



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Characterization of a pectic heteropolysaccharide isolated from persimmon peel and its anti-inflammatory activity

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ARTICLE INFO

Article history:

Received 2 September 2024

Received in revised form 30 September 2024

Accepted 1 December 2024

Keywords:

Persimmon peel polysaccharide

Inflammation

Gut microbiota

ABSTRACT

A main component, pectic polysaccharide (PPP-4), was isolated from persimmon peel. PPP-4 had the molecular weight of 56.48 kDa and primarily consisted of galacturonic acid, arabinose, galactose, rhamnose with the molar ratios of 60.80:32.12:3.67:2.20. The backbone of PPP-4 was formed by $\rightarrow 4$ - α -D-GalpA-(1 $\rightarrow$ and $\rightarrow 2,4$)- α -L-Rhap-(1 $\rightarrow$ with branches of $\rightarrow 3$)- α -L-Araf-(1 $\rightarrow$, and $\rightarrow 3$)- β -L-Galp-(1 $\rightarrow$, $\rightarrow 5$)- α -L-Araf-(1 $\rightarrow$, $\rightarrow 3,5$)- α -L-Araf-(1 $\rightarrow$ residues and terminals of 4- β -D-GalpA and T- α -L-Araf residues. Besides, PPP-4 consumption could relieve colonic pathological damage, inhibit the expression of proinflammatory cytokines (IL-1 β , IL-6, and TNF- α) in serum, increase the fecal short chain fatty acids (SCFAs) and improve antioxidant capacity in dextran sodium sulfate (DSS)-induced colitis mice. Simultaneously, PPP-4 was revealed to regulate flora disorder, reduce the relative abundance of harmful microbe (*Dubosiella*, *Odoribacter*, *Parasutterella*, and *Turicibacter*, etc.) and increase the relative abundance of beneficial microbe (such as *Akkermansia* and *Lactobacillus*) in colitis mice. Overall, these data would furnish new theoretical basis for the structural information of PPP-4 and highlight its potentiality to be considered as a natural anti-inflammation reagent.

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1. Introduction

Pectic polysaccharides, found in the primary plant cell walls, are complex 1,4-linked α -galacturonic acid (GalA)-rich heteropolysaccharides that are primarily formed by GalA, arabinose, galactose, and rhamnose. Homogalacturonan (HG) and rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) are common structural forms of pectic polysaccharides^[1]. The HG structure was composed of 1,4-linked GalA repeated units with the existence of acetyl groups at O-2 or O-3 positions or methyl esterification groups (C-6 position)^[2]. The RG-I structure contains $\rightarrow 4$ - α -D-GalpA-(1 $\rightarrow 2$)- α -L-Rhap-(1 $\rightarrow$ attached with the various residues at C-4 position of rhamnose, such as 1,4-linked galactan,

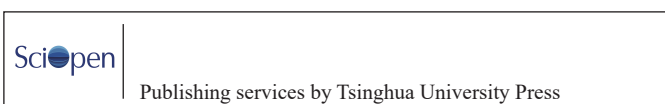
1,5-linked arabinan or other galactoarabinan^[3]. Notably, branched structure of RG-II, formed by galactose, arabinose, xylose, etc., was at C-2 or C-3 positions of 1,4-linked GalA glycosidic linkage in contrast with RG-I^[4].

Persimmon, also known as *Diospyros kaki* L., is widely grown and considered as an important cash crop in East Asian countries, such as China, Japan, and Korea. Significantly, the annual persimmon output of China ranked first referred to a report (FAO) in 2020^[5]. Persimmon was usually processed into delicious and storable persimmon/D. *kaki* cake. However, approximately 10% of the persimmon peel was generated during the production process of persimmon cake^[6]. Persimmon peel was regarded as an appropriate source of pectin, colour, tannin, and polyphenols^[7-8]. Concerning persimmon peel pectin (PPP), only methods of *Aspergillus terreus* and hot-acid were applied to the extraction of PPP^[9-10]. No further purification and corresponding structure identification of the pectic polysaccharide fraction in PPP were investigated. PPP was successfully extracted

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Peer review under responsibility of Beijing Academy of Food Sciences.



by subcritical water (SCW) in our previous study, while limited structural information and biological activity regarding the pectic polysaccharide after purification were reported.

Recently, some studies revealed that natural pectic polysaccharides from different sources, such as *Smilax china* L.^[11], *Saururus chinensis* leaves^[12], lemon, pomelo, and potato, etc., showed improvement effects on mice with ulcerative colitis (UC). Dysbiosis of intestinal floras occurred during the development of UC in mice, resulting that the increase of some harmful bacteria and decrease of several beneficial bacteria, such as decreasing of *Bacteroides* and increasing *Escherichia/Shigella*^[13]. Dietary polysaccharides was reported to reduce UC via regulating colonic microbial dysbiosis, such as *Flammuliana velutipes* polysaccharides^[14] and *Ulva prolifera* polysaccharides^[15]. Similarly, we hypothesized that pectic polysaccharide from persimmon peel could also show good protective effects on UC in mice. To our knowledge, it is the first research to simultaneously investigate the characterization of pectic polysaccharide fraction obtained from persimmon peel and its effects on acute colitis and gut microbiota *in vivo*.

The purpose of this present research was to reveal the structural features of a major component pectic polysaccharide from persimmon peel and predict its protective effects on intestinal inflammation, metabolites, gut microbe disturbance and antioxidant activity in acute colitis induced by dextran sodium sulfate (DSS). This study would provide new knowledge of structure characteristics and anti-inflammation bioactivity of pectic hetero-polysaccharide separated from persimmon peel.

2. Material and methods

2.1 Materials and experimental chemical reagents

The raw material (persimmon peel) was the waste during the processing process of dried persimmon from Fuping county (Weinan, China). DEAE-Sepharose Fast Flow column as well as Sephacryl S-300 gelfiltration column used in the preparation and verification of PPP-4 was provided by GE (Boston, USA). CH₃I, NaBH₄, DMSO and standard monosaccharides (galacturonic acid (GalA), glucose (Glc), rhamnose (Rha), glucuronic acid (GlcA), xylose (Xyl), fucose (Fuc), mannose (Man), arabinose (Ara), and galactose (Gal)) used in determination of monosaccharide composition were purchased from Sigma (St. Louis, USA). Short chain fatty acids (SCFAs, including isovaleric acid, acetic acid, isobutyric acid, propionic acid, valeric acid, and butyric acid) and deuterium oxide (D₂O; Beijing, China) used in nuclear magnetic resonance (NMR) assay were provided by Macklin Biochemical Technology Co., Ltd. (Shanghai, China). The remaining reagents used in this work were of analytical grade purity.

2.2 Purification of PPP-4

The PPP used in this part was prepared according to our previous process^[16]. A total of 450 mg PPP was subjected to stepwise elution based on DEAE-Sepharose Fast Flow column referred to our previous method^[17]. Then, the fourth component (PPP-4) with the highest content was selected for further study. Subsequently, to verify whether it was a single component, PPP-4 was further eluted by distilled water (1 mL/min) based on a gelfiltration column of Sephacryl S-300.

2.3 Characterizations of PPP-4

2.3.1 Assay of carbohydrate, soluble protein and total phenol

In this current part, the contents of total carbohydrate, soluble protein and polyphenols in PPP-4 were investigated *via* phenol-sulfuric acid method referred to previous reports^[18], Bradford method^[19], and Folin-Ciocalteu method^[20], respectively, with *D*-glucose, bovine serum albumin and gallic acid as the testing standards. In addition, the total phenol content in PPP-4 was shown as gallic acid equivalents percentage (GAE^p, mg GAE/mg PPP-4 × 100%) in this present study.

2.3.2 Detection of monosaccharide composition and molecular weight

Monosaccharide composition in PPP-4 was performed according to the reported method *via* gas chromatography (GC)^[16]. Briefly, PPP-4 was hydrolyzed with trifluoroacetic acid (TFA), reduced by NaBH₄, further derived and dissolved in dichloromethane. The GC conditions are as follows: flame ionization detector (FID) equipped with DB-17 capillary column (0.25 mm × 30.00 m × 0.25 μm); programmed temperature from 180 °C (held for 2 min) to 210 °C at 5 °C/min and 210 °C for 2 min, then to 215 °C at 0.3 °C/min and 210 °C for 20 min, then to 240 °C at 8 °C/min and keeping for 17 min at 240 °C. Carrier gas speeds: N₂ (1.5 mL/min), H₂ (60 mL/min) and air (450 mL/min). Monosaccharide standards were analyzed according to above derivatization method. In addition, the molecular weight (*M_w*) of the obtained PPP-4 was measured *via* high-performance gel permeation chromatography (HPGPC)^[17].

2.3.3 Ultraviolet (UV) and infrared spectroscopy (IR) spectroscopy assay

The UV absorption feature (200–600 nm) of PPP-4 was recorded *via* a UV-2550 ultraviolet spectrophotometer produced by Shimadzu (Tokyo, Japan) at the concentration of 0.5 mg/mL. The Fourier transform infrared spectroscopy (FTIR) spectrum of PPP-4 was detected by a Vetex 70 FTIR spectrometer provided by Bruker (Karlsruhe, Germany). In brief, PPP-4 and KBr were fully dried and mixed at the ratio of 1:100 (*ml/m*). Followed by the treatment of abrade and extrusion, the resulting thin slice was used to collect the scanning spectrum (4 000–400 cm⁻¹).

2.3.4 Crystal property and helical conformation assay

A D8 ADVANCE A25 X-ray diffractometer, produced by Bruker (Karlsruhe, Germany), was applied to investigate the crystal property of PPP-4. In brief, PPP-4 was dissolved in pure water, transferred to a plastic cell culture plate (6-holes) and subjected to fully freeze-drying treatment. The prepared PPP-4 was transferred on experimental pallet for further recording between 5° and 50°. The investigation of helical conformation was conducted by Congo red assay according to previous research^[21].

2.3.5 Determination of thermal property

The thermodynamic property of PPP-4 was revealed *via* TG/DTG and differential scanning calorimeter (DSC) assay based on a simultaneous

thermal analysis platform produced by Netzsch (STA 449F3, Selb, Germany). In brief, 5 mg PPP-4 was transferred to an aluminum crux and thermal characteristic scanning curves were obtained at the temperature from 40 to 600 °C (10 °C/min). In addition, N₂ was provided, and a vacuum aluminum furnace was considered as the control.

