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Structural characterization and antioxidant activity of a heteropolysaccharide from *Ganoderma capense*

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ABSTRACT

In this work, crude polysaccharide extracts were obtained from mycelia of the edible fungus *Ganoderma capense* (Lloyd) Teng. After removal of proteins by the Sevage method, fractionation and purification by anion-exchange and gel-permeation chromatography, a polysaccharide (GCPB-3) was isolated. The relative molecular weight of GCPB-3 was 124 kDa determined by high performance gel permeation chromatography (HPGPC). The homogeneous polysaccharide was composed of D-xylose and L-arabinose in the ratio of 1:1, and showed a specific optical rotation of $[\alpha]_D^{25} = +145^\circ$ (c 1.0, H_2O). Its structural features were determined by monosaccharide analysis, partial acid hydrolysis, methylation analysis, periodic acid oxidation, gas chromatography-mass spectrometry (GC-MS), Fourier transform-infrared spectroscopy (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy (^1H, ^{13}C, HMQC and HMBC). The results characterized GCPB-3 as a heteropolysaccharide with backbone consisting of β-L-Ara*p* and β-D-Xyl*p*, linked with 1→4 sugar bonds. Interestingly, GCPB-3 showed some DPPH•- and hydroxy-radical scavenging activities.

1. Introduction

Ganoderma is one of the most important edible fungi, and belongs to the class of basidiomycetes and the family *Polyporaceae* (Sun et al., 2006). Fungi of the genus *Ganoderma* have been used widely in many Asian countries such as China, Japan and Korea for thousands of years (Yang, Wang, Xie, Sun, & Wang, 2010). They are called 'Lingzhi' in Chinese and 'Reishi' in Japanese, and have been collected, cultivated and used as healthy functional food, herb of longevity, as well as traditional medicine. The most commonly used species include *G. capense*, *G. lucidum* and *G. Japonicum*. *Ganoderma* is famous and precious for containing a wealth of valuable polysaccharides, triterpenoids (Chen et al., 2012), proteins, amino acids, alkaloids, steroids, lactones, nucleotides, fatty acids, enzymes and lectins (Berovič et al., 2003; Han et al., 2012). Indeed, these chemical compounds have been isolated from the mycelia and fruiting bodies of *Ganoderma* mushrooms, and showed various pharmacological activities against aging, malignant tumors, hypertension, hypercholesterolemia, lupus erythematosus, hepatitis B, sclerema adultorum, alopecia reata, myodystrophy, insomnia and atopic dermatitis (Ngai & Ng, 2004; Li, Yan, Hua, & Zhang, 2013; Pan, Jiang, Liu, Miao & Zhong, 2013). As healthy functional food, *Ganoderma* tastes bitter and is appetizing with most nutrients easily obtained by ordinary cooking methods. *G. capense* is a *Ganoderma* species that morphologically resembles *G. lucidum* to a certain extent. In contrast to *G. lucidum*, fewer studies have focused on *G. Capense*, and mostly assessed glycopeptides. As a tradition Chinese medicine, *G. capense* is used to slow down the aging of organs and prolong life (Shi, Yang, Hu & Zhang, 2014). It was hypothesized that the antioxidant activity of *G. capense* should be one of the reasons for their anti-aging activity. In our previous studies, the four crude polysaccharides of *G. capense* had DPPH• radicals scavenging activities (Li, Yan, Hua, & Zhang, 2013; Yan, Kong, Zhang, & Cui, 2013), and the crude polysaccharide PB has noticeable effect at a high concentration, which was similar closed to the positive control (vitamin C). Therefore, it is essential and meaningful to investigate the exact structures of polysaccharides in PB and their antioxidant activities (Zhang, Liu, Park, Xia, & Kim, 2012).

Polysaccharides are polymers that consist of more than 10 monosaccharides linked by glycosidic bonds. They are widely found in plants, microorganisms, algae and animals, and show unique biological activities for cardiovascular diseases, hepatitis and cancer prevention and treatment, and in the regulation of glucose metabolism (Xu et al., 2009). With the continuous progress of modern science and technology, further analytical methods have been developed to improve the study of polysaccharides. In recent years, a deeper understanding of natural and effective methods has promoted studies dealing with the physical and chemical

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characterization of polysaccharides. To further research and development of homogeneous polysaccharides, a special focus on their structural analysis and biological activities is of great value.

In all living organisms, oxidation is an essential process that produces the energy necessary for biological processes (Duan, Zhang, Li, & Wang, 2006; Kong et al., 2010). However, uncontrolled and over-production of oxygen-derived free radicals cause many diseases such as degenerative processes, rheumatoid arthritis, atherosclerosis and cancer (Mau, Lin, & Song, 2002). Indeed, free radicals are able to damage numerous biological substances, including DNA, proteins and lipid membranes (Tsai, Song, Shih, & Yen, 2007). Meanwhile, it was reported that synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylated hydroxyquinone (TBHQ) promoted tumor formation (Cheung, Cheung, & Ooi, 2003). Interestingly, polysaccharides are somewhat resistant to oxidation and can be used as new antioxidants for their nontoxicity (Chen, Xie, Nie, Li, & Wang, 2008).

2. Materials and methods

2.1. Materials and chemicals

Submerged fermentation mycelium powder of *G. capense* was provided by Huai'an Yutu *Ganoderma* Co. Ltd. (Jiangsu Province, China). DEAE cellulose-52 was obtained from Whatman Ltd. Sephadex G-75 gel was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). T-series dextrans, trifluoroacetic acid (TFA), DPPH· (1,1-diphenyl-2-picrylhydrazyl) and standard monosaccharides were purchased from Sigma-Aldrich (St. Louis, USA). Phenol, glucose and sulfuric acid were supplied by Guangzhou Reagent Co. (Guangzhou, China). All other chemicals and reagents were of analytical grade.

