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Purification, Physicochemical Characterization, and Bioactivities of Polysaccharides from Puerh Tea

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Abstract Two fractions of polysaccharides, named PTPS-1 and PTPS-2, was extracted and purified from puerh tea by Sephacryl S-300 column chromatography. The physicochemical properties of these two polysaccharides were investigated by high-performance liquid chromatography (HPLC), Fourier Transform IR spectra, scanning electron microscope (SEM) and atomic force microscope (AFM). Analysis of chemical compositions (protein, neutral sugars, uronic acid and monosaccharide composition) suggested that they were both kinds of acid heteropolysaccharides bound with protein, and contained seven monosaccharides with different molar ratio. Meanwhile, evaluation of antioxidant activities by in vitro assays of DPPH, ABTS and FRAP showed that PTPS-1 demonstrated stronger antioxidant ability than PTPS-2. Similarly, PTPS-1 exhibited remarkable inhibitory potential on α-glycosidase in vitro, significantly stronger than that of PTPS-2 and acarbose. Moreover, the results from animal test indicated PTPS-1 possessed significant inhibition on postprandial hyperglycemia in diabetic mice compared with the model group.

Keywords: *puerh tea polysaccharides, purification, antioxidant activity, α-glycosidase inhibition, physicochemical characterization*

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1. Introduction

Tea (*Camellia sinensis*) has a long history of medicinal and dietary application in Asian countries such as China, Japan, India and Thailand as ancient as 5,000 years ago (Chopade, Phatak, Upaganlawar, & Tankar, 2008). Puerh tea, as a kind of post-fermented tea, together with green tea (unfermented), oolong tea (semi-fermented), black tea (fermented), puerh tea (post-fermented) are the main types of Chinese tea (*Camellia sinensis*), according to the degree of fermentation during manufacturing (Lin & Lin-Shiau, 2006). Puerh tea is originally produced in a few cities (such as Dali, Lincang, Xishuangbanna, Simao and Dehong) in Yunnan province of China, using the sun-dried crude green tea as the raw materials (H. P. Lv, Lin, Tan, & Guo, 2013), and can be stored for a long period (10 years or more) (Yang, Chou, Ueng, Chou, Yang, Lin-Shiau, et al., 2014). In recent years, puerh tea has attracted a growing attention for its unique flavor and health benefits (Jie, Lin, Zhang, Lv, He, & Zhao, 2006; Wu, Yen, Wang, Chiu, Yen, Chang, et al., 2007), which is closely associated with its microorganism-involved post-fermentation process, namely wet-storage or wet piling (Xu, Chen, Wang, Hochstetter, Zhou, & Wang, 2012).

Tea polysaccharides (TPS) is one of the main bioactive components in tea, which has been reported to possess various bioactivities, such as antidiabetic, antioxidant, anti-cancer, immunomodulatory and hypolipidemic, of which antidiabetic effect is most concerned (Chen, Zhang, Qu, & Xie, 2008; Nie & Xie, 2011). Since the early 1990s, synthetic α-glycosidase inhibitors such as acarbose, miglitol and voglibose are widely used for the treatment of patients with type 2 diabetes as oral antidiabetic drugs. However, they also cause various side-effects like flatulence, diarrhea and abdominal discomfort. Therefore, safer natural antidiabetic agents are desired and many potential compounds have been reported from natural products, such as marine algae (Eom, Lee, Yoon, Jung, Jeon, Kim, et al., 2012; Heo, Hwang, Choi, Han, Kim, & Jeon, 2009), tea (Aloulou, Hamden, Elloumi, Ali, Hargafi, Jaouadi, et al., 2012), and tournefortia hartwegiana (Ortiz-Andrade, Garcia-Jimenez, Castillo-Espana, Ramirez-Avila, Villalobos-Molina, & Estrada-Soto, 2007). In our previous research, tea polysaccharides had been found to present potential antidiabetic property and were suggested to be the main active component in puerh tea (Xu, Wu, Zhang, Chen, & Wang, 2014). Therefore, further studies on the purified polysaccharides from puerh tea were necessary.

In the present study, two fractions of polysaccharides were purified from puerh tea, and their chemical compositions, structure, *in vitro* antioxidant activities and α-glycosidase inhibition, as well as the effect on postprandial hyperglycemia in diabetic mice were investigated.

2. Materials and Methods

2.1. Materials

Puerh tea (product number 7572), produced in 2008, was obtained from Yunnan Dayi Tea Industry Co. (Yunnan, China). Dialysis membrane (7000 Da), galacturonic acid, dextran, acarbose, butylatedhydroxytoluene (BHT), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), seven standard monosaccharides (L-rhamnose, D-galacturonic acid, L-arabinose, D-xylose, D-mannose, D-glucose and D-galactose) and baker's yeast α-glycosidase (EC 3.2.1.20) were obtained from Sigma Chemical Co. (Missouri, USA). Coomassie brilliant blue G-250 and bovine serum albumin were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG) was obtained from Xibao Co. (Shanghai, China). All other chemicals were analytical grade and purchased from Shanghai Boer Chemical Reagent Co. (Shanghai, China).

