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Effects of different extraction methods on the structural, antioxidant and hypoglycemic properties of red pitaya stem polysaccharide

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ABSTRACT

Processing conditions can change the compositions and microstructures of polysaccharides, resulting in favorable and unfavorable effects on their chemical characteristics and bioactivites. Here, this study comparatively evaluated the effects of the commonly used hot water, alkaline, acidic, enzymatic, ultrasonic and hot water-alkaline extractions on the structural features and antioxidant and hypoglycemic properties of pitaya stem polysaccharides. Nuclear magnetic resonance spectroscopy showed six polysaccharides had similar glycosyl types. Scanning electron microscopy exhibited the surface morphology of the extracted six polysaccharides differed significantly. Polysaccharide obtained by hot water showed better antioxidant and hypoglycemic properties than that of the other polysaccharides. These data suggested that alkaline, acidic, enzymatic, ultrasonic and hot water-alkaline extractions have various influences on the degradation of polysaccharides without varying the major structure in comparison with hot water extraction. Additionally, monosaccharide composition and molecular weight of polysaccharides are two chief factors affecting the bioactivity of pitaya stem polysaccharides.

1. Introduction

Pitaya (*Hylocereus polyrhizus*, commonly known as dragon fruit), family Cactaceae, has been widely distributed in subtropical and tropical regions. The fruits have played a remarkable role as medicine, food and ornament. After the pitaya fruits are harvested, pitaya stems are commonly discarded as wastes. Each hectare of pitaya farmland can produce around 60 tons of wasted stems, which creates serious environmental problems (Nurul & Asmah, 2014). However, pitaya stem can be either consumed fresh after removing sharp spines, or served as animal feed, or processed into flour, vinegar, juice and ice cream (Soedjatmiko, Chrisnasari, & Hardjo, 2019). Additionally, pitaya stem is a rich source of polysaccharides, phytosterols and flavonoids (Jaafar, Rahman, Mahmod, & Vasudevan, 2009). Plant polysaccharides are more prominent groups among the bioactive chemicals in pitaya stem, accounting for approximately 7 % of the dry pitaya stem's weight (Nerd, Sitrit, Kaushik, & Mizrahi, 2002). Polysaccharides, served as the appreciable active components with plentiful sources and without poisonousness, have become a hot spot of researchers due to their extraordinary physicochemical properties, as well as due to their multiple pharmacological activities for the developing novel pharmaceuticals and remedies of chronic illness (Jiang et al., 2019). Pitaya stem polysaccharides have been proved with remarkable antioxidant, antibacterial, anti-hyperglycemic and moisturizing-like effects (He, Li, Zhao, Song, Zhu, & Dong, 2011).

Extraction is the fundamental procedure for characterization and exploitation of the active polysaccharides from plant sources. Processing conditions are greatly correlated with diversified compositions and microstructures of polysaccharides, resulting in favorable and unfavorable effects on their chemical characteristics and bioactivites. The extraction of interesting plant polysaccharides, which can be ingested or utilized for functional product purposes, depends on compatible

Abbreviations: AGI, α-Glucosidase inhibitory; DSC, Differential scanning calorimetry; FT-IR, Fourier transform-infrared spectroscopy; M_W, Molecular weight; NMR, Nuclear magnetic resonance; PSP, Pitaya stem polysaccharide; SEM, Scanning electron microscopy; SEC-MALLS-RI, Size exclusion chromatography combined with multi-angle laser light scattering and refractive index detectors; TGA, Thermogravimetric analysis; T-AOC, Total antioxidant capacity.

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methods with economically viable yields. Generally, hot water, alkali-assisted, acid-assisted, enzyme-assisted, ultrasonic-assisted and hot water-alkali assisted extractions have been used to separate polysaccharides from natural materials. Currently, certain studies have demonstrated that the monosaccharide composition, molecular weight distribution and glycosidic linkage of the polysaccharides from fruits and their byproducts such as grapefruit, orange, mulberry and longan obtained by different extraction methods were considerably different (Wang et al., 2015; Chen et al., 2019; Huang et al., 2019). However, there is limited information in comparison with the structural characteristics and bioactivity of pitaya byproduct polysaccharides extracted by different methods.

Based on the aforementioned, in this research, the objective was to discuss the impacts of a few universal extraction methods involving hot water, alkaline, acidic, enzymatic, ultrasonic and hot water-alkaline extraction, on the yields, structural characteristics, surface morphological features, conformation behaviors, and thermal stability properties, as well as antioxidant and hypoglycemic effects *in vitro* of polysaccharides obtained from red pitaya stem. The cytotoxicity of pitaya stem polysaccharide was further determined. The research findings can offer useful references in the development of *H. polyrhizus* byproduct polysaccharides for functional products.

2. Materials and methods

2.1. Material and chemical reagents

RIN-m5F (ATCC CRL-11605) cells were purchased from Tongpai Biotechnology Co., Ltd. (Shanghai, China). Fetal bovine serum (FBS, Australia Origin) and Cell Counting Kit-8 were arranged from Invigentech Inc. (Irvine, CA, USA). Gibco RPMI medium 1640 basic was arranged from ThermoFisher Biochemical Products (Beijing) Co. (Beijing, China). α-Glucosidase, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and *p*-nitrophenyl glycopyranoside (PNPG) were obtained from Sigma Chemical Co., Ltd (St. Louis, MO, USA). Beijing Solarbio Science and Tech. Co. (Beijing, China) provided penicillin–streptomycin solution (penicillin 10 ku/mL and streptomycin 10 mg/mL), phosphate buffer solution (pH 7.2–7.4), trypsin-EDTA solution, and total antioxidant capacity (T-AOC) assay kit. Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) provided hydroxyl free radical assay kit and inhibition and produce superoxide anion assay kit. All compounds were analytical grade, unless otherwise specified.

2.2. Extraction of pitaya stem polysaccharides (PSP) by different methods

2.2.1. Pretreatment of pitaya stem

Raw red pitaya stems were harvested in March 2021 on the farmland of Jinsui Ecological Technology Group Co., Ltd. located in Guangxi Province, China. The stems were washed and cut, and then ground into powder after lyophilizing using Labconco Corporation freeze-drier (Kansas City, MO, USA). The stem powder was refluxed with 90 % ethanol to remove pigments and lipids (Chen et al., 2019). The residue was collected by filtration and lyophilized to obtain depigmented pitaya stem powder.

2.2.2. Hot water extraction (HWE)

The depigmented stem powder was mixed with distilled water in a ratio of 1:50 (g/mL) and subjected to a water bath at 90 °C for 2 h (Xu, Zhang, & Wang, 2016). After centrifugation and filtration, protein in the supernatant was removed by Sevag reagent (chloroform: *n*-butanol = 4: 1, v/v). The mixture was concentrated and mixed with absolute ethanol in a ratio of 1:3 (v/v) at 4 °C overnight. The precipitate was collected, followed by dialysis with the Minimate TFF capsule 3 K Omega membrane (Pall Corporation, Port Washington, NY, USA) for 3 days and then lyophilized to acquire pitaya stem polysaccharide (PSP-h).

2.2.3. Alkali-assisted extraction (AAE)

The depigmented stem powder was mixed with NaOH solution (pH = 10.0) in a ratio of 1:50 (g/mL) at 50 °C for 2 h. The following steps were the same as mentioned in HWE procedures to acquire pitaya stem polysaccharide (PSP-a).

