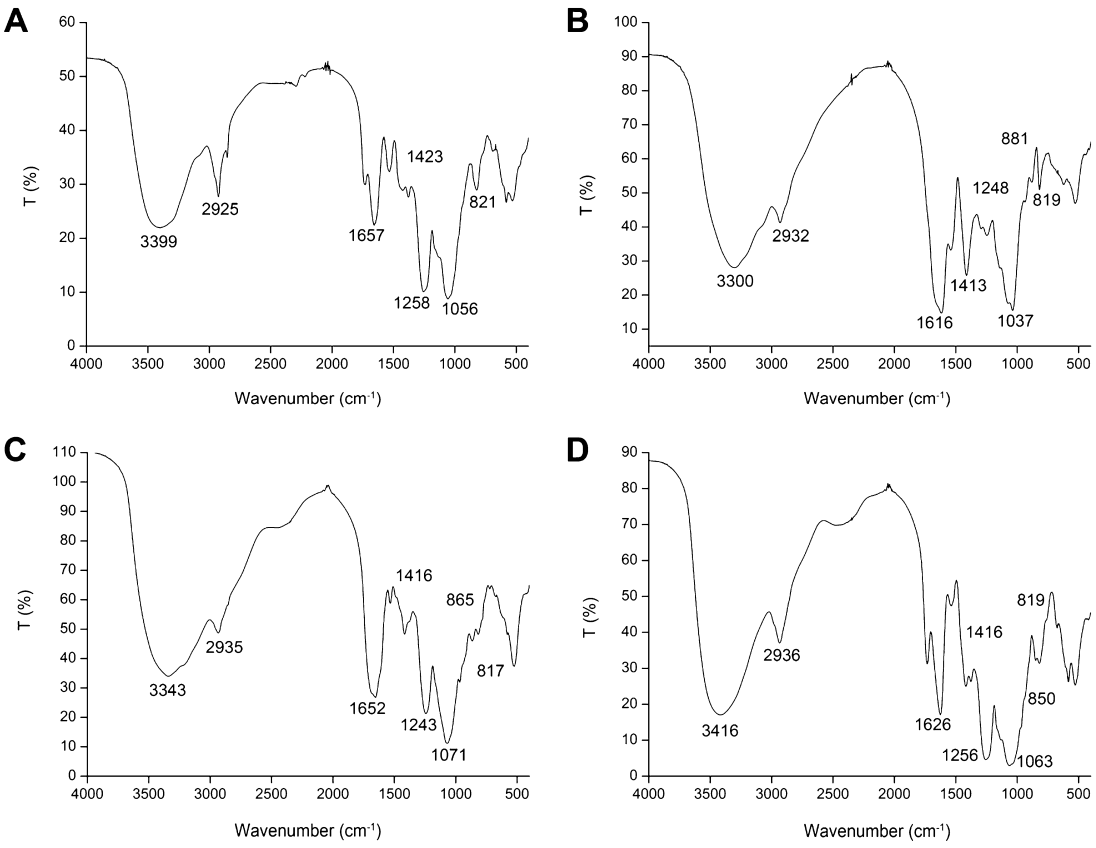

Fig. 3. IR spectra of fucoidan fractions: (A) F1, (B) F2, (C) F3, and (D) F4.

($P < 0.01$) suppressed the increase in the AST level in the serum induced by the CCl_4 injection.

MDA is the end product of lipid peroxidation and an important indicator of liver injury. MDA determination often cooperates with SOD activity, which reflects the body's ability to scavenge oxygen free radicals. The CCl_4 injection caused a marked increase in MDA but a decrease in the serum SOD activity. Most of the treated-treated groups exhibited some inhibition of the serum MDA increases and an enhancement in SOD activity, and a positive correlation was found between dose and efficacy.

