

## Research Article

# Structure Analysis and Antioxidant Activity of a Novel Polysaccharide from Katan Seeds

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In the present work, a novel water-soluble polysaccharide (LWSP) was purified from Katan seeds. Polysaccharide was structurally characterized by NMR spectroscopic analysis, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR) analysis, X-ray diffraction (XRD), and UV absorption. TLC and HPLC showed that LWSP was a polysaccharide consisted mainly of glucose, mannose, xylose, and arabinose. The FTIR spectrum and UV absorption proved polysaccharide characteristic of LWSP. According to XRD, LWSP presented a semicrystalline behavior. The molecular weight was estimated as 64.56 kDa. Results obtained through <sup>13</sup>C and <sup>1</sup>H nuclear magnetic resonance (NMR) indicated that LWSP is consisted of four monosaccharide residues with  $\alpha$  and  $\beta$  anomers. Physicochemical and antioxidant properties of LWSP were also investigated. Results revealed that LWSP exhibited interesting 1,1-diphenyl-2-picrylhydrazyl (DPPH) ( $IC_{50} = 4.48$  mg/ml) and chelating activity ( $IC_{50} = 4.79$  mg/ml), and it displayed moderate reductive capacities. Overall, the findings suggested that LWSP is a promising source of natural additives in various industries fields.

## 1. Introduction

Natural polymers as polysaccharides are generally obtained from marine organisms, fungal, bacterial, and vegetal sources. Plant polysaccharides may correspond to storage polymers or to molecules involved in the cell wall structure. Polysaccharides are a group of carbohydrates formed of monosaccharide units coupled by glycosidic linkage ( $\alpha$  or  $\beta$  configuration). They are formed of high molecular-weight polymers [1]. They have found various applications in several industries due to their multifunctional bioactivities and physicochemical characteristics. Antioxidant molecules are able to inhibit the action of free radicals causing. These molecules are inadequate to prevent radical-induced damages [2]. In fact, some synthetic antioxidants such as butylated hydroxyl toluene (BHT) or butylated hydroxyl anisole (BHA) used in

dietary supplements or in cosmetics have been suspected of being responsible for liver damage and carcinogenesis [2, 3]. The use of natural antioxidants extracted from plants in pharmaceutical and/or food application is more active than those obtained from chemical synthesis like BHT, BHA, or vitamin E.

Concerning pharmaceutical application, polysaccharides possess various biological activities including anticoagulant, anti-inflammatory, antitumor, antiviral, antipathogenic, and antioxidant activities and immune modulating [4]. These biological molecules presented many benefits for human health by reducing the risk of several diseases including heart disease, arthritis, and cancer [5]. Moreover, they are added in various food reformulations to obtain safety products with good quality [6]. In fact, they are used as natural preservatives in terms of antimicrobial and antioxidant

agents, also as foaming and emulsifying ingredients, and they are added into diet foods due to their dietary fibers, mimetic fats, and prebiotic effects [7]. Many researchers have investigated the potential uses of polysaccharides extracted from vegetable processing waste such as potato starch waste [8], onion (*Allium cepa*) solid waste [9], and garlic (*Allium sativum* L.) [10] and from various plants such as fenugreek [11], chickpea [12], sorgho [6], and watermelon rinds [13].

Katan seeds are a member of the *Linaceae* family. The plant is native corps to West Asia and the Mediterranean. Katan is the seed obtained from the flax plant which named *Linum usitatissimum* L. [14]. Katan seeds presented high nutritional value due to dietary fibers, protein, alpha-linolenic fatty acids, and micronutrients [15]. In fact, chemical analysis of Katan seeds averaged 30–40% fat, 20–28% total dietary fibers, 20–25% protein, 3–4% ash, and 4–8% moisture and contain also vitamins such as A, B, D, and E, minerals, and amino acids [14]. Recently, many researchers have highlighted the essential roles of fiber viscosity as a factor that determines the gastrointestinal handling, essentially carbohydrate digestibility and absorption rates, which as a result impacts the glycemic response [15].

In this study, LWSP was purified and characterized by TLC, HPLC, RMN, X-ray diffraction, and UV visible. The physicochemical properties, the molecular weight, and antioxidant activities were also studied.

## 2. Materials and Methods

**2.1. Material and Reagents.** Katan seeds used in this study were purchased from the local market at Sfax city in Tunisia. Seeds were crushed in a Moulinex blender LM 241. The obtained powder was stored in clean and hermetic glass until use.

**2.2. Extraction of Water-Soluble Polysaccharide (LWSP).** LWSP was extracted by the hot water technique as described by Liu et al. [16] with some modifications. Briefly, Katan seed powder was preextracted with 95% ethanol at room temperature to eliminate small molecules and impurities. The dry residue was extracted twice with 20 volumes of deionized water at 90°C while stirring for 4 h. The extract was combined and filtered, and filtrates were then evaporated under vacuum. The obtained liquid was precipitated with 95% (v/v) ethanol at 4°C for 24 h and then centrifuged (4500 × g) using a refrigerated centrifuge for 30 min (Hettich Zentrifugen, ROTINA 380R, Germany). Afterward, the precipitate was dried at 60°C for 3 h to obtain LWSP, and the polysaccharide yield (% w/w) was calculated.

**2.3. Physicochemical Characteristics.** Various physicochemical properties like moisture, ash, fat, color, carbohydrate, protein, and pH (1% solution at 25 ± 0.5°C) of the extracted LWSP were determined.

The carbohydrate content was determined by phenol-sulfuric acid colorimetric method [17]. A standard curve was obtained using glucose standard (Sigma Aldrich, USA) at 5, 25, 50, 100, and 150 µg/ml. The moisture, ash, and fat contents of LWSP were evaluated according to the AOAC

methods [18]. Crude fat was determined gravimetrically after Soxhlet extraction of dried samples with hexane.

The pH of the 1% aqueous solution of extracted LWSP was measured by using a digital pH meter (Systronics Instruments, India) by immersing completely the glass electrode into the solution.

The sample CieLab parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) were read using a Color Flex spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA) and reported as  $L^*$ ,  $a^*$ , and  $b^*$  values, in which  $L^*$  is a measure of lightness,  $a^*$  represents the chromatic scale from green to red, and  $b^*$  represents the chromatic scale from blue to yellow.

The average molecular weight was evaluated using a high-pressure gel filtration chromatograph equipped with a refractive index detector using Zorbax PSM 300 column (6.2 × 250) as previously described [19]. The average molecular weight (Mw) of the LWSP was determined by the comparison of its retention time with the calibration curve using different dextran with known molecular weights.

