Journal of Holistic Integrative Pharmacy 4 (2023) 157–165

Contents lists available at ScienceDirect

Journal of Holistic Integrative Pharmacy

journal homepage: www.keaipublishing.com/en/journals/journal-of-holistic-integrative-pharmacy

Dioscoreae persimilis polysaccharide ameliorates DSS-induced ulcerative colitis in mice through modulation of microbiota composition

Qian Zhang [a,b,*,1], Guorong Wu [a,1], Shumin Shen [a], Chong Li [c,**]

[a] School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, 510006, China
[b] Guangdong Provincial Key Laboratory of Advanced Drug Delivery, Guangdong Provincial Engineering Center of Topical Precise Drug Delivery System, Guangdong Pharmaceutical University, Guangzhou, 510006, China
[c] CAS Key Laboratory of Regenerative Biology, Joint School of Life Sciences, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, 510530, China

ARTICLE INFO

Keywords:
Polysaccharide
Ulcerative colitis
Mice
Dioscoreae persimilis
Microbiota composition

ABSTRACT

Ulcerative colitis (UC) is a non-specific inflammatory bowel disease that has a high rate of recurrence, development of novel therapeutic approaches with high efficacy and few adverse effects are still needed. *Dioscoreae persimilis* is an edible plant that has been widely consumed as a remedy for gastrointestinal diseases in traditional Chinese medicine. Polysaccharides have been proven to have protective effects on UC. However, the role of polysaccharides from *D. persimilis* in UC has not been studied yet. The refined *D. persimilis* Polysaccharide (DP), which consists of glucose and galactose, was extracted and purified using three-phase partitioning (TPP) method. The primary chemical and structural characteristics of DP were investigated by UV, FT-IR, molecular weight, and monosaccharide composition. Based on dextran sulfate sodium (DSS) induced UC in mice, the alleviatory effect of DP on UC was explored. DP was found to alleviate histopathological changes of colon, improve colonic antioxidant capacity and ameliorate inflammation response in colitis mice. Moreover, 16S rDNA sequencing of fecal revealed that DP could restore the diversity and composition of gut microbiota, especially up-regulates the abundance of *Acetatifactor*, *Lachnospiraceae*, and *Lactobacillus*, and increase the ratio of Firmicutes/Bacteroidetes. According to this study, DP has the potential to serve as an effective nutritional supplement for improving colitis.

1. Introduction

Ulcerative colitis (UC), as one of the main forms of inflammatory bowel disease (IBD), is characterized by chronic inflammation and ulcerative lesions in the intestinal mucosa of the rectum and colon.[1] Clinical symptoms of UC include diarrhea, bloody stools and weight loss. Available evidence indicates that with the changing dietary habits in recent years, the incidence of UC has shown an upward trend in modern countries.[2] The current drugs used to treat UC, such as 5-aminosalicylates, immunosuppressants, corticosteroids and antibacterial, can be challenging to effectively control the exacerbation of the disease and may even lead to some side effects with long-term use.[3,4] Therefore, it is crucial to investigate new drugs that have a proven effectiveness in treating UC with fewer adverse effects in order to improve the quality of life for patients.

Dioscorea persimilis Prain et. Burk., commonly known as Guang yam in Chinese, is a plant from the Dioscoreaceae family. Its tubers have been utilized as a food source and for medicinal purposes, particularly for gastrointestinal ailments, in traditional folk medicine for several millennia.[5] *D. persimilis* is a species commonly found in Guangxi, Guangdong province of China. It has been utilized in traditional Chinese medicine (TCM) to treat various ailments including diarrhea, intestinal disease, long-term dysentery, metritis and kidney failure.[6] Additionally, *D. persimilis* has other related species (Dioscoreaceae family), such as *Dioscorea opposite*, *Dioscorea japonica* and *Dioscorea alata*, which were also used as yam. Previous studies have primarily concentrated on *D. opposite* and have demonstrated its various pharmacological functions, such as anti-inflammatory, antioxidant, antitumor, estrogenic and gastrointestinal effects.[7–10] Additionally, polysaccharides are recognized as important components of *D. opposite* and contributes to numerous biological

