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Characterization of a heteropolysaccharide isolated from diploid *Gynostemma pentaphyllum* Makino

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ABSTRACT

A novel water-soluble polysaccharide (GPP), with a molecular mass of 7.1×10^3 Da, was isolated from the defatted whole-plant of diploid *Gynostemma pentaphyllum* Makino. Monosaccharide composition analysis indicated that GPP was a heteropolysaccharide mainly containing mannose, glucose, galactose and arabinose, at a molar ratio of 1.00:77.33:4.81:1.83. The detailed structure analysis revealed that GPP consisted of a (1→4)-α-D-glucoside backbone with a 1→)-α-D-glucopyranosyl branch at the C-6 position of (1→4,6)-linked-α-D-glucopyranosyl on every 5 monosaccharide residues, with a few mannose, galactose and arabinose terminal residues. GPP exhibited scavenging capacities against hydroxyl, peroxyl and DPPH radicals *in vitro*, and had a greater bile acid-binding ability than psyllium on a per weight basis. These results suggested a potential application of GPP in functional foods and dietary supplements.

1. Introduction

Gynostemma pentaphyllum Makino (*Cucurbitaceae*), a perennial liana herb, has been used in beverage, food and traditional herb medicine. The botanical is widely distributed in China, Korea, Japan and Southeast Asia. *G. pentaphyllum* Makino has been used to prevent and treat cardiovascular disease, hyperlipidaemia, hypertension, hepatitis, diabetes and cancer (Lu et al., 2008). The known chemical constituents of *G. pentaphyllum* Makino include but are not limited to saponins (Zhang et al., 2010b), carotenoids (Liu, Kao, & Chen, 2004), flavonoids (Xie et al., 2011), chlorophylls (Huang, Hung, Wu, & Chen, 2008), lignin (Wang, Zhang, Chen, Wang, & Wen, 2009), polysaccharides (Yang, Zhao, Yang, & Ruan, 2008). Among them, saponins, well known as gynosaponins or gypenosides, are believed to be the functional components responsible for its diverse biological properties (Bai et al., 2010). However, recent studies suggested that the polysaccharide components exhibited significant bioactivities, such as antioxidant (Wang & Luo, 2007), anti-exercise fatigue (Chi, Chen, Wang, Xiong, & Li, 2008), antitumor (Chen et al., 2011) and immunostimulatory activities (Yang et al., 2008). Polysaccharides may have greater bioavailability because they may be water soluble and could be soaked out easily into tea infusion compared with saponins and essential oil (Yang et al., 2008). To date, very few studies investigated the chemical structure and bioactivities of *G. pentaphyllum* polysaccharides.

Oxidation is essential to many living organisms for energy production through biological processes. However, the uncontrolled production of oxygen-derived free radicals may cause lipid peroxidation, DNA damage and protein oxidation, which may lead to impaired living cells. There are growing evidences showed that lipid peroxidation and toxicity associated with free radicals may induce cancer, atherosclerosis and aging process. Potent natural antioxidants with low cytotoxicity are in high demand for reducing the risk of chronic human diseases because of the potential toxicity of synthetic antioxidants such as BHA (Yuan, Zhang, Fan, & Yang, 2008). Many polysaccharides have showed antioxidant capacities (He, Yang, Jiao, Tian, & Zhao, 2012; Wang et al., 2012; Wu, Zhu, Zhang, Yang, & Zhou, 2012; Ye & Huang, 2012). Polysaccharides may also bind bile acids and fats in the intestine and enhance their excretion, which stimulates the conversion of cholesterol to bile

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acids in liver, and consequently reduce plasma and LDL cholesterols (Liu et al., 2010a,b; Niu et al., 2012).

In this study, a novel polysaccharide, named GPP, was purified from *G. pentaphyllum* Makino. Its monosaccharide composition and chemical structure were characterized by chemical and instrumental analyses. The *in vitro* antioxidant activity and bile acid binding property of the GPP were also determined. The results are important for developing *G. pentaphyllum* polysaccharides derived nutraceutical and functional food products.

2. Materials and methods

2.1. Materials

Diploid whole-plant *G. pentaphyllum* Makino, grown in the Dabashan area of Shaanxi province of China in 2009, was gifted by the Asian Citrus Holdings Limited (Hong Kong, China). After being washed using tap water, the sample was dried at 70–76 °C, then pulverized and kept in zip lock bags. The ploidy was identified by root tip chromosome checking.

