Effects of steam on polysaccharides from Polygonatum cyrtonema based on saccharide mapping analysis and pharmacological activity assays

Zherui Chen (mc05840@umac.mo)
University of Macau Institute of Chinese Medical Sciences https://orcid.org/0000-0002-6602-8407
Baojie Zhu
University of Macau Institute of Chinese Medical Sciences
Jing Zhao
University of Macau Institute of Chinese Medical Sciences
Zhixin Chen
University of Macau Institute of Chinese Medical Sciences
Wen Cao
University of Macau Institute of Chinese Medical Sciences
Junqiao Wang
Nanchang University
Shao-ping Li
University of Macau https://orcid.org/0000-0003-4255-6229

Research Article

Keywords: Polygonatum cyrtonema, Polysaccharides, Steam, Chemical composition, Bioactivity

Posted Date: June 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1645421/v1

Abstract

Background: *Polygonatum cyrtonema,* one of origins of Polygonata Rhizoma (*HuangJing* in Chinese), is traditionally steamed repeatedly before being used as herbal medicine in China. However, there had no standardization of steaming of *HuangJing*. Therefore, comprehensive study for effects of steam on polysaccharide from *Polygonatum cyrtonema* based on saccharide mapping, a powerful method developed for polysaccharides analysis, and pharmacological activity are still necessary in order to explore the effect of steam on the physiochemical and biological activities of its polysaccharides and determine the standard for *Polygonatum cyrtonema*.

Methods: To explore the effect of steam on the physiochemical and biological activities of *P. cyrtonema* polysaccharides (PCP), six polysaccharides named PCP0, PCP1, PCP2, PCP3, PCP4 and PCP5 were extracted from the herb consecutively steamed for 0 to 5 times, respectively. Their molecular weight distribution, monosaccharide composition and PACE fingerprints were investigated through HPSEC-MALLS-RID, HPAEC-PAD and saccharide mapping based on polysaccharide analysis by using carbohydrate gel electrophoresis (PACE) and HPTLC, respectively. In addition, their antioxidant ability and immunostimulatory activities on RAW 264.7 cells in term of NO production and phagocytosis were compared.

Results: Results suggested that molecular weights could be changed during steam, which increased by first steaming and then decreased with further steaming though all polysaccharides molecular weight were 10^5-10^7 Da. They all showed irregularly spherical conformation in aqueous solution based on AFM imaging. Monosaccharide composition and PACE fingerprints was significantly different after steaming, i.e., galactose increased while glucose and mannose decreased, and β-1,4-Gal*p* appeared while β-1,4-man*p* increased after steaming. Steamed PCP significantly increased scavenging activity against ABTS radicals, while PCP0 had better immunostimulatory effect on RAW 264.7 in terms of NO production and phagocytosis.

Conclusions: In summary, steam significantly affected the chemical composition and bioactivities of polysaccharides from *P. cyrtonema*. Considering the conflict results of steaming on antioxidant and immunopotentiation activities of PCP, 2 times of continuously steam is the optimal choice under the modern process condition.

Introduction

Polygonatum cyrtonema Hua is one of origins of Polygonata Rhizoma (*HuangJing* in Chinese), which has a long history of use in China as a tonic. It has high medicinal and edible value in medicine and food, and is beneficial to lowering blood sugar [1], blood lipids [2] and immune potentiation [3]. Polysaccharides are considered as its major active components [4], which showed the effects of anti-oxidation [2, 5–7], anti-aging [8] and immunomodulation [9–14]. According to the theory of traditional Chinese medicine, the processing of Chinese raw materials is usually essential which can improve the efficacy or reduce the toxicity of crude drugs [15]. According to *Ben Cao Gang Mu*, *HuangJing* should be processed with “nine steaming and nine drying” (*Jiu-Zheng-Jiu-shai* in Chinese), which means repeatedly steaming and drying until the herb turns black inside before being used as medicine. *Yi Lin Zhuan Yao*, a medical book published in Qing dynasty, recorded that *HuangJing* had the adverse effect of "stinging the throat". The process of “nine steaming and nine drying” was always used to eliminate or alleviate the adverse reactions, reduce irritation to throat, and improve its efficacy.

However, there had no standardization of steaming of *HuangJing*. In ancient China, people believed the number of steaming times is judged based on its color and shape.

Recently, small molecular components in *P. sibiricum* (another plant origin of *HuangJing*), including aldehydes and alkanes decreased and ketones, nitrogen heterocycles increased during steaming process [16]. Though effect of steam on compositional monosaccharides, molecular weights, antioxidant and immunomodulatory activities of polysaccharides from *HuangJing* has been investigated [17–20], comprehensive study for effects of steam on polysaccharide from *Polygonatum cyrtonema* based on saccharide mapping, a powerful method developed for polysaccharides analysis, and pharmacological activity are still necessary.

Consider to the complexity of polysaccharides, the establishment of simple and fast qualitative and quantitative methods with good accuracy and high specificity has been the key and bottleneck for the quality control of polysaccharides from traditional Chinese medicines. The analytical methods of polysaccharides, including conventional quantitative methods (i.e., colorimetric assays) [21] or recent qualitative methods (i.e., HPLC, GC, FT-IR) [22, 23], show low specificity and poor accuracy for quantification or barely revealed limited structural information. In view of this, our research group proposed the strategy of saccharide mapping for qualitative analysis and quantitative detection of polysaccharides [24, 25]. For this method, high performance size exclusion chromatography coupled with multi angle laser light scattering and refractive index detection (HPSEC-MALLS-RID) as one of the most powerful techniques can quickly and accurately determine the content and relative molecular weight of natural polysaccharides and their different components based on their universal refractive index increment (dn/dc) [25]. In addition, saccharides mapping based on polysaccharide analysis using carbohydrate gel-electrophoresis (PACE) is simple, reproducible, high resolution and high throughput. It has been proved to be one of the most effective methods for the quality control of natural resources polysaccharides [24, 26, 27].

In this study, six polysaccharides were extracted and obtained from *P. cyrtonema* consecutively steamed for 0 to 5 times, respectively, and their molecular weights and distributions, UV absorption, saccharide mapping based on polysaccharide analysis by using carbohydrate gel electrophoresis (PACE) and HPTLC, as well as antioxidant and immunostimulatory activities were investigated and compared.

Materials And Methods

Materials and chemicals

Fresh cultivated rhizomes of *P. cyrtonema* were collected from Jinzhai Senfeng Agricultural Technology Development Co., Ltd., Anhui, China. After removing fibrous root, the rhizomes of *P. cyrtonema* were cleaned, dried and then cut into thin slices (3 mm ± 1 mm). Species identification was performed by Professor SP Li, one of corresponding authors. Voucher specimens of these samples were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China.