**2.4. Scanning Electron Microscopy.** The surface micromorphology of LWSP was observed using a scanning electron microscope system (JSM-5400, JEOL, Japan) with an accelerating voltage of 10.0 kV under 50x, 95x, and 250x magnifications. The double-sided adhesive coated aluminum SEM stub was used to fix the samples. After being frozen under liquid nitrogen, it was fractured, mounted, and sputtered with gold using a sputter coater (JFC-1100, JEOL, Japan) for conductivity. The LWSP samples were then photographed with an angle of 90° to the surface [20].

### 2.5. Monosaccharide Composition of LWSP

**2.5.1. Thin-Layer Chromatography (TLC).** LWSP (2 mg) was hydrolyzed in 250 µl trifluoroacetic acid (4M) for 8 h at 100°C. The hydrolysis LWSP was analyzed by TLC. The developing solvent was a mixture of chloroform/acetic acid/water (6:7:1). The revelation is obtained by spraying 5% (v/v) H<sub>2</sub>SO<sub>4</sub> in ethanol followed by incubation and drying in oven at 105°C for 10 min. The standards used were as follows: glucose, fructose, sucrose, mannose, arabinose, and galactose at a concentration of 10 g/l.

**2.5.2. High-Performance Liquid Chromatography (HPLC).** The monosaccharide compositions were analyzed by HPLC using an Aminex HPX-87H column with a mobile phase of 0.001N H<sub>2</sub>SO<sub>4</sub>. The sample was hydrolyzed by dissolved 2 mg of LWSP in 250 µl of 4M trifluoroacetic acid (TFA) at 100°C for 8 h. Then, 20 µl of obtained hydrolysate was added to 980 µl of H<sub>2</sub>O and filtered through a 0.45 µm pore size filter. Monosaccharide composition was analyzed at a flow rate of 0.4 ml/min and at 60°C. Glucose, fructose, sucrose, gluconic acid, mannose, arabinose, galactose, and xylose were used as standard monosaccharide. The monosaccharide composition assays were performed in two independent experiments.

### 2.6. Structural Analysis of LWSP

**2.6.1. Fourier-Transformed Infrared Spectroscopy (FTIR) Analysis.** The structure groups of the extracted LWSP were

identified using Fourier-transformed infrared spectrophotometer (Nicolet FTIR spectrometer) equipped with a horizontal attenuated total reflection (ATR) accessory. 1 mg of dried sample was grounded with KBr powder and then pressed into 1 mm pellets for FTIR measurement from 4000 to 400  $\text{cm}^{-1}$ . The data were analyzed by the OPUS 3.0 data collection software program (Bruker, Ettlingen, Germany).

**2.6.2. Nuclear Magnetic Resonance (NMR) Analysis.** The structural analysis of LWSP was carried out by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR using a Bruker 600 M spectrometer (Rheinstetten, Germany) at 25°C. The 30 mg of powdered samples were dissolved in 1 ml 99.9%  $\text{D}_2\text{O}$ .  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded at a frequency of 300 and 75.5 MHz (field of 7.1 T), respectively. Data analysis was carried out using the MestRe Nova 5.3.0 (Mestrelab Research S.L.) software. The chemical shift was expressed in parts per million.

**2.6.3. X-Ray Diffraction.** The physical characteristic of LWSP was studied to obtain the X-ray diffraction (XRD) pattern. It was employed using an X-ray diffractometer (Siemens D5000, Bruker, Germany). The data were collected in the  $2\theta$  range 5–80° with a step size of 0.02° and a counting time of 0.78 s/step.

**2.6.4. UV Absorption Peak Detection.** UV-visible spectra were determined using TU-1900 spectrophotometer at 25°C in the wavelength range of 200–800 nm [21]. The LWSP sample was dissolved in ultrapure water to a final concentration of 0.05%.

## 2.7. Antioxidant Activities of LWSP

**2.7.1. DPPH Radical-Scavenging Assay.** The DPPH radical-scavenging activity of LWSP was determined according to the method described by Bersuder et al. [22]. Briefly, LWSP powder was dissolved in ultrapure water at different concentrations (0–10 mg/ml). 500  $\mu\text{l}$  of each sample was mixed with 375  $\mu\text{l}$  of 99.5% ethanol and 125  $\mu\text{l}$  of DPPH (0.02% in ethanol). Then, the mixtures were incubated in the dark at room temperature for 1 h, and the reduction of DPPH radical was measured in absorbance of 517 nm. BHT was used as a positive control. The DPPH radical-scavenging activity was calculated as follows:

$$\text{DPPH radical-scavenging activity (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} * 100, \quad (1)$$

where  $A_{\text{control}}$  is the absorbance of the control reaction and  $A_{\text{sample}}$  is the absorbance of LWSP. All experiments were done in triplicate.

**2.7.2. Measurement of Reducing Power.** The reducing power activity was determined by testing the reducing power of iron according to the method reported by Yildirim and Mavi [23]. Samples of LWSP were dissolved in 0.2 mol/l phosphate buffer (pH 6.6) at a series of concentrations (0–15 mg/ml). 2.5 ml of each sample was mixed with 2.5 ml of 10 mg/ml potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 100 mg/ml trichloroacetic

TABLE 1: Physicochemical analysis of LWSP.

Parameters	LWSP
Yield (%)	14.76 ± 0.99
pH	7.00 ± 0.01
Moisture (%)	3.83 ± 0.24
Ash (%)	7.61 ± 0.13
Fat (%)	0.3 ± 0.01
Carbohydrates (%)	76.03 ± 0.06
Mw	64.56 kDa
Color	
$a^*$	0.53 ± 0.01
$b^*$	14.56 ± 0.48
$L^*$	66.23 ± 0.02

$L^*$  is a measure of lightness,  $a^*$  represents the chromatic scale from green to red, and  $b^*$  represents the chromatic scale from blue to yellow.

acid was added to the mixture. After centrifugation, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%, w/v). The mixture was incubated at room temperature for 10 min, and the absorbance was measured at 700 nm. Ascorbic acid was used as a positive control.

