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Characterization of a Novel Polysaccharide from Tetraploid *Gynostemma pentaphyllum* Makino

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ABSTRACT: A novel heteropolysaccharide (GPP-TL) was isolated from tetraploid *Gynostemma pentaphyllum* (Makino) leaf by hot water extraction and anion-exchange and gel permeation chromatography approaches. GPP-TL had a molecular weight of 9.3×10^3 Da and was primarily composed of glucose, galactose, and arabinose, with a molar ratio of 43:5:1, respectively. The chemical structure of GPP-TL was characterized using chemical and instrumental analyses. The results indicated the presence of (1→4)-α-D-glucopyranosyl, (1→4)-β-D-galactopyranosyl, (1→4,6)-linked-α-D-glucopyranosyl, and terminal 1→)-α-D-glucopyranosyl moieties in a molar ratio of 5.7:1:1.5:1, respectively. The results indicated that GPP-TL had glucose and galactose residues in the main chain with (1→6)-linked branches at glucose residues. In addition, GPP-TL exhibited scavenging capacities against hydroxyl, peroxyl, and DPPH radicals in vitro and had a stronger bile acid-binding ability than psyllium on a same-weight basis.

KEYWORDS: *polysaccharide, structure analysis, antioxidant activity, bile acid-binding capacity*

INTRODUCTION

Gynostemma pentaphyllum is a perennial liana plant growing in southern China, Japan, India, and Korea.[1] *G. pentaphyllum*, known as jiaogulan in China, has been used in food or supplemental products for more than 500 years. Nowadays, many commercial jiaogulan products are available in the United States, China, and several other Asian and European countries, and the most popular one is "jiaogulan tea".[2] *G. pentaphyllum* and its extract may also be used in sport or health beverages, mineral water, colas, wine, beer, biscuits, or noodles[3] for its potential in reducing the risk of cardiovascular diseases,[4] diabetes,[5] gastric ulcer,[6] and cancer.[7]

Cardiovascular disease is a leading cause of death and disability in the world and becomes a health concern for younger populations.[8] Many studies have suggested that oxidation of low-density lipoproteins (LDL) might play an important role in the development of cardiovascular diseases. Additionally, free radicals and lipid peroxides in cells may have a direct toxic effect on endothelial cells by reacting with proteins and lipids in the cell membrane.[9] Population-based and animal studies indicated that a diet supplemented with antioxidants might reduce the risk of cardiovascular disease.[10] Lin et al. reported that *G. pentaphyllum* extract had antioxidant effects.[11] Wang and Luo reported that polysaccharides extracted from *G. pentaphyllum* had strong antioxidant capacities.[12] Many other polysaccharides were also reported to have antioxidant activities.[13,14] Polysaccharides may have greater bioavailability because they may be water-soluble and may be easily leached into tea infusion compared with essential oils and saponins.[15] Besides the antioxidant mechanism, polysaccharides may also bind bile acids and increase their excretion.[16,17] This intestinal depletion of bile acids may reduce serum cholesterol levels by accelerating the conversion of cholesterol into bile acids in the liver.[18] The reduction of plasma cholesterol concentration may lower the risk of coronary heart diseases.[19] To date, little is known about the chemical properties and potential health properties of polysaccharides in the tetraploid *G. pentaphyllum*, which is a new genotype bred from diploid *G. pentaphyllum*.

In this study, a novel water-soluble polysaccharide (GPP-TL) was isolated from the tetraploid *G. pentaphyllum* leaves for the first time and characterized for its chemical structure. The in vitro antioxidant activity and bile acid-binding property of the GPP-TL were also determined as a preliminary evaluation of its possible application in functional foods and dietary supplements for reducing the risk of cardiovascular diseases.

MATERIALS AND METHODS

Materials. Tetraploid *G. pentaphyllum* leaves were a gift from Asian Citrus Holdings Limited (Hong Kong, China). They were harvested in the Dabashan area of Shaanxi province of China in 2009. After washing by tap water, the dried sample was obtained using a tea dryer at 70−76 °C and pulverized by a conventional pulverizing machine and then kept in zip-lock bags at ambient temperature for about 2 weeks before analysis. The ploidy was confirmed by root tip chromosome checking.

Dextrans with different molecular weights were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Standard monosaccharides (arabinose, fucose, galactose, glucose, glucuronic acid, mannose, rhamnose, and xylose), inositol, sodium borohydride, acetonitrile, and 3-methyl-1-phenyl-2-pyrazolin-5-one (PMP) were purchased from Acros Organics (Pittsburgh, PA, USA). Diethylaminoethyl cellulose (DEAE-32) was

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purchased from Whatman International Ltd. (Kent, UK). Cholic and chenodeoxycholic acids, diphorase, nicotinamide adenine dinucleotide, 3-α-hydroxysterol dehydrogenase, 30% hydrogen peroxide, nitroblue tetrazolium chloride (NBT), fluorescein (FL), iron(III) chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,2-diphenyl-1-picrylhydrazyl 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 chemicals and reagents were of analytical grade without further purification.