2.3.6 Atomic force microscope (AFM) and scanning electron microscope (SEM) analysis

The ultramicrostructure of PPP-4 was measured *via* a Multimode-8 AFM tester produced by Bruker. The prepared PPP-4 was first dissolved in pure water at the concentration of 20 µg/mL with shaking in water bath at 60 °C for 2 h. Subsequently, 10 µL solution was removed to mica sheet and naturally dried for about 12 h at room temperature. Finally, surface topography (2D/3D) of PPP-4 was observed at the mode of tapping.

After completely drying, PPP-4 was fixed on a working stage and the process of gold spraying was carried out. Finally, the surface structures of PPP-4 at magnifications of 200× and 2 000× were captured by SEM (S-3400N, Tokyo, Japan).

2.3.7 Methylation analysis

Before the treatment of methylation, uronic acid in PPP-4 was reduced by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as well as NaBD₄ (Sigma) for 3 times, referred to previous report^[22]. The sample was methylated according to the detailed methods^[17].

2.3.8 NMR analysis

A total of 40 mg of PPP-4 was dissolved in 2 mL of D₂O (99.9% D) with stirring keeping for about 4 h at room temperature, followed by the treatment of lyophilization. The above process was repeated for another 2 times. Finally, the deuterated PPP-4 was re-dissolved in 0.6 mL D₂O for further measurement of NMR spectra (1D/2D, including ¹H, ¹³C, ¹H-¹³C heteronuclear single quantum coherence (HSQC), ¹H-¹H correlation spectroscopy (COSY), ¹H-¹³C heteronuclear multiple bond coherence (HMBC), and ¹H-¹H nuclear overhauser effect spectroscopy (NOESY) spectra) based on Bruker AVANCEIII spectrometer (500 MHz, Karlsruhe, Switzerland) at the temperature of 300 K.

2.4 Design of inflammation experiment

2.4.1 Mice

In this present study, thirty male C57BL/6J mice (18–20 g) were obtained from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China). All experimental mice in this study were bred in the animal center of Northwest A&F University (Yangling, China, Animal license No. SCXK 2022-0107) and administered with a standard diet. Conditions of mice house was under the temperature of (21 ± 4) °C and 12 h light/dark circulate. In addition, all mice handling procedures were in line with all relevant ethical and moral principles and got permission from Northwest A&F University Animal Care Committee.

2.4.2 Colitis induction and PPP-4 treatment

DSS (*Mw* between 36.00 and 50.00 kDa) produced by MP Biomedicals (Santa Ana, USA), was applied to induce an acute colitis in this study. The procedures of colitis induction and PPP-4 treatment were showed as follows: after 7 days of acclimation, the 30 male animals were randomized into 3 groups, including the control group (Control), the DSS group (DSS), and DSS + 100 mg/kg PPP-4 group (PPP-4). Mice from Control and DSS groups were fed with saline and PPP-4 group was administered with 100 mg/kg PPP-4 per day for 4 weeks. Moreover, mice in DSS and PPP-4 groups were administered with 2.5% DSS (*m/V*) dissolved in drinking water during the 4th week.

The disease activity index (DAI) and body weight changes were recorded during the whole 4th week. The DAI was calculated per day according to the method in Table S1. At the 7th day of DSS treatment, all the animals were sacrificed. The processed serum, feces and colorectal tissues of all experimental mice were collected and stored in refrigerator (−80 °C). In addition, the colorectal length was recorded and partial of colorectal tissues were removed for further histological analysis.

2.5 Histopathological analysis

About 1 cm colonic tissue was removed and fixed overnight with 4% paraformaldehyde. Then, paraffin embedding and hematoxylin and eosin (H&E) staining were applied to investigate the histopathological changes of colorectal tissue. The pathological state of colorectal in mice was obtained *via* NP27 light microscopy produced by Olympus (Tokyo, Japan). Three different fields of vision (magnification of 200×) in each sample were randomly recorded.

2.6 Biochemical analysis

The serum levels of IL-1β, IL-6, IL-10, TNF-α, and lipopolysaccharides (LPS) were investigated *via* commercial ELISA kits produced by Shanghai Kexing Trading Co., Ltd. (Shanghai, China). The colonic level of glutathione peroxidase (GSH-Px), myeloperoxidase (MPO), total antioxidant capacity (T-AOC), malondialdehyde (MDA), catalase (CAT), and total superoxide dismutase (T-SOD) were investigated *via* commercial assay kits produced by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All testing procedures were conducted in line with the reference instructions.

2.7 Measurement of fecal SCFAs

SCFAs (isobutyric acid, propionic acid, isovaleric acid, valeric acid, butyric acid, and acetic acid) were extracted from mice feces and detected by GC and flame ionization detector (FID) according to previous reported method^[23]. Concisely, approximately 100 mg of feces was homogenized with 2 mL distilled water, followed by the process of centrifugation for 5 min (10 000 r/min, 4 °C). Then, 1 mL supernatant was fully mixed with 0.2 mL 50% H₂SO₄ (*V/V*) and 1 mL diethyl ether. The above mixture was incubated at 4 °C keeping for 20 min, followed by centrifugation (10 000 r/min, 4 °C, 5 min). The supernatant (organic phase) was filtrated by 0.45 µm organic system filter membrane. Finally, the levels of fecal SCFAs were

measured *via* Shimadzu GC-2014C equipped with an DB-FFAP capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 mm) produced by Agilent (Santa Clara, USA). The injection volume, injection temperature and detector temperature were 3 μ L, 250 $^{\circ}$ C and 250 $^{\circ}$ C, respectively. The carrier gas was N₂ (1.5 mL/min), H₂ (60 mL/min), and air (450 mL/min), respectively. The starting temperature (50 $^{\circ}$ C, held for 1 min) was firstly raised to 120 $^{\circ}$ C (15 $^{\circ}$ C/min), and then increased to 170 $^{\circ}$ C (5 $^{\circ}$ C/min), and finally raised to 240 $^{\circ}$ C (held for 3 min) with the rate of 15 $^{\circ}$ C/min. The same processing flow was conducted on SCFAs standards.

2.8 Gut microbiota analysis

Total bacterial DNA in mice faeces was extracted *via* the PowerSoil DNA commercial isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA). After the quality control of total DNA, the 16S rRNA gene, located in V3–V4 regions of hypervariable regions, was obtained *via* PCR amplification and then then sequenced on the Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA). The obtained raw reads in this present research were further processed by Biomarker Technologies Co, Ltd. (Beijing, China) based on BMK cloud platform (<http://www.biocloud.net/>). Effective reads were obtained after the quality filtering, double ended sequence splicing and removal of chimera. Operational taxonomic units (OTUs) were generated after cluster analysis (97% similarity) based on UPARSE (version 7.1) and then and annotated referred to SILVA database (version 123). The α - and β -diversity of gut microbiota was analyzed by QIIME2 (<https://qiime2.org/>). The biomarkers with statistically different were obtained by linear discriminant analysis coupled with effect size (LEfSe, LDA scores > 4.0).

2.9 Statistical analysis

In this study, data were expressed as means $\pm$ SD. Data were analyzed with *t* tests *via* GraphPad Prism software (8.0.1, USA). $P < 0.05$ was considered as significantly different. Correlation analysis was conducted by R software (4.2.2) using packages of psych, pheatmap and reshape2.

3. Results and discussion

3.1 Preparation, physicochemical composition and M_w determination of PPP-4

The PPP, extracted by subcritical water, was firstly obtained from persimmon peel. The obtained PPP was eluted by DEAE-Sephacrose FF ion exchange column. The fourth elution peak (PPP-4), with the highest yield ((59.55 $\pm$ 3.83)% recovery rate relative to all recovered samples), was prepared according to our previous report^[17]. The content of PPP-4 accounts for approximately 44% of PPP. To verify whether it is a single component, PPP-4 was subjected to a Sephacryl S-300 gelfiltration column taking the pure water as the eluent (Fig. 1A). As was showed in Fig. 1B, PPP-4 exhibited a signal symmetrical peak, indicating that PPP-4 was a homogeneous polysaccharide fraction. Subsequently, the M_w of PPP-4 was investigated *via* HPGPC and its result was displayed in Fig. 1B. The retention time of PPP-4 was 19.017 min and its average M_w was estimated as 56.48 kDa according to calibration curve, higher than the M_w of a galacturonic acid-rich polysaccharide from persimmon peel (PPP-2, 12.28 kDa)^[24].

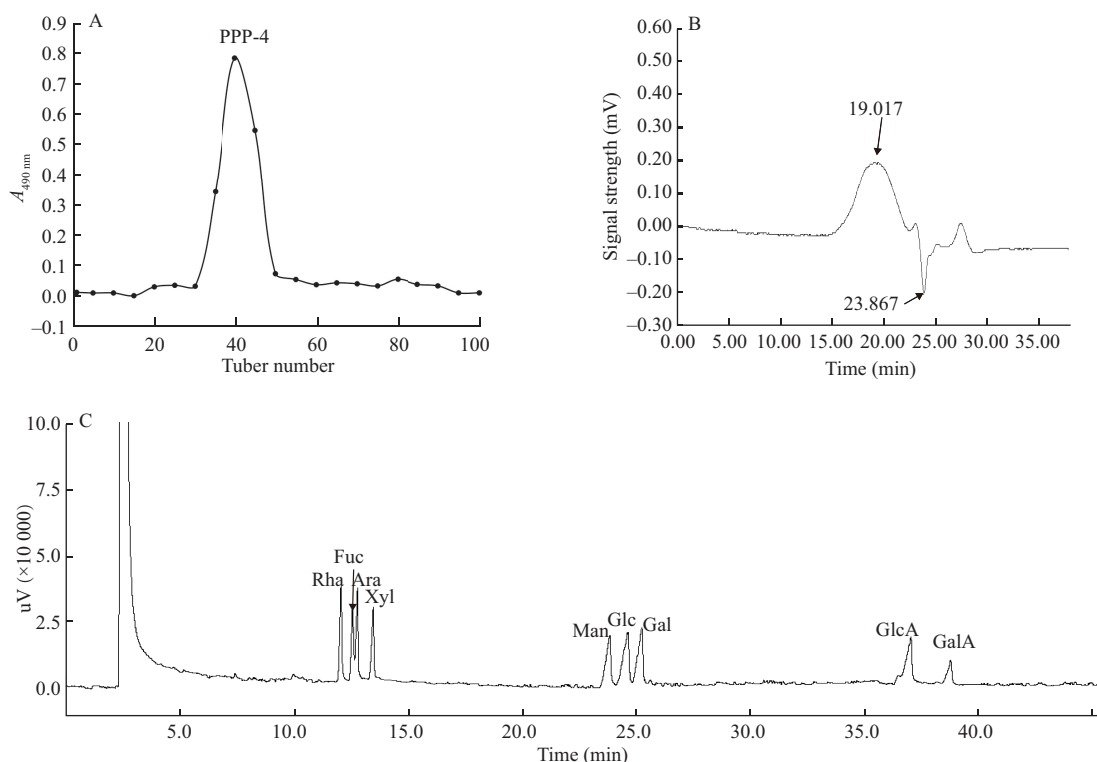


Fig. 1 The elution curve of PPP-4 on (A) Sephacryl S-300 gelfiltration column and (B) HPGPC, the determination of monosaccharide composition of (C) monosaccharide standards and (D) PPP-4.

