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Antioxidant and antihyperlipidemic activities of purified polysaccharides from *Ulva pertusa*

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Abstract

Several studies have reported that ulvan from the alga *Ulva pertusa* (Chlorophyta) exhibits substantial antioxidant and antihyperlipidemic activities; however, which group of heteropolysaccharides play roles in these activities remains unknown. To identify the components of ulvan that possess biological activity, we used a DEAE-Sepharose fast-flow column. Two purified polysaccharides (F1 and F2) were detected and their physicochemical properties, antioxidant and antihyperlipidemic activities were analyzed. F1 (average molecular weight of 190 kDa) was composed of rhamnose, xylose, and glucose; F2 (average molecular weight of 83 kDa) was composed of rhamnose and xylose. Both polysaccharides showed significant antioxidant activities especially superoxide radical-scavenging power against ascorbic acid the half maximal inhibitory concentration (IC_{50}) of which was 3.5 mg mL^{-1} ($P < 0.05$). Treatment of hyperlipidemic Kunming mice with F1 and F2 resulted in a significant decrease in serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol, and a significant increase in high-density lipoprotein cholesterol. The results suggested that purified polysaccharides conferred greater biological activity than raw polysaccharides extracted from ulvan. These results indicated that F1 and F2 may be potential sources of natural antioxidants and therapeutic agents for hyperlipidemia.

Keywords DEAE-Sepharose fast-flow · *Ulva pertusa* · Chlorophyta · Antioxidant · Hyperlipidemia

Introduction

Polysaccharides are one of the four major biomacromolecules (the others being polynucleotides, lipids, and proteins). Polysaccharides are a variety of carbohydrates composed of numerous (generally > 10) monosaccharides linked in branched or unbranched chains by glycosidic bonds and are found in many plants, animals, and microorganisms (Thetsrimuang et al. 2011; Xie et al. 2016). In recent years,

research on polysaccharides has focused on their complicated structures and biological activities, including their potential roles as anticancer and anti-proliferation agents (Chien et al. 2015; Jia et al. 2015), antioxidants (Xie et al. 2015), antihyperlipidemia agents (Liu et al. 2012), and immune system stimulators (Cui et al. 2015; Berri et al. 2016).

Fractionation and purification technologies have been important to find new effective substances beyond what we have from raw polysaccharides (Luo et al. 2010; Wei et al. 2010; Zhao et al. 2010; Jiang et al. 2016). The biological activities of polysaccharides depends on factors besides molecular structure, molecular weight, monosaccharide composition, water solubility, glycosidic bond of the main chain, degree of branching, and the conformation type and degree of substitution of the main chains (Ma et al. 2012). As a consequence of polysaccharide function being dependent on so many factors, fractionation and purification will provide opportunities to acquire new products with specific functions (Luo et al. 2010; Wei et al. 2010; Zhao et al. 2010; Jiang et al. 2016).

Ulva pertusa (Chlorophyta) is a green alga that contains abundant vitamins, dietary fiber, and trace elements. It is a

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low-calorie food (Lahaye and Jegou 1993) and had been a recommendation for people with hyperlipidemia in Traditional Chinese medicine. Ulvan, a polysaccharide from *U. pertusa*, belongs to a group of sulfated heteropolysaccharides, and its main disaccharide units are [β-D-GlcpA-(1→4)-α-L-Rhap3s] and [α-L-IdopA-(1→4)-α-L-Rhap3s] (Yu et al. 2003b; Lahaye and Robic 2007; Alves et al. 2013). Qi et al. (2005a, b) studied the antioxidant effects of different sulfate content derivatives of ulvan and showed that high sulfate content derivatives showed a significant antioxidant effect in vitro. Yu et al. (2003a) studied the antihyperlipidemic activities of sulfated polysaccharides from ulvan and reported that polysaccharides with low molecular weight (151.6–28.2 kDa) were more beneficial in reducing hyperlipidemia compared with those of high molecular weight. Many reports (Yoon et al. 2015; Zahid et al. 2016; Ali et al. 2017) have isolated active substances from *U. pertusa* with strong biological activities. However, few studies have reported on ulvan from *U. pertusa* that has been purified using ion exchange chromatography. In this study, ulvan extracted from *U. pertusa* was purified by anion-exchange chromatography to obtain two polysaccharides (F1 and F2). To compare the purified polysaccharides with the unpurified ulvan (U), we analyzed the physicochemical properties of U, F1, and F2, including total sugar, sulfate and uronic acid contents, molecular weight, monosaccharide composition, FT-IR, and ^{13}C nuclear magnetic resonance (NMR) analyses. The antioxidant properties of U, F1, and F2 were evaluated and compared using a superoxide radical-scavenging assay, reducing power assay, and DPPH radical-scavenging assay. Antihyperlipidemic activity analyzed in vivo in Kunming mice included serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-C), and high-density lipoprotein cholesterol (HDL-C).