Male ICR mice (SCXK 2008-0033, 18-20 g) were obtained from Experimental Animal Center of Zhejiang Chinese Medical University.

2.2. Extraction and Purification of PTPS

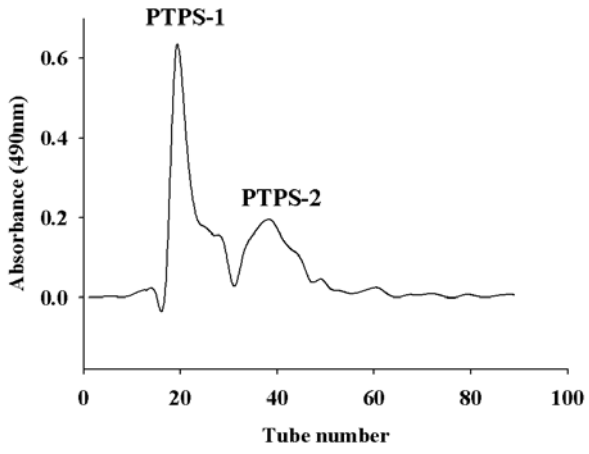

Figure 1. Elution profile of the puerh tea polysaccharides on Sephacryl S-300 column (2.6×60 cm)

The puerh tea polysaccharides (PTPS) were isolated by the modified procedure as described previously (Chen, Zhang, & Xie, 2004). Briefly, dry meshed tea powders (100 g) were mixed with 1 L of ethanol (80%, v/v) for 24 h to remove most of the polyphenols and monosaccharides. After the supernatant was removed, the residues were dried in air and then extracted with hot water at 70°C for 60 min (3 times). The aqueous extracts were concentrated and then precipitated with 4-fold vol of 95% ethanol. The precipitate that formed was collected by centrifugation at 3000 ×g for 10 min and repeatedly washed sequentially with ethanol, acetone, and ether, respectively for 3 times. The precipitate was dissolved in hot water (70°C) and excluded protein with Sevag method (1938), and dialyzed against distilled water for 48 h with dialysis tubing (molecular weight cut-off, 7000 Da) to remove low-molecular weight matters, and then concentrated and precipitated with 4-fold vol of 95% ethanol to obtain the polysaccharide fraction. The fraction was dissolved in water (60°C) to remove the rest of ethanol in a rotary evaporator under reduced pressure, and lyophilized to yield PTPS, finally. Crude PTPS was then separated on Sephacryl S-300 column (2.6× 60 cm), eluted with H_2O at a flow rate of 0.6 ml/min and collected at 5 ml/tube. Fractions were analyzed for total carbohydrate content and peaks for PTPS-1and PTPS-2 were pooled and freeze-dried (Figure 1).

2.3. Composition Analysis

Neutral sugar content was measured by the anthrone-sulfuric acid method (Morris, 1948), using D-glucose as standard. Uronic acid content was determined by the carbazole-sulfuric acid method using galacturonic acid as standard (Bitter & Muir, 1962). Protein was analyzed by the method of Bradford (1976) using bovine serum albumin as the standard (Bradford, 1976).

2.4. Monosaccharide Composition Analysis

PTPS-1 and PTPS-2 were hydrolyzed and derivatized to monosaccharides according to the method of Lv and others (2009). Then, the analysis of the monosaccharides were carried out on an Prominence LC-20A (Shimadzu, Japan) equipped with an Elite-17 ms column (30 m × 0.32 mm × 0.25 μm) and a SPD-20A detector (FID). The wavelength for UV detection was 250 nm. Elution was carried out at a flow rate of 0.8 ml/min at 35°C. The mobile phase A consisted of acetonitrile and the mobile phase B was 0.045% KH_2PO_4–0.05% triethylamine buffer (pH 7.0) using a gradient elution of 80–71–70–66% B by a linear decrease from 0–15–30–35 min. The injection volume was 20 μl.

2.5. Infrared Spectral Analysis

The IR spectra of the PTPS-1 and PTPS-2 were determined using a Fourier transform infrared spectrophotometer (Avatar 370, Thermo Nicolet, Madison, USA) equipment. The polysaccharides were grounded with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000-400 cm^{-1}.

2.6. Scanning Electron Microscopy (SEM)

SEM images were obtained at 15.0 kV on a Hitachi S-4700 (Hitachi, Tokyo, Japan) field emission scanning electron microscope after gold plating. Images at 3 different magnifications (1000×, 4000×, and 7000×) were collected for each sample (Han, Yu, Shi, Xiong, Ling, & He, 2011).

2.7. Atomic Force Microscopy (AFM)

AFM topography images were obtained with a Pico Scem 2100 AFM (U.S. MEDICAL Inc., alpharetta, Ga., U.S.A.) in tapping mode at ambient temperature. PTPS-1 and PTPS-2 were diluted to the concentration of 5 μg/ml with distilled water. About 10 μl of solution was dropped on the surface of glass solid and successively air-dry under ambient temperature (Foschiatti, Hearshaw, Cescutti, Ravenscroft, & Rizzo, 2009).

