

Article

Extraction, Characterization, and Antioxidant Activity of Polysaccharides from Ajwa Seed and Flesh

Manel Dhahri ^{1,*}, Salim Sioud ² , Shuruq Alsuhaymi ³, Fatimah Almulhim ³ , Ali Haneef ⁴, Abdelhamid Saoudi ⁴, Mariusz Jaremko ⁵ and Abdul-Hamid M. Emwas ^{2,*} 

- ¹ Biology Department, Faculty of Science Yanbu, Taibah University, Yanbu El-Bahr 46423, Saudi Arabia
 - ² Core Labs, King Abdullah University of Science and Technology (KAUST), Thuwal 23955, Saudi Arabia
 - ³ Division of Biological and Environmental Sciences and Engineering (BESE), King Abdullah University of Science and Technology (KAUST), Thuwal 23955, Saudi Arabia
 - ⁴ King Abdullah International Medical Research Center, Jeddah 21423, Saudi Arabia
 - ⁵ Smart-Health Initiative (SHI) and Red Sea Research Center (RSRC), Division of Biological and Environmental Sciences and Engineering (BESE), King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia
- * Correspondence: manel.dhahri@gmail.com (M.D.); abdelhamid.emwas@kaust.edu.sa (A.-H.M.E.)

Abstract: The date palm has been cultivated in dry and hot areas of the planet for much of human history. In the Kingdom of Saudi Arabia, dates are the main crop used as a source of food. Among several species of date fruits, the Ajwa AL-Madinah date is unique, growing only in Al-Madinah geographical region. The Ajwa date is used in traditional medicine due to its abundant active components and therapeutic properties. This study investigates the structural properties and the antioxidant effects of water-soluble polysaccharides extracted from Ajwa flesh and seed. The polysaccharides were isolated by two techniques including hot water and ultrasonic extraction. After isolation and partial purification, the physicochemical properties of four samples of polysaccharides extracted from flesh and seed were studied by several techniques including FTIR, solid-state NMR, elemental analysis, and mass spectrometry. Several radical scavenging experiments were combined to study the antioxidant activity of the polysaccharide compounds. FTIR and NMR results showed a structure typical of heterogeneous polysaccharides. Mass spectrometry revealed that the polysaccharide samples were composed mainly of mannose, glucose, galactose, xylose, arabinose, galacturonic acid, and fucose. In addition, the physicochemical properties and composition of polysaccharides extracted from flesh and seed were compared. The extracted polysaccharides showed antioxidant activity, with 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, Fe chelating ability, hydroxyl free radical scavenging ability, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. These results highlight their potential to be a useful nutritional element or supplemental medication.

Keywords: date fruit; palm; Ajwa; date; polysaccharides; antioxidant activity



Citation: Dhahri, M.; Sioud, S.; Alsuhaymi, S.; Almulhim, F.; Haneef, A.; Saoudi, A.; Jaremko, M.; Emwas, A.-H.M. Extraction, Characterization, and Antioxidant Activity of Polysaccharides from Ajwa Seed and Flesh. *Separations* **2023**, *10*, 103. <https://doi.org/10.3390/separations10020103>

Academic Editor: Paraskevas D. Tzanavaras

Received: 27 December 2022

Revised: 18 January 2023

Accepted: 25 January 2023

Published: 2 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Throughout the Middle East and North Africa, the date fruit has long been treasured as a source of nutrition. Its reputation as an outstanding product has grown over time, supported by recent scientific research proving its great health advantages [1,2]. Dates offer a wide range of necessary nutrients and significant health advantages, making them a nearly ideal food in many aspects on all continents [3]. The nutritional and therapeutic properties of the date fruit are linked to its chemical composition which differs according to the ripening stage, cultivar, and growing climate [4,5]. Numerous studies demonstrated the antioxidant, antimutagenic, gastroprotective, anti-inflammatory, hepatoprotective, nephroprotective, and anti-cancer properties of dates [4,5]. Polysaccharides are some of the most interesting and abundant chemical compounds found in date palm fruit. They act as biological carriers and have medicinal value due to their complex chemical

structure and diverse biological activities [6,7]. For example, polysaccharides are well-known anticoagulant agents [8,9]. Several studies reported that polysaccharides have the potential to act as protective agents from neurological diseases [6]. Although seed polysaccharides have been identified as potential pharmacological agents, they remain not well explored due to the cost incurred on processes such as extraction, isolation, and purification.

Antioxidants, which oversee removing free radicals, can reduce oxidative stress, which damages the intracellular architecture and DNA of cells. Antioxidant research, particularly the discovery of effective natural molecules from plants with minimal cytotoxicity, has emerged as an important area of biomedicine [10]. According to research, date fruit scavenges free radicals and prevents macromolecular alterations in biological systems [11]. Date palm fruit (DPF), which consists of a seed encased in a supple and delicious pericarp, is a common meal in most Arab nations [12]. In dry and semi-arid regions of the world for the past six millennia, the date palm (*Phoenix dactylifera* L.) has been grown for its delectable edible fruits. According to Food and Agriculture Organization data, the production of date fruit in Saudi Arabia has climbed 14.8% year on year since 2014, reaching 1,310,015 metric tons in 2019 [13]. Dates are a historic and commercially valuable crop throughout North Africa and the Middle East, where they play a crucial role in people's daily meals [14]. However, date seeds and date residue are currently considered a waste rather than a resource [14]. Another type of date waste is the overall loss of dates in Saudi Arabia during the date marketing process, which was calculated at 7.8%. This confirms that over 698,000 SAR worth of dates totaling 60,000 tons were lost each year, according to a recent report [13]. Nevertheless, this waste contains a variety of elements, including pigments, dietary fiber, sugars, and bioactive substances, which were shown to have positive effects on health. Hence, various date palm parts have long been used in folk medicine to cure a variety of diseases [2]. Considering their richness in bioactive molecules and their low toxicity, different types of date waste should be valued. Ajwa date is one of the most important varieties of date palm, grown in the Al Madinah area of western Saudi Arabia. This date variety is reported to have therapeutic properties [15]. Ajwa dates are mentioned in "Hadith" and Islamic literature history because it is thought that eating them can heal numerous chronic diseases. Ajwa dates are used not just for their food value, but also for their therapeutic properties against a variety of diseases [16]. Phytochemical investigations have shown that Ajwa shell and seed are high in phenolics and flavonoids, which have several positive effects on human health due to their powerful antioxidant activities. In addition, their richness in phenolics and flavonoids are crucial for decreasing cholesterol levels and preventing cardiovascular diseases [16]. Recently, an extract of Ajwa fruit was determined to be protective against acute diclofenac toxicity-induced colopathy [17]. In an interesting recent study, the Ajwa effect was studied on microbial infections in children with cancer in the form of an integrated therapy in the diet. The results suggested that using Ajwa might be a safe and cost-efficient strategy in pediatric cancer care [18]. In addition, via lowering apoptosis and oxidative stress, extracted compounds from Ajwa date fruit were shown to reduce the hepatotoxicity caused by carbon tetrachloride in Sprague-Dawley rats [19]. Interestingly, previous studies confirmed that by slowing down the process of cholesterol absorption of the intestine, which prevents its deposition in arteries and hence disorders such as coronary heart disease, stroke, and hyperlipidemia, soluble polysaccharides in the dietary fiber of date palm prevent atherosclerosis [20]. S. Khalid et al. reviewed the health benefits of Ajwa date fruit and seed which were determined to exhibit anti-microbial activity, anti-inflammatory effects, as well as antitoxic, antitumor and hypolipidemic hepatoprotective effects [16]. Phytochemical and pharmacological studies of Ajwa have been more concentrated on the phenolic and flavonoids compounds that were prepared mostly by organic extraction using several solvents including methanol [21], acetone [22], dichloromethane [23], and ethanol [24]. There has not been much interest in the pharmacological potential of water-soluble compounds from Ajwa date, and more specifically water-soluble polysaccharides. There is little information about the properties and bioactivities of polysaccharides extracted from date fruit and seed. Plant polysaccha-

rides have received a lot of interest due to their strong immunomodulatory, anticancer, antioxidant, and antiviral properties [25]. The biological activity of plant polysaccharides is directly connected to structural properties, and affected by monosaccharide content, glycosidic bond type and linking style, spatial arrangement, and method of extraction [26]. The aim of the current study is to isolate polysaccharides from Ajwa date flesh and seed by two cost-effective and green techniques including hot water extraction and ultrasonic extraction. We then explore their antioxidant activities. To the best of our knowledge, no prior work has investigated the antioxidant activities of polysaccharides extracted from Ajwa date flesh and seeds.

2. Materials and Methods

2.1. Extraction of Polysaccharides

Ajwa date was bought from several Saudi markets. Ajwa shell and flesh were dried separately and then ground up using a mill grinder to obtain a fine powder. Liposoluble compounds and impurities were removed by 95% ethanol treatment two times at 60 °C. The solvent was eliminated, and the powder was then oven dried at 50 °C. As the procedures used for plant polysaccharide extraction have a direct impact on the structure of the polysaccharides, which in turn has an impact on their biological functions [27], we tested several techniques including hot water extraction, ultrasonic-assisted extraction, microwave-assisted extraction, cellulase assisted extraction, and amylase assisted extraction to extract polysaccharides from Ajwa date flesh and seed. However, in this study, we will focus on hot water extraction and ultrasonic-assisted extraction techniques since they produce polysaccharides with the highest solubility.

2.1.1. Hot Water Extraction

A total of 100 g of dried Ajwa powder (flesh and seed) was extracted with 1.5 L distilled water at 80 °C (2×2 h at 80 °C) by mechanical stirring. The supernatant was recuperated by centrifugation at 4000 rpm for 15 min. The supernatants were collected and concentrated, and polysaccharides were precipitated by adding four volumes of ethanol. After incubation overnight at 4 °C, polysaccharides were recuperated by centrifugation at 4000 rpm for 15 min.

2.1.2. Ultrasonic Extraction

A total of 100 g of Ajwa flesh and seed powder was ultrasonically extracted in an ultrasonic bath with a power of 200 W at 80 °C for 1 h [28]. The supernatant was collected by centrifugation for 15 min at 4000 rpm, concentrated, and precipitated with 95% ethanol. The polysaccharides were recuperated by centrifugation and then the precipitate was successively washed with 95% ethanol and acetone.