The monopolysaccharide compositions obtained in the present and previous studies indicate that different cultivation areas could induce structural changes in fucoidan (Imbs et al., 2009, 2011). Imbs et al. (2009) determined the composition of the water-soluble fucoidan-containing fraction from mature *Costaria costata* and determined that the molecular weight of the fucoidan ranged from 20 to 300 kDa with sugar monomers in the molar ratio of Fuc:Gal:Man:Rha:Xyl:Glc = 617:179:48:37:44:74. These researchers discovered that the fucose content was relatively higher for mature alga and that was increased by approximately 5-fold from April to July as the algae grew to maturity. Further work performed by Imbs et al. (2011) on the fucoidan composition of *C. costata* collected from Troits Bay (Sea of Japan) demonstrated the differences induced by different cultivation areas. The monosaccharide composition of *Costaria costata* cultivated in Dalian in northern China exhibits a relatively low content of fucose compared with the fucoidan obtained from Primorsky Krai in Russia (Imbs et al., 2009). Such differences are potentially due to the differences in the temperature and nutritional contents of the sea water between the Dalian coast of China and Primorsky Krai, Russia. Studies using IR spectroscopy indicated that the F1, F3, and F4 fractions demonstrate characteristics of the O=S=O stretching vibration of sulphate esters, as determined through the observation of strong absorption bands at 1260–1640 cm^{-1}. Synytsya et al. (2010) studied fucoidan extracts from the sporophyll of *U. pinnatifida* and revealed a very intense and broad IR band at 1256 cm^{-1}, corresponding to a strong Raman band at 1269 cm^{-1}, which was attributed to the asymmetric O=S=O stretching vibration of sulphate esters with some contribution from the COH, CC, and CO vibrations. The IR band of the F3 and F4 fractions also exhibited adsorptions at approximately 817 and 850 cm^{-1}, which indicates the presence of sulphate groups at positions 2 and 4, respectively. Bilan et al. (2006) obtained similar findings for the fucoidan extracted from *F. serratus* L. The ^{13}C NMR spectra of F1–F4 were complex. Intense signals were observed in the anomeric C1 region, and the spectra of F1, F3, and F4 also appeared to have signals in the high-field region of 16.5–18.5 ppm, which are due to α-L-fucopyranoside (Bilan et al., 2006; Vishchuk et al., 2011). This finding agrees with the results obtained by Imbs et al. (2009), who illustrated that the ^{13}C NMR spectrum of the fucoidan from *Costaria costata* exhibits a signal at 16.9 ppm, which was assigned to the CH_3 group of fucopyranose. The spectra of F2 were different from those of F1, F3, and F4, and no signal was observed in the high-field regions; instead, these spectra exhibited a signal at 100.4 ppm, which may indicate the presence of mannopyranose (Bilan et al., 2006; Zhang, 1999). F1, F3, and F4 showed complex signals of pyranoid ring carbons C2–5 in the region of 65–83 ppm, which indicates the complex structural patterns of the monomer composition and their glycosylation, sulphation, and/or O-acetylation substitutions. In contrast, the corresponding signals of F2 appeared to exhibit much clearer peaks, indicating the lack of sulphation substitution.

Fucoidan has been reported to have antioxidant activity, and the fractions obtained in the present study exhibited significant antioxidant activities against ·OH (Fig. 5) in a concentration-dependent manner, which is consistent with the results reported by Wang, Zhang, Zhang, and Li (2008) and Wang et al. (2009). Interestingly,