2.8. DPPH Assay

The DPPH free radical scavenging activities of the PTPS-1 and PTPS-2 were determined by the method of Mohsen and Ammar (2009), with a slight modification. One ml of the tested samples at various concentrations (62.5-1000 μg/ml) was added to 3 ml of ethanolic DPPH solutions (0.1 mM). Discolorations were measured at 517 nm after incubation for 30 min at 30°C in the dark. BHT was used as the positive control. The DPPH scavenging effect was calculated as follows:

$$\text{DPPH scavenging effect}(\%) = \left(1 - \text{A}_{\text{samp}} / \text{A}_{\text{cont}}\right) \times 100$$

where A_{samp} and A_{cont} were defined as absorbance of the sample and the control, respectively.

2.9. ABTS Assay

ABTS assay was carried out according to the method of Cai et al. (2004). The ABTS cation radical solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulphate and incubating in the dark at room temperature for 12 h. The ABTS cation radical solution was then diluted with water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. ABTS cation radical solution (3 ml) was added to 0.1 ml of the test samples with various concentrations (62.5-4000 μg/ml) and mixed vigorously. The absorbance was measured at 734 nm after standing for 6 min. BHT was used as the positive control. The ABTS scavenging effect was calculated as follows:

$$\text{ABTS scavenging effect}(\%) = \left(1 - \text{A}_{\text{samp}} / \text{A}_{\text{cont}}\right) \times 100$$

where A_{samp} and A_{cont} were defined as absorbance of the sample and the control, respectively.

2.10. Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed according to a modified method of Benzie and Strain (1999). Briefly, the working FRAP reagent was prepared by mixing 10 vol of 300 mM acetate buffer (pH 3.6) with 1 vol TPTZ (10 mM) in HCl (40 mM) and with 1 vol of $FeCl_3$ (20 mM). Freshly prepared FRAP reagent was warmed at 37°C, and a reagent blank reading was taken at 593 nm. Subsequently, 0.6 ml of test samples was added to the FRAP reagent (4.5 ml). A second reading at 593 nm was performed after 8 min. The initial blank reading with the FRAP reagent alone was subtracted from the final reading of the FRAP reagent with the sample to determine the FRAP value of the sample. A standard curve was prepared using different concentrations (50-2500 μM) of $FeSO_4 \cdot 7H_2O$. BHT was used as the positive control. The reducing ability of the OTPS was expressed as the equivalent to that of 1 μM $FeSO_4 \cdot 7H_2O$.

2.11. α-Glycosidase Inhibition Assay

The α-glycosidase inhibitory activities of the PTPS-1 and PTPS-2 were determined according to the method described by Apostolidis and Lee (2010) with a slight modification. A mixture of 50 μl of sample and 100 μl of 0.1 M phosphate buffer (pH 6.9) containing α-glycosidase solution (1 U/ml) was incubated in 96 well plates at 25°C for 10 min. After preincubation, 50 μl of 5 mM *p*NPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Before and after incubation, absorbance was recorded at 405 nm by microplate reader (SpectraMax M5, Molecular Devices, California, USA). Acarbose was used as the positive control. The α-glycosidase inhibitory activity was expressed as inhibition percent and was calculated as follows:

$$\text{Inhibition}(\%) = (1 - \text{DA}_{\text{samp}} / \text{DA}_{\text{cont}}) \times 100$$

where A_{samp} and A_{cont} were defined as absorbance of the sample and the control, respectively.

2.12. Animal Experiment

The animal experiment was performed as previously described by Huang et al. (2013). Male ICR mice (SCXK 2008-0033) were housed in plastic cages at room temperature (22 ± 1°C) under a 12 h light–dark cycle, and provided with a diet of commercial pellets and water ad libitum. The mice were allowed to adapt to diet and general conditions of vivarium for 1 week before the experiment. All experiments were performed in accordance with the institutional ethical guidelines.

Diabetes was induced in 16-h fasting mice by a single injection of freshly prepared alloxan (80 mg/kg BW, dissolved in 0.9% normal saline). Whole blood samples were obtained from the tail vein of the overnight fasted mice 72 h after alloxan injection, and BG levels were measured with OneTouch Ultra blood glucosimeter (Johnson & Johnson, New Brunswick, USA). The mice with BG levels over 11.1 mM were considered diabetic and used for the study.

A total of 30 diabetic mice were used and divided into three groups of 10 each. All mice were fasted for 8-h with free access to water before the experiment. Model group (MG): the fasted diabetic mice were administrated orally soluble starch (2 g/kg BW) alone; Treatment groups: the fasted diabetic mice were administrated orally soluble starch (2 g/kg BW) with the PTPS-1 (50 mg/kg BW); Acarbose group: the fasted diabetic mice were administrated orally soluble starch (2 g/kg BW) with acarbose (50 mg/kg BW). Blood samples were taken from the tail vein at 0, 30, 60, 120 min. Blood glucose was measured with OneTouch Ultra blood glucosimeter. Area under the curve (AUC) was calculated using Sigma Plot (Version 11.0; Systat Software Inc., Chicago, USA).