ABTS, potassium persulfate and ascorbic acid (≥ 99%) were purchased from International Laboratory (San Bruno, CA), Fluka (Selzer, Germany) and Aladdin (Shanghai, China), respectively. Polygalacturonic acid (PGA), galacturonic acid (GA), konjac glucomannan (KG), dextran (DEX), pectinase (EC 3.2.1.15), endo-1,4-β-D-mannanase (EC 3.2.1.78) and endo-1,4-β-D-galactanase (EC 3.2.1.89) were purchased from Megazyme (Wicklow, Ireland). 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Griess reagent, lipopolysaccharides (LPS) and fluorescein isothiocyanate-dextran (FITC-Dextran) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Endotoxin detection specific limulus test kit was purchased from Bioendo TEShnology (Xiamen, China). Cell counting kit 8 (CCK8) was purchased from MedChemExpress. Phosphate-buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin (P/S) were purchased from Gibco-Invitrogen (Paisley, Scotland, UK). Nylon membrane filters (0.22/0.45 μm) were purchased from Millipore (Billerica, MA). Deionized water was prepared using a Millipore MilliQ-Plus system (Millipore, Billerica, MA). All the other reagents were of analytical grade.

Preparation of P. cyrtonema **and polysaccharides**

Steam of P. cyrtonema

P. cyrtonema slices were steamed in an autoclave at 115 ℃ for 2 h (0.07 MPa) each time, followed by vacuum drying to be collected as the sample named as PC1. Afterwards, PC1 was further steamed and dried under the same condition to obtain the second sample named as PC2. Similarly, PC3, PC4 and PC5 were prepared, respectively. All those samples included the raw material without steaming (PC0) were grounded and sieved.

Extraction of the polysaccharides

Each sample (PC0-PC5) of 50.0 g was soaked at room temperature for 2 h, followed by hot water extraction (95 ºC) for further 2 h, material-to-liquid ratio was 1:15. The water extracts were precipitated with ethanol at final concentration of 75% to harvest polysaccharides. The supernatant (PCS) and precipitate were collected. Then small molecular weight substances (less than 3 KDa) were removed after ultrafiltration. The polysaccharides were dried with freeze-drier to obtain PCP0, PCP1, PCP2, PCP3, PCP4 and PCP5, respectively.

Sample pretreatment of PCP

Partial and complete acid hydrolysis of PCP

According to a previously reported method with minor modification [28], PCP solution (2 mg/mL) was treated with trifluoroacetic acid (TFA) at a final concentration of 1.0 mol/L and incubated at 80°C for 2 h to gather partial acid hydrolysates (PAH). At the same time, the PCP solution (4 mg/mL) of each sample was mixed with equal volume of 4 mol/L TFA for complete acid hydrolysis at 105 ºC for 4 h to gather complete acid hydrolysates (CAH).

Enzymatic hydrolysis of PCP

Three enzymes of pectinase, β-1,4-galactanase and β-1,4-mannanase were selected to depolymerize PCP (2 mg/mL) at 40°C for 12 h. After incubation, enzymes were inactivated at 80°C for 20 min. As for PACE analysis, the enzymatic PCP hydrolysates should be freeze-dried and derivatized with ANTS. While no additional treatment is required for HPTLC and monosaccharides analysis. Polysaccharide standards including PGA, GA and KG were treated with those enzymes, respectively, under the same conditions. PCP solution without TFA or enzymes treatment was used as blank control.

Physicochemical characterization of polysaccharides

Molecular weights and chain conformation analysis

The molecular weights and their distribution of PCP were determined by HPSEC-MALLS-RID according to our previous report [29]. HPSEC-MALLS-RID detection method is composed of multi-angle light scattering (MALS) detectors (Wyatt Technology Co., Santa Barbara, CA, USA), Agilent 1260 series LC/DAD system (Agilent Technologies, Palo Alto, CA, USA) and a refractometer (RID, Optilab rEX, Wyatt Technology Co.) in series at 35 ºC. The chromatographic columns are TOSOH gel columns TSK-GEL G5000PWXL (300 mm × 7.8 mm) and TSK-GEL G3000PWXL (300 mm × 7.8 mm), and the mobile phase is 0.9% NaCl solution. The flow rate is 0.5 mL/min with 100 μL injection volume. All the PCP were dissolved in mobile phase at 2 mg/mL and filtered through 0.45 μm filter membrane before injection. ASTRA 7.3.2 software was used to process the data.

Compositional monosaccharides analysis

The complete acid and pectinase hydrolyzed samples as well as PCS were analyzed by HPAEC-PAD system (Thermo Scientific™ Dionex™ ICS-5000^{+}, Dionex, USA). Ten monosaccharide standards including Fuc, Ara, Rha, Gal, Glc, Xyl, Man, Fru, GalA and GlcA were used to calculate the content of each monosaccharide in the samples. All the samples were filtered through 0.45 μm membrane before analysis. The mobile phase consisted of 88% deionized water and 12% 10mM NaOH, running for 22 minutes at a flow rate of 0.4mL/min under a CarboPac PA200 (3 mm × 250 mm) analytical column with a system temperature of 25°C (Supplementary Table 1).

PACE analysis of partial acid and enzymatic hydrolysates

PACE was performed according to previous report [30]. Briefly, all lyophilized derivatized hydrolysates of PCP were redissolved in isovolumetric urea (6 mol/L), and separated by Mini-Protean Tetra System, a vertical slab gel electrophoresis apparatus from Bio-Rad. Gels were imaged using an In-Genius LHR CCD camera system (Syngene, Cambridge, UK) under UV 365 nm. Quantity-One software (Ver4.6.2, BioRad) and Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Matlab version, Ver1.315, developed by the Research Center of Modernization of Chinese Herbal Medicine, Central South University and the Hong Kong Polytechnic University) were used for similarity analysis.

HPTLC analysis of complete acid and pectinase hydrolysates

Merck silica gel 60 plates pre-washed with methanol were used for HPTLC analysis. The method was modified according to previous report [30]. In brief, the PCP0-PCP5 samples after complete acid and pectinase hydrolysis were prepared into 8 mg/mL, respectively. SP-III electric thin-layer strip sampler (KEZHE SHANGHAI, China) was used for semi-automatic sampling. The bands were 7 mm wide, 5 mm distance, and 10 mm from the bottom edge. Then the plate was firstly developed to 90 mm with 1-butanol/isopropanol/acedic acid/water, 7:5:1:2 (v/v/v/v) as developing reagent at room temperature. Then the plate was dried and placed in the same chamber to develop 95 mm with the same developing reagent as described above. Finally, the developed plates were dried and colorized with aniline-diphenylamine-phosphoric acid solution, 10% sulfuric acid ethanol solution and 0.2% ninhydrin solution, respectively, then heated at 105 ºC and photographed under white light.