* Corresponding author. School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, 510006, China.
** Corresponding author.
E-mail addresses: zhangqian1990122@163.com (Q. Zhang), li_chong688@163.com (C. Li).
[1] These authors contributed equally to this manuscript and worked as co-first authors.

https://doi.org/10.1016/j.jhip.2023.09.004
Received 30 June 2023; Received in revised form 18 September 2023; Accepted 18 September 2023

activities, including antihypoglycemic, antimutagenic, antioxidant, growth-promoting, immune regulation and intestinal flora modulation.[11–17] Despite being considered a valuable remedy, and even the overall toxicity is lower than that of *D. opposite* in toxicity studies,[18] the chemical constituents and biological activities of *D. persimilis* have yet to be fully understood. Previous studies have identified phenanthrenes, phenolic constituents, and several secondary metabolites in *D. persimilis*.[5,6] However, there have been no reports on *D. persimilis* polysaccharide.

It is currently believed that the pathogenesis of UC is associated with the disruption of the bacterial community.[19] The gut microbiota plays a crucial role in shielding the gut from harm, and a stable and dynamic balance of the intestinal community structure can help maintain overall health and prevent inflammation by resisting the colonization of pathogenic bacteria.[20] Clinical and experimental studies have demonstrated that UC patients experience dysbiosis, resulting in changes to the composition and function of their gut microbiota.[21] As a result, researchers have identified the gut microbiota as a new therapeutic target for UC.

Recent studies have indicated that polysaccharides have a variety of pharmacological effects, including protective effects on gastrointestinal flora.[22–25] Research studies have shown that polysaccharides extracted from the sporoderm-broken spores of *Ganoderma lucidum* (BSGLP) have the ability to suppress obesity and inflammation through regulating inflammation, gut microbiota, and gut barrier function.[26] Similarly, SP2-1, a homogeneous polysaccharide obtained from *Scutellaria baicalensis* Georgi has been found to improve intestinal barrier function and modulate gut microbiota, thereby ameliorating DSS-induced ulcerative colitis.[27] The pectic polysaccharides found in *Aconitum carmichaelii* leaves have shown to provide protection against DSS-induced UC in mice. This is achieved by influencing the metabolomic profiles in the serum that are related to bacterial changes and regulating the composition of gut microbiota.[28] The modulation of intestinal flora has also been observed with polysaccharides from *D. oppositifolia*. In a study, a mannoglucan (CYP-1) isolated from *D. oppositifolia* was found to shape the gut microbiota by decreasing the abundances of *Alistipes*, *Helicobacter* and *Enterobacteriaceae* in DSS-induced colitis mice.[16] However, the effects of polysaccharides from *D. persimilis* on UC have not been investigated yet.

The oral DSS induced UC model exhibits clinical symptoms and histological features that closely resemble human pathogenesis. By adjusting the dose, researchers can establish models of acute or chronic colitis. As a result, UC models induced by DSS induction have been primarily used to study polysaccharide prevention.[29,30] In the present study, the refined *D. persimilis* Polysaccharide (DP) was extracted and purified using three-phase partitioning (TPP) method. The primary chemical and structural properties of DP were analyzed using UV, FT-IR, molecular weight and monosaccharide composition. The study focused on the beneficial effects of DP on histopathological changes in colonic tissue lesions, oxidative stress, and inflammatory responses in UC mice. Furthermore, based on 16S rDNA sequencing, the impacts of DP on the composition of gut microbiota were explored. This study may aid in elucidating the role of DP in colitis and provide insight into the relationship between the host, microbe, and polysaccharide. It suggested that DP may be used as a nutritional supplement to ameliorate colitis.

