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Research Article

Purification and Structural Characterization of a Novel Water-Soluble Neutral Polysaccharide from *Cantharellus cibarius* and Its Immunostimulating Activity in RAW264.7 Cells

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Polysaccharide is one of the important active ingredients of *Cantharellus cibarius*. The aims of this work were to analyze preliminary characterization and to investigate immunostimulating activity of a novel water-soluble neutral polysaccharide named JP1, which was purified from the fruiting body of *Cantharellus cibarius* using DEAE-FF chromatography and Sephadex G-100 chromatography. The characteristics of JP1 were determined by HPGPC, FT-IR spectra, gas chromatography, and Congo Red Method. Immunostimulating activity of JP1 was investigated in RAW264.7 cells. Results indicated that JP1 consisted of L-Arabinose, D-Mannose, D-Glucose, and D-Galactose in a molar ratio of 1 : 1.06 : 1.95 : 1.17 with a molecular weight of 336 kDa. JP1 is nontoxic to RAW264.7 cells at this concentration range (62.5–1000 μg/mL). Furthermore, JP1 can promote mouse peritoneal macrophages to secrete NO and enhance the secretion of macrophages' cytokines IL-6 in RAW264.7 cells. These results suggested that JP1 could have potential immunostimulating activity applications as medicine or functional food.

1. Introduction

Chronic inflammation has been strongly related to a wide range of progressive diseases, such as cancer, neurological disease, metabolic disorder, and cardiovascular disease [1, 2]. Polysaccharides are natural polymers that exist widely in plants, animals, and microorganisms [3–5]. Previous studies have demonstrated that the polysaccharides play a vital role in the life of living organisms [6–8]. In particular, plant polysaccharides have attracted much more attention from experts in the food and biomedical fields, due to their biological activities [9–11].

Special attention was payed to mushrooms over recent decades, which could be used as food or medical materials. As reported, many constituents isolated from mushrooms have been confirmed to have the bioactivities like antitumor, antiinfection, immunomodulating, and blood sugar controlling [12–14]. These active constituents include proteins, polysaccharides, and protein–polysaccharide complexes. Among them, the polysaccharide occupies an important position. For example, the polysaccharides of edible and medicine mushroom have the potential application as biological response modifier (BRM) [15, 16]. The research of both the structure and physiological effects of polysaccharides receives more and more attention.

Cantharellus cibarius, one kind of popular edible macrofungi (mushrooms), is commonly known as chanterelle, yellow silk fungus, or golden chanterelle [17]. It is cultivated mainly in North America, Northern Europe, and China. It incrementally attracted attention of researchers in the past decade for its high nutritional and medical value. It is rich in protein, carbohydrates, carotene, vitamin C, vitamin D, potassium, calcium, phosphorus, and other nutrients [18–20].

As a traditional medicine, it is believed that chanterelles can benefit the stomach, purge toxins in liver, clear the lung, and improve the eyesight. The function of antitumor, reducing blood glucose, antioxidation, curing the diseases caused by VA deficiency, like nyctalopia, xerodermia, and

keratomalacia, and preventing the infection of respiratory and digestive tract have come forward by modern medicine [18, 21–23]. It is also proofed that a protein bound polysaccharide fraction from *Cantharellus cibarius* possessed immunemodulating function that observably promotes the proliferation of mouse splenocytes with the dose-dependent manner [24, 25]. Moreover, the antioxidation and hypoglycemic functions of polysaccharides extracted from *C. cibarius* have been studied [26].

The properties of polysaccharides are depending on the structure. The primary structure, for example, the composition, linkage pattern, and sequence of the monosaccharide, would have significantly impacted on the bioactivities. What is more, the molecular conformation and stereoconfiguration are interwovenness with the biological function of polysaccharides. Since the heterogeneity of the polysaccharide involved may complicate the understanding of the underlying mechanisms, the in-depth study of the polysaccharide is difficult to carry on. The existing researches on polysaccharides of *C. cibarius* are confined to *in vitro* simulating and simple cell experiments [27].

Macrophage is one kind of the phagocyte that plays vital roles in the innate immune responses, embryogenesis, tumorigenesis, cutaneous wound healing, and hematopoiesis. Macrophages can serve as an antigen-presenting cells (APCs) that present antigen to T lymphocytes and, then, induce the adaptive immune response when pathogenic organisms invade the host. So macrophage is always considered as an deal cell model to examine the immunomodulatory characteristics of bioactive compounds.

