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Bioactivity of β-1,3-xylan Extracted from *Caulerpa lentillifera* by Using *Escherichia coli* ClearColi BL21(DE3)-β-1,3-xylanase XYLII

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Abstract Oligosaccharides extracted from algae exhibit many bioactivities and are used as food additives and dietary supplements. In this study, β-1,3-xylan was extracted from the green algae *Caulerpa lentillifera*; this compound was hydrolyzed by β-1,3-xylanase XYLII to produce mixed < 3 kDa β-1,3-xylooligosaccharide (XOS_{mix}), which was mainly composed of β-1,3-xylose, β-1,3-xylobiose, and β-1,3-xylotriose. The antioxidant and anticoagulant activities of XOS_{mix} were then examined. Results revealed that the 2,2-diphenyl-1-pikryl-hydrazyl scavenging activity, reducing power, and total antioxidant status of 20 mg/mL XOS_{mix} was equivalent to those of 8.7, 115.1, and 157.3 μg/mL trolox, respectively; whereas the ferrous ion chelating activity of 20 mg/mL XOS_{mix} was equivalent to that of 64.3 μg/mL EDTA. Regarding the anticoagulant activity, XOS_{mix} delayed the activated partial thromboplastin time. These results suggest that XOS_{mix} exhibits potential for application in the food industry.

Keywords: *pseudomonas vesicularis MA103, β-1,3-xylanase, β-1,3-xylooligosaccharide, bioactivity*

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1. Introduction

Poly- and oligosaccharides obtained from marine algae exhibit many bioactivities, such as anticlotting, antioxidation, antiviral, antiinflammatory, and anticancer activities [1,2,3,4]; these activities are affected by the molecular weight (MW) and bonding [5]. Algal polysaccharides are easily extractable bioingredient, and they abundantly vary in molecular chemistry. In recent years, oligosaccharides degraded from algal polysaccharides have been applied in chronic disease therapy [4]; however, comprehensive studies on such algal polysaccharides are required. The bioactivity of algal oligosaccharides also must be investigated.

Xylooligosaccharide can generally be obtained from acidic and enzymatic hydrolysis; both methods degrade xylan to xylooligosaccharides or xylose, thus increasing the availability and economic value [6,7,8]. Acidic hydrolysis is conducted under high temperature and pressure, and the cost of product recovery and instruments is exorbitant. Moreover, this method produces byproducts during processing, thus reducing the hydrolysis product of xylan [9,10]. The enzymatic hydrolysis method provides high specificity, requires a mild processing condition, and yields easily recoverable products. This method is applied for producing xylooligosaccharides [10] and employed in the food and cosmetics industries, medical biotechnology, agriculture, environmental protection, and sewage treatment.

β-1,3-xylan is a component of D-xylose cell wall polysaccharides composed of β-1,3 bonds [11], and it is mainly observed in macroalgae, such as *Caulerpa*, *Bryopsis*, *Bangia*, *Porphyra*, and *Palmaria* spp. [12,13]. Some reports have revealed antiinflammatory, antiviral, and anticancer activities of β-1,3-xylan [13,14,15]; however, few studies have addressed the bioactivity of β-1,3-xylooligosaccharide generated from β-1,3-xylan through enzymatic hydrolysis. In a previous study, the β-1,3-xylanase-producing marine bacteria *Pseudomonas vesicularis* MA103 was isolated, and the β-1,3-xylanase-producing gene was transferred to *Escherichia coli* ClearColi BL21(DE3), which hydrolyzed β-1,3-xylan on insertion (data not shown). This study aimed to evaluate the availability on β-1,3-xylooligosaccharide; therefore, the hydrolysis products of β-1,3-xylooligosaccharide were collected, and their antioxidant and anticlotting activities were evaluated.

2. Materials and Methods

Materials

Activated partial thromboplastin time-soluble activator (APTT–SA) reagent kit was purchased from Helena Laboratories (Beaumont, TX, USA). Arabinose, 2,2-diphenyl-

1-pikryl-hydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), trolox, galactose, glucose, heparin, mannose, rhamnose, xylose (X_1), and other chemicals were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). *C. lentillifera* was kindly provided by East Green BIO Corporation (Hualian, Taiwan). Furthermore, pure β-1,4-xylobiose (X_2), β-1,4-xylotriose (X_3), β-1,4-xylotetraose (X_4), β-1,4-xylopentaose (X_5), and β-1,4-xylohexaose (X_6) were purchased from Qingdao BZ Oligo Biotech Co., Ltd (Qingdao, China). Rabbit coagulase plasma and all media for bacterial cultivation were purchased from Becton, Dickinson and Company (Sparks, MA, USA).