2.13. Statistical Analysis

All the experiments were carried out in triplicate. The results were expressed as means ± SD and evaluated by analysis of variance (ANOVA) followed by Turkey's studentized range test carried out on the SAS system, and $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Chemical Composition of the Purified Puerh Tea Polysaccharides

The yield, protein, neutral sugar, uronic acid contents and monosaccharides composition of PTPT-S and PTPS-2 are summarized in Table 1. The yield of PTPS-1 was 0.46%, much higher than that of PTPS-2 (0.14%). Moreover, the PTPS-1 had a higher protein content (7.97% in PTPS-1, and 2.24% in PTPS-2), but lower neutral sugar (31.66% in PTPS-1, and 36.15% in PTPS-2) and uronic acid contents (48.17% in PTPS-1, and 54.85% in PTPS-2) than PTPS-2. Analysis of monosaccharide compositions by HPGPC (Figure 2) revealed that both polysaccharides contained seven monosaccharides e.g. L-rhamnose, D-mannose, D-glucose, D-galactose, L-arabinose, D-xylose and D-Galacturonic acid with different molar ratios. The highest content was L-arabinose followed by D-galactose and L-rhamnose.

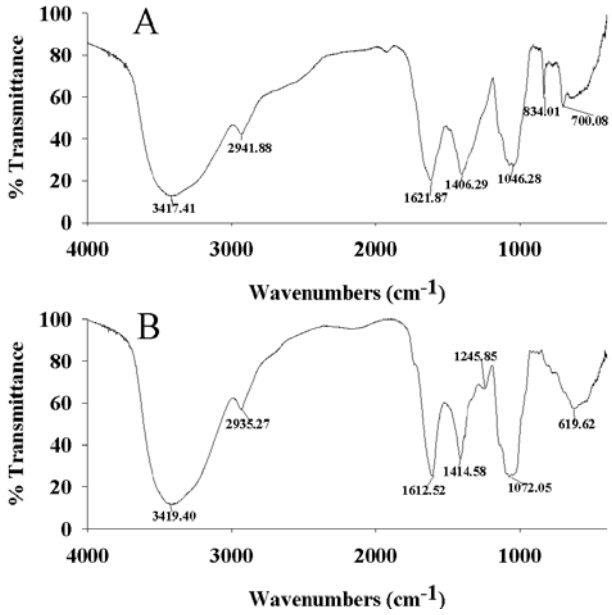

Figure 2. IR spectra of PTPS-1 (A) and PTPS-2 (B)

Table 1. Chemical analysis of the purified puerh tea polysaccharides

	PTPS-1	PTPS-2
Yeild (%)	0.46	0.14
Protein （%）	7.97 ± 0.38 [b]	2.24 ± 0.15 [a]
Neutral sugar （%）	31.66 ± 0.43 [a]	36.15 ± 0.94 [b]
Uronic acid （%）	48.17 ± 0.62 [a]	54.85 ± 0.28 [b]
Neutral sugar composition (mol ratio)		
L-rhamnose	2.49	0.92
D-mannose	0.51	0.23
D-glucose	0.15	0.13
D-galactose	2.80	3.26
L-arabinose	3.69	4.70
D-xylose	0.07	0.12
D-Galacturonic acid	0.29	0.65

Mean values followed by different letters are significantly different at $p < 0.05$.

3.2. FT-IR Spectra of PTPSs

As shown on Figure 2, PTPS-1 and PTPS-2 had similar FT-IR spectra, which exhibited a broadly stretched peak at around 3420 cm^{-1}, a characteristic absorption of hydroxyl group and one weak C-H band at around 2940 cm^{-1}. The relatively strong peak nearby 1620 cm^{-1} and 1400 cm^{-1} indicated the presence of proteins (carboxylate groups) and carbohydrate (C-H bands) respectively. Furthermore, the absorptions at 1100-1000 cm^{-1} range were the characteristic absorption bands of pyran-glycosides. The FT-IR spectra of PTPSs showed that PTPS-1 and PTPS-2 both possessed the characteristic absorption of polysaccharides.

3.3. SEM Images of PTPSs

Scanning electron micrographs of PTPS-1 and PTPS-2 are illustrated in Figure 3. Though the preparation of polysaccharides might cause damage to the samples as some rigid fragments appeared in the micrographs, it could still be observed the difference between PTPS-1 and PTPS-2. At low magnification, the PTPS-1 showed 3-dimensional mesh structure, while the PTPS-2 displayed irregular branched shape. The surface of PTPS-1 was smooth and flaky under higher power lens, while the PTPS-2 revealed rough and pothole surface. The smooth surface indicated that PTPS-1 had stronger intermolecular forces which could contribute to more stable structure, and the 3-dimensional mesh structure could be hypothesized to show stronger interaction with other molecules.