Polysaccharides from mushrooms have been found to improve the viability of macrophage against pathogenic microorganisms and tumorigenesis through increasing the secretion of nitric oxide (NO) and the production of cytokines, such as tumor necrosis factor α (TNF-α) and interleukin (IL-1, IL-2, IL-6, IL-8, IL-10, and IL-12) [28]. Previous study showed that polysaccharides could bind to specific membrane receptors of macrophage and activate the immune response [7]. However, the mechanisms involved in the activation of macrophages by polysaccharides are still not clear. Thus, it is useful to explore the structural characterizations, biological functions, and molecular mechanisms of the polysaccharides from *Cantharellus cibarius*.

In this present study, the main purpose of the present work was to isolate and purify a novel water-soluble neutral polysaccharide (denoted as JP1) from the fruit body of *Cantharellus cibarius* by DEAE-FF chromatography and Sephadex G-100 chromatography, and then we determined the structural characteristics and conformation using UV, IR, and GC–MS techniques methods. Furthermore, we investigated the immunostimulating activities in mouse macrophage cell line RAW264.7. Based on these results, the mechanism of immunostimulating activity of JP1 was also discussed. These results from our study might provide useful information to understand better the chemical structure and immunomodulatory activities of botanical polysaccharides from *Cantharellus cibarius*.

2. Materials and Methods

2.1. Materials. Dried *Cantharellus cibarius* fruiting body was purchased from Kunming Jianhang Trading Co., Ltd. Dialysis tube of molecular weight cut-off (MWCO) = 3000 kDa was purchased from Guangzhou Qiyun Biological Technology Co., Ltd. Chloroform and N-butyl alcohol were acquired from Guangzhou Dongju Experimental Apparatus Co., Ltd. DEAE-Sepharose Fast Flow: S8800 was obtained from Pharmacia 17-0709-10. EDTA disodium was purchased from Jian Yang Biotechnology Co., Ltd. The DEAE-FF chromatography and Sephadex G-100 chromatography were purchased from GE Healthcare Life Science (Piscataway, NJ). The murine macrophage cell line RAW 264.7 was acquired from American Type Culture Collection (ATCC, Rockville, MD). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate-buffered saline (PBS, pH 7.4) were purchased from Gibco Life Technologies (Grand Island, NY). Trypsin digestion EDTA was acquired from Evergreen Co., Ltd. MTT reagent (B.R.) and lipopolysaccharide (LPS, B.R.) were obtained from America Sigma Companies. Dimethylethylene SA was acquired from Tianjin Fu Yu Fine Chemical Co., Ltd. NO kit was acquired from Pik days Ltd. All of the other chemical reagents applied in the work were of analytical grade.

2.2. Extraction and Preparation of JP1. The purchased fruit body of *Cantharellus cibarius* was dried by hot air at 60°C and crushed into powder using a grinder. In this experiment, hot water extraction was applied. The polysaccharides were extracted in boiling water bath at a ratio of 1 : 20 (w/v) for 2 h with continuous stirring. After 2 rounds of extraction, the mixture was centrifuged at 3500 rpm for 15 min; then the combined supernatants were concentrated to 200 mL using the vacuum rotatory evaporator at 55°C, followed by deproteination using the Sevag method. The process was repeated 30 times until no chloroform-protein gel existed. Three volumes of 75% ethanol were added to the resulting solution and kept for 24 h at 4°C. Then they were separated with centrifuge and discarded the supernatant. The deposit was dried with electrothermal air drying oven at 40°C for 2 h, followed by redissolving with distilled water. Put the resulting solution into the ultralow temperature freezer (−80°C) for two days to prefreeze and then it was freeze-dried at −50°C for 48 h to obtain the water-decocting extract, which is always called crude polysaccharide [29, 30].

DEAE-Sepharose Fast Flow column chromatography was applied to further separate the polysaccharide [31]. The crude polysaccharide was dissolved with distilled water to prepare the sample solution (10 mg/mL). Firstly, 10 mL of the sample solution was pipetted into the column and then eluted with distilled water. In this study, the eluent rate was 1.2 ml/min and the collection time was 5 min per tube. After 30 tubes, the eluents were detected by phenol-sulfuric acid method [32]. An orange yellow compound would be found if the polysaccharide exists in the eluent and then the multimode microplate reader was used to detect the absorbance at 490 nm. Afterwards, the elution curve was drawn using the value of absorbance and tube number, combining the eluents

with the same peak. The eluents were stored in the plastic bottles and frozen at 20°C. Eluents with the same peak were combined and concentrated at 50°C and then freeze-dried, which we called CJP1.

After that, the CJP1 fraction was further purified by Sephadex G-100 column chromatography. A total of 20 mg of CJP1 was redissolved in 10 mL of distilled water and loaded onto a Sephadex G-100 column. The sample was eluted with distilled water at a speed of 1.2 ml/min, and then the eluent was collected and detected using the phenol–sulfuric acid method. JP1 fraction was collected after gel filtration, dialysis, and freeze-drying. The JP1 powder was white in color.