2.2.4. Acid-assisted extraction (CAE)

The depigmented stem powder was added with HCL solution (pH = 3.0) in a ratio of 1:50 (g/mL) at 50 °C for 2 h. The following steps were the same as mentioned in HWE procedures to acquire pitaya stem polysaccharide (PSP-i).

2.2.5. Enzyme-assisted extraction (EAE)

The depigmented stem powder (10 g) was added with 500 mL distilled water containing cellulase (50 mg) and pectinase (50 mg). The pH of the suspension was adjusted to either pH 4.5–5.0 for enzymes. The mixture was incubated at 50 °C for 2 h, and thereafter heated at 100 °C for 5 min to inactivate enzymes. The following steps were the same as mentioned in HWE procedures to acquire pitaya stem polysaccharide (PSP-e).

2.2.6. Ultrasonic-assisted extraction (UAE)

The depigmented stem powder was added with distilled water in a ration of 1:50 (g/mL), and extracted using an ultrasonic cleaning bath (JAC-5020, KODO, Hwaseong, Korea) at 50 °C for 2 h (Jalili Safaryan, Ganjloo, Bimakr, & Zarringhalami, 2016). The ultrasonic power was 200 W. The following steps were the same as mentioned in HWE procedures, the PSP sample (PSP-u) was obtained.

2.2.7. Hot water-alkaline assisted extraction (HAE)

The depigmented stem powder was extracted with distilled water in a ratio of 1:50 (g/mL), and the pH of the suspension was adjusted to pH 8.0 using NaOH solution. The extraction was conducted at 90 °C for 2 h. The following steps were the same as mentioned in HWE procedures, the PSP sample (PSP-c) was obtained.

2.3. Physicochemical characterization

2.3.1. Chemical composition analysis

Anthrone-sulfuric acid method was used to determine the sugar content of each PSP (Tang et al., 2021), and polysaccharide extraction yield (%) and carbohydrate content (%) were calculated by the following equations, respectively:

$$\text{Polysaccharide extraction yield}\,(\%) = \frac{\text{Weight of the extracted polysaccharide}\ (\text{g})}{\text{Weight of pretreated stem powder}\ (\text{g})} \times 100$$

$$\text{Carbohydrate content}\,(\%) = \frac{\text{Sugar content of extract}\ (\text{g})}{\text{Weight of pretreated stem polysaccharide powder}\ (\text{g})} \times 100$$

Additionally, residual protein content in each PSP was determined using the method of Bradford (1976).

2.3.2. Monosaccharide composition analysis

Each PSP in the sealed tubes was mixed with 2 M trifluoroacetic acid (TFA) and hydrolyzed at 105 °C for 6 h. After that, the hydrolyzed PSP samples were evaporated three times by using rotary vacuum evaporation and co-distillation with methanol to eliminate TFA. The hydrolyzed PSPs, or standard solutions were dissolved in distilled water and filtered through a 0.45 μm membrane. The chromatographic system adopted Dionex ICS 5000 chromatography system (Thermo Fisher Scientific, Waltham, MA, USA) with Dionex™ CarboPac™ PA10 (250 × 4.0 mm, 10 μm) liquid chromatography column by following the previously reported method (Wang, Zhang, Xiao, Huang, Li, & Fu, 2018). The mobile phase consisted of 0.1 M NaOH solution (A) and 0.1 M NaOH solution with 0.2 M NaAc (B) at a flow rate of 0.5 mL/min. The gradient elution was conducted as follows: 0 min (95 % A); 30 min (80 % A); 30.1 min (60 % A); 45 min (60 % A); 45.1 min (95 % A); 60 min (95 % A). The column temperature was fixed at 30 °C. Monosaccharides were identified by their elution times relative to mixes of sugar standards, including fucose (Fuc), D-galactosamine hydrochloride (D-GalN), rhamnose (Rha), arabinose (Ara), glucosamine(GluN), galactose (Gal), glucose (Glu), xylose (Xyl), mannose (Man), fructose (Fru), ribose (Rib), galacturonic acid (GalA), guluronic acid (GulA), glucuronic acid (GluA) and mannuronic acid (ManA).

2.3.3. Fourier transform-infrared spectroscopy (FT-IR) and ultraviolet (UV) analysis

The FT-IR spectra of PSP samples were recorded on Nicolet iS50 FTIR spectroscopy (Thermo Fisher Scientific, Waltham, MA, USA) in the frequency range of 4000–400 cm^{-1} for determining the functional groups (Tang et al., 2021). A total of 1 mg of dry sample and 100 mg of dry potassium bromide (KBr) crystals were mixed and pressed into pellets, and the data were collected with the FTIR spectrophotometer. The UV spectra were scanned on a Genesys 10S UV–Visible spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) from 200 to 400 nm for identifying nucleic acid and protein in PSP samples.

2.3.4. ^{1}H and ^{13}C NMR spectroscopy

A total of 30 mg of each PSP sample was diluted in deuterium oxide (D_2O) for NMR analysis. A Bruker AM-500 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) was utilized to record ^{1}H and ^{13}C NMR spectra of the PSP samples.

2.4. Characterization of surface morphology

A Zeiss Merlin Compact scanning electron microscope (ZEISS Group, Oberkochen, Germany) was used to observe surface morphology of different PSPs at a voltage of 5.0-kV with image magnification of 1000×. Each PSP sample was sputtered with gold to make the sample conductive before observation.

2.5. Characterization of chain conformation

2.5.1. Congo red test

Congo red test was used to examine the conformational transition of polysaccharides as the previous procedure (Tang et al., 2021). In total, 1 mL of the sample solution (1 mg/mL) was diluted with an equal ratio of Congo red solution (80 μM). Then, various levels of 1 M NaOH solution were added to the final volume (0–0.5 M). The final volume of mixture was 4 mL. The maximum absorption wavelength of each PSP with Congo red solution and NaOH was determined by Genesys 10S UV–Visible spectrophotometer in 400–600 nm range. As a control, Congo red solution was mixed with the same concentration of NaOH.

2.5.2. Measurement of SEC-MALLS-RI

The molecular weight and chain conformational parameters of PSP samples were measured using size exclusion chromatography combined with multi-angle laser light scattering and refractive index detectors (SEC-MALLS-RI) as previously reported (Zheng, Huang, & Ling, 2019). The weight-average molecular weight (M_w), and polydispersity index (M_w/M_n) for six polysaccharides in 0.1 M $NaNO_3$ aqueous solution containing 0.02 % NaN_3 at 45 °C were detected on a DAWN HELEOS-II laser photometer (He-Ne laser, λ = 663.7 nm, Wyatt Technology Co., Santa Barbara, CA, USA), combined with Shodex OH-pak SB-805 HQ, 804 HQ and 803 HQ columns (300 × 8 mm, Showa Denko K.K., Tokyo, Japan). The basic light-scattering equation is as follows:

$$\frac{Kc}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{16\pi^2 n^2}{3\lambda_0^2}\langle S^2\rangle_z \sin^2\left(\frac{\theta}{2}\right)\right) + 2A_2 c + \ldots$$

$$K = \frac{4\pi^2 n^2 (dn/dc)^2}{N_A \lambda_0^4}$$

where K is the optical constant; n is the refractive index of the solvent; λ_0 is the wavelength of laser in vacuum; N_A is the Avogadro's number; R_θ is the Rayleigh ratio; A_2 is the second viral coefficient; c is the mass concentration; dn/dc is the refractive index increment. Additionally, a differential refractive index detector (Optilab T-rEX, Wyatt Technology Co., Santa Barbara, CA, USA) was simultaneously connected to give the concentration of fractions and the dn/dc value. The dn/dc value of the sample in 0.1 M $NaNO_3$ aqueous solution containing 0.02 % $NaNO_3$ was determined to be 0.141 mL/g. ASTRA6.1 software (Wyatt Technology Co., Santa Barbara, CA, USA) was used to collect and process the data.