Materials and methods

Materials and chemicals

Ulva pertusa was washed, air dried and saved in plastic boxes at room temperature after being gathered on the coast of Qingdao, China. The DEAE-Sepharose fast-flow column was from GE Healthcare Bio-Sciences AB (Sweden). Dialysis membrane was from Spectrum Co. (USA), and molecular weight was cutoff at 500 Da. Analysis kits for nicotinamide adenine dinucleotide-reduced (NADH), phenazine methosulfate (PMS), nitro blue tetrazolium (NBT), hydrogen peroxide (H$_2$O$_2$), ethylene diamine tetra-acetic acid (EDTA), ferrozine, and trichloroacetic acid (TCA) were provided by Solarbio Chemical Co (Beijing, China). The assay kits for serum TC, TG, LDL-C, and HDL-C were purchased from Nanjing Jiancheng (China). Other chemicals were of analytical grade.

Extraction of polysaccharides

Ulvan polysaccharides were obtained through hot-water extraction and ethanol precipitation based on the method of Yu et al. (2003a, b).

Isolation and purification of polysaccharides

Ulvan polysaccharides were dissolved in water and centrifuged at ×7155*g* for 8 min. After centrifugation, the supernatant was run through a DEAE-Sepharose fast-flow column (5.0 cm × 20 cm) that had been equilibrated with ten multiples of deionized water. Column volume was approximately 400 mL. The sample was run with a flow rate of 10 mL min^{-1} and eluted with a stepwise gradient of NaCl aqueous solutions (0.5 and 1 M). Fractions were combined according to the total carbohydrate content measured using the phenol–sulfuric acid method and yielded the F1 and F2 fractions. After dialyzing, freeze-drying and concentrating, F1 and F2 were obtained.

General methods of characterization

The total sugar content was quantified by the phenol–sulfuric acid colorimetric method, with rhamnose as standard (Yu et al. 2009). Uronic acid content was calculated by the sulfuric acid carbazole colorimetric method by determining the absorbance at 530 nm with D-glucuronic acid as a standard (Yu et al. 2003b). The sulfate content was expressed by the barium chloride–gelatin method (Kawai et al. 1969).

Molecular weight measurement

High-performance gel-permeation chromatography (HPGPC) was used to measure molecular weights using an Agilent1200 system with a SHODEX SB-807 HQ column and Waters 2414 refractometer (Waters Corp., USA). Column temperature was 35 °C; the mobile phase was 0.1 M NaCl and the flow rate was 0.5 mL min^{-1}. Dextran T-series standards of different molecular weights were used to construct the calibration curve to calculate the molecular weights of samples.

Monosaccharide measurement

Each sample was dissolved in 2 M trifluoroacetic acid (TFA) at 100 °C for 4 h in a sealed glass tube. After being cooled to room temperature, the hydrolyzed products were evaporated until dryness by adding 2 mL of methanol. This process was repeated three times to fully remove the TFA. The process of derivation with the polysaccharides was executed according to the method by Zhang et al. (2015). After derivatization, the derivations were measured by high-performance gel-permeation chromatography (HPGPC-20AT, Shimadzu, Japan).

Fourier transform-infrared (FT-IR) spectra

The dried polysaccharides (1 mg) mixed with 100 mg of potassium bromide powder were pressed into pellets for a Perkin-Elmer FT-IR spectrometer measurements range from 400 to 4000 cm^{-1}.

Nuclear magnetic resonance (NMR)

The ^{13}C-NMR spectra of the polysaccharide solutions in D_2O were recorded at 27 °C on a Brucker AV-500 spectrometer.

Antioxidant activity of superoxide radical-scavenging assay

The superoxide radical-scavenging effect was quantified for each sample using a previously reported method (Xu et al. 2016) with minor modifications. Gradient concentrations of samples (0.006–0.2 mg mL^{-1}), Tris–HCl (16 mM, pH 8.0), NADH (145 μM), NBT (50 μM), and PMS (24 μM) were mixed over ice then incubated at room temperature for 5 min and then absorbance was read at 560 nm against Tris–HCl buffer as a blank. The superoxide radical-scavenging effect was derived from the following equation:

$$\text{Scavenging effect } (\%) = (1 - A_s / A_i) \times 100$$

where A_i is the absorbance of the Tris–HCl buffer and A_S is the absorbance of the sample (or ascorbic acid).

Antioxidant activity of reducing power assay

The reducing power of samples was assessed according to the method of Qi et al. (2006) with some modifications. First, 2-mL gradient concentrations of samples (0.8–4.0 mg mL^{-1}) were dissolved in a phosphate buffer (0.2 M, pH 6.6). Next, 2 mL of potassium ferricyanide (0.5% w/v) was added, and then the samples were incubated at 50 °C for 20 min. Then, 2 mL of TCA solution (10% w/v) performed a termination. After mixing with a ferric chloride solution (0.1% w/v), absorbance was determined at 700 nm. The reducing power of samples was assessed as the percentage of the activity compared with a 0.13 mM solution of ascorbic acid.