2.3. Determination of Molecular Weight. The molecular weight of JP1 was measured by high-performance gel permeation chromatography (HPGPC). It was performed on a Waters instrument equipped with TSK-GEL G-5000 PWXL column (300 mm × 7.8 mm inner diameter, 10 μm) and TSK-GEL G-3000 PWXL column (300 mm × 7.8 mm inner diameter, 6 μm) connected in series and eluted with 0.02 mol/L KH_2PO_4 at a flow rate of 0.6 mL/min as previous study with some modifications [33].

2.4. Periodate Oxidation–Smith Degradation. 25 mg of JP1 was dissolved in 12.5 mL of distilled water, and 12.5 mL of $NaIO_4$ (30 mmol/L) was then added in the dark at room temperature. During the incubation period, 0.1 mL of aliquots was withdrawn at 6, 12, 24, 36, and 48 h intervals, diluted to 100 mL with distilled water, and read using a spectrophotometer at 223 nm, until the optical density value became stable. Glycol (2 mL) was used to stop periodate oxidation. The solution of periodate product (2 mL) was titrated to calculate the production of formic acid by 0.01 mol/L NaOH, and the rest was extensively dialyzed against tap water and distilled water for 48 h. The residue was concentrated and reduced with $NaBH_4$ (70 mg). The solution was placed at room temperature for 24 h, neutralized to pH 6.0–7.0 with 50% acetic acid, dialyzed as described above, and concentrated to a volume of 10 mL followed by freeze-drying. Subsequent treatments were performed as described in previous study [34].

The residues (10 mg) were hydrolyzed with 4 mL of 2 mol/L trifluoroacetic acid in a sealed glass tube at 110°C for 6 h. Acetylation was carried out with 10 mg of hydroxylamine hydrochloride and 0.5 mL of pyridine for 30 min at 90°C. Next, 0.5 mL of acetic anhydride was added with continuous heating. The acetate derivative was analyzed using an Agilent gas chromatograph 6890N system with a HP-45 column (30 m × 0.25 mm i.d., 0.25 μm) and a flame ionization detector. The temperature program was set to increase to 220°C from 80°C atan increment of 2°C/min, then elevate to 250°C at an increment of 5°C/min, and hold for 5 min at 250°C. The detector was set at 250°C. The injection volume was 1.0 μL. Glucose, galactose, glycol, glycerol, and erythrite were used as standards.

2.5. Determination of Monosaccharide Composition. Gas chromatography (GC) can be used to determine the composition of neutral monosaccharide as previous study [35, 36]. The acetylation was carried out with 10 mg JP1, 10 mg hydroxylamine hydrochloride, and 0.5 mL pyridine at 90°C for 30 min. After cooling down, 0.5 mL of acetic anhydride was added at 90°C for 0.5 hours. Water and chloroform were added to extract three times after cooling. Then the chloroform layer was evaporated and the residue dissolved in chloroform. It was filtered with 0.22 μm vacuum filter and analyzed by GC. The percentages of monosaccharides in the sample were calculated from the peak areas using response factors.

The ultraviolet-visible waveband scanning was used to evaluate the purity of the polysaccharides. The polysaccharides were redissolved with distilled water and prepared to 1 mg/mL solution. 5 mL of the solution is enough for the experiment. The scanning range was 200 nm to 800 nm and the interval was 1 nm.

The infrared spectrum can detect the glycosidic linkage conformation and the presence of functional group on the polysaccharide. The samples were submitted to Analytical and Testing Center of Jinan University. An FT-IR spectrophotometer was used to determine the IR spectra of samples within the range of 4000 cm^{-1} to 400 cm^{-1} [5].

2.6. Determination of Tertiary Structure Using Congo Red Method. We determined the tertiary structure of JP1 using Congo Red Method as previous study with some modifications [34]. 1 mg/L solution of JP1 polysaccharide was mixed with 0.1 mmol/L Congo Red solution and then 1 mol/L NaOH solution was added to make the final concentration of 0.05, 0.1, 0.15, 0.20, 0.30, 0.35, and 0.40 mol/L. They were placed for 10 min at room temperature. At last, they were scanned within 600–400 nm to get the relation between the maximum absorption wavelength of Congo Red and the concentration of NaOH. Besides, 0.1 mmol/L Congo Red solutions mixing with NaOH solution was taken to make a final concentration of NaOH which is consistent with the above and was also scanned within 600–400 nm.