Extraction, Isolation, and Purification. Ground *G. pentaphyllum* (100 g) was treated with methanol and refluxed with 80% ethanol at 50 °C for 2 h to remove small molecular impurities. After filtration, the dry residue was extracted in hot water (1:20, w/v) twice at 80 °C, 2 h each time. The combined supernatants were condensed to about 200 mL with rotary evaporators and centrifuged at 6000 rpm for 5 min. Four volumes of pure ethanol was added into the solution slowly with stirring to precipitate the polysaccharide, and the mixture was kept at 4 °C overnight. The resulting precipitate was collected by centrifugation, repeatedly washed with pure ethanol three times, and dried under nitrogen, giving crude polysaccharides. Crude polysaccharides were treated with 30% hydrogen peroxide at 50 °C for 7 h and then dialyzed against distilled water using dialysis tubes (molecular weight cutoff = 3500 Da), concentrated, and lyophilized. After the purification by DEAE column (2.5 × 30 cm) and Sephacryl S-400 chromatography (1.0 × 100 cm), the collected fraction, eluted with distilled water, was concentrated using rotary evaporators and then lyophilized to give a purified polysaccharide, named GPP-TL.

Homogeneity and Molecular Weight Determination. The homogeneity and molecular weight were determined using a size exclusion HPLC system (Shimazu, Kyoto, Japan). Sample was dissolved in distilled water and filtered through a 0.45 μm filter, applied to a gel filtration column (Shodex SUGAR KS-805, 8 mm i.d. × 300 mm, Showa Denko, Japan), eluted by deionized water at a flow rate of 1.0 mL/min, and detected with a differential refractometric detector. The molecular weight of GPP-TL was calculated using a standard curve prepared using dextrans with different molecular weights (2500, 4600, 7100, 10000, and 21400 Da, respectively).

Monosaccharide Composition Analysis. GPP-TL (5 mg) was completely hydrolyzed to monosaccharides in 2 M trifluoroacetic acid (TFA). After heating at 100 °C for 8 h, the excess acid was removed through codistillation with methanol.[20] The residual was dissolved in 1 mL of distilled water and used for further derivatization.

Gas chromatography (GC) was used for identification and quantification of the monosaccharides.[21] After being reduced with $NaBH_4$ at 65 °C for 1 h, monosaccharides were converted into the alditol acetates by treatment with pyridine and acetic anhydride at 100 °C for 1 h. The resulting alditol acetates were analyzed using a Shimadzu GC-2010 gas chromatograph (Shimadzu, Columbia, MD, USA) equipped with a HP-5 capillary column (30 m × 0.32 mm, 0.25 μm). The column temperature was from 110 to 220 °C at a rate of 5 °C/min, held for 2 min, then increased to 240 °C at a rate of 2 °C/min, kept for 2 min, and finally increased to 280 °C in 4 min. The products were identified by their characteristic retention times.

The released monosaccharides were labeled with PMP according to a protocol described previously.[22] Briefly, 50 μL of monosaccharide standard mixture or the hydrolyzed polysaccharide sample was mixed with 50 μL of 0.6 M sodium hydroxide and 100 μL of 0.5 M PMP in methanol. The mixture was kept at 70 °C for 30 min, then cooled to room temperature, and neutralized with 100 μL of 0.3 M hydrochloric acid, followed by extraction with chloroform (1.0 mL) three times. The aqueous layer was filtered through a 0.45 μm pore membrane filter for HPLC (Shimadzu LC-20A) analysis at 250 nm. A C18 column (4.6 mm × 250 mm, 5 μm, Phenomenex, Torrance, CA, USA) was used, and the mobile phase consisted of ammonium acetate solution (pH 5.5) and acetonitrile with the volume ratio of 22:78.

Infrared Spectral Analysis. The Fourier transform infrared (FI-IR) spectroscopy spectrum was recorded using a Jasco FT-IR-4100 spectrometer with an ATR PRO450-S single-reflection ATR accessory (Jasco, Easton, MD, USA) in the range of 4000−700 cm^{-1}.

Methylation Analysis. Ten milligrams of GPP-TL was dissolved in dimethyl sulfoxide and methylated four times with methyl iodide according to the method of Needs and Selvendran.[23] The fully methylated polysaccharide was converted to alditol acetates by depolymerization with formic and trifluoroacetic acids, followed by reduction with $NaBH_4$ and acetylation with acetic anhydride. The resulting alditol acetates were subjected to gas chromatography−mass spectrometry (GC-MS) analysis. Linkages were identified according to the relative retention time and fragmentation patterns. The monosaccharide molar ratio was calibrated using the peak areas and response factor in GC.

Nuclear Magnetic Resonance Spectroscopy (NMR). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVIII-600 MHz NMR spectrometer at 30 °C, with D_2O as the solvent.