Antioxidant activity of DPPH radical-scavenging assay

To evaluate the free radical-scavenging capacity of samples, we used a previously reported method (Wang et al. 2014) with minor modifications. A 1-mL aqueous solution of sample was added at gradient concentrations to 2 mL of DPPH ethanol solution (0.1 mM). After incubation at 37 °C in the dark for 10 min, absorbance was measured at 517 nm. Ascorbic acid was used as a positive control and deionized water was the blank control. DPPH radical scavenging was calculated using the following equation:

$$\text{Scavenging activity } (\%) = [(A_i - A_s) / A_i] \times 100$$

where A_i is the absorbance of the deionized water and A_S is the absorbance of the sample (or ascorbic acid).

Animals and experimental design

Eighty male Kunming mice weighing from 18 to 22 g were provided by the Animal Lab Center of Shandong University (animal license SCXK (Lu) 20140007, China). All protocols were approved by the Ethics Committee of Weifang Medical University for animal studies. The mice were housed in stainless steel cages on a 12-h light-dark cycle at room temperature (21 °C ± 2 °C) and had free access to water and food. After 3 days to accommodate animal facilities, animals were weighed and randomly assigned to eight groups of ten mice. Group 1 was the normal control; group 2 was the hyperlipidemic control; group 3 was given ulvan (250 mg kg^{-1} body weight); groups 4–6 were given F1 in doses of 500, 250, and 125 mg kg^{-1}; group 7 treated with F2 (250 mg kg^{-1} body weight); and group 8 was fed the standard drug cholestyramine (500 mg kg^{-1}) as a positive control. During the 28-day experiment, group 1 was fed the common commercial mice chow, and all other groups were fed a cholesterol-rich diet (8.0% lard, 2.0% cholesterol, 0.3% sodium cholic acid, and 89.7% commercial chow) by oral administration for 28 days.

At the end of the experimental period (28 days), food was withheld for at least 12 h, and then the mice were weighed and 1-mL blood samples were collected from the eyeballs to measure serum TC, TG, HDL-C, and LDL-C levels.

Statistical analysis

Statistical analysis was performed by the Statistical Analysis Systems software package. The results are presented as means of three determinations ± SD and followed by Student's t test. Values of $P < 0.05$ were considered statistically significant.

Results

Isolation, purification, and physicochemical properties of polysaccharides

Ulvan was further purified through anion-exchange chromatography according to differences in the existence of ionic groups. Two fractions eluted with the 0.5 and 1 M sodium chloride solutions were collected and afforded F1 and F2 after dialysis and concentration. The physicochemical properties of

Table 1 Components of monosaccharides and physicochemical properties of samples from *Ulva pertusa*

Samples	U	F1	F2
Color	Light yellow	Yellow	White
Total sugar (w/w %)	62.16	51.07	72.09
Uronic acid (w/w %)	16.49	14.27	24.09
Sulfate (w/w %)	20.17	15.56	23.99
Average molecular weights (Da)	143,234	190,368	83,094
Sugar components			
Rhamnose (mol %)	21.55	15.20	23.10
Xylose (mol %)	7.20	5.87	8.52
Glucose (mol %)	4.66	3.98	nd*

*Not detected

the samples are shown in Table 1. The total sugar of F1 was 51.07%, which was lower than the other two samples. Pigments and other impurities may lead to differences in color. The uronic acid to total sugar ratios in the samples were 0.27, 0.28, and 0.33; thus, we obtained polysaccharides with stronger ionic strength in elution as we increased the concentration of eluent. We also obtained greater sulfate contents. The sulfate to total sugar ratios were 0.32, 0.30, and 0.33 indicating that the sulfate content was greater in F2 compared with F1. As shown in the HPGPC analysis, F2 had the lowest average molecular weight. Monosaccharide analysis indicated that all the three samples were heteropolysaccharides and were mainly composed of the monosaccharides rhamnose and xylose. Glucose was present in U and F1, but undetected in F2. The rhamnose content in the three samples followed the order: F2 > U > F1. The rhamnose content in F1 was 15.20%, far less than other two polysaccharides.