2. Materials and methods

2.1. Source and model of the instrument

D. persimilis was acquired from Guangdong Shizhen Pharmaceutical Co., Ltd. (license: Yue 20180642, Guangdong Province, China). DSS (molecular weight 36–50 kDa) was obtained from Dalian Meilun Biotechnology Co., Ltd. (Liaoning Province, China); The tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 ELISA kits were purchased from Shanghai Enzyme Biotechnology Co., Ltd. (Shanghai, China); The BCA, myeloperoxidase (MOP), SOD, GSH and MDA activity kits were purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China). Dextrans, standard monosaccharides and trifluoroacetic acid (TFA) were supplied by Macklin Biochemical Technology Co., Ltd. (Shanghai, China). D-Glucose, sulfuric acid and phenol were purchased from Guangzhou Reagent Co. (Guangzhou, China). All other agents were of analytical grade.

2.2. Extraction and refinement of DP

Powder of *D. persimilis* (0.5 Kg) was soaked with ethanol (95%) for 12 h twice at room temperature, to remove ethanol-soluble substances. The obtained powder was dried and extracted thrice with deionized water (1:10 w/v, 80 °C), each time for 2 h. The combined aqueous extracts were filtered and concentrated to 100 mL under reduced pressure at 65 °C to obtain crude extract. Afterwards, the TPP method was carried out to refine polysaccharide from *D. persimilis* according to a previous report[31] with minor modifications. Briefly, 20% (w/v) $(NH_4)_2SO_4$ was added to crude extract (100 mL) and vortexed gently, followed by the additional of t-butanol (150 mL). To ensure a complete and quick phase separation, the mixture was placed into a separating funnel. The separating funnel was shaken gently and kept in stand for 30 min to separate the three phases and the lower phase was collected, which was mainly composed of $(NH_4)_2SO_4$ and polysaccharide. The obtained lower phase was further dialyzed (cut-off Mw 1000 Da) against distilled water for 48 h to remove salts and concentrated, after which the solution was performed for ethanol precipitation. The solution was precipitated by adding 95% ethanol to a final concentration of 80% ethanol for 24 h at 4 °C. The precipitate after centrifugation (3500 r, 10 min) was collected and lyophilized. Ultimately, the refined *D. persimilis* polysaccharide (10.5 g) was obtained, denoted as DP.

2.3. Primary characterization of DP

The extraction yield (%) of DP was estimated according to the following equation: extraction yield (%, w/w) = [weight of the dried DP (g)/weight of the original *D. persimilis* powder (g)] × 100%. The total carbohydrate content of DP was determined by phenol-sulfuric acid method using D-glucose as a standard.[32]

The monosaccharide of DP was analyzed by high-performance liquid chromatography (HPLC) through pre-column derivatization with 1-phenyl-3methyl-5-pyrazolone (PMP) method.[33,34] Polysaccharide sample was hydrolyzed with 2 M TFA (w/v=8.0 mg: 2.0 mL) and hydrolyzed at 120 °C for 4 h. The solution was concentrated and then washed with methanol for three times. After remove excess TFA, the polysaccharide sample and standard monosaccharides were derivatized with PMP methanol solution. The derivatives were analyzed by an Agilent 1260 HPLC system (Agilent Technologies Corporation, Santa Clara, CA, USA) coupled with an Agilent ZORBAX Eclipse XDB C_{18} column (4.6 × 250 mm, 5 μm) and an ultraviolet detector. The mobile phase was composed of 0.1 M phosphate buffer (pH 6.9)-acetonitrile (v/v = 83:17). The injection volume was 20 μL and was detected at 250 nm.

The molecular weight of *D. persimilis* polysaccharide was investigated by high-performance gel-permeation chromatography (HPGPC), which was performed on a HPLC system equipped with a TSK-GEL G-3000 PWXL gel column (Tosoh Biosep, Yamaguchi, Japan) and a refractive index detector (RID-10A, Shimadzu, Japan). The 0.02 M monopotassium phosphate solution (KH_2PO_4) was used as the mobile phase with a flow rate of 0.5 mL/min. The column was calibrated with T-series Dextran (T1000, T500, T70, T40, T10, and T5) as standards.