2.7. Preparation of JP1 Samples for Cell Culture. The polysaccharide sample JP1 should be dubbed into seven different doses with sterile and low sugar DMEM; thus the concentrations are 62.5 μg/mL, 125 μg/mL, 250 μg/mL, 500 μg/mL, and 1000 μg/mL.

The vials containing cells were taken out from the liquid nitrogen tank and thawed for 1 min in the 37°C water bath immediately. In the meantime, shake it constantly to make it melt. After spraying alcohol into the clean bench, the solution was poured into 15 mL centrifuge tube and then the prepared DMEM medium was added 10 times. It was mixed and then centrifuged for 5 min (1000 rpm/min). Discarding the supernatant, 10 mL fresh medium was mixed in and was pipetted repeatedly with Pasteur pipette until it became the suspension. The total number of cells was counted by cell counting board and adjusted to the concentration of 5×10^4 cells/mL. Put it into a flask, add about 3 mL medium, and then put it in the CO_2 incubator (37°C, 5%) for 24 h. After the original broth drained, rinse it with PBS for 1-2 times and then add 3 mL fresh medium [10].

RAW 264.7 cells were seeded in 96-well cell culture plate (100 μL/well) in the concentration of 10^6 cells/mL. Then they

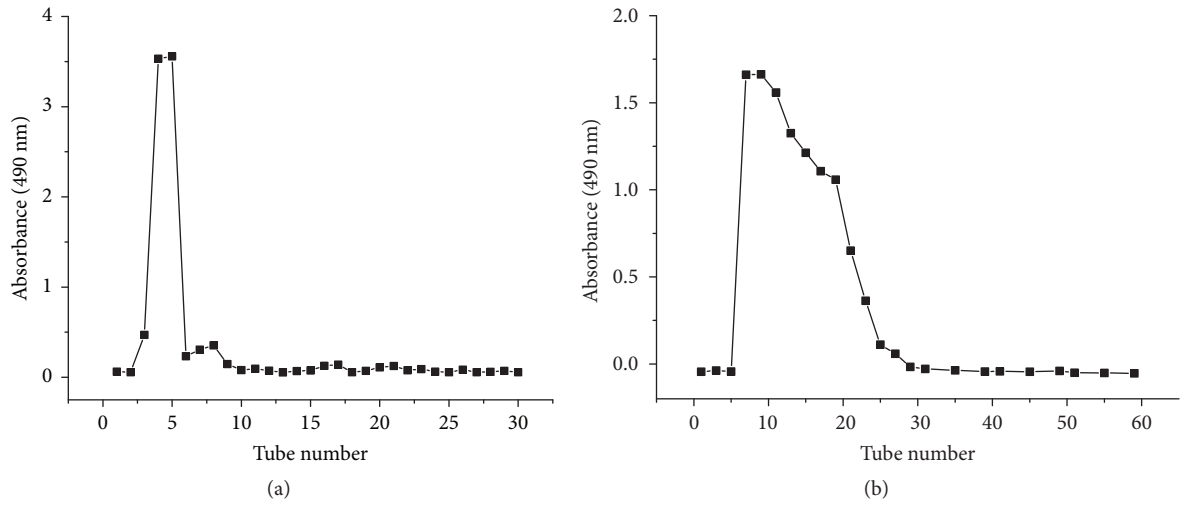

Figure 1: H_2O elution curve of crude polysaccharide with DEAE-FF chromatography (a) and CJP1 with Sephadex G-100 column (b).

were cultivated in 37°C in 5% carbon dioxide incubator. After 24 h, the supernatant was removed for fresh culture medium, adding 20 μL in 5% carbon dioxide incubator. As positive control, culture was continued. It was removed after 24 h and 5 mg/mL MTT solution 20 μL was added and continued to cultivate for 4 h. The supernatant was removed, and 150 μL DMSO was added. The microplate was oscillated for 10 min. Measure the value of absorbance by enzyme-linked immunosorbent assay at the wavelength of 490 nm [37]. Calculate the relative degree of proliferation of the cells called relative growth rate (RGR) according to the formula. The reaction of the sample should be less than level 1 reaction (RGR = 80–99%) and the positive control group at least level 3 reactions (RGR = 30–49%):

$$\text{RGR}\,(\%) = \frac{\text{average absorbance value of the test group}}{\text{mean absorbance value of the control group}} \times 100\%. \quad (1)$$

2.8. Analysis of NO Production and IL-6. NO concentration in the culture medium supernatant of RAW 264.7 cells was detected as total nitrite using the Griess method [33, 38]. RAW 264.7 cells and samples (100 μL/well) were cultured in 96-well plates for 24 h. Then one hundred microliters of supernatant was mixed with 100 μL of Griess reagents. After incubation for 10 min at room temperature, absorbance was determined at 540 nm. A standard curve was prepared using sodium nitrate (0–100 μmol/L). 50 μg/mL was used as the positive control.