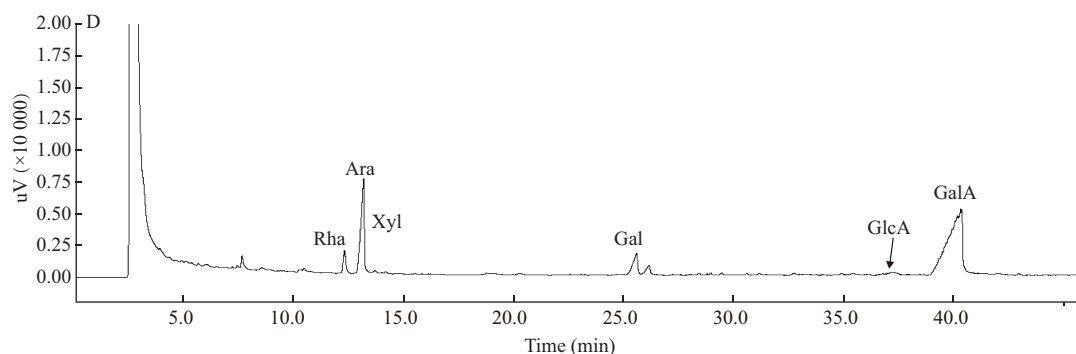


Fig. 1 (Continued)

The contents of total sugar, phenol, and soluble protein in PPP-4 were $(94.99 \pm 0.75)\%$, $(0.10 \pm 0.02)\%$ GAE^p and $(0.12 \pm 0.04)\%$, respectively, indicating that the main component of PPP-4 was carbohydrate. In addition, the monosaccharide composition assay with precolumn derivatization and the results (Figs. 1C and D) indicated that PPP-4 was formed by GalA, Ara, Gal, Rha, GlcA, and Xyl at the molar ratios of 60.80:32.12:3.67:2.20:0.84:0.37, demonstrating that PPP-4 was proved to be a typical pectic polysaccharide. The high contents of GalA or Ara were also expected in pectin samples.

3.2 The scanning spectra of UV and FTIR

As shown in Fig. 2A, no visible absorption peaks (260 and 280 nm) were observed in the UV scanning curve, demonstrating that nucleic acid and protein were absent in PPP-4, this result was in line with that of protein composition determination^[25].

As was displayed in Fig. 2B, the characteristic O–H stretching vibration absorption peak and O–H deformation vibration absorption signal of polysaccharide samples were identified at 3 404 and 2 939 cm^{-1} in the infrared scanning curves^[26]. More importantly, there were obvious absorbance signals at 1 743, 1 611, and 1 423 cm^{-1} in FTIR curve, indicating that PPP-4 was a typical acidic polysaccharide with the existence of uronic acid^[27], which was in conformity with obtained analyzing result of monosaccharide composition. The sharp peaks at 1 102 and 1 018 cm^{-1} were C–O–H and C–O characteristic absorption signals of pyranose, suggesting that pyranose was abundant in PPP-4. In addition, the obvious C–O–C symmetric

and asymmetric stretching vibration peak of *D*-pyranose at 919 and 763 cm^{-1} were also distinguished, indicating that the existence of pyranose in PPP-4. The signals at 831 and 894 cm^{-1} , revealed the presence of α -glycosidic linkage and β -glycosidic linkage in PPP-4^[28]. Taken together, PPP-4 was a typical acidic polysaccharide with the existence of α -glycosidic linkage and β -glycosidic linkage.

3.3 Congo red and XRD analysis

Fig. 2C shows the utmost light absorption wavelength of PPP-4-Congo red complex in different concentrations of NaOH solutions. In contrast with Congo red solution, there was redshift phenomenon in PPP-4-Congo red complexes. These testing result revealed that triple helical conformation occurred in obtained PPP-4, which was similar to BJP-4 (an acidic polysaccharide) obtained from blackened jujube^[29] but was different from a pectic polysaccharide named MDP1 from *Milletia dielsiana*^[30]. PPP-4 might have some specific bioactivities attributed to the existence of carboxyl groups or triple helix structure, it needs to be proved in future study^[31]. X-ray diffraction (XRD) was an effective technology to investigate the structural information about crystalline or amorphous in polysaccharides samples. In this present study, the crystal characteristic of PPP-4 was measured *via* an X-ray diffractometer at the scanning range 5° to 50° . As was showed in Fig. 2D, a strong reflection at 12.6° and shoulder peak at 27.8° revealed the existence of crystalline and amorphous portions in PPP-4, which was consist with BSF-20 from barley water-soluble fiber^[32].

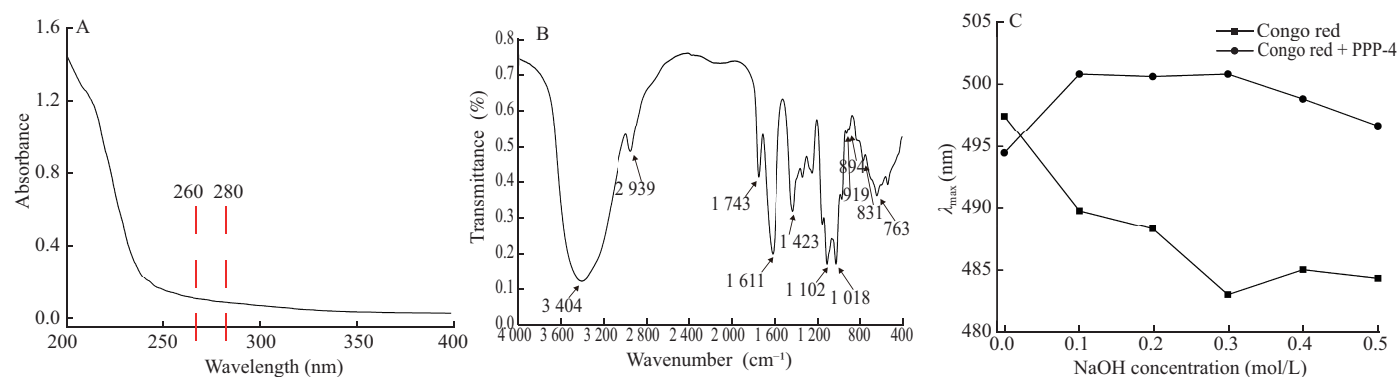


Fig. 2 The structural features of PPP-4. (A) UV curve; (B) FT-IR scanning spectrum; (C) Congo red assay; (D) XRD spectrum; (E) TG/DTG test curves and (F) DSC scanning curve.

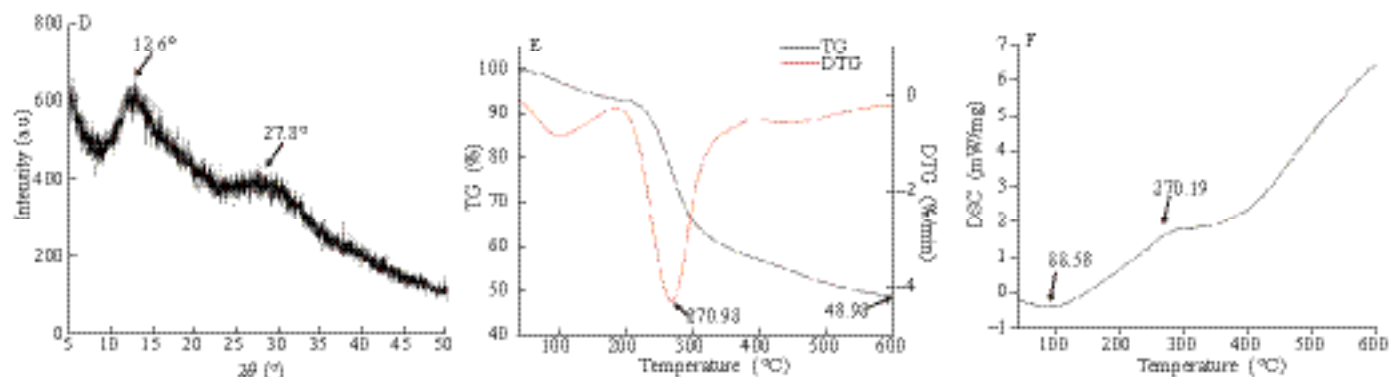


Fig. 2 (Continued)

3.4 Thermal characteristic analysis

The record results data of TG/DTG of PPP-4 was displayed in Fig. 2E, the first observed stage of weightlessness around at 106.43 °C might be attributed to moisture loss, including free water and bound water^[33]. Clearly, the observed maximum weightlessness period occurred between 200 and 400 °C, and the maximum weight loss rate of PPP-4 was appeared at 270.98 °C, which might be the result of thermal depolymerization and degradation of PPP-4^[34]. In addition, the weight loss rate of PPP-4 was up to 51.07% at 600 °C, lower than that of PPP1-1 (weight loss rate reached to 95.45%) in our previous study^[17], indicating that the thermal stability of PPP-4 was better than that of PPP1-1. DSC curve of PPP-4 was shown in Fig. 2F. There was a wide endothermic peak at 88.58 °C, demonstrating the loss of water. In addition, PPP-4 showed a weak exothermic peak appeared at 280.19 °C, indicating the degradation of polysaccharide, which was consistent with TG/DTG assay.

3.5 Micromorphology analysis

The morphologies of PPP-4 were presented in Figs. 3A and B at the magnifications of 200× and 2 000×, respectively. PPP-4 had

lamellar, synaptic structures with holes and smooth and compact structure on the surface, which were the typical morphological features of pectic polysaccharides^[35]. The thick sheet-like structure indicated a certain molecular aggregation in PPP-4. Significantly, the synaptic and layered honeycomb structures may help enhance the antioxidant activity of PPP-4. In addition, the appearance of synaptic and sheet-like special structures increases the surface area of PPP-4 to a certain extent, resulting in improved solubility characteristics.