2.2. Extraction and purification

G. capense mycelium powder (3.8 kg) was placed in round bottom flask and soaked with petroleum ether (five times v/w) overnight. The powder was dried and extracted three times with 38 L water at 70 °C for 3 h each time, thereafter, the extract was filtrated. Then, the residual *G. capense* mycelium powder was immersed in 0.3 M NaOH solution (w/v 1:15). A centrifugation at 5000 r/min for 5 min was carried out after stirring for 2 h at room temperature. The resulting supernatant was neutralized by 0.5 M HCl, followed by addition of ethanol to a final concentration of 70%. The pellet obtained after centrifugation was named PB. The polysaccharides samples (PB) were deproteinized by Sevage reagent (1-butanol/chloroform, v/v = 1:4). One liter water solution of polysaccharide and 200 mL Sevage reagent were mixed together in a separating funnel. After vibrating for 10 min and placing for 2 h, denatured protein was outflowed from the separating funnel. Repeat adding Sevage reagent and vibrating until little protein was left. The crude polysaccharide extracts were obtained after dialysis (M_W cut off: 3000 Da) and lyophilization.

The crude polysaccharide extracts were dissolved in distilled water, centrifuged and filtrated. The supernatant was then fractionated on a DEAE cellulose-52 column (ϕ2.6 × 40 cm), with 0.05, 0.1 and 0.25 M sodium chloride as elution gradient. An automated step-by-step fraction collector was used for collection of test tubes. All fractions were analyzed for carbohydrate content using the phenol-sulfuric method (Dubois, Gilles, Hamilto, Rebers, & Smith, 1956). Three peaks were obtained at 490 nm and the main fractions containing carbohydrates were collected, concentrated, dialyzed, lyophilized and denoted as PB-1, PB-2 and PB-3, respectively. PB-3 was then applied to a Sephadex G-75 gel-filtration column (ϕ1.6 × 100 cm), and eluted with deionized water at a flow rate of 0.3 mL/min for further purification. At this time, only one sharp peak was obtained by measuring absorbance at 490 nm, and this fraction was named GCPB-3.

2.3. Optical rotation identification

GCPB-3 was dissolved in deionized water completely. After addition of ethanol to a final concentration of 50% for 12 h in 25 °C and centrifugation at 3500 r/min for 5 min, a precipitate was obtained. Subsequently, using the same method, ethanol was added to the supernatant to a final concentration of 70%. These two precipitates were dissolved in distilled water to the same concentration after lyophilization. Optical rotation was determined with a P8000 Kruss polarimeter (Germany).

2.4. Homogeneity and molecular weight determination

High performance gel permeation chromatography (HPGPC) (Sun et al., 2010a) was used for the determination of homogeneity and molecular weight of GCPB-3. This was performed on a high performance liquid chromatography system equipped with TSK-GEL G-5000PW$_{XL}$ and G-3000PW$_{XL}$ gel columns in series (Tosoh Biosep, Japan), using 0.02 M monopotassium phosphate solution as eluent at a flow rate of 0.6 mL/min. Detection was by a Waters 2414 refractive index detector (Massachusetts, USA). The column was calibrated with the Dextran T-series standard of different molecular weights (Dextran T1000, T500, T70, T40, T10 and T5) and the column was kept at 35 °C during the experiment. The following standard curve equation was obtain by Breeze GCP Soft, Log $M_W = 8.17e+001 - 1.34e+001\,V + 7.87e-001\,V^2 - 1.58e-002\,V^3$, where V was elution volume (Geng, Chen, & Xu, 2009). Finally, the molecular weight of GCPB-3 was estimated using the calibration curve prepared above.

2.5. Analysis of monosaccharide composition

Identification and quantification of the monosaccharides of GCPB-3 were carried out by gas chromatography (GC). The sample (5 mg) was hydrolyzed with 2 mL TFA (2 M) at 120 °C for 6 h in a sealed glass tube. After evaporation to dryness, methanol was added to the sample, and the resulting solution was evaporated repeatedly to dryness till neutral pH was obtained. Afterward, hydroxylamine hydrochloride (10 mg) and 1 mL pyridine were added for 30 min at 90 °C for acetylization. Acetic anhydride (1 mL) was then added with continuous heating, and alditol acetate derivatives were obtained and analyzed on an Agilent 6820 GC system (Agilent, USA) equipped with an OV-17 capillary column. The temperature program of the column was set as follows: initial temperature of 200 °C was increased to 220 °C at a rate of 15 °C/min, and kept for 10 min, then, it was increased from 220 °C to 240 °C at 10 °C/min, and kept for 10 min at 240 °C. The injection temperature was 250 °C finally.

2.6. Fourier transform-infrared (FT-IR) spectrometry

GCPB-3 (2 mg) was incorporated, grounded and pressed into a pellet with potassium bromide. Spectra were recorded at absorbance mode from 4000 to 400 cm^{-1} with a Perkin-Elmer FT-IR spectrometer.

2.7. Periodate oxidation and Smith degradation

GCPB-3 (30 mg) was oxidized with 30 mL sodium periodate ($NaIO_4$, 0.015 M) in darkness at 4 °C. The reaction was completed when absorbance did not decrease as monitored at 223 nm every