FT-IR analysis

The FT-IR spectra of U, F1, and F2 were recorded at the range of 400–4000 cm^{-1} as shown in Fig. 1. A strong peak near 3350 cm^{-1} that was due to the O–H stretching vibration was present in all three fractions, but its location differed. In F1, the strong peak occurred at 3284 cm^{-1}, which was lower than other three fractions (U, 3350 cm^{-1}; F2, 3387 cm^{-1}). The bands in the region of 2920 cm^{-1} were due to C–H stretching vibration. This vibration is a characteristic of
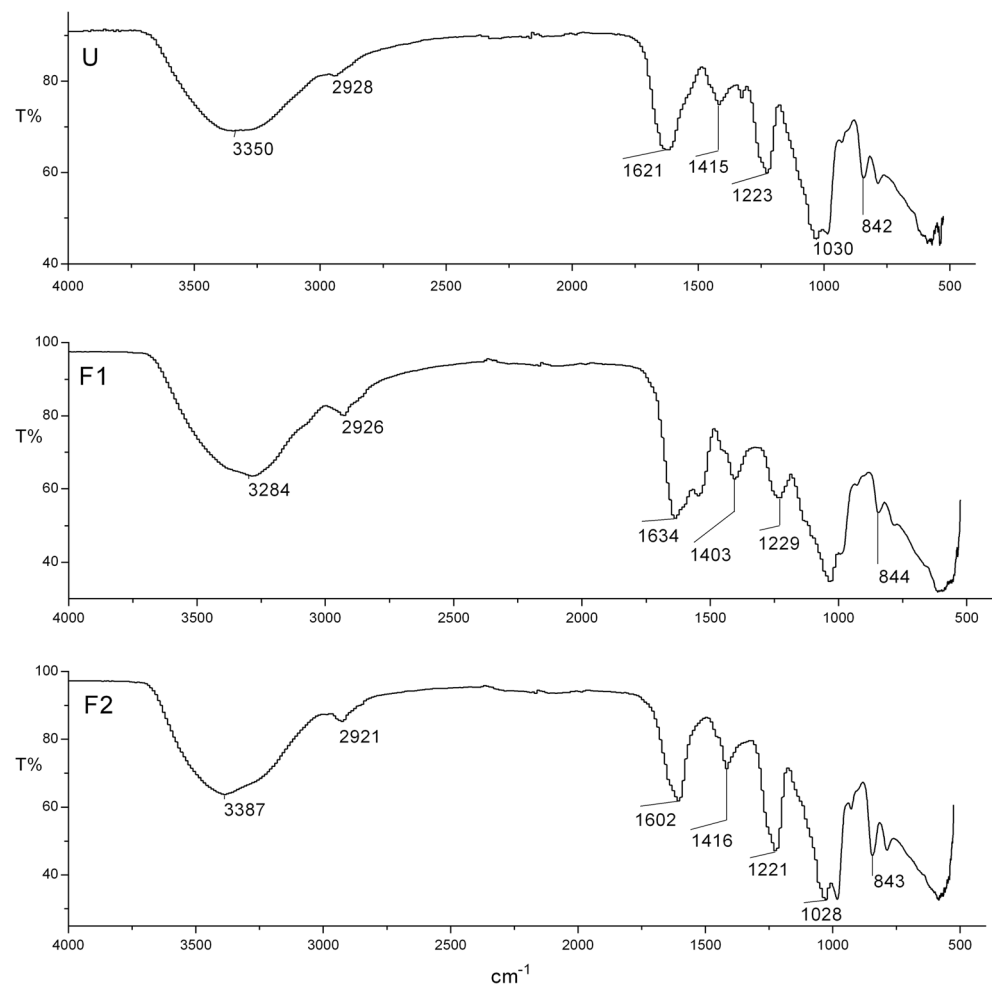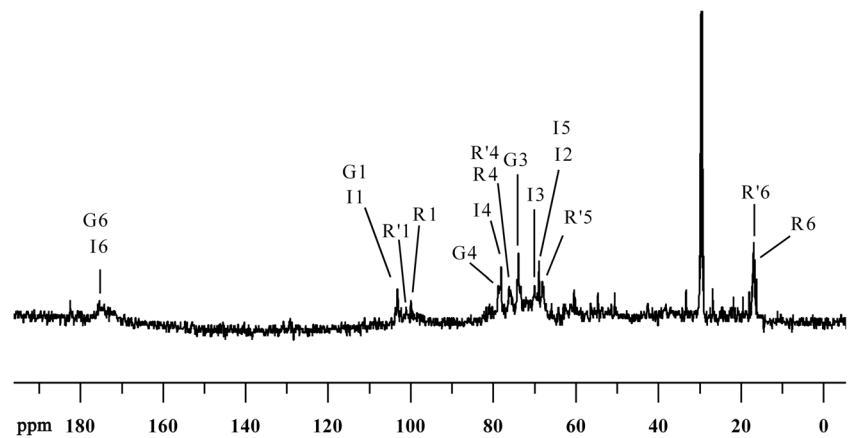
Fig. 1 The FT-IR spectra of U, F1, and F2

Fig. 2 ^{13}C NMR spectra of F2

polysaccharides and U, F1, and F2 all had these bands. The comparatively strong absorption peak at 1600–1650 cm^{-1} signified the existence of C=O. The bands near 1416 and 1602 cm^{-1} related to the stretching vibration of carboxylate groups. These two absorption peaks seen in FT-IR spectra were in line with the preceding results (Table 1) that showed there were uronic acid components in all three samples. The F1 peak was at 1403 cm^{-1}, which was lower than other two fractions (U, 1415 cm^{-1}; F2, 1416 cm^{-1}). Considering the signals of F1 at 3284 and 1403 cm^{-1}, we can deduce that F1 may possess stronger electron-donating ability than U and F2. The absorption band from 1300 to 800 cm^{-1}, called the "finger print" region, is the result of surface structure and the conformation of the molecule. The peaks at 950–1200 cm^{-1} reveal the existence of C–O–C and C–O–H link bonds.