2.1.3. Deproteinization

The Sevag method was used to remove protein, a liquid–liquid extraction that isolates proteins in the interphase between the aqueous and organic phases. To 100 mL of polysaccharides solution (in the conical flask), 20 mL of chloroform was added, followed by the addition of n-butanol (4 mL) to prevent foaming. The mixture was vigorously stirred for 20 min and centrifuged at 2500 rpm for 10 min. The deproteinized polysaccharides in the upper layer were collected and the procedure was repeated until no interphase was observed. The collected aqueous layer was collected and precipitated with ethanol. The precipitate was dissolved, dialyzed against ultrapure water utilizing a 3.5 kDa cut-off membrane for 72 h at 4 °C to remove small molecules, and lyophilized [29]. The polysaccharides extracted from flesh and seed by hot water extraction and ultrasonic extraction were named AFP-HWE, AFP-US, ASP-HWE, and ASP-US, respectively.

2.2. Polysaccharide Characterization

2.2.1. Scanning Electron Microscopy (SEM) Analysis

The surface morphology of the extracted polysaccharides was analyzed by scanning electron microscopy (Carl Zeiss SMT, Oberkochen, Germany) at an accelerating voltage of 5 kV. All samples were coated with platinum for a 5.0 nm thick coating for 40 s before analysis.

2.2.2. Colorimetric Assay

The amount of total sugar and uronic acid was determined by phenol–sulfuric acid assay and carbazole method [30]. Carbohydrate and uronic acid content were estimated by using the slopes of curves of standards. The results were represented as weight % of the dry sample.

2.2.3. Elemental Analysis

The molar ratio of carbon, hydrogen, nitrogen, and sulfur in polysaccharides extracted from flesh and seed was estimated by the organic elemental analyzer OEA Flash 2000 from Thermo Scientific, Waltham, MA, USA [8].

2.2.4. Infrared Spectroscopy

AFP-HWE, AFP-US, ASP-HWE, and ASP-US were mixed with KBr, pressed, and then analyzed with the FT-IR spectrometer (Nicolet FTIR iS10, Thermo Scientific, Waltham, MA, USA) with scanning between 400 and 4000 cm^{-1} . OMNIC Spectra software was used for data treatment [8,31].

2.2.5. Solid State NMR

Solid-state ^{13}C NMR was employed to characterize and check the purity of polysaccharides extracted by using the WB Bruker 400 AVANCE III spectrometer equipped with a 4 mm double-resonance CP MAS Bruker Probe. The spectrum was recorded at room temperature using a cp pulse program from the Bruker pulse library as described before [32,33]. All spectra were collected and processed by Bruker TopSpin 3.5pl7 software (Bruker, Billerica, MA, USA) [8].

2.2.6. Polysaccharide Hydrolysis and Derivatization

Hydrolysis of the polysaccharides was established according to the reported Wu et al. protocol [34]. A total of 50 mg of polysaccharides (AFP-HWE, AFP-US, ASP-HWE, and ASP-US) was treated with 4 M TFA (4 mL) in a sealed flask for 15 min at ambient temperature. After that, 1 mL of distilled water was added, and the mixture was maintained in a boiling water bath for 2 h. Then, 2 mL of distilled water was added, and the mixture was maintained in a hot water bath for 1 h. The reaction mixture was centrifuged for 10 min at 3000 rpm after it was cooled to room temperature. Under low pressure, the supernatant was collected and dried [34].

A mixed standard solution containing 1 mg/mL of monosaccharides standard was prepared and serially diluted to concentrations of 0.0001 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$. Both hydrolyzed polysaccharides and standard mixture were subjected to derivatization using 1-phenyl-3-methyl-5-pyrazolone (PMP). The derivatization was conducted according to Xu et al. [35]. Briefly, 50 μL of polysaccharides sample or monosaccharide standards were mixed with 200 μL of ammonia solution (28.0–30.0%) in water and 200 μL 0.2 M PMP solution in methanol. After 30 min of reaction, the mixtures were dried by vacuum centrifugation at 70 $^{\circ}\text{C}$. Then, 500 μL of water was used to reconstitute the dried samples and washed twice with 500 μL of chloroform. The aqueous layer from each sample or standard was recuperated and injected for full monosaccharide analysis [35].

2.2.7. High-Resolution Mass Spectrometry (HR-MS)

The Orbitrap ID-X was used to determine the m/z of the studied molecules. The Orbitrap ID-X spectrometer could reach a high resolution (>120,000) and reliable mass accuracy

(<5 ppm mass error). Electrospray ionization in positive mode (ESI⁺) was applied for the studied compounds. The mass spectrometer was calibrated using a purchasable “Calibration Mix ESI (Thermo Scientific, Waltham, MA, USA)” by following the manufacturer’s guidelines. The PMP-derivatized monosaccharides and the unknown polysaccharides were automatically infused (5 µL each) through the UHPLC system (Vanquish, Thermo Scientific, Waltham, MA, USA) with the use of column (Acquity UPLC HSS C18, 2.1 × 100 mm, 1.8 µ) for the separation. The mobile phase was composed as follows: A = Water + 0.1% formic acid + 25 mM Ammonium Formate and B = Acetonitrile + 0.1% formic acid. The flow rate was set to 0.5 mL/min. The separation gradient was applied for the separation as follows: 5%B (0–1 min), 5%B to 99%B (1–7 min), 99%B (7–9 min), and 5%B (9.1–10 min). In addition, the quantitation of the individual sugar was evaluated using pure monosaccharide standards (mannose, galacturonic acid, rhamnose, glucose, galactose, xylose, arabinose, and fucose) with known concentrations (0.01, 0.1, 1, and 10 µg/mL). The calibration curve was built using Quan Browser (Xcalibur software, Thermo Scientific, Waltham, MA, USA).

2.3. Antioxidant Activities

2.3.1. ABTS Radical Scavenging Assay

ABTS (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) reagent was prepared by dissolving ABTS in water to prepare a 7 mM solution, as previously reported [36]. Furthermore, ABTS radical cation (ABTS)^{•+} was created by reacting the ABTS solution with potassium persulfate (2.45 mM) and then followed by incubation in the dark at room temperature before use. To achieve an absorbance of 0.7 at 734 nm, the ABTS^{•+} solution was diluted with PBS (pH 7.4) after 12 to 16 h. To perform the assay, 10 µL of samples at various concentrations (250, 500, 1000, 2000, 5000 µg/mL) was added to 96-well plates followed by the addition of 190 µL ABTS reagent. The absorbance at 734 nm was measured after shaking the plate for 10 s at medium speed and incubating it for 5 min in the dark. Ascorbic acid at various concentrations (0–100 µg/mL) was used as a reference [36]. The following equation was used to calculate the degree of scavenging:

$$\text{Scavenging effect (\%)} = \{(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}\} \times 100.$$

2.3.2. Fe Chelating Ability

A total of 50 µL of samples at various concentrations (250, 500, 1000, 2000 µg/mL) was placed in 96-well microplates, followed by the addition of 160 µL of deionized water and 20 µL of FeSO₄ solution (0.30 mmol/L) in each well, and the plate was incubated for 5 min at room temperature. Then, 30 µL of ferrozine solution (0.80 mmol/L) was added to each well. After 15 min of incubation, the absorbance was measured at 562 nm. A decrease in absorbance corresponds to an increase in iron chelating ability. Disodium ethylenediamine tetra-acetic acid (EDTA-Na₂) at varying concentrations (0–1000 µg/mL) was used as a reference [37].

The percentage of an iron chelating ability was calculated as

$$\text{Scavenging effect (\%)} = \{(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}\} \times 100.$$

2.3.3. Hydroxyl Free Radical Scavenging Ability

The hydroxyl free radical scavenging ability was determined for polysaccharides Ajwa samples as described by Ji et al. Successively, 1 mL of each sample at various concentrations (250, 500, 1000, 2000, 5000 µg/mL) was mixed with 2 mL of 9 mM ferrous sulfate solution and 2 mL of 9 mM salicylic acid–ethanol solution. A total of 2 mL of 8.8 mM hydrogen peroxide solution was added, mixed thoroughly, and then maintained at 37 °C for 60 min. Then, the absorbance was determined at 510 nm. The hydroxyl free radical scavenging effect (%) was estimated as below [38].

$$\text{Scavenging effect (\%)} = \{1 - (\text{absorbance of sample} - \text{absorbance of blank}) / \text{absorbance of control}\} \times 100.$$

2.3.4. DPPH Radical Scavenging Assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of Ajwa polysaccharides was determined using the described method [39]. Briefly, 50 µL of polysaccharide samples at various concentrations (250, 500, 1000, 2000, 5000 µg/mL) were pipetted into a 96-well plate, followed by 100 µL of deionized water and 25 µL of methanolic DPPH solution (0.4 mM). The absorbance (Abs) was measured at 517 nm after 30 min of incubation at ambient temperature in the dark. As a positive control, ascorbic acid (0–100 µg/mL) was employed. The following equation was used to calculate the polysaccharide sample's capacity to scavenge DPPH free radicals:

$$\text{DPPH radical scavenging effect (\%)} = (1 - (\text{Abs1} - \text{Abs2}) / \text{Abs0}) \times 100,$$

where Abs1 is the absorbance of the polysaccharides sample, Abs0 is the absorbance of the control (water instead of the sample) and Abs2 is the Abs of the sample in the same way as Abs1 but with methanol instead of DPPH solution [39].

Statistical Analysis

Analysis was carried out in triplicate and results are presented as mean standard deviation (SD). Data were analyzed using an ANOVA test and differences are statistically significant if values of $p < 0.05$.

