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Structural characterization and hypoglycemic activity of a polysaccharide isolated from the fruit of *Physalis alkekengi* L.

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Abstract

A water-soluble polysaccharide isolated with hot water from the fruit of *Physalis alkekengi* L. which is a traditional Chinese medicine herb was fractionated with different concentration of ethanol and purified by Sepharose CL-6B gel filtration chromatography. The structural characterization and hypoglycemic activity of the purified polysaccharide fraction (designated PPSB) were evaluated in this paper. PPSB ($M_w = 27$ kDa) is an acid heteropolysaccharide consisting of Ara, Gal, Glc and GalA in ratio of 2.6:3.6:2:1 and α-configuration. It has a backbone composed of (1→5)-linked Ara, (1→6)-linked Gal with three branches attached to O-3 of (1→6)-linked Gal and terminated with either Gal or Gal and Glc, and all of Glc and the majority of GalA are distributed in branches. Pharmaceutical experiments showed PPSB administered orally in alloxan-induced diabetic mice can significantly reduce blood glucose levels and water intake, and increase the body weight of diabetic mice compared with alloxan-induced diabetic control group. The results suggest PPSB could be considered as a potential candidate for developing a new anti-diabetic agent.

Keywords: *Physalis alkekengi* L.; Polysaccharide; Structure analysis; Hypoglycemic activity

1. Introduction

Physalis alkekengi L. of the family of Solanaceae is a traditional Chinese herbal plant distributed abundantly in the northeast region of China. It has been reported to have many ethnopharmacological properties including anti-inflammatory, anti-cold, anti-cough and anti-fungal activities (Wang & Yang, 1993). Some active components from *P. alkekengi*, such as physalin, alkaloids and flavone, have been investigated (Gong & Shan, 2002; Keith & Jack, 1973; Mahmood, Mojgan, & Fatemeh, 1996; Vessal, Mehrani, & Hossein, 1991; Zhou & Wang, 1997). However, no specific studies on polysaccharides isolated from *P. alkekengi* fruit have been carried out. We therefore specifically focused on the structure of the polysaccharide fraction (designated PPSB below) isolated from *P. alkekengi* fruit and evaluate the hypoglycemic effects of PPSB in alloxan-induced diabetic mice. The results showed that PPSB ($M_w = 27$ kDa) which is acid heteropolysaccharide consists of Ara, Gal, Glc and GalA in the ratio of 2.6:3.6:2:1. Pharmaceutical experiments indicated that PPSB can significantly reduce blood glucose levels and water intake, and increase the body weight in alloxan-induced diabetic mice, and has potential use as an anti-diabetic agent.

2. Experimental

2.1. Materials and chemicals

The mature fruits of *P. alkekengi.* were collected in Jilin province (northeast of China) in September 2004, and identified by Prof. Hongxing Xiao (School of Life Science, Northeast Normal University, Changchun, China).

Sepharose CL-6B was purchased from Amersham Pharmacia Co. (Sweden). T-series Dextrans, Trifluoroacetic

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acid (TFA) and 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluene-sulphonate (CMC) were purchased from Fluka. Alloxan monohydrate, dimethyl sulfoxide (DMSO), and standard sugars were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Blood glucose reagent kit was purchased from Beijing BHKT clinical Reagent Co. (China). Xiangke Pill was produced by Guangzhou ZhongYi Pharmaceutical Company Ltd. Xiaoke Pill, which is composed of Chinese herbal medicines (Kudzuvine root, Rehmannia, *Astragalus mongholicus*, etc.) and chemical composition (Glibenclamide), is an effective compound used for treating diabetes in China. All other chemical reagents used were of analytical grade.

2.2. General methods

Evaporation was performed at around 40 °C under reduced pressure, and the products were dried by lyophilization. Optical rotation was measured at 20 °C with a WZZ-T_1 polarimeter (Shanghai Physical Optics Instrument Co.). Spectrophotometer (Shimadzu MPS-200) was used to detect UV–Vis absorption spectra. Infrared (IR) spectrum (KBr pellets) was recorded on SPECORD in a range of 400–4000 cm^{-1}. Gas chromatography (GC), used for identification and quantification, was performed on a Vavian 3400 (Hewlett-Packard Component, USA) equipped with DM-2330 capillary column (30 m × 0.32 mm × 0.2 μm) and flame-ionization detector (FID). The column temperature was kept at 120 °C for 2 min, and increased to 250 °C (maintained for 3 min) at a rate of 8 °C/min. The injector and detector heater temperature were 250 and 300 °C, respectively. The rate of N_2 carrier gas was 1.2 ml/min. Gas chromatography–mass spectrometry (GC–MS) was run on the instrument HP5890(II) (Hewlett-Packard Component, USA) with a HPS quartz capillary column (25 m × 0.22 mm × 0.2 μm), and at temperatures programmed from 120 °C (maintained for 2 min) to 260 °C (kept for 40 min) at a rate of 15 °C/min. The concentration of polysaccharide was measured by the phenol–sulfuric acid method using D-glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein was measured by the Micro-Kjeldahl method (Wang, Qin, Gao, & Yan, 1999). Uronic acid content was determined according to a *m*-hydroxydiphenyl colorimetric method by using D-galacturonic acid as the standard (Filisetti-Cozzi & Carpita, 1991).

