Structural Characterization of Mannoglucan Isolated from Ophiocordyceps sobolifera and Its Antioxidant Activities

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ABSTRACT: A novel polysaccharide structure (PS-T80) was collected from Ophiocordyceps sobolifera biomass and characterized via a combination of chemical and spectral analyses. Employing high-performance gel permeation chromatography (HPGPC), the average molecular weight is proven to be $7.4 \times 10^4$ Da. Furthermore, a sugar composition analysis of the obtained polysaccharide suggests two main sugars, $\beta$-D-glucose and $\alpha$-D-mannose, at a molar ratio of 2:1, respectively, in the backbone. The structure analysis unveils that PS-T80 is a mannoglucan, possessing the repeating unit of $[\beta$-$\text{D-Glcp}(1 \rightarrow 3)-\alpha$-$\text{D-Manp}(1 \rightarrow 3)-\beta$-$\text{D-Glcp}(1 \rightarrow \cdots)]_n$. Such a configuration could be considered a novel polysaccharide. Impressively, in vitro antioxidant tests revealed that PS-T80 has a promising antioxidant activity. These results demonstrate that the obtained PS is a potential bioactive material for biomedical applications.

1. INTRODUCTION

The genus Cordyceps has been tracked in nature in Asian countries (e.g., China, Japan, Korea, Thailand, and Vietnam)\(^1,2\) and is known as a special entomophagous medicinal fungus\(^3,4\) that has been utilized as the best tonic during the development of human society. Indeed, some Cordyceps spp. have proven the capability to boost immunity, resist fatigue, prolong longevity, and associated antioxidant performance of polysaccharides of several diseases.\(^5-9\) The genus Cordyceps spp. have proven the biological activities in fungi, and it has been collected and is known as a special entomophagous medicinal fungus\(^3,4\) rooted in its pharmacological activities from respiratory, renal, hepatic, neurological, and cardiovascular diseases to antitumor, pharmacological activities from respiratory, renal, hepatic, neurological, and cardiovascular diseases to antitumor, oxidative stress, and renal injury in endotoxemic rats.\(^10\) However, the structure and activation of extraction. In previous investigations, we investigated the structure of PS-T100 from Ophiocordyceps sobolifera. The molecular mass of the obtained polysaccharide, extracted at a temperature of 100 °C for 3 h and in triplicate, is in the range of $\sim 2.29 \times 10^3$ Da. Its glucose to mannose molar ratio was 3:2 in the structure.\(^10\) However, such a high temperature facilitates the extraction of the polysaccharide possessing a high molecular mass. Consequently, there is insufficient understanding of the chemical structure and antioxidant activity of O. sobolifera-derived small-molecular-mass polysaccharides.

Herein, we explore the idea that a novel structure of PS could be extracted from O. sobolifera. Thus, a mild condition is employed to extract and purify polysaccharides (denoted as PS-T80). Structural characterizations of the extracted PS-T80 unveil repeating units of $[\beta$-$\text{D-Glcp}(1 \rightarrow 3)-\alpha$-$\text{D-Manp}(1 \rightarrow 3)-\beta$-$\text{D-Glcp}(1 \rightarrow \cdots)]_n$ associated with a molar glucose to $O. sobolifera$ are insufficient, and it is significant to explore the chemical structure—antioxidant activity correlation of various PSs. For these reasons, the extraction and purification of PSs from O. sobolifera species in Vietnam are pursued with utmost urgency. Moreover, it should be noted that the differences in original sources of samples, cultivation conditions, and species could cause distinctions between the components, structure, and activation of extraction. In previous investigations, we investigated the structure of PS-T100 from Ophiocordyceps sobolifera. The molecular mass of the obtained polysaccharide, extracted at a temperature of 100 °C for 3 h and in triplicate, is in the range of $\sim 2.29 \times 10^3$ Da. Its glucose to mannose molar ratio was 3:2 in the structure.\(^10\) However, such a high temperature facilitates the extraction of the polysaccharide possessing a high molecular mass. Consequently, there is insufficient understanding of the chemical structure and antioxidant activity of O. sobolifera-derived small-molecular-mass polysaccharides.