**2.7.3. Ferrous Ion Chelating Activity.** The chelating of ferrous ions by LWSP was measured according to the previously reported method Decker and Welch [24]. Briefly, 3 ml of LWSP sample at different concentrations (0–10 mg/ml) was mixed with 100  $\mu\text{l}$  of  $\text{FeCl}_2$  (2 mM) and incubated at room temperature for 5 min. The reaction was initiated by the addition of 0.4 ml of 5 mM ferrozine solution. The mixture was shaken and incubated at room temperature for 10 min. The absorbance was measured at 562 nm using EDTA as a positive control. Analyses of all samples were run in triplicate and averaged. The chelating effect was calculated using the following equation:

$$\text{Ferrous ion - chelating activity (\%)} = \frac{(A_{\text{control}} + A_{\text{blank}} - A_{\text{sample}})}{A_{\text{control}}}, \quad (2)$$

where  $A_{\text{control}}$  is the absorbance of the control (without LWSP),  $A_{\text{blank}}$  is the absorbance of the LWSP (without ferrozine), and  $A_{\text{sample}}$  is the absorbance of the mixture of LWSP and ferrozine.

## 3. Results and Discussion

**3.1. Physicochemical Analysis of LWSP.** The chemical composition of the LWSP is presented in Table 1. The pH of 1% LWSP solution recorded 7.00 ± 0.01. In addition, the results proved that carbohydrates presented the most interesting part (76.03 ± 0.06%) of the extract. The same result was obtained for the *sorghum* polysaccharides and *Opuntia Ficus Indica* cladode polysaccharides which recorded 78.84% and

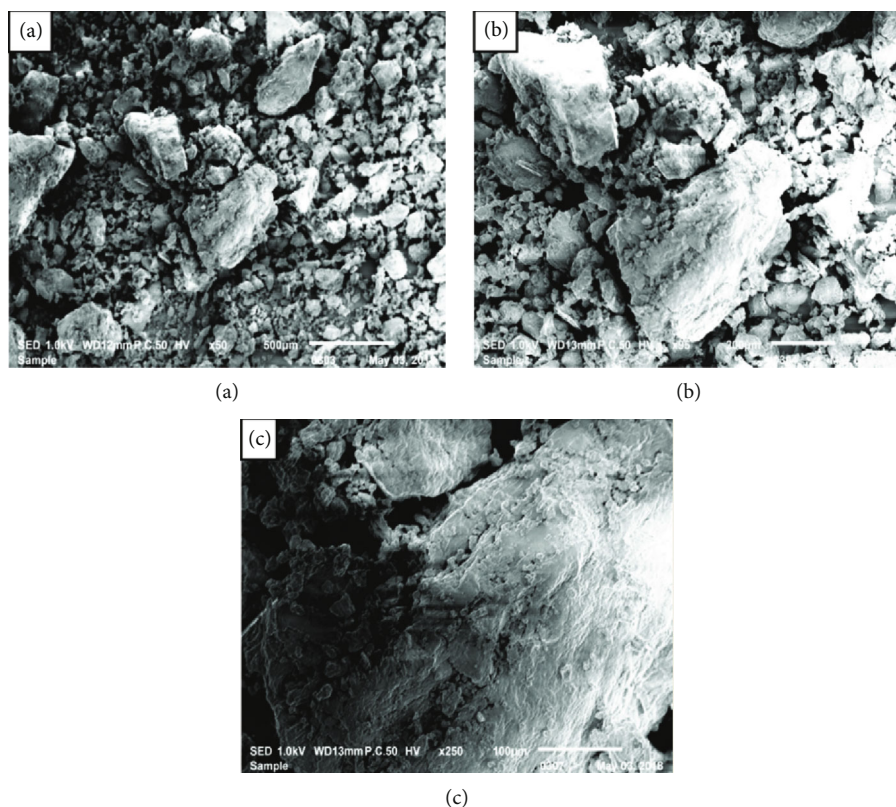


FIGURE 1: Scanning electron micrographs of the LWSP at magnifications of (a) 50x, (b) 95x, and (c) 250x, respectively.

85.31%, respectively [6, 25]. The ash and fat contents calculated for LWSP were 7.61 and 0.3%, respectively (Table 1). The sample was characterized by a relatively low moisture (3.83%).

The molecular weight of LWSP was investigated by the gel filtration high-performance liquid chromatography. LWSP samples showed two peaks which the major was determined to be approximately 64.56 kDa (Table 1). The presence of the minor peaks could be caused by the degradation of the molecules by the high temperature used for the dissolution of LWSP [25].

As presented in Table 1, LWSP showed a high value of  $L^*$  66.23. The  $b^*$  and  $a^*$  values were recorded at 14.56 and 0.53, respectively (Table 1). Similar results were obtained by Ktari et al. [11] who reported that polysaccharide extracted from fenugreek displayed the lighting yellow color. The incorporation of polysaccharides in food usually has an effect on the color of the final product [11]. This characteristic enhanced their suitability to be added in food and nonfood formulations.

**3.2. Scanning Electron Microscopy.** The scanning electron microscopy (SEM) has been widely used to assess the surface morphology and complex 3D microstructure of polysaccharides for its application in different products. It was employed to monitor the morphological properties of the extracted polysaccharides. It is the most powerful tool in the study in structural morphology such as porosity, size, and shape of macromolecules [25–27]. SEM analysis of poly-

saccharide LWSP is presented in Figure 1. It is revealed as a sponge-like structure containing numerous cavities. Such structure makes the LWSP suitable for a variety of industrial applications. Also, this structure with cavity distribution allows the LWSP to absorb a large amount of water, when solubilized in water-based solutions. Such characteristic makes it a fast swelling system in several applications like gelling and emulsifying agents.

Previous works reported that the presence of numerous cavities on the surface of polysaccharides leads to an improvement in the various physical and functional characteristics, such as solubility, water/oil retention capacities, and emulsion properties. These characteristics are required for the application of such polysaccharides in various applications specifically in the food sector [27, 28]. In the other works, the scanning electron micrographs of gum polysaccharide extracted from Katan seeds revealed a splendor and shiny surface. In addition, the structure and surface morphology of polysaccharides could be influenced by different preparation methods: extraction and purification [29].

**3.3. Monosaccharide Composition.** The analysis of the hydrolysis LWSP obtained by TLC showed the presence of four plugs emerged with a retention factor of 0.55, 0.60, 0.63, and 0.69, respectively, similar to the standard monosaccharide glucose, mannose, arabinose, and xylose (Figure 2(a)). The obtained results showed that LWSP was a heteropolysaccharide, composed of glucose, mannose, xylose, and arabinose.