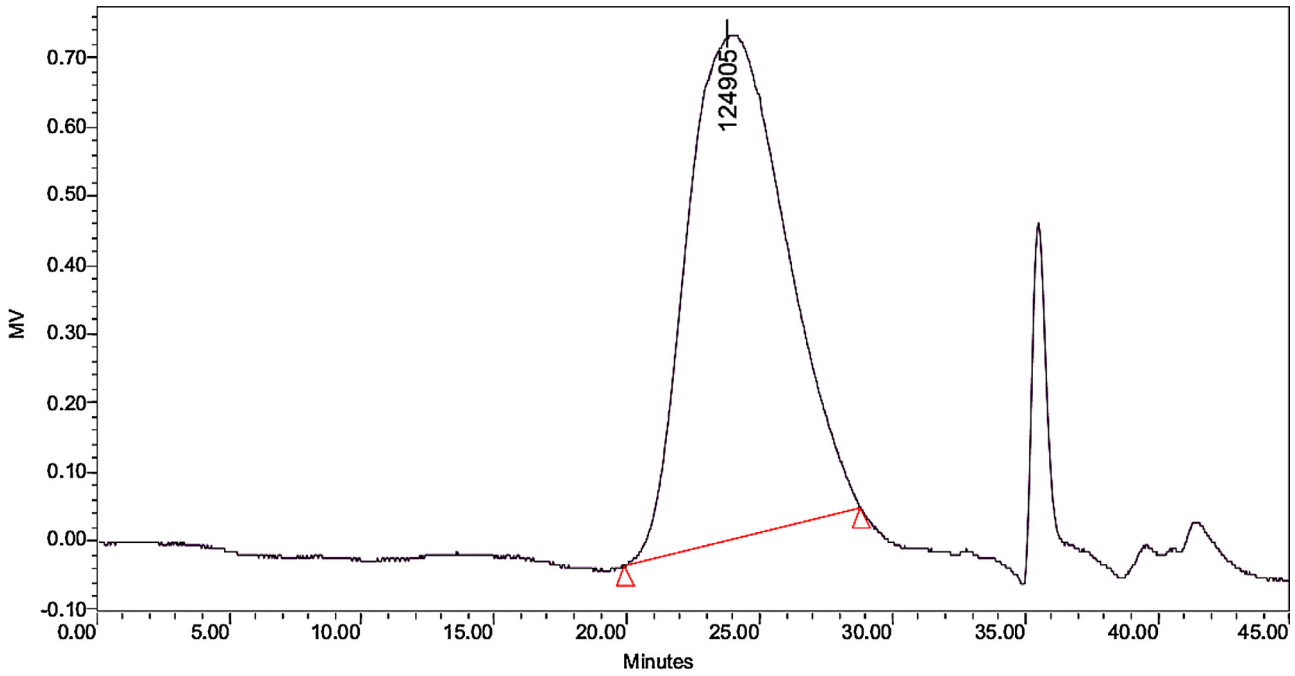

Fig. 1. HPGPC profile of GCPB-3.

6 h. The production of formic acid (HCOOH) was determined by titration with sodium hydroxide. To remove the excess $NaIO_4$, 2 mL ethylene glycol was added. The oxidation products were extensively dialyzed (M_w cut off: 3000 Da) against tap and distilled water, respectively, for 48 h. Then, sodium borohydride ($NaBH_4$, 80 mg) was added for 12 h at 25 °C as reducing agent. Subsequently, the excess $NaBH_4$ was neutralized by addition of acetic acid (Rout, Mondal, Chakraborty, & Islam, 2008; Li et al., 2008). After concentration, the solution was subjected to complete hydrolysis with 2 M TFA, and the acid was removed by co-distillation with methanol under vacuum. The acetylated products were analyzed by GC.

2.8. Methylation analysis

GCPB-3 (10 mg) was methylated according to the method of Ciucanu and Kerek (Ciucanu & Kerek, 1984) with minor modifications. IR spectroscopy was used to characterize the methylated products. The absorption peak corresponding to the hydroxyl group was absent, indicating complete sample methylated. For hydrolysis 2 M TFA was added to the methylated products, and the excess acid was evaporated by co-distillation with distilled water. Finally, the hydrolysates were acetylated with equivalent amount of acetic anhydride-pyridine after being reduced with $NaBH_4$. The partially methylated alditol acetates were analyzed by GC-MS (GC-MS-QP 2010, Shimadzu, Kyoto, Japan). The acetylated derivatives were injected into a HP-1 capillary column, and the following temperature program was set: the initial column temperature of 150 °C was increased to 180 °C at the rate of 10 °C/min. The rate was changed to 15 °C/min from 180 °C to 260 °C. Afterward, the temperature was kept for 5 min at 260 °C. Injection was performed at 220 °C. The ion source of the mass spectrometer was set at 200 °C. The sample (1 μL) was injected with a split ratio of 50:1.

2.9. Nuclear magnetic resonance (NMR) spectroscopy

The ^1H and ^{13}C NMR spectra of GCPB-3 were generated with a Bruker AV-500 spectrometer (Germany), operating at 500 and 125 MHz for ^1H NMR and ^{13}C NMR, respectively, at 27 °C. Two-dimensional spectra (^1H-^1H COSY, HMBC and HMQC) were recorded using standard Bruker procedures, with lyophilized GCPB-3 sample dissolved in D_2O. The delay time was 2 s and chemical shifts were expressed in ppm.

2.10. Assessment of DPPH• radical scavenging activity

The DPPH• radical scavenging activity was measured according to a previously reported method (Wang, Gao, Zhou, Cai, & Yao, 2008) with a few modifications. A 2 mL aqueous solution of GCPB-3 at various concentrations were mixed with 2 mL of 0.1 mM methanolic solution of DPPH• radicals. After incubation at 37 °C in the dark for 30 min, absorbance was measured at 517 nm. Ascorbic acid (Vc) and distilled water were used as control and blank, respectively. The scavenging effect was calculated according to the following equation: Scavenging rate $\% = (1 - A_S/A_0) \times 100\%$, where A_S is the absorbance obtained for a sample (GCPB-3 or Vc) and A_0 is the absorbance of the blank.

2.11. Assessment of hydroxy radical scavenging activity

The scavenging capacity of hydroxyl radical was measured according to a previously described method (Sun, Li, & Liu, 2010), which takes advantage of the 1, 10- phenanthroline-Fe^{3+}-H_2O_2 system. The reaction mixture consisted of 2 mL phosphate buffer, 1 mL 1, 10-phenanthroline, 1 mL $FeSO_4$ and variable concentrations of GCPB-3. Finally, H_2O_2 (1 mL) was added to the mixture to start the

Table 1
GC-MS analysis of GCPB-3 methylated products.