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mannose ratio that was found to be 2:1. The molecular mass is located in the range of $7.4 \times 10^4$ Da. Such a chemical structure is a novel PS. Interestingly, the in vitro antioxidant tests suggest that the obtained PS-T80 could function as a natural antioxidant. This exploration further contributes to filling in the puzzle of *O. sobolifera*-isolated PSs and discloses a novel pathway toward the employment of the Vietnam *O. sobolifera*-collected PSs for potential biomedical applications.

2. RESULTS AND DISCUSSIONS

2.1. Structure Characterizations. 2.1.1. Determination of Average Molecular Weight. Figure 1 shows a PS-T80 gel permeation high-performance liquid chromatogram (see the Experimental Section for details), revealing two distinguished $M_w$ peaks located at $1.81 \times 10^4$ and $1.53 \times 10^5$ Da, separately. The PS-T80 average molecular weight is roughly $7.40 \times 10^4$ Da. The polydispersity index is approximately 1.83, implying a broad molecular weight distribution of the isolated PS-T80. In other words, the PS-T80 is a multidispersion PS.

The PS-T80 average molecular weight is lower than isolated PS from the same species introduced by Lu et al. (2016), which is $1.53 \times 10^4$ kDa. Furthermore, the obtained value is higher than those extracted from *Cordyceps sinensis* (*C. sinensis*) and *Cordyceps militaris* (*C. militaris*), ranging from 12.9 to 460 kDa.30–33

2.1.2. FT-IR Investigation of Polysaccharides. Fourier-transform infrared spectroscopy (FTIR) was employed to investigate crucial vibrations of the isolated PS-T80 sample, as depicted in Figure 2, in which four primary absorption bands could be observed. The intense peak centered at $\sim 3383$ cm$^{-1}$ indicates the O–H stretching vibrations of hydroxyl groups. The bands at 2893 and 2924 cm$^{-1}$ could be attributed to the C–H stretching vibration. The appearance of the peak located at 1637 cm$^{-1}$ could be induced by associated water.34,35 The notable absorption band at 1408 cm$^{-1}$ is caused by C=O stretching vibrations. It should be noted that the bands at 1100 ± 1000 cm$^{-1}$ could be affected by an axial and equatorial (OH) side group effect. The maxima ascribed to the side group and ring vibrations can be associated with the polysaccharide spectrum. Hence, the characteristic absorption at 1076 and 1043 cm$^{-1}$, indicated one axial (OH) group, located in either position C-2 or C-4 of mannose and β-configurations in glucan, respectively.36 Furthermore, the notable absorption bands at 889 cm$^{-1}$ could be induced by β-type glycosidic linkages.36

2.1.3. Methylation Analysis. The methylation was conducted to investigate the monosaccharide composition and sugar linkage positions in the PS-T80 sample (see the Experimental Section). In this circumstance, the pieces of monosaccharide after methylation were determined by a GC-MS analysis. It turns out that the PS-T80 is a heteropolysaccharide consisting of two primary methylated sugar derivatives, which are 3-O-substituted glucopyranosyl (i.e., 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylglucitol) and 3-O-substituted mannopyranosyl (i.e., 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylmannitol). These findings indicate that monosaccharides in PS-T80 are located in the pyranose ring form. Such a characteristic is rooted in the existence of D-mannose and D-glucose with a molar ratio of 1:2 in the backbone, which is found to be consistent with the HPLC analysis, as shown in Figure S2. Hence, two distinct linkages, which are (1 $\rightarrow$ 3)-
linked glucosyl and (1 → 3)-linked mannosyl, exist in PS-T80. It can be said that the obtained monosaccharide composition of PS-T80 is found to be different from those in previous reports, owing to the varieties in cultivation conditions, sample sources, and species.\textsuperscript{3,11,33,37,38}