2.3. Isolation and purification of PPSB

3.5 kg of fresh *P. alkekengi* fruits were extracted with distilled water (6 l) at 100 °C for three times and 3 h for each time. The whole extract was filtered and centrifuged to remove water-insoluble fractions. The supernatant was precipitated with three volumes of ethanol at 4 °C overnight after concentration by evaporation at 45 °C under reduced pressure. The crude polysaccharide was recovered by centrifugation, and dried at 45 °C under reduced pressure after washing successively with ethanol and ether. The polysaccharide (5 g) was dissolved in 100 ml distilled water and frozen at −20 °C, thawed and centrifuged at 10,000 rpm for 20 min to remove insoluble materials. Crude polysaccharide was precipitated with 50% ethanol, and supernatant was recovered by centrifugation. Polysaccharide fraction named PPSA was obtained from the supernatant mentioned above by precipitating with 70% ethanol. PPSA was deproteinated by a combination of proteinase and Sevag method (Staub, 1965). PPSA was further purified on a Sepharose CL-6B column (2.6 × 90 cm) eluted with 0.15 mol/L NaCl at a flow rate of 0.5 ml/min, and the main polysaccharide fraction (PPSB) was collected, dialyzed and lyophilized. PPSB was used for structural analysis and activity assessment.

2.4. Homogeneity and molecular weight

The homogeneity and molecular weight of PPSB were evaluated by high performance liquid chromatography(HPLC) (Shimadzu, Japan) equipped with a TSK-GEL G3000 PWXL column (7.8 × 300 mm) and a RID-10A Refractive Index Detector. HPLC was performed on 0.5% PPSB (20 μl) dissolved in distilled water with 0.7% Na_2SO_4 as the mobile phase at 0.5 ml/min and 40 °C. The columns were calibrated with T-series Dextran (T-200, T-80, T-40, T-20 and T-10) as standards.

2.5. Analysis of monosaccharide composition

The monosaccharide of PPSB was analyzed by GC. Polysaccharide was hydrolyzed and acetylated according to Johnes and Albersheim (1972). Briefly, PPSB (10 mg) was hydrolyzed with 2 M TFA (2 ml) at 120 °C for 2 h, and the excess acid was completely removed by co-distillation with ethanol. The hydrolyzed product was reduced with KBH_4 (30 mg), followed by neutralization with dilute acetic acid and evaporated at 45 °C after adding 1 mg myo-inositol and 0.1 M Na_2CO_3 (1 ml) at 30 °C with stirring for 45 min. The residue was concentrated by adding methanol. The reduced products (alditols) were added with 1:1 pyridine–propylamine at 55 °C with stirring for 30 min, and acetylated with 1:1 pyridine–acetic anhydride in a boiling water bath for 1 h. The acetylated products were analyzed by GC, and identified and estimated with myo-inositol as the internal standard.

2.6. Partial acid hydrolysis

PPSB (80 mg) was hydrolyzed with 0.05 M TFA (3 ml) for 16 h at 80 °C, and acid was removed by addition of ethanol repeatedly. The hydrolyzed PPSB was centrifugated to remove the precipitate ($PPSB_1$), and the supernatant was dialyzed against distilled water for 48 h in a dialysis sack (molecular weight cut-off of 3 kDa). The solution in or out of sack was collected, respectively, for further analysis. Ethanol was added to the solution in the sack after dialysis,

and the precipitate and supernatant designated as $PPSB_2$ and $PPSB_3$, respectively, were recovered after centrifugation. The fraction out of dialysis sack (PPSB4) and all other fractions ($PPSB_1$, $PPSB_2$ and $PPSB_3$) were dried for GC analysis (Wang, Luo, & Liang, 2004).