3. Results and Discussions

3.1. Polysaccharide Extraction

The most common pharmacological research about Ajwa has been concentrated on the extraction of phenolic and flavonoid compounds and studying their bioactivities. Numerous solvents such as methanol, acetone, and ethanol have been employed commonly to isolate a wide spectrum of antioxidant chemicals found in the flesh and seed of Ajwa dates [15,22,40] but there is not much research that studies the antioxidant effects of water-soluble compounds in date (*Phoenix dactylifera*), especially Ajwa date. In this study, we are interested in isolating water-soluble polysaccharides from Ajwa date flesh and seed. We have previously tested several techniques such as cellulase-assisted extraction, amylase-assisted extraction, and microwave-assisted extraction (supplementary material). However, in this study, we will present the results of two extraction techniques, including hot water extraction and ultrasonic-assisted extraction, as they produce polysaccharides that exhibit higher water solubility when compared to other techniques of extraction. The hot water extraction method is a kind of solid–liquid extraction method which takes advantage of the characteristics of solubility of date polysaccharides in water and transfers the polysaccharide in flesh or seed to the solvent water phase. It is also the most widely used extraction method at present [25,41]. Due to its low cost and simple requirements for experimental equipment, it is a relatively economic and green extraction method [41]. In addition, the ultrasonic approach has recently been used to isolate polysaccharides from a variety of plant components [25,42]. By destroying cell walls, ultrasonic treatment increased mass transfer between the plant and the solvent and improved the yield of extracted polysaccharides [43]. A very recent study was focused on polysaccharide isolation from the fruit (flesh) of Ajwa in addition to four other commonly utilized Arabian varieties of date palm by a simple technique that includes decoction and centrifugation [44]. In our study, the polysaccharides were extracted from the seed by hot water extraction and ultrasonic-assisted extraction in percentages of 1.5% and 1.03%, respectively. The ratios of flesh polysaccharides obtained by hot water extraction and ultrasonic extraction were 5.02% and 4.77%, respectively (Table 1). These ratios are comparable to those obtained from two palm species fruits; *Caryota mitis* (2.65%) and *Chamaerops humilis* (3.89%) [45]. The amounts of neutral sugar and uronic acid in the different polysaccharide samples were comparable. The composition of Ajwa date flesh and seed polysaccharides obtained by hot water extraction was approximately 56.84% and 31.2% of neutral sugar and 34.93% and 8.36% of uronic acid, respectively

(Table 1). The amount of neutral sugar and uronic acid in the polysaccharides obtained by ultrasonic extraction was, respectively, 67.35% and 36.46% in Ajwa date flesh and 34.40% and 6.55% in Ajwa date seed (Table 1). Mrabet et al. studied 10 varieties of Tunisian dates and determined that they contain neutral sugar ranging from 15.56% to 25.71% and a low quantity of uronic acid that varies from 4.76% to 7.26 % [46]. Khatib et al. reported a percentage of 32.9% of galacturonic acids in the polysaccharide PF1 fraction extracted from Ajwa flesh [44], which is comparable to our results. Regarding the element analysis results, the amount of carbon, nitrogen, and hydrogen was comparable for the four polysaccharide samples as shown in Table 1.

Table 1. Physicochemical characterization of polysaccharides extracted from Ajwa seed (ASP-HWE and ASP-US) and flesh (AFP-US and AFP-HWE).

Samples	ASP-HWE	ASP-US	AFP-HWE	AFP-US
Ratio (%)	1.5	1.03	5.02	4.77
Neutral sugar (%)	31.2	34.4	56.84	67.35
Uronic acid (%)	8.36	6.55	34.93	36.46
N (%)	1.56	2.012	1.261	1.105
C (%)	31.152	36.38	31.798	31.303
H (%)	5.658	5.967	5.364	5.217
Monosaccharide Composition (Molar Ratio)				
Mannose	1.13	1.67	0.04	0.01
Galacturonic Acid	0.06	0.18	0.62	0.87
Rhamnose	0.03	ND ^a	ND ^a	0.14
Glucose	2.57	3.80	0.10	0.05
Galactose	0.95	1.40	0.02	0.01
Xylose	5.05	1.12	3.12	4.42
Arabinose	5.01	1.11	3.09	4.38
Fucose	0.04	ND ^a	1.10	0.09

^a not detected.

3.2. Infrared Spectroscopy

FTIR spectroscopy is a potent and rapid approach for obtaining structural information on natural polysaccharides. As shown in Figure 1, all the FTIR spectra show a basic polysaccharide backbone. The four samples exhibited a broad peak in the region between 3500 and 3200 cm^{-1} , characteristic of stretching vibration of the OH group (SAP-HWE: 3373 cm^{-1} ; SAP-US: 3373 cm^{-1} ; FAP-HWE: 3421 cm^{-1} ; FAP-US: 3416 cm^{-1}). Additionally, the characteristic band of polysaccharides at approximately 2935 cm^{-1} was assigned to C–H stretching vibration [14]. Ajwa seed polysaccharides present a characteristic band of the CO group of the carboxylic acid group at 1650 cm^{-1} . In addition, the bands at 1384 and 1416 cm^{-1} are correlated to O–C=O bending and may be linked to the presence of uronic acids. Additionally, the absorbance at 1744 cm^{-1} in FAP-HWE and FAP-US confirmed the presence of uronic acid [47], which is in agreement with the results of the carbazole assay that demonstrate a high amount of uronic acid in FAP-HWE and FAP-US. In the 1150–1010 cm^{-1} region, different polysaccharide samples have distinct bands. Ring vibrations dominate this area, which is overlapping with the (C–O–C) glycosidic bond vibration and stretching vibrations of (C–OH) side groups [48]. Bands at 812 cm^{-1} and 893 cm^{-1} may be related to the presence of α - and β -glycosidic bonds [49].

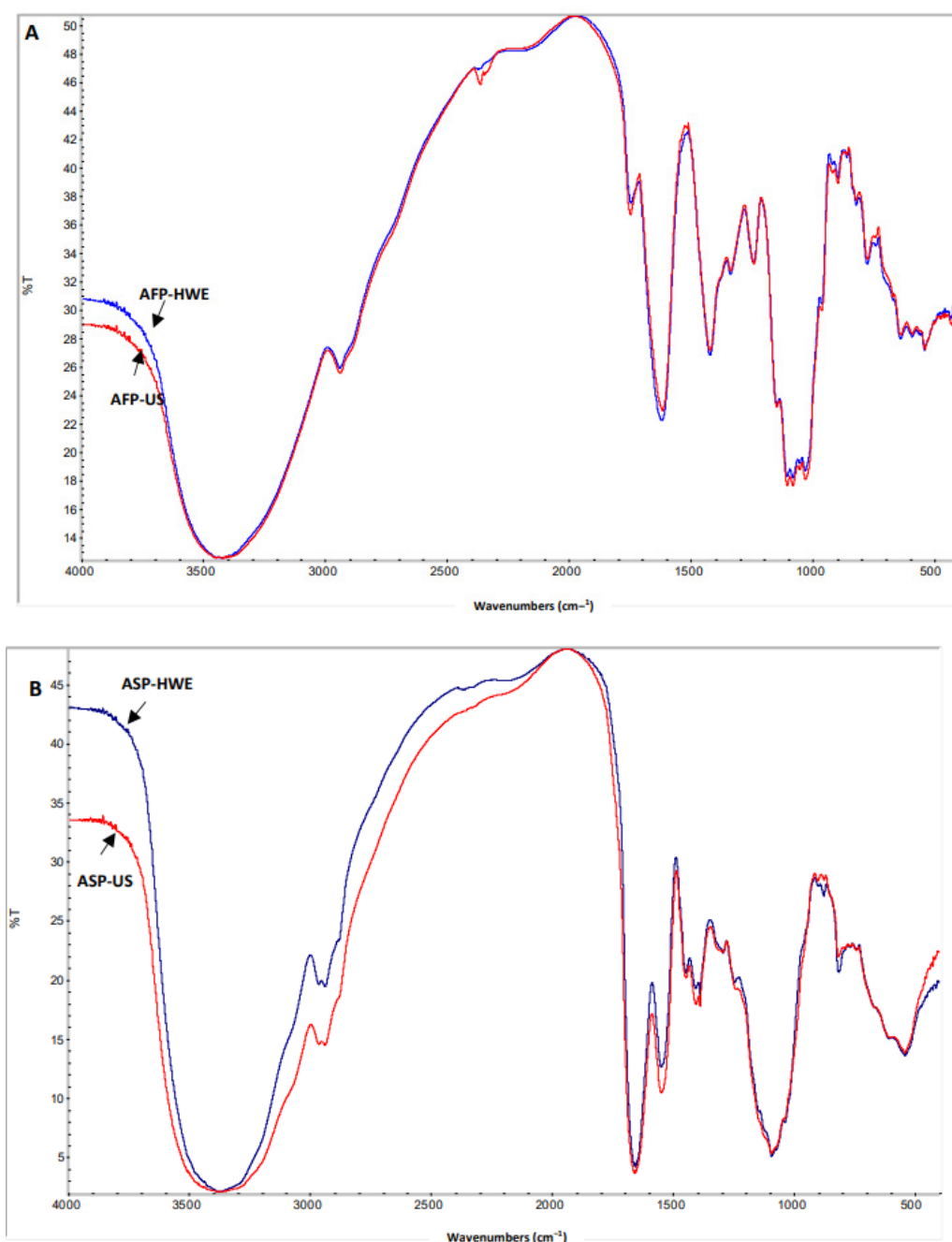


Figure 1. Infrared spectroscopy of polysaccharides extracted from Ajwa flesh (A) and Ajwa seed (B).

3.3. Solid State NMR

NMR is one of the most potent tools for determining the structure of polysaccharides [8]. Particularly, solid-state NMR is an excellent choice for polysaccharide characterization since samples can be analyzed directly in their native state as a solid. The preparation is crucial for studying polysaccharides using solution-state NMR and requires three cycles of solubilization/lyophilization in deuterium oxide (D_2O) leading to the exchange of exchangeable protons in the polysaccharide with deuterium [50], a long process. In this study, ^{13}C CP/MAS NMR spectroscopy was employed to study the chemical structure of polysaccharides extracted from flesh and seed. This approach employs high-resolution ^{13}C solid-state NMR through magic-angle spinning (MAS) and cross-polarization (CP) technology to measure the intensity of ^{13}C signals. [8]. The obtained spectrum (Figure 2) indicated that polysaccharides extracted from Ajwa were typical heteropolysaccharides.

Anomeric carbon in the α -configuration was responsible for the resonance in the range of 97.0–101 ppm, while anomeric carbon in the β -configuration was responsible for the resonance in the range of 103–107 ppm [51,52]. The polysaccharides extracted from Ajwa flesh contained sugar residues in an α -configuration, as indicated by the presence of the anomeric carbon signal at 99.4 ppm while Ajwa seed polysaccharides included sugar residues in a β -configuration. Based on previous data, chemical shift at 103.7 ppm (ASP-HWE), 102.2 ppm (ASP-US) and 99.4 ppm (AFP-HWE, AFP-US) corresponded to C1. The 80–70 ppm peaks belonged to C5, C3, C2, and the 65–60 ppm peak belonged to C4 and C6, respectively [53]. The signal around 174 ppm for ASP and 175 ppm for AFP was assigned to the carboxyl group present in uronic acids [8], which is in concordance with mass spectroscopy and FTIR results. Furthermore, the polysaccharide ASP-US was found to include 1,4-D-galacturonan with an anomeric carbon resonance at 102.2 ppm, according to previous work [54]. The C4 of 1,4-d-galactopyranosyluronan is attributed to the strong signal at 72.4 ppm [54]. According to previous polysaccharide research, the range of 25 to 40 ppm refers to the CH₃, CH₂, and CH groups [55,56]. For Ajwa seed polysaccharides (ASP-HWE and ASP-US), the interval of 0–30 ppm and 120–140 ppm showed additional small and more or less resolved resonances, and they most likely matched minor metabolites (free sugars, amino acids, short peptides, phenolic compounds) [57].