Chemical analyses

The sulfate content was determined using the barium chloride–gelatin method with Na_2SO_4 as standard [16]. The total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as standard [17]. The protein concentration was measured using the Lowry method with bovine serum albumin as the standards [18]. Furthermore, the recombinant β-1,3-xylanase XYLII activity was measured by determining the amount of reducing sugars released form β-1,3-xylan through the dinitrosalicylic acid method [19] with X_1 as standards. The enzyme activity was assayed at 35°C for 10 min by using 0.45% β-1,3-xylan as substrate in 20 mM phosphate buffer (pH 7.5). One unit (U) of β-1,3-xylanase XYLII activity was defined as the amount of enzyme required to release 1 μmol of reducing sugars from β-1,3-xylan in 1 min.

Preparation of mixed β-1,3-xylooligosaccharide

β-1,3-xylan was extracted from *C. lentillifera* according to the method published by Iriki *et al*. [11]. β-1,3-xylanase XYLII was extracted from *P. vesicularis* MA103 and was transferred to *E. coli* ClearColi BL21(DE3) pET-39b(+)-*xyl*II prepared in our laboratory, which was induced by 0.0125 mM isopropyl-β-d-thiogalactopyranoside at 18°C for 24 hr. After induction, the solution was centrifuged at 6000 ×*g* for 30 min, and the pellet was collected, ultrasonicated (200 on–off cycles of 10 s each) on ice by using a Qsonica Q125 sonicator (Newtown, CT, USA), and centrifuged at 12,000 ×*g* for 30 min. Moreover, the supernatant (mainly contained β-1,3-xylanase XYLII) was filtered through a 30 kDa filter (MWCO 30 kDa, Millipore, NH, USA) and washed with a phosphate buffer (20 mM, pH 7.5) three times. The residues, which had a mass higher than 30 kDa, were collected, identified as β-1,3-xylanase XYLII (activity: 10.9 U/mL, MW = 91 kDa), and stored at –20°C until for further use.

The < 3 kDa mixed β-1,3-xylooligosaccharide (XOS_{mix}) sample was prepared using the following steps. A 450 mL solution of 20 mM phosphate buffer (pH 7.5) containing 0.5% β-1,3-xylan was hydrolyzed using 50 mL of β-1,3-xylanase XYLII (10.9 U/mL) at 35°C for 72 hr. The solution was then filtered through a 3 kDa filter (MWCO 3 kDa, Millipore, NH, USA); the filtrate was considered XOS_{mix}, which was stored at –20°C for further use. Figure 1 shows the flow diagram corresponding to this preparation of XOS_{mix}.

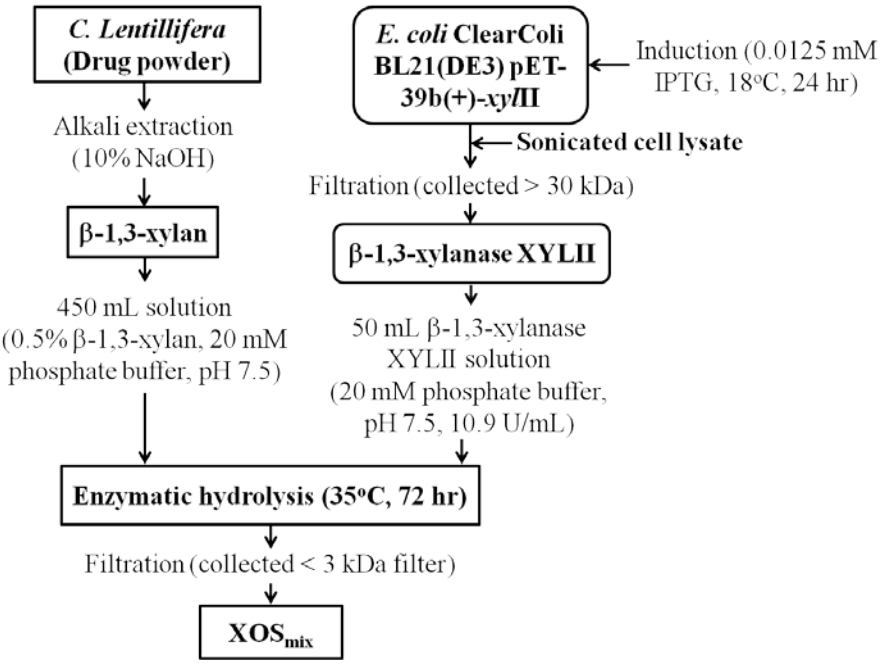

Figure 1. Schematic diagram of the preparation of XOS_{mix}

Monosaccharide composition and degrees of polymerization assay

The monosaccharide composition was examined using a method described by Konishi *et al*. [20]; 25 mg of sample was mixed with 2 mL 2 M trifluoroacetic acid (TFA) and hydrolyzed at 121°C for 3 hr under vacuum. The hydrolyzed solution was vacuum dried and neutralized using double-distilled water (ddH_2O) for eliminating TFA. The neutralized monosaccharide was diluted to 5 mg/mL, and the monosaccharide composition was analyzed through high performance liquid chromatography (HPLC). The HPLC system comprised a pump PU-2080 (Jasco, Tokyo, Japan), a Carbo Sep CHO-682 Pb column (7.8 × 300 mm, 7 μm; Transgenomic, Inc., Omaha, NE), and an ERC-7515 A RI detector (ERC Inc., Saitama, Japan). The mobile phase was ddH_2O with a constant flow rate of 0.4 mL/min at 80°C. Six monosaccharides (glucose, X_1, rhamnose, galactose, arabinose, and mannose) were used as the standards.