Diethylaminoethyl Cellulose (DE-32) was purchased from Whatman International Ltd. (Kent, UK). Ambelite IRA-93 was purchased from Polysciences, Inc. (Warrington, PA, USA). Dextrans with different molecular weights were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Standard monosaccharides (arabinose, glucose, mannose, glucuronic acid, galactose, rhamnose, fucose, and xylose), inositol, sodium borohydride, 3-methyl-1-phenyl-2-pyrazolin-5-one (PMP) and acetonitrile were purchased from Acros Organics (Pittsburgh, PA, USA). Deuterated water (D_2O) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Thirty percent hydrogen peroxide, nitroblue tetrazolium chloride (NBT), cholic and chenodeoxycholic acids, diphorase, nicotinamide adenine dinucleotide, 3-α hydroxysterol dehydrogenase, fluorescein (FL), iron (III) chloride, 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,2-diphenyl-1-picryhydrazyl radical ($DPPH^{\bullet}$) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 2,2′-Azinobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA, USA). Other reagents and chemicals were all analytical grade.

2.2. Extraction, isolation and purification of GPP

Ground *G. pentaphyllum* Makino (100 g) was treated with methanol and refluxed with 80% ethanol at 50 °C for 2 h to remove oligosaccharides. After filtering, the dried residues were extracted with hot water twice at 80 °C for 2 h. The combined supernatants were concentrated at 80 °C and centrifuged at 6000 × *g* for 5 min, and precipitated using four times volume of ethanol (190 proof) at 4 °C over 8 h. The precipitate was collected by centrifugation, washed twice with ethanol (200 proof), and then dried with nitrogen, giving crude polysaccharides. Crude polysaccharides were treated with 30% hydrogen peroxide at 50 °C for 7 h, and then dialyzed against running water for 48 h. After treating with macroporous resin, the eluent was concentrated and applied to a DEAE column (2.5 cm × 30 cm) and eluted with 200 mL of distilled water at a rate of 16 mL/h. The progress of elution was monitored by the phenol–sulfuric acid procedure. The collected fraction was concentrated and then lyophilized to give a purified polysaccharide, named GPP.

2.3. Characterization of GPP

2.3.1. Purity and molecular weight determination

The purity and molecular weight of GPP were determined using a high performance size-exclusion chromatography (Shimazu, Kyoto, Japan). Sample was firstly dissolved in distilled water and passed through 0.45 μm filter, then applied to gel-filtration column (Shodex SUGAR KS-805, 8 mm ID × 300 mm, Showa Denko, Japan) and eluted at a flow rate of 1.0 mL/min with deionized water and detected with a refractive index detector. Column calibration was performed with standard dextrans with different molecular weights (2500, 4600, 7100, 10,000 and 21,400 Da, respectively). The standard curve represented the linear relationship of the retention time and the logarithm of their respective molecular weights. The molecular weight of GPP was calculated by comparing with the standard dextrans with different molecular weights.

2.3.2. Analysis of monosaccharide composition by GC and HPLC

GPP (5 mg) was completely hydrolyzed with 1 mL of 2 M trifluoroacetic acid (TFA) at 100 °C for 8 h. The excess acid was removed through co-distillation with methanol (Yang et al., 2005). The residual was dissolved in 1 mL distilled water and used for further derivatization.

Monosaccharide composition was analyzed by GC according to a previously described procedure with minor modification (Englyst & Cummings, 1984). The solution of 6 monosaccharide standards or the hydrolyzed polysaccharide was reduced with $NaBH_4$ at 65 °C for 1 h, and then acetylated with pyridine and acetic anhydride at 100 °C for 1 h. The alditol acetates were analyzed by Shimadzu GC-2010 gas chromatography (Shimadzu, Columbia, MD, USA) equipped with a HP-5 capillary column (30 m × 0.32 mm, 0.25 μm) and a flame-ionization detector, and the program of oven temperature was: initially from 110 to 220 °C at 5 °C/min, held for 2 min, then increased by 2 °C/min until 240 °C where it was held for 2 min, then increased by 10 °C/min to a final temperature of 280 °C.

Pre-column derivatization with PMP was carried out according to a protocol described previously (Fu & Oneill, 1995). Briefly, 50 μL seven monosaccharide standard mixtures or the hydrolyzed polysaccharide sample was mixed with 50 μL of 0.6 mol/L sodium hydroxide and 100 μL of 0.5 mol/L PMP in methanol. The mixture was kept at 70 °C for 30 min, then cooled to ambient temperature and neutralized with 100 μL of 0.3 mol/L hydrochloric acid. The resulting solution was mixed with H_2O (1.0 mL) and then extracted with chloroform (1.0 mL) for three times. The aqueous layer was combined and filtered through a 0.45 μm pore membrane filter for HPLC analysis with Shimadzu LC-20A HPLC system equipped a C18 column (4.6 mm × 250 mm, 5 μm, Phenomenex, Torrance, CA, USA). The wavelength of UV detection was 250 nm. The mobile phase consisted of ammonium acetate solution (pH 5.5) and acetonitrile with the volume ratio of 22:78.