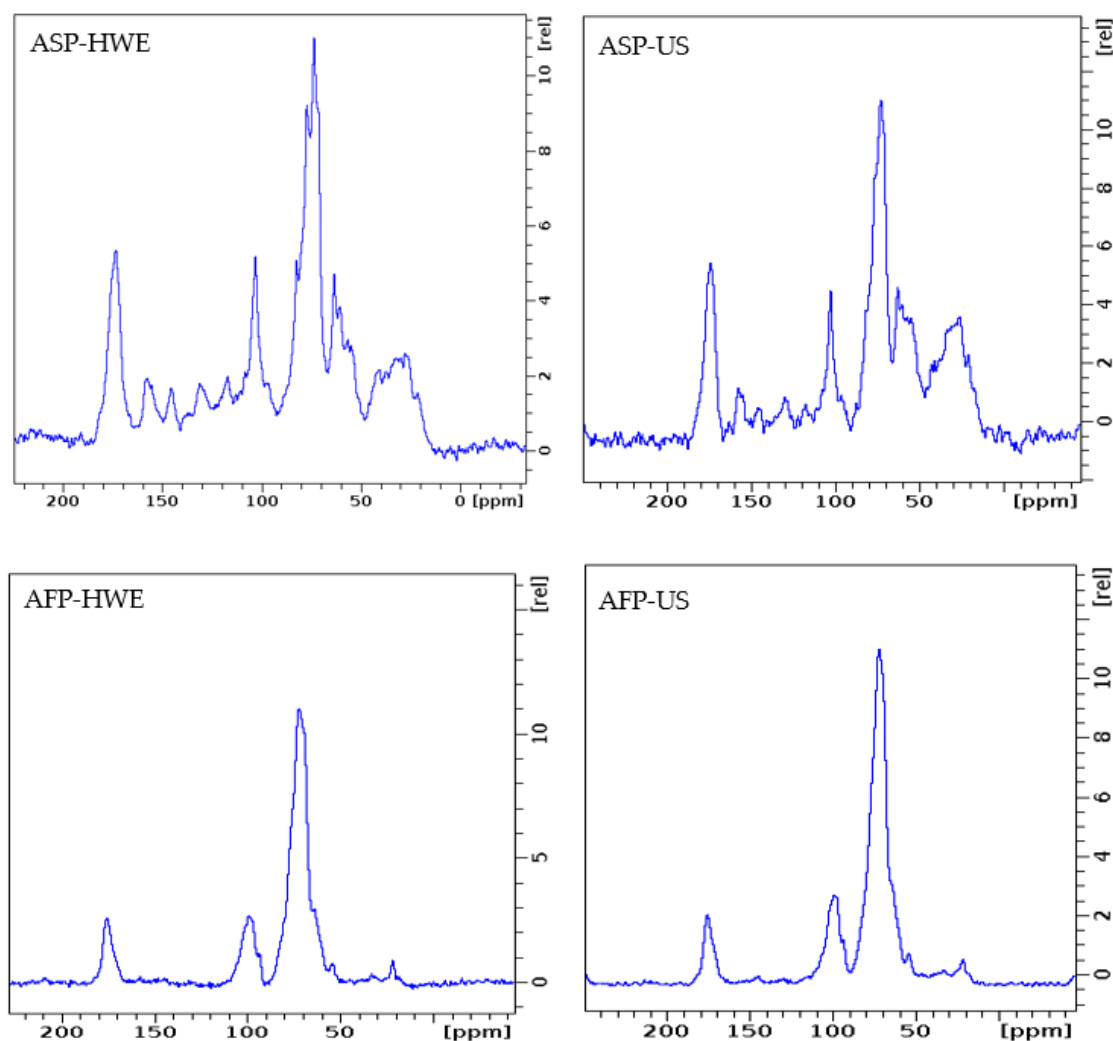


Figure 2. The ^{13}C NMR spectra of polysaccharides extracted from Ajwa seed (ASP) and flesh (AFP) by hot water extraction (HWE) and ultrasonic extraction (US).

3.4. High-Resolution Mass Spectrometry (HR-MS)

Natural polysaccharides have a very complex structure. The procedures employed for the elucidation of the primary structure of polysaccharides are divided into two kinds: chemical analysis and instrumental analysis [41]. In this study, we employed hydrolysis by TFA followed by derivatization. Because monosaccharides have a low ionization efficiency, derivatization of monosaccharides is required for very sensitive detection. A well-known label that reacts with reducing carbohydrates, needing no acid catalyst and resulting in no desialylation or isomerization, is 1-phenyl-3-methyl-5-pyrazolone (PMP), which was first developed in 1989 [34]. Regarding the instrumental method, we employed an ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC-IDX Orbitrap-MS). The results showed a heterogeneous composition of polysaccharides and revealed the presence of the following main monosaccharides components: mannose, glucose, galactose, xylose, arabinose, galacturonic acid, and fucose (Table 1). We constated a varying proportion in monosaccharides composition between the different extracted polysaccharides that are influenced by the source and the technique of extraction. For instance, the most represented sugars in seed were mannose, glucose, xylose, and arabinose. Xylose and arabinose were present in greater amount in ASP-HWE than in ASP-US. For Ajwa flesh polysaccharides, the most abundant monosaccharides were xylose and arabinose. The monosaccharide residues detected in Chinese date polysaccharides were rhamnose, galactose, glucose, fructose, arabinose, mannose, xylose, galacturonic acid, and xylose [41]. Khatib et al. show the occurrence of six main monosaccharide residues. The most abundant were galactose and xylose, in addition to glucose, arabinose, rhamnose, and fucose [44]. Rhamnose and arabinose are present in most Chinese date polysaccharides, while galacturonic acid is more prevalent in some acidic varieties [41].

3.5. Scanning Electron Microscopy (SEM) Analysis

SEM is an effective and useful tool for determining the morphological features of biopolymers such as polysaccharides. Figure 3 shows SEM micrographs of the extracted polysaccharides at magnifications of 500 and 10,000 [58]. Polysaccharides obtained with ultrasonic methods have a compact structure with an irregular spherical and coated-like shape. Polysaccharides obtained by the hot water extraction technique presented a loose and multi-branched organization and low degree of aggregation. As shown, the morphology of extracted polysaccharides from flesh and seed are different, varying with the protocol of extraction.

3.6. Antioxidant Activities

The body will create reactive oxygen free radicals with high oxidation throughout the metabolic oxidation process. Excess reactive oxide species lead to oxidative stress which frequently causes several cell defects such as reduced ATP levels, increased cytosolic Ca^{2+} , damage to DNA, malfunction of biological functions in lipid bilayers, and more [59]. These effects will eventually result in a variety of illnesses such as immune function decrease, aging, and tumors [60]. Antioxidants protect against free radical-induced oxidative damage by various mechanisms [61]. Therefore, combining several radical scavenging experiments was essential to confirm the antioxidant activity of polysaccharides [62,63].

The antioxidant activities of polysaccharides extracted from flesh and seed by hot water extraction and ultrasonic techniques were studied by several tests including ABTS radical scavenging, Fe chelating ability, Hydroxyl free radical scavenging ability, and DPPH radical scavenging assays.

3.6.1. ABTS Radical Scavenging Assay

ABTS is a technique widely used to evaluate the antioxidant activity of polysaccharides. The radical $\text{ABTS}^{\bullet+}$ is produced by oxidizing ABTS with potassium persulfate and it is reduced in the presence of hydrogen-donating antioxidants [64]. The results shown in Figure 4 illustrate the potent ABTS radical scavenging properties of all polysaccha-

rides extracted in this study. The scavenging capacity augments with a concentration in the range of 0–5000 $\mu\text{g/mL}$. ABTS radical scavenging activity at 5 mg/mL was $89.15\% \pm 0.08\%$ and $91.60\% \pm 0.21\%$ for Ajwa seed polysaccharides extracted by hot water extraction and ultrasonic-assisted extraction, respectively, which was significantly higher than the antioxidant effect of an aqueous extract of Ajwa pits reported by Arshad et al. that exhibited an ABTS radical scavenging ability of 26.90% [22]. Date seed extracts from Khalas obtained from water extraction presented lower antioxidant activity when compared to that obtained from acetone and ethanol extraction, which showed the highest DPPH and ABTS radical scavenging effects related to the phenolic and flavonoid contents [65]. The control, ascorbic acid demonstrated the highest antioxidant effect with $86.79\% \pm 2.11\%$ capacity to scavenge $\text{ABTS}^{\bullet+}$ at $100 \mu\text{g/mL}$.