2.6. Thermal stability properties

The thermal properties of PSPs were characterized *via* thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) using a Perkin Elmer STA6000 (PerkinElmer Inc., Waltham, Massachusetts, USA) as mentioned previously (Wang et al., 2018). The heating rate was 10 °C/min, and the temperature range was 30–1000 °C.

2.7. Functional activities in vitro of PSPs with different extraction methods

2.7.1. Assay for antioxidant activity

T-AOC, as well as DPPH, hydroxyl (•OH) and superoxide anion radicals ($O_2^{\bullet -}$) scavenging activities were utilized to analyze the antioxidant properties of PSPs. T-AOC was determined using the total antioxidant capacity (T-AOC) assay kit. The T-AOC value was calculated using micromoles of Fe^{2+} equivalents (Fe) per mL of PSP and a calibration standard curve of $FeSO_4{\cdot}7H_2O$ solution. DPPH radical scavenging activity was measured by Tang et al. (2019). In brief, different levels (0.1–2.5 mg/mL) of PSP samples (0.1 mL) were mixed with DPPH-ethanol solution (0.1 mL and 0.1 mM) and left for 30 min in the dark. An Epoch™ microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) was used to record the absorbance at 517 nm. The percentage of DPPH discoloration (%) = $[A_{blank} - (A_{sample} - A_{control})]/A_{blank} \times 100$, where A_{sample} represented the absorbance of the solution with DPPH and PSP; $A_{control}$ was the absorbance of the solution with PSP and ethanol; and A_{blank} represented the absorbance of the solution with DPPH and ethanol. The scavenging effect on •OH was analyzed using a hydroxyl free radical assay kit. The inhibitory capacity on superoxide anion radical was determined by inhibition and produce superoxide anion assay kit. Ascorbic acid (Vc) was used as a positive control.

2.7.2. Assay for hypoglycemic activity

α-Glucosidase inhibitory (AGI) effect of PSPs was measured using PNPG as a substrate according to the modified method of Tang et al. (2019). A total of 20 μL of PSP sample or 1 mg/mL acarbose (as positive control) or distilled water (as black) was mixed with 50 μL of 0.1 U/mL α-glucosidase. After incubating for 10 min at 37 °C, 50 μL of 5 mM PNPG solution was added. The reaction was terminated by adding 100 μL of 0.2 M Na_2CO_3 solution after incubated for 15 min at 37 °C. Enzymatic inhibition data were quantified by measuring the *p*-nitrophenol released from PNP-glycoside at 405 nm against distilled water. Enzymatic inhibition data were calculated as the percentage of inhibition (%) = $[1 - (A_{sample\ or\ acarbose} - A_{sample\ blank})/A_{blank}] \times 100$, where A_{blank} was the absorbance of blank, $A_{sample\ or\ acarbose}$ was the absorbance of sample or positive control, and $A_{sample\ blank}$ was the absorbance of sample without α-glucosidase and PNPG.

Table 1
Extraction yields and chemical compositions of pitaya stem polysaccharides obtained by different extraction methods.

Item	PSP-h	PSP-a	PSP-i	PSP-e	PSP-u	PSP-c
Yield (%)	6.35 ± 0.21^{b}	6.43 ± 0.25^{b}	3.78 ± 0.18^{e}	4.60 ± 0.28^{c}	4.18 ± 0.08^{d}	7.25 ± 0.17^{a}
Chemical characteristics						
Carbohydrate (%)	69.69 ± 2.90^{a}	63.46 ± 2.75^{b}	41.10 ± 3.98^{e}	46.06 ± 2.99^{d}	55.87 ± 2.06^{c}	58.52 ± 3.38^{c}
Protein (%)	–	1.71 ± 0.24	–	–	–	2.54 ± 0.37
Main monosaccharide composition (molar ratio, %)						
Galactose	29.03	57.49	57.49	44.78	52.51	32.76
Glucose	43.76	6.68	6.68	5.14	6.50	39.21
Rhamnose	15.20	15.16	19.98	28.36	19.40	13.63
Arabinose	3.98	11.61	5.10	9.59	10.07	5.75
Xylose	1.09	3.70	1.62	1.18	3.63	1.71
Galacturonic acid	6.95	5.35	9.32	10.96	7.89	6.94

Values represent the mean ± SD (n = 3). The different superscript letters indicate a significant difference ($P < 0.05$) within the row based on Duncan's test. "–": Not detected.

2.8. Cytotoxicity assay

The effect of pitaya stem polysaccharides obtained by hot water on the viability of RIN-m5F cells was measured by the CCK-8 assay, as described previously (Tang et al., 2021). RIN-m5F cells (100 μL/well) were inoculated in a 96-well plate and cultured for 24–48 h (37 °C) in the incubator. The PSP-h solutions at the concentrations of 100, 500 and 1000 μg/mL were added, respectively. In the incubator, the culture plate was incubated for 24 h and 48 h at 37 °C. Each hole was placed in 10 μL of CCK-8 solution and incubated for 1 h (37 °C). The absorbance was noted at 450 nm with the Epoch™ microplate spectrophotometer according to the CCK-8 manufacturer's instruction.

2.9. Statistical analysis

All the experiment results were repeated at least three times and expressed as means with standard deviations. Statistically significant differences were conducted *via* one-way analysis of variance followed by Duncan's test ($P < 0.05$) and the correlation analysis was estimated using Pearson correlation coefficient (*r*) in the SPSS 25.0 software (IBM, Chicago, IL, USA). Origin 2019 software (OriginLab, Northampton, Massachusetts, USA) was used for graphical processing.

3. Results and discussion

3.1. Physicochemical characterization

3.1.1. Extraction yields and chemical compositions

The yields and chemical compositions of the pitaya stem polysaccharides extracted using six methods are listed in Table 1. The extracted polysaccharide yields reduced in the order of PSP-c > PSP-a = PSP-h > PSP-e > PSP-u > PSP-i ($P < 0.05$). Pitaya stems are considered to have chemically and structurally heterogeneous rigid walls, leading to strict limitations to extraction efficiency of the intracellular and wall compounds; hence, the degradation of their cell walls is an essential requirement for active ingredients released. The extracted PSPs' yields were highly influenced by extraction method. Hot water combining with alkaline condition exerted strong damage on the cell wall, thus promoting the release of polysaccharides. Enzyme-assisted extraction could weaken or disrupt cell walls structure and also break down complex interior storage compounds, releasing intracellular compounds such as polysaccharides (Wang et al., 2010). Acid condition could hydrolyze cell walls, but PSP belongs to acidic polysaccharide, which could be the reason for its least extraction yield.

The carbohydrate content in PSP-h, PSP-a, PSP-c, PSP-u, PSP-e and PSP-i were 69.69 ± 2.90 %, 63.46 ± 2.75 %, 58.52 ± 3.38 %, 55.87 ± 2.06 %, 46.06 ± 2.99 % and 41.10 ± 3.98 %, respectively, pointing that carbohydrate was the primary constituent in the six polysaccharides. Additionally, only PSP-a and PSP-c contained protein. This result was consistent with the previous reports that proteins are chemically correlated with amide bones and easily hydrolyzed by alkaline condition, thus the protein content in extracts was plentiful under alkaline media (Pérez-Martínez, Sánchez-Becerril, Ornelas-Paz, González-Chávez, Ibarra-Junquera, & Escalante-Minakat, 2013).