2.7. Periodate oxidation and Smith degradation

PPSB (25 mg) dissolved in 12.5 ml of distilled water were mixed with 12.5 ml of 30 mM $NaIO_4$, and the mixture was kept in darkness at 4 °C. 0.1 ml aliquots were withdrawn from the mixture at 3–6 h intervals and read in a spectrophotometer at 223 nm (Dixon & Lipkin, 1954) after dilution 250× with distilled water. Ethylene glycol (2 ml) was added to terminate the periodate oxidation reaction after 3 days. Some of the periodate-oxidized product (2 ml) was used to assess the amount of formic acid by titration with 0.00488 M sodium hydroxide, and the rest was extensively dialyzed against tap water and distilled water for 24 h, respectively. The content inside was concentrated and reduced with $NaBH_4$ (80 mg) for 16 h at 25 °C, neutralized with 50% acetic acid, dialyzed as described above and re-concentrated to 10 ml. One-third of the solution mentioned above was freeze-dried and fully hydrolyzed for GC analysis; others were added to the same volume of 1 M sulfuric acid, kept for 40 h at 25 °C, neutralized to pH 6.0 with $BaSO_4$, and filtered for analysis by Smith degradation. The filtrate was dialyzed (molecular weight cut-off of 3 kDa), and the content out of dialysis sack was desiccated for GC analysis; the content inside the dialysis sack was diluted with ethanol, the supernatant and precipitate were also dried out for GC analysis after centrifugation (Zhang, 1987).

2.8. Carboxyl-group reduction

The carboxyl-group of the polysaccharide was reduced by the method of Taylor and Conrad (1972). PPSB (40 mg) dissolved in 37.5 ml of distilled water was mixed with 250 mg of 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulphonate (CMC) and incubated at room temperature for 3 h at pH ~4.8 adjusted by adding 0.01 M HCl constantly. 37.5 ml of 2 M $NaBH_4$ prepared freshly was added slowly during the next 60 min. The pH of the mixture was maintained at 7.0 with 4.0 M HCl and the reaction mixture was dialyzed against distilled water. The dialyzate was lyophilized, and carboxyl-group reduced product was recovered and analyzed by GC–MS.

2.9. Methylation analysis

PPSB (20 mg) and reduced PPSB (20 mg) were methylated twice according to the method of Ciucanu and Kerek (1984). The methylated products were extracted by chloroform. No absorption peak in the region 3600–3300 cm^{-1} was detected by IR spectrum and this indicated that the methylated products were completely methylated. The product was hydrolyzed with formic acid and 2 M TFA, and excess acid was evaporated by co-distillation with distilled water. The hydrolyzed product was reduced with $NaBH_4$ for 24 h, and acetylated with acetic anhydride–pyridine (1:1) at 100 °C for 2 h. The alditol acetates of the methylated sugars were analyzed by GC–MS.

2.10. Assay of hypoglycemic activity

2.10.1. Experiment animal

Male adult Kunming mice (body weight 20 ± 2 g) used for experiments were purchased from Jilin University (China). The mice were acclimatized for a period of 2–3 days before being used for the experiment. Before and during the experiment the mice were fed with a standard laboratory diet, given tap water and maintained under a constant 12 h light and dark cycle at 21–23 °C.

2.10.2. Induction of experimental diabetes mice

The hyperglycemia Kunming mice were induced by the tail vein injection of alloxan prepared freshly at a dose of 70 mg/kg body weight. Sera were collected for measurement of blood glucose from the tail vein 72 h after injecting alloxan, mice that were marked hyperglycemia (fasting blood glucose > 11.1 mmol/l) were used as the diabetic mice for further study (El-Demerdash, Yousef, & Abou El-Naga, 2005; Rajesh et al., 2005).

2.10.3. Experimental design

Alloxan-induced diabetic mice (mentioned above) were divided into five groups (10 mice per group), and normal mice was used as the control.

Group 1: Normal control (NC), normal mice treated with distilled water.

Group 2: Diabetic control (DC), diabetic mice treated with distilled water.

Group 3: PPSB-L, diabetic mice treated with 50 mg/kg of PPSB.

Group 4: PPSB-H, diabetic mice treated with 100 mg/kg of PPSB.

Group 5: Diabetic mice treated with 60 mg/kg of Xiangke Pill.