2.3.3. Infrared spectral analysis

The FT-IR spectrum was recorded using a JASCO FT-IR-4100 spectrometer with ATR PRO450-S single reflection ATR accessory (JASCO, Easton, MD, USA) in the range of 4000–700 cm^{-1}.

2.3.4. Methylation analysis

Ten milligram of GPP, dissolved in DMSO, was methylated four times with methyl iodide according to the method of Needs and Selvendran (Needs & Selvendran, 1993) with minor modification. The methylated polysaccharide was depolymerized by heating with formic acid (3 mL) at 100 °C for 6 h, and then further hydrolyzed with 2 mol/L trifluoroacetic acid (2 mL) at 100 °C for 2 h. After being reduced with $NaBH_4$, methylated products were acetylated with acetic anhydride. The resulting products of alditol acetates were identified according to the relative retention time in GC and fragmentation pattern in EI-MS. The molar ratios of them were calibrated from peak areas and response factor in GC.

2.3.5. Nuclear magnetic resonance spectroscopy

1D and 2D NMR spectra were recorded on a Brucker AVIII-600 MHZ NMR Spectrometer at 30 °C. D_2O was used as the solvent.

2.4. Antioxidant activity of GPP

2.4.1. Relative DPPH• scavenging capacity (RDSC)

The RDSC assay was determined according to a laboratory protocol (Cheng, Moore, & Yu, 2006) using a Victor^3 plate reader (Perkin-Elmer, Turku, Finland). Trolox was used as the antioxidant standard. Briefly, 100 μL of 0.208 mmol/L DPPH• solution was added into each well containing 100 μL GPP, standard or blank. The absorbance at 515 nm was recorded every minute for 40 min and each sample was tested in triplicate. The relative scavenging capacity (RDSC) was quantified using the area under the curve (AUC) calculation and expressed as μmoles of Trolox equivalents (TE) per gram of GPP.

2.4.2. Hydroxyl radical scavenging capacity (HOSC)

The HOSC was detected using a laboratory protocol reported previously (Moore, Yin, & Yu, 2006). Fluorescein (FL) was used as the molecular probe and Trolox as the antioxidant standard. 30 μL of GPP, standard or blank was added into 170 μL of 9.28×10^{-8} mol/L FL solution which was prepared in 75 mM sodium phosphate buffer (pH 7.4). Then 40 μL of 0.20 mol/L hydrogen peroxide and 60 μL of 3.43 mol/L iron (III) chloride were added successively. The fluorescence was read every 4 min for 4 h, using a Victor^3 multilabel plate reader (Perkin-Elmer, Turku, Finland). The assay was conducted in triplicate for each sample and the HOSC value was reported in μmol TE/g GPP, based on AUC calculation.

2.4.3. Oxygen radical absorbance capacity (ORAC)

The ORAC value was examined with Fluorescein (FL) as the fluorescent probe and Trolox as the antioxidant standard (Moore et al., 2005). In brief, the initial reaction mixture included 225 μL of 8.16×10^{-8} mol/L FL solution and 30 μL of GPP, standard or blank, which were heated at 37 °C for 20 min in a Victor^3 multilabel plate reader (Perkin-Elmer, Turku, Finland). Then 25 μL of 0.36 mol/L AAPH was added into each well and the fluorescence was measured every 2 min for 2 h at 37 °C. The excitation and emission wavelengths were 485 and 535 nm, respectively, and the result was calculated using the same method as HOSC assay.

2.5. Bile acid-binding capacity

The bile acid-binding capacities were detected following a laboratory protocol reported previously (Liu et al., 2010a). Briefly, for simulating gastric condition, 25 mg of each sample was treated with 0.25 mL 0.01 mol/L HCl, and was incubated for 60 min at 37 °C with continuous shaking. Then 25 μL of 0.1 mol/L NaOH was added into the solution to bring pH to 7.0, and mixed with 1.25 mL 400 μmol/L bile acid stock solution dissolved in 0.01 mol/L phosphate buffer (pH 7.0) to simulate the intestinal condition. The mixture was incubated at 37 °C for another 60 min and then centrifuged at $6000 \times g$ for 10 min. The supernatant and bile acid standards (100 μL of each) were mixed with 125 μL of 1.22 mmol/L nicotinamide adenine dinucleotide and 5 mmol/L NBT, 100 μL of 625 units/L diphorase and 100 μL of 3-α hydroxysterol dehydrogenase solution (625 units/L). The mixture was incubated at ambient temperature for 60 min, and then 100 μL 1.33 mol/L phosphoric acid was added to terminate the reaction. The absorbance of the mixture was recorded at 530 nm. The levels of unbound bile acids were calculated according to the standard curves of two pure bile acids, cholic and chenodeoxycholic acids, which were selected because of their solubility in the experimental solvent. The bile acid-binding capacity (mg/g sample) was determined against a reagent blank, with cholestyramine resin as the positive control. Duplicate tests were conducted for each sample.