The degree of polymerization (DP) was analyzed. XOS_{mix} was hydrolyzed using the aforementioned steps, and the products were analyzed though HPLC on the same column. The mobile phase was ddH_2O with a constant flow rate of 0.4 mL/min at 90°C, and X_1–X_6 were used as the standards.

Fourier transform infrared and electrospray ionization mass spectrometry

The chemical groups of all compounds were analyzed using a Fourier transform infrared spectrometer (FTIR; FTS 155 Win-ir, Bio-Rad, CA, USA). Infrared spectra of potassium bromide (KBr) and sample mixtures were obtained over the frequency range of 400 to 4,000 cm^{-1} at a resolution of 8 cm^{-1}. The sample was thoroughly mixed with KBr (100:1, v:v), dried, ground, and pressed to obtain a sample disk [21].

The XOS_{mix} fraction was analyzed through electrospray ionization mass spectrometry (ESI-MS) using ESI-Orbitrap MS (Exactive, Thermo Scientific, Bremen, Germany) at the Instrumentation Center of National Taiwan University (Taipei, Taiwan).

Antioxidation methods

Total antioxidant status assay

The total antioxidant status (TAS) of each extract was tested using TAS kit (Randox Labs, Crumlin, UK) according to the manufacturer's protocol. TAS of varying concentrations of XOS_{mix} (1, 3, 5, 10, and 20 mg/mL) were expressed as μg/mL of trolox equivalent.

α,α-diphenyl-β-picrythydrazyl assay

The free radical scavenging activity of each extract was tested using $DPPH^{\bullet}$ as described by Shimada *et al*. [22]. Furthermore, 200 μL of varying concentrations of XOS_{mix} (1, 3, 5, 10, and 20 mg/mL) was mixed with 200 μL of 0.1 mM DPPH, reacted at room temperature for 30 minute, and then measured at 517 nm. Trolox was used as the standard. The percentage of DPPH scavenging activity (%) was calculated as follows:

DPPH scavenging activity (%) = $[(A_0 - A_1)/(A_0 - A_2)] \times 100\%$, where A_0 and A_1 are the absorbance of the control and sample, respectively, and A_2 is the 100% DPPH scavenging absorbance.

The trolox equivalent of XOS_{mix} was calculated using the following equation:

Trolox equivalent (μg/mL) = $(A_1 - 0.003)/-0.00217$, where $R^2 = 0.9942$.

Chelating effects on ferrous ions

The ferrous ion (Fe^{2+}) chelating activity for each extract was calculated a modification of a method published by Dinis *et al*. [23]. Furthermore, 250 μL of varying concentrations of XOS_{mix} (1, 3, 5, 10, and 20 mg/mL) was mixed with 925 μL of methanol and 25μL of $FeCl_2 \cdot 4H_2O$ (2 mM) for 30 s. After the reaction, 50 μL of ferrozine (5 mM) was added and reacted for 10 min; the absorbance was tested at 562 nm. EDTA was used as the standard, and the percentage of the chelating effect (%) was calculated as follows.

Chelating effect (%) = $[(A_0 - A_1)/(A_0 - A_2)] \times 100\%$, where A_0 and A_1 are the absorbance of the control and sample, respectively, and A_2 is the 100% Fe^{2+} chelating absorbance.

The EDTA equivalent of XOS_{mix} was calculated using the following equation:

EDTA equivalent (μg/mL) = $(A_1 - 0.0059)/-0.0085$, where $R^2 = 0.9991$.

Reducing power

The reducing power of each extract was determined according to the method described by Wang *et al*. [24]. Moreover, 250 μL of varying concentrations of XOS_{mix} (1, 3, 5, 10, and 20 mg/mL) was mixed with 250 μL of 0.2 M phosphate buffer (pH 6.6) and 250 μL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and reacted at 50°C for 20 min. After the reaction, the solution was cooled to room temperature, and 1 mL of 10% trichloroacetic acid and 100 L of 0.1% $FeCl_3 \cdot 6H_2O$ were added. After reaction for 10 min in the dark, the absorbance of the test sample was measured at 700 nm. Trolox was used as the standard to evaluate the equivalent of each extract, which was calculated using the following equation:

Trolox equivalent (μg/mL) = $(OD_{700nm} + 0.0014)/0.0067$, where $R^2 = 0.998$.