All groups were administered orally by gastric intubation once a day. The consumption of diet and water, and body weight were recorded daily. After 7 days' treatment with PPSB and Xiaoke Pill, sera from fasting mice were collected to detect blood glucose levels by glucose oxidase–peroxidase enzymatic method (GLU kit, Beijing BHKT clinical Reagent Co. Ltd.).

2.10.4. Statistical analysis

All results were expressed as mean ± SEM. Data were analyzed by one-way ANOVA using SPSS. P values less than 0.05 were considered significant.

3. Results and discussion

3.1. Isolation and structural analysis

The yield of the crude water-soluble polysaccharide extracted with hot water from the fruit of *P. alkekengi* was 0.71% of the fresh material. The main fraction (PPSB) was purified with Sepharose CL-6B by a yield of 23% after fractioned with ethanol precipitation from the crude polysaccharide. PPSB was collected for further analysis of structure and activity. PPSB appeared as a white powder, $[\alpha]_D^{20} + 148^\circ$ (*c* 0.2, H_2O). It had a negative response to the Kjeldahl test. The fact that no absorption was detected by the UV spectrum at either 280 or 260 nm indicated the absence of protein and nucleic acid. The average molecular weight of PPSB was determined as 27 kDa by HPLC. The HPLC profile (Fig. 1) also demonstrated that PPSB had a single and symmetrically sharp peak revealing that PPSB was a homogeneous polysaccharide. Results from phenol–sulfuric acid assay showed that PPSB contained 97.5% carbohydrate. PPSB contained 12% uronic acid as evaluated by *m*-hydroxydiphenyl colorimetric method and GC analysis. Analysis by GC indicated that PPSB was composed of Ara, Gal, Glc, GalA with a relative molar ratio of 2.6:3.6:2:1 (Fig. 2).

The IR spectrum of PPSB (Fig. 3) revealed a typical major broad stretching peak around 3423.08 cm^{-1} for the hydroxyl group, and a weak band at 2925.53 cm^{-1} showing the C–H stretching vibration. The absorbance at 1739.50 cm^{-1} indicated the presence of uronic acid. The broad band at 1618.01 cm^{-1} was due to the bound water. The band at 841.57 cm^{-1} was ascribed to α-pyranoses in the polysaccharide (Park, 1971).

All the fractions including $PPSB_1$, $PPSB_2$, $PPSB_3$ and $PPSB_4$ recovered after partial acid hydrolysis of PPSB were subjected to GC analysis. Results from GC analysis shown in Table 1 indicated that Ara, Gal and trace of GalA in $PPSB_1$ and $PPSB_2$ can be the backbone structure of PPSB, and Glc, Gal and GalA in $PPSB_3$ and $PPSB_4$ can be the branched structure of PPSB.

Results from periodate oxidation showed 0.979 mol periodate was consumed and 0.276 mol formic acid was produced per sugar residue, indicating the presence of monosaccharides which are 1→linked or (1→6)-linked. The fact that the amount of periodate consumption was more than amount of formic acid (0.276 mol × 2) demonstrated there were other linkages oxidized by periodate, such as (1→4) or (1→2).

The periodate-oxidized products were fully hydrolyzed and analyzed by GC analysis (Table 2). The presence of Gal and GalA revealed some residues of Gal and GalA were (1→3)-linked, (1→2,3)-linked, (1→2,4)-linked, (1→3,4)-linked, (1→3,6)-linked or (1→2,3,4)-linked that

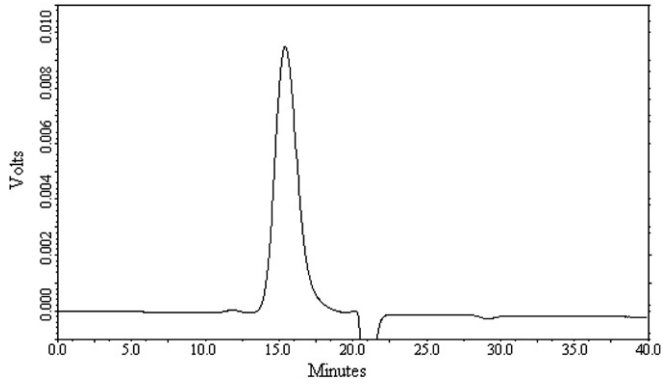

Fig. 1. HPLC profile of PPSB.