3.1.2. Monosaccharide composition

As shown in and Fig. 1A and Table 1, galactose, glucose, rhamnose, arabinose and xylose existed with different molar ratios in PSP-h (29.03 %, 43.76 %, 15.20 %, 3.98 %, 1.09 %), PSP-a (57.49 %, 6.68 %, 15.16 %, 11.61 %, 3.70 %), PSP-i (57.49 %, 6.68 %, 19.98 %, 5.10 %, 1.62 %), PSP-e (44.78 %, 5.14 %, 28.36 %, 9.59 %, 1.18 %), PSP-u (52.51 %, 6.50 %, 19.40 %, 10.07 %, 3.63 %) and PSP-c (32.76 %, 39.21 %, 13.63 %, 5.75 %, 1.71 %). The galacturonic acid content in PSP-h, PSP-a, PSP-i, PSP-e, PSP-u, and PSP-c were 6.95 %, 5.35 %, 9.32 %, 10.96 %, 7.89 %, and 6.94 %, respectively. Extraction methods with high temperature such as HWE and HAE appeared to be more conductive to reduce

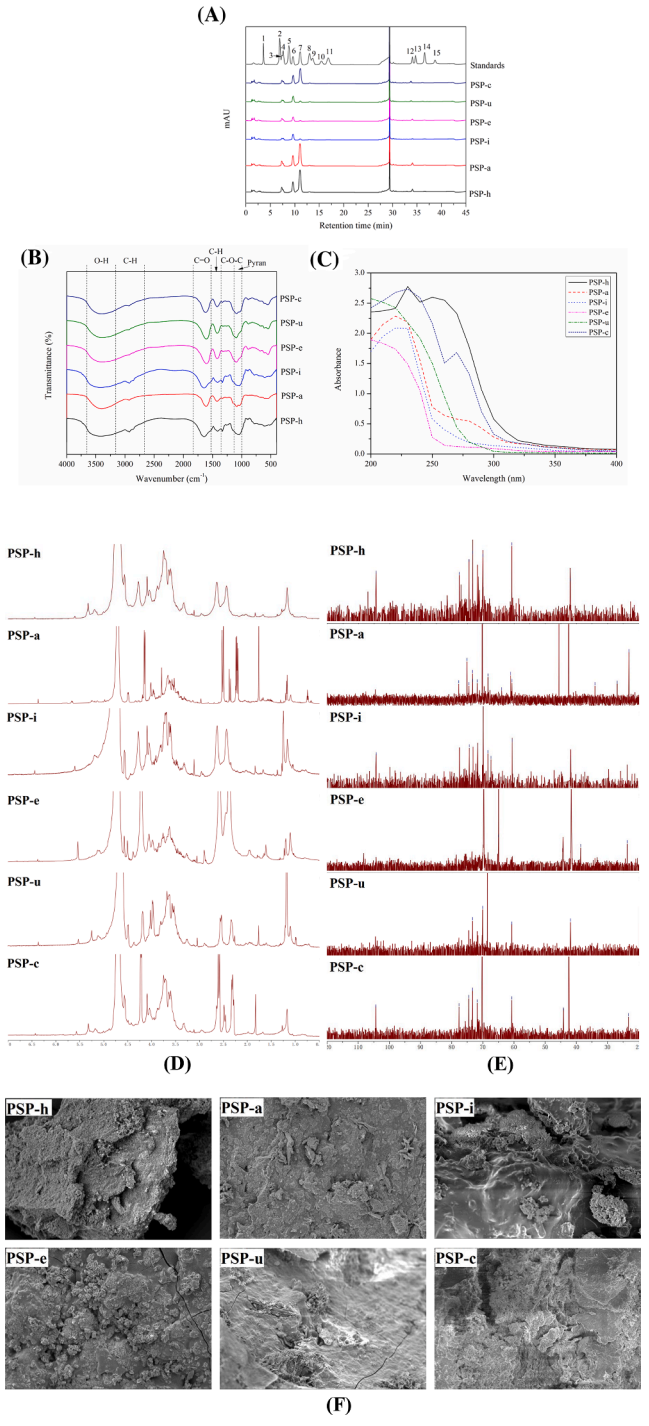

Fig. 1. The physicochemical and morphological characterization of pitaya stem polysaccharides obtained by different extraction methods. (A) ion chromatograms of monosaccharide composition (1. Fuc, 2. D-GalN, 3. Rha, 4. Ara, 5. GluN, 6. Gal, 7. Glu, 8.Xyl, 9. Man, 10. Fru, 11. Rib, 12. GalA, 13. GulA, 14. GluA and 15. ManA), (B) FT-IR spectrum, (C) UV spectrum, (D) ^{1}H NMR spectra, (E) ^{13}C NMR spectra, and (F) scanning electron micrographs (magnification 1000 ×).

galactose and galacturonic acid contents, whereas to increase glucose content. Enzyme-assisted extraction had a positive effect on rhamnose and arabinose contents in PSPs. Hence, the monosaccharide composition of polysaccharides were not significantly changed by different extraction methods, but their content were significantly affected, which was consistent with that reported by Wang, Yang and Wei (2010).

3.1.3. FT-IR and UV analysis

The FT-IR spectrum of six PSPs is presented in Fig. 1B, indicating that extraction methods had not obvious impact on the chemical functional groups. In the region of 3600–3200 cm^{-1}, the strong broadbands were assigned to O—H stretching vibration, and the weak bands at 2900–2800 cm^{-1} were assigned to the C—H tensile vibration. Absorption peaks from 1700 cm^{-1} to 1600 cm^{-1} were related to antisymmetric and symmetric C═O, representing that the existence of uronic acid (Cai et al., 2016). This result was consistent with the results of monosaccharide composition analysis, confirming that six PSPs contained galacturonic acid. The signals at 1100–1000 cm^{-1} implied the existence of pyranose rings (Kan, Chai, Li, & Zhao, 2020). The bands at around 920 cm^{-1} were related to the absorption of D-glucopyranosyl. The absorption peak at 920 cm^{-1} was the least intensive in PSP-a, suggesting that PSP-a had the lowest glucose content. Additionally, Fig. 1C shows PSP-a and PSP-c had distinct absorption peaks near the 260 nm and 280 nm wavelengths in the UV spectrum, indicating that they contained proteins and nucleic acids. The two polysaccharides may be protein/polysaccharide or protein/polysaccharide/nucleic acid conjugates, which was in agreement with the results of chemical composition analysis.