Anticoagulant activity assay

The anticoagulant activity was calculated as described by Matsubara *et al*. [25]. Rabbit plasma (90 μL) was mixed with varying concentrations of XOS_{mix} (1, 3, 5, 10, and 20 mg/mL) and was reacted at 37°C for 1 min. The solution was then mixed with 100 μL of APTT (preheated at 37°C for 10 min) for 5 min; 100 μL of $CaCl_2$ (0.025 M) was added, and the absorbance was tested at 660 nm in different clotting time assays. Heparin and ddH_2O were used as positive and negative control, respectively. The heparin equivalent of XOS_{mix} was calculated using the following equation:

Heparin equivalent (μg/mL) = [clotting time (s) − 14.516)/3.3871], where $R^2 = 0.9876$.

Statistical analyses

Data were presented as the mean ± standard deviation. The differences between the mean values were analyzed using the one-way analysis of variance followed by the Duncan test at $p = 0.05$. Statistical analysis was performed using the SPSS 12.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

XOS_{mix} characterization

β-1,3-xylan is mainly extracted using alkali treatments [11,12]. In this study, β-1,3-xylan was extracted from *C. lentillifera* by using alkali, and the chemical composition of XOS_{mix} was analyzed. Table 1 presents the obtained yield of β-1,3-xylan (24.93%) and XOS_{mix} (46.07%). The sulfate content of β-1,3-xylan and XOS_{mix} were 0.69% and 0.74%, respectively. Jiao *et al*. [1] indicated that algal polysaccharide generally contain a sulfate group. In accordance, in our study, β-1,3-xylan extracted from *C. lentillifera* contained a sulfate group. After enzymatic hydrolysis, the final product XOS_{mix} retained the sulfate group, and the total phenolic content was not detected in this study. The protein content of XOS_{mix} was 2.43%; however, phenol was not detected in β-1,3-xylan, possibly because of protein degradation during enzymatic hydrolysis.

The monosaccharide composition indicated that β-1,3-xylan and XOS_{mix} were mainly composed of glucose and X_1. The glucose content of β-1,3-xylan and XOS_{mix} were 2.58% and 3.45%, respectively, whereas the X_1 content of β-1,3-xylan and XOS_{mix} were 97.42% and 96.55%,

respectively (Table 1). Previous studies have revealed that the polysaccharide extracted from *Caulerpa* spp. not only contained xylose but also glucose and galactose [14,20,26]. These results suggest that the chemical composition of algal polysaccharides varies depending on the species, region, and season as well as environmental factors [27]. Extracts obtained from the same algal species by using the same procedures exhibited difference chemical compositions depending on the region [20].

Structural properties of XOS_{mix}

Figure 2 shows the DP of XOS_{mix} determined using HPLC; three major peaks were observed in the spectrum. In contrast to the components of β-1,4-xylooligosaccharide (X_1−X_6), the main oligomer products in XOS_{mix} were identified as xylose, xylobiose, and xylotriose, with some xylotetraose. Yamaura *et al*. [28] analyzed β-1,3-xylan hydrolyzed by the marine bacteria *Pseudomonas* sp. PT-5, which produced β-1,3-xylanase, and indicated that xylose and xylobiose were produced after 6 hr of hydrolysis.

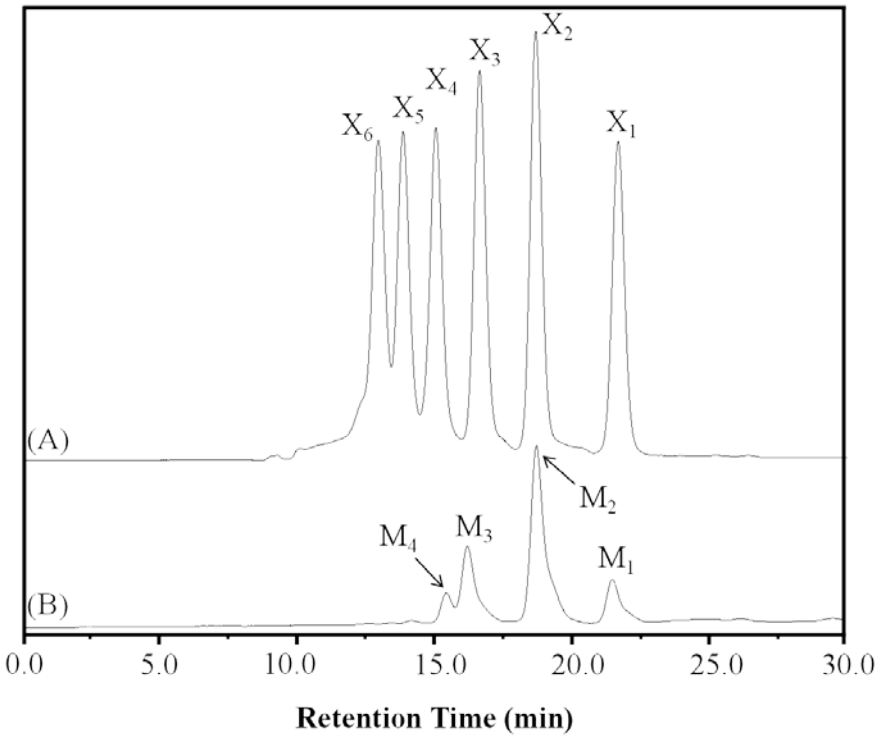

Figure 2. Analysis of mixed < 3 kDa β-1,3-xylooligosaccharide through high-performance liquid chromatography.