Methylated sugar	Molar ratios	Mass fragments (*m*/*z*)	Type of linkage
2,3-Me_3-Ara	1.11	53,59,69,87,100,111,129,168,247	→4)-Ara-(1→
2,3-Me_3-Xyl	1.00	59,69,87,100,111,129,142,168,187,217	→4)-Xyl-(1→

reaction. After incubation at 37 °C for 60 min, absorbance (A_1) was measured at 536 nm. In parallel, absorbance (A_2) was obtained with 2 mL phosphate buffer, 1 mL 1, 10-phenanthroline, 1 mL $FeSO_4$, 1 mL distilled water and H_2O_2 (1 mL), while the absorbance of standard control group (A_3) was obtained with a reaction mixture consisting of 2 mL phosphate buffer, 1 mL 1, 10-phenanthroline, 1 mL $FeSO_4$ and 2 mL distilled water. Ascorbic acid (Vc) was used as positive control. And the scavenging effect was derived as follows: scavenging rate % = $(A_1 - A_2)/(A_3 - A_2) \times 100\%$.

3. Results and discussion

3.1. Isolation and purification of GCPB-3

The crude polysaccharide extract (PB) was obtained from *G. capense* mycelium powder by derosination, NaOH extraction, ethanol precipitation, deproteinization and lyophilization. Anion-exchange chromatography (DEAE cellulose-52) and Sephadex G-75

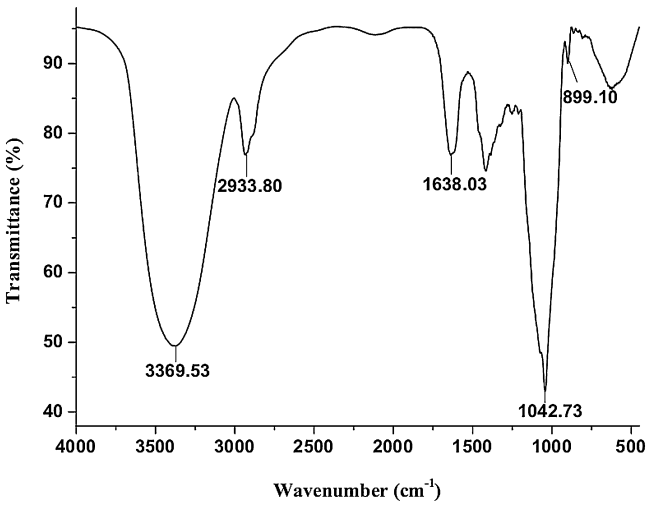

Fig. 2. IR spectrum of GCPB-3.

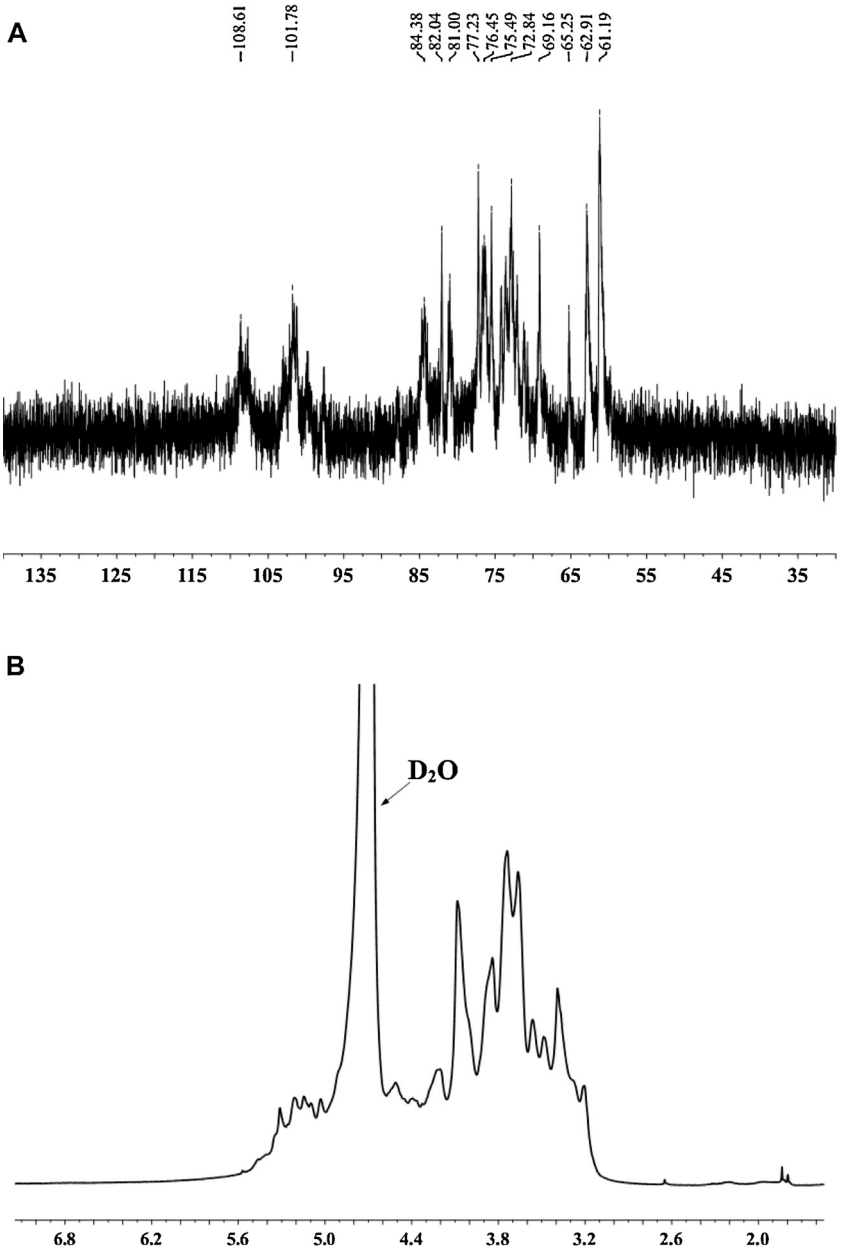

Fig. 3. NMR data of GCPB-3. (A) ^{13}C NMR spectrum of GCPB-3; (B) ^{1}H NMR spectrum of GCPB-3.