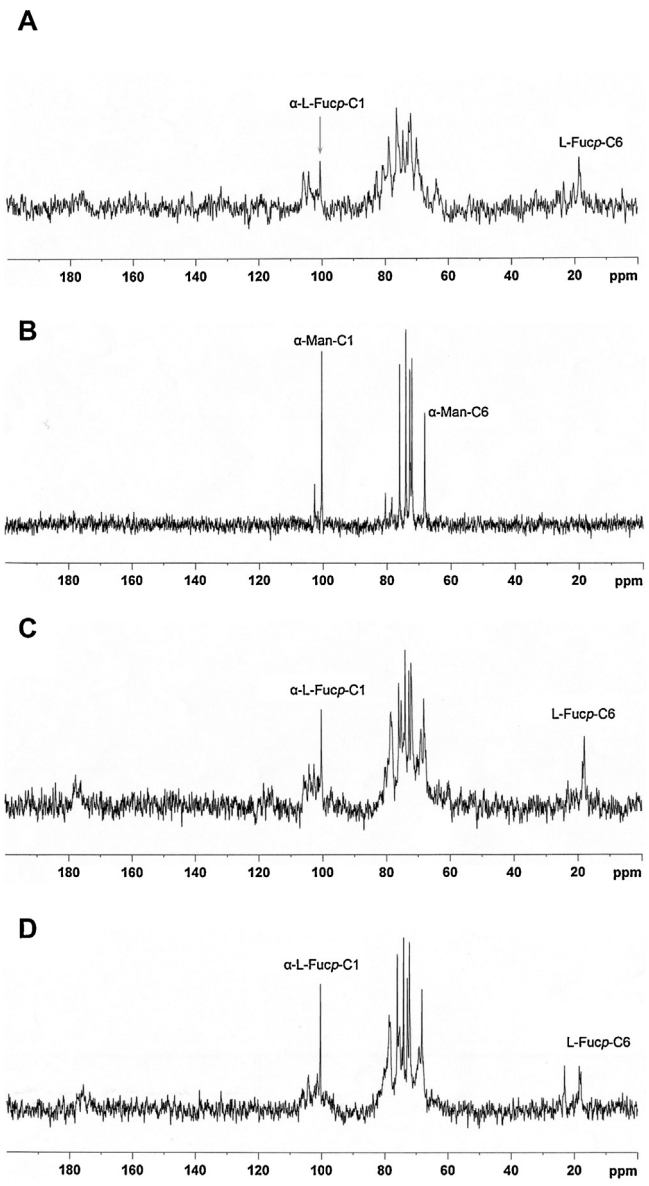

Fig. 4. ^{13}C NMR spectra of fucoidan fractions: (A) F1, (B) F2, (C) F3, and (D) F4.

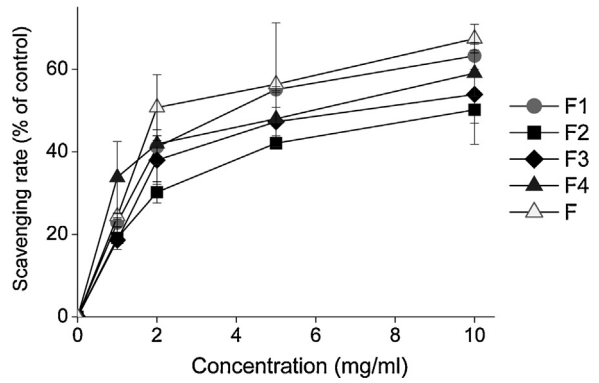

Fig. 5. Hydroxyl radical scavenging activities of fucoidan mixture F and fractions F1–F4.

crude fucoidan demonstrated the highest scavenging rate of 67.4% at 10 mg/mL, whereas F1, F3, and F4 showed scavenging rates of 63.3%, 53.9%, and 59.1%, respectively, at 10 mg/mL. This finding is consistent with the results reported by other researchers, who found that crude sample has higher scavenging activities compared with the corresponding purified samples. It was previously thought that other small polysaccharides, pigments, protein, and peptides may affect the scavenging activities of the crude samples (Chattopadhyay et al., 2010). Patel, Mulloy, Gallagher, O'Brien, and Hughes (2002) found that crude commercial fucoidan is more active than purified fucoidan for the inhibition of the proliferation of vascular smooth muscle cells. Thus, these researchers proposed that crude fucoidan displays a specific structure, which may affect its biological activities. However, crude fucoidan and its purified fractions are potent scavengers and may be potentially related with the reductones in crude fucoidan, which may react with radicals to stabilise the molecules (Chattopadhyay et al., 2010). Importantly, the scavenging activities of F1, F3, and F4 were not related with their fucose and low sulphate contents. Xue et al. (2001) investigated the antioxidant activities of the low-molecular-weight fucoidan from *L. japonica* on free radicals of active superoxide anion and hydroxyl and found that the fraction with a high amount of glucuronic acid but a low sulphate content displayed the highest scavenging effect on free radicals of active oxygen (Xue et al., 2001).

It has been reported that antioxidant activities suppress the activation of hepatic stellate cells and thus prevent liver fibrosis (Canbay et al., 2002; Song et al., 2003). Hayashi et al. (2008) reported that commercial fucoidan can partially prevent CCl_4-induced liver fibrosis and reduces CCl_4-induced lipid peroxidation. Fucoidan also prevents Con A-induced liver injury by mediating the endogenous production of IL-10 and the inhibition of proinflammatory cytokines in mice (Saito et al., 2006). In our study, the potential anti-oxidant activities of crude fucoidan and the purified fractions indicate that fucoidan may demonstrate anti-fibrotic activity to protect against liver injury. As shown in Fig. 6, the intraperitoneal injection of mice with CCl_4 significantly increased the serum ALT and AST levels, which increased to 261.45 μ/L and 280.42 μ/L from 23.62 and 45.98 μ/L, respectively. The intragastric administration of F and F1–F4 at different doses ranging from 300 to 500 mg/kg d decreased the levels of serum ALT and AST after seven days. Moreover, the ALT and AST levels of the mice administered bifendate (300) were significantly lower compared with those obtained for the CCl_4 group, and all of the doses of F3 tested showed significant liver protection for suppressing the increases of ALT and AST. The three different doses of F4 resulted in significantly different serum ALT and AST levels, with the exception of the serum ALT value obtained for the low dose. As shown in Table 1, The F2 fraction had very low or almost no sulphate content (1%) compared to the other fractions and this might be the reason for none significant effect on the serum ALT and AST activities with the exception of the ALT content obtained with the highest dose. Compared with F3 and F4, the sulphate content of F1 is lower and may be potentially related with its relatively weak activities for decreasing the serum AST and ALT levels. The results indicate that the MDA contents in the liver tissue of mice treated with the intermediate and high doses of F, F1, F2, F3 and F4 were all significantly decreased compared with those obtained in the mice treated only with CCl_4, as shown in Fig. 6. In contrast to the MDA levels, the tissue contents of SOD reflect the scavenging activities in the serum. As illustrated in Fig. 6, all of the doses of crude fucoidan and F1 with a relatively low content of sulphate exhibited a significant effect in the promotion of the scavenging activities (SOD) of free radicals. The administration of F3 and F4 at the intermediate and high doses tested significantly increased the SOD levels. In addition, F2 had no effect in the promotion of SOD activity. These findings may indicate that the antioxidant activities of fucoidan are sulphate-dependent but