3.1.4. NMR spectroscopy

The structural information, including α- or β- anomeric configurations and glycosyl types of PSPs, was further evaluated using NMR, as exhibited in Fig. 1D and E. The NMR spectra of PSP-h, PSP-a, PSP-i, PSP-e, PSP-u and PSP-c seemed to be almost resembled each other. The NMR spectra of six PSPs were crowded in a narrow region ranging from 3.0 to 5.5 ppm and 60 to 120 ppm, which were typical signals of polysaccharides. As shown in PSP-h ^{1}H and PSP-h ^{13}C, the signal of H/C at 5.33/104.35 ppm was assigned to the anomeric regions of H1 and C1, and the multitudes of signals at 3.9–5.1 ppm and 60–80 ppm were attributed to the atoms of H2-H6 and C2-C6, respectively. On the basis of literature and monosaccharide composition analysis in this study, these signals may indicate the main glycosyl type in PSP-h including α-L-Rha*f*-(1→ (Zhang, Zou, Zhao, Qiu, Regenstein, & Yang, 2021). The same signals in PSP-a, which occurred in the region of the ^{1}H NMR spectrum at 5.26, 4.49, 4.16, 4.02 and 3.94 ppm and the ^{13}C signals at 104.48, 77.79, 75.16, 73.38 and 71.75 ppm, were assigned to the H1/C1, H2/C2, H3/C3, H4/C4 and H5/C5. The ^{1}H signals of PSP-i at 5.19, 4.83, 4.28, 4.04 and 3.85 ppm assigned to H-1, H-2, H-3, H-4 and H-5 while its ^{13}C signals at 104.36, 74.50, 70.01, 68.53 and 67.53 ppm were assigned to the C-1, C-2, C-3, C-4 and C-5. Additionally, the PSP-e ^{1}H NMR spectrum at 5.12, 4.55, 4.37 and 4.20 ppm were assigned to the H-1, H-2, H-3 and H-4, and the PSP-e ^{13}C NMR spectrum at 106.63, 69.86, 65.15 and 65.09 ppm were assigned to the C-1, C-2, C-3 and C-4. The ^{1}H signals at 5.24, 5.10, 4.48, 4.36, 4.18 and 4.02 ppm were assigned to the H-1, H-2, H-3, H-4, H-5 and H-6 while the ^{13}C signals at 104.16, 74.33, 73.30, 70.06, 68.51 and 60.74 ppm were assigned to the C-1, C-2, C-3, C-4, C-5 and C-6 in PSP-u. Further, the corresponding H-1, H-2, H-3, H-4, H-5 and H-6 chemical shifts of PSP-c were shown at 5.18, 4.22, 4.09, 4.04, 3.76 and 3.61 ppm, while its ^{13}C signals at 104.34, 77.64, 74.50, 73.29, 70.25 and 60.73 ppm were assigned to the C-1, C-2, C-3, C-4, C-5 and C-6. The other five PSPs had almost identical signals of the anomeric region when compared with PSP-h in the ^{1}H and ^{13}C NMR spectra. Therefore, the different extraction methods including HWE, AAE, CAE, EAE, UAE and HAE have diverse effects merely on the degradation of polysaccharides without varying the main glycosyl type, which was similar to the results of monosaccharide composition and FT-IR.

3.2. Surface morphological characterization

Fig. 1F demonstrates the surface morphology of PSPs, as recorded by scanning electron microscopy (SEM) images. All PSPs viewed the fragment appearances without uniform size and shape. The surface of PSP-h showed a reticular layer and irregular layered structure. The branches

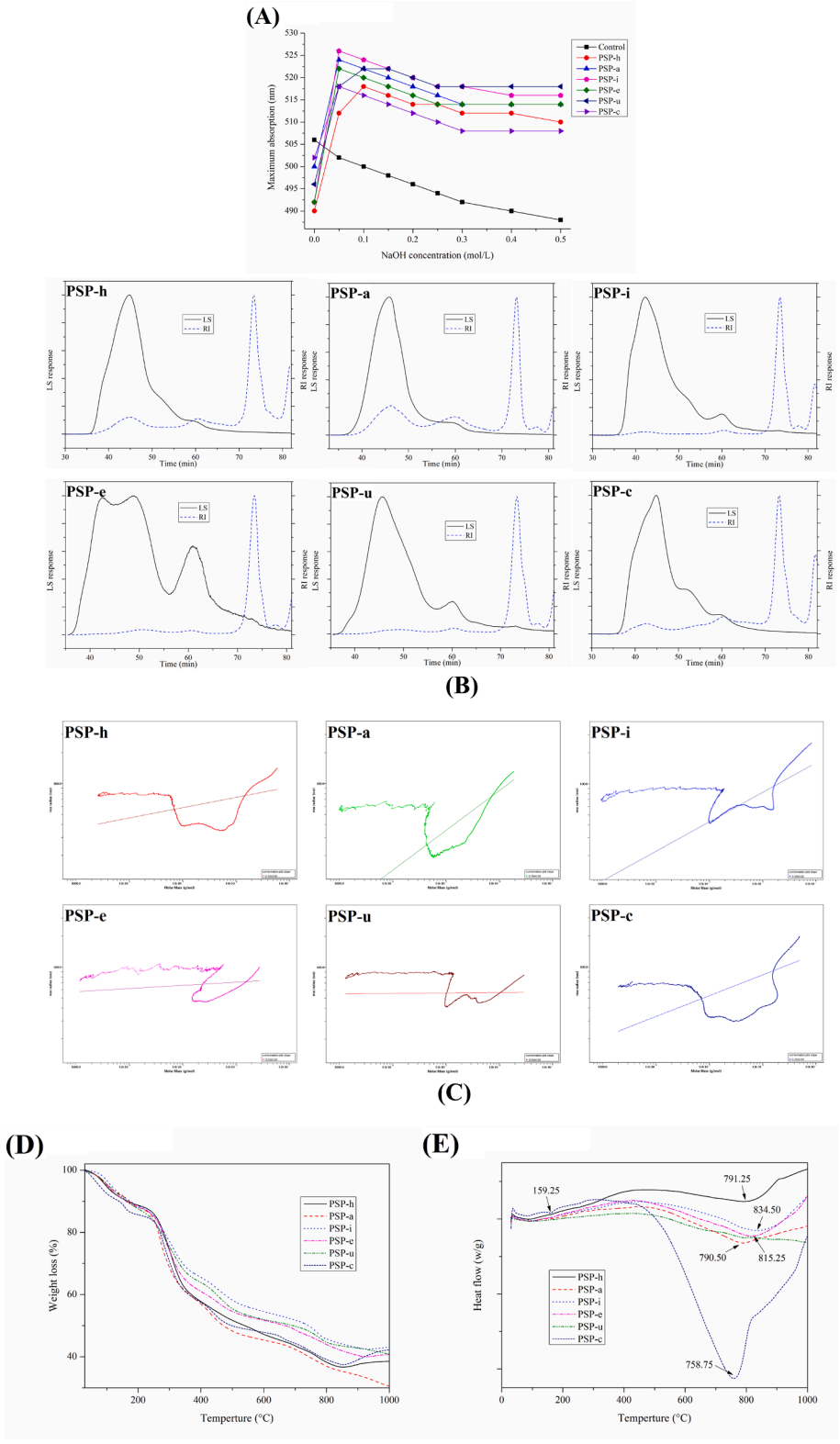

Fig. 2. The chain conformation and thermal stability properties of pitaya stem polysaccharides obtained by different extraction methods. (A) Congo red analysis, (B) SEC-MALLS-RI chromatograms, (C) plots of $< S^2 >_z^{1/2}$ *vs* M_w, (D) thermogravimetric (TGA) analysis, and (E) differential scanning calorimetry (DSC) analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and network structures of polysaccharides may be the reason for this appearance. PSP-a not only had a tight structure, but also presented a rough surface with irregular rod-like structure. Compared with PSP-h and PSP-a, PSP-c showed a tight structure and rough appearance with a large number of interspaces, which may be due to high temperature extraction under alkaline condition. PSP-i presented relatively smooth surface with composed of many small lumpish particles, whereas PSP-e showed as small irregular particles looked like snow, becoming more irregular and distorted surface. PSP-u had relatively even and some cavities of surface. The phenomena may result from the cavitation of

polysaccharides induced by ultrasound (Yin, Fan, Fan, Shi, & Gao, 2018). These findings confirmed that the different extraction methods induced different surface topography and appearance of the PSPs, resulting from different degree of hydrolysis of the sugar chains.