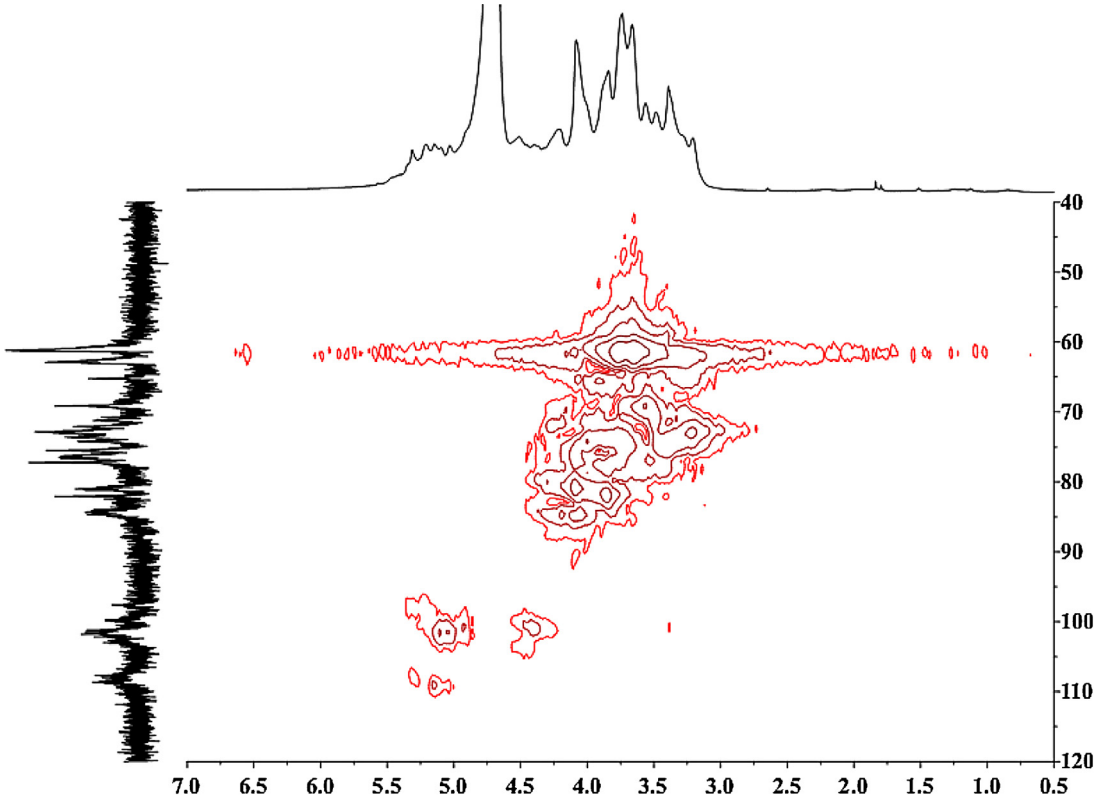

Fig. 4. HMQC spectrum of GCPB-3.

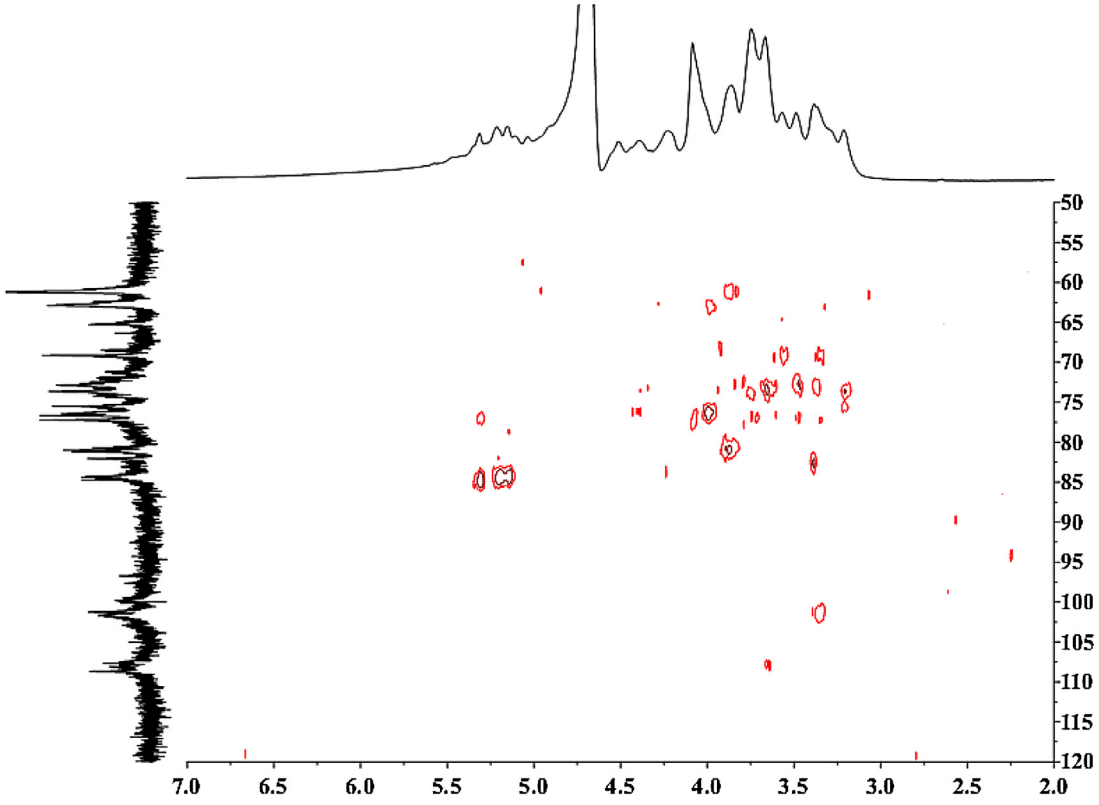

Fig. 5. HMBC spectrum of GCPB-3.

gel-filtration columns were used to purified PB. The purified product was named GCPB-3 for further structure characterization. No absorption was detected in UV spectra at both 260 and 280 nm, indicating the absence of nucleic acids and proteins. The average molecular weight of GCPB-3 was determined as 124 kDa, and a single and symmetric peak obtained in the HPGPC profile suggested GCPB-3 was a homogeneous polysaccharide (Fig. 1).

