Carbohydrate Polymers 114 (2014) 12–20

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Effect of ultrasonic treatment on structure and antitumor activity of mycelial polysaccharides from *Cordyceps gunnii*

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ARTICLE INFO

*Article history:*
Received 7 January 2014
Received in revised form 21 July 2014
Accepted 23 July 2014
Available online 10 August 2014

*Keywords:*
*C. gunnii*
Mycelial polysaccharide
Ultrasonic, Antitumor activity
Structure

ABSTRACT

Taking mycelial polysaccharides from *Cordyceps gunnii* (*C. gunnii*) as the study subject, the effect of ultrasonic power, time and concentration of polysaccharides on antitumor activity of the polysaccharides was investigated. The ultrasonic processing condition of the polysaccharides was optimized by using orthogonal test design, and determined to be 400 W, 15 min and 1 g/L. The change of structures of polysaccharides before and after ultrasonic treatment was also studied. Results show that ultrasonic treatment did not change the characteristic attribute of polysaccharides from *C. gunnii*. The composition of monosaccharide residues and the category of glycosidic bond have not been changed. But the molecular weight and intrinsic viscosity was reduced, and the alpha-helicity was enhanced after ultrasonic treatment. It was possible that ultrasonic treatment is an effective way for enhancing antitumor activity of polysaccharides.


## 1. Introduction

*C. gunnii*, well known as the Chinese rare caterpillar fungus, is widely found in China. Polysaccharides from the mycelium of *C. gunnii* have a variety of physiological activities (Xiao, Fang, Qi, Liu, & Liang, 2004), such as immunoregulatory, anticancer, antiviral, and antidotal (Liu et al., 2008b).

In recent years, a lot of attention has been paid to biological activities of polysaccharides and their chemical diversity. The appropriate structure transformation can improve and increase the biological activity of polysaccharides (Liu & Sun, 2005). The modification methods of polysaccharides include chemical, physical and biological approach (Liu, Wang, & Liu, 2009). Comparing with other methods, the emerging ultrasonic method has advantage of saving energy and time, reducing not only the consumption of organic solvents, but also the waste produced by chemical reaction on the environment and so on (Kuijpers, Kemmere, & Keurentjes, 2002). Mycelial polysaccharide from *C. gunnii* using ultrasound to enhance its immunoregulatory activity has been reported (Xiao et al., 2005).

In this study, the mycelial polysaccharides from *C. gunnii* were isolated and purified. In order to get polysaccharide of higher antitumor activity, the polysaccharide was treated by ultrasonic, and the ultrasonic processing condition of polysaccharide was optimized by using orthogonal test design. The structures of the polysaccharides before and after ultrasonic treatment were analyzed. The fundamental information obtained from this work is beneficial to the interpretation in the relationship of the structure of polysaccharides and its biological functions.

## 2. Materials and methods

### 2.1. Materials

The *C. gunnii* and H22 cell were obtained from the Key Laboratory of biological resources and functional food, Tianjin University of Science and Technology, Tianjin, China.

The standard monosaccharides (D-glucose, D-xylose, D-galactose, L-rhamnose, D-mannose, and D-arabinose) and Sephadex G-100 were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

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http://dx.doi.org/10.1016/j.carbpol.2014.07.068
0144-8617/

**Table 1**
Orthogonal factor level table and orthogonal test result.

(a) $L_9(3^3)$ orthogonal design on optimization of ultrasonic processing condition of polysaccharide

| Level | Factors | | |
|---|---|---|---|
| | Ultrasonic power (W) | Ultrasonic time (min) | Concentration of polysaccharide (g/L) |
| −1 | 350 | 15 | 0.5 |
| 0 | 400 | 20 | 1 |
| 1 | 450 | 25 | 1.5 |

(b) The results of orthogonal test according to orthogonal design $L_9(3^3)$

| Number | Error term | Power (W) | Time (min) | Concentration (g/L) | Inhibition ratio (%) |
|---|---|---|---|---|---|
| 1 | 1 | 350 | 15 | 0.5 | 68.5 |
| 2 | 1 | 350 | 20 | 1 | 75.2 |
| 3 | 1 | 350 | 25 | 1.5 | 60.4 |
| 4 | 2 | 400 | 15 | 1 | 83.3 |
| 5 | 2 | 400 | 20 | 1.5 | 78.5 |
| 6 | 2 | 400 | 25 | 0.5 | 72.3 |
| 7 | 3 | 450 | 15 | 1.5 | 68.1 |
| 8 | 3 | 450 | 20 | 0.5 | 44.9 |
| 9 | 3 | 450 | 25 | 1 | 50.9 |
| K1 | | 68.033 | 73.3 | 61.9 | |
| K2 | | 78.033 | 66.2 | 69.8 | |
| K3 | | 54.633 | 61.2 | 69 | |
| R | | 23.4 | 12.1 | 7.9 | |

### 2.2. Fermentation of the mycelium of C. gunnii

The mycelium of *C. gunnii* was cultivated by the liquid fermentation in shake flasks (Liu & Zhu, 2013). The environmental conditions: the fermentation temperature was 25 °C, the inoculation quantity was 8%, the inoculating age was 3 d, fermentation time was 5 d, and the speed of rotary shaker was 150 r/min. The biomass of the mycelium of *C. gunnii* was 12.3 g/L.

### 2.3. Extraction, purification and fractionation of the polysaccharides

The dried mycelium of *C. gunnii* was extracted three times by distilled water at 80 °C for 2 h. The supernatant was mixed with 4 volumes of EtOH to obtain crude polysaccharides (Liu, Leung, & Wu, 2008; Zhu et al., 2011), named CPS80. The protein was removed from CPS80 with the Sevage method (Staub, 1965). The obtained polysaccharides then were purified by Sephadex G-100 (2 cm × 50 cm) with distilled water. The collected fractions containing polysaccharides were assayed by the phenol-sulfuric acid method (Dubois, Gillis, Hamilton, Rebers, & Smith, 1956). Each fraction showed only one main peak, and the first fraction was collected and freeze dried, named MPCG-1. The total sugar content of MPCG-1 and CPS80 was determined by the phenol–sulfuric acid method.