The AFM has been used for imaging the morphology of polysaccharides and individual polysaccharide chains. Pectic polysaccharides rarely exhibit single chains, but aggregation can be observed. The AFM scanning data of planar and 3D graphics of PPP-4 are presented in Figs. 3C and D. The heights of PPP-4 ranged from −9.5 to 8.5 nm, while the height range of single-chain polysaccharide was from 0.1 to 1.0 nm^[24]. The spotted-like structures revealed the presence of molecular aggregation as well as intertwined molecules in PPP-4, which was in accordance with obtained SEM data.

3.6 Linkage analysis of PPP-4

After uronic acid reduction and methylation treatment, GC/MS analysis was used to further demonstrate glycosidic bond existed in PPP-4.

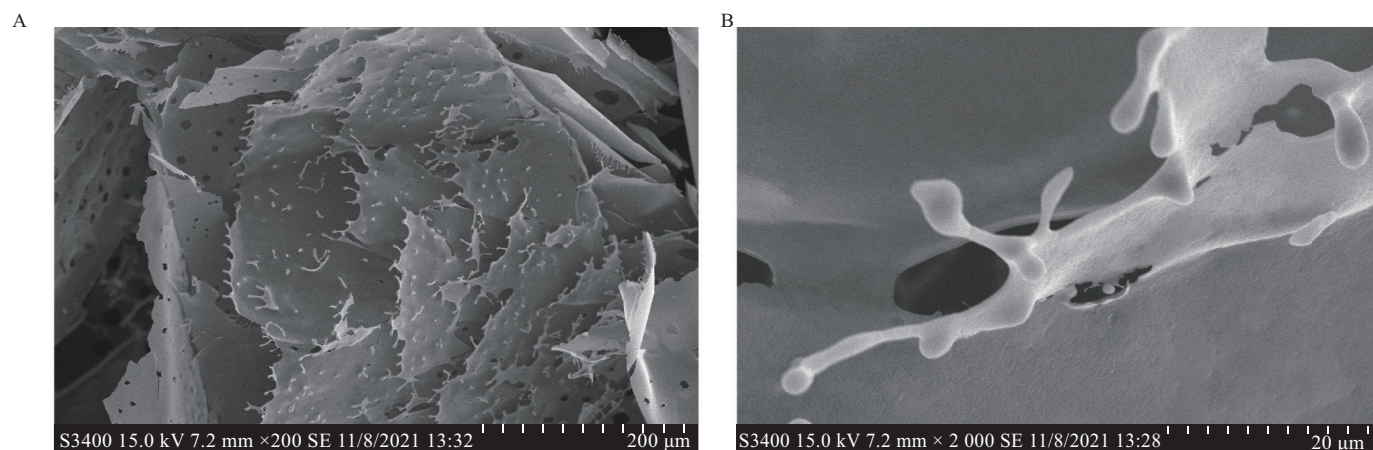


Fig. 3 SEM results of PPP-4 at magnification of (A) 200× and (B) 2 000×, and atomic force micrographs of PPP-4 at scanning of (C) planar and (D) 3D.

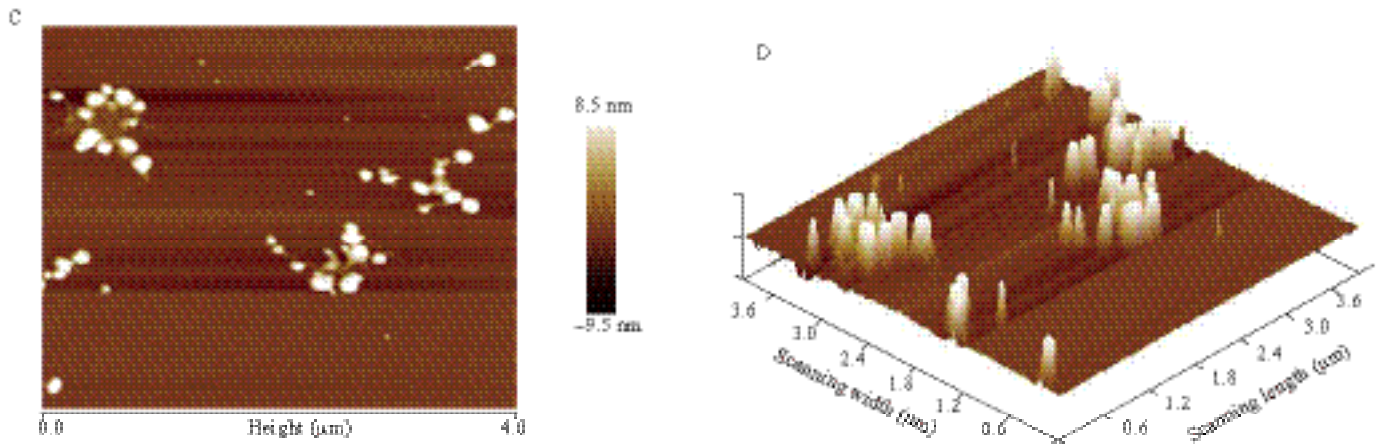


Fig. 3 (Continued)

Total ion chromatogram of partial methylated alditol acetate (PMAA) and ion fragmentation peak spectrum of PPP-4 were shown in Figs. S1 and S2. The linkage analysis of PPP-4 by GC-MS revealed 9 derivatives (Table 1), including T-*L*-Araf (1.99%), $\rightarrow$ 3)-*L*-Araf-(1 $\rightarrow$ (7.76%), $\rightarrow$ 5)-*L*-Araf-(1 $\rightarrow$ (23.22%), $\rightarrow$ 3,5)-*L*-Araf-(1 $\rightarrow$ (0.96%), $\rightarrow$ 2)-*L*-Rhap-(1 $\rightarrow$ (0.92%), $\rightarrow$ 2,4)-*L*-Rhap-(1 $\rightarrow$ (1.23%), $\rightarrow$ 3)-*D*-Galp-(1 $\rightarrow$ (3.86%), $\rightarrow$ 4)-*D*-GalpA (7.81%), and $\rightarrow$ 4)-*D*-Galp-(1 $\rightarrow$ / $\rightarrow$ 4)-*D*-GalpA-(1 $\rightarrow$ (52.25%), which was approximately in line with the testing data of monosaccharide composition. In addition, the ratio of terminal residue and branched residues is about 1:1, which was similar to the fact that terminal linkages were generally equal to the numbers of branched residues in polysaccharides samples. However, the detailed structures of PPP-4 need to be further revealed combined with NMR spectra analysis.

3.7 NMR spectra assay

The 1D and 2D NMR scanning spectra results of PPP-4 were shown in Fig. 4. The obvious signals of δ 3.83 in ^1H NMR spectrum (Fig. 4A) and δ 52.98 in ^{13}C NMR spectrum (Fig. 4B) and revealed the existence of $-\text{OCH}_3$ [36]. The signals $\delta_{\text{H}}/\delta_{\text{C}}$ at 5.11/107.54, 5.17/107.18, 5.13/107.47, and 5.28/106.96 can be assigned to $\rightarrow$ 5)- α -*L*-Araf-(1 $\rightarrow$ (A), $\rightarrow$ 3)- α -*L*-Araf-(1 $\rightarrow$ (B), $\rightarrow$ 3,5)- α -*L*-Araf-(1 $\rightarrow$ (J), and T- α -*L*-Araf (K). The signals $\delta_{\text{H}}/\delta_{\text{C}}$ at 4.67/104.55 and 5.18/99.24 belonged to $\rightarrow$ 3)- α -*D*-Galp-(1 $\rightarrow$ (C) and $\rightarrow$ 2,4)- α -*L*-Rhap-(1 $\rightarrow$ (L) [37–38].

In addition, the signals $\delta_{\text{H}}/\delta_{\text{C}}$ at 4.98/100.49, 4.94/100.24, 5.15/99.55, 5.13/99.08, 4.63/96.28 and 5.35/92.25 could belong to H-1/C-1 of $\rightarrow$ 4)- α -*D*-GalpA-6-OMe-(1 $\rightarrow$ (a) (D), $\rightarrow$ 4)- α -*D*-GalpA-6-OMe-(1 $\rightarrow$ (b) (E), $\rightarrow$ 4)- α -*D*-GalpA-(1 $\rightarrow$ (a) (F), $\rightarrow$ 4)- α -*D*-GalpA-(1 $\rightarrow$ (b) (G), $\rightarrow$ 4)- β -*D*-GalpA (H), and $\rightarrow$ 4)- α -*D*-GalpA (I) [39–43]. From the COSY and NOESY spectrum, the strong signals at δ 3.73, 4.02, 4.48 and 5.18 could be deduced to H2–H5 of D/E residues, respectively. In cross peaks of C/H in HSQC spectrum, δ_{C} at 67.94, 68.65, 79.03 and 70.57 could be assigned to C2–C5 of D/E residues, respectively. The δ_{H} at 3.76, 4.04, 4.42 and 4.75 could be assigned to H2, H3, H4, and H5 in the F/G residues in COSY spectrum, respectively. Then, the corresponding signals of C2–C5 were δ 68.01, 68.55, 78.82, and 71.58, respectively. Similarly, the $\delta_{\text{H}}/\delta_{\text{C}}$ 3.51/ 68.72, 3.75/68.57, 4.30/84.74 and 4.13/74.41 could be assigned to the H2/C2, H3/C3, H4/C4, and H5/C5 of $\rightarrow$ 1)- β -*D*-GalpA. The $\delta_{\text{H}}/\delta_{\text{C}}$ at 3.83/81.38, 3.97/82.09, 4.34/81.36, and 4.10/66.06 could be assigned to the H2/C2, H3/C3, H4/C4, and H5/C5 of $\rightarrow$ 4)- α -*D*-GalpA. In addition, the δ_{C} existed at 170.82, 170.78, 175.13, 175.07, 175.33, and 174.34 belonged to C-6 (carboxyl) of D, E, F, G, H, and I residues in ^{13}C NMR spectrum, respectively, which was in line with the observed characteristic signal at 1743 cm^{-1} in FTIR scanning spectrum. The assignments for the $^1\text{H}/^{13}\text{C}$ of other residues in PPP-4 could be further assigned by the ^1H - ^1H COSY (Fig. 4C), HSQC (Fig. 4D), NOESY (Fig. S3) spectra and results were listed in Table 2.

Table 1
GC-MS analysis of PPP-4.