3.2. Monosaccharide identification and quantification of GCPB-3

An accurate content of sugars in GCPB-3 was obtained by GC analysis, after complete hydrolysis. The results showed that GCPB-3 was composed of arabinose and xylose. GCPB-3 appeared as white powder, $[\alpha]_D^{25} = +145^\circ$ (c 1.0, H_2O).

3.3. FT-IR spectra analysis of GCPB-3

A typical major broad stretching peak at 3369.53 cm^{-1} for hydroxyl group and a weak band at 2933.80 cm^{-1} for the C–H stretching vibration were identified in the GCPB-3 FT-IR spectrum (Fig. 2). The presence of carbonyl group was indicated by the absorbance at 1638.03 cm^{-1}. In addition, the main absorptions of C–O stretching (1042.73 cm^{-1}) suggested that the characteristic sugar moieties were of pyranose configuration. Finally, the band at 899.10 cm^{-1} was attributed to β-configuration in the polysaccharide (Li, Fan, & Ding, 2011).

3.4. Periodic acid oxidation and Smith degradation of GCPB-3

After periodic acid oxidation, most arabinose and xylose moieties were oxidized. These findings indicated the existence of 1→2 or 1→4 linked bonds in GCPB-3. Some arabinose and xylose left demonstrated the presence of some linked bonds, which could not be oxidized. Meanwhile, large amounts of glycerinum were produced, further illustrating that the monosaccharides oxidized in GCPB-3 mainly had 1→2 or 1→4 linked bonds.

3.5. Analysis of GCPB-3 methylation

The fully methylated GCPB-3 was hydrolyzed with acid, converted into alditol acetates, and analyzed by GC-MS. As summarized in Table 1, the methylation results showed the presence of the two derivatives 2,3-di-O-methyl-arabinose and 2,3-di-O-methyl-xylose, in molar ratios of 1.11:1. These data indicated that GCPB-3 consisted of (1→4)-L-arabinopyranosyl and (1→4)-D-xylopyranosyl residues. The ratio of almost 1:1 suggested that they could be linked one to another. Importantly, the characteristics of the GCPB-3 monomer were consistent with periodate oxidation and Smith degradation data.

Table 2
^{13}C and ^{1}H NMR chemical shifts of GCPB-3.

Sugar residues		Chemical shifts, δ (ppm)				
		1	2	3	4	5
→4)-β-L-Ara-(1→	H	5.15	3.83	3.84	3.40	3.85
	C	108.61	73.89	77.69	74.97	81.86
→4)-β-D-Xyl-(1→	H	5.10	4.07	3.57	3.64	3.22
	C	101.78	80.80	61.43	69.14	72.92

3.6. NMR spectroscopy analysis of GCPB-3

The ^{1}H and ^{13}C NMR spectra of GCPB-3 are shown in Fig. 3. There were two anomeric carbons from 90 to 110 ppm in the ^{13}C NMR spectrum and HMQC (Fig. 4). The signals at 108.61 and 101.78 ppm represented the anomeric carbons in arabinose and xylose, respectively (Chandra, Ghosh, Ojha, & Islam, 2009; Boyko et al., 2012). The proton signal of arabinose residue of GCPB-3 was 5.15 ppm (Faber, Haaster, Kamerling, & Vliegenthart, 2002) and the chemical shifts of C_3 and C_5 were 77.69 and 81.86 ppm, respectively (Bergstrom, Nair, Weintraub, & Jansson, 2002), which prove that arabinose was in pyranose form. The anomeric proton signal in arabinose residue of GCPB-3 was 5.15 ppm, which indicated it was in L form (Chandra et al., 2009; Qi et al., 2012). According to the above data, the arabinose residue was confirmed to be β-L-arabinopyranose residue. The HMBC spectrum (Fig. 5) further confirmed some of the correlations between carbon and proton signals within the sugar residues. For instance, the anomeric carbon signal of β-L-Ara at 108.61 ppm was correlated with the H-4 signal of β-D-Xyl at 3.64 ppm. The cross peak representing the anomeric carbon signal of β-D-Xyl at 101.78 ppm was correlated with the H-4 signal of β-L-Ara at 3.40 ppm. From the HMBC data, we proposed that →4)-β-L-Ara-(1→ and →4)-β-D-Xyl-(1→ were linked together. Others signals were summarized in Table 2.

After acid hydrolysis, periodic acid oxidation, Smith degradation, FT-IR spectroscopy, GC-MS and NMR spectroscopy, GCPB-3 was identified as heteropolysaccharide with backbone consisting of β-L-Ara*p* and β-D-Xyl*p*, with 1→4 sugar bonds. The suggested GCPB-3 repeat unit was concluded (Fig. 6).

3.7. DPPH•- and hydroxy-radical scavenging activity of GCPB-3

Scavenging of DPPH• radicals is the basis of a common antioxidant assay. Antioxidants can protect against the damage caused by

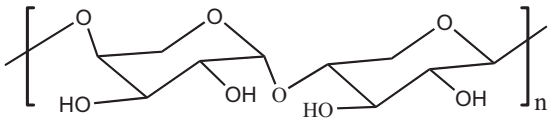

Fig. 6. Predicted structure of the repeating unit of GCPB-3.