(A) The standards (X) were xylose (X_1), β-1,4-xylobiose (X_2), β-1,4-xylotriose (X_3), β-1,4-xylotetraose (X_4), β-1,4-xylopentaose (X_5), and β-1,4-xylohexaose (X_6). (B) The mixed < 3 kDa β-1,3-xylooligosaccharide products were xylose (M_1), xylobiose (M_2), xylotriose (M_3), and xylotetraose (M_4)

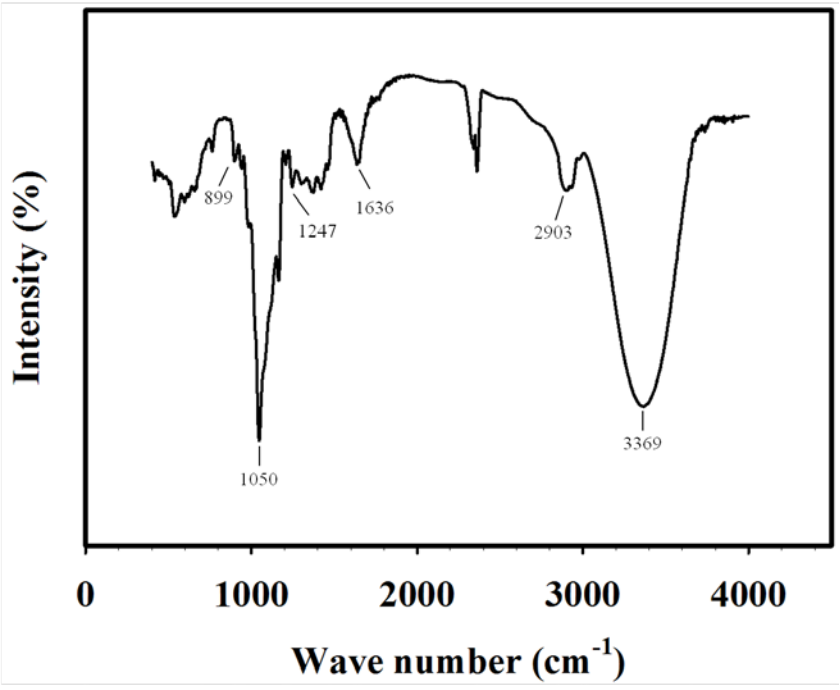

Figure 3. Fourier transform infrared spectroscopy of mixed < 3 kDa β-1,3-xylooligosaccharide

β-1,3-Xylan hydrolyzed by β-1,3-xylanase purified from *Vibrio* sp. XY-214 yielded xylose, xylobiose, and xylotriose [29]. In this study, β-1,3-xylanase XYLII extracted from *P. vesicularis* MA103-transformed *E. coli* ClearColi BL21(DE3) hydrolyzed β-1,3-xylan and produced β-1,3-xylooligosaccharid. XOS_{mix} was then analyzed through FTIR, and the spectrum is shown in Figure 3. The major peaks observed in the spectrum were located in 899, 1050, 1247, 1636, 2903, and 3369 cm^{-1}. Furthermore, the absorbance of 3600−3000 cm^{-1} represented the O–H group, whereas the absorbance of approximately 1166−1000 cm^{-1} represented the C–O, C–C or C–OH group in hemicelluloses [30]. Samanta *et al.* [31] used FTIR to analyze alkali-extracted xylan from corncob and reported a spectrum similar to that observed in the present study, suggesting that XOS_{mix} contains the xylan group. The FTIR spectrum of XOS_{mix} also revealed peaks at 2903 cm^{-1} and 1636 cm^{-1}, indicating a C–H group, and the peak at 899 cm^{-1} represented the β-glycosidic bonds between molecules. These results are in accordance with those previous studies [32, 33]. Jayapal *et al.* [30] and Ayoub *et al.* [32] indicated that a peak at 1642 cm^{-1} represents water molecules in the xylan structure. Gómez-Ordóñez and Rupérez [34] indicated that apeak at 1220–1260 cm^{-1} represents the S=O group. In this study, XOS_{mix} exhibited similar features in the FTIR spectrum.

XOS_{mix} was purified through HPLC, and the X_1–X_3 fractions were collected. These fractions were analyzed in the positive ion mode of ESI-MS, and the DP and MW of each fraction were detected (Figure 4). In the ESI-MS spectrum, the *m/z* ratios of X_3 (Figure 4A), X_2 (Figure 4B), and X_1 (Figure 4C) were 173.0, 305.1, and 437.1, respectively. These results are in concordance with those of previous studies [26,35], indicating that XOS_{mix} contains β-1,3-xylose, β-1,3-xylobiose, and β-1,3-xylotriose.