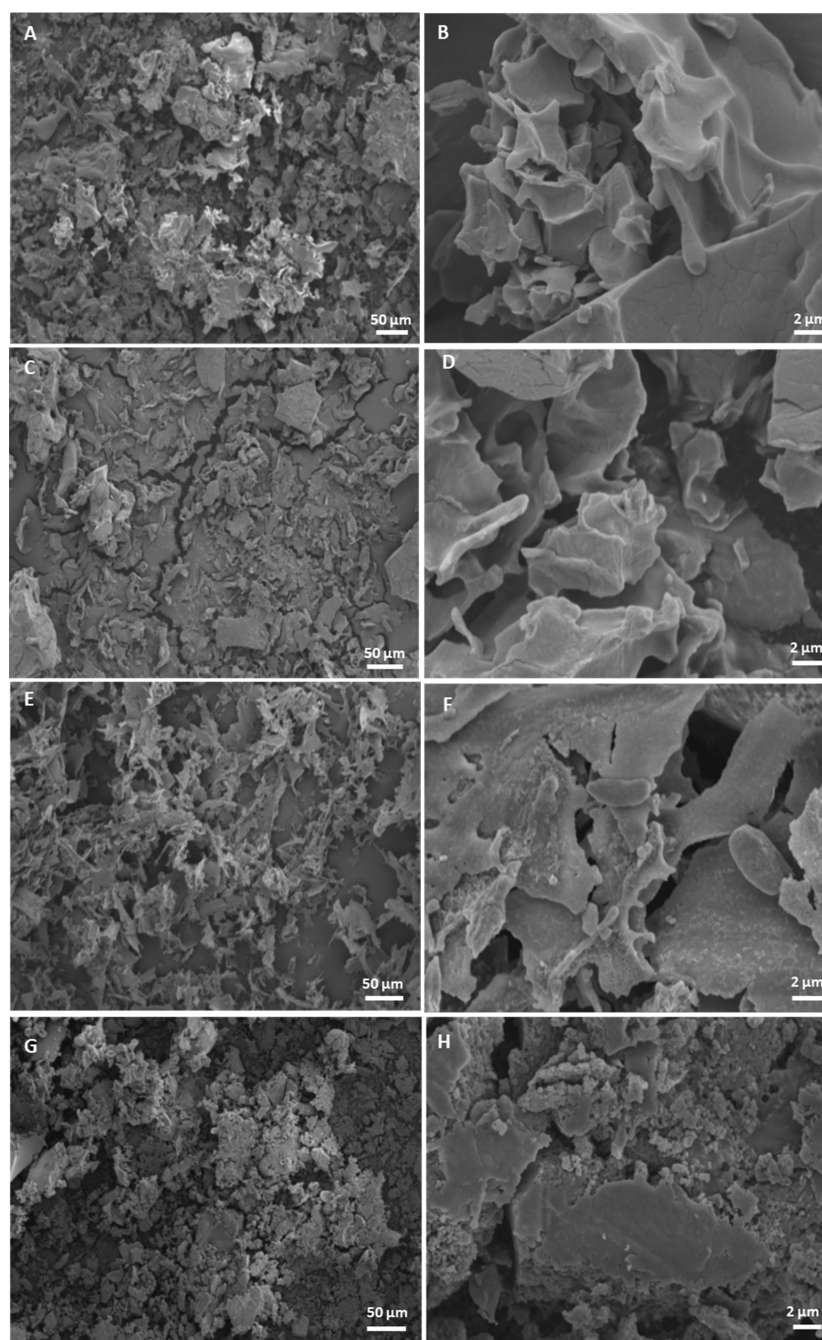


Figure 3. Scanning electron microscopy (SEM) images of polysaccharide fractions isolated from Ajwa flesh and seed: FAP-HWE (A,B), FAP-US (C,D), SAP-HWE (E,F) and SAP-US (G,H).

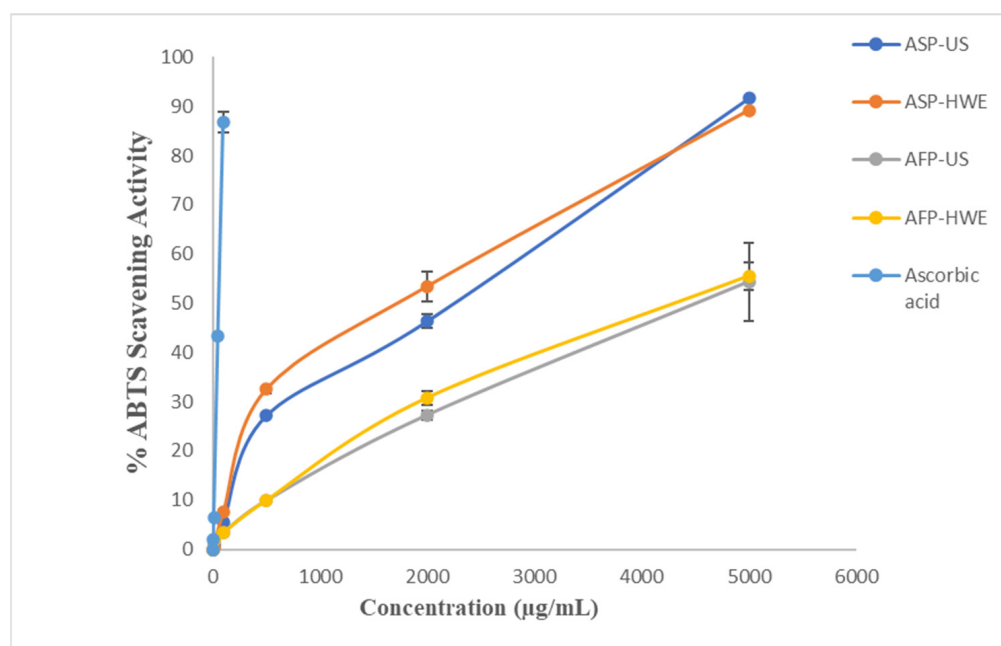


Figure 4. The ABTS scavenging ability of polysaccharides extracted from Ajwa seed and flesh. Results are presented as means $\pm$ standard deviations ($n = 3$). Differences are statistically significant if $p < 0.05$ when compared to the control.

For flesh polysaccharides, the ability of AFP-US and AFP-HWE to scavenge ABTS $^{\bullet+}$ at 5 mg/mL was $54.32\% \pm 7.92\%$ and $55.52\% \pm 2.79\%$, respectively. The scavenging activities of AFP-HWE and AFP-US were less than those of ASP-US and ASP-HWE, and the differences were most significant at 5 mg/mL concentration ($p < 0.05$). These findings suggested that polysaccharides extracted from seed should be investigated as a potential antioxidant due to their significant ABTS radical scavenging ability.

3.6.2. Fe Chelating Ability

The Fenton free radical reaction, which is triggered by ferrous ions, can produce reactive oxygen species and cause oxidative damage to cells. Accordingly, the chelating effect on ferrous ions has recently become a popular method for evaluating part of the antioxidant activity of polysaccharides. The capacity of polysaccharides to chelate iron may be due to the development of cross-bridges between the uronic acid carboxyl group and the divalent ions [66,67]. The chelating capacity of Ajwa polysaccharides increased with concentration, as expected (Figure 5). The Fe^{2+} chelating activities at 2 mg/mL of the four polysaccharides ASP-HWE, ASP-US, AFP-HWE, and AFP-US were $83.06\% \pm 3.32\%$, $82.11\% \pm 1.04\%$, $76.13\% \pm 0.76\%$, and $73.42\% \pm 1.21\%$, respectively. The control EDTA- Na_2 demonstrated the highest antioxidant effect with $93.39\% \pm 0.10\%$ capacity to chelate ferrous at 100 $\mu\text{g/mL}$. With the different radical scavenging experiments, Ajwa polysaccharides showed an antioxidant effect, while their impact on ferrous ions was more noticeable, especially for Ajwa seed polysaccharides. From Chinese dates, Chang et al. isolated four different types of polysaccharides that present a high antioxidant effect. They exhibited a larger scavenging impact on superoxide anions than they did on hydroxyl radicals, whereas the acidic polysaccharide's chelation effect on ferrous ions was more pronounced [68]. According to previous studies, the number of accessible hydroxyl groups in the fractions may influence the chelating capacity of polysaccharides [69,70].

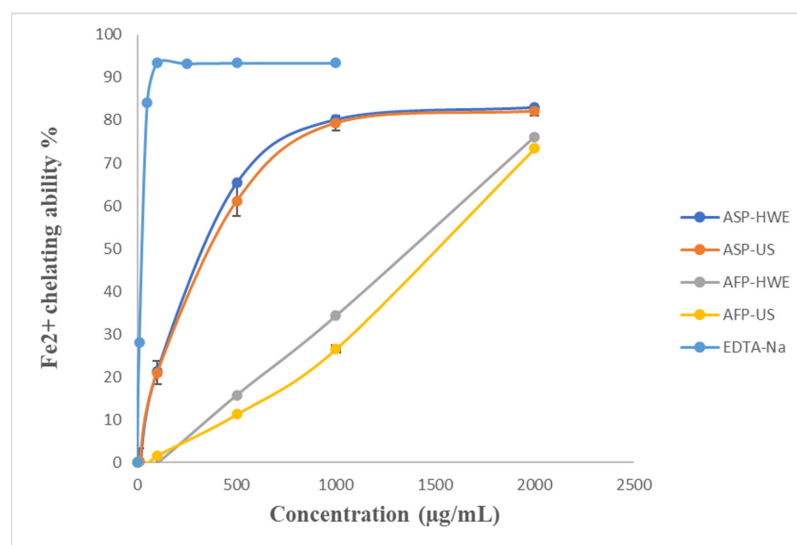


Figure 5. The Fe²⁺ chelating ability of polysaccharides extracted from Ajwa seed and flesh. Results are presented as means $\pm$ standard deviations ($n = 3$). Differences are statistically significant if $p < 0.05$ when compared to the control.

3.6.3. Hydroxyl Free Radical Scavenging Ability

Hydroxyl radicals (OH) are a kind of free radical with a high oxidation capacity. They have a high oxidation efficiency and a quick reaction rate, allowing them to easily oxidize a variety of biological macromolecules. It is a free radical that causes tissue lipid peroxidation, nucleic acid degradation, and protein and polysaccharide breakdown [71]. Figure 6 shows the evaluation of the capacity of extracted polysaccharides to scavenge hydroxyl radicals at doses varying from 0.1 to 5 mg/mL. The scavenging abilities of ASP-HWE, ASP-US, AFP-HWE, and AFP-US at 5 mg/mL were 41.29% $\pm$ 2.17%, 39.55% $\pm$ 1.16%, 25.59% $\pm$ 1.50%, and, 26.62% $\pm$ 3.79%, respectively, which was comparable to recent research in which the scavenging activities of polysaccharides extracted from Chinese yam such as CYCP, CYP1, CYP2, and CYP3 were 30.9%, 32.6%, 44.21% and 53.1%, respectively, at 1 mg/mL, and did not increase at higher concentrations [28]. Ajwa seed polysaccharides had a greater capacity for hydroxyl radical scavenging than flesh polysaccharides at the experimental maximum concentration of 5 mg/mL, but there was no significant difference between ASP-HWE and ASP-US and between AFP-HWE and AFP-US.

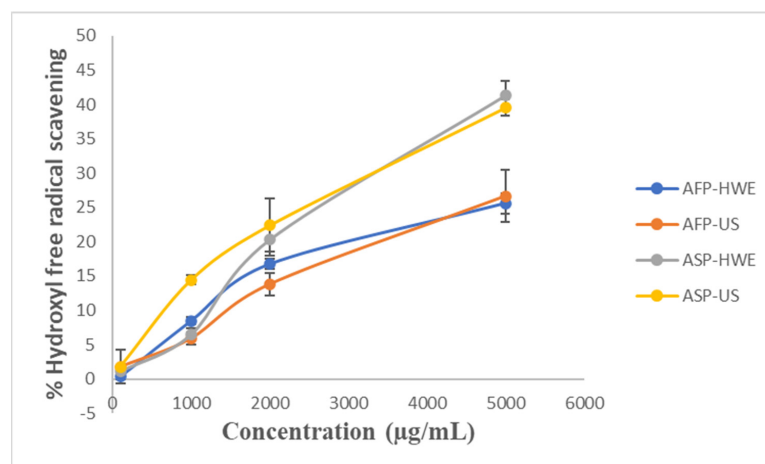


Figure 6. Hydroxyl free radical scavenging ability of polysaccharides extracted from Ajwa seed and flesh. Results are presented as means $\pm$ standard deviations ($n = 3$). Differences are statistically significant if $p < 0.05$ when compared to the control.