Table 2 ^{13}C NMR spectral data of samples from *Ulva pertusa*

Samples	Residues[a]	Chemical shifts (ppm)					
		C-1	C-2	C-3	C-4	C-5	C-6
U	G	103.1	73.8	73.8	78.7	76.2	175.4
	R	97.3	68.9	78.6	77.0	67.5	18.5
	I	101.8	70.2	73.9	77.9	69.3	175.4.
	R'	98.0	68.9	80.5	77.0	67.5	18.7
F1	G	103.0	73.7	73.7	78.7	75.1	175.3
	R	99.9	69.9	69.5	75.7	67.9	16.3
	I	102.4	68.8	69.6	78.7	68.8	174.1
	R'	100.6	69.9	69.5	75.7	67.9	16.8
F2	G	103.1	73.6	73.6	78.7	75.4	175.2
	R	100.2	69.6	69.5	75.7	67.7	17.2
	I	102.3	68.5	68.5	78.1	68.5	173.4
	R'	101.0	69.6	69.5	75.7	67.7	17.3

[a] G: (1→4)-linked β-D-glucuronic acid; R: (1→4)-linked α-L-rhamnose-3-sulfate (linked with β-D-glucuronic acid); I: (1→4)-linked α-L-iduronic acid; R': (1→4)–linkedα-L-rhamnose-3-sulfate (linked with α-L-iduronic acid)

Nuclear magnetic resonance (NMR) analysis

Figure 2 shows the ^{13}C-NMR spectra of F2. According to Lahaye et al., signals from 90 to 110 ppm shown in the ^{13}C NMR are ascribed to anomeric carbons. The signals G1 (103.0 ppm), I1 (102.4 ppm), R'1 (100.6 ppm), and R1 (99.9 ppm) were assigned to β-D-glucuronic acid, α-L-idulonic acid, 1,4-linked α-L-rhamnose-3-sulfate (linked with α-L-iduronic acid), and 1,4-linked α-L-rhamnose (linked with β-D-glucuronic acid), respectively. The signals at 175.3 and 174.1 ppm were assigned to the C–6 stretching vibration of carboxyl carbons. Methyl signals at 16.8 and 16.3 ppm were observed, revealing that α-L-rhamnose residues were involved. The ring-carbon signals (55–85 ppm) were assigned as indicated in Fig. 2 and presented in Table 2. The details of ^{13}C NMR chemical shifts of U and F1 also are summarized in Table 2.

Antioxidant activity of superoxide radical-scavenging activity

As shown in Fig. 3a, the scavenging effect was enhanced when gradient concentrations were below 0.05 mg mL^{-1}. The scavenging effect reached almost 80% and enhanced slowly over the 0.10 mg mL^{-1}, and increases in concentration for U, F1, and F2. F2 showed a stronger inhibiting effect than F1 and U. As shown in Fig. 3b, the IC$_{50}$ value of ascorbic acid was 3.6 mg mL^{-1}. Meanwhile, the IC$_{50}$ values of U, F1, and F2 were 40, 52, and 20 μg mL^{-1}, respectively (Fig. 3a). Three samples exhibited significantly better superoxide radical-scavenging effects than ascorbic acid ($P < 0.05$).