Relative DPPH$^{\bullet}$ Scavenging Capacity (RDSC). The GPP-TL was examined for its radical DPPH scavenging capacity according to a laboratory protocol reported previously.[24] Briefly, 100 μL of 0.208 mM DPPH$^{\bullet}$ solution was mixed with 100 μL of GPP-TL, standard (Trolox), or blank to initiate the radical−antioxidant reaction. The absorbance at 515 nm was measured every minute for 40 min using a Victor^3 multilabel plate reader (Perkin-Elmer, Turku, Finland), and each sample was tested in triplicate. The RDSC was calculated according to the area under the curve (AUC) and expressed in micromoles TE (Trolox equivalents) per gram of GPP-TL.

Hydroxyl Radical Scavenging Capacity (HOSC). The HOSC of GPP-TL was analyzed following a laboratory protocol reported previously using a Victor^3 multilabel plate reader (Perkin-Elmer).[25] The sample solution (30 μL) was mixed with 170 μL of 9.28 × 10^{-8} M fluorescein (FL) solution, which was used as molecular probe. Then hydrogen peroxide (40 μL, 0.20 M) and iron(III) chloride (60 μL, 3.43 M) were added successively prior to the record of fluorescence every 4 min for 4 h. The result was calculated using AUC, and all of the tests were performed in triplicate.

Oxygen Radical Absorbance Capacity (ORAC). The ORAC assay was conducted using FL as the fluorescent probe and Trolox as the antioxidant standard, according to a previously described protocol.[26] In brief, the final assay mixture included 225 μL of 8.16 × 10^{-8} M FL solution, 30 μL of GPP-TL, standard, or blank solution, and 25 μL of 0.36 M AAPH. The sample and the standard were run in triplicate simultaneously using a Victor^3 multilabel plate reader (Perkin-Elmer). The fluorescence was measured every 2 min for 2 h at ambient temperature. Excitation and emission wavelengths were 485 and 535 nm, respectively, and the result was calculated using the relative area under the curve for the sample compared with the Trolox standard curve.

Bile Acid-Binding Capacity Assay. The bile acid-binding properties were evaluated using a laboratory protocol.[17] Briefly, 10 mg of each sample was digested with 0.1 mL of 0.01 M HCl at 37 °C for 60 min with continuous shaking to simulate gastric conditions. Then the solution was brought to pH 7.0 with 10 μL of 0.1 M NaOH. For simulating the intestinal condition, the mixture was incubated at 37 °C for another 60 min after the addition of 0.5 mL of 0.01 M phosphate buffer (pH 7.0) and 0.5 mL of 400 μM bile acid solution. Samples were quantified after centrifuging at 6000 rpm for 10 min. The supernatant and bile acid standards (100 μL of each) were mixed with 125 μL of 1.22 mM nicotinamide adenine dinucleotide and 5 mM NBT, 100 μL of 625 units/L diphorase, and 625 units/L 3-α hydroxysterol dehydrogenase. The mixture was incubated at ambient temperature for 60 min. The reaction was terminated by the addition of 100 μL of 1.33 M phosphoric acid. The absorbance of each mixture was read at 530 nm with cholestyramine resin as the positive control. The amount of bile acids bound was calculated according to the standard curves of two pure bile acids, cholic and chenodeoxycholic acids. Duplicate tests were performed for each sample against each bile acid.

Statistical Analysis. All values were expressed as the mean ± SD for each experiment. 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, USA). $P \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Isolation and Purification of GPP-TL. After hot water extraction and ethanol precipitation, the crude polysaccharide was obtained from *G. pentaphyllum* powder, with a yield of