2.1.4. 1D and 2D NMR Spectroscopy Investigations. The nuclear magnetic resonance (NMR) technique was employed to deeply investigate the structure of the sample PS-T8, as displayed in Figures 3−6. The one-dimensional NMR spectrum at 500 MHz of the PS-T80 sample reveals two anomeric proton signals at 5.15 ppm (d, $J_{H-1,H-2} = 4$ Hz) and 4.56 ppm (d, $J_{H-1,H-2} = 8$ Hz), respectively, implying the presence of disaccharide repeating units and the existence of $\alpha$- and $\beta$-anomeric configurations in the isolated PS-T80 sample. It should also be noted that the 3.16−3.80 ppm location could be assigned to other sugar protons. These achievements are consistent with the FT-IR and $^1$H NMR findings of described polysaccharides.\textsuperscript{39−41} The anomeric proton-driven chemical shifts, of less than 5.0 ppm, could be attributed to $\beta$-linked residues\textsuperscript{39−41} while the values exceeding 5 ppm indicate $\alpha$-type configurations.

Figure 4 shows a one-dimensional $^{13}$C NMR spectrum at 125 MHz of PS-T80 that depicts two different anomeric signals, peaking at 95.9 and 92.1 ppm, respectively, corresponding to $\beta$-linked glucopyranosyl and $\alpha$-D-mannopyranosyl. The one-dimensional NMR result signifies that the PS-T80 repeat unit is composed of glucose and mannose at a molar ratio of 2:1. This result is very consistent with both the methylation and HPLC analysis, in which the sugar composition analysis signifies the existence of $\beta$-glucose and $\beta$-mannose.

The 2D NMR investigation further brings out the chemical structure of PS-T80 from \textit{Ophiocordyceps sobolifera}, as depicted in Figure 5. The $^1$H−$^{13}$C HSQC spectrum shows that 12 cross-peaks are positioned at 3.16−5.15 and 60.6−95.9 ppm, thereby confirming an anomeric resonance region. The signals at 4.56/95.9, 3.33/75.8, 3.63/72.8, 3.65/60.8, 3.80/60.6, and 3.32/69.7 ppm, separately, imply residue A. Furthermore, the signals at 5.15/92.1, 3.45/71.5, 3.16/74.2, 3.42/76.0, 3.75/71.5, and 3.38/69.6 are attributed to residue B. The monosaccharides derived from the hexose form could be unveiled through the cross-peak number belonging to the methylene group region.

The proton $^1$H−$^1$H COSY spectra indicate the interaction of an adjacent hydrogen within A and B, which could be interpreted as follow: between A H-2/A H-3, A H-4/A H-3/A H-5, A H-5/A-6; and between B H-3/B H-2/B H-4, B H-5/B H-4/B H-6, as displayed in Figure 6. Such information unveils the carbon linkage sequence within the PS-T80 monosaccharides. The protons and adjacent carbon interactions ($^1$H → $^{13}$C) could be verified in the HMBC spectrum, as shown in Figure 7. The couples A H-1/A C-2; A H-2/A C-3; A H-3/A C-4/A C-2; A H-4/A C-5/A C-3; and A H-5/A C-6; and B H-1/B C-2; B H-3/B C-4/B C-2; and B H-5/B C-4/B C-6 were determined. These outcomes disclose the hydrogen and carbon bonding order in the PS-T80 sugar components (Table 1). An anomeric proton and carbon cross-linking bearing at the A H-1 and B C-3 and B H-1 and A C-3 linkages could be identified. These linkages highlight the existence of A(1 → 3)B and B(1 → 3)A bonding, which is consistent with the GC−MS result.
2.2. Antioxidant Activity Properties. 2.2.1. Total Antioxidant Capacity. The total antioxidant capacity was evaluated via the phospho-molybdenum method (see the Experimental Section for more details). In principle, the antioxidant compounds induce the reduction of Mo(VI) into Mo(V) at a low pH, associating with maximal absorbance at 695 nm. The antioxidant capacity could be measured through the number of equivalents of either ascorbic acid or gallic acid. In the current situation, the standard curve equations of gallic acid and ascorbic acid are found to be $Y = 0.7820 \times X_{GA} + 0.1648$, $R = 0.9966$; and $Y = 4.5974 \times X_{AS} - 0.3231$, $R = 0.9952$; respectively. Here, $Y$, $X_{GA}$ and $X_{AS}$ stand for optical density and concentration, respectively. The total antioxidant capacity of PS-T80 is found to be $0.1460 \pm 0.0011$ mg GA/g or $0.1222 \pm 0.0003$ μmol AS/g at the concentration of 1.5 mg/mL. This result suggests that the polysaccharide possesses an antioxidant capacity.