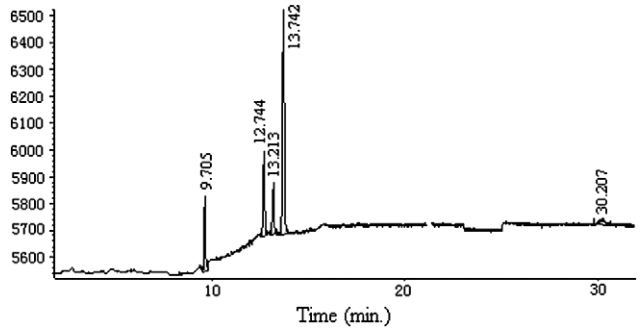

Fig. 2. GC profile of PPSB. Peaks from left to right: Ara, Gal, Glc, Internal standard and GalA.

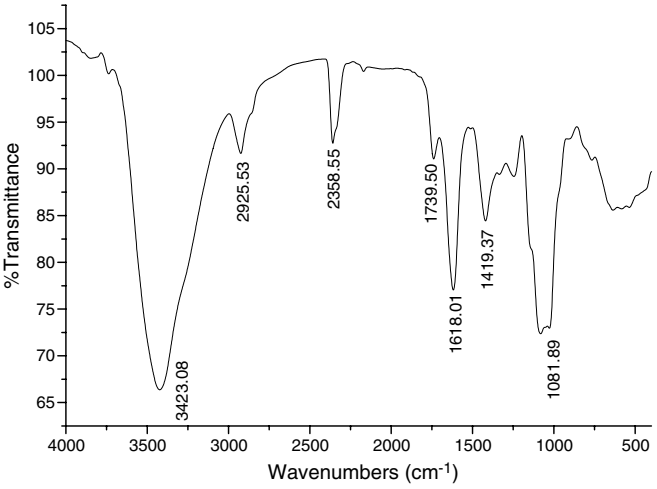

Fig. 3. IR spectrum of PPSB.

Table 1
GC analysis for fractions from partial acid hydrolysis

Fractions	Molar ratios			
	Ara	Gal	Glc	GalA
$PPSB_1$[a]	0.93	1		tr.
$PPSB_2$[b]	1.86	1		
$PPSB_3$[c]		1	1.24	0.92
$PPSB_4$[d]		1	1.09	

tr., trace.
[a] Precipitation after hydrolysis.
[b] Precipitation in the sack.
[c] Supernatant in the sack.
[d] Fraction out of sack.

Table 2
GC analysis for periodate-oxidized products and Smith degradation of PPSB

Fractions	Molar ratios						
	Gly[a]	Ery[b]	EryA[c]	Gal	Ara	GalA	Glc
Full acid hydrolysis	4.05	1.21		1		0.67	
Smith degradation							
Out of sack	3.97	1.13		1		0.63	
Supernatant in sack							
Precipitation in sack							

[a] Glycerol.
[b] Erythritol.
[c] Erythric acid.

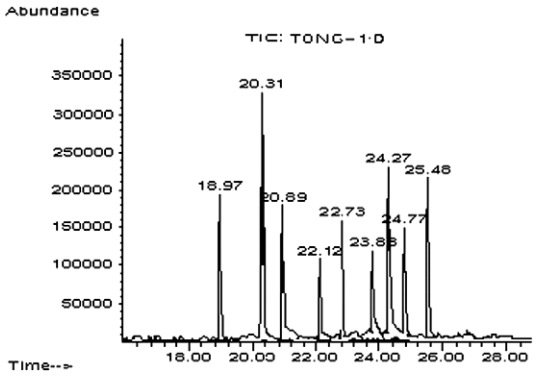

Fig. 4. GC profile of methylated PPSB on GC–MS.

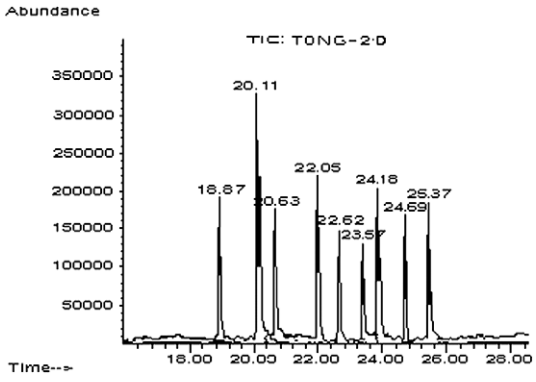

Fig. 5. GC profile of methylation of reduced PPSB on GC–MS.

can not be oxidized, respectively. No Ara and Glc were observed and large amounts of Gly and Ery were obtained, demonstrating that Ara and Glc were all linkages (discussed below) which can be oxidized by periodate.