AFM analysis

PCP0-PCP5 (1 mg/mL) were fully dissolved in ultrapure water and diluted to a concentration of 1×10^{-2} μg/mL. Using droplet deposition method, pipette 5μL of solution onto the surface of newly cut mica sheet and dry it at room temperature. After the sample was dry, used BioScope Resolve AFM (Bruker Co., Santa Barbara, USA) for AFM measurement. NanoscopeAnalysis 1.8 software was used for image analysis.

Antioxidation of PCP against ABTS radicals

In brief, 7 mmol/L ABTS aqueous solution and 2.5 mmol/L potassium persulfate aqueous solution were mixed in a ratio of 1:1, and then stand in the dark for 12 hours. This solution was diluted with deionized water to reach a 0.7 ± 0.05 absorbance value at 734 nm and obtained the ABTS working solution. PCP0-PCP5 solution (0.5, 1, 2, 4, 8 mg/mL) and ascorbic acid (0.015, 0.03, 0.06, 0.12, 0.24, 0.5, 1, 2, 4, 8 mg/mL) were prepared. In a 96-well plate, 200 μL of ABTS working solution and 10 μL of the sample solution were added to each well, and the reaction was kept in the dark for 6 min. After the reaction, the absorbance was measured, and the ABTS clearance rate formula was as followed:

$$C\ (\%) = [1-(A_1-A_2)/A_0] \times 100$$

C was the clearance rate, A_0 is the control absorbance, A_1 is the sample absorbance, and A_2 was the background absorbance, which was to eliminate the interference of tested solution.

Effects of PCP on macrophage functions

Cell culture

RAW 264.7 cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in Dulbecco's modified eagle medium supplemented with 10% FBS, 1% P/S at 37°C in a humidified atmosphere of 5% CO_2.

Cytotoxicity assay

RAW 264.7 cells (5×10^3 cells/well) were cultured in 96-well microplates overnight, and then treated with LPS (0.4 μg/mL) and a series of concentrations of PCP for 24 h, respectively. Equal volume of culture medium was used as blank control. Subsequently, the original culture medium was discarded and stained with 100 μL of culture medium containing 10% CCK8 for 4 h in dark. The absorbance values were read at 450 nm and the cell viability was calculated as the ratio of absorbance values between sample and vehicle control group.

Nitric oxide determination

RAW 264.7 cells (5×10^4 cells/well) were seeded in 96-well microplates overnight, and then cells were treated with a series of concentrations of PCP and LPS (0.4 μg/mL) for 24 h, respectively. Equal volume of culture medium was used as vehicle control. Subsequently, 75 μL of supernatants were collected and mixed with an equal volume of modified Griess reagent at room temperature for 15 min. The absorbance was measured at 540 nm. NO production was expressed as ratio of absorbance values between sample and LPS treated group.

Phagocytic activity test

FITC-dextran was used for phagocytic assay. RAW 264.7 cells (1×10^5 cells/well) were cultured in 24-well plates overnight and then incubated with culture medium, LPS (0.4 μg/mL) and a series of concentrations of PCP for 18 h, respectively. Then the cells were treated with FITC-dextran (0.1 mg/mL in culture medium) and incubated at 37°C for additional 1 h in dark. After incubation, the cells were collected with cold PBS after washed for three times. BD Accuri™ C6 Cytometer (BD Biosciences, San Jose, CA, USA) was used to analyze. The percentage of phagocytosis was expressed as ratio of phagocytic rate between treatment and control cells.

Determination of endotoxin contamination

Endotoxin detection specific limulus test kit was used for avoiding endotoxin contamination. The results showed that PCP0-PCP5 had no endotoxin contamination in the samples.

Statistical analysis

GraphPad Prism 8.0.2 was used to analyze and process the data. Data were presented as mean ± SEM from at least three independent experiments for each sample. Statistical significance between the experimental groups was determined by Student's t-test, and *p* values less than 0.05 were considered as statistically significant.

Results And Discussion

Appearance of P. cyrtonema and its polysaccharides

After steam treatment, the color of *P. cyrtonema* slices deepened gradually. The raw material (PC0) was milky white, after first steam the color turned reddish brown with lighter color in center (PC1). Then, for the second time of steam treatment, slice PC2 changed to dark brown. Slice PC3 changed into black and began to appear luster, the color of PC4 and PC5 almost no change. The color of the polysaccharide powders was also changed accordingly, similar to the raw materials (Fig. 1). Traditionally, the number of steam treatment according to the color of materials. The color of PC5, same to those of PC3 and PC4, was black. Therefore, 5 times were selected for the steam of *P. cyrtonema*.

Molecular weight distribution of PCP

HPSEC-MALLS-RID is a useful method for determining the absolute molecular weights (Mw), the dispersibility index (DPI) and radius of gyrations (Rg) of polysaccharides without standards [31]. Figure 2 showed that HPSEC-MALLS-RID chromatograms of PCP in 0.9% NaCl aqueous solution at 35°C, and molecular mass distribution results of PCP0-PCP5 were summarized in Table 1. To facilitate the comparison of the effect of steaming treatment on the molecular weight distribution of PCP, the same peak division was performed on PCP1-PCP5 according to that of PCP0, as shown in Fig. 2A, which was divided into three peaks. In PCP0, Peak3 had the highest content and a molecular weight of ~ 7.85×10^3 Da, with Mw/Mn of 1.16. After the first steaming treatment, Mw of PCP1 increased dramatically to 1.01×10^7 Da, and gradually decreased with the increase of steaming times (Fig. 2B). This indicated that steam could change the extraction and/or molecular weight distribution of PCP. In addition, the detection at UV 280 nm showed that PCP0 had a very low signal, but steamed PCP showed obviously high signals. With the increase of steaming times, the peak height of protein signal increased firstly, PCP2 to the highest, and then decreased gradually (Fig. 2D). This implied that steaming treatment induced Maillard reaction of polysaccharides in *P. cyrtonema*. And it gradually degraded since the third steaming treatment.