3.6.4. DPPH Radical-Scavenging Activity

DPPH solution that has a purple color presents maximal adsorption at 517 nm. The radicals are scavenged in the presence of reducing substances and transform into a non-radical form, which has a yellow color and decreased adsorption at 517 nm [72]. Figure 7 shows the scavenging capacity against the DPPH radical of the extracted polysaccharides from flesh and seed by the two techniques. The DPPH radical scavenging effects of the samples varied with the concentration, origin of the polysaccharides (seed or flesh), and the protocol of extraction. The IC₅₀ values obtained for AFP-US, AFP-HWE, ASP-US and ASP-HWE were 1.73 ± 0.01 , 1.92 ± 0.08 , 3.29 ± 0.13 and 3.39 ± 0.26 mg/mL, respectively. The antioxidant activity of Ajwa seed polysaccharides is higher than that of polysaccharides extracted from Tunisian date seed that present a maximum DPPH radical scavenging activity of 36.3% at a concentration of 1 mg/mL [48], whereas extracted polysaccharides from Ajwa seed and flesh were less effective than ascorbic acid (IC₅₀ = 43.57 ± 1.63 µg/mL). The highest DPPH radical scavenging effect of polysaccharide samples was observed for AFP-US, which presents the highest amount of uronic acid (Table 1). Wang et al. convincingly showed that acidic polysaccharides have a greater antioxidative impact than neutral polysaccharides, owing to galacturonic acid's capacity to bind metal ions and so scavenge DPPH radicals [73]. Furthermore, uronic acid-rich polysaccharides have considerable antioxidant activity since the uronic acid residues modify the characteristics of polysaccharides and the solubility of the linked polysaccharide conjugates, according to Ji et al. [69,74]. Notably, the highest scavenging capacity was detected with polysaccharides extracted from Ajwa flesh that correlated favorably with the quantity of uronic acid. In addition, variable polysaccharides may have different DPPH radical scavenging capacities due to variations in surface morphology and monosaccharide content [75]. In addition, Zhang et al. extracted antioxidant polysaccharides from *Zizyphus jujuba* cv. *Muzao* and when compared to homogeneous polysaccharides fractions (HJP1, HJP2, and HJP3), the heterogenous polysaccharide HJP has a higher DPPH radical scavenging and reducing effect [76].

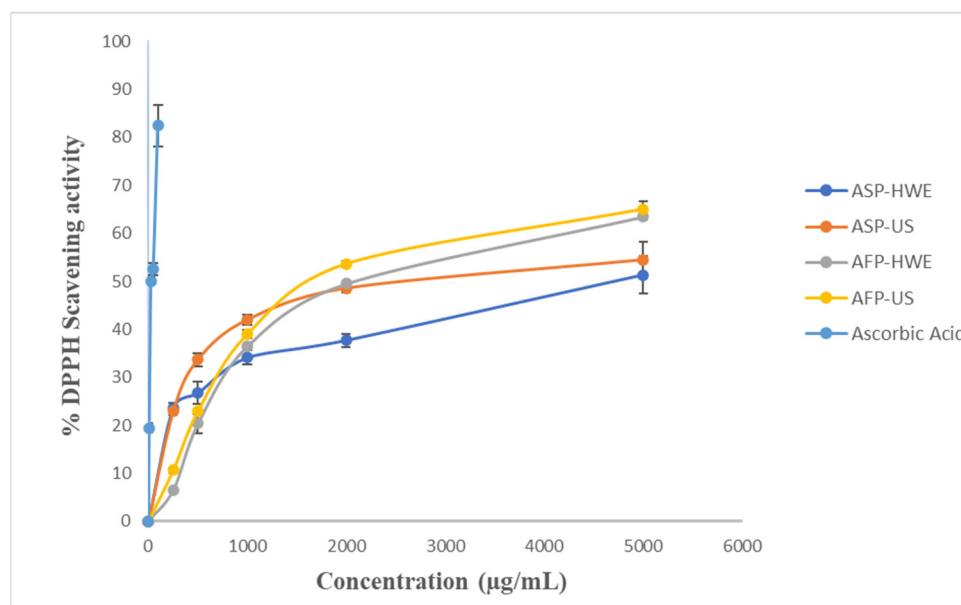


Figure 7. The DPPH scavenging ability of polysaccharides extracted from Ajwa seed and flesh. Results are presented as means $\pm$ standard deviations ($n = 3$). Differences are statistically significant if $p < 0.05$ when compared to the control.

4. Conclusions

This study investigated bioactive polysaccharides extracted from Ajwa date. We extracted interesting water-soluble antioxidant polysaccharides from Ajwa date seed and flesh. Several analytical methods including elemental analysis, FTIR, NMR, and MS

assessment revealed that the extracted compounds have a typical heteropolysaccharide chemical structure. The physicochemical properties of these polysaccharides were different, which influences their antioxidant activities.

The four polysaccharides extracted by hot water and ultrasonic extraction (ASP-HWE, ASP-US, AFP-HWE and AFP-US) possess an interesting antioxidant activity, assessed by different tests including ABTS radical scavenging, Fe chelating ability, hydroxyl free radical scavenging ability, and DPPH radical scavenging assay. The impact on ferrous ions was most noticeable for Ajwa seed polysaccharides. We further suggest that the antioxidant differences between the polysaccharides are due to variances in uronic acid, monosaccharide composition, glycosidic linkages, and other factors. Further investigations of the molecular mechanism of the antioxidant activity of Ajwa polysaccharide should be carried out. These findings provide a scientific basis for the further use of polysaccharides extracted from Ajwa seed and flesh.

Author Contributions: Conceptualization, M.D. and A.-H.M.E.; methodology, M.D.; validation, S.S., A.H. and A.S.; data curation, S.A. and F.A.; writing—original draft preparation, M.D. and S.S.; writing—review and editing, A.-H.M.E. and M.J.; project administration, A.H. and A.-H.M.E.; funding acquisition, M.J. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Taibah university and King Abdullah University of Science and Technology (under fund number BAS/1/1085-01-01) to M.J.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Al-Dashti, Y.A.; Holt, R.R.; Keen, C.L.; Hackman, R.M. Date palm fruit (*Phoenix dactylifera*): Effects on vascular health and future research directions. *Int. J. Mol. Sci.* **2021**, *22*, 4665. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Echegaray, N.; Gullón, B.; Pateiro, M.; Amarowicz, R.; Misihairabgwi, J.M.; Lorenzo, J.M. Date fruit and its by-products as promising source of bioactive components: A review. *Food Rev. Int.* **2021**, 1–22. [\[CrossRef\]](#)
3. Idowu, A.T.; Igiehon, O.O.; Adekoya, A.E.; Idowu, S. Dates palm fruits: A review of their nutritional components, bioactivities and functional food applications. *AIMS Agric. Food* **2020**, *5*, 734–755. [\[CrossRef\]](#)
4. Tang, Z.X.; Shi, L.E.; Aleid, S.M. Date fruit: Chemical composition, nutritional and medicinal values, products. *J. Sci. Food Agric.* **2013**, *93*, 2351–2361. [\[CrossRef\]](#)
5. Mohamed, R.M.; Fageer, A.S.; Eltayeb, M.M.; Mohamed Ahmed, I.A. Chemical composition, antioxidant capacity, and mineral extractability of Sudanese date palm (*Phoenix dactylifera* L.) fruits. *Food Sci. Nutr.* **2014**, *2*, 478–489. [\[CrossRef\]](#)
6. Dhahri, M.; Alghrably, M.; Mohammed, H.A.; Badshah, S.L.; Noreen, N.; Mouffouk, F.; Rayyan, S.; Qureshi, K.A.; Mahmood, D.; Lachowicz, J.I. Natural Polysaccharides as Preventive and Therapeutic Horizon for Neurodegenerative Diseases. *Pharmaceutics* **2021**, *14*, 1. [\[CrossRef\]](#)
7. Alshawwa, S.Z.; Alshallash, K.S.; Ghareeb, A.; Elazzazy, A.M.; Sharaf, M.; Alharthi, A.; Abdelgawad, F.E.; El-Hossary, D.; Jaremko, M.; Emwas, A.-H. Assessment of Pharmacological Potential of Novel Exopolysaccharide Isolated from Marine *Kocuria* sp. Strain AG5: Broad-Spectrum Biological Investigations. *J. Life* **2022**, *12*, 1387. [\[CrossRef\]](#)
8. Dhahri, M.; Sioud, S.; Dridi, R.; Hassine, M.; Boughattas, N.A.; Almulhim, F.; Al Talla, Z.; Jaremko, M.; Emwas, A.M. Extraction, Characterization, and Anticoagulant Activity of a Sulfated Polysaccharide from *Bursatella leachii* Viscera. *ACS Omega* **2020**, *5*, 14786–14795. [\[CrossRef\]](#)
9. Badshah, S.L.; Riaz, A.; Muhammad, A.; Tel Çayan, G.; Çayan, F.; Emin Duru, M.; Ahmad, N.; Emwas, A.-H.; Jaremko, M. Isolation, characterization, and medicinal potential of polysaccharides of *Morchella esculenta*. *Molecules* **2021**, *26*, 1459. [\[CrossRef\]](#)
10. Wang, H.; Liu, Y.; Qi, Z.; Wang, S.; Liu, S.; Li, X.; Wang, H.; Xia, X. An overview on natural polysaccharides with antioxidant properties. *Curr. Med. Chem.* **2013**, *20*, 2899–2913. [\[CrossRef\]](#)
11. Shahbaz, K.; Asif, J.A.; Liszen, T.; Nurul, A.A.; Alam, M.K. Cytotoxic and Antioxidant Effects of *Phoenix dactylifera* L. (Ajwa Date Extract) on Oral Squamous Cell Carcinoma Cell Line. *BioMed Res. Int.* **2022**, *2022*, 5792830. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Ahmad Mohd Zain, M.R.; Abdul Kari, Z.; Dawood, M.A.O.; Nik Ahmad Ariff, N.S.; Salmuna, Z.N.; Ismail, N.; Ibrahim, A.H.; Thevan Krishnan, K.; Che Mat, N.F.; Edinur, H.A.; et al. Bioactivity and Pharmacological Potential of Date Palm (*Phoenix dactylifera* L.) Against Pandemic COVID-19: A Comprehensive Review. *Appl. Biochem. Biotechnol.* **2022**, *194*, 4587–4624. [\[CrossRef\]](#) [\[PubMed\]](#)

