Food Chemistry 119 (2010) 1626–1632

Contents lists available at ScienceDirect

Food Chemistry

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

Isolation, chemical composition and antioxidant activities of a water-soluble polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja

Jian-Hua Xie, Ming-Yong Xie *, Shao-Ping Nie, Ming-Yue Shen, Yuan-Xing Wang, Chang Li

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

ARTICLE INFO

Article history:
Received 17 July 2008
Received in revised form 20 July 2009
Accepted 15 September 2009

Keywords:
Cyclocarya paliurus (Batal.) Iljinskaja
Polysaccharide
Purification
Monosaccharide composition
Antioxidant activity

ABSTRACT

A water-soluble polysaccharide was isolated from the water extract of *Cyclocarya paliurus* (Batal.) Iljinskaja, which is a well-known native health tea in China. This polysaccharide was named as CPP-1. The molecular weight of CPP-1 was determined by high-performance gel permeation chromatography, with an average molecular weight of about 1167 kDa. The analysis of monosaccharide composition in the polysaccharide by gas chromatography revealed that it was a heteropolysaccharide and consisted of D-xylose, L-arabinose, D-glucose, D-galactose, L-rhamnose and D-mannose in a molar ratio of 1.00:9.67:9.65:4.96:3.29:2.70. Furthermore, CPP-1 contains 8.44% of protein and 17 general amino acids, and it is rich in glutamic acid, asparagic acid, leucine, glycine, arginine, tyrosine and alanine. The antioxidant activity of CPP-1 was also evaluated. It was found that CPP-1 exerted significant scavenging effects on DPPH radicals with a value of around 91.4%, compared to the reference controls of BHT (91.2%) and ascorbic acid (98.9%) at a concentration of 400 μg/ml, and with EC_{50} values of 52.3 μg/ml.

1. Introduction

Cyclocarya paliurus (Batal.) Iljinskaja (*C. paliurus*), a Chinese native plant, belongs to the genus *Cyclocarya* Iljinskaja (Juglangdaceae), which is the sole species in its genus and is mainly found at 420–2500 m elevation in the mountainous regions of Anhui, Fujian, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Guizhou, and Zhejiang provinces (Xie & Li, 2001). It is commonly called "sweet tea tree" because of the taste of its leaves (Fang, Wang, Wei, & Zhu, 2006). The leaves of *C. paliurus* have traditionally been used in China, both as drug formulations in traditional Chinese medicine (TCM), and as an ingredient in functional foods or dietary supplements for trace elements (Li et al., 2000; Xie, Li, Nie, Wang, & Lee, 2006). Significant attention has recently been drawn to the use of *C. paliurus* for developing functional food, as *C. paliurus* produces a great variety of nutrients that are essential for human health. *C. paliurus* health tea, the aqueous extract of *C. paliurus* leaves, is already known as a functional health food, has been become the first FDA-approved health tea of China in 1999 (Xu & Song, 2004). Recently, epidemiological researches showed that *C. paliurus* is beneficial in the prevention of hypolipidemic and diabetes mellitus. Results from a recent study (Kurihara, Asami, et al., 2003) showed that *C. paliurus* inhibits α-glucosidase, a disaccharide-degrading enzyme in the small intestinal mucosa, leading to a decrease in the absorption of glucose into the blood and a subsequent lowering of the blood glucose level. A pilot study indicated that a daily dietary supplement of *C. paliurus* could prevent dyslipidemia in rats and hamsters after a chronic high fat diet treatment (Kurihara, Asami, et al., 2003; Kurihara, Fukami, et al., 2003). In addition, many other therapeutic effects of *C. paliurus*, such as the enhancement of mental efficiency, antihypertensive action and immunomodulation, have been reported (Jiang, Zhang, Zhou, Qiu, & Chen, 2006; Shu, Xu, Li, & Yu, 1995; Xie & Li, 2001).

To date, the constituents responsible for action against various types of illnesses related to hyperlipoidemia, hypertensive and immune response and molecular mechanisms underlying these biological activities are unknown. Most studies on *C. paliurus* were concerned about the extract activities, and low molecular weight substances, such as triterpenoids, flavonoids, steroids, saponins and other compounds present in this plant (Jiang et al., 2006; Kennelly et al., 1995; Shu et al., 1995; Xie et al., 2006). Recently, polysaccharides have emerged as an important class of bioactive natural products (Zhao, Kan, Li, & Chen, 2005). A wide range of polysaccharides has been found to exhibit a variety of biological activities, such as anti-tumour activity (Nie, Xie, Zhou, & Cao, 2007; Saima, Das, Sarkar, Sen, & Sur, 2000; Sheng et al., 2007), free radical-scavenging activity (Chen, Xie, Nie, Li, & Wang, 2008; Liu, Ooi, & Chang, 1997; Tsai, Song, Shih, & Yen, 2007), heparinoid-activity (Maeda, Uehara, Harada, Sekiguchi, & Hiraoka, 1991) and immunomodulation activity (Tzianabos, Wang, & Kasper, 2003). However, less attention has been paid to the polysaccharides present in *C. paliurus*. Among the bioactive constituents, polysaccharides may play an important role in the field of antilipidemic and

* Corresponding author. Tel.: +86 791 3969009; fax: +86 791 3969069.
E-mail address: myxie@ncu.edu.cn (M.-Y. Xie).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.09.055

antihypertensive effects and enhancement of immunity. In our early research, a crude polysaccharide from the leaves of *C. paliurus* was obtained, which is effective in reducing blood glucose and improving the capacity of glucose tolerance in diabetic mice (Xie et al., 2006). However, there are no references available in the literature to studies performed on the separation and purification of polysaccharides from *C. paliurus* to the best of our knowledge.