Table 1
The molecular weight, polydispersity index (Mw/Mn) and contents of PCP0-PCP5

Sample	Peak1				Peak2				Peak3				Tota Con
	Mw, kDa (error%)	Mw/Mn (error%)	Rz, nm	Content%	Mw, kDa (error%)	Mw/Mn (error%)	Rz, nm	Content%	Mw, kDa (error%)	Mw/Mn (error%)	Rz, nm	Content%	%
PCP0	1.08×10^4(± 2.8%)	1.43(± 5.1%)	56.7 (± 4.2%)	0.1	70.4 (± 2.4%)	3.64(± 5.6%)	21.9 (± 18.5%)	20.7	7.85 (± 2.6%)	1.16(± 6.4%)	17.9 (± 29.3%)	49.5	70.3
PCP1	1.01×10^4(± 3.9%)	1.70(± 6.2%)	65.8 (± 4.2%)	1.4	173 (± 2.8%)	3.55(± 6.4%)	34.4 (± 9.1%)	48.6	102 (± 3.3%)	1.18(± 7.2%)	39.2 (± 8.7%)	12.5	62.5
PCP2	5.87×10^4(± 4.0%)	1.44(± 5.7%)	52.0 (± 6.4%)	0.7	381 (± 3.6%)	3.15(± 4.8%)	29.0 (± 15.1%)	54.0	146 (± 4.2%)	1.18(± 5.3%)	31.5 (± 15.3%)	23.9	78.6
PCP 3	9.28×10^4(± 4.5%)	1.88(± 5.4%)	42.3 (± 10.4%)	0.9	788 (± 3.3%)	2.15(± 5.3%)	37.2 (± 9.2%)	41.3	169 (± 3.7%)	1.18(± 6.0%)	39.7 (± 9.2%)	28.4	70.6
PCP 4	7.93×10^4(± 4.6%)	3.84(± 4.9%)	50.3 (± 7.6%)	0.5	408 (± 4.2%)	1.85(± 4.1%)	30.7 (± 16.0%)	40.3	798 (± 5.1%)	1.22(± 4.8%)	33.4 (± 16.6%)	34.5	75.3
PCP 5	2.92×10^4(± 3.8%)	1.16(± 4.0%)	44.7 (± 7.9%)	0.3	206 (± 3.6%)	2.59(± 3.5%)	23.0 (± 24.7%)	32.4	364 (± 4.5%)	1.06(± 3.5%)	22.2 (± 32.8%)	36.5	69.2

Monosaccharide composition

After complete acid hydrolysis, monosaccharide composition of PCP was determined by HPAEC-PAD (Supplementary Fig. S2). Results showed that PCP without steam treatment was mainly composed of GalA, Man and Glc. After steaming, all PCP were mainly composed of Gal, Man and GalA, with small amount of Ara, Rha and Glc. The molar ratio of Gal, GalA, Man, Rha, Ara and Glc in PCP0 was 3:6:16:1:3:8, and PCP1-PCP5 were 7.3:5:6:1:2.4:1, 7.9:3.1:4.3:1:1.6:0.6, 7.4:2.6:2.7:1:0.8:0.4, 6:2.1:2.6:1:0.6:0.5 and 5.9:1.7:2.6:1:0.3:0.5, respectively (Supplementary Table 2). Obviously, the monosaccharide composition of PCPs changed dramatically after the first steaming. The content of Man and Glc decreased significantly while the content of Gal increased obviously. As the steaming times increased, the content of Man and Glc remained stable starting from PCP3 while Gal decreased in PCP4 and PCP5. At the same time, GalA and Ara also decreased. According to previous studies [32], PCP contained mainly Man, Glc, Gal and Ara, which is consistent with our results.

Interestingly, according to the study by Li et al.[18], Glc and Ara increased with the steam treatment which was inconsistent with our research results, which might attribute to different samples and/or steaming conditions.

Besides, previous studies have shown that PCP contain a lot of fructose [33], while the traditional complete acid hydrolysis method can cause fructose loss. Therefore, we also degraded the samples with pectinase and observed changes in monosaccharide composition. The results showed that the molar ratio of Gal, GalA, Man, Rha, Ara, Glc and Fru in PCP0-PCP5 was 7.5:35:7.9:1:7.9:7.7:55.4, 11.5:16.9:2.6:1:3.8:0.6:0.8, 14:10.7:2.1:1:2.7:0.6:0.3, 11.1:7.8:1.5:1:1.1:0.6:0.2, 9.6:6.2:1.7:1:0.7:0.6:0.2 and 8.3:4.6:1.8:1:0.4:0.3:0.1, respectively (Supplementary Table 3). The result of pectinase hydrolysis was similar to that of complete acid hydrolysis, except the content of Fru which was very different. The content of Fru was decreased dramatically after the first steam treatment, and almost disappear in PCP1-PCP5. This indicated that the steam processing changes the polysaccharide structure and lose a large amount of Fru.

At the same time, we examined changes in Fru and Glc in 75% ethanol supernatant during ethanol precipitation, and the contents of Fru and Glc in the supernatant increased with steaming. The results showed that the composition of polysaccharide changed with the increase of steaming times, and the contents of monosaccharides such as fructose and glucose or oligosaccharides in free state increased. As the polysaccharide is relatively stable under the condition of normal temperature, however, under the steaming condition of high pressure and high temperature, various monosaccharide components can undergo dehydration and degradation, and Maillard reaction [34] can occur under the common existence of other components such as amino acids. Polysaccharides are composed of various monosaccharides that are degraded during processing and undergo further reaction changes that alter the monosaccharide composition of polysaccharides.

PACE profiles of PCP

Saccharide mapping based on PACE has been proven to be one of powerful methods for the routine analysis of oligosaccharides derived from polysaccharides [30]. Therefore, both partial acid hydrolysates and enzymatic hydrolysates of PCP were compared using saccharide mapping based on PACE analysis (Fig. 3A) and their similarity was conducted using Quantity-One software and Similarity Evaluation System. The HPSEC-MALLS-RID chromatogram and ion chromatogram of PCP0-PCP5 after treat with pectinase, β-1,4-Galactanase, β-1,4-Mannanase or TFA were used to detect glycoside bond variations and sugar composition to determine glycoside bonds with significant differences in the steaming process (Supplementary Figs. 3 & 4) Their hydrolysates had high similarity except the partial acid hydrolysates of PCP0 and the β-1,4-manannase hydrolysates of PCP1 and PCP2 (Table 2). In addition, the hydrolysates obtained by different hydrolysis methods showed different similarity after steaming, especially the β-1,4-galactanase hydrolysates of PCP. PACE fingerprints of pectinase hydrolysates from PCP showed that the content of GalA in PCP0 was lower than in other PCP. The fingerprints of β-1,4-galactanase hydrolysates indicated that no β-1,4-Gal*p* were detected in PCP0. However, after steaming treatment, β-1,4-Gal*p* appeared in PCP1-PCP5. In the case of β-1,4-manannase hydrolysates, it indicated that PCP contained small number of polysaccharides with β-1,4-Man*p*, and after steaming, they could be easily extracted, and the level of β-1,4-Man*p* in PCP increased. For the partial acid hydrolysates analysis, PCP0 showed an obviously different PACE fingerprints compared with other PCP. All these results indicated that steaming treatment had a huge impact on PCP.

Table 2
The correlation coefficient of PCPs to their simulative mean chromatogram.