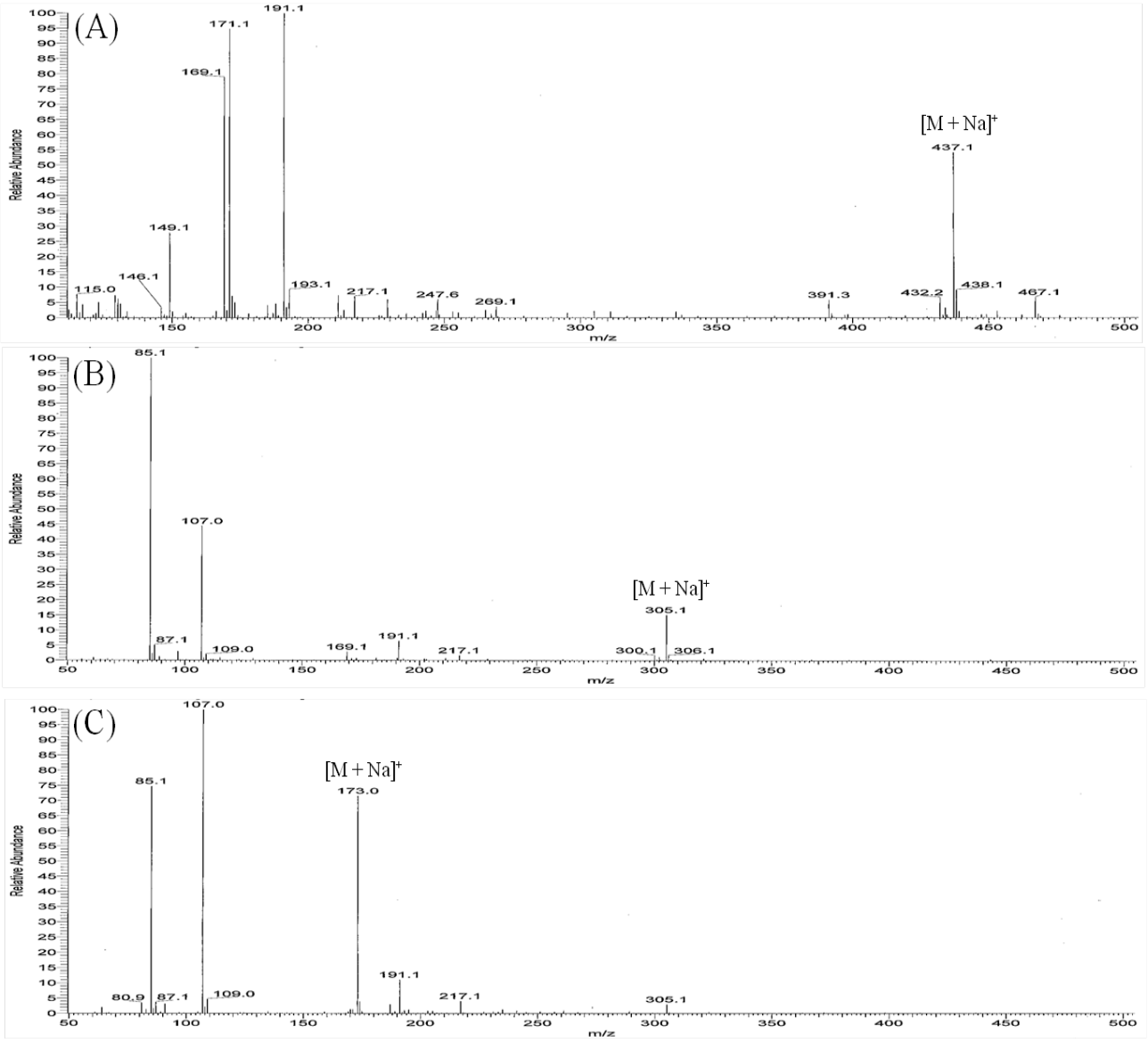

Figure 4. Electrospray ionization mass spectrometry spectrum of mixed < 3 kDa β-1,3-xylooligosaccharide

The sample was prepared through high-performance liquid chromatography: (A) xylotriose (M_3), (B) xylobiose (M_2), and (C) xylose (M_1).

Antioxidant assay

The total antioxidant status assay is used for testing the ABTS radical cation ($ABTS^{\bullet+}$) scavenging activities. As can be seen in Figure 5A, the total antioxidant status was 0, 114.4, 166.9, 146.4, and 157.3 μg/mL for 1, 3, 5, 10, and 20 mg/mL sample concentrations, respectively. The 5 mg/mL sample concentrations exhibited the highest TAS

activity. The ABTS scavenging activities of 20 mg/mL XOS_{mix} was equivalent to that of 157.3 μg/mL trolox.