Peak No.	Retention time (min)	Methylated sugar	Linkages types	Mass fragments (<i>m/z</i>)	Relative percentage ^a (%)
1	41.84	2,3,5-Me ₃ -Araf	T- <i>L</i> -Araf-(1 $\rightarrow$	43, 71, 101, 117, 129, 145, 161	1.99
2	46.08	2,3,5-Me ₃ -Araf	$\rightarrow$ 3)- <i>L</i> -Araf-(1 $\rightarrow$	43, 71, 87, 117, 129, 201	7.76
3	49.54	2,3-Me ₂ -Araf	$\rightarrow$ 5)- <i>L</i> -Araf-(1 $\rightarrow$	43, 71, 87, 117, 130, 161, 189	23.22
4	50.25	3,4-Me ₂ -Rhap	$\rightarrow$ 2)- <i>D</i> -Rhap-(1 $\rightarrow$	43, 71, 87, 101, 129, 131, 189	0.92
5	52.66	2-Me-Araf	$\rightarrow$ 3,5)- <i>L</i> -Araf-(1 $\rightarrow$	43, 58, 99, 117, 141, 159, 201, 261	0.96
6	55.50	3-Me-Rhap	$\rightarrow$ 2,4)- <i>L</i> -Rhap-(1 $\rightarrow$	43, 71, 87, 101, 129, 159, 189, 201	1.23
7	58.45	2,4,6-Me ₃ -Galp	$\rightarrow$ 3)- <i>D</i> -Galp-(1 $\rightarrow$	73, 71, 101, 117, 129, 130, 161, 203	3.86
8	61.27	1,2,3,6-Me ₄ -Galp	$\rightarrow$ 4)- <i>D</i> -GalpA	43, 71, 87, 117, 129, 161, 189, 203, 232	7.81
9	61.72	2,3,6-Me ₃ -Galp	$\rightarrow$ 4)- <i>D</i> -GalpA-(1 $\rightarrow$ / $\rightarrow$ 4)- <i>D</i> -Galp-(1 $\rightarrow$	43, 71, 99, 117, 131, 173, 187, 203, 233	52.25

Note: ^a Values of PMAAs are calculated based on the peak area.

The linkage sequence of these 12 residues (A–L) in PPP-4 was deduced referred to the scanning data of NOESY spectrum (Fig. S3) as well as the HMBC spectrum in Fig. 4E. The residual cross peaks of D,E C-1/D,E H-4 and G H-1/D,E C-4 in HMBC spectrum, as well as cross-peaks of D,E H-1/D,E H-4, F,G H-1/F,G H-4, and E,F H1/D,E H4 in NOESY spectrum were found, indicating the existence of

D/E-D/E, D/E-F/G, and F/G-F/G links in PPP-4. The correlations of A H-1/C-3, B H-1/C-3, B H-1/L C-4, F H-1/L C-2, were identified in HMBC spectrum. In addition, the cross signals of L H-1/F,G H-4 were distinguished in NOESY spectrum, indicating the presence of A-A, A-C, F-L, and L-F/G links. The correlations of F,G H-1/H,I H-4, were identified in NOESY spectrum, which confirmed the existence

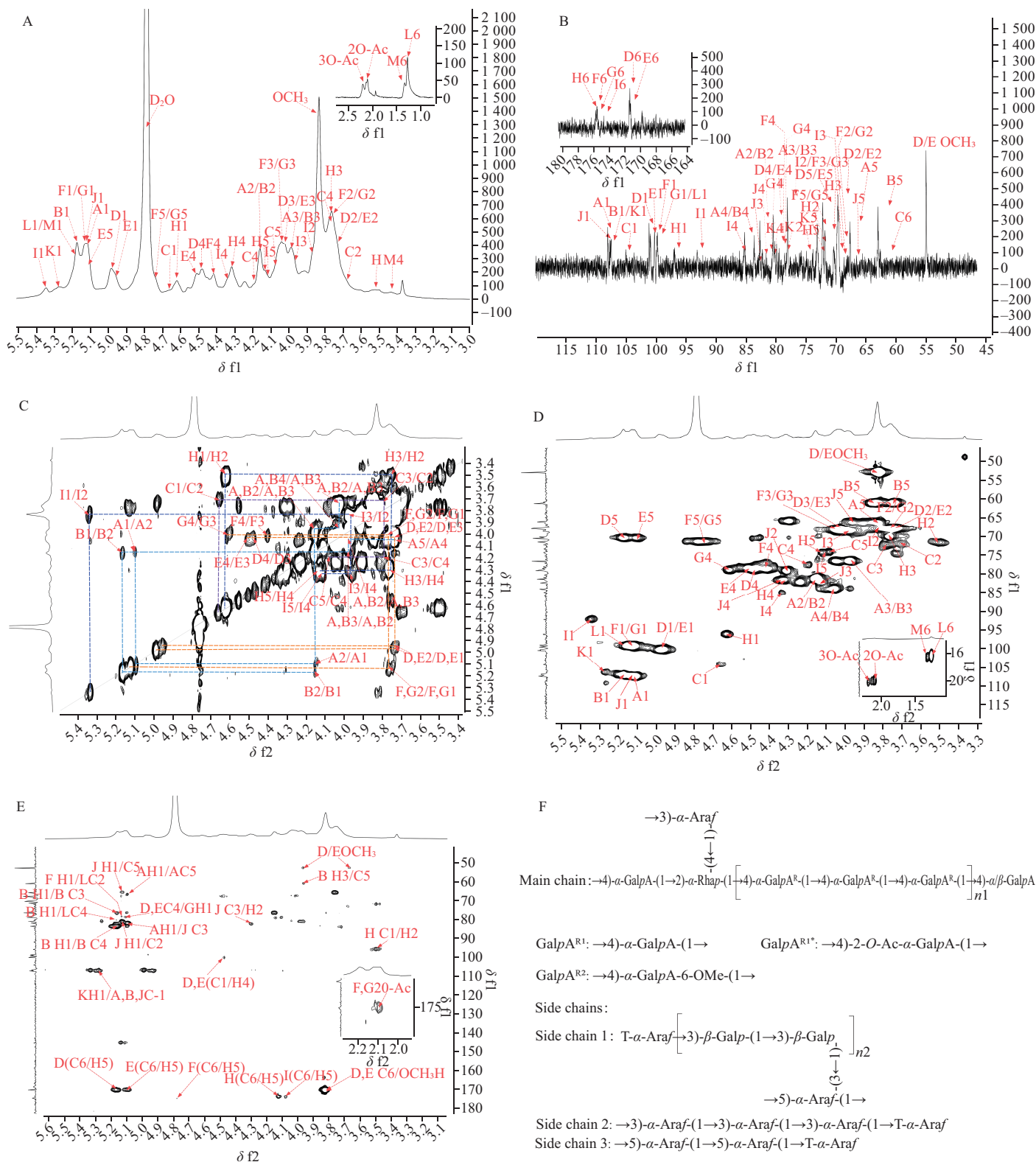


Fig. 4 NMR scanning spectra of PPP-4 dissolved in D₂O. (A) ¹H, (B) ¹³C, (C) COSY, (D) HSQC, (E) HMBC and (F) deduced structure.

Table 2Chemical shift assignments of ^{13}C and ^1H NMR for PPP-4.

Label	Residue		1	2	3	4	5	6	OCH_3
A	$\rightarrow 5)\text{-}\alpha\text{-L-Araf-}(1\rightarrow$	C	107.54	81.38	76.66	83.98	66.45		
		H	5.11	4.16	3.98	4.06	3.82		
B	$\rightarrow 3)\text{-}\alpha\text{-L-Araf-}(1\rightarrow$	C	107.18	81.25	76.66	83.98	61.21		
		H	5.17	4.16	3.98	4.06	3.97/3.85		
C	$\rightarrow 4)\text{-}\alpha\text{-Galp-}(1\rightarrow$	C	104.55	71.88	72.45	78.87	74.15	60.74	
		H	4.67	3.69	3.78	4.19	4.09	–	
D	$\rightarrow 4)\text{-}\alpha\text{-D-GalpA-6-OMe-}(1\rightarrow (\text{a})$	C	100.49	67.94	68.65	79.03	70.57	170.82	3.83
		H	4.98	3.73	4.02	4.48	5.18		52.98
E	$\rightarrow 4)\text{-}\alpha\text{-D-GalpA-6-OMe-}(1\rightarrow (\text{b})$	C	100.24	67.94	68.65	79.03	70.57	170.78	3.83
		H	4.94	3.73	4.02	4.52	5.11		52.86
F	$\rightarrow 4)\text{-}\alpha\text{-D-GalpA-}(1\rightarrow (\text{a})$	C	99.55	68.01	68.55	78.82	71.58	175.13	
		H	5.14	3.76	4.04	4.42	4.75		
G	$\rightarrow 4)\text{-}\alpha\text{-D-GalpA-}(1\rightarrow (\text{b})$	C	99.08	68.01	68.55	78.43	71.58	175.07	
		H	5.13	3.76	4.04	4.61	4.79		
H	$\rightarrow 4)\text{-}\beta\text{-D-GalpA}$	C	96.28	71.77	70.19	81.28	74.60	175.33	
		H	4.63	3.51	3.75	4.30	4.13		
I	$\rightarrow 4)\text{-}\alpha\text{-D-GalpA}$	C	92.25	68.72	68.57	84.74	74.41	174.34	
		H	5.35	3.83	3.97	4.34	4.10		
J	$\rightarrow 3,5)\text{-}\alpha\text{-L-Araf-}(1\rightarrow$	C	107.47	81.38	82.09	81.36	66.06		
		H	5.13	4.31	4.13	4.36	3.95		
K	T-Araf	C	106.96	–	–	–	–		
		H	5.28	–	–	–	–		
L	$\rightarrow 2,4)\text{-}\alpha\text{-Rhap-}(1\rightarrow$	C	99.24	77.98	68.18	80.77	71.61	–	
		H	5.18	4.00	3.81	3.66	3.80	1.27	

Note: – not detected.

of F,G-H and F,G-I links in PPP-4. Considering all above analytical data, the deduced structure of PPP-4 was shown in Fig. 4F. Taken together, the backbone of PPP-4 appeared to be mainly composed of $\rightarrow 4)\text{-}\alpha\text{-D-GalpA-6-OMe-}(1\rightarrow$, $\rightarrow 4)\text{-}\alpha\text{-D-GalpA-}(1\rightarrow$, $\rightarrow 2,4)\text{-}\alpha\text{-L-Rhap-}(1\rightarrow$, and $4\text{-}\beta\text{-D-GalpA}$ residues with some side chains consisting of $\rightarrow 5)\text{-}\alpha\text{-L-Araf-}(1\rightarrow$, $\rightarrow 3,5)\text{-}\alpha\text{-L-Araf-}(1\rightarrow$, $\rightarrow 3)\text{-}\beta\text{-D-Galp-}(1\rightarrow$, $\rightarrow 3)\text{-}\alpha\text{-L-Araf-}(1\rightarrow$ residues and terminal of T- $\alpha\text{-L-Araf}$ residue.