### 2.4. MTT method

The H22 cells were seeded at a concentration of $1 \times 10^5$ cell/mL in a volume of 0.1 mL in 96-well plates. Cells were incubated with polysaccharide at concentrations of 50, 100, 200, 400 and 800 μg/mL in RPMI medium 1640 supplement with 10% fetal calf serum, 100 mg/L streptomycin, and 100 mU/L penicillin in a humidified atmosphere of 5% $CO_2$ at 37 °C. After 44 h, each well was added 20 μL of 5 mg/mL of MTT and incubated for another 4 h. Then the culture media were removed and DMSO (100 μL) was added to each well. At the same time, the parallel and negative control groups were set. Absorbance at 490 nm was detected by microplate ELISA reader. The inhibition ratio of H22 cell proliferation was calculated as follows: $\phi = \frac{OD_0 - OD}{OD} \times 100\%$

$OD_0$ is the absorbance value of negative control group, and OD is that of sample group (Liu, Lin, Gao, Ye, & Xi, 2007).

### 2.5. Ultrasonic treatment

Ultrasonic treatment under 20 kHz and different ultrasonic power, ultrasonic time and the concentration of polysaccharides, was carried out using an ultrasonic cell disrupter (SCIENTZ-IID), respectively. A 20 mL amount of the MPCG-1 solution was placed into a reaction vessel (a 20 mL beaker) and kept at a stable temperature 0 °C, and the ultrasonic probe dipped into the solution about 20 mm. Thus the solution of ultrasonic treated polysaccharides were obtained, and lyophilized.

### 2.6. Optimization of the ultrasonic technological conditions

The antitumor activity of ultrasonic treated polysaccharides was measured by MTT method which was described above. With the ultrasonic power, ultrasonic time and concentration of polysaccharide as independent variables, and antitumor activity of polysaccharide as the index, the technological conditions of ultrasonic processing were optimized by studying orthogonal design test ($L_9(3^3)$), as shown in Table 1(a). The polysaccharide named MPCG-2 was obtained under the optimal condition.

### 2.7. Determination of the intrinsic viscosity [η]

MPCG-1 and MPCG-2 were dissolved in distilled water to a concentration of 2 g/L, respectively. A 10 mL of sample was placed in an ubbelohde capillary tube (type $\phi$ 0.5–0.6 mm), and immersed in a thermostat bath at 25 ± 0.1 °C. The one-point method (Yu, 1988) was adopted to determine the descent time ($t$) of polysaccharide solution flowing through the capillary tube. Distilled water was used to determine $t_0$. The procedure was performed three times and the mean values were used. The relative viscosity ($\eta_r$) was got from $\eta_r = \eta/\eta_0 = t/t_0$, and the specific viscosity ($\eta_{sp}$) was obtained from $\eta_{sp} = (\eta - \eta_0)/\eta_0 = \eta_r - 1$. The intrinsic

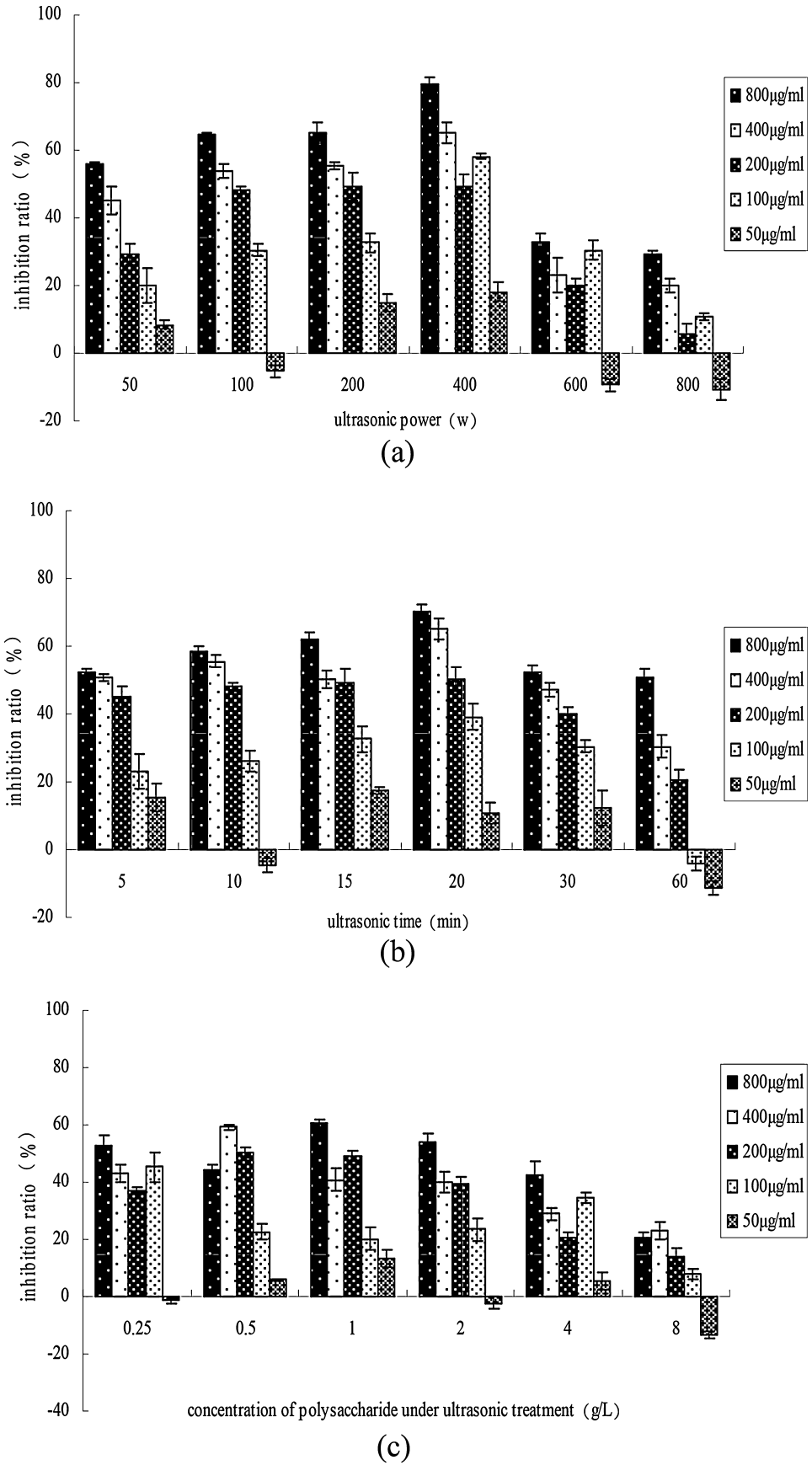



**Fig. 1.** Analysis of ultrasonic treated conditions affecting of polysaccharide antitumor activity against H22 cells ((a) ultrasonic power; (b) ultrasonic time; (c) polysaccharide concentration of ultrasonic treatment).