2.6. Statistical analysis

All data were expressed as mean ± SD. Differences between means were determined by analysis of variance (ANOVA) with Tukey's test, using SPSS (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, IL). $P \leq 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Isolation and purification

The crude polysaccharide with a yield of 2.57 g/100 g was isolated by hot water extraction from dry *G. pentaphyllum* Makino powder. After purification through DEAE-cellulose-32 column, GPP was obtained as the one major peak eluted with distilled water. The total carbohydrate content of GPP was 98.7%.

HPLC analysis (Data not shown) showed a single, symmetric and sharp peak, which indicated that GPP was homogeneous, with a purity of 97.3%. No significant absorbance was observed at 260 nm or near 280 nm in the UV spectrum, indicating that GPP contained no protein or nucleic acid (data not shown).

3.2. Molecular weight of GPP and monosaccharide composition

The average molecular weight of GPP was determined by high-performance gel permeation chromatography. The average molecular weight of GPP was estimated 7.1×10^3 Da based on the equation of the standard curve. GC is a preferred method in quantitative and qualitative determination of monosaccharide composition because of its accuracy, efficiency and convenience. According to the retention time of alditol acetate derivatives in GC, GPP consisted of four different monosaccharides, including mannose, glucose, galactose and arabinose, with the molar ratio of 1.00:77.33:4.81:1.83 (Data not shown). However, the derivative method used in GC analysis was not suitable for uronide-containing polysaccharides. Because uronic acids have unusual resistance to acid hydrolysis, and lactonization and decarboxylation would occur if uronic acids were liberated from the polymers (Jones & Albersheim, 1972). Therefore, PMP derivatization was carried out to determine uronic acid qualitatively. The result (data not shown) showed that GPP contained no uronic acid but only mannose, glucose, galactose and arabinose, which agreed to the result of GC analysis.

3.3. Structure characterization of GPP

IR spectrum of GPP (data not shown) displayed a broadly-stretched peak at 3358 cm^{-1} indicating the presence of hydroxyl groups. The weak peak at 2925 cm^{-1} was assigned to C-H stretching bands of CH_2. No absorbance band at 1700 cm^{-1} for carboxylic acid groups was observed, which was in good agreement with the result of PMP derivatization. Three intense stretching peaks at 1148, 1065 and 1025 cm^{-1} were ascribed to the presence of C—O bonds and pyranose ring in the monosaccharide blocks of GPP. The weak peaks at 932 and 759 cm^{-1} were due to non-symmetrical and symmetrical stretching vibration of glucopyranose ring, respectively. The characteristic band at 859 cm^{-1} resulted from the α-anomeric configuration.

The fully methylated GPP was depolymerized with acid and converted into partially methylated alditol acetates which were then

Table 1
Methylation analysis of GPP.

Methylated sugar	Retention time	Linkage type	Molar ratio	Mass fragments (*m*/*z*)
2,3,4,6-Me_4 Glc	17.26 min	1-linked Glc	0.6	43,71,101,129,161,205
2,3,6-Me_3 Glc	19.50 min	1,4-linked Glc	5	43,71,87,99,101,113,117,129,131,161,173,233
2,3-Me_2 Glc	20.86 min	1,4,6-linked Glc	1	43,85,101,117,142,159,201,261

analyzed by GC–MS. The GC–MS analysis obtained three homogeneous peaks, which were identified as 2,3,4,6-tetra-*O*-methyl-glucitol, 2,3,6-tri-*O*-methyl-glucitol and 2,3-di-*O*-methyl-glucitol in the molar ratio of 0.6:5:1 (Table 1) (Cao et al., 2006; Chakraborty, Mondal, Pramanik, Rout, & Islam, 2004; Zhang et al., 2010a). This result implied that GPP probably had a (1 → 4)-linked-glucan main chain with (1 → 6)-linked-glucan and few other monosaccharides like mannose, galactose and arabinose as side chains every 5 monosaccharide residues.