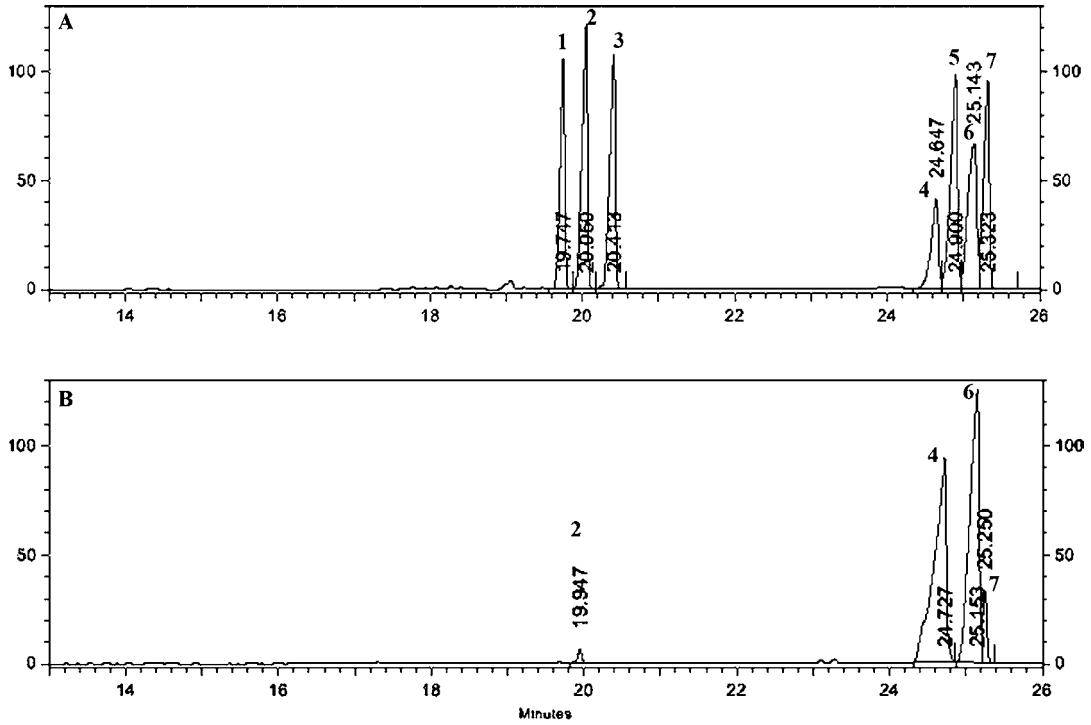

Figure 1. GC chromatograms of (A) standard monosaccharides and (B) monosaccharide composition of GPP-TL. GPP-TL was hydrolyzed with TFA at 100 °C for 8 h and then derivatized by acetyl. Peaks: 1, rhamnose; 2, arabinose; 3, xylose; 4, inositol; 5, mannose; 6, glucose; 7, galactose.

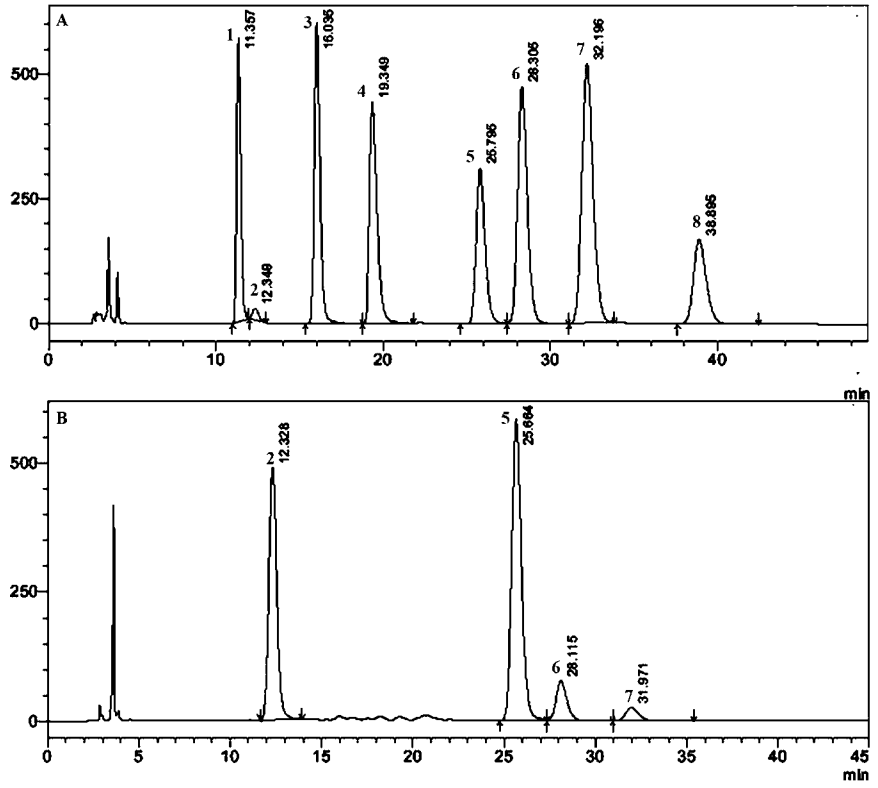

Figure 2. HPLC chromatograms of PMP derivatives of (A) standard monosaccharides and (B) component monosaccharides of GPP-TL. GPP-TL was hydrolyzed with TFA at 100 °C for 8 h and then labeled with PMP. Peaks: 1, mannose; 2, PMP; 3, rhamnose; 4, glucuronic acid; 5, glucose; 6, galactose; 7, arabinose; 8, fucose.

approximately 4.88 g/100 g. After the H_2O_2 treatment and DEAE- 32 chromatography, a major peak eluting with distilled water from the Sephacryl S-400 column, named GPP-TL, was obtained. The total carbohydrate content of GPP-TL was 99.4%.