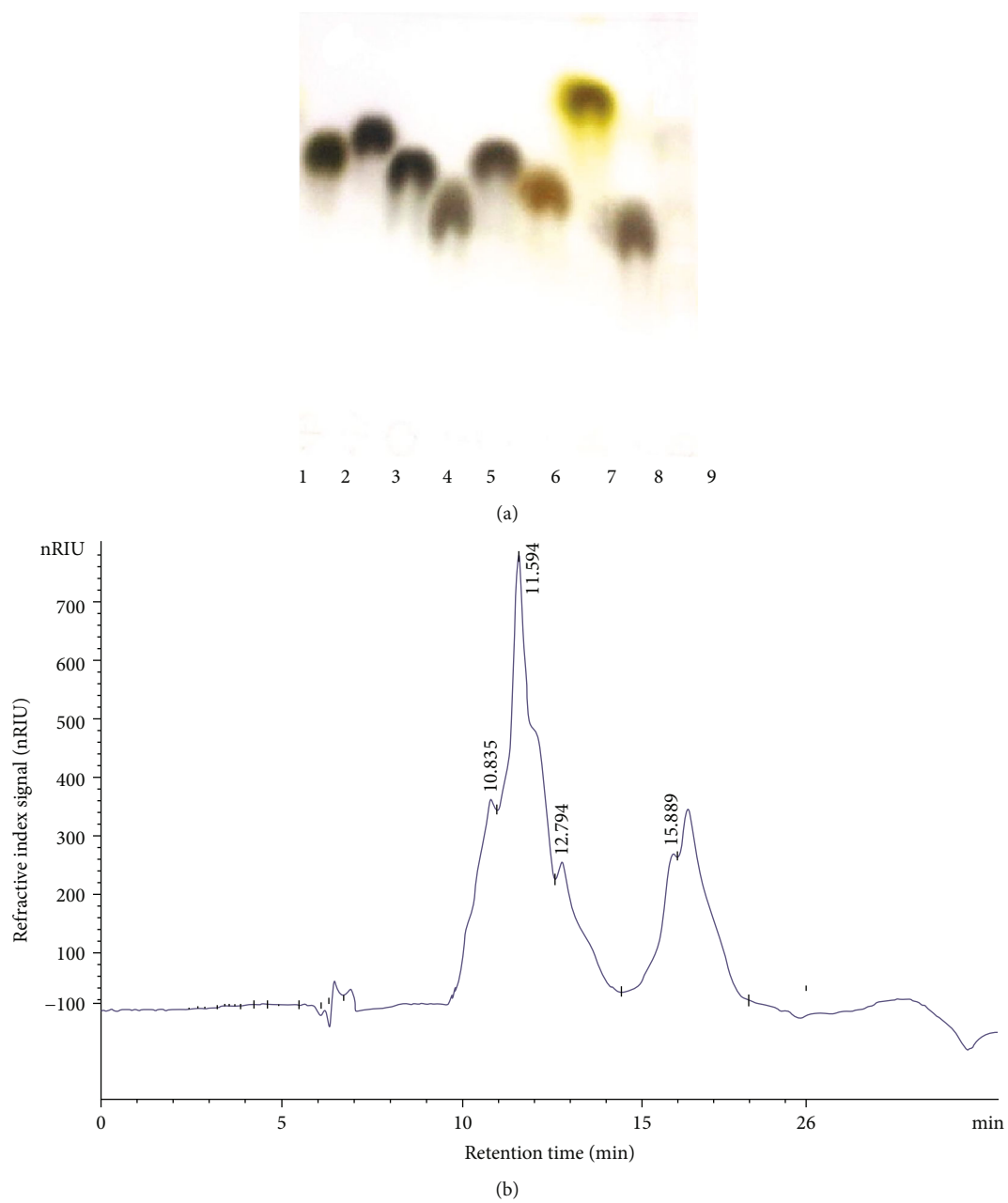
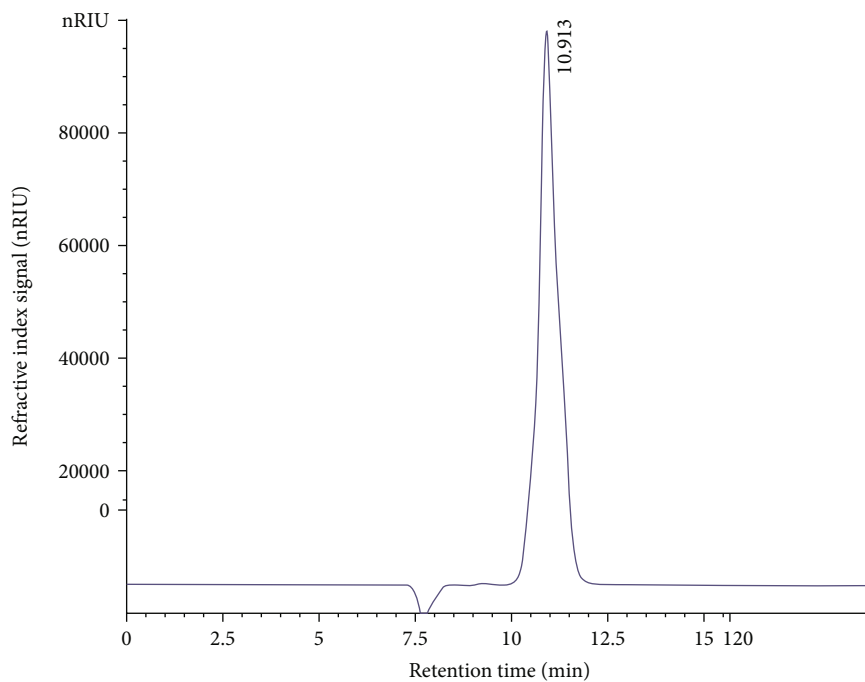
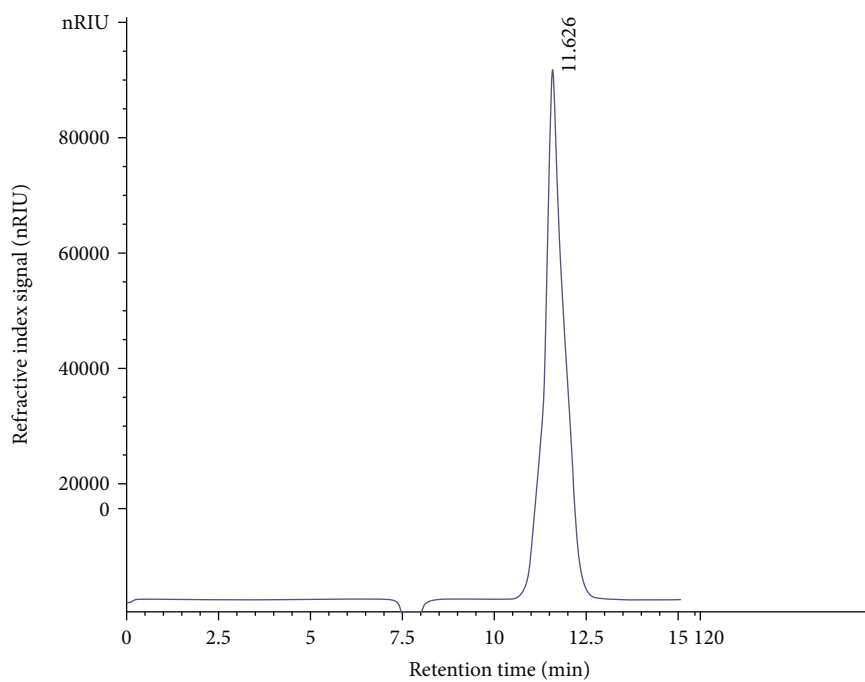


FIGURE 2: Continued.