The ^{1}H and ^{13}C NMR spectra of GPP were shown in Fig. 1. The anomeric proton signals at δ 5.38, 5.35 and 4.96 ppm corresponded at H-1 of →4)-Glc*p*-(1→, →4,6)-Glc*p*-(1→ and Glc*p*-(1→ residues, respectively. The anomeric carbon signals for the →4)-Glc*p*-(1→, →4,6)-Glc*p*-(1→ and Glc*p*-(1→ residues were respectively

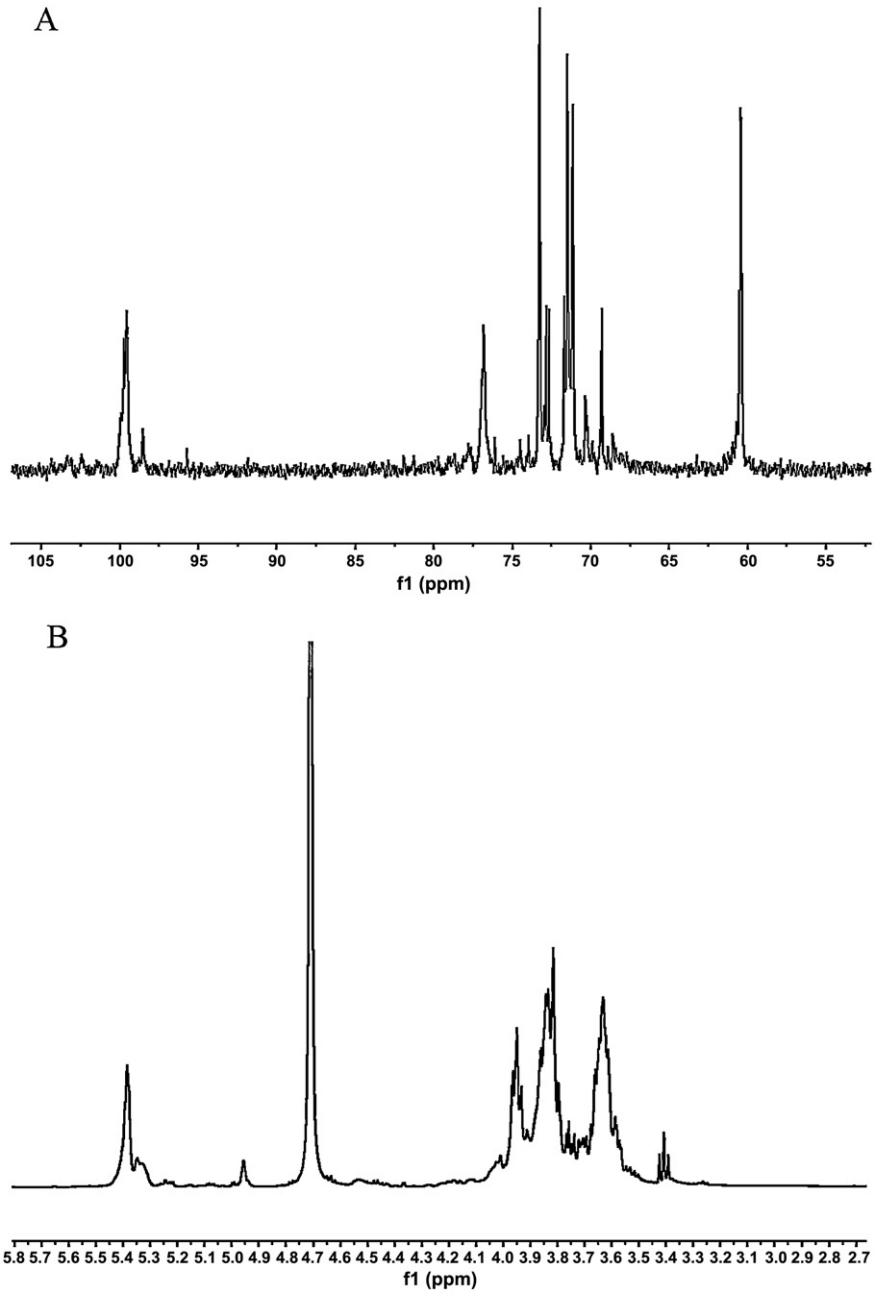

Fig. 1. ^{13}C and ^{1}H NMR spectra of GPP. (A) ^{13}C NMR spectrum, and (B) ^{1}H NMR spectrum. GPP from the whole-plant of diploid *Gynostemma pentaphyllum* Makino was dissolved in D_2O and examined on a Varian 600 NMR spectrometer. Numerical value is in *d* (ppm).

Table 2
Chemical shift of resonances in the ^{13}C and ^{1}H NMR spectra of GPP.