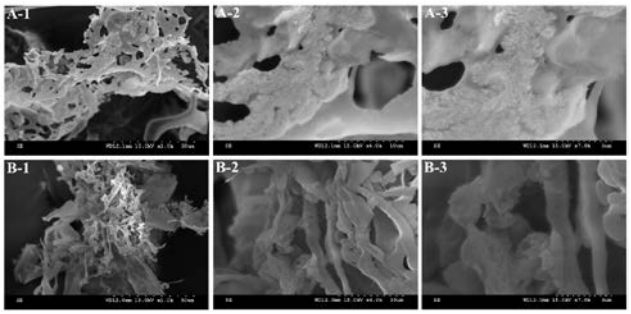

Figure 3. SEM of PTPS-1(A) and PTPS-2 (B) at 1000 (1), 4000 (2) and 7000 (3) fold magnification

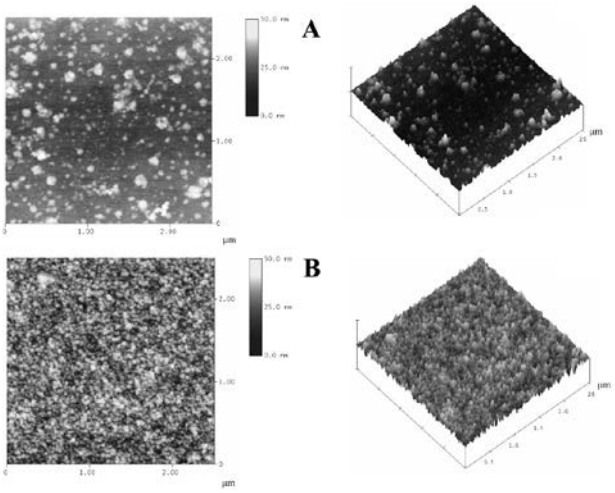

Figure 4. AFM height images of molecular structure of the puerh tea polysaccharide fractions: PTPS-1 (A), PTPS-2 (B)

3.4. AFM Images of PTPSs

AFM opens up exciting avenues for analyzing the molecular elasticity and spatial distribution of single polysaccharides and proteins (Marszalek & Dufrene, 2012). It works by scanning a sharp tip over the surface of a sample while sensing the near-field physical interactions

between the tip and the sample (Muller & Dufrene, 2011). The surface morphologies of PTPS-1 and PTPS-2 are visualized by AFM at an area of 2.5×2.5 μm^2 (Figure 4.). The average surface roughness (RMS) for PTPS-1 and PTPS-2 was 23.4 nm and 32.7 nm, respectively. The diameter of spherical lumps of PTPS-1 was in the range of 50-200 nm, while that of PTPS-2 ranged from 30 nm to 100 nm. In general, the width of polysaccharide chain was in the range of 0.1 to 1 nm (Ding, Feng, Cao, Li, Tang, Guo, et al., 2010; Tao, Biao, Yu, & Ning, 2008), which was much smaller than that of PTPSs. These results suggested that PTPS-1 and PTPS-2 contained more than one chains and they were branched and entangled with each other, which in agree with the hypothesis of Han et al. (2011).