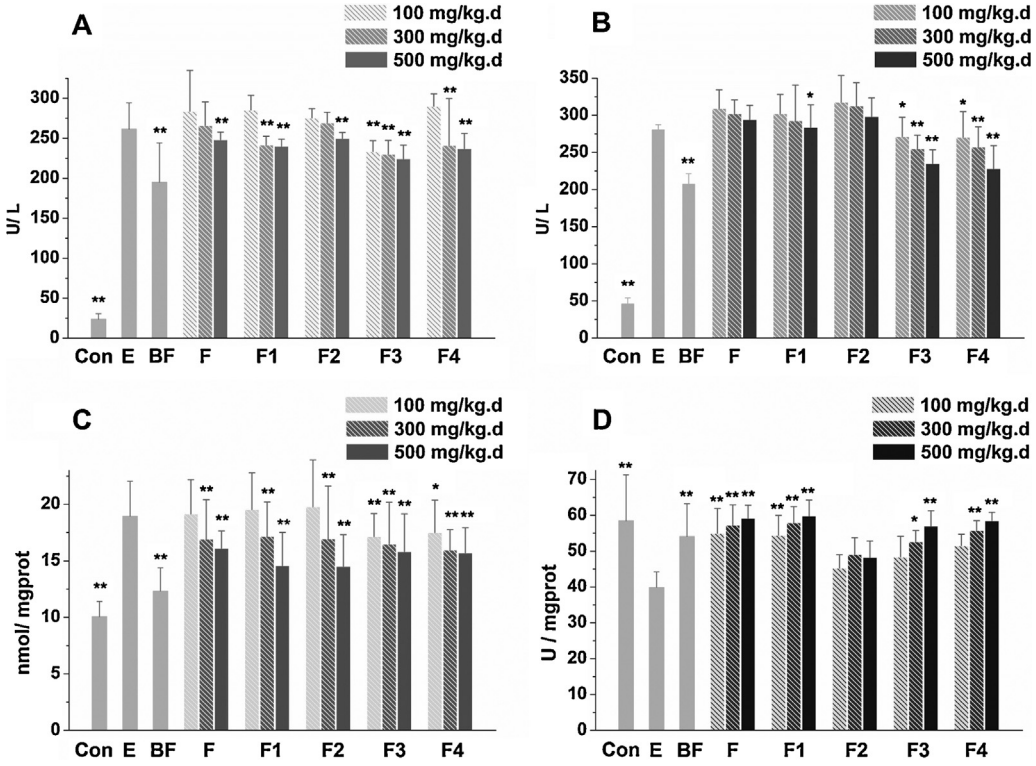

Fig. 6. Protective effects of fucoidan fractions against carbon tetrachloride-induced acute liver injury in mice. (A) Serum ALT level; (B) serum AST level; (C) MDA concentration in liver tissue; and (D) SOD activities in liver tissue. Abbreviations: Con, control group, CCl_4-negative; E, experimental group, CCl_4-positive; BF, bifendate-treated group. $^*P < 0.05$; $^{**}P < 0.01$.

are not positively correlated with the sulphate content. As inferred from the relevant literature, the biological activities of fucoidan are closely related with its structure, which varies depending on the seaweed species, cultivation areas, and cultivation season, among other factors.

In summary, the fucoidan extracted from *Costaria costata* cultivated in northern China may be a potential antioxidant for the scavenging of free radicals and protection against liver injury. The present study provides the first investigation of the *in vivo* protective activities of fucoidan from *Costaria costata* against CCl_4-induced liver injury. Thus, fucoidan may be developed as an ingredient of functional foods to protect against liver damage.

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