Residues	Chemical shift (δ, ppm)					
	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6
(A) →4)-α-Glc*p*-(1→	99.6/5.38	71.5/3.63	73.3/3.95	76.8/3.63	71.2/3.84	60.4/3.80
(B) →4,6)-α-Glc*p*-(1→	99.7/5.35	71.5/3.57	72.8/3.68	76.8/3.83	71.2/3.65	69.3/3.41
(C) α-Glc*p*-(1→	99.5/4.96	71.5/3.58	73.3/3.65	70.3/3.57	71.2/3.66	60.4/3.82

assigned at δ 99.6, 99.7 and 99.5 ppm. The appearance of the respective anomeric proton and carbon signals indicated that both Glc*p* moieties exhibited a α-anomeric configuration (Chakraborty et al., 2004), which was in good agreement with the presence of the IR band at 859 cm^{-1}. The carbon signal at δ 76.8 ppm should be C-4 signal of the →4)-Glc*p*-(1→ and →4, 6)-Glc*p*-(1→ residues, which was shifted about 6 ppm downfield compared with the signal of Glc*p*-(1→ at δ 70.3 ppm due to the glycosylation. Similarly, the C-6 signal at δ 69.3 ppm for →4, 6)-Glc*p*-(1→ appeared almost 9 ppm downfield compared with the signal of →4)-Glc*p*-(1→ and Glc*p*-(1→ at δ 60.4 ppm (Bao, Wang, Dong, Fang, & Li, 2002). The assignment of all other signals in ^{1}H and ^{13}C NMR spectra were summarized in Table 2, by taking together with the results of H-H-COSY (Fig. 2), HSQC (Fig. 3) and HMBC (Fig. 4) experiments and previously reported data (Bubb, 2003; Cao et al., 2006; Cui et al., 2008; Duus, Gotfredsen, & Bock, 2000; Huang & Zhang, 2009).

From the H-H-COSY spectrum, H-1 signal for →4)-α-Glc*p*-(1→ at δ 5.38 ppm might correlate with H-2 at δ 3.63 ppm. Similarly, H-3 (δ 3.95 ppm), H-4 (δ 3.63 ppm), H-5 (δ 3.84 ppm) and H-6 (δ 3.80 ppm) were assigned successively. Taking together these results with the HSQC spectrum, C-2, C-3, C-4, C-5 and C-6 of →4)-α-Glc*p*-(1→ were assigned to δ 71.5, 73.3, 76.8, 71.2 and 60.4 ppm, respectively. Signals of →4, 6)-α-Glcp-(1→ and α-Glcp-(1→ residues were assigned by a similar procedure.

HMBC spectrum could further confirm the information obtained from the HSQC and COSY experiments and observe more assignments about the sequence of glycoside residues. From the HMBC spectrum (Fig. 4), cross peaks H-1-C-4 and H-4-C-1 of →4)-α-Glc*p*-(1→ suggested the linkages of two 1,4-α-Glc*p* residues. The signals of mannose, galactose and arabinose were not clear in methylation analysis result and the NMR spectra perhaps because of their relatively low content.

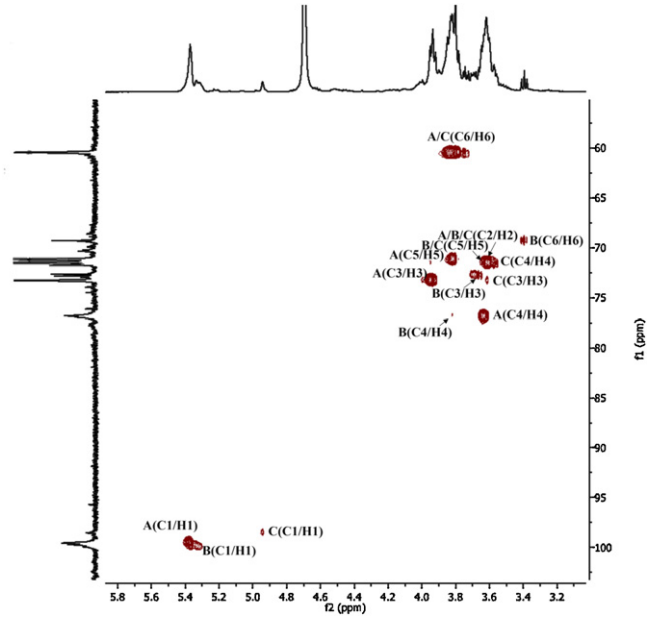

Fig. 3. 600 MHz HSQC spectrum of GPP in D_2O solution at 30 °C. (A) →4)-α-Glc*p*-(1→, (B) →4,6)-α-Glcp-(1→, and (C) α-Glcp-(1→.