Samples	The simulative mean chromatograms					
	PACE				HPTLC	
	SMC-PAH	SMC-GA	SMC-MA	SMC-PE	SMC-PE	SMC-CAH
PCP0	100.00	100.00	100.00	100.00	100.00	100.00
PCP1	0.75	0.00	0.81	0.96	0.76	0.51
PCP2	0.76	0.00	0.77	0.96	0.77	0.51
PCP3	0.76	0.00	0.88	0.96	0.77	0.51
PCP4	0.75	0.00	0.89	0.96	0.77	0.51
PCP5	0.72	0.00	0.88	0.96	0.76	0.50

HPTLC fingerprints of PCP

HPTLC showed the samples of complete acid hydrolysis and pectin-decomposing PCP had verified the experimental results of monosaccharide composition to some extent. According to the results of phenylamine-phosphoric acid coloration, pectin enzymatic samples contain more oligosaccharides than completely hydrolyzed samples (Fig. 3B), and according to 10% ethanol sulfuric acid colorization, the complete acid hydrolysis sample of PCP0 might contain little of small molecular substances. In other samples, bands of monosaccharides and oligosaccharides can be clearly observed. Colorization of ninhydrin coloration showed that there almost were no amino acids (Fig. 3B & 3C). HPTLC profiles similarity was shown in Table 2, which indicated that no significant difference between pectinase and complete acid hydrolysates of PCP.

Morphology of PCP

The biological activity of natural polysaccharide is also closely related to its chain conformation besides molecular weight [35]. Therefore, it is very important to study the chain conformation of PCP in aqueous solution to understand the effect of steam treatment on its structure and biological activity. The conformation of polysaccharide can be analyzed according to the theory of dilute polymer solution. Generally, the chain conformation of polysaccharides in

aqueous solution is determined by the double logarithmic plot of Rg *vs* the molecular mass of polysaccharides according to Mark-Houwink equation $Rg = kMw^{v}$ [36]. According to the polymer solution theory, the exponent (*v*) is 0.2–0.4 for branched polymers with a compact helical chain conformation, 0.3 for spheres, 0.5–0.6 for flexible polymers in good solvents and 0.6-1.0 for semi-flexible chains [37]. According to the calculation results of HPSEC-MALLS/RI, the *v* index of PCP0-PCP5 was concentrated between 0 and 0.3 (Supplementary Fig. S1). The results showed that PCP0-PCP5 appeared as irregular monodisperse spheres in 0.9% aqueous sodium chloride solution.

Atomic force microscopy (AFM) has become a powerful tool to directly characterize the structure and properties of polymers [38]. The planar images with height and diameter (scanned at 3×3 μm) of PCP0-PCP5 in aqueous solution obtained by AFM were shown in Fig. 4. An irregular monodisperse spherical shape of all PCP were observed, consistent with the results of HPSEC-MALLS-RID. Their molecular height was in the range of 1 to 1.5 nm, and the diameter was ranged from 15 to 20 nm. Specifically, with the steaming times increased, the height of PCP increased, and the diameter decreased gradually. There was almost no significant difference after the second steaming, except PCP4 with the height and diameter of 1.4 nm and 20.0 nm, respectively. This is likely to be related to the change of polysaccharides structure and/or fractions caused by steaming.

ABTS scavenge ability of PCP

ABTS scavenge ability of PCP was shown in Fig. 6A, and all PCP showed scavenge ability against ABTS radicals in different extents, and PCP0 showed the lowest capacity. Generally, steamed PCP had higher dose-dependent free radical scavenge ability, and IC_{50} values of PCP1-PCP5 were 4.89, 1.81, 1.79, 2.21, 3.04 mg/mL, respectively. Change of steamed PCP in antioxidant capacity may attribute to Maillard reaction of polysaccharides during steam processing [39], which was supported by UV 280nm absorption and molecular weights increased after steaming (Fig. 2). Though the ability of PCP scavenging ABTS radicals increased with the number of steaming times [18], IC_{50} showed that antioxidant activity of PCP3 reached to the strongest, and then decreased with the following steam treatment. The variation might be due to the different steam treatment conditions. Anyway, steaming significantly enhances antioxidant capacity of PCP, which is beneficial to its efficacy in delaying aging [8], lowering blood sugar [6, 40, 41] and regulating blood lipids [2]. The significance of steaming to health beneficial effects *P. cyrtonema* should be further well investigated.

Immunostimulatory activity of PCP

Macrophages play an indispensable role in the innate and adaptive immunity of the human body [42]. Studies have shown that high levels of NO are associated with immune responses during antitumor and antiviral processes, which can trigger cell proliferation, apoptosis, signal transduction, immune defense and other physiological processes [43]. Phagocytosis is a basic cellular process that plays an important role in the immune system [44]. In this study, RAW 264.7 cells were treated with a series of concentrations of PCP and their effects on NO production and phagocytic activity were investigated. Effects of PCP on NO production of macrophages were shown in Fig. 6C, though viability of RAW 264.7 cells was not significantly affected in the ranges of investigated concentration (Fig. 6B). Steaming reduced the effect of PCP on NO production of macrophages. As a result, effect of PCP0 was the best, while steamed PCP was reduced with increasing steam times, PCP3-PCP4 were only effective at the highest concentration (200 μg/mL), and PCP5 showed no such effect.

Flow cytometry was used to determine the fluorescence intensity in cells after RAW 264.7 cells devoured FITC-dextran. The results showed that LPS (0.4 μg/mL) and PCP could promote the phagocytic activity of macrophages in a dose-dependent manner (Fig. 6D). With the increase of steam times, their ability on phagocytosis was weakened, and after the third steam treatment (PCP3-PCP5), they showed no effect on FITC-dextran phagocytose compared with that of blank control group.

Conclusions

Steaming treatment significantly influenced the physicochemical properties and bioactivities of polysaccharides from *P. cyrtonema*, one of the origins of *HuangJing* considered as well-known tonic herb. In brief, steaming could significantly increase the molecular weights, UV absorption and antioxidant activity of polysaccharides from *P. cyrtonema*. Polysaccharides with glycosidic linkages such as β-1,4-Gal*p* and β-1,4-man*p* obviously increased after steaming, but fructan could be completely degraded. In addition, steaming could signification decrease the immunopotentiation activity, such as NO release and phagocytosis of RAW 264.7 cells of polysaccharides from *P. cyrtonema*. Considering the conflict results of steaming on antioxidant and immunopotentiation activities of PCP, 2 times of continuously steam is the optimal choice. However, further study is still necessary to well understand the beneficial effect of steaming on *HuangJing*.