GPP-TL showed a single, symmetric, and sharp peak on high-performance gel permeation chromatography (HGPC), indicating its homogeneity, with a purity of 98.4% (data not shown). In addition, GPP-TL contained no protein or nucleic acid, as

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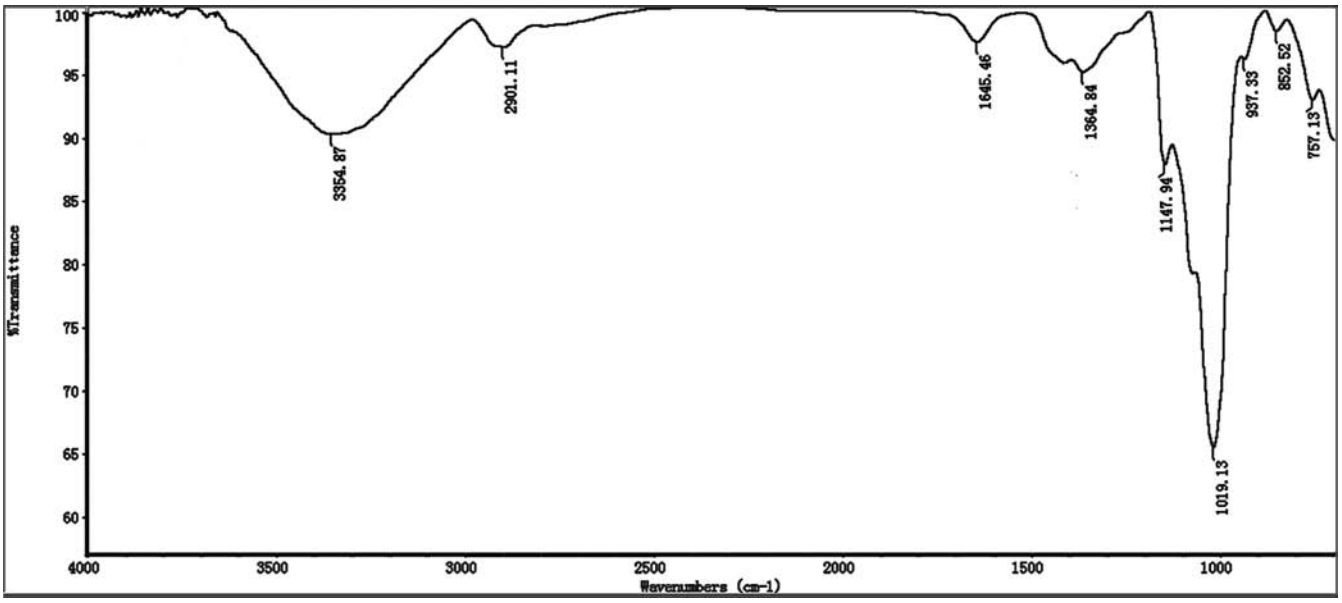

Figure 3. IR spectrum of GPP-TL, recorded in the range of 4000−700 cm^{-1}.

evidenced by a lack of absorbance near 260 and 280 nm (data not shown).

Molecular Weight of GPP-TL and Monosaccharide Composition. The average molecular weight of GPP-TL was 9.3×10^3 Da based on HGPC data. As shown in Figure 1, the peaks of alditol acetate derivatives suggested the presence of glucose, galactose, and arabinose, with the molar ratio of 43:5:1. However, the derivative method used in GC analysis was not suitable for the detection of uronide-containing polysaccharides because uronic acids had the unusual resistance to acid hydrolysis, and decarboxylation and lactonization would occur when uronic acids were liberated from the polymer.[27] Therefore, PMP derivatization was carried out to qualitatively determine the uronic acid and accurately confirm the result of GC analysis. Figure 2 indicates that GPP-TL did not contain uronic acid but only glucose, galactose, and arabinose, which was in good agreement with the result of GC analysis. Taken together, GPP-TL was a heteropolysaccharide primarily containing glucose and galactose.

Structure Characterization of GPP-TL. The different absorption bands of the FT-IR spectrum (Figure 3) were assigned according to the literature.[28,29] The broad band centered at 3355 cm^{-1} arose from OH stretching vibrations of hydroxyl groups. The weak band at 2901 cm^{-1} was due to the C−H stretching vibrations of CH_2. No absorbance band near 1700 cm^{-1} for carboxylic acid groups was observed, which was in good agreement with the result of PMP derivatization. Strong overlapped IR bands in the region of 1200−1020 cm^{-1} were ascribed to C−O stretching vibrations of pyranose ring. The weak peaks at 937 and 757 cm^{-1} were due to nonsymmetrical and symmetrical stretching vibrations of glucopyranose ring, respectively. The absorption band centered at 852 cm^{-1} was characteristic of α-anomeric configuration.

Methylation analysis by GC-MS was used to provide more structural information for GPP-TL. Four homogeneous peaks of the partially methylated alditol acetates were obtained from GC analysis. The peaks were identified as 2,3,4,6-tetra-*O*-methylglucitol, 2,3,6-tri-*O*-methylglucitol, 2,3,6-tri-*O*-methylgalactitol, and 2,3-di-*O*-methylglucitol in the molar ratio of 1:5.7:1:1.5 (Table 1) according to their retention time and comparison with mass spectrum patterns from the literatures. Therefore, it was