The ultraviolet spectrum of the polysaccharide was scanned with a SP-756P ultraviolet spectrophotometer from 200 to 400 nm. For Fourier transform-infrared (FT-IR) spectrum analysis, polysaccharide sample (2 mg) and potassium bromide powder (100 mg) were mixed, pressed into a disk by a mold, and analyzed with a PerkinElmer FT-IR spectrometer in the 4000-400 cm^{-1} region.[35]

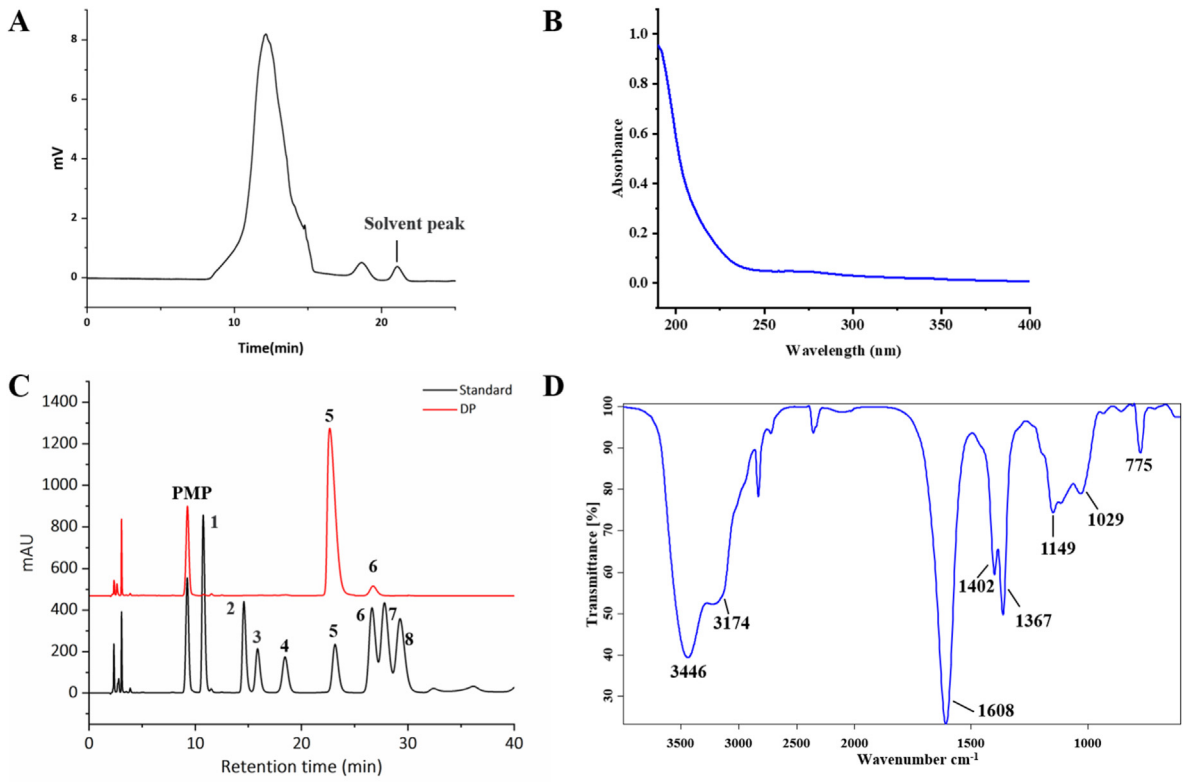

Fig. 1. Characterization of DP. (A) HPGPC spectrum of DP. (B) UV spectrum of DP. (C) PMP derivative of monosaccharide standards and DP (1, Man; 2, Rha; 3, GluA; 4, GalA; 5, Glc; 6, Gal; 7, Xyl; 8, Ara). (D) FT-IR spectrum of DP.

