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

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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

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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,