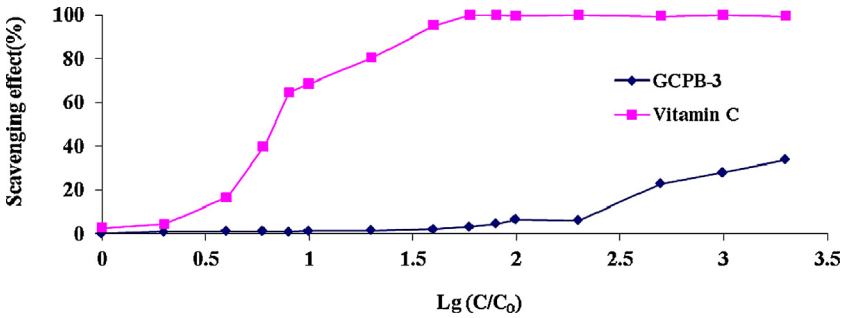

Fig. 7. DPPH• radical scavenging effect of GCPB-3. The X-axial is shown as $\lg(C/C_0)$ while C_0 is the minimum concentration in the scavenging activity of experiment of GCPB-3 and Vitamin C. *C* was measuring concentration.

free radicals, which have been implicated in the etiology of various major diseases. As shown in Fig. 7, the scavenging rate of GCPB-3 was 33.8%, when used as 20 mg/mL (C_0 is the minimum concentration in the scavenging activity experiment of GCPB-3 and Vitamin C). GCPB-3 also displayed concentration dependent radical scavenging effects for hydroxy radicals: a scavenging rate of 15.0% was obtained for GCPB-3, when used at 20 mg/mL.

4. Conclusions

In this work, crude polysaccharide extracts of *G. capense* were obtained by NaOH extraction and ethanol precipitation. Then, DEAE Cellulose-52 chromatography and Sephadex G-75 gel filtration were used to purify GCPB-3, which has a relative molecular weight of 124 kDa and is composed of arabinose and xylose at 1:1 molar ratio. GCPB-3's specific optical rotation is $[\alpha]_D^{25} = +145^\circ$ (c 1.0, H_2O) and its structure comprises a backbone of 1→4 linked β-L-Ara and β-D-Xyl. Importantly, GCPB-3 displays DPPH•- and hydroxyl-radical scavenging activities.

The assay of DPPH• radicals scavenging showed that the scavenging effects of GCPB-3 was increased with increasing concentrations. Inoxidizability could reduce uncontrolled and over-production of oxygen-derived free radicals. This may have some relationships with the effects of slowing down the ageing process, anti-aging and prolong life. The antioxidant activity of the genus *Ganoderma* can be also considered as one of the reasons of its wide use as functional foods in some Asian countries. However, the antioxidant mechanism of GCPB-3 is still not clear and because of the complex mechanism of antioxidant activity, only these tests are normally not enough to evaluate precisely the antioxidant activity of the potential antioxidant. So different antioxidant activities of *Ganoderma capense* polysaccharide should be further investigated to find out its mechanism in future work.

In conclusion, the polysaccharides were found to be the major active components in *G. capense* mycelia powder in our work (Shi, Zhang & Yang, 2013). All these experimental results made the chemical basis for the benefits of *G. capense* toward anti-aging and antioxidant activities and lay the foundation for further research on underlying mechanisms.

Acknowledgements

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References

Bergstrom, N., Nair, G. B., Weintraub, A., & Jansson, P.-E. (2002). Structure of the O-polysaccharide from the lipopolysaccharide from *Vibrio cholerae* O6. *Carbohydrate Research*, *337*, 813–817.

Berovič, M., Habijanič, J., Zore, I., Wraber, B., Hodžar, D., Boh, B., & Pohleven, F. (2003). Submerged cultivation of *Ganoderma lucidum* biomass and immunostimulatory effects of fungal polysaccharides. *Journal of Biotechnology*, *103*, 77–86.

Boyko, A. S., Dmitrenok, A. S., Fedonenko, Y. P., Zdorovenko, E. L., Konnova, S. A., Knirel, Y. A., & Ignatov, V. V. (2012). Structural analysis of the O-polysaccharide of the lipopolysaccharide from *Azospirillum brasilense* Jm6B2 containing 3-*O*-methyl-D-rhamnose (D-acofriose). *Carbohydrate Research*, *355*, 92–95.

Chandra, K., Ghosh, K., Ojha, A. K., & Islam, S. S. (2009). Chemical analysis of a polysaccharide of unripe (green) tomato (*Lycopersicon esculentum*). *Carbohydrate Research*, *344*, 2188–2194.

Chen, Y., Xie, M. Y., Zhang, H., Wang, Y. X., Nie, S. P., & Li, C. (2012). Quantification of total polysaccharides and triterpenoids in *Ganoderma lucidum* and *Ganoderma atrum* by near infrared spectroscopy and chemometrics. *Food Chemistry*, *135*, 268–275.

Chen, Y., Xie, M. Y., Nie, S. P., Li, C., & Wang, Y. X. (2008). Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma atrum*. *Food Chemistry*, *107*, 231–241.

Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, *81*, 249–255.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Duan, X. J., Zhang, W. W., Li, X. M., & Wang, B. G. (2006). Evaluation of antioxidant property of extract and fractions obtained from a red alga *Polysiphonia urceolata*. *Food Chemistry*, *95*, 37–43.

Dubois, M., Gilles, 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.

Faber, E. J., Haaster, D. J., Kamerling, J. P., & Vliegenthart, J. F. G. (2002). Characterization of the exopolysaccharide produced by *Streptococcus thermophilus* 8S containing an open chain nononic acid. *European Journal of Biochemistry*, *269*, 5590–5598.

Geng, A. J., Chen, J., & Xu, X. F. (2009). Determination of Lentinan content and its molecular weight by GPC. *Modern Food Science and Technology*, *25*, 458–460.