2.2.2. ABTS Radical Scavenging Activity. The ABTS$^+$ scavenging activities of PS-T80 rise along with the increasing concentration of PS-T80. Indeed, the ABTS$^+$ scavenging activities are 34.16%, 43.18%, 52.11%, and 59.54%, corresponding to the concentration range from 2 to 6 mg/mL, as shown in Figure 8. The IC$_{50}$ of PS-T80 is found to be 4.83 mg/mL. The scavenging rates of PS-T80 reach around 60% at 6 mg/mL, which is considerably lower than that of ascorbic acid at the same scavenging rate (i.e., 98.46%). The ABTS radical scavenging activity of the presented polysaccharides is greater than those of both C. militaris polysaccharides (IC$_{50}$: from 7.264 to 6.966 mg/mL).13

![Figure 6](https://pubs.acs.org/doi/10.1021/acsomega.1c06651)  
Figure 6. 2D $^1$H–$^1$H COSY spectra of the isolated PS-T80 sample from O. sobolifera: (a) overall spectrum; (b) close-up spectrum.

![Figure 7](https://pubs.acs.org/doi/10.1021/acsomega.1c06651)  
Figure 7. HMBC spectrum resulting from the isolated PS-T80 sample.

### Table 1. NMR Chemical Shifts ($\delta$, ppm) and Correlations Involving Atoms Observed in the HSQC, HBMC, and COSY spectra of PS from O. sobolifera Recorded in D$_2$O

| sugar residue position | $\delta_H$ | $\delta_C$ | HSQC ($^1$H $\rightarrow ^1$C) | COSY ($^1$H $\rightarrow ^1$H) | HMBC ($^1$H $\rightarrow ^1$C) |
|-----------------------|------------|------------|-------------------------------|-------------------------------|-------------------------------|
| A: $\rightarrow 3$)-$\beta$-$\delta$-glucopyranoside(1$\rightarrow $) | 1 | 4.56 | 95.9 | 4.56/95.9 | 4.56/75.8 |
|                       | 2 | 3.33 | 75.8 | 3.33/75.8 | 3.33/63.6 |
|                       | 3 | 3.63 | 72.8 | 3.63/72.8 | 3.63/63.6/3.33 |
|                       | 4 | 3.65 | 60.8 | 3.65/60.8 | 3.65/63.6/3.80 |
|                       | 5 | 3.80 | 60.6 | 3.80/60.6 | 3.80/3.32 |
|                       | 6 | 3.32 | 69.7 | 3.32/69.7 | 3.32/3.80 |
| B: $\rightarrow 3$)-$\alpha$-$\delta$-mannopyranoside(1$\rightarrow $) | 1 | 5.15 | 92.1 | 5.15/92.1 | 5.15/71.5 |
|                       | 2 | 3.45 | 71.5 | 3.45/71.5 | 3.45/3.16 |
|                       | 3 | 3.16 | 74.2 | 3.16/74.2 | 3.16/3.45/3.42 |
|                       | 4 | 3.42 | 76.0 | 3.42/76.0 | 3.42/3.16/3.75 |
|                       | 5 | 3.75 | 71.5 | 3.75/71.5 | 3.75/3.42/3.38 |
|                       | 6 | 3.38 | 69.6 | 3.38/69.6 | 3.38/3.75 |

https://doi.org/10.1021/acsomega.1c06651
2.2.3. DPPH Radical Scavenging Activity Study. The antioxidant activity from biological extracts, which are generally associated with high levels of bioactive compounds, could be evaluated through the DPPH free radical. As shown in Figure 8, the DPPH radical scavenging activities also improve with an increase in PS-T80 concentration. The scavenging rate at concentrations of 0.4, 0.6, 0.8, 1.0, 2.0, and 2.5 mg/mL are 16.52%, 23.79%, 40.68%, 51.36%, 78.75%, and 93.85%, respectively. The IC50 values are approximately 0.97 mg/mL and the antioxidant activity of PS-T80 is slower than that of ascorbic acid, which is 4.58.