Table 1. Methylation Analysis of GPP-TL

methylated sugar (as alditol acetates)	molar ratio	mass fragments (m/z)	linkage pattern
2,3,4,6-Me_4 Glc	1	43, 71, 101, 129, 161, 205	1-linked Glc
2,3,6-Me_3 Glc	5.7	43, 71, 87, 99, 101, 113, 117, 129, 131, 161, 173, 233	1,4-linked Glc
2,3,6-Me_3 Gal	1	43, 45, 87, 99, 101, 113, 117, 233	1,4-linked Gal
2,3-Me_2 Glc	1.5	43, 85, 101, 117, 142, 159, 201, 261	1,4,6-linked Glc

suggested that the repeating unit of GPP-TL consisted of 1,4-linked glucose, 1,4-linked galactose, 1,4,6-linked glucose, and 1-linked glucose. The molar ratio of these residues agreed overall with the monosaccharide composition of GPP-TL described above.[30−34]

NMR spectroscopy has become the most powerful technique for the structure analysis of carbohydrates. The ^1H and ^{13}C NMR spectra of GPP-TL are shown in Figure 4. Four signals appeared in the anomeric region of both the ^1H and ^{13}C NMR spectra, suggesting the presence of four different linkage patterns. The anomeric proton signals at δ 5.42, 5.38, and 4.99 and the anomeric carbon signals at δ 99.5, 99.7, and 98.6 corresponded at H-1 and C-1 of (1→4)-α-glucopyranose, (1→4,6)-α- glucopyranose, and (1→)-α-glucopyranose residues, respectively, which was in good agreement with the presence of an IR band at 852 cm^{-1}. The anomeric proton signals at δ 4.66 and the anomeric carbon signals at δ 104.3 could be attributed to H-1 and C-1 of (1→4)-β-galactopyranose. The characteristic absorption of β-configuration near 890 cm^{-1} was not observed in FT-IR analysis, possibly due to the relatively low content of galactose.[35]

The carbon signals of substituted C-4 of (1→4)-α-glucopyranose, (1→4,6)-α- glucopyranose, and (1→4)-β-galactopyranose residues had moved downfield to δ 76.7 and 77.6, respectively. The signals for unsubstituted C-6 of (1→4)-α-glucopyranose, (1→)-α-glucopyranose, and (1→4)-β-galactopyranose residues were at δ 60.4, 60.9, and 60.7, whereas the signal at δ 69.3 for substituted C-6 of (1→4,6)-α-glucopyranose was in the lower field. The assignments of all other proton and carbon signals are summarized in

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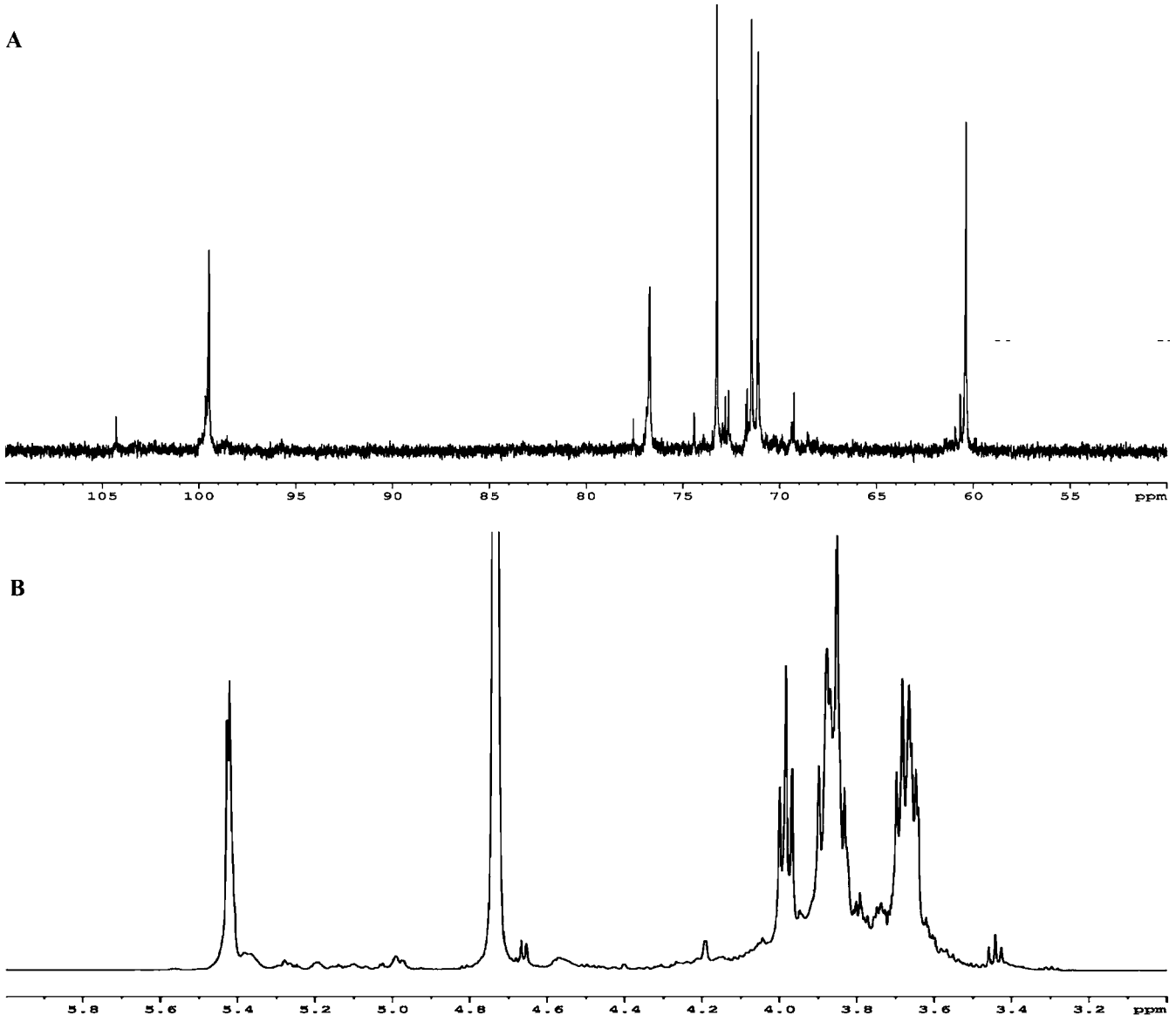