GC analysis for Smith degradation shown in Table 2 indicated there was no precipitation in the dialysis sack and this demonstrated that the backbone of PPSB should be oxidized completely by HIO_4. Hence, it can be concluded that the linkages of backbone are (1→), (1→2), (1→6), (1→2,6), (1→4) and (1→4,6) that can be oxidized producing glycerin and erythritol detected out of sack. The fact that no EryA was detected suggested there were (1→3) and (1→2,3) linkages in GalA.

The fully methylated PPSB and reduced PPSB were hydrolyzed with acid, converted into alditol acetates, and analyzed by GC–MS (Figs. 4 and 5). PPSB (Table 3) and reduced PPSB both showed nine peaks, i.e. 2,3,4,6- Me_4-Glc, 2,3-Me_2-Ara, 2,3,4,6-Me_4-Gal, 2,4,6-Me_3-Gal, 2,3,6-Me_3-Glc, 2,3,6-Me_3-Gal, 2,3,4-Me_3-Gal, 2,3-Me_2-Glc and 2,4-Me_2-Gal. According to the difference in molar ratio of 2,3,4,6-Me_4-Gal, 2,4,6-Me_3-Gal, 2,3,6-Me_3-Gal, 2,3,4-Me_3-Gal and 2,4-Me_2-Gal in PPSB and reduced PPSB (Table 4), it can be deduced the linkage of GalA is (1→3)-linkage.

The results from analysis of GC–MS, which were consistent with the results from partial acid hydrolysis, Periodate oxidation and Smith degradation, indicated that 2,3-Me_2-Ara (1,5-linked Ara) and 2,3,4-Me_3-Gal (1,6-linked Gal) were major components of the backbone structure with 3 branches attached to O-3 of (1→3)-linked Gal; all Glc and the majority of GalA were distributed in branches, and residues of branches terminated with either Gal or Gal and Glc were composed of (1→3)-linked GalA, (1→4)-linked Gal, and (1→4)-linked Glc or (1→4,6)-linked Glc. In addition, small amount of (1→3)-linked Gal and trace of (1→3)-linked GalA was found in one of branches and backbone, respectively. In short, the monomer of PPSB was evaluated as below according to analysis of GC–MS, partial acid hydrolysis, periodate oxidation and Smith degradation.

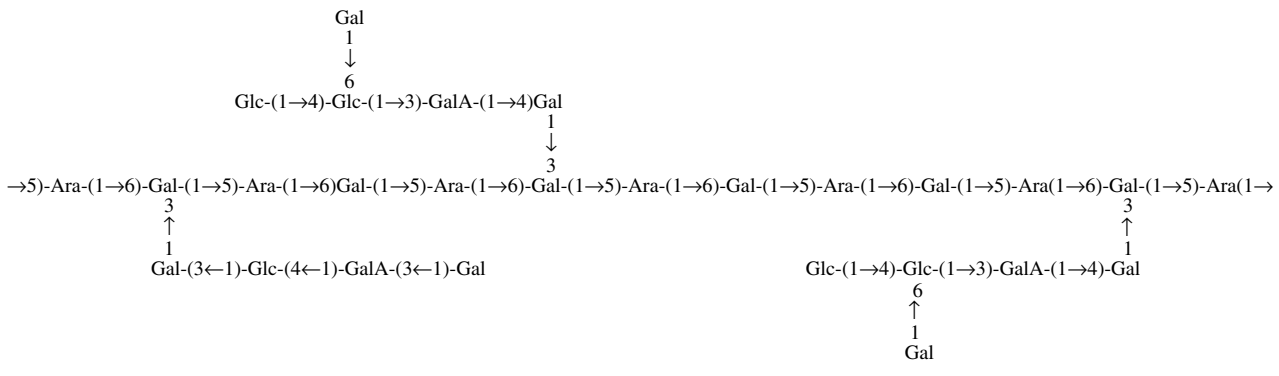

Table 3
GC–MS analysis of methylated PPSB

Methylated sugars (as alditol acetates)[a]	Type of linkage	Molar ratio
2,3,4,6-Me_4-Glc	Terminal Glc	1.17
2,3-Me_2-Ara	1,5-linked Ara	4
2,3,4,6-Me_4-Gal	Terminal Gal	1.02
2,4,6-Me_3-Gal	1,3-linked Gal	0.55
2,3,6-Me_3-Glc	1,4-linked Glc	0.91
2,3,6-Me_3-Gal	1,4-linked Gal	0.67
2,3,4-Me_3-Gal	1,6-linked Gal	1.63
2,3-Me_2-Glc	1,4,6-linked Glc	0.97
2,4-Me_2-Gal	1,3,6-linked Gal	1.49

[a] 2,3,4,6-Me4-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucose, etc. The order of methylated sugars made according to the order of peaks (from left to right) in Fig. 4.