Concentrations of IL-6 were determined using a Mouse IL-6 ELISA Kit according to the manufacturer instructions and previous study [10].

2.9. Statistical Analysis. Data are presented as mean ± SD and were analyzed for statistical differences. The Student's t-tests were used for all statistical analysis between different groups. P values below 0.05 were considered significant.

3. Results and Discussion

3.1. Extraction and Preparation of JP1. Crude polysaccharides were isolated from the fruiting body of *Cantharellus cibarius* with a yield of 5.63%. DEAE-FF chromatography was used to fractionate the extracts. The neutral polysaccharide was eluted by distilled water. Based on Figure 1(a), the main peak is between tubes 3 and 6, and it is symmetrical. There was a small peak behind the main peak, which was regarded as the impure peak and should be dismissed. In the phenol-sulfuric acid method, the concentration of the polysaccharide has a linear relationship with the absorbance. The operation was repeated for three times, and the results were the same as above, which means the reproducibility was good. Therefore, we just collected and combined the eluents in the tubes within the same peak and dismissed others. In the subsequence, tubes could be directly collected and combined without phenol-sulfuric acid assay.

In the light of Figure 1(b), the main peak is between tubes 8 and 24 and the reproducibility was good. So, we just collected directly and combined the eluents in the tubes within the same peak and dismissed others.

3.2. Molecular Weight. Due to the advantages of rapid and reproducible HPGPC, it is widely used to determine the molecular weight of the polysaccharide. Based on HPGPC spectrum (Figure 2) and regression equation, $\log \text{MW} = -0.158t + 12.98$, $R^2 = 0.983$, therefore, we got JP1 = 336 kDa. JP1 showed a single and symmetrical sharp peak on the HPGPC chromatogram, indicating that it was homogeneous polysaccharide [5].

3.3. Periodate Oxidation–Smith Degradation. The chemical analysis results demonstrated that JP1 showed different

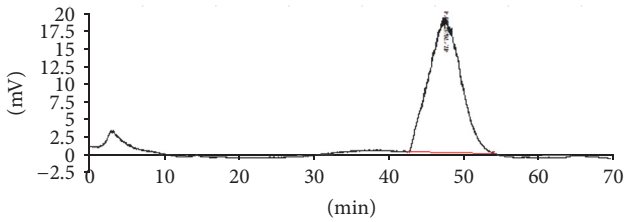

Figure 2: Chromatography of JP1 by HPGPC.

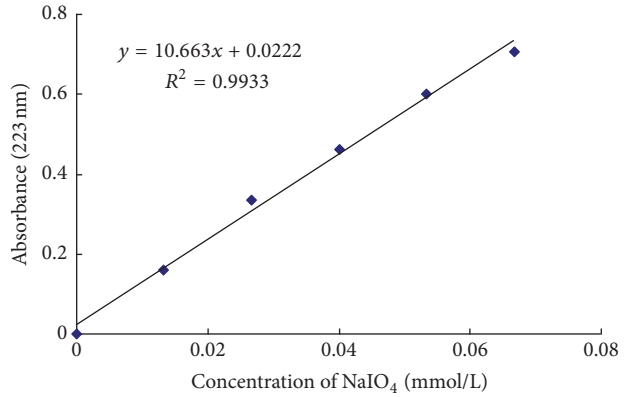

Figure 3: Standard curve of $NaIO_4$ concentration.

monosaccharide composition and branching linkages. In mushrooms, polysaccharides are present as structural components of the cell walls [39]. The position of glycosidic linkages in JP1 was examined. The results of periodate oxidation showed 0.839 mol of $NaIO_4$ produced 0.229 mol of methanoic acid (Figure 3), indicating that there may be $1 \rightarrow 2$ or $1 \rightarrow 4$, $1 \rightarrow 3$ or $1 \rightarrow 6$ glycosidic linkage in the molecule. The periodate-oxidized and Smith-degraded products were further analyzed by GC.

3.4. Monosaccharide Composition. The monosaccharide composition of JP1 was analyzed using GC. As shown in Figure 4(a), four monosaccharides, including L-Arabinose, D-Mannose, D-Glucose, and D-Galactose, were identified according to the elution time of relative monosaccharide standards. According to the internal standard method, the percentages of the four polysaccharides were L-Arabinose 19.31%, D-Mannose 20.45%, D-Glucose 37.67%, and D-Galactose 22.54%, respectively.