The DPPH scavenging assay is used for testing the antioxidant and scavenging activities of peroxy radicals [22]. Figure 5B presents the healthy benefit potential of XOS_{mix} in a concentration-dependen manner. The scavenging activity was 1.4%, 56.3%, 76.7%, 79.7%, and 79.5% for 1, 3, 5, 10, and 20 mg/mL sample concentrations, respectively. These results were equivalent to those of 8.7 μg/mL trolox when the sample concentration was 20 mg/mL.

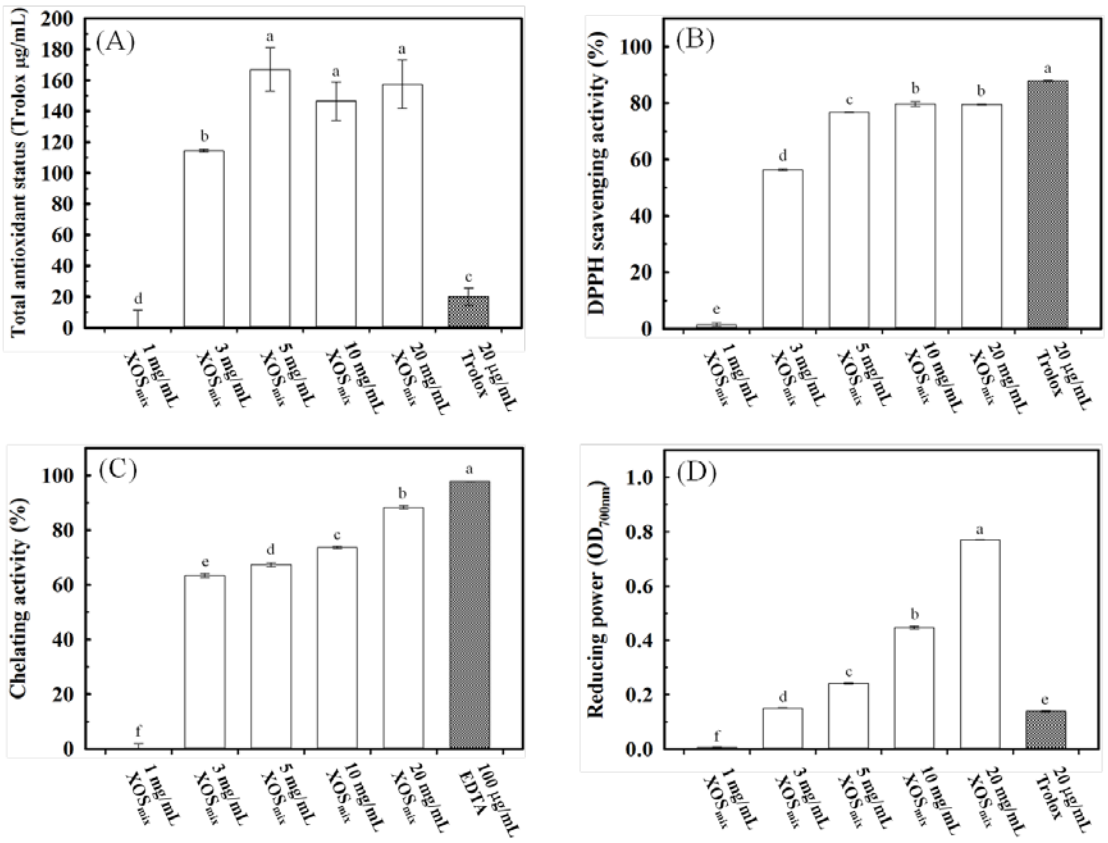

Figure 5. Antioxidant activity of varying concentrations of mixed < 3 kDa β-1,3-xylooligosaccharide.

Panel A: Total antioxidant status of varying concentrations of mixed < 3 kDa β-1,3-xylooligosaccharide (XOS_{mix}). Panel B: The 2,2-diphenyl-1-pikryl-hydrazyl scavenging activity of varying concentrations of XOS_{mix}. Panel C: Chelating effects of varying concentrations of XOS_{mix} on ferrous ions. Panel D: Reducing power of varying concentrations of XOS_{mix}. Each value is the mean ± standard deviation (n = 3). Different superscript letters indicate significantly different values ($p < 0.05$).

Many metal ions accelerate lipid oxidation and act as pro-oxidant. Thus, the chelating activity affects the antioxidation activity of XOS_{mix}. Figure 5C shows the Fe^{2+} chelating activity of XOS_{mix}, which increased with increasing sample concentration and was 63.5%, 67.4%, 73.6%, and 88.3% for 3, 5, 10, and 20 mg/mL sample concentrations, respectively. Chelating activity was not detected when the sample concentration was 1 mg/mL; however, optimal activity was observed when the sample concentration was 20 mg/mL and was equivalent to that of 64.3 μg/mL EDTA.