2.4. Experimental animals and methods

2.4.1. Preparation of UC mouse model

C57BL/6 mice (six-week-old male, SCXK (Yue) 2018-0002) were purchased from Guangdong Medical Experimental Animal Center and had been acclimated for 7 days, during which the mice had free access to food and water. Mice were kept in a 23±2 °C room under conditions of 12 h light/12 h darkness. Mice were divided randomly into three groups, with 8 mice in each group. The DSS (2.0% w/v) was administered ad libitum for 7 days to induce UC in mice.[36] The normal control (NC) and DSS groups received water, DP treatment group (400 mg/kg) received the preventative administration for three days. The body weight of the mice was measured regularly during the modeling period, and the disease activity index (DAI) was calculated according to the weight loss and stool consistency and hematochezia following the criteria in Supplementary Table S1.[37] At day 8, feces in each group were harvested. All mice were anaesthetized by isoflurane, and serum were collected immediately. The mice were fixed on the dissecting table, and the abdomen was cut open with surgical scissors. To ensure the integrity of the colon, the cecum and colon were cut together, the length of the colon was measured using a ruler, and the colon tissue was collected.

2.4.2. Histopathological analysis

After washing the intestinal contents with normal saline, cut longitudinally along the edge of the mesentery, a 1 cm proximal intestinal segment near the anus was fixed with 4% paraformaldehyde (PFA), dehydrated using 50%–100% ethanol, embedded in paraffin, and stained with hematoxylin-eosin (H&E) based on the standard experimental procedure. Finally, the morphological changes of the colon tissue were observed under a 400-power optical microscope.

2.4.3. Assay of biochemical indicators

Colon tissue homogenate samples in each group were prepared by 50 mM ice-cold PBS solution, and BCA kit was used to measure protein concentration. Then, the MPO, T-SOD and MDA activity assay kits were used to determine antioxidant indexes (Jiancheng Bioengineering Institute, Nanjing, China).

Blood was collected into a 1.5 mL centrifuge tube, placed in a refrigerated centrifuge at 4 °C for 30 min, and centrifuged at 3000 r/min for 10 min to separate the serum. The levels of inflammatory cytokines including TNF-α, IL-1β and IL-6, were measured using ELISA kits (Abclonal, Wuhan, China) according to the manufacturer's instructions.

2.4.4. Gut microbiota analyses

Fecal samples from the NC, DSS and the DP-administered group were randomly selected for intestinal flora analysis. Total genome DNA was extracted according to manufacturer's protocols. The bacterial 16S rDNA gene V3–V4 region was amplified using PCR, then the sequencing was demultiplexed and quality-filtered performed using an Illumina MiSeq/Novaseq (Illumina, San Diego, CA, USA) instrument manual. Reads were then processed using the MiSeq Control Software (MCS)/Novaseq Control Software (NCS), which was performed at the GENEWIZ Bio-Pharm Technology Co., Ltd. (Suzhou, China). OTU clustering sequence similarity is set to 97%. α diversity (Shannon and Chao1 index) was calculated using the method of random sampling sample sequences. Besides, Rarefaction curves and Rank-Abundance graph can also reflect the species richness and evenness. Principal coordinate analysis (PcoA) and nonmetric multidimensional scaling (NMDS) which display β diversity was calculated based on the distance between the matrix Brary-Curtis, and UPGMA (Unweighted pair group method with arithmetic mean) clustering tree was constructed. LEfSe analysis was conducted and the criteria for feature selection set as linear discriminant analysis (LDA) score >3.0.