3.3. Characterization of chain conformation

3.3.1. Congo red analysis

Congo red forms a complex with polysaccharides of triple helical structure in alkaline media with different concentrations (Gong et al., 2020). As the concentration of NaOH enhanced, the destruction of triple helical structure would cause a drop in maximum absorption. The chemical conformation of pitaya stem polysaccharides may be influenced by extraction methods to a certain extent, as shown in Fig. 2A. Compared with the pure Congo red solution, the maximum absorption wavelengths of all PSPs-Congo red complexes showed the shift as the NaOH concentration increasing. When the concentration of NaOH achieved around 0.1 mol/L, the highest absorptions of all PSPs-Congo red complexes were obtained. However, with the further increase of the NaOH concentration, the maximum absorption of the polysaccharide solutions decreased gradually, indicating that the triple helical conformation of PSPs had been destroyed. Hence, there was a triple helix conformation for the PSPs obtained by six extraction methods.

3.3.2. Molecular weight and chain conformation

SEC-MALLS-RI analysis was applied to evaluate and detect the molecular weight and conformational parameters of polysaccharides. The SEC-MALLS-RI chromatograms of the PSPs in 0.1 M $NaNO_3$ solution at 45 °C are shown in Fig. 2B. Laser light scattering (LLS) response determined light scattering intensity, which was sensitive to M_w and root-mean-square radius ($<S^2>_z^{1/2}$), while refractive index detector (RI) provided polysaccharide concentration (Hu, Huang, Wong, & Yang, 2017). There were mainly-one peak for PSP-h and PSP-a in the SEC-MALLS chromatograms recorded by LLS, indicating that one major constituent. There were a large broad peak and a small peak for PSP-i and PSP-u, and three peaks for PSP-e detected by LLS. PSP-c had a large broad peak and two small peaks detected by LLS. These indicated the existence of aggregates, higher molecular weight components, and fragments of degraded polysaccharide with lower molecular weight. However, the peaks of the SEC-MALLS chromatograms determined by RI were different from those recorded by LLS. There were one large peak for all PSPs, and another two small peaks in the low retention time region for PSP-h, PSP-a and PSP-c. These suggested a few of aggregates and high molecular weight components in PSPs.

The data of the M_w, $<S^2>_z^{1/2}$ and M_w/M_n of PSPs in 0.1 M $NaNO_3$ solution at 45 °C are summarized in Table 2. The PSPs having diverse M_w could be acquired by six extraction methods. Six PSPs showed a M_w range from 71 kDa to 354.4 kDa, and each polysacharide had not a narrow molecular weight distribution according to the M_w/M_n value. The polysaccharide with higher M_w was prepared by hot water because of its validity of degrading cell wall. The obvious decrease in M_w for PSP-e and PSP-u may be caused by the fact that the enzymatic and ultrasonic conditions degraded the polysaccharide molecules. Additionally, the $<S^2>_z^{1/2}$ value is commonly a measure of how far from the centre of mass and how the mass of the polymer chains is concentrated. The relatively larger $<S^2>_z^{1/2}$ value reflects polymer chains with lower compact conformation (Huang, Huang, Li, & Zhang, 2009). Therefore, pitaya stem polysaccharide obtained by ultrasound existed as more compact conformation in aqueous solution compared with the other PSPs.

Table 2
Results of SEC-MALLS-RI for six PSPs in 0.1 M $NaNO_3$ aqueous solution at 45 °C.

	M_w (kDa)	$<S^2>_z^{1/2}$ (nm)	M_w/M_n	ν
PSP-h	354.41 ± 2.33	75.72 ± 1.68	48.22 ± 0.91	0.10
PSP-a	210.73 ± 4.19	69.81 ± 0.72	58.70 ± 2.32	0.39
PSP-i	186.40 ± 1.79	127.44 ± 2.80	109.65 ± 2.19	0.29
PSP-e	71.05 ± 2.68	69.83 ± 0.51	30.89 ± 0.84	0.03
PSP-u	80.82 ± 3.92	57.16 ± 0.69	32.07 ± 1.56	0.00
PSP-c	327.10 ± 4.86	105.92 ± 2.77	59.04 ± 1.86	0.20

The chain conformation of polysaccharide is evaluated according to calculating the ν value from the equation of $S_z^{2/2} = kM_w^{\nu}$. The ν and k values of the above equation are estimated from many experimental points in the SEC-MALLS chromatograms. Fig. 2C demonstrates the double logarithmic relationship of $S_z^{2/2} = f(M_w)$ of PSPs in 0.1 M $NaNO_3$ solution at 45 °C, and the ν values of PSPs are also listed in Table 2. Generally, the ν values of 0.20–0.40, 0.50–0.60 and 1.0 reflect a branching polymer, a flexible linear polymer and a rigid rod polymer, respectively (Chang et al., 2016). The ν values of PSP-h (0.10), PSP-a (0.39), PSP-i (0.29), PSP-e (0.03), PSP-u (0.00) and PSP-c (0.20) were all in the range of 0.2–0.4, indicating that they had highly branched structures in 0.1 M $NaNO_3$ solution at 45 °C. Additionally, the plots of $S_z^{2/2} = f(M_w)$ for all PSPs presented "U-shaped" curves, further confirming that they were highly branched polymers. This observed phenomenon could be explained by the accompanied occurrence of opposing separation mechanisms (Xu, Xu, & Zhang, 2012). Moreover, the ν value $\leq$ 0.33 displays the polymer molecular shape as a tight uniform sphere (Wyatt, 1993). The ν value of PSP-u was lower than that of other five PSPs, implying that PSP-u showed a more compact globular conformation than that of the other PSPs in aqueous solution.

3.4. Thermal stability properties

The weight-loss curves of PSPs assessed by TGA, presented in Fig. 2D, mainly display-three-stage loss patterns. The initial weight loss occurred at the temperature range of 30–200 °C, which was ascribed to the evaporation of water. Then, a more rapid weight loss occurred between 200 °C and 350 °C, as a result of the structural depolymerisation of polysaccharides (Zhu et al., 2019). As the temperature raised to the third temperature range, little to no change in PSP weight occurred. The weight loss of PSP-i (approximately 43 % of the original weight) was slightly lower than that of the other PSPs, suggesting that the thermal stability of PSP increased under acidic condition.

DSC analysis was completed to further clarify the thermal transition of PSPs, as shown in Fig. 2E. The DSC curves of PSPs mainly showed two endothermic peaks. The first endothermic peak transition of PSPs were in the range of 30–200 °C, which was attributed to the loss of free and bound water. The second endothermic peak transition of PSPs were observed between 700 °C and 850 °C, representing that PSPs were degradated. The thermal characteristics and transition temperature among the PSPs extracted by different methods are predicated on the structural and functional group variations. Specifically, the lower glass-transition temperature afforded PSP-i (159.25 °C) more structural stability and also made it more resistant to higher temperature (Munir, Shahid, Anjum, & Mudgil, 2016), a result similar to the TGA finding.