Abbreviations

AFM
Atomic Force Microscopy
ABTS
potassium persulfate and ascorbic acid
ANTS
8-aminonaphthalene-1,3,6-trisulfonic acid
Ara
Arabinose
CAH
Complete Acid Hydrolysates
CCK8
Cell counting kit 8

CMM
Chinese Materia Medica
DEX
Dextran
DMEM
Dulbecco's Modified Eagle Medium
DMSO
Dimethyl Sulfoxide
dn/dc
refractive index increment
DPI
Dispersibility Index
FBS
Fetal Bovine Serum
FBS
Fetal Bovine Serum
FITC-Dextran
Fluorescein Isothiocyanate-Dextran
FT-IR
Fourier Transform Infrared Spectroscopy
Fuc
Fucose
Gal
Galactose
GA
Galacturonic Acid
GC
Gas Chromatography
Glc
Glucose
GlcA
Glucoronic Acid
HPAEC
High Performance Anion Exchange Chromatography
HPLC
High Performance Liquid Chromatography
HPSEC
High Performance Size Exclusion Chromatography
HPTLC
High Performance Thin Layer Chromatography
KG
Konjac Glucomannan
LPS
Lipopolysaccharides
MALLS
Multi-angle Laser Light Scattering
Man
Mannose
Mw
Molecular Weights
Mw/Mn
Polydispersity Index
NaOH
Sodium Hydroxide
NO
Nitric Oxide
P/S
Penicillin/Streptomycin
PACE
Carbohydrate Gel Electrophoresis

PAH
Partial Acid Hydrolysates
PBS
Phosphate-buffered Saline
PC
Polygonatum cyrtonema
PCP
Polygonatum cyrtonema polysaccharides
PCS
the 75% ethanol supernatant of *Polygonatum cyrtonema* polysaccharides
PGA
Polygalacturonic Acid
Rg
Radius of Gyration
Rha
Rhamnose
RID
Refractive Index Detector
SEC
Size Exclusion Chromatography
SEM
Standard Error of Mean
TCM
Traditional Chinese Medicine
TFA
Trifluoroacetic Acid
TLC
Thin Layer Chromatography
UV
Ultraviolet
Vc
Ascorbic acid
Xyl
Xylose.

Declarations

Ethical approval and consent to participate

This article is compliance with ethical standard and does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Availability of data and materials

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author, Shao-ping Li, upon reasonable request.

Conflicts of interest

The authors declare that they have no conflict of interest.

Funding

The research was partially funded by grants from the National Key R&D Program of China (2019YFC1711300), the Science and Technology Development Fund, Macau SAR (File no. 0017/2019/AKP), the Key-Area Research and Development Program of Guangdong Province (File no. 2020B1111110006) and the University of Macau (File no. MYRG2018-00083-ICMS /MYRG2019-00128-ICMS /CPG2021-00009-ICMS).

Author contributions

SPL and JZ designed the study and offered supervision, project administration and funding acquisition. ZRC conducted the experiments and statistical analyses, drafted the manuscript. BJZ, ZXC and WC helped carry out experiments. SPL and JQW reviewed and revised the manuscript. All authors read and

approved the final manuscript.

Acknowledgements

All herbal materials were provided by Mr. Lei Li of Jinzhai Senfeng Agricultural Technology Development Co., Ltd., Anhui, China. The research was partially funded by grants from the National Key R&D Program of China (2019YFC1711300), the Science and Technology Development Fund, Macau SAR (File no. 0017/2019/AKP), the Key-Area Research and Development Program of Guangdong Province (File no. 2020B1111110006) and the University of Macau (File no. MYRG2018-00083-ICMS /MYRG2019-00128-ICMS /CPG2021-00009-ICMS). And the authors would like to thank the reviewers and also the authors of all references.

CRediT author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Author statement

Zhe-rui Chen: Investigation, Formal analysis, Writing - Original Draft

Bao-jie Zhu: Investigation, Formal analysis

Zhi-xin Chen: Investigation

Wen Cao: Investigation

Jun-Qiao Wang: Writing - Review & Revision

Jing Zhao: Conceptualization, Resources, Writing - Review & Revision, Supervision, Project administration, Funding acquisition

Shao-ping Li: Conceptualization, Resources, Writing - Review & Revision, Supervision, Project administration, Funding acquisition

Declaration of interests

 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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

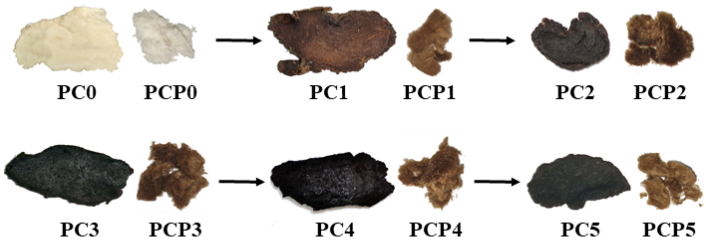
References

1. Deng Y, He K, Ye X, Chen X, Huang J, Li X, *et al*. Saponin rich fractions from *Polygonatum odoratum* (Mill.) Druce with more potential hypoglycemic effects. J Ethnopharmacol. 2012;141(1):228 – 33.
2. Li JX, Shang YX, Wang Y, Gao J, Xue N, Huang C, et al. Hypoglycemic and Hypolipidemic Activity of *Polygonatum sibiricum* Fermented with Lactobacillus brevis YM 1301 in Diabetic C57BL/6 Mice. J Med Food. 2021;24(7):720–31.
3. Chen ZB, Liu JJ, Kong X, Li H. Characterization and Immunological Activities of Polysaccharides from *Polygonatum sibiricum*. Biol Pharm Bull. 2020;43(6):959–67.
4. Cui XW, Wang SY, Cao H, Guo H, Li YJ, Xu FX, et al. A Review: The Bioactivities and Pharmacological Applications of *Polygonatum sibiricum* polysaccharides. Molecules. 2018;23(5):12. doi:10.3390/molecules23051170.
5. Wang WJ, Li S, Song MX. *Polygonatum sibiricum* polysaccharide inhibits high glucose-induced oxidative stress, inflammatory response, and apoptosis in RPE cells. J Recept Signal Transduct Res. 2021. doi:10.1080/10799893202118830 61.
6. Luo JY, Chai YY, Zhao M, Guo QQ, Bao YH. Hypoglycemic effects and modulation of gut microbiota of diabetic mice by saponin from *Polygonatum sibiricum*. Food Funct. 2020;11(5):4327–38.
7. Cai JL, Zhu YL, Zuo YJ, Tong QZ, Zhang ZG, Yang L, et al. *Polygonatum sibiricum* polysaccharide alleviates inflammatory cytokines and promotes glucose uptake in high-glucose- and high-insulin-induced 3T3-L1 adipocytes by promoting Nrf2 expression. Mol Med Rep. 2019;20(4):3951–8.
8. Zheng SY. Protective effect of *Polygonatum sibiricum* Polysaccharide on D-galactose-induced aging rats model. Sci Rep. 2020;10(1):2246. doi:10.1038/s41598020590557.
9. Wang YJ, Liu N, Xue X, Li Q, Sun DQ, Zhao ZX. Purification, structural characterization and in vivo immunoregulatory activity of a novel polysaccharide from *Polygonatum sibiricum*. Int J Biol Macromol. 2020;160:688–94.
10. Sun TT, Zhang H, Li Y, Liu Y, Dai W, Fang J, et al. Physicochemical properties and immunological activities of polysaccharides from both crude and wine-processed *Polygonatum sibiricum*. Int J Biol Macromol. 2020;143:255–64.
11. Liu ZG, Ni HY, Yu L, Xu SW, Bo RN, Qiu TX, et al. Adjuvant activities of CTAB-modified *Polygonatum sibiricum* polysaccharide cubosomes on immune responses to ovalbumin in mice. Int J Biol Macromol. 2020;148:793–801.