2.4.5. Ethics approval

This study was performed in line with the principles of the Institutional Animal Care. Approval was granted by the Ethics Committee of

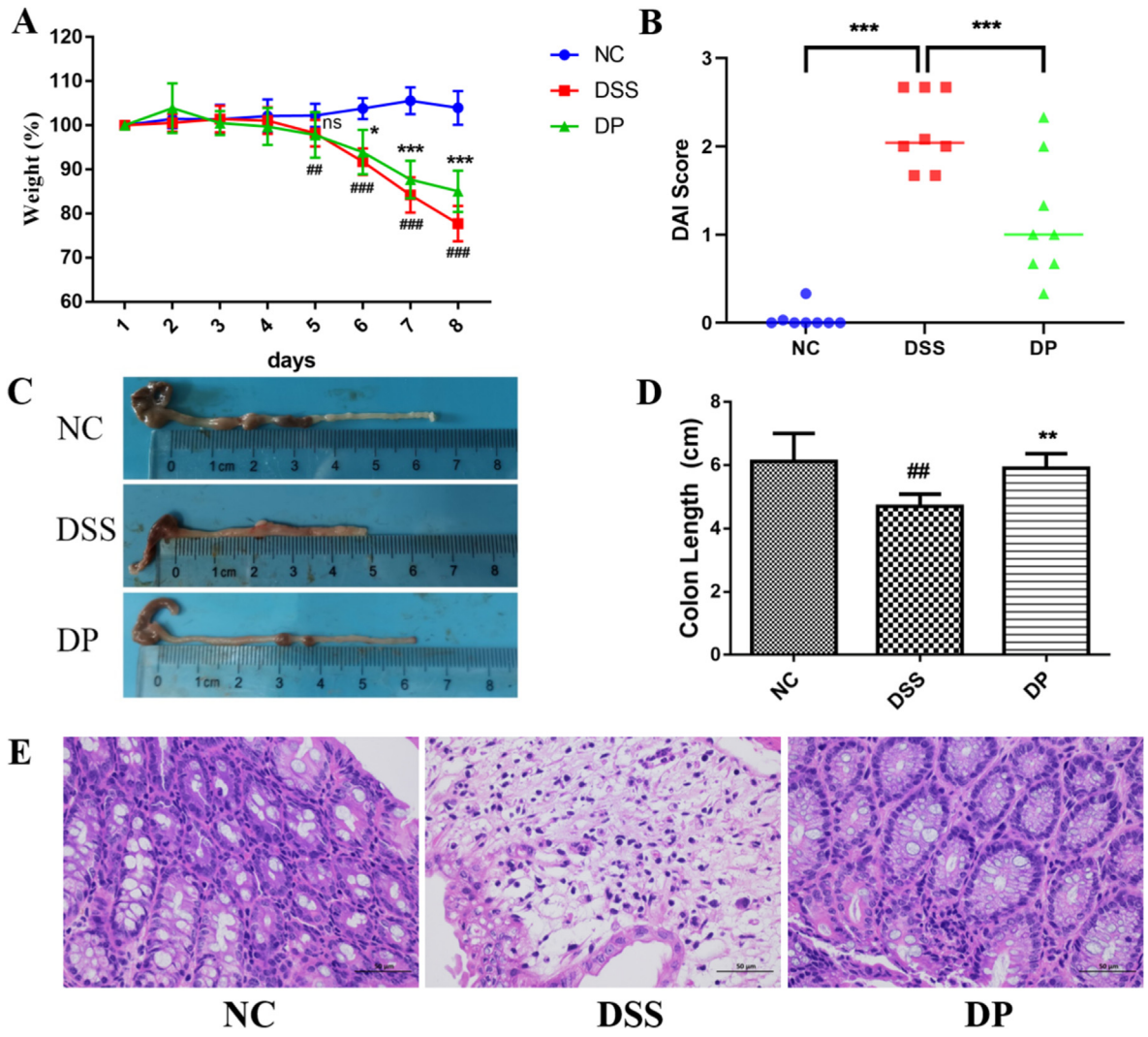

Fig. 2. Effects of DP on body weight, DAI scores, colon lengths and histological changes of UC mice. (A) Effect of DP on body weight of UC mice (n=8); (B) Effect of DP on DAI score of UC mice (n=8); (C and D) Effect of DP on colon length of UC in mice (n=8); (E) H&E staining of intestinal tissue sections (n=3), × 400. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ versus DSS group; $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$ versus NC group; ns, $P > 0.5$.