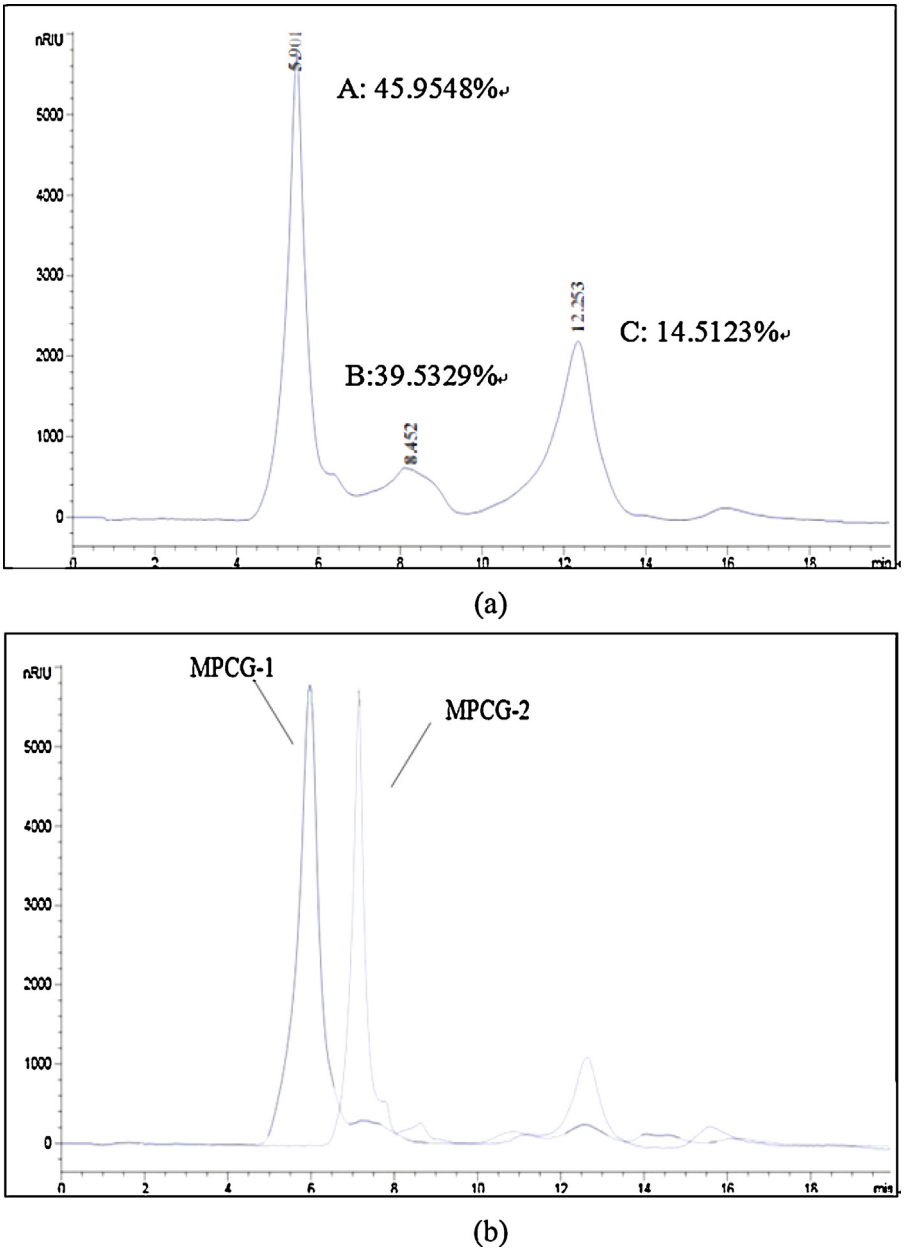



**Fig. 2.** HPLC profile of CPS80 (a), MPCG-1(b) and MPCG-2 (b).

viscosity values were obtained from the one point empirical equation:

$$[\eta] = \frac{\left(\eta_{sp} + 5 \ln \eta_r\right)}{6c}$$

### 2.8. Determination of molecular weight by HPLC

The molecular weight of CPS80, MPCG-1 and MPCG-2 were determined by using an HPLC (Agilent-1200) equipped with a column of TSK-GEL G4000PWXL (7.8 mm × 300 mm, column temperature 30 °C) and refractive index detector (RID, detecting temperature 35 °C). Standard dextrans T-10, T-40, T-70, T-500 and T-2000 were passed through the column equilibrated at a fixed flow rate (0.8 mL/min), and then the retention times were plotted against the logarithms of their respective molecular weight (Zhu et al., 2011). The solution of the polysaccharide (20 μL, 1 g/L) was also pass the column. The retention times of the samples were then plotted in the same graph, and the molecular weights were determined.