Table 4
GC–MS analysis of methylation of PPSB and reduced PPSB

Methylated sugars	Type of linkage	Molar ratios		
		PPSB	Reduced PPSB	GalA[a]
2,3,4,6-Me_4-Gal	Terminal Gal	1.02	0.98	–
2,4,6-Me_3-Gal	1,3-linked Gal	0.55	1.79	1.24
2,3,6-Me_3-Gal	1,4-linked Gal	0.67	0.70	–
2,3,4-Me_3-Gal	1,6-linked Gal	1.63	1.58	–
2,4-Me_2-Gal	1,3,6-linked Gal	1.49	1.51	–

[a] Molar ratio of GalA = molar ratio of PPSB – molar ratio of reduced PPSB.

3.2. Hypoglycemic activity of PPSB

Diabetes mellitus has become a major health problem, affecting approximately 3% of the population worldwide. Currently available drugs for diabetes mellitus have a number of limitations such as adverse effects and high rates of secondary failure (DeFronzo, 1999). These problems have led to the search for alternative therapies that may have a similar degree of efficacy without the troublesome side effects associated with the conventional drug treatment. The identification of compounds from Chinese traditional medicinal herbs with hypoglycemic activity may also provide an opportunity to develop a new class drugs for the treatment of diabetes.

It has been reported that *P. alkekengi* has diverse biological activities and pharmacological functions including the pain-relieving, anti-inflammatory, analgesia, cough-relieving and sputum-relieving effects (Ma, 2002). In contrast, the hypoglycemic effect of polysaccharide isolated from *P. alkekengi* fruit has not been investigated. This is the first reported study showing a hypoglycemic activity of polysaccharide from *P. alkekengi* fruit.

The effects of PPSB on blood glucose level in alloxan-induced diabetic mice were shown in Table 5. The daily administration of PPSB (50, 100 mg/kg) for 7 days in alloxan-induced diabetic mice caused a significant reduction in the blood glucose level when compared with the diabetic control group ($P < 0.01$) and day zero in the respective group ($P < 0.01$). The mean decrease percentage of blood glucose levels caused by PPSB at the dose of 50 and 100 mg/kg were 26.5% and 31.2%, respectively. The results showed that higher dose (PPSB-H) was a little more effective in reducing blood glucose level than lower dose (PPSB-L) on alloxan-induced diabetic mice. However, no significant differences were observed between PPSB-H group and PPSB-L group. The hypoglycemic activity of PPSB at the dose 50 and 100 mg/kg in alloxan-diabetic mice was as effective as the Xiaoke Pill.

Table 5
Blood glucose levels in alloxan-induced diabetic mice before and after 7 days treatment with PPSB and Xiaoke Pill

Treatment groups	Dose (mg/kg)	Blood glucose (mmol/l)	
		Before treatment	After treatment
Normal control	–	6.24 ± 0.34	6.12 ± 0.40
Diabetic control	–	16.62 ± 0.88	16.34 ± 0.70
PPSB-L	50	15.99 ± 0.73	$12.01 \pm 0.83^{a,b}$
PPSB-H	100	16.25 ± 0.72	$11.24 \pm 0.66^{a,b}$
Xiaoke Pill	60	16.43 ± 0.53	$10.92 \pm 0.38^{a,b}$

The results were expressed as mean ± SEM ($n = 10$).
[a] Indicates statistically significant difference when compared to diabetic control ($P < 0.01$).
[b] Indicates statistically significant difference when compared to level of blood glucose before treatment in the respective group ($P < 0.01$).

Furthermore, the effects of PPSB on body weight and water intake in alloxan-induced diabetic mice were assessed. The daily body weight was shown in Fig. 6. The body weights in PPSB-H, PPSB-L and Xiaoke Pill group were increased significantly ($P < 0.01$) when compared with the diabetic control group. After alloxan was injected, the diabetic animals had a significantly increased water intake when compared with the normal control group (Fig. 7). Oral administrations of the PPSB at the dose 50 and 100 mg/kg both can significantly attenuate the increased water intake in diabetic animals when compared with the diabetic control group ($P < 0.01$).

The results suggest that PPSB may be considered as a potential candidate for developing a new anti-diabetic agent.