13. Hamden, Z.; El-Ghoul, Y.; Alminderej, F.M.; Saleh, S.M.; Majdoub, H. High-Quality Bioethanol and Vinegar Production from Saudi Arabia Dates: Characterization and Evaluation of Their Value and Antioxidant Efficiency. *Antioxidants* **2022**, *11*, 1155. [\[CrossRef\]](#)
14. Oladzad, S.; Fallah, N.; Mahboubi, A.; Afsham, N.; Taherzadeh, M.J. Date fruit processing waste and approaches to its valorization: A review. *Bioresour. Technol.* **2021**, *340*, 125625. [\[CrossRef\]](#)
15. Zhang, C.-R.; Aldosari, S.A.; Vidyasagar, P.S.; Nair, K.M.; Nair, M.G. Antioxidant and anti-inflammatory assays confirm bioactive compounds in Ajwa date fruit. *J. Agric. Food Chem.* **2013**, *61*, 5834–5840. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Khalid, S.; Khalid, N.; Khan, R.S.; Ahmed, H.; Ahmad, A. A review on chemistry and pharmacology of Ajwa date fruit and pit. *Trends Food Sci. Technol.* **2017**, *63*, 60–69. [\[CrossRef\]](#)
17. Hassan, S.M.A.; Aboonq, M.S.; Albadawi, E.A.; Aljehani, Y.; Abdel-Latif, H.M.; Mariah, R.A.; Shafik, N.M.; Soliman, T.M.; Abdel-Gawad, A.R.; Omran, F.M.; et al. The Preventive and Therapeutic Effects of Ajwa Date Fruit Extract Against Acute Diclofenac Toxicity-Induced Colopathy: An Experimental Study. *Drug Des. Dev. Ther.* **2022**, *16*, 2601–2616. [\[CrossRef\]](#)
18. Al Jaouni, S.K.; Hussein, A.; Alghamdi, N.; Qari, M.; El Hossary, D.; Almuhayawi, M.S.; Olwi, D.; Al-Raddadi, R.; Harakeh, S.; Mousa, S.A. Effects of *Phoenix dactylifera* Ajwa on infection, hospitalization, and survival among pediatric cancer patients in a university hospital: A nonrandomized controlled trial. *J. Integr. Cancer Ther.* **2019**, *18*, 1–9. [\[CrossRef\]](#)
19. Elsadek, B.; El-Sayed, E.-S.; Mansour, A.; Elazab, A. Abrogation of carbon tetrachloride-induced hepatotoxicity in Sprague-Dawley rats by Ajwa date fruit extract through ameliorating oxidative stress and apoptosis. *Pak. J. Pharm. Sci.* **2017**, *30*, 2183–2191.
20. Gnanamangai, B.; Saranya, S.; Ponnuragan, P.; Kavitha, S.; Pitchaimuthu, S.; Divya, P. Analysis of antioxidants and nutritional assessment of date palm fruits. In *Sustainable Agriculture Reviews 34*; Naushad, N., Lichtfouse, E., Eds.; Springer: Cham, Switzerland, 2019; pp. 19–40.
21. Anwar, S.; Raut, R.; Alsahli, M.A.; Almatroudi, A.; Alfheaid, H.; Alzahrani, F.M.; Khan, A.A.; Allemailem, K.S.; Almatroodi, S.A.; Rahmani, A.H. Role of Ajwa date fruit pulp and seed in the management of diseases through in vitro and in silico analysis. *Biology* **2022**, *11*, 78. [\[CrossRef\]](#)
22. Arshad, F.K.; Haroon, R.; Jelani, S.; Masood, H.B. A relative in vitro evaluation of antioxidant potential profile of extracts from pits of *Phoenix dactylifera* L. (Ajwa and Zahedi dates). *Int. J. Adv. Inf. Sci. Technol.* **2015**, *35*, 28–37.
23. Boulouar, N.; Marouf, A.; Cheriti, A. Antifungal activity and phytochemical screening of extracts from *Phoenix dactylifera* L. cultivars. *Nat. Prod. Res.* **2011**, *25*, 1999–2002. [\[CrossRef\]](#)
24. Ragab, A.R.; Elkablawy, M.A.; Sheik, B.Y.; Baraka, H.N. Antioxidant and tissue-protective studies on Ajwa extract: Dates from Al-Madinah Al-Monwarah, Saudia Arabia. *J. Environ. Anal. Toxicol.* **2013**, *3*, 163. [\[CrossRef\]](#)
25. Huang, G.; Chen, F.; Yang, W.; Huang, H. Preparation, deproteinization and comparison of bioactive polysaccharides. *Trends Food Sci. Technol.* **2021**, *109*, 564–568. [\[CrossRef\]](#)
26. Li, J.; Fan, Y.; Huang, G.; Huang, H. Extraction, structural characteristics and activities of *Zizyphus vulgaris* polysaccharides. *Ind. Crops Prod.* **2022**, *178*, 114675. [\[CrossRef\]](#)
27. Huang, H.; Huang, G. Extraction, separation, modification, structural characterization, and antioxidant activity of plant polysaccharides. *Chem. Biol. Drug Des.* **2020**, *96*, 1209–1222. [\[CrossRef\]](#)
28. Ashfaq, A.K.; Shahzor, G.K.; Ying, L.; Saghir, A.S.; Yan-Feng, W.; Aijaz, H.S.; Xiaoju, T.; Mamoun, A.H.; Wen, H. Optimization of enzyme assisted extraction of polysaccharides from *Poria cocos*. *J. Med. Plants Res.* **2017**, *11*, 331–337. [\[CrossRef\]](#)
29. Guerreiro, B.M.; Freitas, F.; Lima, J.C.; Silva, J.C.; Reis, M.A. Photoprotective effect of the fucose-containing polysaccharide GuoPol. *Carbohydr. Polym.* **2021**, *259*, 117761. [\[CrossRef\]](#)
30. Coelho, M.N.; Soares, P.A.; Frattani, F.S.; Camargo, L.M.; Tovar, A.M.; de Aguiar, P.F.; Zingali, R.B.; Mourão, P.A.; Costa, S.S. Polysaccharide composition of an anticoagulant fraction from the aqueous extract of *Marsippanthes chamaedrys* (Lamiaceae). *Int. J. Biol. Macromol.* **2020**, *145*, 668–681. [\[CrossRef\]](#)
31. Dhahri, M.; Mansour, M.B.; Bertholon, I.; Ollivier, V.; Boughattas, N.A.; Hassine, M.; Jandrot-Perrus, M.; Chaubet, F.; Maaroufi, R.M. Anticoagulant activity of a dermatan sulfate from the skin of the shark *Scyliorhinus canicula*. *Blood Coagul. Fibrinolysis* **2010**, *21*, 547–557. [\[CrossRef\]](#)
32. Chisca, S.; Duong, P.; Emwas, A.-H.; Sougrat, R.; Nunes, S.P. Crosslinked copolyazoles with a zwitterionic structure for organic solvent resistant membranes. *Polym. Chem.* **2015**, *6*, 543–554. [\[CrossRef\]](#)
33. Alkordi, M.H.; Haikal, R.R.; Hassan, Y.S.; Emwas, A.-H.; Belmabkhout, Y. Poly-functional porous-organic polymers to access functionality–CO₂ sorption energetic relationships. *J. Mater. Chem. A* **2015**, *3*, 22584–22590. [\[CrossRef\]](#)
34. Wu, X.; Jiang, W.; Lu, J.; Yu, Y.; Wu, B. Analysis of the monosaccharide composition of water-soluble polysaccharides from *Sargassum fusiforme* by high performance liquid chromatography/electrospray ionisation mass spectrometry. *Food Chem.* **2014**, *145*, 976–983. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Xu, G.; Amicucci, M.J.; Cheng, Z.; Galermo, A.G.; Lebrilla, C.B. Revisiting monosaccharide analysis—Quantitation of a comprehensive set of monosaccharides using dynamic multiple reaction monitoring. *J. Anal.* **2018**, *143*, 200–207. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Somanjana, K.; Sandipta, G.; Krishnendu, A. Simplified Methods for Microtiter Based Analysis of In Vitro Antioxidant Activity. *Asian J. Pharm.* **2017**, *11*. [\[CrossRef\]](#)
37. Zhang, H.; Cai, X.-T.; Tian, Q.-H.; Xiao, L.-X.; Zeng, Z.; Cai, X.-T.; Yan, J.-Z.; Li, Q.-Y. Microwave-Assisted Degradation of Polysaccharide from *Polygonatum sibiricum* and Antioxidant Activity. *J. Food Sci. Technol.* **2019**, *84*, 754–761. [\[CrossRef\]](#)