### 2.9. Determination of specific optical rotation

MPCG-1 and MPCG-2 were dissolved with distilled water, and put into the polariscope tube, respectively. The optical rotation was read directly with digital polarimeter. The determined temperature was 20 ± 0.1 °C. The specific optical rotation was calculated as follows:

$$[\alpha]_{\lambda}^{t} = \frac{\alpha}{L \times C}$$

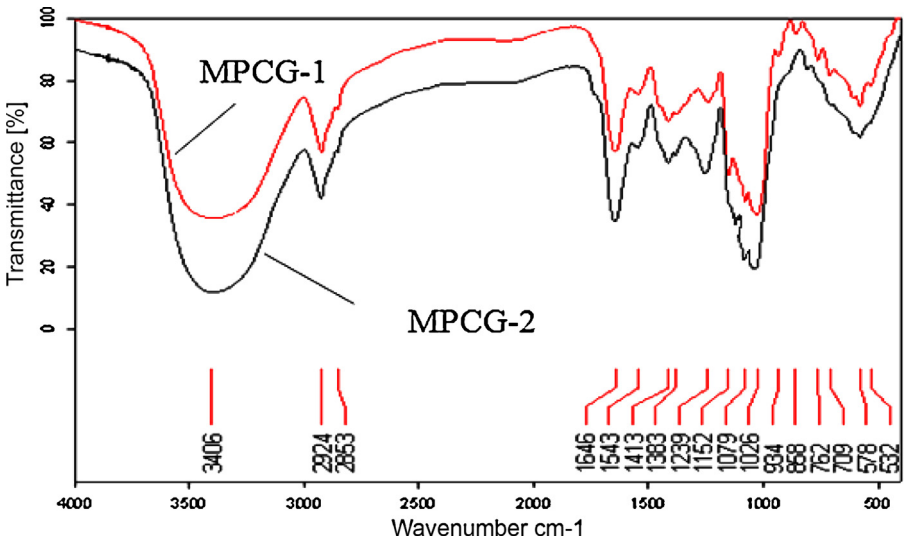



**Fig. 3.** FT-IR spectrum of MPCG-1 and MPCG-2.

$\alpha$ is the optical rotation; $t$ is the temperature; $\lambda$ is the wavelength of light source; $L$ is the length of polariscope tube; $C$ is the polysaccharide concentration (g/mL).

### 2.10. Monosaccharide composition analysis of polysaccharides

About 10 mg amount of polysaccharide sample was hydrolyzed with 4 mL of 2 M TFA at 110 °C for 3 h. The soluble fraction was evaporated to dryness under stream of nitrogen. One part of the hydrolysate was analyzed by paper chromatography (PC). The other was acetylated with $Ac_2O$-Pyridine (1:1, v/v) at 100 °C for 1 h, and was ready for GC analysis. MPCG-1, MPCG-2 and D-Glucose, D-xylose, D-galactose, L-rhamnose, D-mannose, and D-arabinose as standard were all derivatized.

The conditions of GC—chromatographic column: DB-17 (30 m × 0.32 mm × 0.5 m); detector: FID; carrier gas: $N_2$; flow rate:1 mL/min; injection temperature and detector: 280 °C; column temperature: 190 °C.