Han, X. Q., Chan, B. C. L., Dong, C. X., Yang, Y. H., Ko, C. H., Lee, Y. G. G., Chen, D., Wong, C. K., San, L. C. B., Tu, P. F., Shaw, P. C., Fung, K. P., Leung, P. C., Hsiao, W. L., & Han, Q. B. (2012). Isolation, structure characterization, and immunomodulating activity of a hyperbranched polysaccharide from the fruiting bodies of *Ganoderma sinense*. *Journal of Agricultural and Food Chemistry*, *60*, 4276–4281.

Kong, F. L., Zhang, M. W., Liao, S. T., Yu, S. J., Chi, J. W., & Wei, Z. C. (2010). Antioxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of *Litchi chinensis* Sonn. *Molecules*, *15*, 2152–2165.

Li, J. W., Fan, L. P., & Ding, S. D. (2011). Isolation, purification and structure of a new water-soluble polysaccharide from *Zizyphus jujuba cv* Jinsixiaozao. *Carbohydrate Polymers*, *83*, 477–482.

Li, N. S., Yan, C. Y., Hua, D. H., & Zhang, D. Z. (2013). Isolation, purification, and structural characterization of a novel polysaccharide from *Ganoderma capense*. *International Journal of Biological Macromolecules*, *57*, 285–290.

Li, S. G., Wang, D. G., Tian, W., Wang, X. X., Zhao, J. X., Liu, Z., & Chen, R. (2008). Characterization and anti-tumor activity of a polysaccharide from *Hedysarum polybotrys* Hand.-Mazz. *Carbohydrate Polymers*, *73*, 344–350.

Mau, J. L., Lin, H. C., & Song, S. F. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International*, *35*, 519–526.

Ngai, P. H. K., & Ng, T. B. (2004). A mushroom (*Ganoderma capense*) lectin with spectacular thermostability, potent mitogenic activity on splenocytes, and antiproliferative activity toward tumor cells. *Biochemical and Biophysical Research Communications*, *314*, 988–993.

Pan, K., Jiang, Q. G., Liu, G. Q., Miao, X. Y., & Zhong, D. W. (2013). Optimization extraction of *Ganoderma lucidum* polysaccharides and its immunity and antioxidant activities. *International Journal of Biological Macromolecules*, *55*, 301–306.

Qi, X. H., Mao, W. J., Gao, Y., Chen, Y., Chen, Y. L., Zhao, C. Q., Li, N., Wang, C. Y., Yan, M. X., Lin, C., & Shan, J. M. (2012). Chemical characteristic of an anticoagulant-active sulfated polysaccharide from *Enteromorpha clathrata*. *Carbohydrate Polymers*, *90*, 1804–1810.

Rout, D., Mondal, S., Chakraborty, I., & Islam, S. S. (2008). The structure and conformation of a water-insoluble (1→3)-,(1→6)-β-D-glucan from the fruiting bodies of *Pleurotus florida*. *Carbohydrate Research*, *343*, 982–987.

Shi, M., Yang, Y. N., Hu, X. S., & Zhang, Z. Y. (2014). Effect of ultrasonic extraction conditions on antioxidative and immunomodulatory activities of a *Ganoderma lucidum* polysaccharide originated from fermented soybean curd residue. *Food Chemistry*, *155*, 50–56.

Shi, M., Zhang, Z. Y., & Yang, Y. N. (2013). Antioxidant and immunoregulatory activity of *Ganoderma lucidum* polysaccharide (GLP). *Carbohydrate Polymers*, *95*, 200–206.

Sun, L. W., Feng, K., Jiang, R., Chen, J. Q., Zhao, Y., Ma, R., & Tong, H. B. (2010). Water-soluble polysaccharide from *Bupleurum chinense* DC: Isolation, structural features and antioxidant activity. *Carbohydrate Polymers*, *79*, 180–183.

Sun, S. J., Gao, W., Lin, S. Q., Zhu, J., Xie, B. G., & Lin, Z. B. (2006). Analysis of genetic diversity in *Ganoderma* population with a novel molecular marker SRAP. *Applied microbiology and biotechnology*, *72*, 537–543.

Sun, Y. X., Li, T. B., & Liu, J. C. (2010). Structural characterization and hydroxyl radicals scavenging capacity of a polysaccharide from the fruiting bodies of *Auricularia polytricha*. *Carbohydrate Polymers*, *80*, 377–380.

Tsai, M. C., Song, T. Y., Shih, P. H., Shih, P. H., & Yen, G. C. (2007). Antioxidant properties of water-soluble polysaccharides from *Antrodia cinnamomea* in submerged culture. *Food Chemistry*, *104*, 1115–1122.

Wang, H., Gao, X. D., Zhou, G. C., Cai, L., & Yao, W. B. (2008). In vitro and *in vivo* antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. *Food Chemistry*, *106*, 888–895.

Xu, W. T., Zhang, F. F., Luo, Y. B., Ma, L. Y., Kou, X. H., & Huang, K. L. (2009). Antioxidant activity of a water-soluble polysaccharide purified from *Pteridium aquilinum*. *Carbohydrate Research*, *344*, 217–222.

Yan, C. Y., Kong, F. S., Zhang, D. Z., & Cui, J. X. (2013). Anti-glycated and antiradical activities in vitro of polysaccharides from *Ganoderma capense*. *Pharmacognosy Magazine*, *9*, 23–27.

Yang, Q., Wang, S. W., Xie, Y. H., Sun, J. Y., & Wang, J. B. (2010). HPLC analysis of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes activity and Bax, Bcl-2 expression. *International Journal of Biological Macromolecules*, *46*, 167–172.

Zhang, J., Liu, Y. J., Park, H. S., Xia, Y. M., & Kim, G. S. (2012). Antitumor activity of sulfated extracellular polysaccharides of *Ganoderma lucidum* from the submerged fermentation broth. *Carbohydrate Polymers*, *87*, 1539–1544.