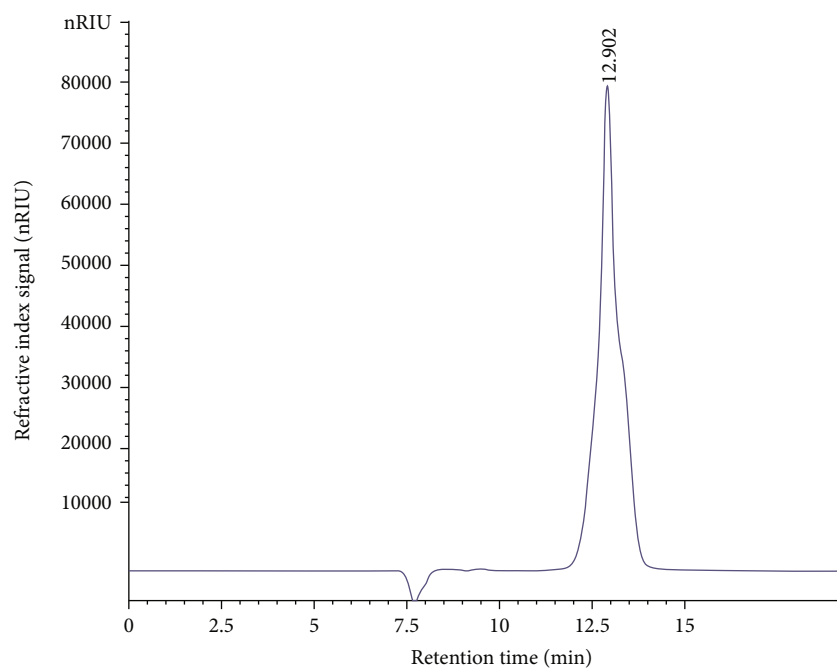


(c)

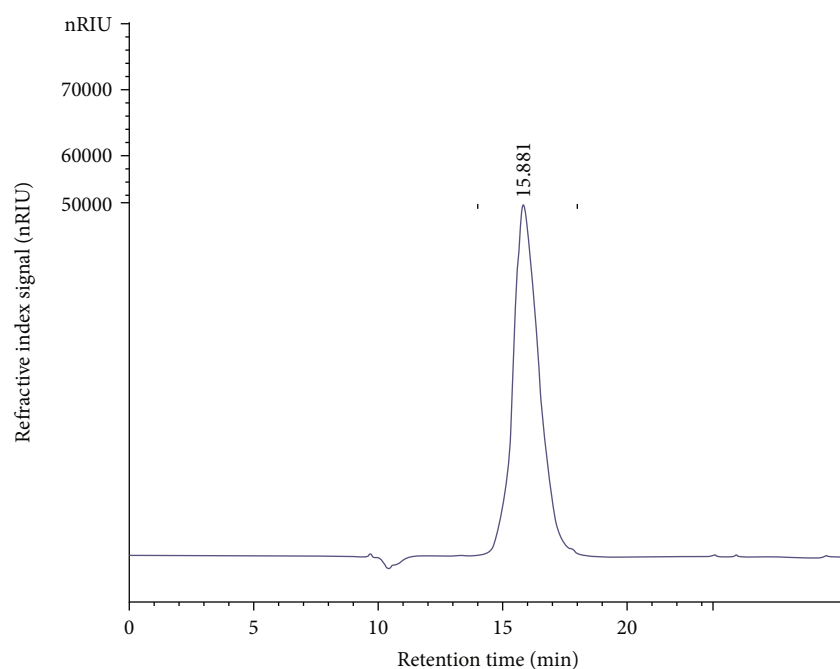


(d)

FIGURE 2: Continued.



(e)



(f)

FIGURE 2: (a) TLC analysis of LWSP. (1) Arabinose, (2) xylose, (3) fructose, (4) glucose, (5) tagatose, (6) mannose, (7) rhamnose, (8) galactose, and (9) hydrolyzed LWSP. (b) HPLC analysis of LWSP hydrolyzed by TFA; (c) HPLC analysis of glucose; (d) HPLC analysis of mannose; (e) HPLC analysis of arabinose; (f) HPLC analysis of xylose.

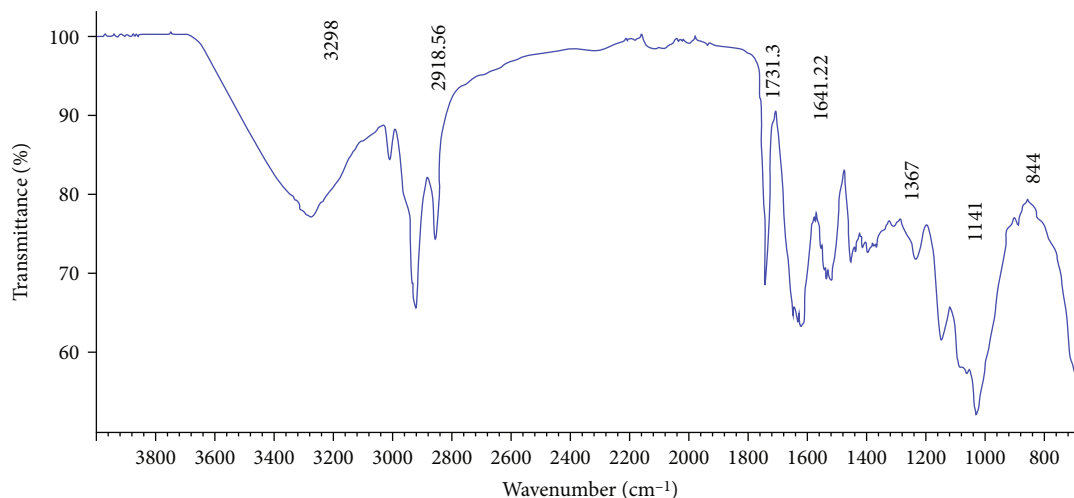


FIGURE 3: FTIR spectra of LWSP determined according to wavenumber ( $\text{cm}^{-1}$ ) and transmittance (%).