The UV-visible spectra were shown in Figure 4(b). There was no absorption peak at 260 nm or 280 nm, which means, in the sample, neither nucleic acid nor protein existed. The measurement range of the experiment was 200–800 nm, while the light source of UV range was deuterium lamp and the one of visible range was tungsten lamp. The serration appeared around 340 nm because the switching point of these 2 lamps was 340 nm.

The IR spectra of JP1 were shown in Figure 4(c) and Table 1. The broad band in the region of 3000~3500 cm^{-1} displayed the characteristic of the hydroxyl group, which means poor degree of methylation. The weak bands in the region of 2930 cm^{-1} were due to C-H stretching vibration and the medium bond in the region of 1400~1200 cm^{-1} indicated the C-H deviational vibration [40, 41]. JP1 was confirmed as polysaccharides by these three peaks. The medium peak at around 1640 cm^{-1} indicated the acetyl (-C=O) group. The sharp and strong absorption in the range of 1300~1000 cm^{-1} displayed the stretching vibration of pyranoid ring, so that JP1 were pyranose.

Congo Red reacts with triple-helix polysaccharides, which shifts the maximum absorption towards the long wavelength in solution. The specific transition from triple-helix conformation to single coil conformation makes the maximum absorption decrease in Congo Red polysaccharide solution [42]. As shown in Figure 5(a), JP1 did not show the specific shift of the maximum absorption wavelength at different concentrations of NaOH, demonstrating the absence of triple-helix conformation in JP1, as in the control.

3.5. Toxic Effects of JP1 on RAW264.7 Cells. The JP1 toxic effects on macrophages were shown in Figure 5(b). From the results of the experiment, with increasing concentration of JP1, the proliferation of macrophages is also growing, indicating that JP1 is nontoxic to cells in this concentration range. The cytotoxicity grade of the positive control LPS is 1, which meets the cytotoxicity range as positive control. Furthermore, its cytotoxicity is stronger than JP1.

3.6. Immunostimulating Activity of JP1 in RAW264.7 Cells. The polysaccharides of edible fungi are important biological macromolecules that have been shown to have antioxidant, antitumor, anti-inflammatory, and antidiabetic activities [8, 43, 44]. Previous studies have indicated that natural polysaccharides can activate immunologic response and enhance the secretion of cytokines including interleukin-6 (IL-6), IL-12, TNF-α, interferon gamma (IFN-γ), and nitric oxide (NO) [45].

Measuring NO according to Griess method, as shown in Figure 3, we get the standard curve line: $Y = 0.008X + 0.082$, $R^2 = 0.999$ (Y = absorbance, X = content).

NO can combine with O^{2-} or Fe, which is in the protein of Fe-S center, to produce Fe-NO complex. This substance has a direct effect on tumor cell destruction. Although NO itself does not generally induce nitration of tyrosine residues in proteins, NO reacts with superoxide to produce peroxynitrite at physiological pH, which can induce the nitration of tyrosine *in vivo* [46]. NO produced by macrophages also has a feedback effect, which mediates its tumor-killing ability indirectly. In addition, NO also plays a role in mouse NK-mediated DNA breakage and cytolytic function [47].

On the range of 0.005–0.5 mg/mL, JP1 can promote mouse peritoneal macrophages to secrete NO and shows certain positive correlation (Figure 5(c)). NO is one of nearly 100 kinds of biologically active substances secreted by activated macrophages, related to a number of immune responses and inflammation [48].

When tissue injury or inflammation occurs, it may accompany the release of IL-6. This cytokine mainly comes from the lesion in macrophages and stromal cells. It can