Therefore, the aim of the present research was to isolate and determine the structural features and antioxidant activity of the polysaccharides present in this dried leaves of *C. paliurus*. In this study, a water-soluble polysaccharide was isolated by anion-exchange chromatography and gel permeation chromatography from the leaves of the *C. paliurus*, which has not been reported previously. We named it *C. paliurus* polysaccharide-1 (CPP-1). The present paper is concerned with the isolation, chemical characterisation and evaluation of the antioxidant activity of CPP-1.

2. Materials and methods

2.1. Materials

The dried leaves of *C. paliurus*, cultivated in Xiushui County, Jiangxi Province, China, were provided by Jiangxi Xiushui Miraculous Tea Industry Co. (Jiangxi, China). All samples were sliced and ground into fine powder in a mill before extraction.

DEAE-Sephadex A-25, Sepharose CL-6B, gel filtration chromatography, and Sephacryl S-400 were purchased from Amersham Biosciences (Uppsala, Sweden) and MW standards of T-series Dextran were obtained from Pharmacia Biotech (Uppsala, Sweden). Pure monosaccharide standards of D-mannose (Man), L-rhamnose (Rha), D-ribose (Rib), D-galactose (Gal), D-xylose (Xyl), D-arabinose (Ara), L-fucose (Fuc) and D-glucose (Glu) were obtained from Merck Co. (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO, USA). Amino acid standards of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-cystine, L-proline, L-serine, L-threonine, L-tyrosine, L-valine, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid and butylatedhydroxytoluene (BHT) were obtained from China Sigma–Aldrich (Shanghai, China). Inositol, hydroxylamine hydrochloride, acetic anhydride, pyridine, and acetic acid were of analytical pure grade, and purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Aqueous solutions were prepared with ultra-pure water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other reagents used were of analytical grade.

2.2. Preparation of crude polysaccharides

The dried leaves of *C. paliurus* powder (105 g) were first weighed and extracted with 1000 ml of 80% ethanol for 24 h to remove the interfering components, such as monosaccharide, disaccharide, oligosaccharide and polyphenol in the samples at 80 °C. The extraction procedure was carried out in the water bath. After filtration, the residue were dried at room temperature and placed in an extraction tube, then extracted twice with ultra-pure water (20:1 weight/volume ratio) at 80 °C for 2 h. The extracts were filtered, while warm, through glass wool and centrifuged at 8400×*g* for 15 min in a high speed centrifuge (model 3K30, Sigma, Germany) to separate the supernatant and the residue. The associated proteins in the extracts were removed, using the Sevag method (Navarini et al., 1999). After removing the Sevag reagent, the water phase were concentrated under reduced pressure at 55 °C and precipitated with four volumes of ethanol, then kept at 4 °C overnight in refrigerator to precipitate polysaccharides. The precipitates formed in the solution were collected and then redissolved in ultra-pure water, centrifuged at 8400×*g* for 15 min. The supernatant was further dialysed for 36 h in natural water and 12 h in ultra-pure water (MW cut-off 14 kDa) before concentration under vacuum evaporator at 55 °C. Lastly, the precipitate was frozen at −40 °C overnight and lyophilised in vacuum freeze dryer (model ALPHA 2–4, Christ, Germany). The crude polysaccharides were obtained.

2.3. Separation and purification of the polysaccharides

The crude polysaccharides from above were redissolved in ultra-pure water, then applied to a DEAE-Sephadex A-25 column (2.4 × 60 cm) for separation. The column was coupled to an ÄKTA Purifier 100 system (Amersham Pharmacia Biosciences). Detailed experimental conditions were as follows: concentration of crude polysaccharides, 3 mg/ml; injection volume, 4 ml; mobile phase, ultra-pure water; flow rate, 0.5 ml/min. Fractions of 5 ml were collected with a Pharmacia LKB Superfrac fraction collector, and the eluent (polysaccharide and protein elution) was monitored with a Shimadzu RID-10A Refractive Index Detector. After fractionation on a DEAE-Sephadex A-25 anion-exchange column, a fraction was obtained in the water eluate. The fraction eluted with water from crude polysaccharides designated as CPP was further purified on a Sephacryl S-400 column (2.4 × 60 cm). The column was coupled to an ÄKTA Purifier 100 system (Amersham Pharmacia Biosciences) for separation. The injection-loop was 5 ml, and 10–20 mg of the isolated fractions were applied onto the column. Sample elution was carried out using ultra-pure water as the eluent, at a flow rate of 0.5 ml/min. The eluent (polysaccharide and protein elution) was monitored with a Shimadzu RID-10A Refractive Index Detector. Two polysaccharide fractions, named as CPP-1 and CPP-2, were separated. The fractions, CPP-1 and CPP-2, were dialysed and lyophilised, respectively. The yields of CPP-1 and CPP-2 were about 76.6% and 23.3% from the crude polysaccharides, respectively. The yield of CPP-2 was low, so the CPP-1 was used in the subsequent studies.

2.4. Homogeneity and molecular weight determination

The homogeneity and molecular weight of CPP-1 were identified by high-performance gel permeation chromatography (HPGPC) with a Waters HPLC apparatus (UK6 injector and 510 HPLC pump, Waters, Milford, MA) equipped with an Ultrahydrogel™-500 column (300 × 7.8 mm), a Waters 2410 RI detector, and UV detector connected in series with a Millennium^{32} workstation. Detailed experimental conditions were as follows: concentration of CPP-1, 1 mg/ml, column and RI detector temperature, 35 °C (column temperature auto-control system); injection volume, 20 μl; mobile phase, ultra-pure water; flow rate, 0.6 ml/min; run time, 30 min, and integral pattern, force baseline to peak. Different weight-average molecular weights of standard dextrans, T-2000, T-500, T-70, T-40, and T-10, were prepared as 0.1% (w/v) solutions and 20 μl of solutions were injected in each run, and then the retention time was plotted against the logarithms of their respective molecular weights. A calibration curve was prepared from the known MW Dextran T system standards.