Antioxidant activity of reducing power activity

The reducing capacity of F1 and U was significant ($P < 0.05$) and changed in a concentration-dependent manner (Fig. 4). In addition, the reducing capacity of F2 was
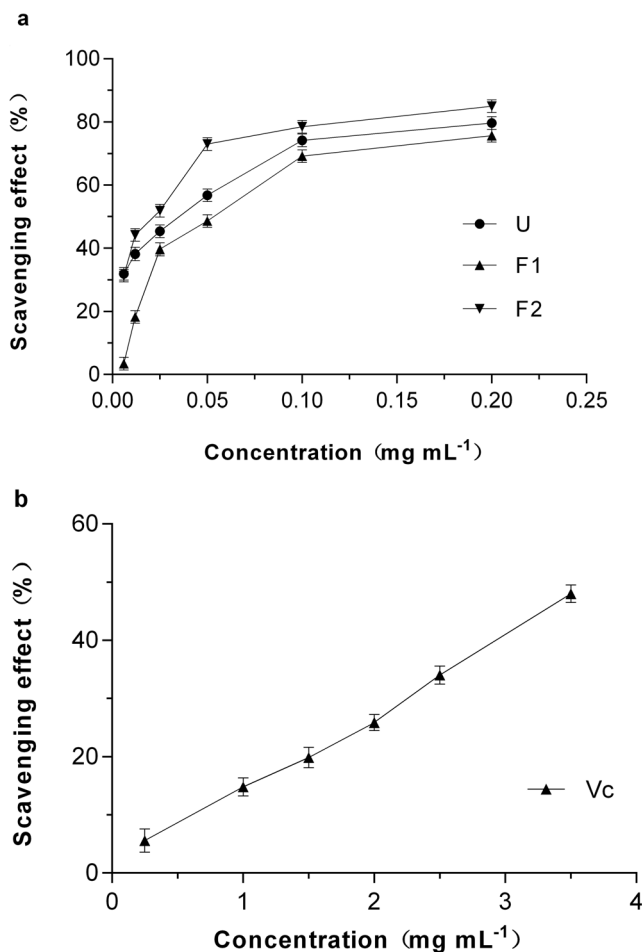
Fig. 3 **a** Scavenging effect of U, F1, and F2 on superoxide radicals. Values are means ± SD ($n = 3$). **b** Scavenging effect of ascorbic acid on superoxide radicals. Values are means ± SD ($n = 3$)

more substantial than that of U. The reducing capacities of the three samples types were in the order F1 > U > F2; yet, all samples were weaker than ascorbic acid.
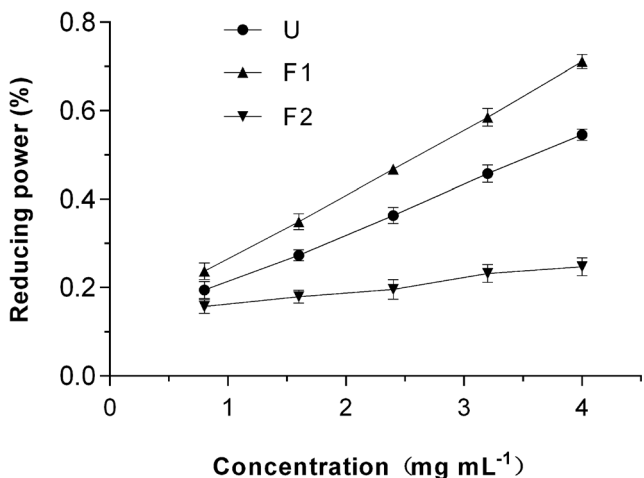

Fig. 4 Reducing power of U, F1, and F2. Values are means ± SD ($n = 3$). Reducing power was expressed as a percentage of the activity shown by a 0.13-mM solution of ascorbic acid

Antioxidant activity of DPPH radical-scavenging activity

Ascorbic acid exhibited significant DPPH radical-scavenging activity and the IC_{50} value was 0.084 mM. When the concentration was over 0.112 mM, the scavenging effect reached almost 70% and enhanced slowly as shown in Fig. 5a. Compared with U and F1, F2 enhanced significantly as the concentration increased. Besides, F2 exhibited much more effective DPPH radical-scavenging activity than U and F1 as shown in Fig. 5b.

Antihyperlipidemic activity in mice

F1 exhibited effective antihyperlipidemic activity in a concentration-dependent manner (Table 3). Compared with the hyperlipidemic group, the F1-treated group (500 mg kg^{-1}) had significant effects ($P < 0.05$) on TC, TG, LDL-C, and HDL-C. A dose of 250 mg kg^{-1} of F1 also had significant effects on TC, TG, HDL-C ($P < 0.05$), and LDL-C ($P < 0.01$).
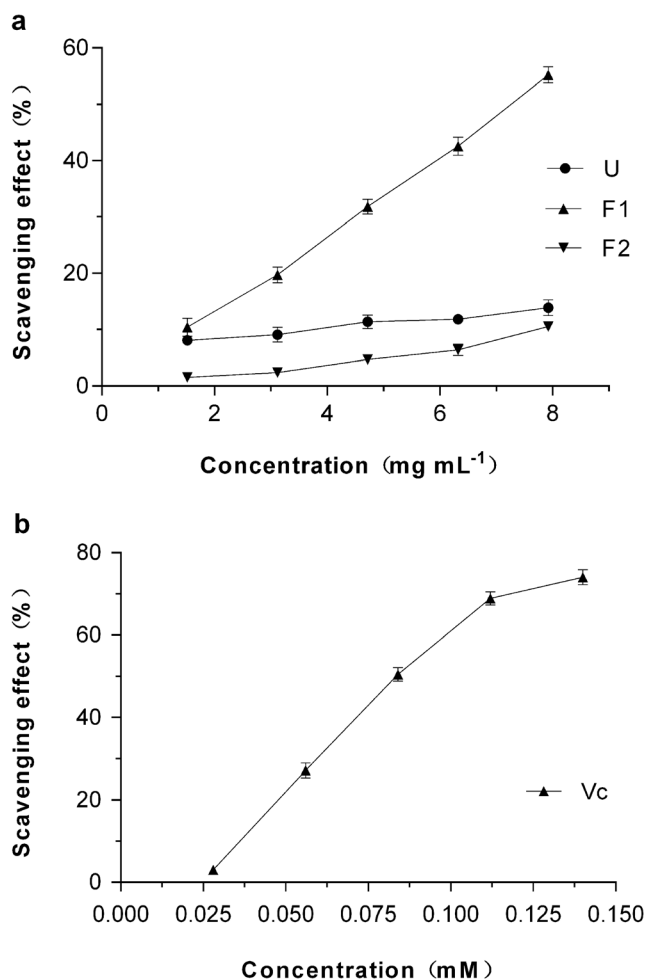