3.8 Effect of PPP-4 consumption on DSS-induced colitis in vivo

3.8.1 PPP-4 mitigated DSS-induced colitis in mice

In this present research, oral administration of 100 mg/kg PPP-4 was applied to investigate the effect of PPP-4 on DSS-induced colitis. The experimental groups and corresponding treatments were shown in Fig. 5A. DAI was recognized as one of the typical indicator evaluating the pathological degrees of colitis. In contrast with Control group, DSS exposure increased the DAI scores, resulting in weight loss, thin stools, diarrhea and even bloody stools, while this phenomenon was reversed by oral administration of PPP-4 (Fig. 5B). As were displayed in Figs. 5C-E, in contrast with those in Control group, body weight and colorectal length of mice in DSS group was significantly decreased. Nevertheless, consumption of PPP-4 obviously relieved body weight loss ($P < 0.001$) and colonic shortening ($P < 0.01$). Moreover, the changes of thymus and spleen were response to immune reaction and inflammation in mice. The higher the inflammation level, the larger the value of the spleen index, and the smaller the value of the thymus index^[44]. Similar to the previous study, compared with Control group, DSS challenge decreased thymus index and increased spleen index^[45]. As shown in Figs. 5F and G, oral administration of PPP-4 obviously decreased

spleen index ($P < 0.0001$) and increased thymus index ($P < 0.001$) when compared with DSS group, indicating that dietary PPP-4 alleviated the inflammatory level of colitis mice.

Subsequently, histopathologic changes were investigated via H&E staining and the corresponding histological scores were also calculated. Histologically, DSS exposure obviously injured the colon structures, resulting in collapse of crypt structure, thickening of muscular layer, deficiency of the goblet cells, damage of surface epithelium, as well as infiltration of inflammatory cells. However, the tissue damage (Fig. 5H) was ameliorated with the intervention of PPP-4. Taken together, our above data indicated that PPP-4 administration could alleviate the clinical symptoms and colorectal tissue damage induced by DSS.

3.8.2 PPP-4 regulated inflammatory reaction and microbial metabolite

The increase of pro-inflammatory cytokines (such as TNF- α , IL-6 and IL-1 β) can aggravate the severity of colitis^[46]. The imbalance of gut microbiota and collapse of gut barrier result to release of LPS^[47]. To further investigate anti-inflammation effect of PPP-4, we measured the levels of endotoxin (LPS), inflammatory cytokines (IL-6, IL-1 β , TNF- α , and IL-10) in the serum of mice and MPO activity in the colorectal tissue. Compared with the Control group, the levels of pro-inflammatory cytokines (IL-6, TNF- α , IL-1 β) and LPS markedly increased and the levels of anti-inflammatory cytokine (IL-10) level significantly decreased in serum, (Figs. 6A-E), indicating that severe inflammatory effects are produced in colitis mice. More importantly, PPP-4 could reduce the levels of LPS and proinflammatory cytokines (IL-6, IL-1 β , and TNF- α) in serum in

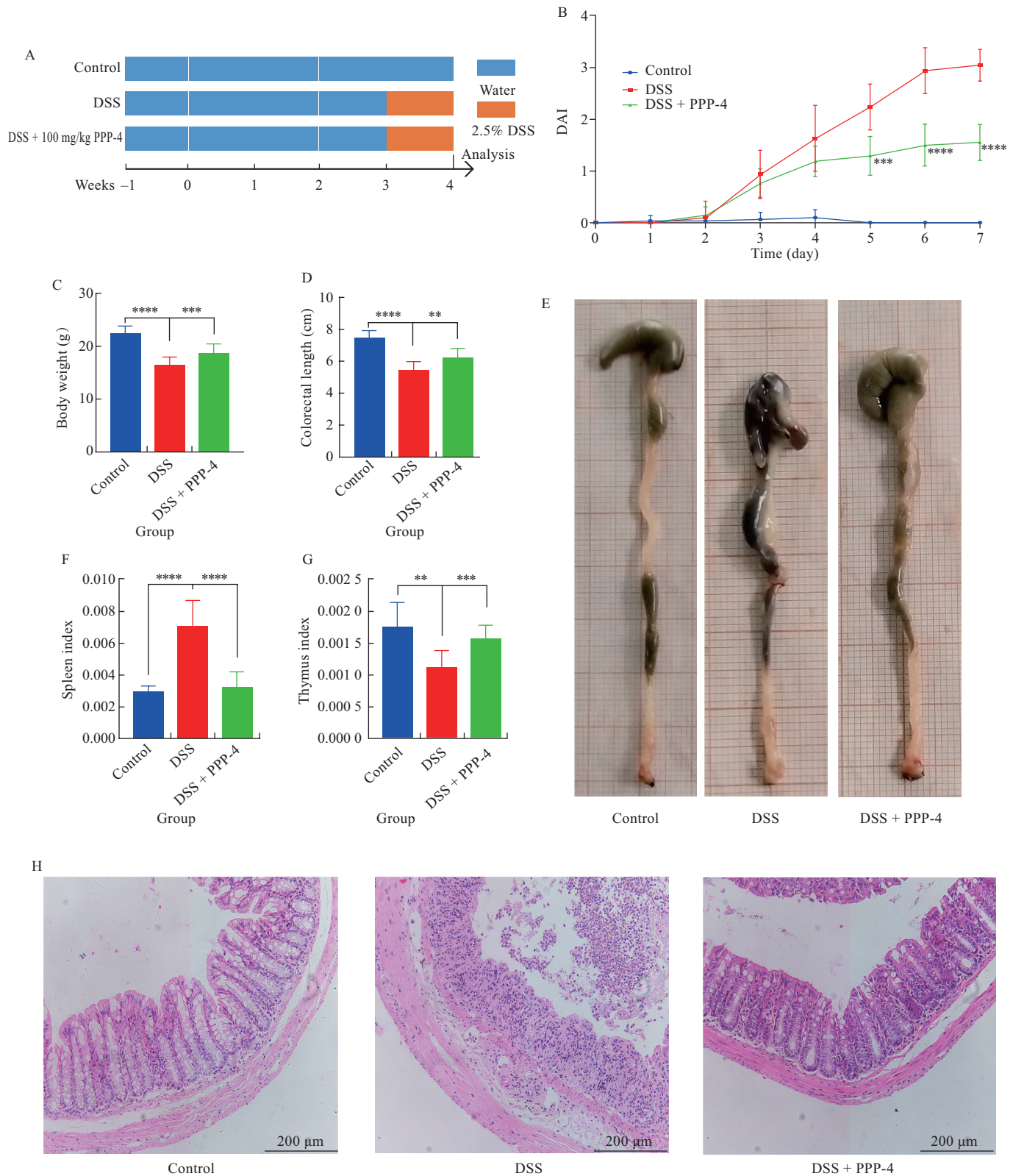


Fig. 5 Oral consumption of PPP-4 mitigated the pathological indicators of colitis induced by DSS in mice. (A) Processing flow of mice experiment, (B) DAI scores during DSS treatment, (C) body weight, (D) colorectal length, (E) representative macroscopic photos of colon, (F) spleen index, (G) thymus index, (H) H&E staining. Data were shown as the mean $\pm$ SD ($n = 6-8$). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

contrast with DSS. Meanwhile, administration of PPP-4 promoted anti-inflammatory cytokines production. The marked decrease in IL-6 production in PPP-4 group could be attributed to the side chains of arabinan in RG-I^[46]. Those obtained results suggested that PPP-4 consumption could significantly regulate the expression levels of some inflammatory cytokines.

SCFAs, a kind of valuable microbial metabolite, were reported to have the bioactivity of relieving inflammation and inhibiting microbial pathogens^[48]. As displayed in Figs. 6F and G, compared with Control group, the SCFAs expression levels (including propionic acid, isobutyric acid, acetic acid, isovaleric acid, butyric acid, and valeric acid) in feces of mice were markedly decreased after DSS exposure ($P < 0.05$). However, this situation was reversed after PPP-4

administration. Taken together, those above results indicated that dietary PPP-4 could decrease serum LPS and improve the levels of SCFAs in faeces of DSS-treated mice, which further revealed that PPP-4 could reduce inflammatory reaction and gut microbiota metabolite dysbiosis induced by DSS.

The activity of MPO could obliquely reflect the degree of inflammation and neutrophil infiltration in colonic tissue^[49]. After treatment of DSS (Fig. 6H), the level of MPO was significantly exaggerated ($P < 0.001$), while PPP-4 could significantly reduce the content of MPO ($P < 0.001$), indicating that the inflammation response in colon were relieved. The anti-inflammation of PPP-4 might be related to pectic polysaccharides rich in galactan in PPP-4^[46].