2.5. Monosaccharide composition

Purified polysaccharide sample (20 mg) was hydrolysed with 2 M H_2SO_4 (5 ml) for 8 h at 110 °C in a sealed glass tube. After removing the residual acid with $BaCO_3$, the hydrolysates were converted to acetylated aldononitrile derivatives according to conventional protocols and analysed by gas chromatography (GC) in a Agilent 6890 system GC (Agilent Technologies, Palo Alto, CA, USA) with myo-inositol as the internal standard (Honda, Suzuki,

Kakehi, Honda, & Takai, 1981). The Agilent 6890 system used above was fitted with a DB-1701 capillary column (30 m × 0.32 mm ID, film thickness 0.25 mm) and a flame-ionisation detector (FID). The operation was performed under the following conditions: H_2, 16 ml/min; air, 150 ml/min; N_2, 20 ml/min; injection temperature, 250 °C; detector temperature, 250 °C. The oven temperature programme was 1 min at 70 °C, 30 °C/min to 220 °C, and 1.5 °C/min to 250 °C. The temperature of the injector and detector was 250 °C. Injections were made in the splitness mode. The molar percentage of the component monosaccharides was calculated using the relative correction factors shown in the equation:

$$f = (W_i/W_s)/(A_i/A_s)$$

where A_i and W_i are the peak area and the weight of the internal standard, respectively, A_s and W_s are the peak area and the weight of the standard monosaccharide, respectively. Quantification was based on the internal standard method. The molar ratio value of the sample was determined according to the following equation:

$$R = f \times (A_1/A_2)/M$$

where A_1 is the peak area of each particular monosaccharide, A_2 is the peak area of the acetyl inositol internal standard, M is the molar weight of the monosaccharide and $f_{i/s}$ is the correction factor in the equation. The results obtained were compared with the following sugar standards: D-mannose, L-rhamnose, D-ribose, D-galactose, D-xylose, D-arabinose, L-fucose and D-glucose.

2.6. Total sugar, uronic acid, protein and amino acid analysis

The total sugars were determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with D-glucose as standard at 490 nm. Uronic acid contents were determined according to Blumenkrantz and Asboe-Hansen's (1973) method by measuring the absorbance at 525 nm using the *m*-hydroxybiphenyl colorimetric procedure and with D-glucuronic acid as the standard (Blumenkrantz & Asboe-Hansen, 1973). Proteins were estimated by the Lowry method, with bovine serum albumin as a reference protein (Lowry, Rosebrough, Lewsfarr, & Randall, 1951). Amino acids were released by hydrolysis with 6 M HCl at 110 °C for 22 h in a sealed tube and the methods of Hou, Sun, He, Zhang, and Wang (2006) were employed.

2.7. Infrared spectral analysis of the polysaccharide

The IR spectrum of CPP-1 was determined, using a Fourier-transform infrared spectrophotometer (FTIR, Nicolet, USA) equipped with an OMNIC workstation. The purified polysaccharide was ground with KBr powder (spectroscopic grade) and then pressed into a 1 mm pellet for FTIR measurement in the frequency range 4000–500 cm^{-1} (Kumar, Joo, Choi, Koo, & Chang, 2004).

2.8. NMR spectroscopy

The ^1H spectrum of the polysaccharide was recorded using Bruker DRX-400 NMR spectrometer (Bruker, Rheinstetten, Germany) (operating frequency of 400.13 MHz). The polysaccharide sample (CPP-1) was deuterium-exchanged by lyophilisation with D_2O and then examined in 99.9% D_2O. Chemical shift was expressed in ppm (Chattopadhyay et al., 2007).

2.9. Assay for antioxidant activities

The free radical-scavenging activity of the purified polysaccharides was measured by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) test, according to the method of Shimada, Fujikawa, Yahara, and Nakamura (1992), with some modifications. The 0.1 mM solution of DPPH· in 95% ethanol was prepared daily before UV measurements. Two ml of various concentrations (15.6, 31.3, 62.5, 125, 250, and 400 μg/ml) of the purified polysaccharides in ultra-pure water were thoroughly mixed with 2 ml of freshly prepared DPPH·. The mixture was shaken vigorously and allowed to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank with an ultraviolet–visible spectrophotometer (TU-1900, Pgenenal, Beijing, China). Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity, which was analysed from the graph plotted of inhibition percentage against compound concentration. Ascorbic acid and butylatedhydroxytoluene (BHT) were used as positive controls. All tests and analyses were carried out in triplicate and averaged. The ability to scavenge the free radical, DPPH· in percent (I%) was calculated using the following equation:

$$I\% = [A_0 - (A_2 - A_1)]/A_0 \times 100$$

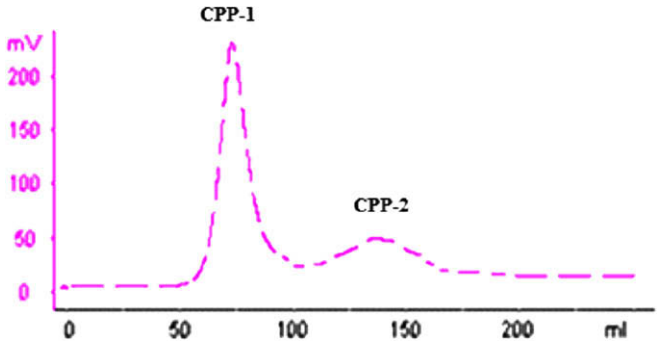

Fig. 1. The chromatography of *C. paliurus* crude polysaccharides on Sephacryl S-400 column by the ÄKTA Purifier 100 system.