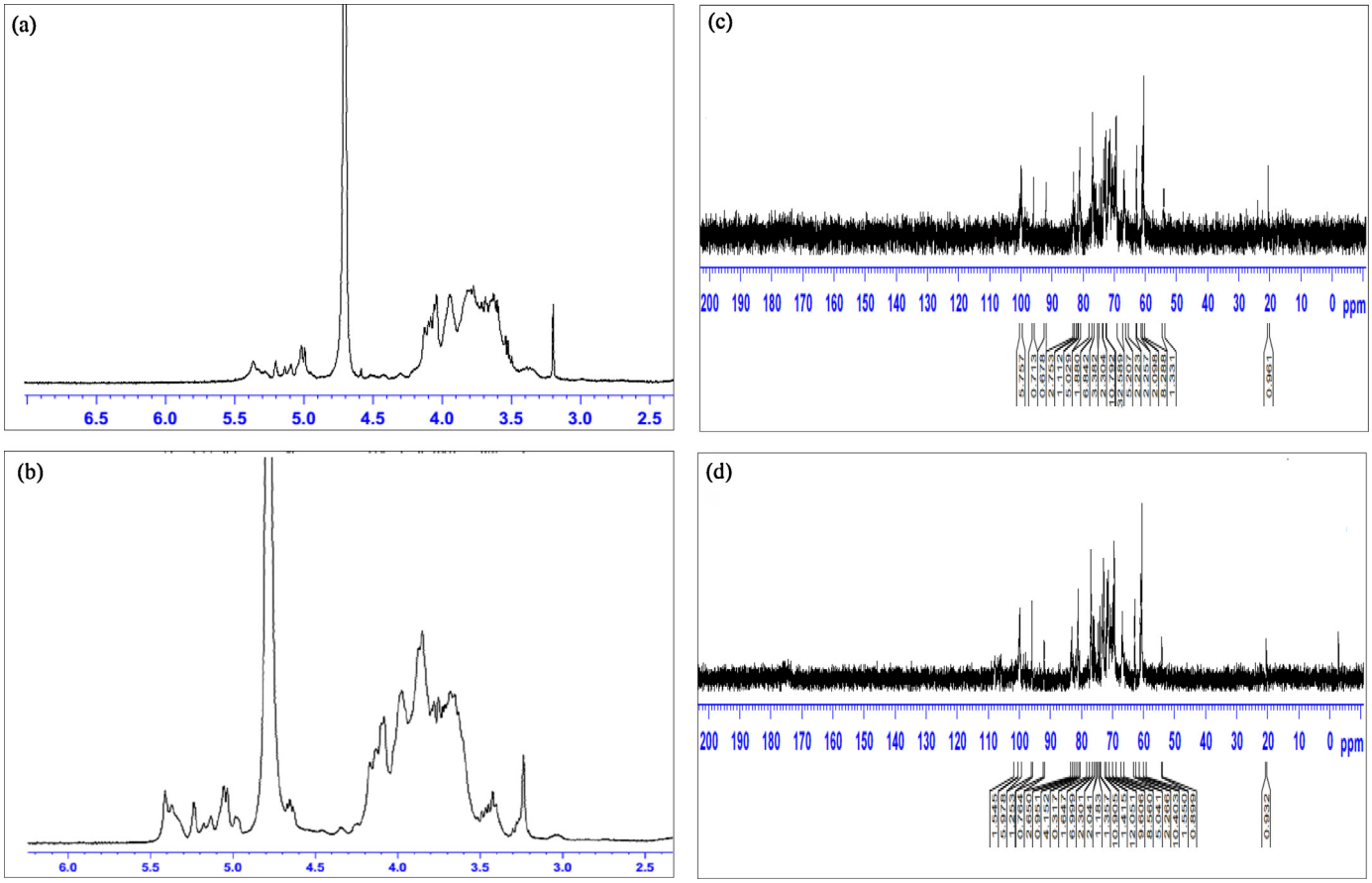



**Fig. 4.** $^{1}H$ NMR and $^{13}C$ NMR spectrum of MPCG-1 and MPCG-2.

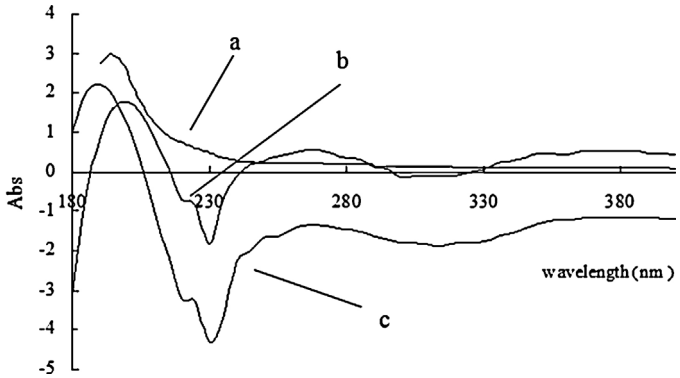
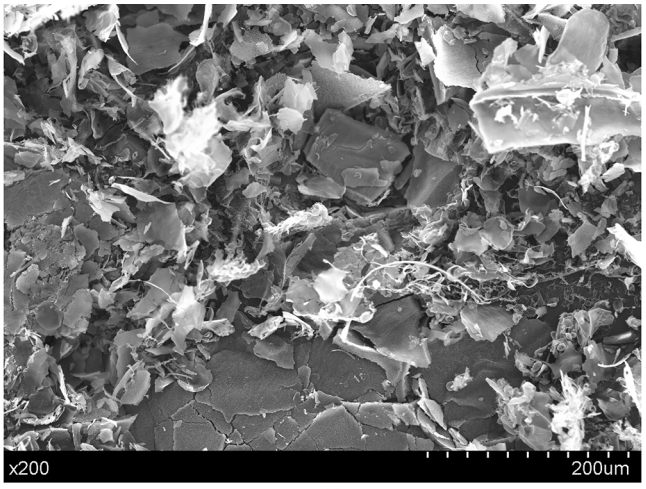


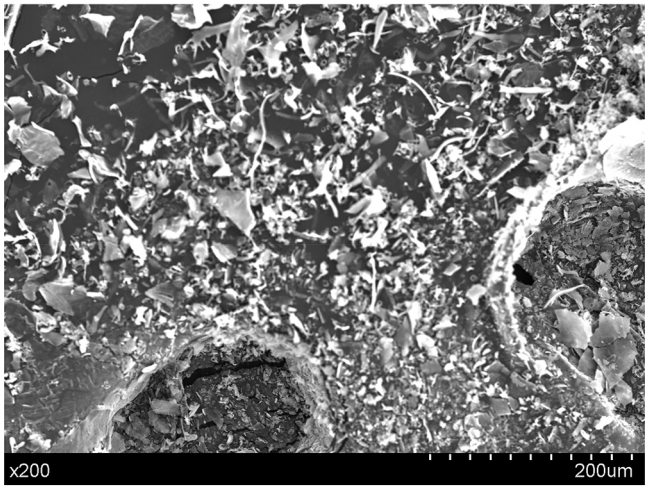

**Fig. 5.** CD spectra of MPCG-1and MPCG-2 ((a) MPCG-1/MPCG-2, (b) MPCG-1 + congo, (c) MPCG-2 + congo).

### 2.11. IR spectroscopy

An amount of 1 mg polysaccharide sample was mixed with 150 mg of dried KBr, and pressed into disk for analysis. The IR spectrum was recorded in the range of 400–4000 $cm^{-1}$ on the Vector 22 FT-IR spectrophotometer. MPCG-1 and MPCG-2 were measured by IR spectroscopy which was described above, respectively.




(a)




(b)

**Fig. 6.** Pictures of MPCG-1(a) and MPCG-2(b) on SEM.

### 2.12. NMR spectroscopy

$^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on Bruker AVANCE DRX-400 NMR spectrometer (600 MHz) at a probe temperature of 298 K. The freeze-dried MPCG-1 and MPCG-2 were exchanged twice with $D_2O$ and measured, respectively.

### 2.13. Periodate oxidation–Smith degradation

*Periodate oxidation* (Bailey & Bourne, 1960)—The polysaccharide sample (10 mg) was mixed with 0.03 M sodium metaperiodate (25 mL) and kept in the dark at 4 °C. 50 μL of aliquots were withdrawn at 8 h intervals, diluted to 5 mL and read in the spectrophotometer at 223 nm (Linker, Evans, & Impallomeni, 2001). The reaction was completed after 72 h and the excess acid was decomposed by ethanediol. Consumption of $HIO_4$ was calculated according to the standard curve of sodium periodate by a spectrophotometric method (Aspinall & Ferrier, 1957). The production of formic acid was determined by titration with 0.01 M NaOH, and bromocresol blue was used as indicator.

*Smith degradation*—The reaction mixture was dialyzed against distilled water, and the nondialysate was reduced with potassium borohydride ($NaBH_4$, 30 mg) for 12 h. The pH of the solution was adjusted to 5.0 by adding acetic acid, and the solution was dialyzed. The nondialysate was dried by disdillation under reduced pressure and hydrolyzed with 2 M TFA (1 mL) at 100 °C for 3 h. Then the hydrolysate, erythritol and glycerol were derivated and analyzed by GC (Zhao, Kan, Li, & Chen, 2005), respectively.

MPCG-1 and MPCG-2 were measured with the above method.

### 2.14. Circular dichroism spectrum (CD)

The solution of polysaccharide (0.5 mg/mL, 2 mL) was mixed with Congo red solution (50 umol/L, 2 mL) and NaOH solution (0.1 g/mL, 80 μL). The mixtures were reacted for 10 min at room temperature, and analyzed by circular dichroism spectrometer. The scanning wavelength range was 190–400 nm.