The DPPH radical scavenging activities of the PS-T80 polysaccharides are also comparable to those of reported medicinal fungi while they are lower than that of C. sobolifera polysaccharides (i.e., 6% at 1 mg/mL) at a polysaccharide concentration of 1.0 mg/mL.43 Moreover, the found value is higher than those of both of the polysaccharides from C. sinensis (i.e., IC50 of 1.23 mg/mL)12 and C. militaris (i.e., IC50 of 1.15 mg/mL).93 Thus, the presented polysaccharides exhibit potent antioxidant properties.

3. CONCLUSIONS

This work demonstrates the polysaccharides’ structural characterizations and antioxidant activities collected from O. sobolifera species grown in Vietnam. It has been proven that the isolated polysaccharide is heterogeneous and is repeatedly constructed by β-D-glucose and α-D-mannose units. This polysaccharide possesses a molecular mass of roughly 7.40 × 104 Da. The 1D and 2D NMR and FT-IR characterizations and antioxidant activities collected from Ophiocordyceps sobolifera comprising [→3]-β-D-Glcp-(1 → 3)-α-D-Manp-(1 → 3)-β-D-Glcp-(1 →)n units for the first time. In vitro, antioxidant tests revealed that the PS-T80 has the most potent antioxidant activity. The total antioxidant capacity of PS-T80 is 0.1460 ± 0.0011 mg GA/g or 0.1222 ± 0.0003 μmol AS/g at a concentration of 1.5 mg/mL. The results suggest that the PS-T80 polysaccharide could function as an efficient antioxidant to scavenge DPPH and ABTS radicals with IC50 values of 0.97 and 4.83 mg/mL, respectively.

4. EXPERIMENTAL SECTION

4.1. Material Samples. O. sobolifera was supplied by Aloha Medicinals. Its biomass was grown in Nha Trang province, Vietnam. The culture medium contained the following: phosphate buffer pH 7; 50 mL of cicada extract (1 mg/mL) (cicada: Dundubia nagarasingna Distant, 1881); 45 g of glucose; 1.5 g of KH2PO4; and 0.75 g of MgSO4 made up to a 500 mL solution with distilled water and then disinfected at 121 °C for 20 min.

4.2. Chemicals. Sephadex G-100, (CH3)2SO, CH3I, NaBH4, (CH3CO)2O, and trifluoroacetic acid, CHCl3; and n-butanol, DPPH, gallic acid, and ascorbic acid were obtained from Sigma-Aldrich. Dialysis membranes [diethylaminoethyl (DEAE)-cellulose-S2], possessing a Mw cutoff from 8000 to 14,000 Da, were produced by Thermo Fisher. All utilized chemicals were laboratory grade and can be used without further purification, except for ethanol purchased from the local providers.

4.3. Extraction, Isolation, And Puriﬁcation of Poly- saccharides T-80. The steps for extracting PS-T80 from Ophiocordyceps sobolifera (Figure S1) are depicted in Scheme 1.

The dried biomass of Ophiocordyceps sobolifera was crushed into a fine powder and sieved through a 100-mesh sieve. The powder samples (50 g) were defatted, small molecules that were decolorized with 1000 mL of 95% ethanol three times at 78 °C for 3 h. The residue was collected using centrifugation. Afterward, the residue was extracted three times with hot water (80 °C) at a ratio of 1:50 (w/v) for 3 h each time. It should be noted that the selected extraction temperature was the optimum point, originating from the antioxidant activities of samples collected at various extraction conditions from 60 to 100 °C. The mixture was collected through centrifugation at 6000 rpm for 15 min to remove residue after the extraction. The vacuum evaporator removed the supernatant at 60 °C to obtain the extract solution, precipitated by 96% ethanol (4× the extract solution volume) and stored at −10 °C for 1 day. The precipitation was then harvested and washed under cold ethanol, followed by acetone twice. In the end, the product was dried at 40 °C under a vacuum to produce polysaccharide powder. The yield of PS-80 was approximately 5% (w/w).23,44,45