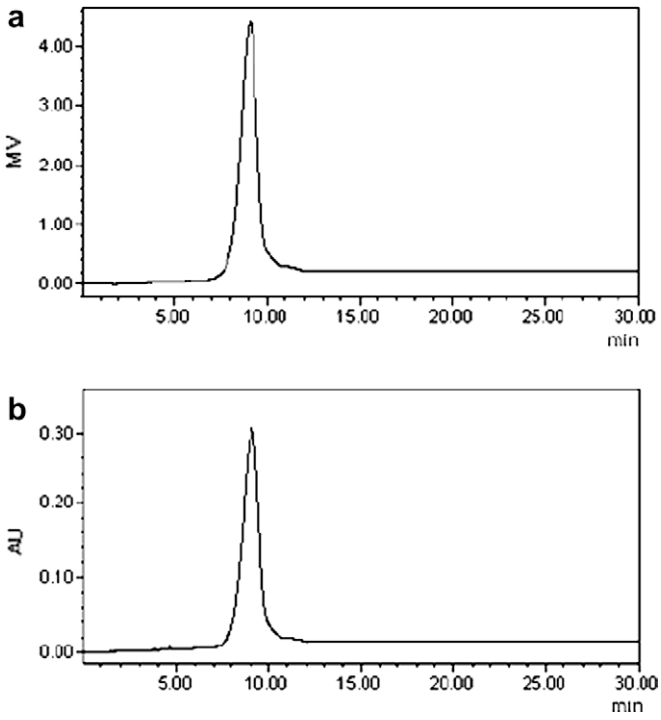

Fig. 2. (a) The profile of the CPP-1 fraction in HPGPC, with RI detection. (b) The profile of the CPP-1 fraction in HPGPC, with UV detection. Detailed experimental conditions are described in the text.

where A_0 is the absorbance of the incubated DPPH· solution without addition of the sample or positive controls, A_1 is the absorbance of the sample without DPPH· solution and A_2 is the absorbance of the incubation mixture containing both the test sample and DPPH· solution.

EC_{50} values were expressed in terms of μg/ml of CPP-1 to show the amount of antioxidant necessary to decrease the initial DPPH· concentration by 50%. The data are presented as mean values ($n = 3$).

3. Results and discussion

3.1. Extraction of crude polysaccharides

Crude polysaccharides (4.74 g) were obtained in a yield of 4.56% of fresh weight of the dried leaves of *C. paliurus* (105 g) after extraction twice with ultra-pure water at 80 °C for 2 h. The yields were 3.72 g from the first extract, and 1.02 g from the second extract. The polysaccharides obtained here were used for the further separation and purification which follows.

3.2. Purification of the polysaccharides

A dialysed fraction of crude polysaccharides from *C. paliurus*, cut-off by 14 kDa membrane, was chromatographed on a DEAE-Sephadex A-25 column to yield a water fraction of polysaccharides, CPP. Then, CPP was subjected to a Sephacryl S-400 column. The results are shown in Fig. 1. After Sephacryl S-400 column separation, the separated CPP-1 fraction was collected as 5 ml fractions by the automated fraction collector, then, concentrated, dialysed and lyophilised. The homogeneity of the water-soluble polysaccharide CPP-1 fraction was proved by high-performance gel permeation chromatography (HPGPC) with a Waters HPLC apparatus equipped with an Ultrahydrogel™-500 column eluted with ultra-pure water at a flow rate of 0.6 ml/min. The molecular weight of CPP-1 was elucidated by high-performance gel permeation chromatography (HPGPC) methods, with the dextran standards used for the calibration curve. The M_w determined is equivalent to the molecular weight of dextran standards, not absolute. Fig. 2 shows a single and symmetrical sharp peak, which indicates that CPP-1 is a homogeneous polysaccharide (Liu et al., 2007). Fig. 2a and b shows the RI