3.5. Functional activities in vitro of PSPs with different extraction methods

3.5.1. Antioxidant activity

Excessive reactive oxygen species (ROS) can lead to oxidative stress (Zheng, Dong, Chen, Cong, & Ding, 2015). Elevated ROS levels can lead to the production of free radicals, which may have harmful effects on nucleic acids, proteins, and lipids. Antioxidants play a potential role in the pharmaceutical and functional food industry. Previous studies found that polysaccharides could protect from ROS-induced oxidative damage by scavenging free radicals (Wang et al., 2014). Hence, it is necessary to evaluate the antioxidant abilities of PSPs obtained by different extraction techniques. In this research, the antioxidant abilities of PSPs were analyzed by determining the T-AOC, as well as DPPH, hydroxyl and superoxide anion radicals scavenging activities.

The total antioxidant capacity in this study is based on

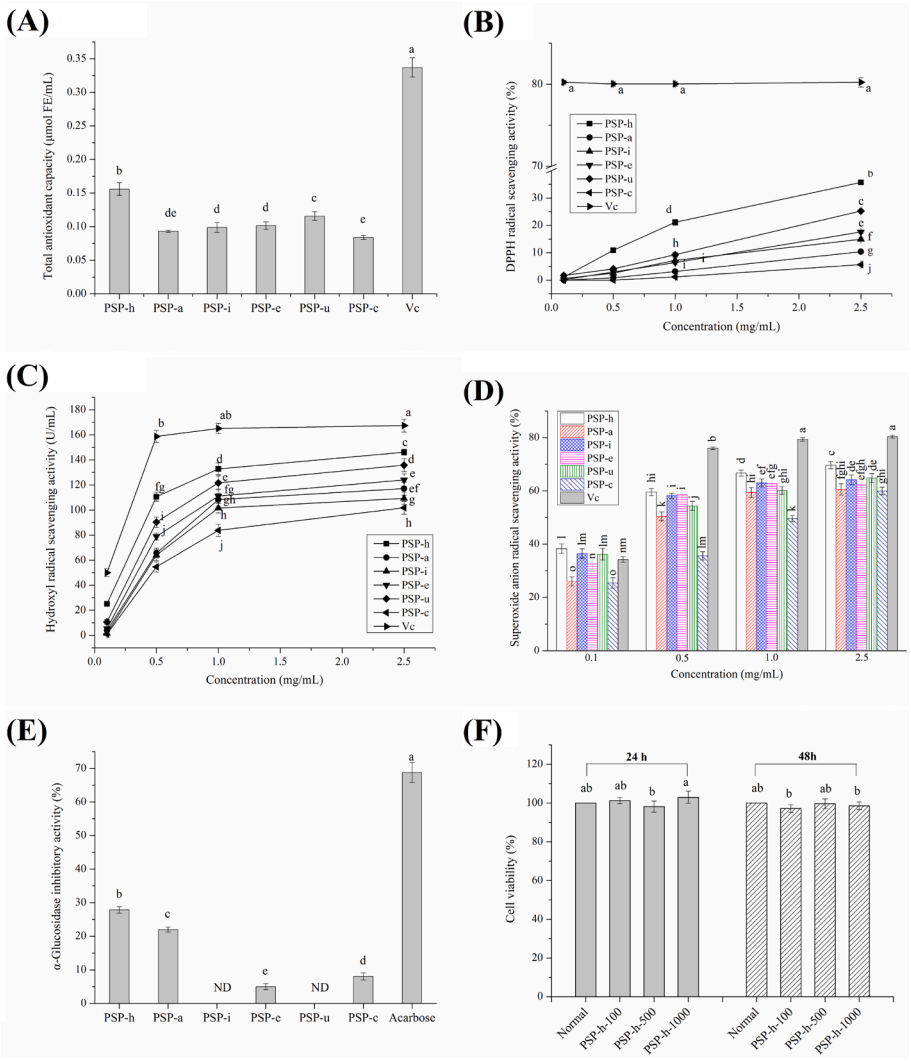

Fig. 3. Antioxidant, hypoglycemic and cytotoxic effects of PSPs *in vitro*. (A) total antioxidant capacity (T-AOC), (B) DPPH radical scavenging activity, (C) hydroxyl radical (•OH) scavenging activity, (D) superoxide radicals ($O_2^{\bullet -}$) scavenging activity, (E) α-glucosidase inhibitory activity, and (E) cytotoxicity on RIN-m5F cells. Values within each sample, denoted by various letters in the diagram, were statistically different, $P < 0.05$.

polysaccharides' ability to convert ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. Fig. 3A shows all different PSPs displayed different Fe^{3+}-reducing power at the concentration of 1 mg/mL. Obviously, the Fe^{3+}-reducing power of six PSPs decreased in the order of PSP-h > PSP-u > PSP-e = PSP-i > PSP-a > PSP-c ($P < 0.05$). The above values were lower than that of Vc (0.34 ± 0.01 μmol Fe^{2+} equivalents/mL). DPPH, a stable free radical with lipophilic properties, has been widely used to assess the antioxidant property of nature compounds such as polysaccharide. The DPPH radical scavenging activities of six PSPs were investigated at different concentrations (0.1–2.5 mg/mL) and the results are summarized in Fig. 3B. PSP-h, PSP-u and PSP-e showed significantly increased scavenging activities in the concentration ranged from 0.1 to 2.5 mg/mL compared with PSP-i and PSP-a, as well as an unobvious increasing scavenging rate was observed in PSP-c and Vc, respectively. PSP-h at the concentration of 2.5 mg/mL had the strongest DPPH radical scavenging ability of 35.74 ± 0.74 %, followed by PSP-u (25.26 ± 0.54 %) and PSP-e (17.61 ± 0.54 %), then by PSP-i (14.95 ± 0.51 %) and PSP-a (10.40 ± 0.39 %), eventually by PSP-c (5.67 ± 0.25 %) ($P < 0.05$). The scavenging rate of Vc (80.23 ± 0.56 %) was dramatically higher than that of all PSPs at the same concentration. Hydroxyl radicals, a highly aggressive radical species, could easily induce oxidative damage to DNA and cell membrane. The hydroxyl radical scavenging activities of different PSPs and Vc are presented in Fig. 3C. The results indicated that six PSPs and Vc showed •OH scavenging activities with a manner of concentration-dependent from 0.1 to 2.5 mg/mL. The •OH scavenging activities of PSP-h, PSP-a, PSP-i, PSP-e, PSP-u and PSP-c at the concentration of 2.5 mg/mL were 146.18 ± 2.33, 117.11 ± 5.23, 109.32 ± 4.89, 124.01 ± 4.93, 135.87 ± 5.12, and 101.78 ± 5.11 U/mL, respectively. PSP-h showed the highest scavenging capacity of •OH, while PSP-c had the lowest value ($P < 0.05$). Notably, the •OH scavenging activity of PSP-h at the concentration of 2.5 mg/mL was comparable to that of Vc (158.78 ± 4.85 U/mL) at the concentration of 0.5 mg/mL. Superoxide radicals, a kind of weak oxidants, could be transformed to H_2O by superoxide dismutase. Fig. 3D shows all the obtained PSPs and Vc had the potential ability to scavenge $O_2^{\bullet -}$ and the scavenging rates increased with higher concentrations. PSP-h exhibited a more potent $O_2^{\bullet -}$ scavenging rate than that of the other PSPs at all the tested concentrations ($P < 0.05$), while the scavenging properties of all the tested PSPs were lower than that of ascorbic acid at the same concentration ($P < 0.05$). According to above findings, pitaya stem polysaccharides extracted by six methods revealed different antioxidant capacities. All PSPs have antioxidant capacities and possibly, could be served as sources of active ingredients, especially pitaya stem polysaccharide obtained by hot water.