The monosaccharide composition of LWSP was also evaluated by comparing the retention time against standards using HPLC. The obtained chromatograms were presented in Figures 2(b)–2(e). It was revealed that LWSP was a polysaccharide composed of glucose, mannose, arabinose, and xylose. Mannose is the predominant peak with a retention time of 11.5 min followed by glucose (10.85 min). Arabinose and xylose are the minor components of LWSP. Accordingly, HPLC analysis confirms TLC.

In a previous study, it was reported that polysaccharide extracted from mature and ripe Katan seeds is composed of D-xylose, D-galacturonic acid, L-galactose, L-arabinose, L-rhamnose, and conceivably, some traces of D-glucose [30].

### 3.4. Structural Analysis of LWSP

**3.4.1. FTIR Spectroscopy.** The FTIR spectrum was shown in Figure 3, a strong peak around  $3298\text{ cm}^{-1}$  for hydroxyl groups stretching vibrations due to inter- and intramolecular hydrogen bands [31]. The peak around  $2918.56\text{ cm}^{-1}$  showed an absorption for C-H stretching vibrations of the free sugars; the band around  $1731.33\text{ cm}^{-1}$  showed an absorption for carboxyl groups stretching vibrations [32]; a peak around  $1641.22\text{ cm}^{-1}$  was occurred due to the associated water [33]. Weak absorption peaks between  $800$  and  $1200\text{ cm}^{-1}$  attributed to the presence of carbohydrate fingerprints and the identification of functional groups characterizing polysaccharides as stretching (C-O-C), bending (O-H), and deforming ( $\text{CH}_3$ ) vibrations [34]. The bands below  $1000\text{ cm}^{-1}$  also reported the visible bands' presence and/or possible linkages between molecules of monosaccharide [35]. In fact, the observed peak approximately at  $844\text{ cm}^{-1}$  was attributed to be a characteristic of  $\alpha$ -configuration in this polysaccharide [36].

**3.4.2. NMR Spectroscopy Data of LWSP.** The structure of polysaccharide LWSP was elucidated through  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Figure 4). In  $^1\text{H}$  NMR spectrogram (Figure 4(a)), it showed a cramped region ranging between

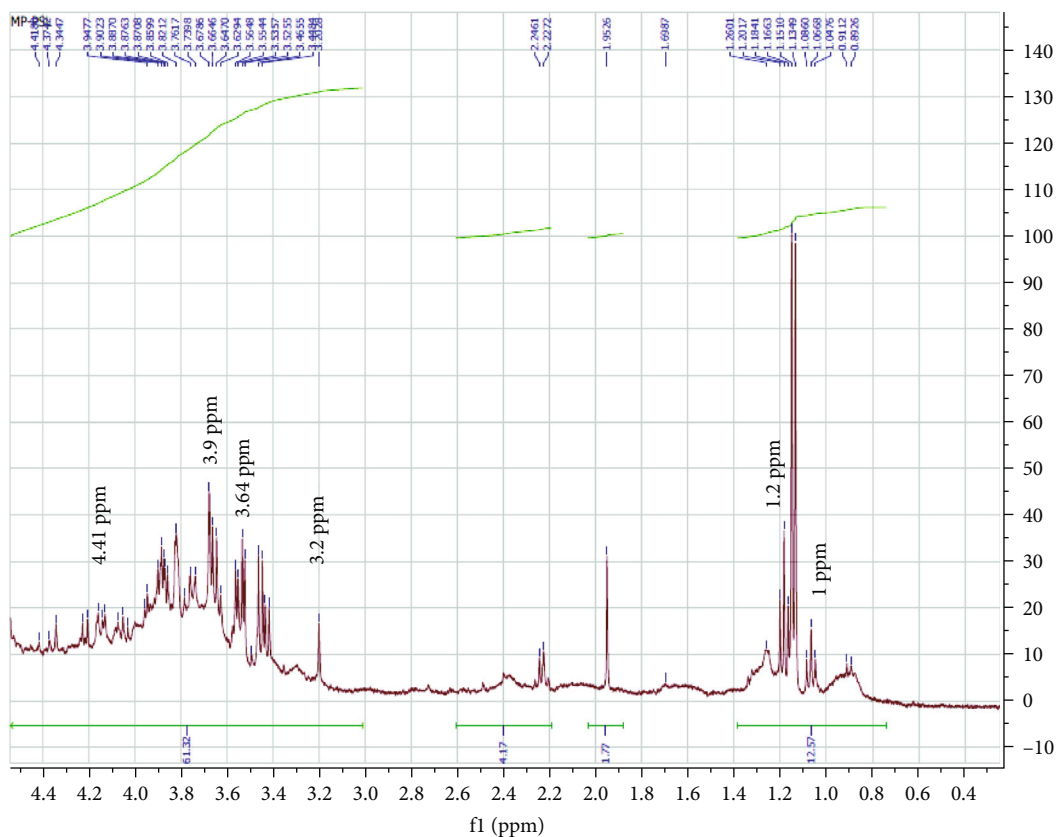
$3.2$  and  $4.41\text{ ppm}$ , indicating the presence of many similar sugar residues which confirm the presence of polysaccharides [37, 38]. Four proton resonance signal peaks can be observed in the anomeric proton region at  $4.3$ ,  $3.9$ ,  $3.7$ , and  $3.2\text{ ppm}$ , indicating that LWSP is consisted of four monosaccharide residues with  $\alpha$  and  $\beta$  anomers. In fact, it was generally believed that the chemical shift values of  $\alpha$ -anomeric protons were mostly larger than  $4.0\text{ ppm}$  while the signals less than  $4.0\text{ ppm}$  correspond to  $\beta$ -anomeric proton [39]. In previous data, it was reported that signals between  $3.2$  and  $3.9\text{ ppm}$  could be attributed to the characteristics of H2-H5 resonate. Therefore, the absorption signal between  $3.64$  and  $3.94\text{ ppm}$  was provoked by protons on sugar rings [40]. Intense peak signal observed at  $1.0$  and  $1.2\text{ ppm}$  and identified the carbon group (R- $\text{CH}_2$ - $\text{CH}_3$ ).