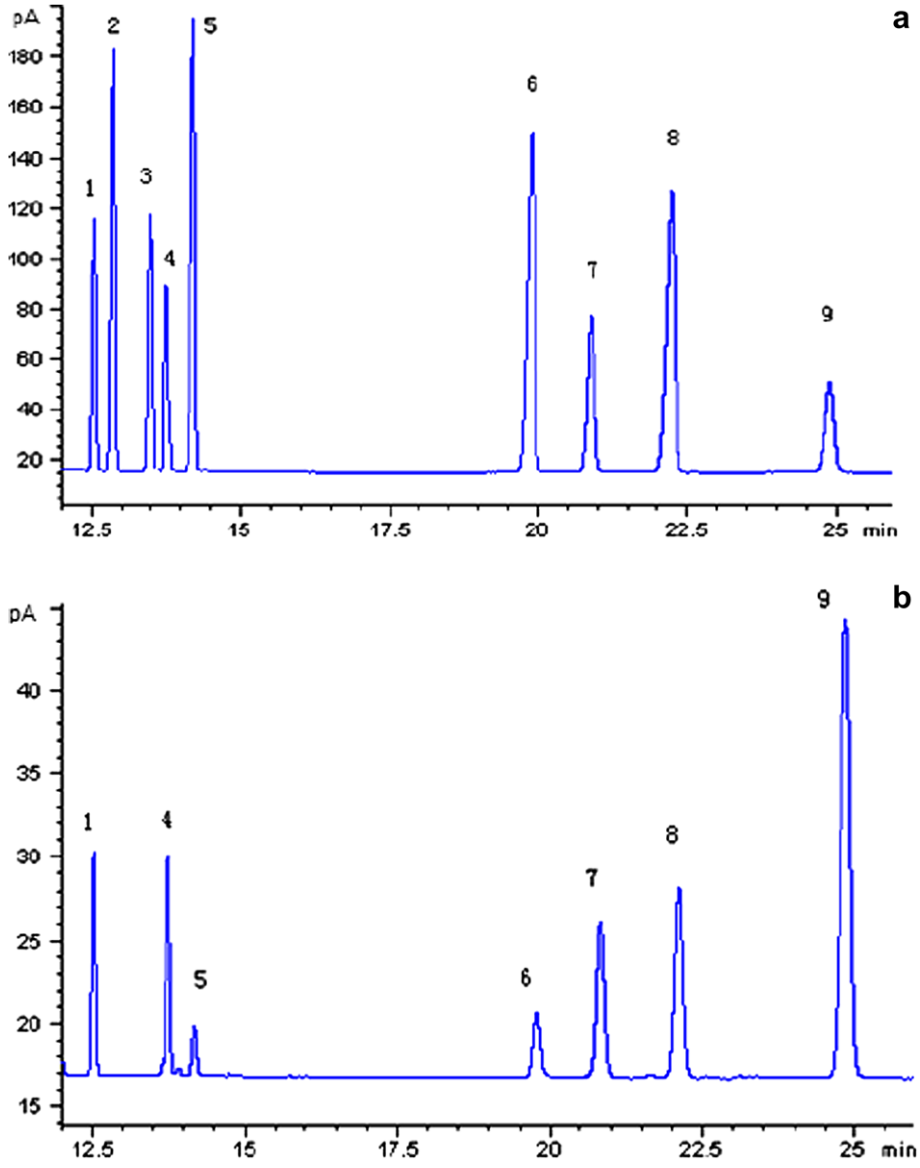

Fig. 3. (a) Gas chromatograms of acetate-derivatized standard monosaccharides. (b) Gas chromatograms of the monosaccharide compositions of polysaccharide CPP-1 purified from *C. paliurus*. (1) L-rhamnose (Rha), (2) D-ribose (Rib), (3) L-fucose (Fuc), (4) L-arabinose (Ara), (5) D-xylose (Xyl), (6) D-mannose (Man), (7) D-glucose (Glu), (8) D-galactose (Gal), and (9) acetyl inositol (the internal standard).

and 280 nm UV absorption chromatograms, respectively. A single and symmetrical peak was observed in the RI response and in the UV chromatogram at a similar retention time, confirming that CPP-1 was a homogeneous component.

3.3. Molecular weight of the purified polysaccharide

High-performance gel permeation chromatography (HPGPC) was applied to elucidate the molecular weights (M_w) of CPP-1, with the dextran standards (T-2000, T-500, T-70, T-40, and T-10) used for the calibration curve. The equation of the standard curve was: Log $M_w = 8.7928 - 0.3062t$ (where M_w represents the molecular weight, while t represents retention time) with a correlation coefficient of 0.991. The average molecular weight value of the purified polysaccharide CPP-1 from *C. paliurus* was estimated to be 1167 kDa.

3.4. Monosaccharide composition of the purified polysaccharide

The monosaccharide composition of CPP-1 fraction, obtained after ion-exchange and gel permeation chromatography, analysed by gas chromatograph (GC), is shown in Fig. 3b. Fig. 3b shows all the peaks of eluents when the CPP-1 acetate derivatives were analysed by GC. The peaks of all monosaccharides were sharp and symmetrical. Compared with the monosaccharide standards (D-mannose, L-rhamnose, D-ribose, D-galactose, D-xylose, D-arabinose, L-fucose and D-glucose) (Fig. 3a), the peaks of the CPP-1 acetate derivatives were identified as L-rhamnose, L-arabinose, D-xylose, D-mannose, D-glucose and D-galactose. The results indicated that L-arabinose (31.23%) was the most abundant monosaccharide in the CPP-1 fraction, and D-glucose (31.16%) was the second most abundant. In CPP-1, there were D-galactose constituted about 16.0%, L-rhamnose 10.6% and D-mannose of 8.72%. In addition, D-xylose was also present at a lower level (3.23%). The data obtained showed that CPP-1 was a heteropolysaccharide and consisted of D-xylose, L-arabinose, D-glucose, D-galactose, L-rhamnose and D-mannose in a molar ratio of 1.00:9.67:9.65:4.96:3.29:2.70.

Table 1
Amino acid composition of the polysaccharide CPP-1 fraction obtained from the leaves of *C. paliurus*.

Amino acid	Content [a] (%)
Aspartic acid	0.243 ± 0.011
Serine	0.091 ± 0.003
Glutamic acid	0.359 ± 0.024
Glycine	0.233 ± 0.019
Histidine	0.031 ± 0.003
Arginine	0.176 ± 0.011
Threonine	0.059 ± 0.006
Alanine	0.172 ± 0.008
Proline	0.113 ± 0.010
Cystine	0.012 ± 0.001
Tyrosine	0.174 ± 0.016
Valine	0.116 ± 0.013
Methionine	0.037 ± 0.004
Lysine	0.146 ± 0.009
Isoleucine	0.155 ± 0.012
Leucine	0.237 ± 0.019
Phenylalanine	0.031 ± 0.008

[a] Means of three determinations ± SD.

3.5. Total sugar, uronic acid, protein content and amino acid composition

CPP-1 had a high total content of carbohydrate (79.6%). The uronic acid content was measured according to Blumenkrantz and Asboe-Hansen's method using D-glucuronic acid as the standard. The uronic acid content in CPP-1 was 20.2%. The protein content of the polysaccharide CPP-1 fraction was 8.44% (w/w) and the amino acid composition of CPP-1 was analysed (Table 1). Table 1 shows that the abundant amino acids were glutamic acid (relative mass 0.359%) and aspartic acid (relative mass 0.243%). The other prominent amino acids were glycine (relative mass 0.233%), leucine (relative mass 0.237%) and arginine (relative mass 0.176%).