3.5.2. *Hypoglycemic activity*

α-Glucosidase plays a crucial role on the degrading process of

Table 3
Matrix for correlation analysis.

	Rha	Ara	Gal	Glc	Xyl	GalA	M_w
T-AOC	-0.124	-0.500	-0.652*	0.428	-0.187	0.155	0.471
DPPH	-0.155	0.242	-0.225	0.021	0.706*	-0.503	-0.394
•OH	-0.121	-0.639	-0.726*	0.700*	-0.500	-0.077	0.532
$O_2^{\bullet -}$	-0.023	0.584	-0.062	-0.104	0.559	-0.523	-0.174
AGI	-0.279	-0.904*	-0.587	-0.507	-0.911*	0.105	0.857*

Notes: *, Correlation is significant at the 0.05 level; Rha, rhamnose; Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; GalA, galacturonic acid; M_w, molecular weight; T-AOC, total antioxidant capacity; DPPH, DPPH scavenging activity; •OH, hydroxyl radical scavenging activity; $O_2^{\bullet -}$, superoxide radical scavenging activity; AGI, α-glucosidase inhibition activity.

complex carbohydrates to glucose, which has been recognized as the therapeutic targets for controlling of postprandial hyperglycaemia (Teng & Chen, 2017). Previous studies found that natural polysaccharides affect blood glucose levels by improving insulin resistance, promoting insulin secretion and regulating the activity of related enzymes (Chen, Chen, Wang, & Kan, 2020). However, there have no reports about the α-glucosidase inhibitory activity of pitaya stem polysaccharides. This research demonstrated that PSPs had α-glucosidase inhibitory activity and different extraction methods could differently influence the α-glucosidase inhibitory activity of PSPs. The inhibitory effects on α-glucosidase of PSPs obtained by HWE, AAE, CAE, EAE, UAE, and HAE are presented in Fig. 3E. The percentage inhibition of different PSPs for α-glucosidase at the concentration of 1 mg/mL were determined as PSP-h (27.89 ± 0.94 %) > PSP-a (22.00 ± 0.75 %) > PSP-c (8.06 ± 1.04 %) > PSP-e (5.01 ± 0.88 %) ($P < 0.05$). The pitaya stem polysaccharides obtained by CAE and UAE had no inhibitory effects on α-glucosidase, while arcabose was tested in around 68.8 % inhibition at the concentration of 1 mg/mL.

3.5.3. Correlation analysis

It was obvious that different extraction techniques had shown notable effects on the antioxidant and hypoglycemic properties of polysaccharides according to the analysis of Section 3.5.1 and 3.5.2. Actually, various factors including the monosaccharide composition and molecular weight could affect the antioxidant and hypoglycemic properties of polysaccharides (Zhang, Zhao, Li, Wu, Liu, & Liang, 2019). In order to elucidate the possible mechanism that the extraction methods affect the antioxidant and hypoglycemic properties of polysaccharides, the correlation analysis was applied to analyze the relationship between the bioactivity and physicochemical characteristics of PSPs in this study. The results are listed in Table 3.

The monosaccharide composition of Ara, Gal and Glc are correlated with T-AOC (Pearson correlation coefficient are r = -0.500, r = -0.652 and r = 0.428, respectively). There is the correlation existed between the DPPH scavenging activity and the monosaccharide composition of Xyl and GalA (Pearson correlation coefficient are r = 0.706 and r = -0.503, respectively). The monosaccharide composition of Ara, Gal, Glc and Xyl are correlated with the •OH scavenging activity (Pearson correlation coefficient are r = -0.639, r = -0.726, r = -0.700 and r = -0.500, respectively). The monosaccharide composition of Ara, Xyl and GalA are correlated with the $O_2^{\bullet -}$ scavenging activity (Pearson correlation coefficient are r = 0.584, r = 0.559 and r = -0.523, respectively). The higher Xyl, Ara and Glc contents may have the stronger antioxidant properties for pitaya stem polysaccharides, which was consistent with that reported by Sun et al (2018). Uronic acid content is identified as another considerable indicator for polysaccharides having antioxidant activity. The antioxidant activities of polysaccharides are related to their electron- or hydrogen-donating capacities. Uronic acid can react with the hydrogen atom in the anomeric carbon (Wu, Shang, Guo, Zhang, & Wu, 2020). Additionally, polysaccharides with molecular weight over 90 kDa commonly own advanced conformation and triple helical structure, which are crucial for high bioactivity (Liu et al., 2013). Hence, the strong antioxidant properties of PSP-h might have resulted due to its highest glucose and moderate galacturonic acid contents, as well as maximum molecular weight.

The monosaccharide composition of Ara, Gal, Glc and Xyl are related to the AGI activity (Pearson correlation coefficient are r = -0.904, r = -0.587, r = -0.507 and r = -0.911, respectively). The high contents of Ara, Gal, Glc and Xyl may conduce more to hypoglycemic activity than other compositions in pitaya stem polysaccharides. The molecular weight is related to the AGI activity (Pearson correlation coefficient is r = 0.857). The higher molecular weight may contribute more to AGI activity, explaining why PSP-h with the maximum molecular weight had exhibited the strongest AGI activity. The lower AGI activities of PSPs from acidic and ultrasonic extractions than that from hot water extraction may be due to the breaking process of sugar chains in those polysaccharides, which was similar to that reported by Zhao et al (2017).

3.6. Cytotoxicity assay

Polysaccharides exhibit great antioxidant and hypoglycemic activities and can be developed as alternative of chemotherapeutic agents with minimal toxic side effects (Gong et al., 2020). The cytotoxic property *in vitro* of pitaya stem polysaccharide obtained by hot water on RIN-m5F cells was determined. As shown in Fig. 3F, PSP-h at the concentration range of 100–1000 μg/mL exhibited no significant toxicity on RIN-m5F cells ($P > 0.05$) relative to the cells treated without PSP-h solution (normal groups) after incubating for 24 h and 48 h. This result indicated that pitaya stem polysaccharide belongs to bioactive ingredient with no or low toxicity.

4. Conclusion

In this study, water, alkaline, acidic, enzymatic, ultrasonic and hot water-alkaline extractions had caused changes in the yield, structural features and bioactivity of PSP, which was evaluated to acquire an appropriate approach to extract PSP for preventing and treating oxidative stress and diabetics. The results indicated that six PSPs had diverse monosaccharide composition, surface morphology, chain conformations and thermal stability, whereas the similar characteristic FT-IR and NMR spectra of pitaya stem polysaccharides. Additionally, PSP-h had the second most yield, maximum glucose and moderate uronic acid contents, and maximum molecular weight than the other PSPs, as well as it presented the most interesting antioxidant and hypoglycemic properties. Further, PSP-h had nontoxicity on RIN-m5F cells. Hence, hot water extraction to obtain polysaccharides from *H. polyrhizus* stem seemed to be more beneficial option with higher yield and stronger bioactivity. The structure of purified pitaya stem polysaccharides, and antioxidant and hypoglycemic potentials in food formulations need to be investigated further.

CRediT authorship contribution statement

Yayuan Tang: Writing – original draft. **Xuemei He:** Writing – review & editing. **Guoming Liu:** Software. **Zhen Wei:** Data curation. **Jinfeng Sheng:** Supervision. **Jian Sun:** Writing – review & editing. **Changbao Li:** Investigation. **Ming Xin:** Software. **Li Li:** Data curation. **Ping Yi:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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