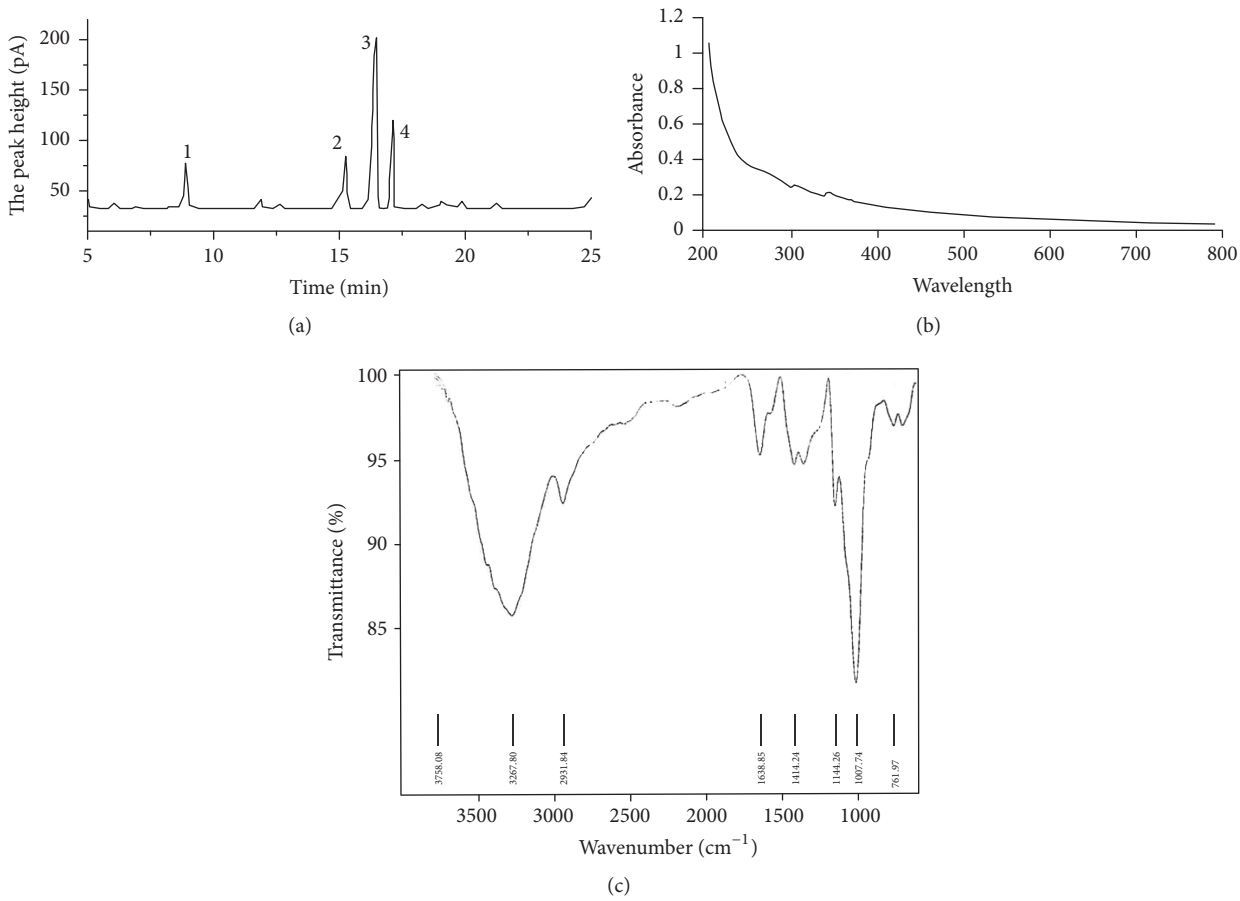

Figure 4: Gas chromatography, UV-visible spectra, and FT-IR spectrum of JP1.

Table 1: FTIR spectrum of JP1.

Wavenumber (cm^{-1})	Strength	Functional group	Indication
3000–3500	Strong	O-H stretching vibration	Degree of methylation
Near 2900	Weak	C-H stretching vibration	Characteristic peak of saccharides
1600–1730	Medium	C=O stretching vibration	Existence of acetyl
1400–1200	Medium	C-H deviational vibration	Characteristic peak of saccharides
1300–1000	Strong	Stretching vibration of pyranoid ring	Pyranoid ring
Near 810 and 870	Weak	Mannose	Characteristic peak of mannose

be applied to various parts of the body and play a role in the immune response, acute reaction, and hematopoietic and nervous systems. IL-6 is capable of inducing T cells' activation, proliferation, and differentiation, protecting neurons against viruses CTL's (cytotoxic lymphocyte) differentiation and promoting neutrophil's differentiation and activation [10, 49]. The result (Figure 5(d)) indicates that the concentration of IL-6 secreted by RAW264.7 (treated by JP1) increased when the concentration of JP1 increased, which has significant differences compared to the PBS control group. It described that JP1 can enhance the secretion of macrophages' cytokines IL-6 (Figure 5(d)).