Guangdong Pharmaceutical University, Guangdong, China [Approval No. SPF2017510].

2.5. Statistics and analysis

Statistical analysis was performed using GraphPad Prism software version 8.3.0 (GraphPad Software, San Diego, CA, USA), and all data were presented as the mean ± standard deviation (SD). Comparisons between groups were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's range test. A P value less than 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Primary characterization of DP

DP was isolated by water extraction and refined by TPP method combine ethanol-precipitation. The extraction yields of DP was 2.1% and the carbohydrate content of the refined DP was found to be 73.8% based on the calibration curve presented in Fig. S1. Results from HPGPC spectrum showed that the refined DP was closely to a single, symmetric peak, indicating that it with relatively high purity (Fig. 1A), and the mean Mw of DP was found to be around 96.44 kDa. The UV spectra of DP as presented in Fig. 1B, lack of absorption at 260 nm and 280 nm revealed that rarely protein and nuclear acid was found in DP. The monosaccharide composition of DP was performed by using PMP method and HPLC system (Fig. 1C). DP consisted of 2 monosaccharides, including glucose (94.92%) and galactose (5.08%). The FT-IR spectrum of DP has the typical absorption peaks of a polysaccharide (Fig. 1D). The wide and strong absorption at 3446 cm^{-1} and 3174 cm^{-1}, mainly due to the stretch vibration of hydroxyl groups and attributed to the presence of intermolecular hydrogen bonds in polysaccharide.[38] The strong absorption band at 1608 cm^{-1} was attributed to the scissoring vibration of bound water molecules.[39] The absorption peaks at 1402 cm^{-1} and 1367 cm^{-1} were the result of C–H deformation vibrations and C–O–H bending vibrations.[40–42] The absorption peaks at 1149 cm^{-1} and 1029 cm^{-1} indicated the presence of the sugar ring C–O–C and pyranose.[27,43] Additionally, the small absorption peak around 750 cm^{-1} also was characteristic of pyranose.[44] This is consistent with the monosaccharide composition that DP was composed of Glc and Gal which are all pyranose.

3.2. Effect of DP on weight loss, colon length and DPI of UC mice

As shown in Fig. 2A, the weight loss was exacerbated remarkably in comparison with the NC group mice, which was significantly impaired by DP treatment. Meanwhile, compared to the NC group, the DAI score was