12. Zhang JZ, Liu N, Sun C, Sun DQ, Wang YJ. Polysaccharides from *Polygonatum sibiricum* Delar. ex Redoute induce an immune response in the RAW264.7 cell line via an NF-kappa B/MAPK pathway. RSC Adv. 2019;9(31):17988–94.
13. Yelithao K, Surayot U, Park W, Lee S, Lee DH, You S. Effect of sulfation and partial hydrolysis of polysaccharides from *Polygonatum sibiricum* on immune-enhancement. Int J Biol Macromol. 2019;122:10–8.
14. Li B, Wu PP, Fu WW, Xiong Y, Zhang L, Gao YB, et al. The Role and Mechanism of miRNA-1224 in the *Polygonatum sibiricum* Polysaccharide Regulation of Bone Marrow-Derived Macrophages to Osteoclast Differentiation. Rejuvenation Res. 2019;22(5):420–30.
15. Zhao Z, Liang Z, Chan K, Lu G, Lee EL, Chen H, et al. A unique issue in the standardization of Chinese materia medica: processing. Planta Med. 2010;76(17):1975–86.
16. Cheng X, Ji H, Cheng X, Wang D, Li T, Ren K, et al. Characterization, Classification, and Authentication of *Polygonatum sibiricum* Samples by Volatile Profiles and Flavor Properties. Molecules. 2021;27(1):18. doi:10.3390/molecules27010025.
17. Wu WJ, Huang NW, Huang JP, Wang LL, Wu LL, Wang Q, et al. Effects of the steaming process on the structural properties and immunological activities of polysaccharides from *Polygonatum cyrtonema*. J Funct Foods. 2022;88:104866.
18. Li Q, Zeng J, Gong P, Wu Y, Li H. Effect of steaming process on the structural characteristics and antioxidant activities of polysaccharides from *Polygonatum sibiricum* rhizomes. Glycoconj J. 2021;38(5):561–72.
19. Fan BL, Wei GL, Gan XF, Li TT, Qu ZY, Xu S, et al. Study on the varied content of *Polygonatum cyrtonema* polysaccharides in the processing of steaming and shining for nine times based on HPLC-MS/MS and chemometrics. Microchem J. 2020;159:105352.
20. Jin J, Lao J, Zhou RR, He W, Qin Y, Zhong C, et al. Simultaneous Identification and Dynamic Analysis of Saccharides during Steam Processing of Rhizomes of *Polygonatum cyrtonema* by HPLC-QTOF-MS/MS. Molecules. 2018;23(11):14. doi:10.3390/molecules23112855.
21. Zhang CH, Yun YH, Fan W, Liang YZ, Yu Y, Tang WX. Rapid analysis of polysaccharides contents in Glycyrrhiza by near infrared spectroscopy and chemometrics. Int J Biol Macromol. 2015;79:983–7.
22. Lv Y, Yang X, Zhao Y, Ruan Y, Yang Y, Wang Z. Separation and quantification of component monosaccharides of the tea polysaccharides from *Gynostemma pentaphyllum* by HPLC with indirect UV detection. Food Chem. 2009;112(3):742–6.
23. Xie PS, Leung AY. Understanding the traditional aspect of Chinese medicine in order to achieve meaningful quality control of Chinese materia medica. J Chromatogr A. 2009;1216(11):1933–40.
24. Guan J, Li SP. Discrimination of polysaccharides from traditional Chinese medicines using saccharide mapping-enzymatic digestion followed by chromatographic analysis. J Pharm Biomed Anal. 2010;51(3):590–8.
25. Cheong KL, Wu DT, Zhao J, Li SP. A rapid and accurate method for the quantitative estimation of natural polysaccharides and their fractions using high performance size exclusion chromatography coupled with multi-angle laser light scattering and refractive index detector. J Chromatogr A. 2015;1400:98–106.
26. Wu DT, Xie J, Hu DJ, Zhao J, Li SP. Characterization of polysaccharides from *Ganoderma* spp. using saccharide mapping. Carbohydr Polym. 2013;97(2):398–405.
27. Wu DT, Cheong KL, Wang LY, Lv GP, Ju YJ, Feng K, et al. Characterization and discrimination of polysaccharides from different species of *Cordyceps* using saccharide mapping based on PACE and HPTLC. Carbohydr Polym. 2014;103:100–9.
28. Wu DT, Meng LZ, Wang LY, Lv GP, Cheong KL, Hu DJ, et al. Chain conformation and immunomodulatory activity of a hyperbranched polysaccharide from *Cordyceps* sinensis. Carbohydr Polym. 2014;110:405–14.
29. Zhu BJ, Yan ZY, Hong L, Li SP, Zhao J. Quality evaluation of *Salvia miltiorrhiza* from different geographical origins in China based on qualitative and quantitative saccharide mapping and chemometrics. J Pharm Biomed Anal. 2020;191:113583.
30. Wu DT, Cheong KL, Deng Y, Lin PC, Wei F, Lv XJ, et al. Characterization and comparison of polysaccharides from *Lycium barbarum* in China using saccharide mapping based on PACE and HPTLC. Carbohydr Polym. 2015;134:12–9.
31. Deng Y, Li M, Chen LX, Chen XQ, Lu JH, Zhao J, et al. Chemical characterization and immunomodulatory activity of acetylated polysaccharides from *Dendrobium devonianum*. Carbohydr Polym. 2018;180:238–45.
32. Yelithao K, Surayot U, Lee JH, You S. RAW264.7 Cell Activating Glucomannans Extracted from Rhizome of *Polygonatum sibiricum*. Prev Nutr Food Sci. 2016;21(3):245–54.
33. Fan BL, Wei GL, Gan XF, Li TT, Qu ZY, Xu S, et al Study on the varied content of P*olygonatum cyrtonema* polysaccharides in the processing of steaming and shining for nine times based on HPLC-MS/MS and chemometrics. Microchemical Journal. 2020;159.
34. Ellis GP. *The Maillard Reaction*, in *Advances in Carbohydrate Chemistry*, M.L. Wolfrom, Editor. 1959, Academic Press. pp. 63–134.
35. Casu B, Lindahl U, *Structure and biological interactions of heparin and heparan sulfate*, in *Advances in Carbohydrate Chemistry and Biochemistry*. 2001, Academic Press. p. 159–206.
36. Liu W, Wang H, Yu J, Liu Y, Lu W, Chai Y, et al. Structure, chain conformation, and immunomodulatory activity of the polysaccharide purified from Bacillus Calmette Guerin formulation. Carbohydr Polym. 2016;150:149–58.
37. Wang J, Ma Z, Zhang L, Fang Y, Jiang F, Phillips GO. Structure and chain conformation of water-soluble heteropolysaccharides from *Ganoderma lucidum*. Carbohydr Polym. 2011;86(2):844–51.
38. Wang J, Nie S. Application of atomic force microscopy in microscopic analysis of polysaccharide. Trends Food Sci Technol. 2019;87:35–46.
39. Brands CMJ, Alink GM, van Boekel M, Jongen WMF. Mutagenicity of heated sugar-Casein systems: Effect of the Maillard reaction. J Agric Food Chem. 2000;48(6):2271–5.