Fig. 5 **a** DPPH radical-scavenging effect of U, F1, and F2. Values are means ± SD ($n = 3$). **b** DPPH radical-scavenging effect of ascorbic acid. Values are means ± SD ($n = 3$)

Table 3   Effects of U, F1, F2, and cholestyramine on serum lipid profiles in mice supplemented with a cholesterol-rich diet

Group	Dose (mg kg^{-1})	TC (mmol L^{-1})	TG (mmol L^{-1})	LDL-C (mmol L^{-1})	HDL-C (mmol L^{-1})
Normal control	–	2.26 ± 0.41	1.25 ± 0.29	0.96 ± 0.19	1.19 ± 0.12
Hyperlipidemia	–	3.30 ± 0.52[#]	1.94 ± 0.16[##]	1.61 ± 0.54[#]	0.80 ± 0.21[##]
U	250	2.77 ± 0.22	1.65 ± 0.26[*]	1.09 ± 0.32[*]	0.91 ± 0.13
F1 (high dose)	500	2.48 ± 0.38[*]	1.55 ± 0.39[*]	1.01 ± 0.30[*]	1.13 ± 0.25[*,Δ]
F1 (middle dose)	250	2.65 ± 0.36[*]	1.54 ± 0.50[*]	1.01 ± 0.19[**]	1.12 ± 0.25[*]
F1 (low dose)	125	2.73 ± 0.54	1.56 ± 0.33[*]	1.14 ± 0.26	0.90 ± 0.12
F2	250	2.24 ± 0.42[**, Δ]	1.47 ± 0.17[**]	1.06 ± 0.19[*]	1.07 ± 0.12[*,Δ]
Positive control	500	2.38 ± 0.54[*]	1.29 ± 0.21[**,Δ]	0.91 ± 0.16[**]	1.08 ± 0.24[*]

The results are presented as mean ± SD, $N = 10$ (10 mice in each group)

[#] $P < 0.05$: compared with the normal control group

[##] $P < 0.01$: compared with the normal control group

[*] $P < 0.05$: compared with the hyperlipidemia control group

[**] $P < 0.01$: compared with the hyperlipidemia control group

[Δ] $P < 0.05$: Compared with ulvan group

The F2-treated group exhibited optimal effects on TC and TG ($P < 0.01$) and significant effects on HDL-C and LDL-C ($P < 0.05$). Compared with the U-treated group, the F1-treated group (500 mg kg^{-1}) significantly increased (24.2%; $P < 0.05$) HDL-C. And the F2-treated group (250 mg kg^{-1}) significantly decreased (19.1%; $P < 0.05$) TC and increased (17.6%; $P < 0.05$) HDL-C.

Discussion

Superoxide anion, generated from molecular oxygen by receiving one electron, was designated an original free radical. The mitochondrial electron transport systems form hydroxyl radicals, singlet oxygens, and hydrogen peroxide, all of which damage biomolecules, such as proteins, DNA, and enzymes (Blokhina et al. 2003). The superoxide radicals are formed from dissolved oxygen in the PMS/NADH system and evaluated by the reduction of NBT in the PMS/NADH model (Luo and Fang 2008). The scavenging effect of U, F1, and F2 on the superoxide radical is shown in Fig. 3a. The scavenging activity of all polysaccharides was significant ($P < 0.05$), and the magnitude of scavenging activity was dependent on concentration. Therefore, U, F1, and F2 were efficient scavengers for superoxide radicals, and they might be protective against the damage induced by superoxide anion radicals.