The PS-T80 sample was dissolved in water before being subjected to dialyzation and evaporation to result in a concentrated solution for purification. In order to remove proteins, Sevag agent (a mixture of chloroform and n-butanol; 4:1 v/v) was introduced into this solution prior to being...
loaded into an anion-exchange chromatography column. The matrix is diethylaminoethyl (DEAE)-cellulose-52 (26 mm × 500 mm). Deionized water and NaCl solution at various concentrations (e.g., 0.1, 0.3, and 0.5 M) were separately employed to elute the sample at a flow rate of 1 mL/min. The polysaccharide content was monitored by the phenol–sulfuric method.46 Samples, which appeared at the same elution polysaccharide content, were regrouped, dialyzed, concentrated, and then named as follows: Fraction I corresponds to distilled water; fractions II–IV are associated with NaCl concentrations of 0.1, 0.3, and 0.5 M, respectively. After undergoing exchange chromatography, the largest water-eluted fraction (i.e., neutral carbohydrates) was collected and loaded into a Sephadex G-100 column (10 mm × 600 mm) with a 0.2 mL/min flow rate for purification. Deionized water and NaCl concentrations (i.e., 0.1 and 0.3 M) were employed. Finally, the pure polysaccharide samples were collected for further investigations. The molecular weight of the lower fraction was selected for structural analysis.47

4.4. Structure Characterization of PS-T80. 4.4.1. Determination of the Homogeneity and Molecular Weight. The average molecular weight (Mw), number average molecular weight (Mn), and polydispersity index (Mw/Mn) of the PS-T80 were analyzed by gel permeation chromatography (GPC, Agilent 1100), as described by Wang et al. (2014)48 with several modifications. Briefly, the sample dispersed in the 0.1 M NaNO3 solution and filtrated by 0.45 μm cellulose acetate filters was injected into the system (Agilent 1100 Series, microTOF-QII Bruker) for analysis. The running program (e.g., injection volume, column temperature, and flow rate) was maintained. Pullulan with molecular masses located in the range 5–800 kDa was utilized as the standard, loaded by an ultrahydrogel column (7.8 mm × 300 mm, 10 μm, 500 Å). Elution conditions were employed as follows: 0.1 M NaNO3 at 40 °C and a flow rate of 1.0 mL/min. The elution volumes versus the logarithms of their respective molecular weights were then plotted.

4.4.2. Methylation and the Identification of Monosaccharide Composition. Methylation of Polysaccharides. The methylation process was performed several times to transform completely free –OH groups into –OCH3. The polysaccharide samples were methylated through the utilization of iodomethane and solid NaOH for 1 day at 60 °C in dimethyl sulfoxide (DMSO) under a stream of N2.

PS Hydrolysis. This step is to convert the glycosidic –O– linkages interconnecting monosaccharides into new free –OH and –CHO groups, which are then turned into –CH2–O–COCH3 and –COCH3 groups, presented in methylated monosaccharide alditol acetates. Particularly, a certain amount of the methylated PS (5 mg) underwent hydrolyzation with trifluoroacetic acid (TFA, 4 mL) at 120 °C for 2 h. The obtained product was then treated under N2 gas. Afterward, the excess TFA was eliminated by MeOH-mediated coevaporation under a N2 atmosphere.

To this end, a 0.25 M solution of NaBH4 in NH3 was introduced for 30 min at room temperature to transform the reduction of partially methylated monosaccharides. A solution (5 mL) of acetic acid (10%) in MeOH was added to naturalize the sample. Excess boric acid was then removed by coevaporation with MeOH under a N2 atmosphere. A mixture (2 mL) of (CH3CO)2O/pyridine (1:1, v/v) was added to promote acetylation at 100 °C for 20 min. After being dried under the N2 flow, the resulting products were dispersed in CHCl3 for the GC–MS analysis (Shimadzu 2010). The running program was established as 150 °C for 1 min, 250 °C for 10 min, and 280 °C for 5 min.