40. Wang Y, Lan CJ, Liao X, Chen D, Song WG, Zhang QL. *Polygonatum sibiricum* polysaccharide potentially attenuates diabetic retinal injury in a diabetic rat model. J Diabetes Investig. 2019;10(4):915–24.
41. Wang Y, Qin SC, Pen GQ, Chen D, Han C, Miao CR, et al. Potential ocular protection and dynamic observation of *Polygonatum sibiricum* polysaccharide against streptozocin-induced diabetic rats' model. Exp Biol Med (Maywood). 2017;242(1):92–101.
42. Hao NB, Lü MH, Fan YH, Cao YL, Zhang ZR, Yang SM. Macrophages in tumor microenvironments and the progression of tumors. Clin Exp Immunol. 2012;2012:11.
43. Boscá L, Zeini M, Través PG, Hortelano S. Nitric oxide and cell viability in inflammatory cells: a role for NO in macrophage function and fate. Toxicology. 2005;208(2):249–58.
44. Greenberg S, Grinstein S. Phagocytosis and innate immunity. Curr Opin Immunol. 2002;14(1):136–45.

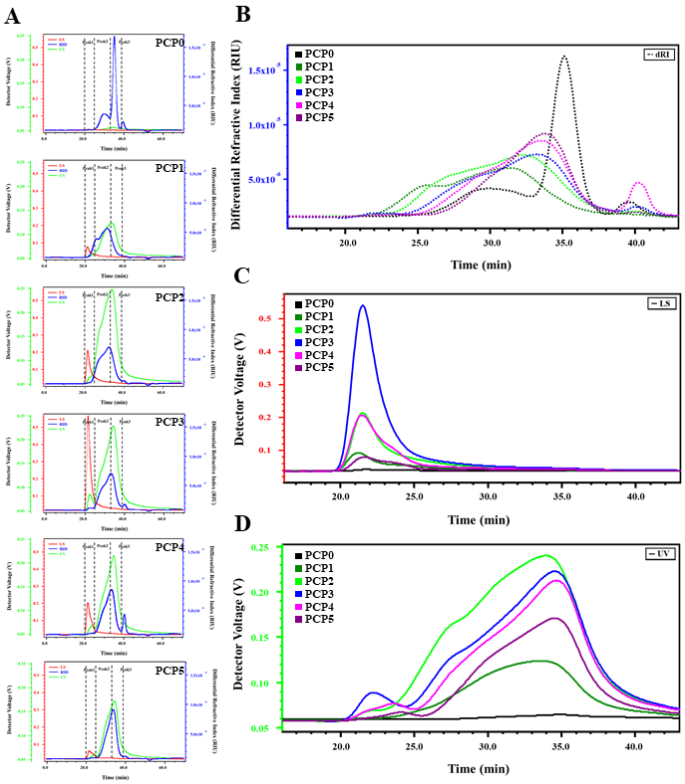
Figures



Chen, *et al*. 2022
Figure 1

Figure 1

Pictures of *Polygonatum cyrtonema* slices steamed 0-5 times and *P. cyrtonema* polysaccharide powder.



Chen, *et al*. 2022
Figure 2

Figure 2

HPSEC-MALLS-RID chromatograms (**A**), comparative chromatograms on RI (**B**), LS (**C**) and UV **(D)** chromatograms of PCP0-PCP5.

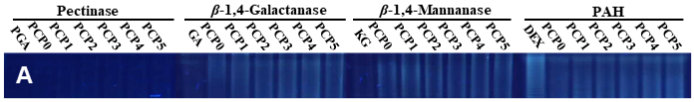
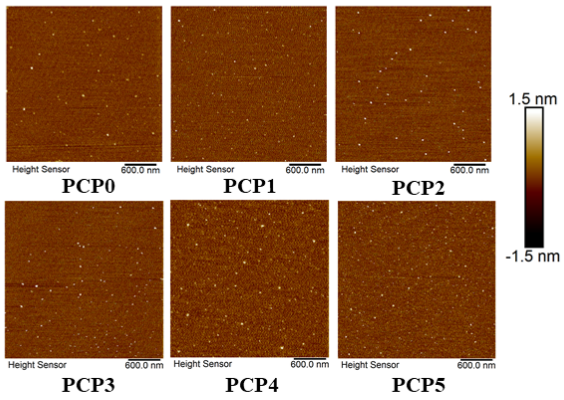


Figure 3

PACE profiles of partial acid hydrolysis (PAH) and enzymatic digestion of PCP (**A**), HPTLC profiles of complete acid hydrolysis (CAH) (**B**) and pectinase hydrolysis (**C**) of PCPs, colorized with aniline-diphenylamine-phosphoric acid solution, sulfuric acid ethanol solution and ninhydrin solution from left to right.

PGA is polygalacturonic acid, **GA** is galacturonic acid, **KG** is konjac glucomannan, **DEX** is dextran. **S1** is Rha, Man and Fru from top to bottom, **S2** is Glc, Gal and GalA from top to bottom respectively, **PE** is the blank control of pectinase.



Chen, *et al*. 2022
Figure 4

Figure 4

Planar view of 0.01 μg/mL PCP0-PCP5 observed under atomic force microscope.

Figure 5

Effects of PCP0-PCP5 on ABTS free radical scavenging activity (**A**), as well as cell viability **(B)**, NO production **(C)** and phagocytosis **(D)** of RAW 264.7 macrophages. All values were expressed as mean ± SEM of three independent experiments. * p <0.05, ** p <0.01, *** p <0.001 *vs* control group, unmarked results indicate no significant difference *vs* control group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ChenCM2022Supplementarydata.docx