3.6. FTIR spectroscopy

The infrared (IR) spectrum of the purified CPP-1 fraction is shown in Fig. 4. It can be seen that the IR spectrum of the purified polysaccharide CPP-1 displayed a broad stretching characteristic intense peak at around 3423 cm^{-1} for hydroxyl and amine groups, and a weak C–H stretching band at 2931 cm^{-1}. The bands in the region of 2371 cm^{-1} also indicated aliphatic C–H bonds. Further, an asymmetrical stretching peak was noticed at 1620 cm^{-1} and a weak symmetrical stretching peak near 1430–1390 cm^{-1}, suggesting the presence of carboxyl groups. Protein structures could also

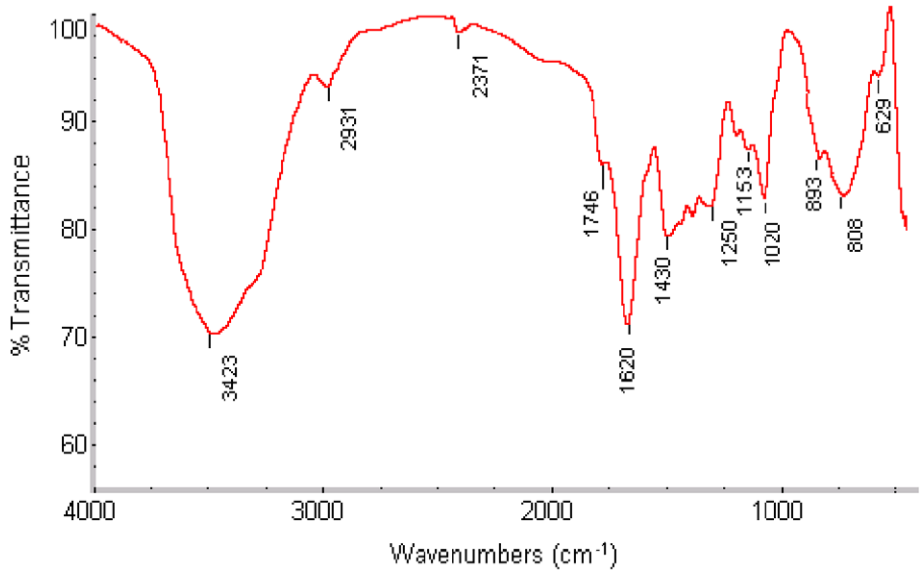

Fig. 4. FT-IR spectra of the polysaccharide of the CPP-1 fraction purified from *C. paliurus*.

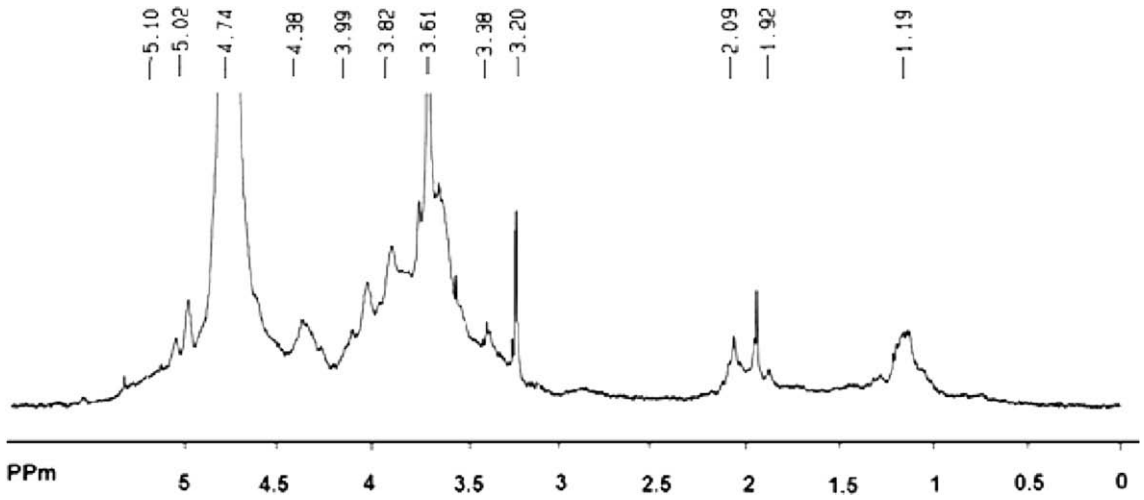

Fig. 5. ^{1}H NMR spectra for CPP-1.

be represented by the absorption at 1242 cm^{-1}. Specifically, the peaks in the 1000–1125 cm^{-1} range and 1171 cm^{-1} region ascertain the presence of glucuronic acid, mannuronic acid, and *o*-acetyl ester (Gonzaga, Ricardo, Heatley, & Soares, 2005). These attributions lead us to speculate that a glucan–protein complex may be present in the CPP-1 fraction. Two stretching peaks at 1020 and 1153 cm^{-1} suggest the presence of C–O bonds. The peak at around 893 cm^{-1} is characteristic of β-D-mannose.

3.7. ^{1}H NMR analysis

In the ^{1}H NMR spectrum of CPP-1 (Fig. 5), signals at 5.10, 3.82, 3.99, and 4.38 ppm were assigned to H-1 to H-4 of (1 → 4) α-galacturonic acid residues, respectively (Hokputsa et al., 2004). The chemical shift at 5.02 ppm of anomeric H-1 indicated a form of L-arabinofuranosyl unit (Yang et al., 2009). The signal at 1.19 ppm was assigned to the CH_3 of α-L-rhamnopyranose units. The signal at 2.09 ppm from acetyl protons was found (Chattopadhyay et al., 2007).