Figure 4. ^{13}C (A) and ^{1}H (B) NMR spectra of GPP-TL.

Table 2. Chemical Shift of Resonances in the ^{13}C and ^{1}H NMR Spectra of GPP-TL

	chemical shift (δ)					
residue	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6
→4)-*α*-Glc*p*-(1→	99.5/5.42	71.5/3.66	73.2/3.98	76.7/3.66	71.1/3.88	60.4/3.83
→4,6)-*α*-Glc*p*-(1→	99.7/5.38	71.5/3.61	72.8/3.71	76.7/3.87	71.1/3.69	69.3/3.44
→4)-*β*-Gal*p*-(1→	104.3/4.66	71.7/3.65	73.2/3.83	77.6/4.19	74.4/3.69	60.7/3.85
α-Glc*p*-(1→	98.6/4.99	71.5/3.62	73.2/3.69	69.4/3.61	71.1/3.70	60.9/3.85

Table 2, according to the literature.[36−38] The signal for arabinose was not observed in either methylation or NMR analysis, which might be explained by its low content (<5%) and the interference of the baseline noises, as well as the poor water solubility of GPP-TL, which limited the final concentration of testing sample for both analyses.[39]

In conclusion, GPP-TL was identified as a kind of galactoglucan, with a backbone mainly composed of (1→4)-linkage-*α*-glucose and possibly (1→4)-linked-*α*-galactose, with a branch chain of (1→4,6)-linked-*α*-D-glucose based on the experimental results. The terminal residue was generally 1→)-*α*-D-glucose and arabinose.

Antioxidant Activity of GPP-TL. Although antioxidant activity was usually associated with small molecular weight compounds, such as phenols and carotenoids, Tian et al. reported that polysaccharide was one of the major active ingredients responsible for antioxidant effect of *Houttuynia cordata*.[40] Increasing evidence indicates that polysaccharides from different sources may possess strong antioxidant properties without concerns of cytotoxicity and may be developed as novel dietary antioxidants.[41,42] The ORAC, HOSC, and DPPH• RDSC were evaluated for GPP-TL.

It is well accepted that DPPH free radical scavenging by antioxidants is due to their hydrogen-donating ability.[43] As shown in Figure 5, GPP-TL had a DPPH• scavenging capacity value of 15.92 μmol TE/g. GPP-TL had greater DPPH radical

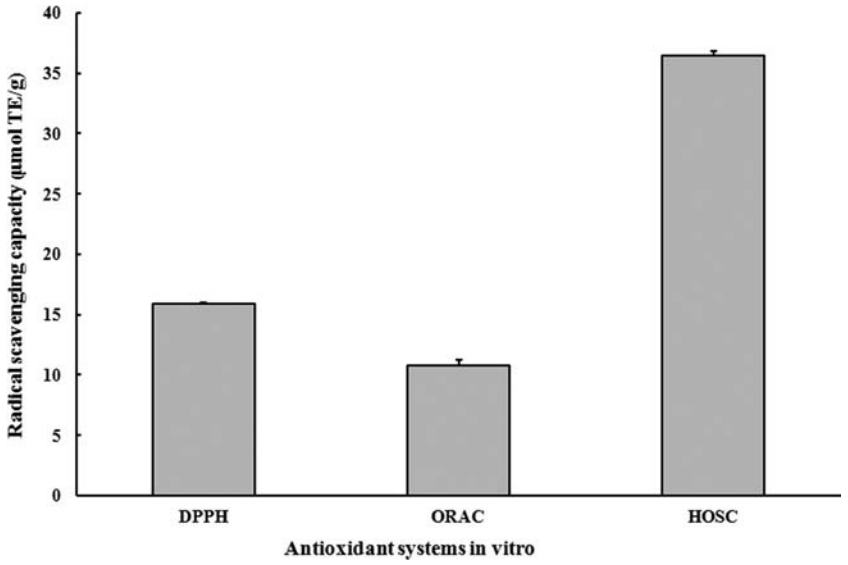

Figure 5. Antioxidant activity of GPP-TL. Data are expressed as Trolox equivalent (TE) in μmol TE/g GPP-TL. Antioxidant capacity was calculated using the area under the curve (AUC) against the Trolox standard. Vertical bars represent SD for triplicate measurements.