4. Conclusion

This study has demonstrated that PPSB isolated from the fruits of *P. alkekengi*, which is an acid heteropolysaccharide consisting of Ara, Gal, Glc, GalA in the ratio of 2.6:3.6:2:1 and α-configuration, has a backbone composed of (1→5)-linked Ara, (1→6)-linked Gal, (1→3)-linked Gal and trace of (1→3)-linked GalA with 3 branches attached to O-3 of (1→6)-linked Gal and terminated with either Gal or Gal and Glc; all Glc and the majority of GalA

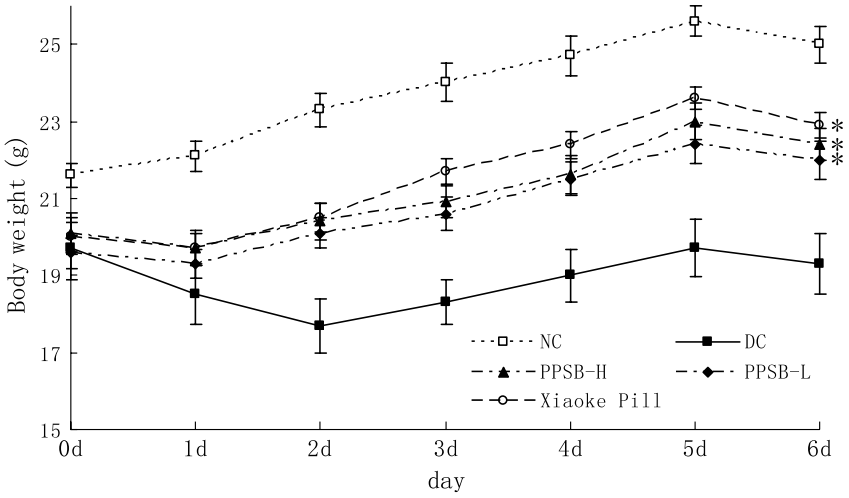

Fig. 6. Effects of PPSB on daily body weight in alloxan-induced diabetic mice. The results were expressed as mean ± SEM (error bars in the figure) ($n = 10$). * Indicates statistically significant difference when compared to diabetic control group on the last day ($P < 0.01$).

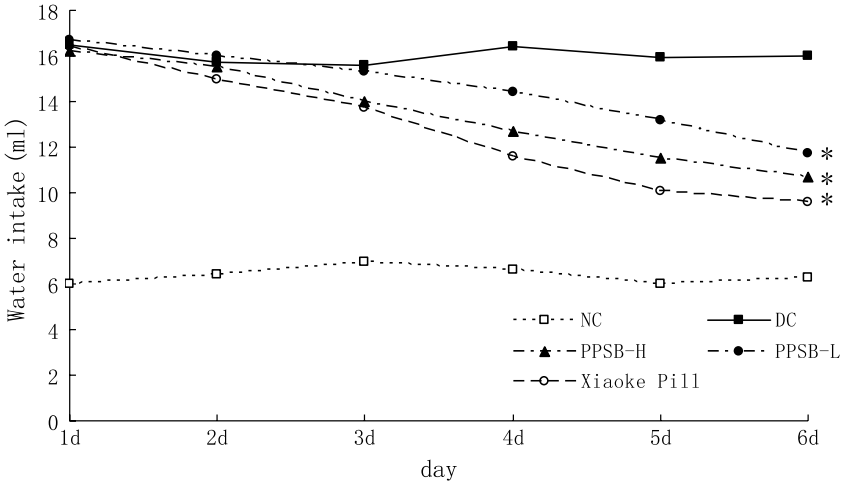

Fig. 7. Effects of PPSB on water intake in alloxan-induced diabetic mice. The results were expressed as mean ($n = 10$). *Indicates statistically significant difference when compared to diabetic control group on the last day ($P < 0.01$).

are distributed in branches, and residues of branches are composed of (1→3)-linked GalA, (1→4)-linked Gal, and (1→4)-linked Glc or (1→4,6)-linked Glc. In addition, there are small amount of (1→3)-linked Gal and trace of (1→3)-linked GalA found in one of branches and backbone, respectively. Preliminary pharmacological assays suggest that PPSB (50, 100 mg/kg) administered orally in alloxan-induced diabetic mice can significantly reduce blood glucose levels and water intake, and increase the body weight. It could be concluded that PPSB may be considered as a potential candidate for developing a new anti-diabetic agent. The correlation between structure and hypoglycemic activity of PPSB will be further investigated in future work.

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