3.8. Assay for antioxidant activities

The model of scavenging the stable DPPH radical is a widely used method for evaluating the free radical-scavenging ability of various antioxidants (Chen et al., 2008; Lai, Chou, & Chao, 2001; Lee, Hwang, Ha, Jeong, & Kim, 2003). The method is based on the reduction of methanolic DPPH radical solution at 517 nm in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations (Sánchez-Moreno, 2002). Herein the above-mentioned model was used to determine inhibitory activities of the water-soluble polysaccharide on DPPH radicals. Fig. 6 depicts the DPPH·-scavenging power of CPP-1. Obviously, the purified polysaccharide scavenging effect was increased with increasing concentrations. At concentrations of 50–125 μg/ml, the scavenging abilities of the CPP-1 from filtrate on DPPH radicals were in the range of 47.6–84.3% (Fig. 6). At the concentration of 400 μg/ml, CPP-1 was observed to possess strong free radical-scavenging effects against DPPH radicals, with a value of around 91.4%, and scavenging effects of ascorbic acid and BHT on the DPPH radical were 98.9% and 91.2%, respectively. Final results were expressed using the term EC_{50}. DPPH radical-scavenging at EC_{50} values of CPP-1 can be indirectly seen in Fig. 6. The EC_{50} value of CPP-1 was found to be 52.4 μg/ml. However, the DPPH free radical-scavenging of CPP-1 was less than that of BHT, a synthetic antioxidant, at the same concentration. These results indicate that CPP-1 has a noticeable effect in scavenging free radicals, especially at high concentration. However, the radical-scavenging activity of CPP-1 was lower than that of vitamin C and BHT used in this study. However, the antioxidant mechanism of CPP-1 is still not fully understood. Therefore, it is suggested that further work could be performed on the possible antioxidant mechanism of CPP-1.

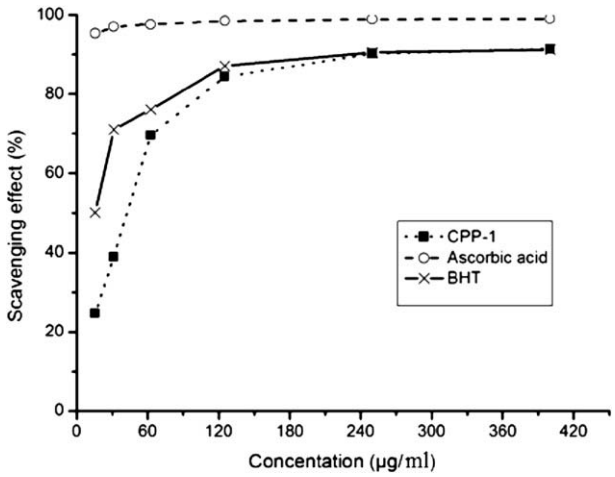

Fig. 6. Free radical-scavenging activity of CPP-1 at different concentrations by DPPH· method. Each sample was assayed in triplicate for each concentration. Experimental results were means ± SD of three parallel measurements.

4. Conclusions

In this work, a purified polysaccharide from the hot water extract of *C. paliurus* was obtained using DEAE-Sephadex anion-exchange and permeation chromatography. The purified polysaccharide was a protein-bound species, with an average molecular weight of about 1167 kDa. It was a heteropolysaccharide and consisted of D-xylose, L-arabinose, D-glucose, D-galactose, L-rhamnose and D-mannose in a molar ratio of 1.00:9.67:9.65:4.96:3.29:2.70. Seventeen amino acids in CPP-1 were identified by amino acid analysis. In addition, the Fourier-transform infrared spectra (FT-IR) of CPP-1 revealed the typical characteristics of polysaccharides. Moreover, the result of the free radical-scavenging activity of CPP-1, measured by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) test, indicated that CPP-1 possessed significant antioxidative activity

and could be possibly developed as a potential natural antioxidant functional ingredient in the food industry.

Acknowledgements

The financial support for this study by the Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0540), the Natural Science Foundation of Jiangxi Province (No. 9920029) and Nanchang University Testing Fund (No. 2005002) is gratefully acknowledged.

References

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acid. *Analytical Biochemistry, 54*(2), 484–489.

Chattopadhyay, K., Mandal, P., Lerouge, P., Driouich, A., Ghosal, P., & Ray, B. (2007). Sulphated polysaccharides from Indian samples of *Enteromorpha compressa* (Ulvales, Chlorophyta): Isolation and structural features. *Food Chemistry, 104*(3), 928–935.

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*(1), 231–241.

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*(3), 350–356.

Fang, S. Z., Wang, J. Y., Wei, Z. Y., & Zhu, Z. X. (2006). Methods to break seed dormancy in *Cyclocarya paliurus* (Batal.) Iljinskaja. *Scientia Horticulturae, 110*(3), 305–309.

Gonzaga, M. L. C., Ricardo, N. M. P. S., Heatley, F., & Soares, D. A. S. (2005). Isolation and characterization of polysaccharides from *Agaricus blazei* Murill. *Carbohydrate Polymers, 60*(1), 43–49.

Hokputsa, S., Gerddit, W., Pongsamart, S., Inngjerdingen, K., Heinze, T., Koschella, A., et al. (2004). Water-soluble polysaccharides with pharmaceutical importance from Durian rinds (*Durio zibethinus Murr.*): isolation, fractionation, characterisation and bioactivity. *Carbohydrate Polymers, 56*(4), 471–481.

Honda, S., Suzuki, S., Kakehi, K., Honda, A., & Takai, T. (1981). Analysis of the monosaccharide compositions of total non-dialyzable urinary glycoconjugates by the dithioacetal method. *Journal of Chromatography, 226*(2), 341–350.

Hou, S. M., Sun, J., He, H. B., Zhang, X. D., & Wang, Y. H. (2006). Simultaneous determination of amino acids in soil by reversed phase high performance liquid chromatography by using 6-aminoquinoly-*n*-hydroxysuccinimidyl carbamate as a precolumn derivatization reagent. *Chinese Journal of Analytical Chemistry, 34*(10), 1395–1400.