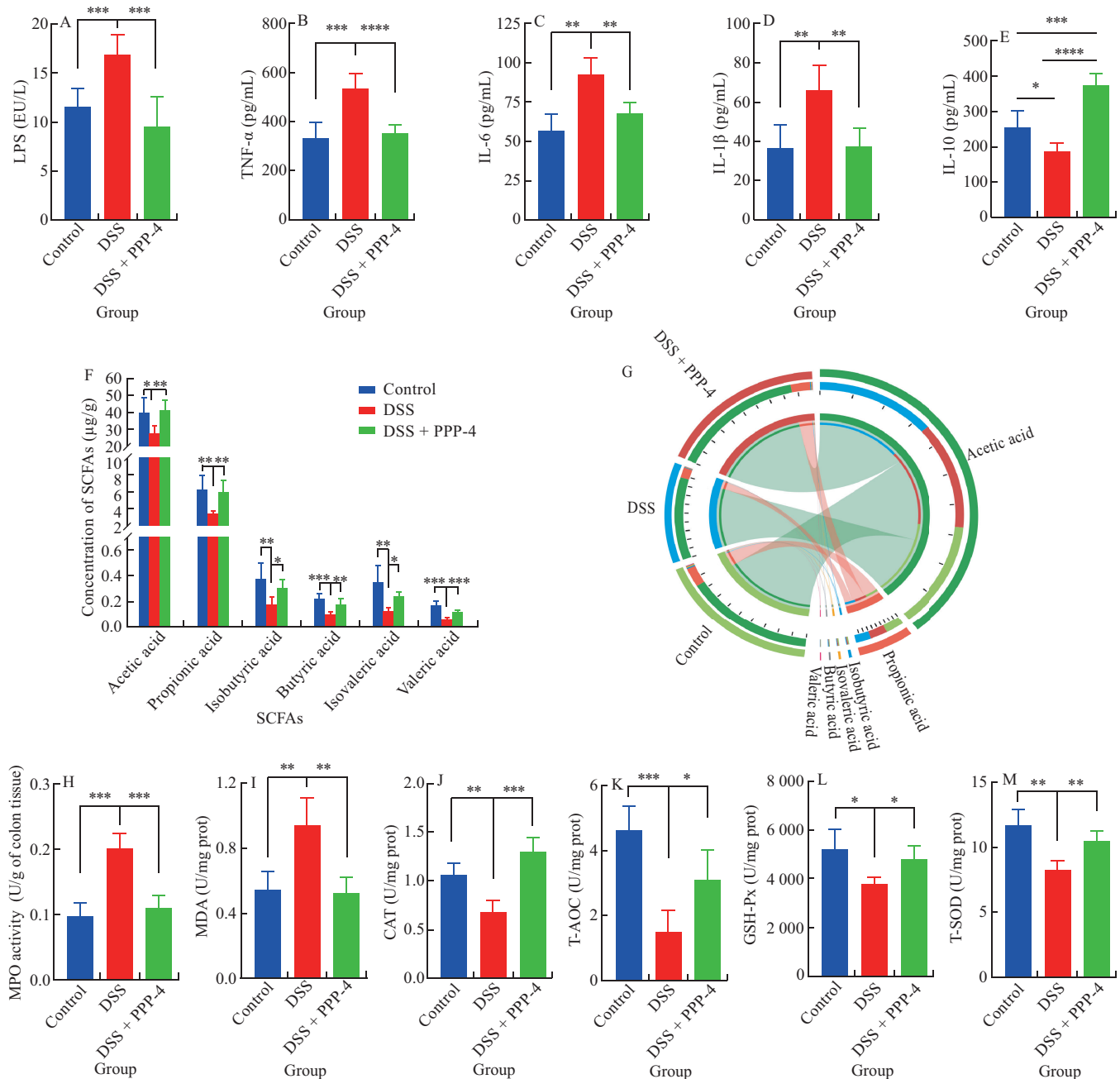


Fig. 6 The colitis-related indexes in serum, faeces and colorectal tissue. The expression levels of (A) LPS, (B) TNF-α, (C) IL-6, (D) IL-1β, and (E) IL-10 in mice serum; (F-G) SCFAs levels of feces; the expression levels of (H) MPO, (I) MDA, (J) CAT, (K) T-AOC, (L) GSH-Px, and (M) T-SOD in colorectal tissue.

Data were shown as the mean ± SD ($n = 4-6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

3.8.3 PPP-4 regulated antioxidant activity in vivo

Patients with colitis have widely increased reactive nitrogen and reactive oxygen species (ROS). Significantly, the increase of ROS may produce lipid peroxidation by-products, such as MDA, which has the risk of mutation and genotoxicity, and may promote the development of colitis^[50]. Normally, the level of MDA increased after treatment of DSS. As expected, the levels of MDA in colonic tissue significantly increased among DSS mice ($P < 0.01$), while the increasing of MDA was obviously suppressed by treatment of PPP-4, as depicted in Fig. 6I. As displayed in Figs. 6J–M, the activities of CAT, T-AOC, GSH-Px, and T-SOD in colorectal tissue were markedly reduced in the DSS group; these data were in line with a previous study^[51]. Interestingly, it can be observed that the levels of CAT, T-AOC, GSH-Px, and T-SOD in colorectal tissue were significantly improved after oral administration of PPP-4 (Figs. 6J–M). Taken together, it was suggested that PPP-4 consumption could decrease the oxidative stress in the colorectal tissues of colitis mice.

3.8.4 Administration of PPP-4 ameliorated gut microbiota

The disorder of gut microbe was occurred during the development of UC. Natural polysaccharide was beneficial to intestinal flora regulation^[52]. Consequently, we examined whether the ameliorative effects of PPP-4 are associated with modulation of gut microbiota via 16S rDNA high-throughput sequencing. Remarkably, the rarefaction curve of OTU numbers as well as shannon index based on each sample/group tended to flatten (Fig. S4), indicating that most of species were covered in current sequencing depth and the OTU numbers in Control group were higher than those in DSS and PPP-4 groups. The effects of PPP-4 on the composition and structure of gut microbes were assessed by α -diversity and β -diversity analysis. Compared with the Control group, α -diversity (Chao1, ACE, Shannon, and Simpson) indexes were markedly decreased in DSS group (Fig. 7A), which was similar to previous study^[53]. However, the diversity indexes of Chao1 and Shannon in PPP-4 group were markedly different from that in DSS group ($P < 0.01$). Likewise, the analysis of beta-diversities, including PCoA (Fig. 7B), hierarchical clustering heatmap of sample (Fig. 7C), upgma tree (Fig. 7D), cluster tree bar (Fig. S5A) basing on bray-curtis distance, and NMDS plot based on unweighted unfrac distance (Fig. S5B), revealed that gut microbiota communities were also different from each other among those 3 different experimental groups. Taken together, these above results revealed that DSS challenge significantly altered the gut microbiota in mice and PPP-4 also significantly regulated richness and diversity of gut microbiota.

The classification of mice gut microbiota of each group at the levels of phylum was shown in Fig. 7E. At phylum level, the relative abundance of Bacteroidota, Firmicutes, Verrucomicrobiota, Proteobacteria, Desulfobacterota, and Actinobacteriota covered about 98% observed bacteria. Remarkably, in contrast with Control group, the higher relative abundance of Actinobacteriota and Proteobacteria as well as the lower relative abundance of Verrucomicrobiota were found in DSS group, which revealed the disturbance of intestinal microbiota^[54], while oral administration of PPP-4 reversed those change. Moreover, DSS consumption increased the relative abundance of Firmicutes and reduced the relative abundance of Bacteroidetes,

these result was in line with previous report^[55], while PPP-4 did not show the improvement on the increasing of Firmicutes and reduce of Bacteroidetes in this study.

In addition, the top 10 bacteria in genus level were displayed in Fig. 7F. Obviously, the relative abundance of *Dubosiella*, Lachnospiraceae NK4A136 group, *Parasutterella*, and *Odoribacter* was increased, while the relative abundance of *Akkermansia* as well as *Ligilactobacillus* was decreased in DSS group in contrast with Control group. The above alterations were alleviated after the treatment of PPP-4. According to the reports, *Ligilactobacillus* and *Akkermansia* could be seen as potential probiotic candidates owing to anti-inflammatory properties^[56–57]. The relative abundance of unclassified Muribaculaceae in PPP-4 and DSS group was lower than that in Control group. By contrast, a higher relative abundance of *Bacteroides* was found in PPP-4 group, which might be attributed to the increased demand of carbohydrate metabolism^[58].

Moreover, LEfSe analysis was conducted to investigate the specific dominant bacteria among three different experimental groups. As was displayed in Figs. 7G and H, a total of 16, 11 and 12 various bacterial taxa enriched in groups of DSS, Control and PPP-4 (LDA score > 4.0), respectively. *Ligilactobacillus* and Unclassified Muribaculaceae at the genus level as well as uncultured *Bacteroidales bacterium* were at species level were the specific dominant bacteria in Control group. By contrast, the obtained relative abundance of unclassified Muribaculaceae and *Ligilactobacillus* in DSS group and PPP-4 group was lower than that in Control group, demonstrating that gut dysbiosis occurred after DSS challenge. In addition, *Dubosiella*, *Odoribacter*, *Parasutterella*, and *Turicibacter* at the genus level as well as *Burkholderiales bacterium* YL45 and unclassified *Alloprevotella* at the level of species were enriched in DSS group. The high abundance of *Dubosiella*, *Parasutterella*, *Turicibacter*, *Alloprevotella*, and *Odoribacter* in DSS group was also found in previous reports^[59–61]. Of note, *Lactobacillus* and *Akkermansia* were dominant genus bacteria in the PPP-4 group. Accordingly, *Akkermansia* was reported a SCFAs-producing bacteria and played an vital part in degradation of mucin in the gut, which were conducive to the balance of intestinal homeostasis^[51]. *Lactobacillus* is generally be regarded as a probiotic bacteria^[62]. Considering above data, we may draw the conclusion that PPP-4 could ameliorate gut microbiota disturbance via reducing the relative abundance of harmful bacteria as well as increasing the relative abundance of beneficial bacteria in mice.

3.8.5 Correlation evaluation

In this part, Pearson's correlation coefficient was applied to study the relationships between gut microbiota with significant changes at genus level in supplementary Table S2 (Control vs. DSS or DSS vs. DSS + PPP-4) and colitis indexes (general indicators, inflammation, antioxidant and SCFAs). As shown in Fig. 8, the gut microbiota was divided into 3 clusters after clustering. Obviously, most of the genera (2/9) in cluster 1 were positively correlated with the LPS, proinflammatory factors (including IL-1 β , IL-6 and TNF- α), MPO, MDA, DAI and spleen index. Simultaneously, they had negative correlations with SCFAs, antioxidant related enzymes, body weight, colorectal length, and thymus index. Considering the increasing relative abundance of genera in cluster 1, they might be considered as harmful microbe aggravating the development of colitis. In cluster 2,