In the reducing power assay the color of the reaction solutions changed from yellow into several shades of blue, which can be attributed to the reducing capacity of antioxidants. Color is a strong indicator that compounds may exhibit potential antioxidant effects. Where reductants exist the Fe^{3+}/ferricyanide is transformed into the ferrous form. As a consequence, the Perl's Prussian blue can be quantified at 700 nm,

which reflected Fe^{2+} and the reduction of Fe^{3+} (Chung et al. 2002). The reducing capacity of F1 and U was significant ($P < 0.05$) and changed in a concentration-dependent manner (Fig. 4), which was in accordance with a previous report (Qi et al. 2005a, b). In addition, the reducing capacity of F2 was more substantial than that of U. The reducing capacities of the three samples types were in the order F1 > U > F2, and all samples were weaker than ascorbic acid. Because antioxidant effects are correlated with reducing capacity, as seen in the results above, F1 may donate electrons to free radicals to convert them into more stable and non-toxin diamagnetic molecules and thus contribute to antioxidant activity (Zha et al. 2009; Kozarski et al. 2012).

The DPPH free radical is a stable free radical that is widely used as a tool to confirm the free radical-scavenging activities of antioxidants. The method of scavenging DPPH is in accordance with a reduction of DPPH$^-$ ethanol solution in the existence of a hydrogen donating antioxidant, which leads to the formation of the non-radical form DPPH-H. In this study, it is likely that U, F1, and F2 were effective electron donors to free radicals, resulting in a more stable product and inhibiting the circulation period of lipid oxidation (Naik et al. 2003; Li et al. 2008). We clearly observed purple change to pale yellow, which can be attributed to antioxidants in the solution. The scavenging activity of U, F1, and F2 on the DPPH radical are shown in Fig. 5a. Compared with U and F2, F1 displayed more scavenging activity on DPPH radical. This result suggested that F1 could donate more hydrogen atoms than U and F2, on the other hand, F1 had scavenging activity on the DPPH radical as the concentration increases.

The structural features of ulvans that are important in their antioxidant activity including molecular weight, monosaccharide composition, sulfation level, and the position of sulfate

groups on the sugar backbone (Jiao et al. 2011)Structure modification of sulfated polysaccharides, such as desulfation, oversulfation, acetylation, and benzoylation, would allow the development of new and possibly more effective derivatives of naturally occurring polysaccharides (Qi et al. 2005a, b, 2006; Jiao et al. 2011). In our antioxidant assay, U, F1, and F2 all had a stronger antioxidant activity in superoxide radical-scavenging activity than ascorbic acid. Besides, F1 exhibited more effective antioxidant activities in reducing power and DPPH radical-scavenging activity than that of U and F2. What is more, considering the data of FT-IR analysis and ^{13}C NMR analysis, we can deduce that F1 donated more electrons or hydrogen atoms than U and F2, leading to a more effective antioxidant activity. These results suggested us some factors, such as degree of branching and conformation of the main chains, could play a more important role in antioxidant activities than molecular weight or sulfate content (Ale et al. 2011).

Hyperlipidemia is a risk factor for atherosclerosis. Substantial increases of TC, TG, and LDL-C levels in serum are known to be indicators of developing of atherosclerosis, whereas HDL-C is a protector against the atherosclerosis (Qi et al. 2012). High TC, TG, and LDL-C levels also increase blood viscosity. Regulating the level of LDL-C, a main carrier of TC, can be an effective way to avoid its migration to the endarterium. The progression of atherosclerosis may be slowed or even reversed by increasing serum HDL-C and reducing serum TG, LDL-C, and phospholipids (Brown 1994). In the antihyperlipidemic assay, the F1 and F2 treatments all showed significant effects, yet F2 exhibited a little bit more effective than F1 by comparing group 5 and group 7. Many studies (e.g., Akbary and Aminikhoei 2018; del Rocío Quezada-Rodríguez and Fajer-Ávila 2017; Cian et al. 2018) have reported that the sulfate group of seaweeds plays an important role in their bioactivities. Chale-Dzul et al. (2017) reported sulfated polysaccharides could protect HepG2 cells against oxidative stress. Berri et al. (2016) studied that sulfated polysaccharides exhibited an antimicrobial activity and stimulated cytokine expression by intestinal epithelial cells. Zhang et al. (2003) showed that the higher the sulfation level of polysaccharides, the stronger the antioxidant activities. The higher content of sulfate in F2 may allow it to associate with the surfaces of animal cells and be more involved in biological activities, such as cell adhesion, cell recognition, or regulation of receptor functions, all of which are of interest in medicine (Ellouali et al. 1993). On the other hand, F2 had a much lower average molecular weight than F1. Qi et al. (2005a, b) reported the lower molecular weight ulvans exhibited stronger antioxidant activities than the others. F2 may have been better able to penetrate the surfaces of animal cells to regulate blood levels in hyperlipidemic mice. Biological activity is the synergistic result of several factors rather than the effect of a single factor (Zhang et al. 2013; Elizondo-Reyna et al. 2016). It is important to clarify the structure–activity relationship of polysaccharides, and all synergistic factors should be taken into consideration comprehensively. Yet, the integrated mechanisms still need further study.

Conclusion

In this study, F1 exhibited strong antioxidant activities and effective antihyperlipidemic activity in a concentration-dependent manner. F2 had signification antihyperlipidemic activity in vivo.

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