scavenging ability than tobacco seed flour with a RDSC value of 2.65−4.37 μmol TE/g[44] and than wheat grains with a RDSC value of 0.91−1.53 μmol TE/g.[45] GPP-TL could donate hydrogen atoms to DPPH radical to terminate the radical-mediated reactions.

Hydroxyl radical is considered to be the most reactive and poisonous free radical in biological systems and may react with almost all biomolecules, including cellular carbohydrates, proteins, lipids, and DNA, and cause tissue damage or cell death.[46] GPP-TL had a HOSC value of 36.42 μmol TE/g, which was comparable to that of tobacco seed flour (25−53 μmol TE/g)[44] and was greater than that of wheat grains (15.7−35.8 μmol TE/g).[45] The HOSC might be explained by hydrogen atom donation ability of the hydroxyl groups in GPP-TL to terminate the free radical-mediated oxidative chain reactions.[47]

Peroxyl radicals are the major oxygen-centered free radicals generated under normal physiological conditions. They may diffuse to cellular loci from the site of their generation and are also able to react with cellular DNA and other macromolecules. GPP-TL showed an ORAC value of 10.83 μmol TE/g under the experimental conditions. This value was lower than those of tobacco seed flour (44−74 μmol TE/g)[44] and wheat grains (18.6−24.5 μmol TE/g).[45]

Bile Acid-Binding Capacity of GPP-TL. Binding bile acids in the intestinal system could accelerate their excretion in feces. It may stimulate the conversion of cholesterol to bile acids in the liver and reduce plasma cholesterol levels.[48,49] This is a widely accepted mechanism for several cholesterol-lowering food polysaccharides such as psyllium. Psyllium is a kind of arabinoxylan derived from the seed husk of the *Plantago* genus. Some researchers suggested that the mechanism of psyllium on reducing the reabsorption of bile acids in the intestinal system is similar to that of resins such as cholestyramine, which increases its excretion.[50] As shown in Figure 6, GPP-TL had a stronger bile acid-binding capacity than psyllium on a same weight basis. Its cholic acid (CA)-binding capacity was about 1.5-fold of that for psyllum, whereas its chenodeoxycholic acid (CDCA)-binding capacity was 2.4-fold of that for psyllium. Cholestyramine resin, a nonadsorbing anion exchange resin capable of binding bile acids in the intestine, was included in the evaluation as a positive control.[51] GPP-TL was not as effective as cholestyramine resin in binding bile acids (Figure 6). Together, the results indicated that

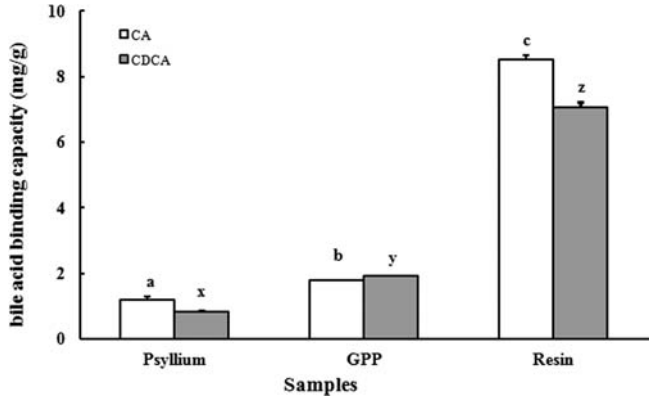

Figure 6. Bile acid-binding property of GPP-TL. CA and CDCA represent cholic and chenodeoxycholic acids, respectively. Data are expressed as the mean ± SD. Vertical bars represent the SD values. Letters a−c represent the significant difference of CA binding capacities, whereas letters x−z indicate the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

GPP-TL had a certain degree of bile acid-binding abilities and might serve as a potential cholesterol-reducing nutraceutical.

In summary, this study isolated and characterized a novel polysaccharide (GPP-TL) from the leaves of tetraploid *G. pentaphyllum*. GPP-TL contained glucose, galactose, and arabinose in a molar ratio of 43:5:1, with a molecular weight of 9.3×10^3 Da. The backbone of GPP-TL was composed of (1,4)-linkage-α-glucose, which occasionally branched at O-6, and terminated with 1-)-α-glucose. GPP-TL could significantly scavenge $HO^{\bullet}$, $O_2^{\bullet -}$, and $DPPH^{\bullet}$ and bind bile acids under physiological conditions, suggesting its possible application in functional foods and dietary supplemental products.

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Notes

The authors declare no competing financial interest.

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