4. Conclusions

In conclusion, the novel polysaccharide isolated from *Cantharellus cibarius* with an average molecular weight of 336 kDa could be considered as materials with natural immunostimulating activity applications. In addition, the structural characterization of JP1 was identified in our study. Based on the results, L-Arabinose, D-Mannose, D-Glucose, and D-Galactose are the effective monosaccharides in the polysaccharide structure. These results suggested that JP1 could be used as an anti-inflammatory ingredient or immunostimulatory functional foods. The isolated polysaccharide

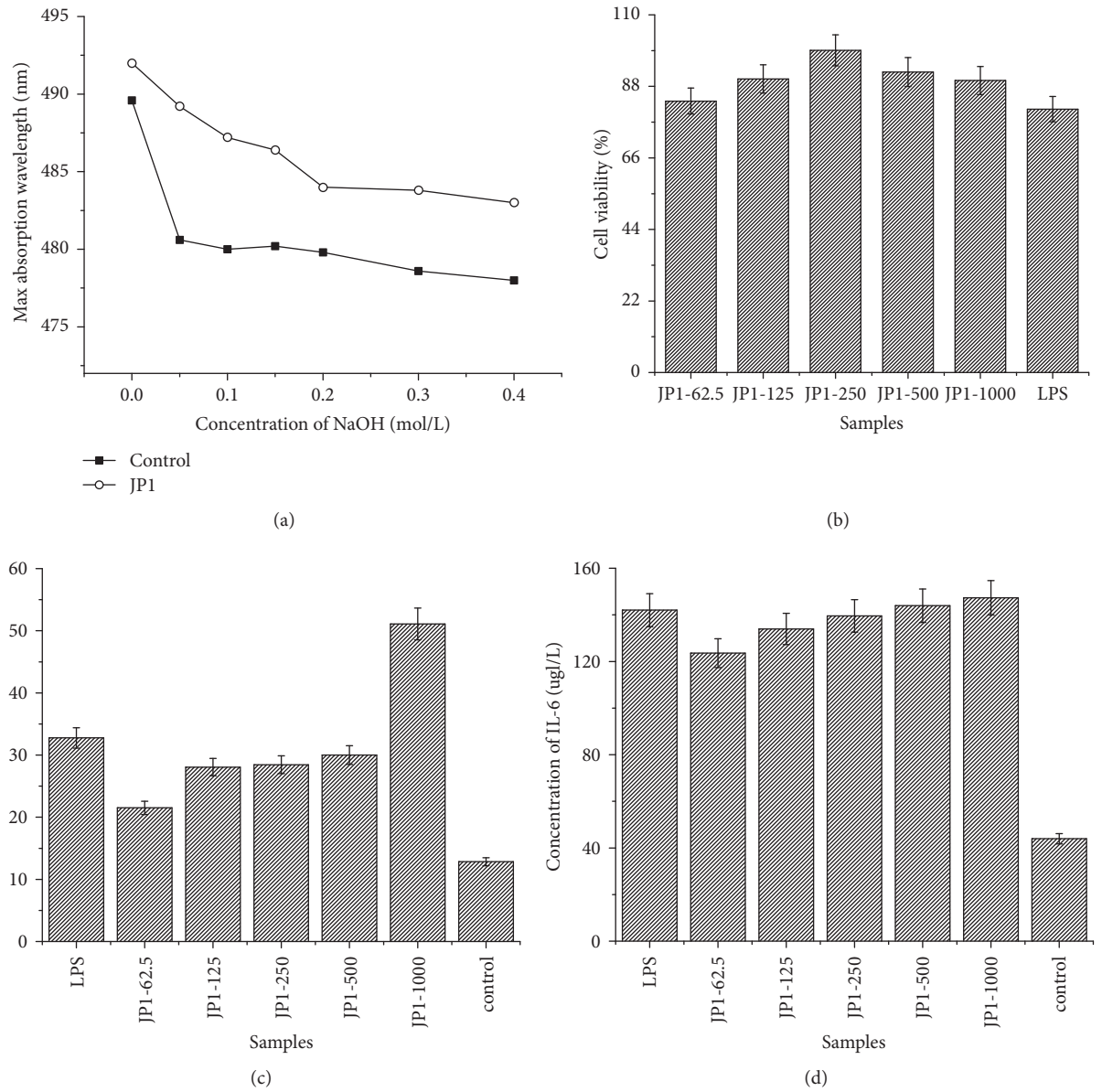

Figure 5: Congo Red test curve of JP1 (a) and its effect on cell viability (b), NO activity (c), and IL-6 (d) in RAW264.7 cells.

needs to be further examined for its potential functions to be unveiled.

Abbreviations

APCs: Antigen-presenting cells
BRM: Biological response modifier
CTL: Cytotoxic lymphocyte
DEAE-FF: DEAE-Sepharose Fast Flow
DMEM: Dulbecco's modified eagle's medium
DMSO: Dimethyl sulfoxide
EDTA: Ethylenediaminetetraacetic acid
FBS: Fetal bovine serum
FT-IR: Fourier transform infrared spectroscopy
GC: Gas chromatography
HPGPC: High-performance gel permeation chromatography
LPS: Lipopolysaccharide
MTT: 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide
RGR: Relative growth rate.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Long Chen and Xichun Peng contributed equally to this work and share first authorship.

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