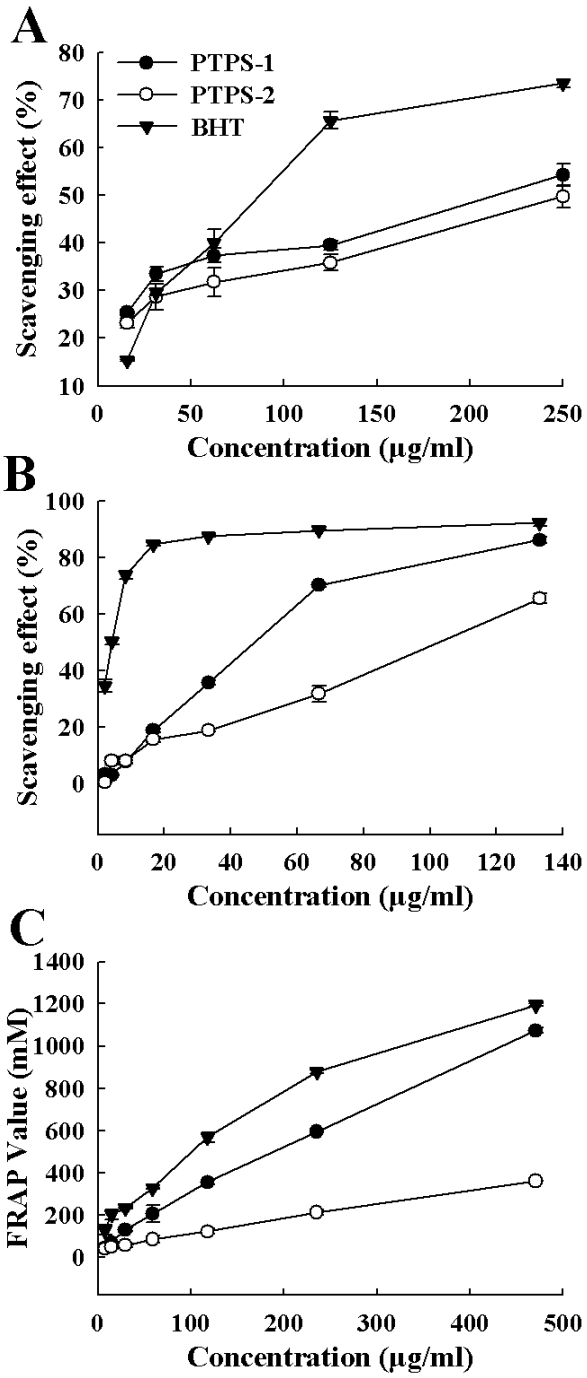

Figure 5. Antioxidant activities of the purified puerh tea polysaccharides. DPPH radical scavenging activity (A), ABTS cation radical scavenging activity (B) and ferric-reducing activity power (C)

3.5. Antioxidant Activities of PTPSs

DPPH free radical is capable of accepting an electron or a hydrogen radical to become a stable diamagnetic molecule (Soares, Dinis, Cunha, & Almeida, 1997), and it has been widely used to test the free radical scavenging ability of various natural products (Giovanelli & Buratti, 2009; Kuda & Ikemori, 2009). The scavenging ability of PTPS-1 and PTPS-2 on DPPH free radical is shown in Figure 5A. All the PTPSs and BHT exhibited effective scavenging ability on DPPH radicals in a dose-dependent manner in the concentrations between 15.63 μg/ml to 250 μg/ml. As shown in Table 2, the IC_{50} (μg/ml) of the PTPS-1, PTPS-2 and BHT on DPPH radical scavenging effect was 206.35, 249.01 and 78.65 respectively. The PTPS-1 demonstrated a higher scavenging ability than that of PTPS-2.

ABTS cation radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radicals, which involve H atom transfer, the reactions with ABTS cation radicals involve an electron transfer process (Kaviarasan, Naik, Gangabhagirathi, Anuradha, & Priyadarsini, 2007). As shown in Figure 5B, the concentration-dependent profile of scavenging ability on ABTS cation radicals was obvious for the PTPSs. In the concentrations ranged from 2.08 μg/ml to 132.89 μg/ml, the scavenging effect of PTPS-1 ranged from 3.36% to 86.12%, meanwhile, that of PTPS-2 from 0.32% to 65.43%. According to the IC_{50} (μg/ml) of PTPSs (44.50 for PTPS-1 and 101.83 for PTPS-2, respectively), PTPS-1 exhibited the significant difference from PTPS-2 (Table 2). Moreover, at the concentration of 132.89 μg/ml, the PTPS-1 showed a strong scavenging power, even closed to the positive control BHT, whose scavenging effect was 92.26%.

FRAP measures the antioxidant effect of any substance in the reaction medium as reducing ability, which is alternatively considered as the ability of a natural antioxidant to donate electrons (Shi, Gong, Liu, Wu, & Zhang, 2009). The results of FRAP assay are illustrated in Figure 5C. It could be determined that all the PTPSs and BHT had a concentration-dependent reducing ability on TPTZ-Fe (III) complex to TPTZ-Fe (II) complex. In this assay, the FRAP value of PTPS-1 increased from 44.24 mM to 1074.08 mM with the concentration increased from 7.35 μg/ml to 470.59 μg/ml, while that of PTPS-2 was from 38.48 mM to 360.47 mM. Apparently, the ferric-reducing power of the PTPSs was PTPS-1 >PTPS-2.

3.6. α-Glycosidase Inhibitory Effects of PTPSs

The α-glycosidase inhibitory effects of PTPSs are shown in Figure 6. All the PTPSs and positive control acarbose presented a dose-response relation between the concentration and inhibition. It can be concluded that PTPS-1 exhibited a much stronger inhibitory effect on α-glycosidase than that of PTPS-2, and even that of acarbose. With the raise of concentration from 7.80 μg/ml to 500 μg/ml, the α-glycosidase inhibition of PTPS-1 increased from 21.93% to 87.34%, while that of PTPS-2 was from 2.30% to 57.63%, and that of acarbose was from 9% to 61%, respectively. There was a significant difference between PTPS-1 and PTPS-2 with the IC_{50} (μg/ml) of 33.60 and 275.23, respectively (Table 2).

