Carbohydrate Polymers 196 (2018) 359–367

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Isolation, purification, characterization and antioxidant activity of polysaccharides from the stem barks of *Acanthopanax leucorrhizus*

Hao-Bin Hu[a,*], Hai-Peng Liang[b], Hai-Ming Li[a], Run-Nan Yuan[c], Jiao Sun[c], La-La Zhang[a], Ming-Hu Han[a], Yun Wu[a]

[a] College of Chemistry & Chemical Engineering, Longdong University, Qingyang 745000, PR China
[b] Department of Oncology, Qingyang First People's Hospital, Qingyang 745000, PR China
[c] College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, PR China

ARTICLE INFO

Keywords:
Acanthopanax leucorrhizus
Polysaccharide
Isolation
Purification
Characterization
Antioxidant activity

ABSTRACT

A novel water-soluble polysaccharide (named ALP-1) was successfully isolated from the stem barks of *Acanthopanax leucorrhizus* by hot-water extraction, and further purified by Cellulose DEAE-52 and Sephadex G-100 chromatography. The structure of ALP-1 was characterized by HPLC, HPGPC, partial acid hydrolysis, periodate oxidation, Smith degradation, methylation, together with UV, IR and NMR spectral analysis. The antioxidant activities also were evaluated *in vitro*. Structural analysis revealed that ALP-1 was a homogeneous galactan with the average molecular weight of 169 kDa, composed of galactose, glucose, mannose and arabinose in a molar ratio of 6.1:2.1:1.1:1.0, owning a backbone structure of 1,6-linked α-D-Gal*p* residues with some branches of α-D-Man*p*-(1 → 3)-α-L-Ara*f* residues at *O*-3 and α-D-Gal*p* residues at *O*-4 of 1,6-linked α-D-Gal*p*. Antioxidant assay showed that ALP-1 exhibited strong DPPH· and HO· scavenging activities, as well as ferric-reducing antioxidant power. These results provide a scientific basis for the further use of polysaccharides from *A. leucorrhizus*.

1. Introduction

Polysaccharides, present in almost all organisms, have many biological functions, such as energy storage, structure support, antigenic determinant, and so on. Because the separation, purification, composition determination, and structural analysis of polysaccharides have made remarkable progress, as well as because the biological functions of polysaccharides are further understood, many bioactive polysaccharides have been widely studying and applying to the biochemical and medical industry (Li, Li, You, Fu, & Liu, 2017). Recently, plant-derived polysaccharides have been recognized as safe, highly stable and effective natural antioxidants in pharmaceutical and food fields due to their relatively nontoxicity and insignificant side-effects (Xu, Yao, Sun, & Wu, 2009). Hence, the exploitation and utilization of plant-derived polysaccharides as potential effective natural antioxidants could be significant and worth to study further.

The genus *Acanthopanax*, which belongs to the family Araliaceae and comprises some 37 species (excluding varieties) in the world, are widespread in Korea, Japan, China, and the far-eastern regions of Russia (Ni & Liu, 2006; Phuong et al., 2006). Their roots and stem barks have traditionally been used as tonic and sedative as well as for the treatment of rheumatoid arthritis and diabetes mellitus, and are popularly used as a health supplement in China and Korea (Yoo, Lee, Jin, & Kim, 2008). *Acanthopanax leucorrhizus* Harms is deciduous shrub of the genus *Acanthopanax* and an endemic medicinal plant growing abundantly in Gansu province of China. Its root and stem bark possess multiple biological functions such as antibacterial, antioxidant, antitumour and so on, especially its stem barks have been used for a long time to treat rheumatism, numbness, contracture, quadribblelegia, hemiplegia, traumatic injury, edema, and itchy skin (Zhao et al., 1987). Numerous studies show that *Acanthopanax* species are rich in polysaccharide, and that many polysaccharides have been isolated from *A. senticosus* (Chen et al., 2011; Fu et al., 2012; Han et al., 2003; Li & Zhou, 2007), *A. obovatus* (Wang, Mao, Ito, & Shimura, 1991; Wang, Tsumura, Ma, Shimura, & Ito, 1993), *A. giraldii* (Lu, Su, & Li, 2002; Wang, Tsumura, Shimura, & Ito, 1992), *A. sessiliflorus* (Lee et al., 2003), *A. brachypus* (Hu, Liang, & Wu, 2015), *A. koreanum* (Kang et al., 2015) and *A. sciadophylloides* (Lee et al., 2015). However, to the best of our knowledge, only few chemical constituents, such as volatile components (Hu, Zheng, & Hu, 2012), sesquiterpenoids (Hu, Zhang, & Wu, 2014) and stilbenoids (Hu et al., 2018), have been isolated from *A. leucorrhizus*. As part of an ongoing investigation of the chemical