38. Ji, X.; Hou, C.; Yan, Y.; Shi, M.; Liu, Y. Comparison of structural characterization and antioxidant activity of polysaccharides from jujube (*Ziziphus jujuba* Mill.) fruit. *Int. J. Biol. Macromol.* **2020**, *149*, 1008–1018. [\[CrossRef\]](#)
39. Chen, G.; Wang, M.; Xie, M.; Wan, P.; Chen, D.; Hu, B.; Ye, H.; Zeng, X.; Liu, Z. Evaluation of chemical property, cytotoxicity and antioxidant activity in vitro and in vivo of polysaccharides from Fuzhuan brick teas. *Int. J. Biol. Macromol.* **2018**, *116*, 120–127. [\[CrossRef\]](#)
40. Jassim, S.A.; Naji, M.A. In vitro evaluation of the antiviral activity of an extract of date palm (*Phoenix dactylifera* L.) pits on a Pseudomonas phage. *Evid. Based Complement. Altern. Med.* **2010**, *7*, 57–62. [\[CrossRef\]](#)
41. Li, J.; Huang, G. Extraction, purification, separation, structure, derivatization and activities of polysaccharide from Chinese date. *Process Biochem.* **2021**, *110*, 231–242. [\[CrossRef\]](#)
42. Ebringerová, A.; Hromádková, Z. An overview on the application of ultrasound in extraction, separation and purification of plant polysaccharides. *Cent. Eur. J. Chem.* **2010**, *8*, 243–257. [\[CrossRef\]](#)
43. Fu, L.; Chen, H.; Dong, P.; Zhang, X.; Zhang, M. Effects of ultrasonic treatment on the physicochemical properties and DPPH radical scavenging activity of polysaccharides from mushroom *Inonotus obliquus*. *J. Food Sci.* **2010**, *75*, C322–C327. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Khatib, M.; Al-Tamimi, A.; Cecchi, L.; Adessi, A.; Innocenti, M.; Balli, D.; Mulinacci, N. Phenolic compounds and polysaccharides in the date fruit (*Phoenix dactylifera* L.): Comparative study on five widely consumed Arabian varieties. *Food Chem.* **2022**, *395*, 133591. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Dawood, D.H.; Elmongy, M.S.; Negm, A.; Taher, M.A. Extraction and chemical characterization of novel water-soluble polysaccharides from two palm species and their antioxidant and antitumor activities. *Egypt. J. Basic Appl. Sci.* **2020**, *7*, 141–158. [\[CrossRef\]](#)
46. Mrabet, A.; Rodríguez-Arcos, R.; Guillén-Bejarano, R.; Chaira, N.; Ferchichi, A.; Jiménez-Araujo, A. Dietary fiber from Tunisian common date cultivars (*Phoenix dactylifera* L.): Chemical composition, functional properties, and antioxidant capacity. *J. Agric. Food Chem.* **2012**, *60*, 3658–3664. [\[CrossRef\]](#)
47. Zhan, R.; Xia, L.; Shao, J.; Wang, C.; Chen, D. Polysaccharide isolated from Chinese jujube fruit (*Zizyphus jujuba* cv. Junzao) exerts anti-inflammatory effects through MAPK signaling. *J. Funct. Foods* **2018**, *40*, 461–470. [\[CrossRef\]](#)
48. Marzouk, W.; Chaouch, M.; Hafsa, J.; LeCerf, D.; Majdoub, H. Antioxidant and antiglycated activities of polysaccharides from Tunisian date seeds (*Phoenix dactylifera* L.). *J. Tunis. Chem. Soc.* **2017**, *19*, 124–130.
49. Zhang, Y.; Zhou, T.; Wang, H.; Cui, Z.; Cheng, F.; Wang, K.-p. Structural characterization and in vitro antitumor activity of an acidic polysaccharide from *Angelica sinensis* (Oliv.) Diels. *Carbohydr. Polym.* **2016**, *147*, 401–408. [\[CrossRef\]](#)
50. Poulhazan, A.; Dickwella Widanage, M.C.; Muszyński, A.; Arnold, A.A.; Warschawski, D.E.; Azadi, P.; Marcotte, I.; Wang, T. Identification and quantification of glycans in whole cells: Architecture of microalgal polysaccharides described by solid-state nuclear magnetic resonance. *J. Am. Chem. Soc.* **2021**, *143*, 19374–19388. [\[CrossRef\]](#)
51. Hu, X.; Xu, F.; Li, J.; Li, J.; Mo, C.; Zhao, M.; Wang, L. Ultrasonic-assisted extraction of polysaccharides from coix seeds: Optimization, purification, and in vitro digestibility. *Food Chem.* **2022**, *374*, 131636. [\[CrossRef\]](#)
52. Tang, G.-H.; Liu, J.-H.; Sun, X.-Y.; Ouyang, J.-M. Carboxymethylation of *Desmodium styracifolium* Polysaccharide and Its Repair Effect on Damaged HK-2 Cells. *Oxidative Med. Cell. Longev.* **2022**, *2022*, 2082263. [\[CrossRef\]](#)
53. Bo, R.; Ji, X.; Yang, H.; Liu, M.; Li, J. The characterization of optimal selenized garlic polysaccharides and its immune and antioxidant activity in chickens. *Int. J. Biol. Macromol.* **2021**, *182*, 136–143. [\[CrossRef\]](#)
54. Akhtar, H.M.S.; Abidin, M.; Hamed, Y.S.; Wang, W.; Chen, G.; Chen, D.; Chen, C.; Li, W.; Mukhtar, S.; Zeng, X. Physicochemical, functional, structural, thermal characterization and α -amylase inhibition of polysaccharides from chickpea (*Cicer arietinum* L.) hulls. *Lwt* **2019**, *113*, 108265. [\[CrossRef\]](#)
55. Maciel, P.D.M.C.; Tavares, M.I.B. Solid state and proton relaxation NMR study of *Dipteryx alata* Vogel. *J. Appl. Polym. Sci.* **2010**, *116*, 50–54.
56. Khan, M.T.; Busch, M.; Molina, V.G.; Emwas, A.-H.; Aubry, C.; Croue, J.-P. How different is the composition of the fouling layer of wastewater reuse and seawater desalination RO membranes? *Water Res.* **2014**, *59*, 271–282. [\[CrossRef\]](#)
57. Wang, J.-Q.; Yin, J.-Y.; Nie, S.-P.; Xie, M.-Y. A review of NMR analysis in polysaccharide structure and conformation: Progress, challenge and perspective. *Food Res. Int.* **2021**, *143*, 110290.
58. Rozi, P.; Abuduwaili, A.; Ma, S.; Bao, X.; Xu, H.; Zhu, J.; Yadikar, N.; Wang, J.; Yang, X.; Yili, A. Isolations, characterizations and bioactivities of polysaccharides from the seeds of three species *Glycyrrhiza*. *Int. J. Biol. Macromol.* **2020**, *145*, 364–371. [\[CrossRef\]](#)
59. Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D. Oxidative stress, aging, and diseases. *Clin. Interv. Aging* **2018**, *13*, 757. [\[CrossRef\]](#)
60. Li, J.; Liu, Y.; Fan, L.; Ai, L.; Shan, L. Antioxidant activities of polysaccharides from the fruiting bodies of *Zizyphus jujuba* cv. Jinsixiaozao. *Carbohydr. Polym.* **2011**, *84*, 390–394. [\[CrossRef\]](#)
61. Aziz, M.A.; Diab, A.S.; Mohammed, A.A. *Antioxidant Categories and Mode of Action*; IntechOpen: London, UK, 2019.
62. Bayar, N.; Kriaa, M.; Kammoun, R. Extraction and characterization of three polysaccharides extracted from *Opuntia ficus indica* cladodes. *Int. J. Biol. Macromol.* **2016**, *92*, 441–450. [\[CrossRef\]](#)
63. Mutailifu, P.; Nuexiati, R.; Lu, C.; Huojiaihemaiti, H.; Abuduwaili, A.; Yili, A. Extraction, purification, and characterization of polysaccharides from *Alhagi pseudoalhagi* with antioxidant and hypoglycemic activities. *Process Biochem.* **2022**, *121*, 339–348. [\[CrossRef\]](#)
64. Ilyasov, I.R.; Beloborodov, V.L.; Selivanova, I.A. Three ABTS^{•+} radical cation-based approaches for the evaluation of antioxidant activity: Fast-and slow-reacting antioxidant behavior. *Chem. Pap.* **2018**, *72*, 1917–1925. [\[CrossRef\]](#)

65. Maqsood, S.; Kittiphattanabawon, P.; Benjakul, S.; Sumpavapol, P.; Abushelaibi, A. Antioxidant activity of date (*Phoenix dactylifera* var. Khalas) seed and its preventive effect on lipid oxidation in model systems. *Int. Food Res. J.* **2015**, *22*, 1180.
66. Wang, N.; Dai, L.; Chen, Z.; Li, T.; Wu, J.; Wu, H.; Wu, H.; Xiang, W. Extraction optimization, physicochemical characterization, and antioxidant activity of polysaccharides from *Rhodospirillum rubrum* sp. SCSIO-45730. *J. Appl. Phycol.* **2022**, *34*, 285–299. [[CrossRef](#)]
67. Wu, Y.-T.; Huo, Y.-F.; Xu, L.; Xu, Y.-Y.; Wang, X.-L.; Zhou, T. Purification, characterization and antioxidant activity of polysaccharides from *Porphyra haitanensis*. *Int. J. Biol. Macromol.* **2020**, *165*, 2116–2125. [[CrossRef](#)]
68. Chang, S.; Hsu, B.; Chen, B. Structural characterization of polysaccharides from *Zizyphus jujuba* and evaluation of antioxidant activity. *Int. J. Biol. Macromol.* **2010**, *47*, 445–453. [[CrossRef](#)]
69. Liu, X.-X.; Liu, H.-M.; Yan, Y.-Y.; Fan, L.-Y.; Yang, J.-N.; Wang, X.-D.; Qin, G.-Y. Structural characterization and antioxidant activity of polysaccharides extracted from jujube using subcritical water. *Lwt* **2020**, *117*, 108645. [[CrossRef](#)]
70. Wang, J.; Hu, S.; Nie, S.; Yu, Q.; Xie, M. Reviews on mechanisms of in vitro antioxidant activity of polysaccharides. *Oxidative Med. Cell. Longev.* **2016**, *2016*, 5692852. [[CrossRef](#)]
71. Fan, J.; Wu, Z.; Zhao, T.; Sun, Y.; Ye, H.; Xu, R.; Zeng, X. Characterization, antioxidant and hepatoprotective activities of polysaccharides from *Ilex latifolia* Thunb. *Carbohydr. Polym.* **2014**, *101*, 990–997. [[CrossRef](#)]
72. Arab, K.; Ghanbarzadeh, B.; Ayaseh, A.; Jahanbin, K. Extraction, purification, physicochemical properties and antioxidant activity of a new polysaccharide from *Ocimum album* L. seed. *Int. J. Biol. Macromol.* **2021**, *180*, 643–653. [[CrossRef](#)]
73. Wang, C.; Chang, S.; Inbaraj, B.S.; Chen, B.-H. Isolation of carotenoids, flavonoids and polysaccharides from *Lycium barbarum* L. and evaluation of antioxidant activity. *Food Chem.* **2010**, *120*, 184–192. [[CrossRef](#)]
74. Ji, X.; Peng, Q.; Yuan, Y.; Shen, J.; Xie, X.; Wang, M. Isolation, structures and bioactivities of the polysaccharides from jujube fruit (*Zizyphus jujuba* Mill.): A review. *Food Chem.* **2017**, *227*, 349–357. [[CrossRef](#)] [[PubMed](#)]
75. Liu, X.; Chen, Y.; Wu, L.; Wu, X.; Huang, Y.; Liu, B. Optimization of polysaccharides extraction from *Dictyophora indusiata* and determination of its antioxidant activity. *Int. J. Biol. Macromol.* **2017**, *103*, 175–181. [[CrossRef](#)]
76. Zhang, L.; Liu, X.; Wang, Y.; Liu, G.; Zhang, Z.; Zhao, Z.; Cheng, H. In vitro antioxidative and immunological activities of polysaccharides from *Zizyphus Jujuba* cv. Muzao. *Int. J. Biol. Macromol.* **2017**, *95*, 1119–1125. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.