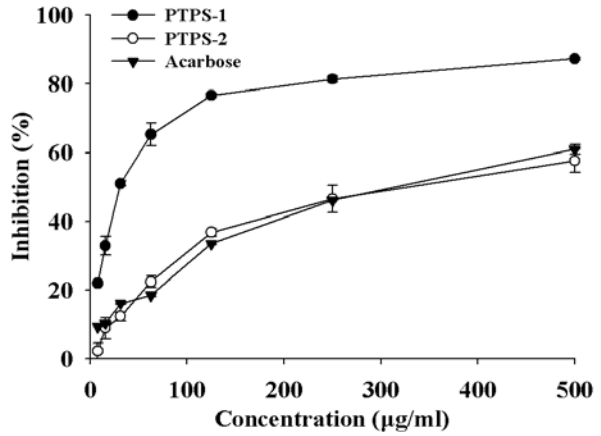

Figure 6. α-Glycosidase inhibitory effect of the purified puerh tea polysaccharides

3.7. Effect of PTPS-1 on Postprandial Blood Glucose *in Vivo*

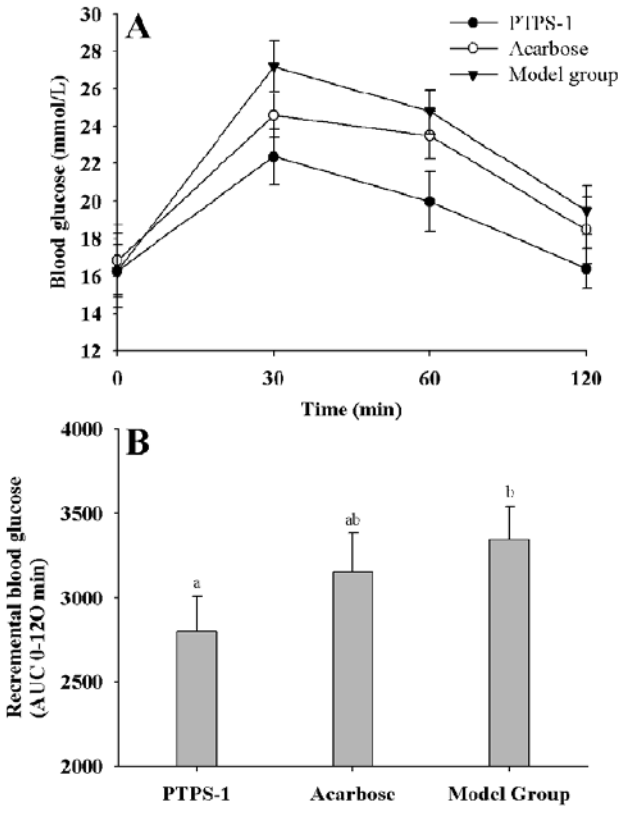

Figure 7. Effects on postprandial glycemic response (A) and Area under the curve (AUC) (B) of PTPS-1, Acarbose and Model group in alloxan-induced diabetic mice. Bars with different letters are significantly different at $p < 0.05$.

Figure 7A presentes the effects of PTPS-1, Acarbose and Model group on postprandial blood glucose in alloxan-induced diabetic mice. It can be observed that postprandial blood glucose levels in the mice administered the purified puerh tea polysaccharides (PTPS-1) increased more slowly than that in Model Group. Pretreatment with PTPS-1 (50 mg/kg BW) showed significantly preventive effect from hyperglycemia induced by glucose at 30 min, compared to the Model Group. Furthermore, the inhibition of PTPS-1 on postprandial blood glucose was even better than that of acarbose in alloxan-induced diabetic mice. The AUC (0-120 min) was given in Figure 7B. It was shown that PTPS-1 had remarkable suppression on postprandial hyperglycemia, which was significantly different from that in Model group. Accordingly, PTPS-1 had improved the glucose tolerance of alloxan-induced diabetic mice and exhibited inhibitory potential on postprandial blood glucose.

4. Discussion

The tea polysaccharides (TPS) were claimed to be produced by partial enzymic digestion of cell wall cellulose, pectin and starch of tea leaves during manufacture processing (Wang, Shao, Xu, Chen, Lin-Shiau, Deng, et al., 2012). It has been found not only to be the main resource of energy in plants but also to play important biological functions in the daily physiological process (Han, Yu, Shi, Xiong, Ling, & He, 2011). In this study, the protein content of PTPS-1 was 7.97%, much higher than that of PTPS-2 (2.24%), while its neutral sugar and uronic acid contents were lower than PTPS-2's. Meanwhile, the PTPS-1 revealed significantly stronger antioxidant activity and α-glycosidase inhibitory effect than PTPS-2. Based on these findings, it could be confirmed that the protein content highly contribute to the biological effects, which was in agreement with Chen's report (2008) that the protein content significantly improved the antioxidant activities of the polysaccharides extracted from green tea. Moreover, our previous research about oolong tea polysaccharides (Wang, et al., 2012) also showed that the higher content of protein was positively associated with the higher antioxidant activities and α-glycosidase inhibitory effect. In addition, one study related to quality control analysis of tea polysaccharides revealed that the differences of tea polysaccharides could not be determined significantly by the total sugar content, but could be convicted of the protein content (Wang, Xian, Xi, & Wei, 2013). That meant the protein content played a key role in TPS which could not only benefit the bioactivities but might also act as an indicator of polysaccharides.