The  $^{13}\text{C}$  NMR spectrum (Figure 4(b)) showed the presence of sugar rings in our polysaccharides. In fact in this spectrogram, we found the existence of two anomeric carbons  $\alpha$  and  $\beta$ -configurations in the regions of  $95.26\text{ ppm}$  and  $110.45\text{ ppm}$ , respectively. This finding confirms the results of  $^1\text{H}$  NMR spectrum. The absorption signals at  $59.74$  and  $61.17\text{ ppm}$  found in the  $^{13}\text{C}$  NMR spectrum can be assigned to an O- $\text{CH}_3$  group [12]. Also, we noticed that the presence of signal in the region ranging from  $57$  to  $87\text{ ppm}$  can be assigned to sugars C2–C6 [41]. The signal appeared between  $69$  and  $77.8$  corresponding to the osidic groups (C2–C5) [42]. The strong signal positioned in the region of  $60$ – $80\text{ ppm}$  was qualified to the pyranose configuration in LWSP.

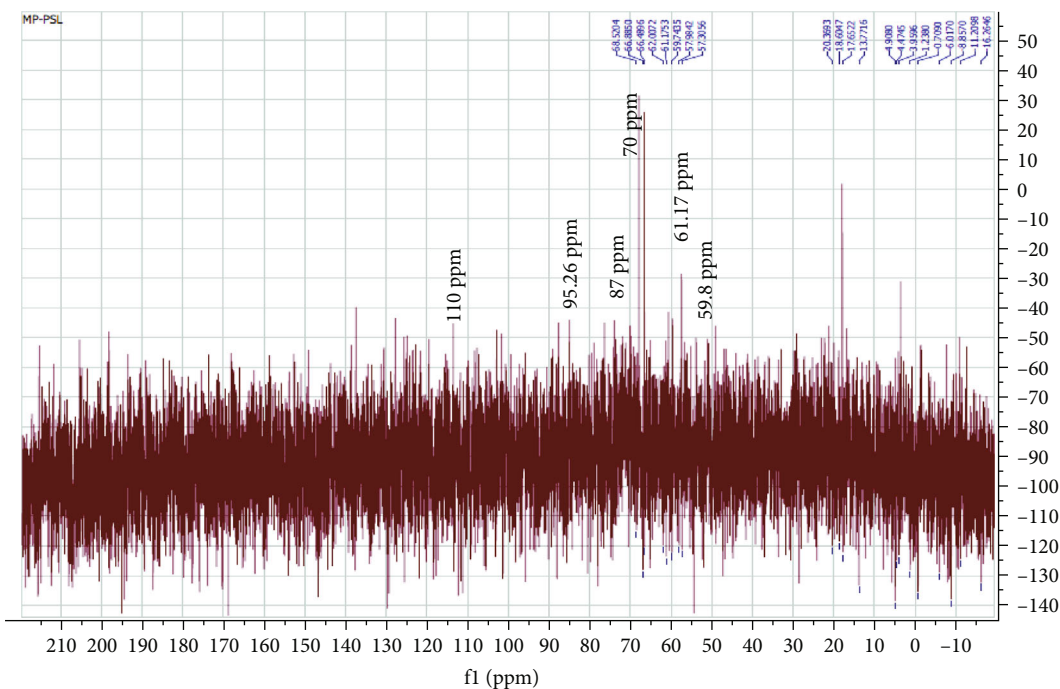
**3.4.3. X-Ray Diffraction Analysis of LWSP.** XRD technique is usually used for semiquantitative and qualitative assessment of amorphous and semicrystalline and crystalline component. Indeed, the crystalline or noncrystalline characteristics of a substance play a major role in the physicochemical properties by influencing the structural arrangements, like solubility, viscosity, and flexibility.

The X-ray diffractogram of LWSP was presented in Figure 5. Various sharp peaks ranging from  $0$  to  $80^\circ$  spectrum





(a)



(b)

FIGURE 4: NMR spectrum of LWSP (a)  $^{13}\text{C}$  NMR spectrum and (b)  $^1\text{H}$  NMR spectrum.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded at a frequency of 300 and 75.5 MHz.

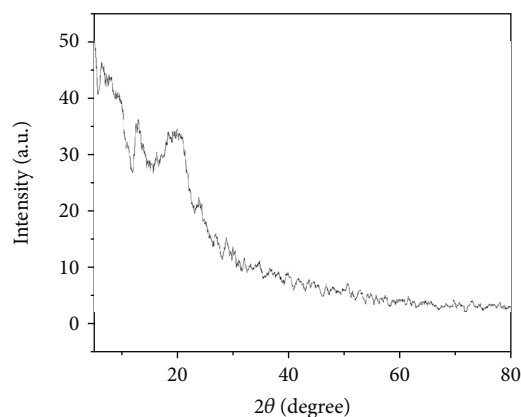


FIGURE 5: X-ray diffraction pattern of LWSP determined according to degree ( $2\theta$ ) and intensity (a.u.).

of  $2\theta$  value were observed demonstrating the semicrystalline nature of LWSP. According to the literature, this finding was similar to the results obtained by Ben Slima et al. [6], and these authors noticed that water-soluble polysaccharides extracted from *Sorghum bicolor* seeds were semicrystalline fibers. In the other study reported by Rashid et al. [43], it showed that gum extracted from Katan seeds depicted amorphous behavior [44].

**3.4.4. UV-vis Spectroscopy.** As presented in Figure 6, LWSP showed a maximum absorption peaks at 210 nm (Figure 6) and did not show any absorbance beyond that range. Thus, LWSP was identified as polysaccharides [45].

### 3.5. Antioxidant Activities of LWSP

**3.5.1. DPPH Radical-Scavenging Assay.** DPPH is a stable free radical used for screening the antioxidant ability of samples. The test mechanism is based on the reduction of DPPH by a proton-donating substrate [46]. The DPPH radical-scavenging capacity of LWSP and BHT (positive control) at different concentrations was determined as shown in Figure 7(a). LWSP activities were lower than that of BHT at the same concentration. The DPPH radical-scavenging activity of the LWSP increased as LWSP concentrations increased, and the highest DPPH radical-scavenging activity was (86.12%) obtained at 10 mg/ml. Our results were similar to those previously reported for other polysaccharides extracted from plants as the sorghum polysaccharides and [6] fenu-greek polysaccharides [11]. The results implied that LWSP exhibits good antioxidant activities by transferring hydrogen atoms or electron to neutralize the free radicals [45]. The radical-scavenging activity of polysaccharides was depended on the molecular weight and monosaccharide composition [47]. In fact, the LWSP radical-scavenging ability could be attributed to the hydroxyl groups and carboxyl group existences in polysaccharides, which can act as quenching of the free radical [48].