### 2.15. Scanning electron microscopy (SEM)

The MPCG-1 and MPCG-2 were fixed onto a copper stub, respectively. After sputtering with a layer of gold, the samples were examined on a SU1510 scanning electron microscope (Lai & Yang, 2007).

### 2.16. Atomic force microscope (AFM)

The dried MPCG-1 and MPCG-2 were dissolved in distilled water to a concentration of $1 \times 10^{-3}$ mg/mL, and a small volume of liquid was deposited onto freshly cleaved mica substrate, respectively. The samples were observed under a JSPM-5200 atomic force microscope when the micas dried.

## 3. Results and discussion

### 3.1. Isolation, purification of polysaccharides

The crude polysaccharides CPS80 were obtained from the cultured *C. gunnii* mycelium by hot water extraction and EtOH precipitation with yield of 12.6%. After purification by Sevage method and fractionation on Sephadex G-100 column, MPCG-1 was obtained from the distilled water eluate. The total content sugar of CPS80 and MPCG-1 was 45.65% and 92.23%, respectively.

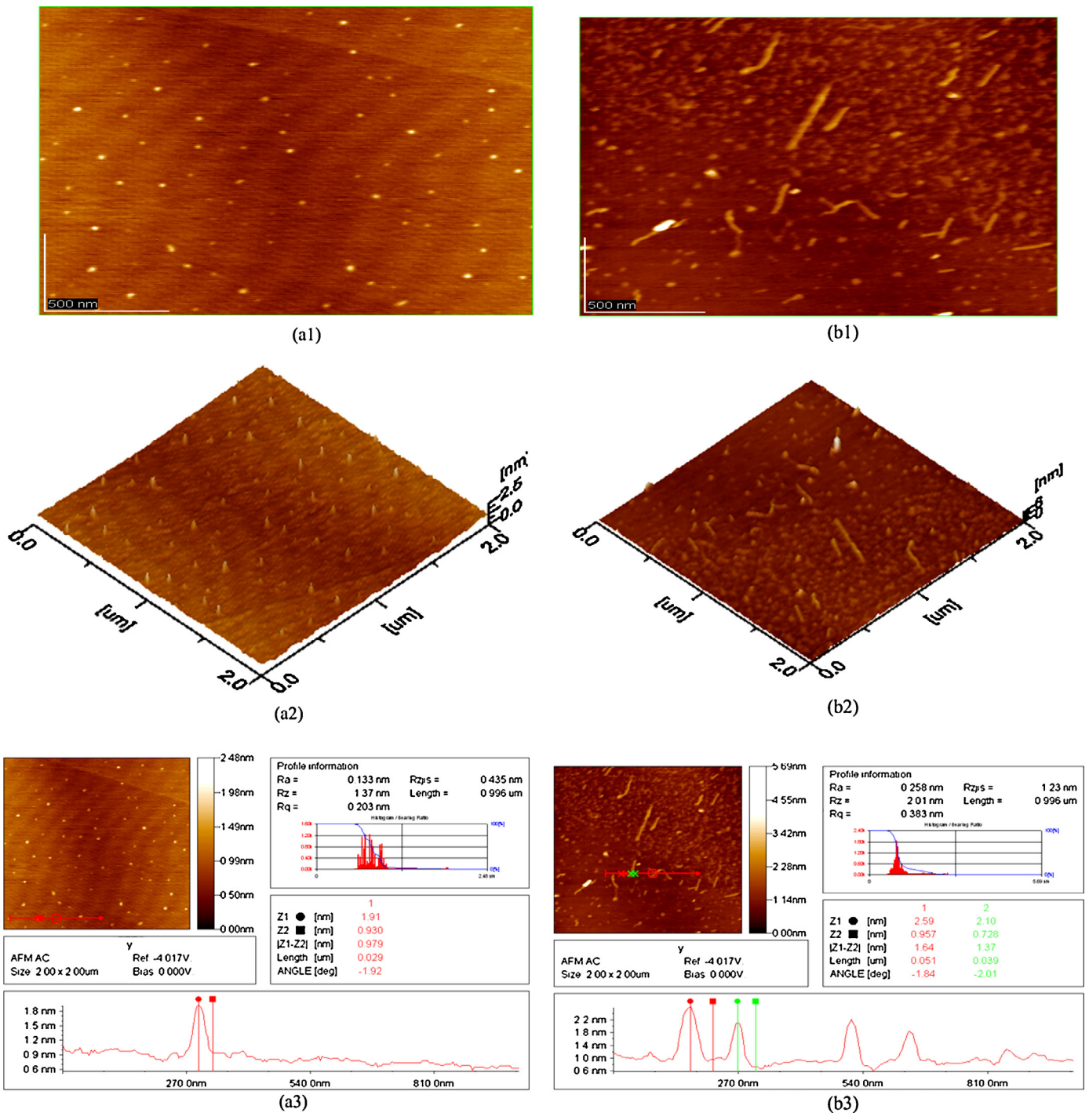



**Fig. 7.** Pictures of MPCG-1 (a) and MPCG-2 (b) on AFM; (1) the plan of AFM; (2) the three-dimensional image of AFM; (3) the linear graph of AFM.

### *3.2. Analysis of ultrasonic treated conditions affecting of polysaccharide antitumor activity*

#### *3.2.1. The result of single factor experiment*

The inhibition ratio on H22 cells of MPCG-1 reached 45.3% at 800 μg/mL. Fig. 1(a)–(c) demonstrated antitumor activity of polysaccharides obtained under different ultrasonic power, ultrasonic time and polysaccharide concentration of ultrasonic treatment, respectively. The inhibition ratio on H22 cells of polysaccharide increased first and then decreased with the increase of ultrasonic power, and reached 79.7%, 70.3% and 69.8% at 800 μg/mL under 400 W, 20 min and 1 g/L, respectively. As a whole, antitumor activity of polysaccharide depended on the concentration of polysaccharide in coculture on H22. Therefore, the best single factors were 400 W, 20 min and 1 g/L.