* Corresponding author.
E-mail address: hhb-88@126.com (H.-B. Hu).

https://doi.org/10.1016/j.carbpol.2018.05.028
Received 18 February 2018; Received in revised form 7 May 2018; Accepted 9 May 2018
0144-8617/

constituents of this plant, a water-soluble polysaccharide (ALP-1) were isolated from the root bark of *A. leucorrhizus* for the first time. Herein, we report on the isolation, purification, structural characterization and antioxidant activities of ALP-1.

2. Materials and methods

2.1. Plant materials

The stem barks of *A. leucorrhizus* was collected in September 2015 from Wushan County (Gansu Province of China), and identified by Prof. Xiao-Qiang Guo (College of Life Science of Longdong University, People's Republic of China). A voucher specimen (No. 20150920012) was deposited in the Herbarium of College of Life Science & Technology, Longdong University, People's Republic of China. The stem barks were washed and dried in the shade. Then, the dried plants were crushed into powder (40 mesh) by a disintegrator.

Cellulose DEAE-52 and Sephadex G-100 were purchased from Hengxin Chemical Reagent Co., (Shanghai, China). All standardes including D-glucose (Glc), D-galactose (Gal), L-rhamnose (Rha), L-arabinose (Ara), D-xylose (Xyl), D-mannose (Man), D-galacturonic acid (GalA), erythritol, glycol and glycerol, as well as dextran series with varying molecular weights of 5, 12, 25, 50, 80, 150, 270 and 410 kDa were purchased from Shanghai Yuanju Bioscience Technology Limited Company (China). Trifluoroacetic acid (TFA) and 1-phenyl-3-methyl-5-pyrazolone (PMP) were purchased from Sinopharm Chemical Reagent (Shanghai, China). 1-Cyclohexyl-3-(2-morpholinoethyl)-carbodiimide *metho-p*-toluenesulfonate (CMC), sodium borodeuteride ($NaBD_4$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid (Vc), and ovalbumin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Other chemicals and reagents used in this study were of analytical grade from Xi'an Chemical Co. (Xi'an, China), and water was ultrapure water.

2.2. Extraction,isolation and purification of ALP-1

The extraction procedure of ALP was performed using the literature method (Chen, Xie, Nie, Li, & Wang, 2008) with some modifications. Briefly, the air-dried powder of *A. leucorrhizus* (300 g) was extracted with 90% ethanol (2 L) for 24 h at room temperature to remove the interfering components, including pigment, monosaccharide, disaccharide, oligosaccharide, volatile compounds and polyphenols. The residues were filtrated and dried with a vacuum freeze dryer, and then extracted three time (each time for 5.0 h) with ultrapure water (30:1, water to material ratio, mL/g) at 80 °C. The combined aqueous extract was concentrated to 25% of the original volume by a rotary evaporator at 60 °C and then centrifuged at 4000*g* for 10 min. Whereafter, the supernatant was collected and precipitated by adding five times of volume of 95% (v/v) ethanol at 4 °C for 24 h. After centrifuging, the separated precipitate was dissolved in appropriate volume of ultrapure water, then deproteinated by the Sevag method (Yang, Huang, Wang, Cao, & Sun, 2008). Briefly, the polysaccharide solution and the Sevag reagent ($CHCl_3$:n-C_4H_9OH = 4:1,v/v) were mixed (3:1, v/v) and shaken vigorously for 30 min at room temperature and centrifuged at 3000*g* for 10 min. Then, the supernatant was collected and dialyzed for 72 h against 100 vol of ultrapure water (cut-off Mw 8000 Da) to further remove the small molecular compounds (e.g., disaccharides or polyphenols). Finally, the dialyzate was lyophilized in vacuum freeze dryer to obtain the crude polysaccharide (ALP, 22.7 g).