Figure 5D presents the reducing power results of XOS_{mix}. In this experiment, the sample exhibited antioxidant activity and reduced $K_3Fe(CN)_6$ to potassium hexacyanoferrate [$K_4Fe(CN)_6$]; $K_4Fe(CN)_6$ interacted with ferric ions to generate Prussian blue, which showed strong absorbance at 700 nm [36]. Furthermore, XOS_{mix} revealed exhibited activity at all concentrations; absorbance was observed at 0.01, 0.15, 0.24, 0.45, and 0.77 nm for 1, 3, 5, 10, and 20 mg/mL sample concentrations, respectively. The reducing power of 20 mg/mL XOS_{mix} was equivalent to that of 115.1 μg/mL trolox.

In previous study, oligosaccharides contained phenol compounds, which represent the DPPH scavenging activity [37]. In this study, soluble polyphenol was not detected in XOS_{mix}; however, effective DPPH scavenging activity was still observed. O'Sullivan *et al*. [38] used methanol for extracting five types of brown algae and examined their DPPH scavenging activity; their results indicated no positive correlation between DPPH scavenging activity and polyphenol concentrations. Previous studies have indicated that -OH, -COOH and some spatial structure in carbohydrates enhance the antioxidant activity [39, 40]; thus, algal polyphenol is not the only substance representing the DPPH scavenging activity. Some researchers exacted polysaccharides from *Enteromorpha prolifera* and indicated that the Fe^{2+} chelating and reducing activity were revesed MWs [41]. As compared with high-MW oligosaccharide, low-MW oligosaccharides have superior activity in chelating transition ion metals, such as cuprous ions or Fe^{2+}, in spatial structures [37]. In addition, Wang *et al*. [42] indicated that low-MW sulfated polysaccharides more efficiently enter cells and contribute H^+ ions.

Anticoagulant activity

Previous studies have indicated that the anticoagulant activity of algal extract is attributable to sulfite ion-containing polysaccharides. In addition to a negative charge, the anticoagulant activity is related to structural specificities, such as the sulfate group position, the

monosaccharide from, and glycosidic bonding [43,44]. Table 1 and Figure 3 illustrate that XOS_{mix} contained sulfate groups; therefore, we further tested the activated partial thromboplastin time by using rabbit plasma against varying XOS_{mix} concentrations.

Table 1. Chemical composition analysis of β-1,3-xylan and mixed < 3 kDa β-1,3-xylooligosaccharide

Sample	Yield (%, w/w)	Sulfate (%)	Total phenol (%)	Total Protein (%)	Monosaccharide composition (%)					
					Glucose	Xylose	Rhamnose	Galactose	Arabinose	Mannose
β-1,3-xylan	24.93% ± 4.91	0.69 ± 0.05	n.d.[1]	n.d.	2.58	97.42	n.d.	n.d.	n.d.	n.d.
XOS_{mix}	46.07% ± 2.96	0.74 ± 0.08	n.d.	2.34 ± 0.29	3.45	96.55	n.d.	n.d.	n.d.	n.d.

[1] n.d., not detected.
Each value is the mean ± standard deviation (n = 3).

Table 2 shows prolonged activated partial thromboplastin times of 27, 30, 26, and 25 s for 3, 5, 10, and 20 mg/mL sample concentrations, respectively. The heparin equivalents were 3.8, 4.2, 3.4, and 3.2 μg/mL for 3, 5, 10, and 20 mg/mL XOS_{mix} concentrations, respectively. By contrast, varying the XOS_{mix} concentration did not significantly affect the prothrombin and thrombin times (data not shown). In general, many factors affect the coagulating mechanism. The sulfate group was a factor, and the underlying mechanism was similar to that of heparin, which stimulated the antithrombin activity to inhibit the inner coagulating factors IXa, XIa, and XIIa, thus delaying thrombosis [45].

Table 2. Anticoagulant activity of varying concentrations of mixed < 3 kDa β-1,3-xylooligosaccharide

Sample	Concentration	APTT (s)
XOS_{mix}	3 mg/mL	27 ± 1.8 ^{bc}
	5 mg/mL	30 ± 0.6 ^{b}
	10 mg/mL	26 ± 1.0 ^{c}
	20 mg/mL	25 ± 1.3 ^{c}
Heparin[1]	2 μg/mL	20 ± 1.7 ^{d}
	20 μg/mL	146 ± 0.8 ^{a}
ddH_2O[2]		16 ± 1.7 ^{e}

[1] Heparin (180 units/mg) is used as the positive control.
[2] ddH_2O was the control.
Each value is the mean ± standard deviation (n = 3).
Different superscript letters in the same column (vertical comparison) indicate significantly different values ($p < 0.05$).

4. Conclusion

In this study, β-1,3-xylan was extracted from *C. lentillifera* through alkali extraction; the compound was then hydrolyzed by *E. coli* ClearColi BL21(DE3)-β-1,3-xylanase XYLII to obtain low-MW XOS_{mix}. XOS_{mix} was composed of β-1,3-xylose, β-1,3-xylobiose, and β-1,3-xylotriose and exhibited antioxidant and anticoagulant activities. This study provides an efficient method for producing XOS_{mix} with a low DP, and XOS_{mix} can be applied in producing dietary supplements and antioxidant and functional nutrient additives.

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