**3.5.2. Reducing Power.** The reducing capacity of sample is depended on the reductones' presence. The latter are capable

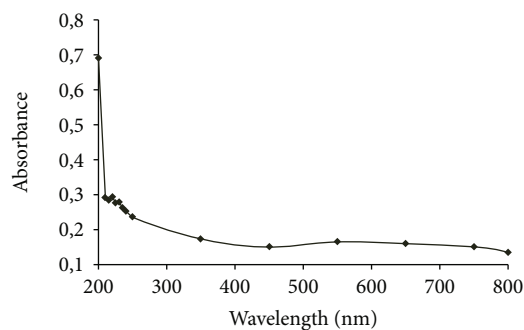


FIGURE 6: The absorbance of the purified LWSP within the wavelength range of 200-800 nm.

to donate electrons to free radicals, break these oxidizing chain reactions, and prevent peroxide formation in order to make samples more stable [42].

The capacity of LWSP to reduce the oxidation form of iron ( $\text{Fe}^{3+}$ ) in ferric chloride to the ferrous form ( $\text{Fe}^{2+}$ ) by antioxidant was investigated. As demonstrated in Figure 7(b), the reducing power of LWSP appeared to be concentration dependent and reached a maximum of 0.408 at 10 mg/ml. Furthermore, LWSP showed lower reducing power than BHT. Our results were similar to those previously reported by Ben Slima et al. [42]. However, it was reported that polysaccharides extracted from *Hohenbuehelia serotina* exhibited reducing power about 0.50 at 10 mg/ml. It was reported that the differences in the monosaccharide components, chemical composition, molecular weight, glycoside bond types, and configuration of polysaccharide could affect its bioactivity [49].

**3.5.3. Ferrous Chelating Activity.** Ferrous chelating assay is one of the essential antioxidant tests that provide significant reflection on the antioxidant activity. The chelating activity of LWSP was lower than EDTA which is a metal ion cheater (used as a positive control) at all concentrations (Figure 7(c)). At a concentration at 1 mg/ml, EDTA could chelate 94%, while the LWSP could chelate 37.6% of  $\text{Fe}^{2+}$ . In the activity of LWSP, a concentration-dependent response reaches a maximum of 72.31% at 10 mg/ml. The chelating activity of LWSP was higher than other plant polysaccharides such as polysaccharides from floral mushroom and *Cystoseira barbata* seaweed which recorded 42.68% at 5 mg/ml and 63% at of 10 mg/ml [30, 38]. It has been reported that the chelating activity is related to the large galactose and mannose quantities and the above functional groups presence in polysaccharide [11, 50].

## 4. Conclusion

The present study is aimed at identifying and characterizing novel polysaccharides extracted from Katan seeds by hot water technique as well as investigating their antioxidant activities. The obtained results from the spectroscopic analyses by SEM, FTIR, XRD, HPLC, and NMR showed that LWSP was a polysaccharide composed of xylose, arabinose,

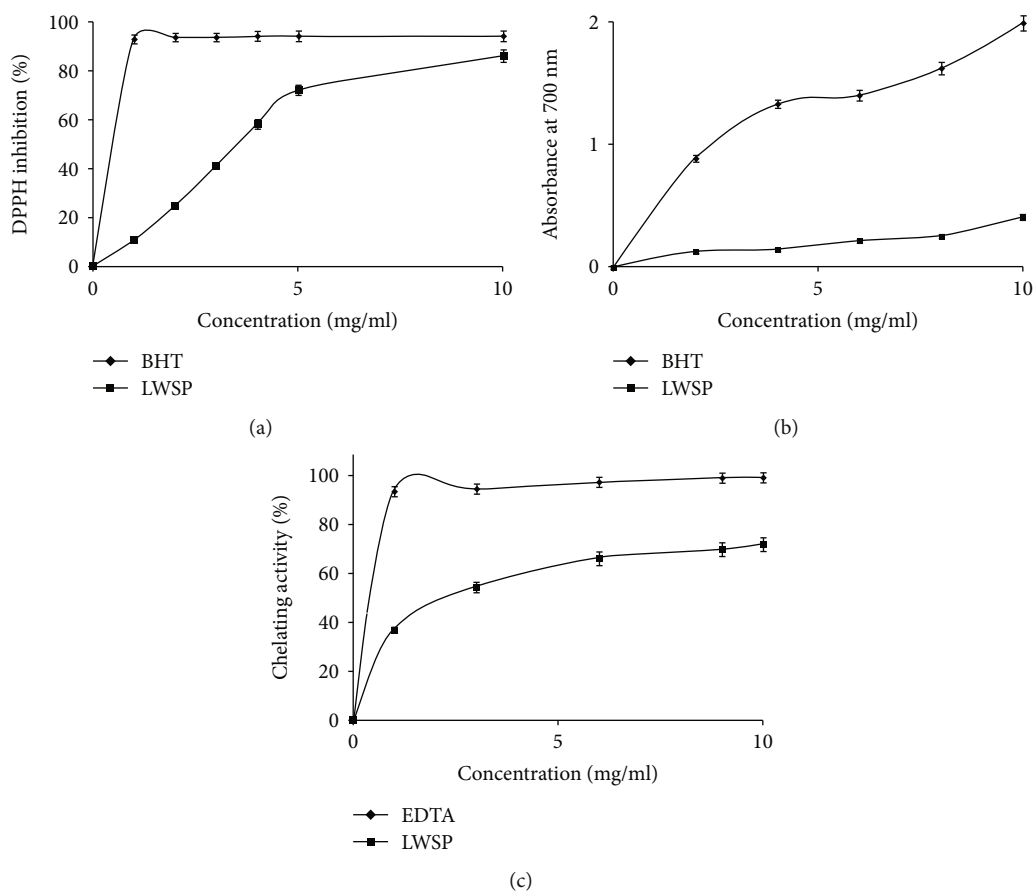


FIGURE 7: Antioxidant activities of LWSP: (a) the percentage of DPPH inhibition; (b) reducing power determined at A 700 nm; (c) the percentage of ferrous chelating activity made at different concentrations (0-10 mg/ml).

galactose, and glucoses with semicrystalline structure. *In vitro*, the reducing power, scavenging activity on DPPH, superoxide, and hydroxyl radical were also studied. Results demonstrated that LWSP exhibited higher antioxidant activity. These findings provided that the novel extracted polysaccharide might find promising values in functional products and therapeutic applications as an antioxidant agent.

### Data Availability

No data were used to support this study.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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