The existence of protein might also affect the structure of TPS. It's reported that TPS were found to be mostly glycoconjugates in which a protein carries one or more carbohydrate chains covalently attached to a polypeptide backbone (Iso, Date, Wakai, Fukui, Tamakoshi, & Grp, 2006). The more protein content the polysaccharides has, maybe the more carbohydrate chains it contains. Our AFM images showed that the polysaccharide granule of PTPS-1 was bigger than that of PTPS-2, meaning PTPS-1 granule might had more carbohydrate chains which could be ascribed to the more protein content. Furthermore, the PTPS-1 SEM images indicated that it had stronger intermolecular forces and interaction with other molecules which might profit from the functional groups in protein. According to Nie and Xie's report (2011), the bioactivities of TPS depend on their physicochemical properties, such as chemical composition, monosaccharide ratio, molecular weight distribution, structure. Therefore, we could make a conclusion that the bound protein in polysaccharides highly affected its physicochemical properties and hence its bioactivities.

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia. The new estimates set by

the International Diabetes Federation (2013) show that an astounding 382 million people suffer from diabetes, with dramatic increases all over the world in 2013. What's worse, there is an increasing trend towards younger and younger people, which means more than 592 million people will be affected with diabetes within a generation, if current demographic patterns continue (Aguiree, Brown, Cho, Dahlquist, Dodd, Dunning, et al., 2013). Effective control of postprandial hyperglycemia is important in early intervention and prevention of diabetic complications for type 2 diabetes management (Ratner, 2001).

In our research, PTPS-1 significantly suppressed the increase of postprandial glucose level in diabetic mice, even better than acarbose, an oral anti-diabetic drug for patients with type 2 diabetes. Furthermore, it is reported that one therapeutic approach for reducing postprandial hyperglycemia in patients with DM is to prevent absorption of carbohydrates after food uptake (Ortiz-Andrade, Garcia-Jimenez, Castillo-Espana, Ramirez-Avila, Villalobos-Molina, & Estrada-Soto, 2007). Then, control of α-glycosidase, the key enzyme responsible for digestion of carbohydrates, can make it work. Measurements of α-glycosidase inhibitory effects of PTPSs *in vitro* also showed that PTPS-1 presented the best inhibitory potential against α-glycosidase in a dose dependent manner. According to the IC_{50} value of the inhibition on α-glycosidase (Table 2), PTPS-2 and acarbose exhibited no significant difference between each other, while PTPS-1 possessed almost 7 times more potence than them. These results suggested that PTPS-1 could act as a potent α-glycosidase inhibitor, which might be the reason for the suppression on postprandial hyperglycaemia.

Table 2. IC_{50} values of the purified puerh tea polysaccharides in different assays

Assay	Test sample	IC_{50} values (μg/ml)	R^2 value
DPPH	PTPS-1	206.35 ± 23.23 [b]	0.720
	PTPS-2	249.01 ± 22.91 [b]	0.879
	BHT	78.65 ± 2.19 [a]	0.955
ABTS	PTPS-1	44.50 ± 0.20 [b]	0.997
	PTPS-2	101.83 ± 2.32 [c]	0.965
	BHT	4.03 ± 0.04 [a]	0.988
α-Glycosidase inhibition	PTPS-1	33.60 ± 3.30 [a]	0.980
	PTPS-2	275.23 ± 51.13 [b]	0.980
	Acarbose	289.18 ± 1.27 [b]	0.989

Mean values followed by different letters are significantly different at $p < 0.05$.

In addition, lots of evidence have implicated that diabetic patients are under oxidative stress (Yao, Sang, Zhou, & Ren, 2010), which plays an important role in initiating beta-cell damage and insulin resistance (Dong, Li, Zhu, Liu, & Huang, 2012). Thus, antioxidants may prevent the progressive impairment of pancreatic beta-cell function and reduce the occurrence of type 2 diabetes (Song, Manson, Buring, Sesso, & Liu, 2005). In our study, antioxidant activities of the PTPSs were evaluated by assays of DPPH, ABTS, and FRAP, respectively. Despite of the different modes of antioxidant activities measured, all of the results obtained showed the same conclusion that PTPS-1 was more potent in the antioxidant activities than PTPS-2, which were correlated with the contents of bond protein in the polysaccharides. Accordingly, PTPS-1 could act like both antioxidant and α-glycosidase inhibitor, and have a potential to be used in prevention of type 2 DM.

5. Conclusion

Based on the results above, it can be found that the purified polysaccharides from puerh tea, PTPS-1 and PTPS-2, were both acid heteropolysaccharides bound with protein. The protein content of PTPS-1 was significantly higher than that of PTPS-2, which was positively associated with their antioxidant activity and α-glycosidase inhibitory effect. Meanwhile, the higher content of protein enhanced the interaction with other molecules resulting in more stable structure and bigger polysaccharide granule. Moreover, PTPS-1 presented inhibitory potential on postprandial blood glucose in alloxan-induced diabetic mice. Thus, PTPS-1 was suggest to possess a higher antidiabetic potential and the bound protein was the key factor that highly affected the polysaccharides' physicochemical properties and hence their bioactivities.

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