In conclusion, GPP probably had a backbone mainly composed of (1 → 4)-linked-α-glucan with a branch chain at the C-6 position of (1 → 4,6)-linked-α-D-glucopyranosyl on every 5 residues,

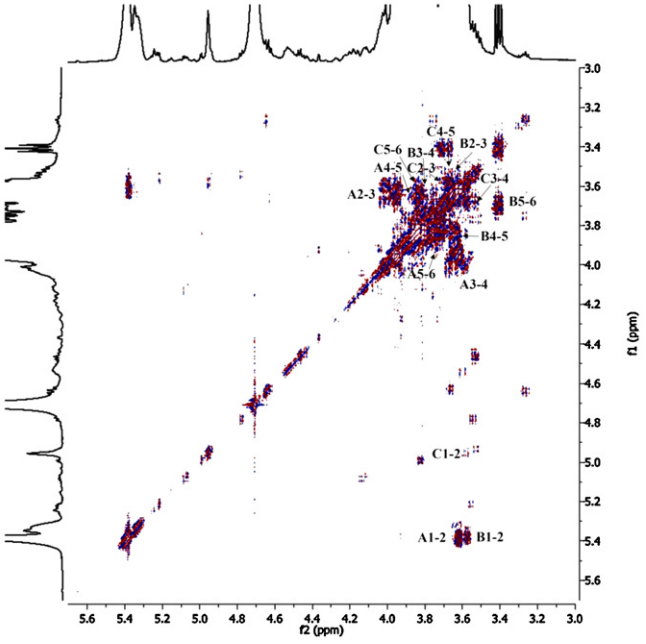

Fig. 2. 600 MHz H-H COSY spectrum of GPP in D_2O solution at 30 °C. (A) →4)-α-Glc*p*-(1→, (B) →4,6)-α-Glcp-(1→, and (C) α-Glcp-(1→.

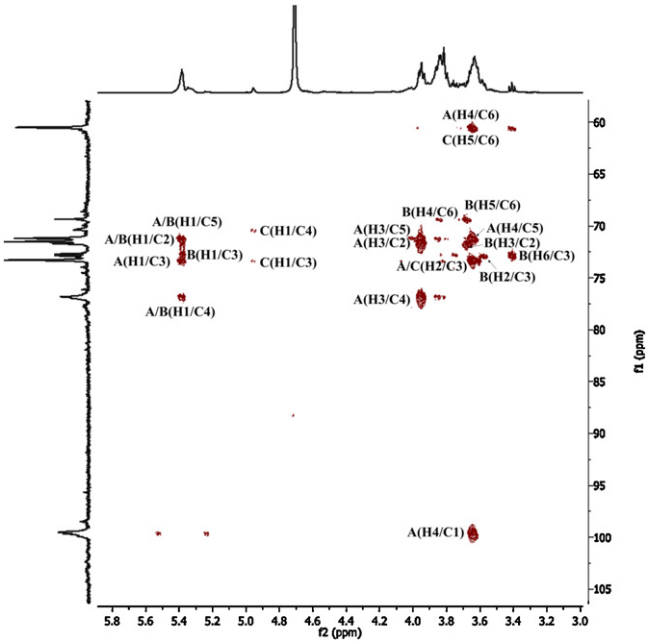

Fig. 4. 600 MHz HMBC spectrum of GPP in D_2O solution at 30 °C. (A) →4)-α-Glc*p*-(1→, (B) →4,6)-α-Glcp-(1→, and (C) α-Glcp-(1→.

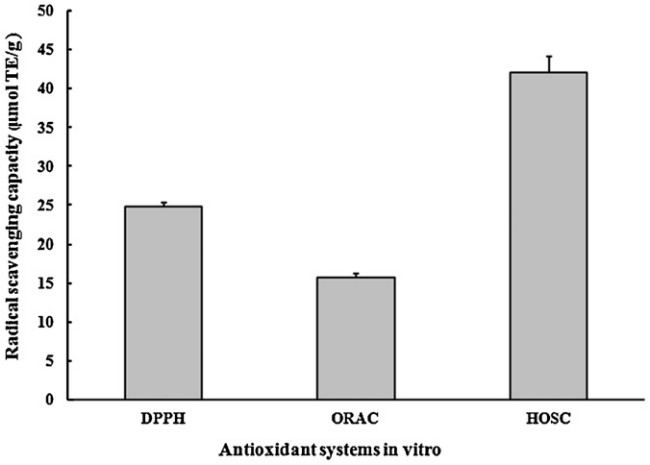

Fig. 5. Radical scavenging capacity of the GPP. Data are expressed as Trolox equivalent (TE) in μmol TE/g GPP. Antioxidant capacity was calculated by area under the curve (AUC) of sample absorbance compared to the absorbance of standards. Vertical bars represent SD.