ALP was redissolved in ultrapure water and purified by Cellulose DEAE-52 column (2.6 cm × 40 cm) equilibrated with ultrapure water. The polysaccharides were fractionated eluting stepwise with ultrapure water, and followed by gradient NaCl solution (0–1.0 M) at a flow rate of 1.0 mL/min. Each fraction (5 mL/tube) was monitored at 490 nm by the phenol-sulphuric acid method (Cuesta, Suarez, Bessio, Ferreira, & Massaldi, 2003). The eluted solution was collected and further purified on Sephadex G-100 gel-filtration column (1.6 cm × 80 cm) using ultrapure water as eluent at a flow rate of 15.0 mL/h. The main fractions obtained was collected, concentrated, dialyzed against ultrapure water, and finally lyophilized to obtain white fluffy pure polysaccharide (4.5 g) named ALP-1 for next analyses.

2.3. Physicochemical characterizations and structural analysis of ALP-1

2.3.1. Physicochemical characterizations of ALP-1

The physical characteristics were analyzed by color and texture observation (Ge, Duan, Fang, Zhang, & Wang, 2009). The total carbohydrate content of ALP was determined by the phenol-sulfuric acid colorimetric method using D-glucose as the standard, and expressed as glucose equivalents (Huang, Tan, Tan, & Peng, 2011). The content of uronic acid was measured by photometry with *m*-hydroxydiphenyl at 525 nm using D-galacturonic acid as the standard (Gao et al., 2015). The protein content was determined by the Bardford's method (Bradford, 1976) using ovalbumin as a standard. ALP-1 was identified for the solubility in water, ethanol, chloroform and acetone according to the British pharmacopoeia (BP) specification (Kannan, Manivannan, Balasubramaniam, & Kumar, 2010). Optical rotation of ALP-1 was confirmed with a CDP-001 digital polarimeter according to the reported method (Yi et al., 2015). The decolorization of polysaccharide was measured using UV-spectrophotometry (Shimadzu UV-2401 spectrometer, Japan) at 420 nm (Xie, Shen, Nie, Li, & Xie, 2011). Gel permeation and anion exchange chromatography were monitored by assaying the total sugar content. D- or L-Configurations of sugars were determined using the Gerwig method (Zhang, Liu, & Lin, 2014). The nature of ALP-1 was verified through the following methods: Fehling's test, α-naphthol reaction, iodination reaction and $FeCl_3$ reaction (Chen & Kan, 2018).

2.3.2. Determination of homogeneity and relative molecular weight

The homogeneity and molecular weight of ALP-1 were identified by high-performance gel- permeation chromatography (HPGPC) on an Agilent 1100 HPLC system equipped with a TSK-GEL G4000PW$_{XL}$ column (7.8 mm × 300 mm) and an Agilent RID-10A refractive index detector. The determination procedure was carried out according to the literature method (Cai, Xie, Chen, & Zhang, 2013). A sample solution (15 μL) was injected for each run, and it was eluted with 0.1 M Na_2SO_4 solution at 40 °C and a flow rate of 0.6 mL/min. Data was analyzed by Agilent GPC soft-ware. According to the peak shape of the HPGPC chromatogram, the homogeneity of ALP-1 can be judged. The molecular weight was estimated by reference to the calibration curve established using the dextrans T-series of known molecular weights (5, 12, 25, 50, 80, 150, 270 and 410 kDa).

2.3.3. Monosaccharide composition analysis of ALP-1

The composition analysis of polysaccharide is an important step to control the quality and got basic information about the polysaccharide. The monosaccharide composition of ALP-1 was determined by the PMP-labeling procedure (Li, Pan, Xia, Zhang, & Wu, 2014) with high performance liquid chromatography (HPLC). Briefly, ALP-1 (5 mg) was hydrolyzed with 2 M TFA (3 mL) at 120 °C for 3 h in a sealed glass tube. Excess acid was removed by evaporating at reduced pressure with the addition of methanol for five times after completing the hydrolysis. Dry hydrolysis product was labeled with PMP through the addition of 0.6 M NaOH (2 mL) and 0.5 M PMP-CH_3OH solution (1 mL). The mixture was reacted at 70 °C for 2 h. Later, the reaction product was neutralized with 0.3 M HCl (1 mL) and extracted with 20 mL chloroform three times. The aqueous phase was finally filtered through 0.45 μm nylon membrane for HPLC analysis. The resulting solution (20 μL) was injected into a RP-C_{18} column (4.6 mm i.d. × 250 mm). The sample was eluted with a mixture of acetonitrile and 0.1 M phosphate buffer (20:80, pH = 6.8) at a flow rate of 1.0 mL/min and the injection volume was 20 μL. Standard monosaccharides were determined by the same procedure. The sugar