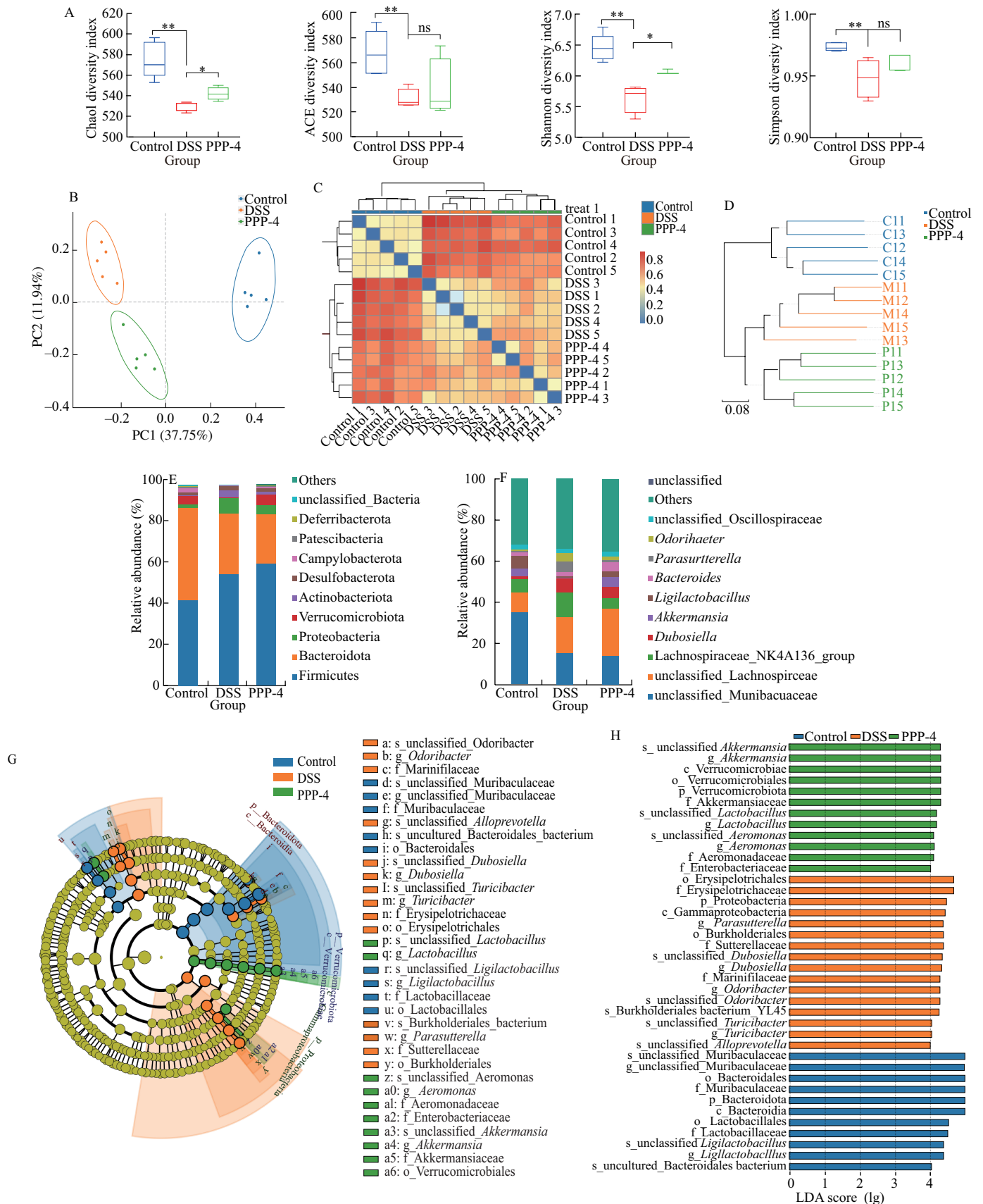


Fig. 7 PPP-4 altered gut microbiota diversity and composition. (A) α -Diversity of Chao1, ACE, Shannon, and Simpson; (B) β -diversity of PCoA plot, (C) hierarchical clustering heatmap of sample, and (D) upgma tree based on bray-curtis distance; Effects of PPP-4 consumption on intestinal microbe composition at the levels of (E) phylum and (F) genus LefSe analysis among 3 experimental groups (LDA score > 4.0). Data were expressed as the mean $\pm$ SD ($n = 5$). * $P < 0.05$, ** $P < 0.01$, ns = no significant difference.

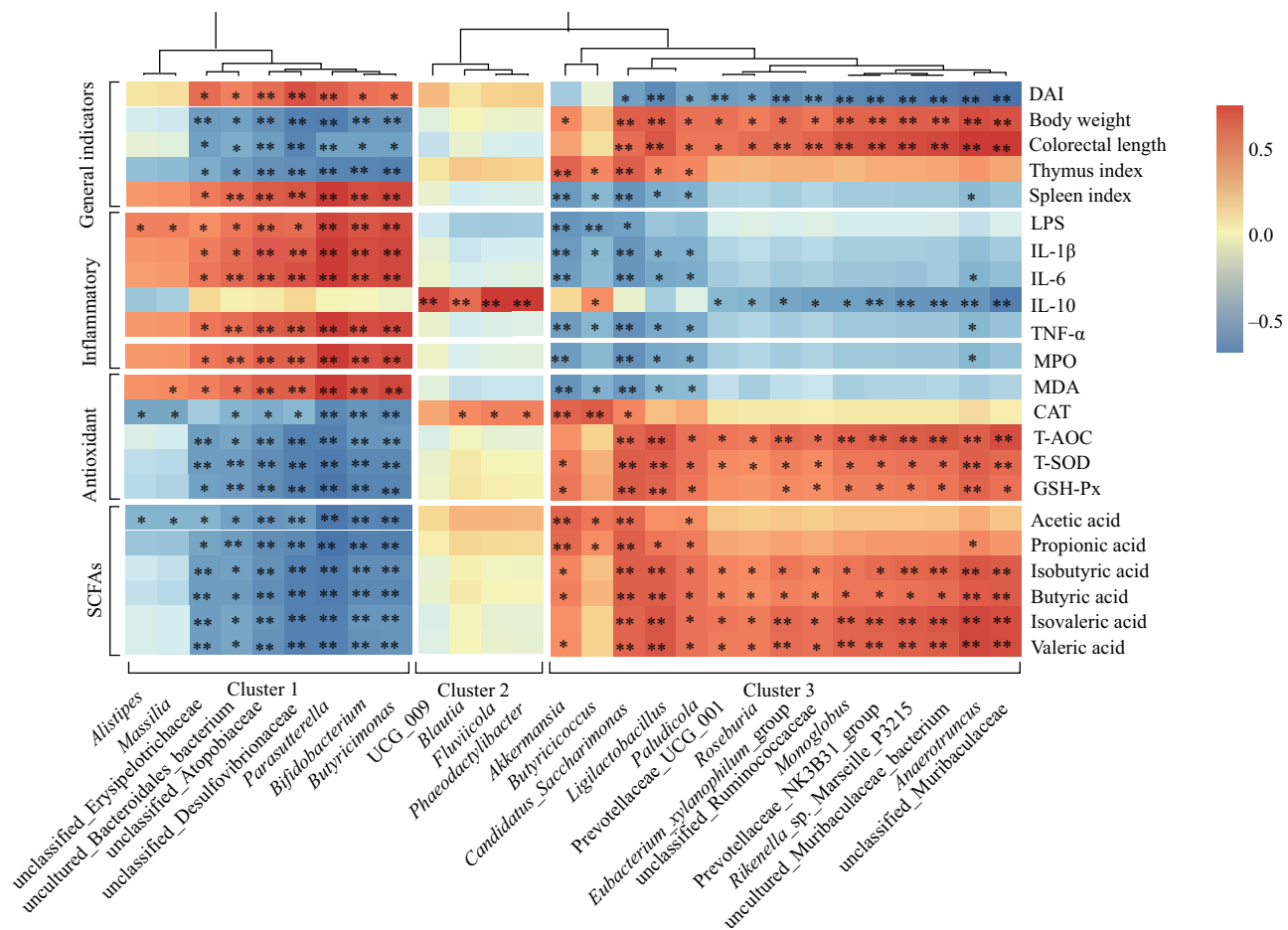


Fig. 8 Clustering heatmap of microbial genera with significant changes (Control vs. DSS or DSS vs. DSS + PPP-4) and colitis-related indexes based on Pearson's correlation coefficient. * $P < 0.05$ and ** $P < 0.01$.

Blautia, *Fluviicola*, *Phaeodactylibacter* and UCG_009 were positively related to IL-10 and CAT (except UCG_009), indicating that *Blautia*, *Fluviicola*, *Phaeodactylibacter*, and UCG_009 had less effect on colitis in mice. In contrast, most of the genera (2/3) in cluster 3 were positively correlated with body weight, colorectal length, SCFAs (isobutyric acid, isovaleric acid, valeric acid and butyric acid) as well as antioxidant related enzymes and had negative correlations with DAI and IL-10. In addition, 1/3 of the genera (such as *Akkermansia* and *Butyricoccus*) in cluster 3 showed negative correlations with LPS, IL-1 β , TNF- α , MDA, MPO as well as spleen index, and had positive correlations with thymus index, CAT, acetic acid, and propionic acid. These above data suggested that *Akkermansia*, *Anaerotruncus*, *Butyricoccus*, *Candidatus_Saccharimonas*, *Ligilactobacillus*, *Monoglobus*, *Paludicola*, *Prevotellaceae* NK3B31 group, *Prevotellaceae* UCG_001, *Rikenella* sp. Marseille P3215, *Roseburia*, *Eubacterium xylanophilum* group, unclassified Muribaculaceae, unclassified_Ruminococcaceae, and uncultured_Muribaculaceae bacterium had good effects on colitis in mice.

Collectively, these observed data illustrated that PPP-4 consumption contributed to relieving the severity of colitis, decreasing the relative abundance of harmful bacteria, increasing the relative abundance of beneficial bacteria, suppressing the expression of pro-inflammatory cytokines, further increasing the secretion level of SCFAs in faeces and enhancing the antioxidant capacity in colorectal tissue of colitis mice.

4. Conclusion

In this research, a bioactive main component, persimmon peel pectic polysaccharide named PPP-4, was purified and its structural characteristics were investigated. PPP-4 (56.48 kDa) was mainly contained GalA, Ara, Gal, Rha with the molar ratios of 60.80:32.12:3.67:2.20. The backbone of PPP-4 was formed by of $\rightarrow 4$ - α -D-GalpA-(1 $\rightarrow$, $\rightarrow 4$)- α -D-GalpA-6-OMe-(1 $\rightarrow$, $\rightarrow 2,4$)- α -L-Rhap-(1 $\rightarrow$, and with some side chains consisting of $\rightarrow 5$ - α -L-Araf-(1 $\rightarrow$, $\rightarrow 3$)- β -L-Galp-(1 $\rightarrow$, $\rightarrow 3,5$)- α -L-Araf-(1 $\rightarrow$, and $\rightarrow 3$)- α -L-Araf-(1 $\rightarrow$ residues and terminals of T- α -L-Araf and 4- β / α -D-GalpA residues. PPP-4 displayed good protective effects on colitis induced by DSS in mice via decreasing pro-inflammatory factor level in serum, relieving oxidative stress in colorectal tissue, increasing the level of SCFAs in faeces and regulating the homeostasis of gut microbiota. This current research provided a theoretical basis that PPP-4 may be considered as a potential candidate in prevention or treatment of colitis. However, the specific deeper anti-inflammatory mechanism and structure-activity relationships of PPP-4 are unclear, needing to further reveal in the future study.

Conflict of interests

The authors listed in this study declared that there are no conflicts of interest in submission and publish of this paper.

Acknowledgements

This present work was supported by the Key-Point Research and Invention Program of Shannxi Province (2021ZDLNY05-06), Chinese Universities Scientific Fund (2452018062), Henan Institute of Science and Technology (103020224001/110) and National Natural Science Foundation of China (32001652). Technical support of NMR assay was provided by Dr. Xiuhuan Li (State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.26599/FSHW.2025.9250473>.

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