#### *3.2.2. The result of orthogonal experiment*

In this experiment, the result of the optimization of ultrasonic processing condition was observed. The result of orthogonal test according to orthogonal design $L_9(3^3)$ was shown in Table 1(b). Based on the variance analysis, effect on antitumor activity of three factors followed the sequences: ultrasonic power > ultrasonic time > polysaccharide concentration. And the optimum ultrasonic processing condition was obtained as follows: ultrasonic power

was 400 W, time was 15 min and polysaccharide concentration was 1 g/mL. The inhibition rate of polysaccharide reached 83.3% at 800 μg/mL.

### 3.3. Structural analysis

The specific optical rotations of MPCG-1 and MPCG-2 were +147° and +95°, respectively. The positive values indicated that the category of glycosidic bond of polysaccharide from *C. gunnii* was α-type and not broken by ultrasonic. And the decrease of value indicated that ultrasonic treatment reduced the asymmetry of polysaccharide. The intrinsic viscosity of MPCG-1 and MPCG-2 obtained by the one-point method were 103 mL/g and 55.4 mL/g, respectively. Obviously, ultrasonic treatment reduced the intrinsic viscosity of polysaccharide. In general, the polysaccharide of lower viscosity has comparatively good bioactivity. Therefore, the enhancement of the antitumor activity of polysaccharide could be caused by the decrease of its intrinsic viscosity.

The result of HPLC (Fig. 2a) indicated that CPS80 mainly contained three fractions, and MPCG-1 had been purified into a relatively homogeneous polysaccharide. The standard curve of molecular weight was $y = -0.4671x + 9.3676$ ($y = \lg M_w$, $x = R_t$), and the estimated average molecular weights of MPCG-1 and MPCG-2 were estimated to $4.1 \times 10^6$ Da and $112.2 \times 10^6$ Da, respectively. The result (Fig. 2b) showed that MPCG-2 significantly had lower molecular weight and distribution than MPCG-1, and also appeared other fractions. The investigation has shown that when the high-molecular-weight polysaccharide is degraded into the low-molecular polysaccharides, the antitumor activity of polysaccharide can be significantly improved. Therefore, the enhancement of the antitumor activity of polysaccharide could be caused by the decrease of its molecular weight.

The PC and GC analysis of monosaccharide all showed that the MPCG-1 and MPCG-2 mainly contained mannose, glucose and galactose. Their molar ratio were 37.69: 86.55: 46.80 and 40.94: 86.47: 47.84, respectively. The result indicated that the ultrasonic treatment had no obvious effect on composition of polysaccharide.

IR spectrum of MPCG-1 (Fig. 3) exhibited bands at 3386, 2927, 1408 $cm^{-1}$ attributing to hydroxyl stretching vibration, C—H stretching vibration and C—H bending vibration, respectively. The three bands above are characteristic absorption bands of polysaccharide. The band at 855 $cm^{-1}$ showed α-polysaccharide (Barker, Bourne, Stacey, & Whiffen, 1954). The three absorption bands at 1026–1152 $cm^{-1}$ indicated the presence of pyranose. Comparing with MPCG-1, the IR spectrum of MPCG-2 had very similar absorption bands, suggesting that ultrasonic treatment had no clear effect on category of glycosidic bond and sugar ring of polysaccharide.

The $^1H$ NMR spectrum of MPCG-1 (Fig. 4a) showed the anomeric proton resonance was over 4.95 ppm, attributing to α-pyranose. There were three main anomeric H at δ5.374–δ4.964 ppm, indicating that MPCG-1 was mainly composed of three types of sugar. In addition, the $^{13}C$ NMR spectrum of MPCG-1 (Fig. 4c) also showed three anomeric C at δ97–δ106 ppm, and the peak at 99.72 ppm corresponded to C-1 of α-D-pyranose. As in the case of IR spectrum, the $^1H$ NMR spectrum (Fig. 4b) and $^{13}C$ NMR spectrum (Fig. 4d) of MPCG-2 had very similar signals when compared with MPCG-1, we believe that ultrasonic treatment didn't damage the characteristic group of polysaccharide.

The results of periodate oxidation demonstrated that the consumption of $NaIO_4$ were 0.168 mmol and 0.165 mmol, respectively, and no formic acid formation was observed for MPCG-1 and MPCG-2, indicating that the two samples all had no 1,6-linkage. The GC analysis of the Smith degradation of the periodate-oxidized MPCG-1 and MPCG-2 all showed that they mainly contained mannose, glucose, galactose and little glycerol, erythritol and ribose, xylose, arabinose, respectively. The molar ratio of mannose, glucose, galactose of MPCG-1 and MPCG-2 were 14.10:45.54:15.32 and 14.01:46.01:14.97, respectively. The results indicated that the chain of MPCG-1 and MPCG-2 were both mainly composed of (1 → 3) glucose, small amount of (1 → 2), (1 → 4) linked monosaccharides and no (1 → 6) linked saccharides. The above results indicated that ultrasonic treatment had no damage on linkage of carbohydrate chain.

CD spectrum of MPCG-1 (Fig. 5b) showed that the absorbance fell substantially at about 200 nm, indicating that MPCG-1 can form complex with congo red, expressing (+) cotton effect. There was a positive peak around 190 nm and two negative peaks at 200–230 nm. With the standard curves published by Greenfield and Fasman (Alder, Greenfield, & Fasman, 1973), the appearances showed that MPCG-1 was α-helix in spatial structure. CD spectrum of MPCG-2 (Fig. 5c) had similar appearances with MPCG-1 indicating it also was α-helix. Comparing with MPCG-1, the maximum absorption peak of MPCG-2 happened blue shift and the peak intensity also increased, indicating that the molecules of polysaccharide were aggregated and their asymmetry was reduced. The results showed that the ultrasonic treatment has some effect on the conformation and stretching of polysaccharide molecules. And the enhancement of the antitumor activity of polysaccharide could be caused by the change of space structure of the polysaccharide.