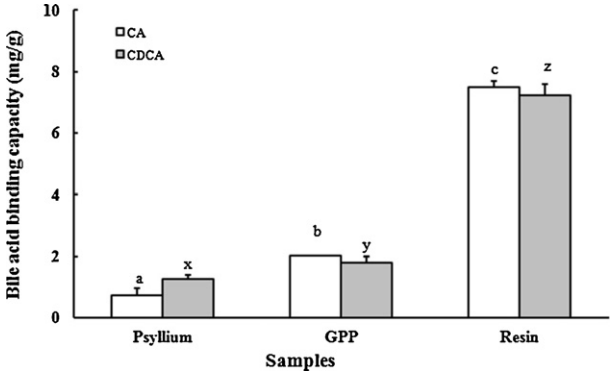

Fig. 6. Bile acid-binding property of GPP. CA and CDCA represent cholic and chenodeoxycholic acids, respectively. Data are expressed as mean ± SD. Vertical bars represent the SD. Letters *a*, *b* and *c* represent the significant difference of CA binding capacities, while letters *x*, *y* and *z* indicate the significant difference of CDCA binding properties among the samples ($P < 0.05$).

on average. And the terminal monosacharide was mainly 1→)-α-D-glucopyranosyl and few other monosaccharides.

3.4. Antioxidant activity of GPP in vitro

There are many assays that can evaluate the antioxidant capacities of polysaccharides *in vitro*. No single assay may accurately evaluate the total antioxidant capacity. Two major mechanisms, single electron transfer (SET) and hydrogen atom transfer (HAT), were involved in the process of radical deactivation. The oxygen radical absorbance capacity assay (ORAC) measures the peroxyl radical scavenging capacity, and the hydroxyl radical scavenging capacity assay (HOSC) measures the scavenging capacity against OH radical generated by the Fenton reaction of Fe(III) and H_2O_2. ORAC and HOSC are two examples of HAT reaction assays, while the DPPH• scavenging capacity (RDSC) is based on measurement of the reducing ability of antioxidants against DPPH•, a SET assay. These three assays were selected in this study to evaluate the antioxidant property of GPP (Huang, Ou, & Prior, 2005; Prior, Wu, & Schaich, 2005).

As shown in Fig. 5, the values of DPPH• scavenging capacity, hydroxyl radical scavenging capacity and oxygen radical absorbing capacity were 24.76, 42.06 and 15.76 μmol TE/g GPP, respectively. Hydroxyl radical is the most reactive oxygen radical, which can cross cell membranes and react with most of biological molecules in cells readily, including DNA, proteins and lipids, and cause further injury to the cells (Yuan et al., 2008). Although peroxyl radical is a relatively weak oxidant, it can decompose to form more toxic oxidative species, such as singlet oxygen and hydroxyl radicals, which can increase local oxidative stress and initiate the chain reaction of lipid peroxidation of the cellular membranes and may cause tissue damage or cell death (Yuan et al., 2005). DPPH is a useful reagent for evaluating the free radical-scavenging activity in a relatively short time. GPP exhibited efficient activity against the three antioxidant systems *in vitro*, which may be attributed to the hydroxyl groups in its molecular structures. It has been reported that polysaccharides with a radical scavenging effect have the similar structure feature containing one or more phenolic or alcohol hydroxyl groups, and their radical scavenging abilities are in proportion to the number of active hydroxyl groups in the molecule structure (Guo et al., 2005; Li, Jiang, Xue, & Chen, 2002).

3.5. Bile acid-binding capacity of GPP

Psyllium is known dietary source for both soluble and insoluble fibers. Psyllium intake may significantly reduce the plasma and LDL cholesterol levels ranging from 10 to 24% and may reduce the risk of coronary heart disease. One of the possible mechanisms has been suggested as its capacity to bind bile acids in the intestinal lumen (Singh, 2007).

GPP had stronger bile acid binding capacity than psyllium on a per same weight basis (Fig. 6). Its cholic acid (CA) binding capacity was about 2.8-fold of that for psyllium, whereas its chenodeoxycholic acid (CDCA) binding capacity was 1.5-fold of that for psyllium. Cholestyramine resin has been commercially used as a cholesterol reducing agent through a bile acid-binding mechanism (McCrindle, O'Neill, Cullen-Dean, & Helden, 1997). Cholestyramine resin was included in the evaluation as a positive control. GPP was not as effective as cholestyramine resin in binding bile acids (Fig. 6). Together, the results indicated that GPP may serve as a potential cholesterol-reducing nutraceutical.

4. Conclusion

A novel bioactive polysaccharide, GPP, was obtained from the whole-plant of diploid *G. pentaphyllum* Makino. Its molecular weight was 7.1×10^3 Da with a 1,4)-linkage-α-glucose backbone, branch chain of -1)-α-glucose-(4,6-linkage and mainly terminal residue of -1)-α-glucose. GPP is an effective HO•, $O_2^{\bullet -}$ and DPPH• scavenging agents and has significant bile acid-binding capacity, suggesting its possible application as nutraceuticals in reducing the risk of heart diseases. Additional research shall focus on its *in vivo* bioactivity and the related mechanism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.11.074.

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