4.4.3. FTIR Study. The FTIR spectrum of the PS-T80 sample was recorded via a spectrophotometer (IRPrestige-21, Shimadzu, Japan). A certain amount of vacuum-dried sample (e.g., 2 mg) was pressed with KBr (100 mg) to form a pellet before being put into an IR machine and scanned at wavenumbers of 400–4000 cm–1.

4.4.4. NMR Method. An NMR spectrometer (Bruker AM500 FT-NMR) performing at 500 and 125 MHz was applied to obtain the 1H and 13C NMR spectra of the polysaccharide samples. 1D NMR was recorded at 353 K. Before the analysis, the PS-T80 samples (20 mg) were partially hydrolyzed, utilizing 5 mL of 2 M TFA at 80 °C for 24 h in a water bath (VNB22, Memmert, Germany), and then lyophilized. Hydrolyzed PS (10 mg) was dissolved in 10 mL of D2O and analyzed. The delay (DI) and acquisition time (AQ) were 1.00 and 3.28 s for 1H NMR spectra and 2.0 and 1.1 s for 13C NMR, respectively. The 2D spectra (HSQC, HMBC, and COSY) were utilized to verify the sugar residues. All chemical shifts were expressed in parts per million (ppm).

4.5. Antioxidant Activity Study. 4.5.1. Total Antioxidant Activity. The total antioxidant activity is evaluated by the phospho-molybdenum method. Briefly, a 0.3 mL aliquot of the sample was mixed with 3 mL of a reagent solution composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was then incubated at 95 °C for 90 min prior to being cooled to 25 °C for measurement. The measurement wavelength was 695 nm. Absorbance was calculated versus a blank, which contained 3 mL of the reagent solution without sample.49 The number of equivalents of gallic acid (GA)50 and ascorbic acid (AS) was utilized to calculate the total antioxidant activity.51

4.5.2. ABTS Radical Scavenging Assay. In this test, ABTS (7 mM) was mixed with potassium persulfate (2.45 mM) in the dark at ambient temperature for 16 h to produce the ABTS radicals.52 A quantity of sample (0.1 mL) possessing the concentration range 2–6 mg/mL was mixed with 3.9 mL of ABTS** solution and was measured at a wavelength of 734 nm. In this circumstance, ascorbic acid was employed as a positive control. The scavenging capability toward ABTS** was calculated as follows:

\[
\text{scavenging rate (\%)} = \left[1 - \frac{A_t}{A_0}\right] \times 100
\]

where \(A_0\) and \(A_t\) are the absorbance of the blank and the sample, respectively; the IC50 value was evaluated through radical scavenging activity.

4.5.3. Investigation of DPPH Radical Scavenging Activity. The DPPH free radical scavenging activity of samples was evaluated with the assistance of a Jasco V-630 spectrophotometer. Particularly, 2 mL of 0.4–2.5 mg/mL extract was dissolved in 1 mL of 100 μM DPPH in ethanol. The reaction mixture was homogenized for 1 min and incubated at ambient temperature for 30 min. Then, its optical density (OD) was measured at a 517 nm wavelength.53 Ascorbic acid was employed as a positive control with concentrations in the range 2–10 μg/mL. Ethanol (3 mL) was used as a blank sample. The scavenging activity of polysaccharides on DPPH radicals was calculated according to the following equation:

\[
\text{scavenging rate (\%)} = \left[1 - \frac{(A_t - A_2)/A_0}\right] \times 100
\]
where A0, A1, and A2 are the absorbances of the blank sample (i.e., 2 mL of distilled water and 1 mL of DPPH solution), polysaccharide sample (i.e., 2 mL of samples and 1 mL of DPPH solution), and control sample (i.e., 2 mL of samples and 1 mL of ethanol), respectively. The IC50 value was utilized to evaluate the radical scavenging activity.

4.6. Statistical Analysis. All of the experiments were repeated three times (n = 3), and the data were displayed as the mean value ± standard deviation (SD), within significance P < 0.05 after an analysis of variance (ANOVA).

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06651.

Photo of Ophiocordyceps sobolifera biomass; and HPLC chromatograms of glucose and mannose in a ratio of 2:1 and PS-T80 (PDF)

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**Notes**

The authors declare no competing financial interest.

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