SEM images showed that MPCG-1 (Fig. 6a) existed in the state of aggregation, its surface showed big lamellar structure, and MPCG-2 (Fig. 6b) was a small lamellar structure, indicating that a part of the polysaccharide aggregates was scattered by ultrasonic treatment. AFM images showed the MPCG-1 (Fig. 7a) formed homogeneous rod-shaped nano aggregates on the mica sheet surface. The height was from 0.930 nm to 1.91 nm and length was 0.996 μm, attributing the entanglement of multiple molecular chains. MPCG-2 (Fig. 7b) appeared some long chain of various sizes, and the aggregates were more compact, indicating that polysaccharide chains were degraded. The micro-morphologies of polysaccharide also proved that ultrasonic treatment made polysaccharide degrade into smaller molecular fragments.

## 4. Conclusion

The results of this work indicated that the ultrasonic treated polysaccharide exhibited stronger antitumor activity. The polysaccharide from *C. gunnii* was mainly D-glucopyranose containing α-(1 → 3)-linked backbone. It expressed (+) cotton effect and α-helix in its spatial structure. Its micro structure showed aggregated state. Comparison of polysaccharide structure before and after ultrasonic treatment indicated that ultrasonic treatment did not change the main structure of polysaccharide in the test conditions. The ultrasonic treated polysaccharide had lower molecular weight and intrinsic viscosity than the natural polysaccharide. And the molecules of polysaccharide were aggregated and asymmetry was reduced. It was likely that molecular weight, intrinsic viscosity and spatial structure had significant effect on antitumor activity of mycelial polysaccharide from *C. gunnii*.

## Acknowledgments

This work was financially supported by the National Sparking Plan Project (2012GA610005), the Foundation of Tianjin University of Science and Technology (No. 20120106), the International Science and Technology Cooperation Program of China (2013DFA31160), and the Foundation of Tianjin Educational Committee (20090604).

## References


Aspinall, G. O., & Ferrier, R. J. (1957). A spectrophotometric method for the determination of periodate consumed during the oxidation of carbohydrates. *Chemical Industry (London)*, *7*, 1216–1221.

Alder, A. J., Greenfield, N. J., & Fasman, G. D. (1973). Circular dichroism and optical rotary dispersion of proteins and polypeptides. *Methods in Enzymology*, *27*, 675.

Barker, S. A., Bourne, E. J., Stacey, M., & Whiffen, D. H. (1954). Infra-red spectra of carbohydrates. Part I. Some derivatives of D-glucopyranose. *Journal of the Chemical Society*, 171–176.

Bailey, R. W., & Bourne, E. J. (1960). Color reactions given by sugars and diphenylamine–aniline spray reagents on paper chromatograms. *Journal of Chromatograph*, *4*, 206–210.

Dubois, M., Gillis, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Kuijpers, M. W. A., Kemmere, M. F., & Keurentjes, J. T. F. (2002). Calorimetric study of the energy efficiency for ultrasound-induced radical formation. *Ultrasonics*, *40*, 675–678.

Linker, A., Evans, L. R., & Impallomeni, G. (2001). The structure of a polysaccharide from infectious strains of *Burkholderia cepacia*. *Carbohydrate Research*, *335*, 45–54.

Lai, L. S., & Yang, D. H. (2007). Rheological properties of the hot-water extracted polysaccharides in Ling-Zhi (*Ganoderma lucidum*). *Food Hydrocolloids*, *21*, 739–746.

Liu, Z. F., & Sun, H. W. (2005). Progress of the research on chemically modifications of polysaccharide. *Journal of Hebei University (Natural Science Edition)*, *25*(1), 104–108.

Liu, C. H., Lin, Q. X., Gao, Y., Ye, L., & Xi, T. (2007). Characterization and antitumor activity of a polysaccharide from *Strongylocentrotus nudus* eggs. *Carbohydrate Polymers*, *67*, 313–318.

Liu, Y. S., Leung, P. H., & Wu, J. Y. (2008). Exopolysaccharide production in batch and semi-continuous fermentation of *Cordyceps sinensis*. *Journal of Biotechnology*, *136*(S1), S301–S302.

Liu, A. J., Zhu, C. M., Zhu, Z. Y., Zhong, Y. R., Zhu, S., & Yu, Y. H. (2008). Effects of *C. gunnii* (*Berk.*) *Berk*. polysaccharide on the senescent mice. *Modern Food Science and Technology*, *3*, 201–203.

Liu, Y. Y., Wang, Q., & Liu, H. Z. (2009). Modification of cell wall polysaccharides from *Saccharomyces cerevisiae*. *Chemical Industry and Engineering Progress*, *28*(4), 686–691.

Liu, X. C., & Zhu, Z. Y. (2013). Optimization of nutritional conditions for promotion of polysaccharide produced by *C. gunnii* in submerged culture. *Science and Technology of Food Industry*, *30*(10), 226–228.

Staub, A. M. (1965). Removal of protein—Sevag method. *Methods in Carbohydrate Chemistry*, *5*, 5–6.

Xiao, J. H., Fang, N., Qi, H., Liu, J. W., & Liang, Z. Q. (2004). Immunosuppressive activity of exopolysaccharide from *Paecilomyces gunnii*. *Traditional Chinese Medicine*, *27*(3), 192.

Xiao, J. H., Liang, Z. Q., Hu, X. J., Xiao, Y., Fang, N., & Wan, W. H. (2005). Immunocompetence of *C. gunnii* polysaccharide and its depolymerizing substance. *Immunological Journal*, *21*(1), 51–54.

Yu, Y. Z. (1988). Determing the intrinsic viscosity by one-point method. *Shanghai Chemical Industry*, *6*, 29–31.

Zhao, G. H., Kan, J. Q., Li, Z. X., & Chen, Z. D. (2005). Structural features and immunological activity of a polysaccharide from *Dioscorea opposita* Thunb roots. *Carbohydrate Polymers*, *61*, 125–131.

Zhu, Z. Y., Liu, R. Q., Si, C. L., Zhou, F., Wang, Y. X., Ding, L. N., Chen, J., Liu, A., & Zhang, Y. M. (2011). Structural analysis and anti-tumor activity comparison of polysaccharides from *Astragalus*. *Carbohydrate Polymers*, *85*, 895–902.