Jiang, Z. Y., Zhang, X. M., Zhou, J., Qiu, S. X., & Chen, J. J. (2006). Two new triterpenoid glycosides from *Cyclocarya paliurus*. *Journal of Asian Natural Products Research, 8*(1–2), 93–98.

Kennelly, E. J., Cai, L. N., Long, L. N., Shamon, L., Zaw, K., Zhou, B. N., et al. (1995). Novel highly sweet secodammarane glycosides from *Pterocarya puliurus*. *Journal of Agricultural and Food Chemistry, 43*(10), 2602–2607.

Kumar, C. G., Joo, H. S., Choi, J. W., Koo, Y. M., & Chang, C. S. (2004). Purification and characterization of an extracellular polysaccharide from haloalkalophilic Bacillus. *Enzyme and Microbial Technology, 34*(7), 673–681.

Kurihara, H., Asami, S., Shibata, H., Fukami, H., & Tanaka, T. (2003). Hypolipemic effect of *Cyclocarya paliurus* (Batal.) Iljinskaja in lipid-loaded mice. *Biological and Pharmaceutical Bulletin, 26*(3), 383–385.

Kurihara, H., Fukami, H., Kusumoto, A., Toyoda, Y., Shibata, H., Matsui, Y., et al. (2003). Hypoglycemic action of *Cyclocarya paliurus* (Batal.) Iljinskaja in normal and diabetic mice. *Bioscience, Biotechnology, and Biochemistry, 67*(4), 877–880.

Lai, L. S., Chou, S. T., & Chao, W. W. (2001). Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. *Journal of Agricultural and Food Chemistry, 49*(2), 963–968.

Lee, S. E., Hwang, H. J., Ha, J. S., Jeong, H. S., & Kim, J. H. (2003). Screening of medicinal plant extracts for antioxidant activity. *Life Sciences, 73*(2), 167–179.

Li, L., Xie, M. Y., Sun, Z. H., Wu, X. H., Sun, D. H., & Wang, X. R. (2000). The studies on the elements transferring characteristics and element speciation in the extract of *Cyclocarya Paliurus* leaves. *Chemical Journal of Chinese Universities – Chinese, 21*(5), 707–709.

Liu, C. H., Lin, Q. X., Gao, Y., Ye, L., Xing, Y. Y., & Xi, T. (2007). Characterization and antitumor activity of a polysaccharide from *Strongylocentrotus nudus* eggs. *Carbohydrate Polymers, 67*(3), 313–318.

Liu, F., Ooi, V. E. C., & Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Science, 60*(10), 763–771.

Lowry, O. H., Rosebrough, N. J., Lewsfarr, A., & Randall, R. J. (1951). Protein measurement with the folin reagent. *Journal of Biological Chemistry, 193*, 265–275.

Maeda, M., Uehara, T., Harada, N., Sekiguchi, M., & Hiraoka, A. (1991). Heparinoid-active sulfated polysaccharides from *Monostroma-nitidium* and their distribution in the chlorophyta. *Phytochemistry, 30*(11), 3611–3614.

Navarini, L., Gilli, R., Gombac, V., Abatangelo, A., Bosco, M., & Toffanin, R. (1999). Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: Isolation and characterization. *Carbohydrate Polymers, 40*(1), 71–81.

Nie, S. P., Xie, M. Y., Zhou, P., & Cao, S. W. (2007). In vitro antioxidative and anticancer activities of tea glycoprotein in green tea. *European Food Research and Technology, 224*(2), 437–442.

Saima, Y., Das, A. K., Sarkar, K. K., Sen, A. K., & Sur, P. (2000). An antitumor pectic polysaccharide from *Feronia limonia*. *International Journal of Biological Macromolecules, 27*(5), 333–335.

Sánchez-Moreno, C. (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International, 8*, 121–137.

Sheng, J. C., Yu, F., Xin, Z. H., Zhao, L. Y., Zhu, X. J., & Hu, Q. H. (2007). Preparation, identification and their antitumor activities in vitro of polysaccharides from *Chlorella pyrenoidosa*. *Food Chemistry, 105*(2), 533–539.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthone on the auto oxidation of soybean in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry, 40*(6), 945–948.

Shu, R. G., Xu, C. R., Li, L. N., & Yu, Z. L. (1995). Cyclocariosides II and III: Two secodammarane triterpenoid saponins from *Cyclocarya paliurus*. *Planta Medica, 61*(6), 551–553.

Tsai, M. C., Song, T. Y., Shih, P. H., & Yen, G. C. (2007). Antioxidant properties of water-soluble polysaccharides from *Antrodia cinnamomea* in submerged culture. *Food Chemistry, 104*(3), 1115–1122.

Tzianabos, A., Wang, J. Y., & Kasper, D. L. (2003). Biological chemistry of immunomodulation by zwitterionic polysaccharides. *Carbohydrate Research, 338*(23), 2531–2538.

Xie, M. Y., & Li, L. (2001). Review in studies on chemical constituents and bioactivities of *Cyclocarya paliurus*. *Chinese Traditional and Herbal Drugs, 32*(4), 365–366.

Xie, M. Y., Li, L., Nie, S. P., Wang, X. R., & Lee, F. S. C. (2006). Determination of speciation of elements related to blood sugar in bioactive extracts from *Cyclocarya paliurus* leaves by FIA-ICP-MS. *European Food Research and Technology, 223*(2), 202–209.

Xu, Q., & Song, Y. J. (2004). Research status on *Cyclocarya paliurus*. *Acta Medicinae Sinica, 17*(3), 451–453.

Yang, B., Jiang, Y. M., Zhao, M. M., Chen, F., Wang, R., Chen, Y. D., et al. (2009). Structural characterisation of polysaccharides purified from longan *(Dimocarpus longan Lour.)* fruit pericarp. *Food Chemistry, 115*(2), 609–614.

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*(2), 125–131.