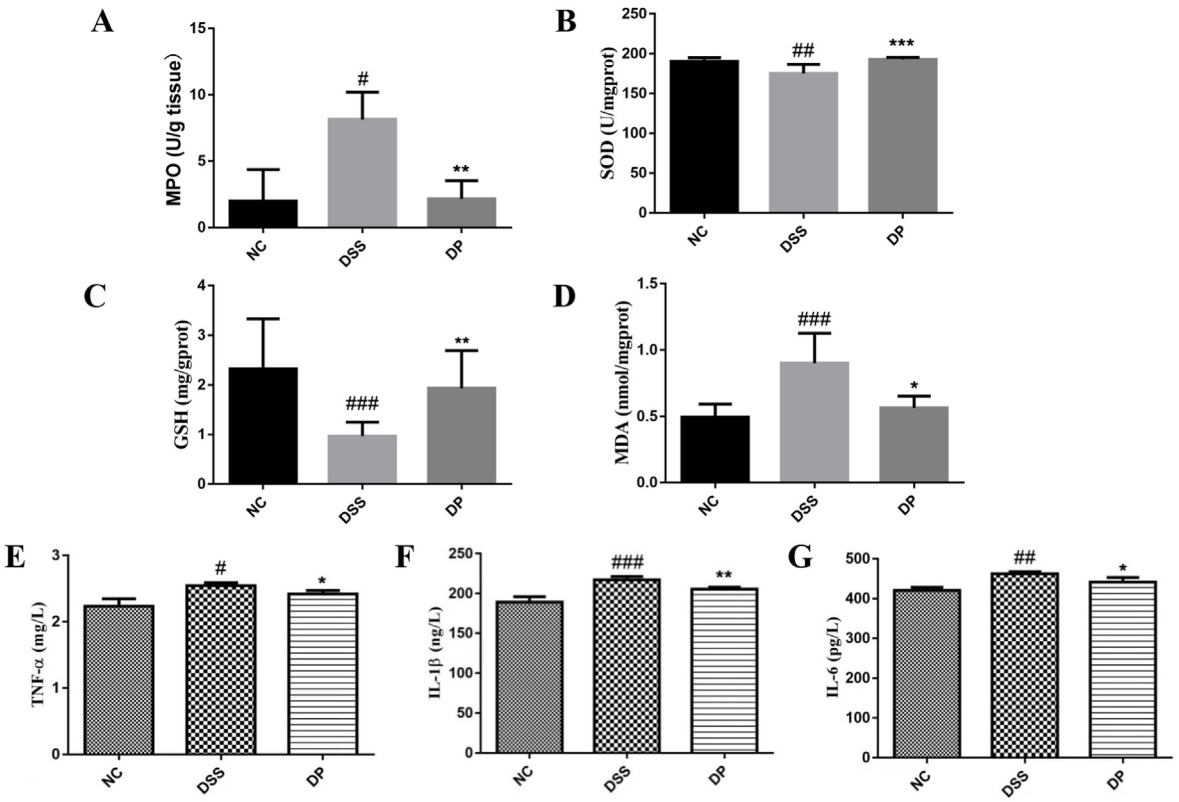

Fig. 3. Effect of DP on oxidative stress and inflammatory cytokines. MOP (A), SOD (B), GSH (C) and MDA (D) in mice colon tissue (n=4). The levels of TNF-α (E), IL-1β (F) and IL-6 (G) in the serum of mice (n=4). (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ DP group vs. DSS group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ DSS group vs. NC group.

greatly increased in the DSS group and DP administration could significant relief this trend ($P < 0.01$) (Fig. 2B). Furthermore, the colon length of DSS-treated mice was obviously shortened ($P < 0.01$) compared to the NC group. Whereas the significant relief of colonic shortening by DP treatment ($P < 0.01$) was displayed in Fig. 2C and D. The above results indicated that the UC model was successfully established,[28] and DP can rescue the exacerbated weight loss, increased DAI index and colon shortening caused by DSS-induced UC mice.

3.3. Effects of DP on the histopathological changes of UC mice

The histopathological changes of colon tissue were evaluated by H&E staining. As shown in Fig. 2E, the significant inflammatory infiltration, the severe damaged colonic mucosa epithelium and the loss of crypts were found in the DSS group. Nevertheless, the lower degree of colon damage was found in the DP treatment group in comparison with the DSS group. DP treatment group mice exhibited the less inflammatory cell infiltration, relatively intact colonic architecture and less mucosal damage, indicating that the DP treatment play a role in mitigating histopathological symptoms induced by DSS in UC mice.

3.4. Effects of DP on inflammation and inflammation-induced oxidation of UC mice

As a local mediator of mucosal healing and the response of inflammation in UC,[45] the expression of MPO in colon tissue was markedly elevated in the DSS group than that of the NC group (Fig. 3A), indicating that a severe inflammatory response in the colon of UC mice. Nevertheless, DP administration could dramatically reduce MPO in colon tissues of UC mice ($P < 0.01$).

Given that oxidative stress accompanies inflammation and exacerbates inflammation-compromised epithelial integrity. Compared with the NC group, the expression of antioxidant enzyme SOD and GSH in the DSS group were significantly decreased ($P < 0.01$). Otherwise, the level of malondialdehyde (MDA) was remarkedly increased in the DSS group in comparison with the NC group (Fig. 3D). The DP treatment reversed the changes of MDA, SOD and GSH in mice with DSS-induced colitis (Fig. 3B–D). These results showed that DP improved the intestinal inflammation-induced oxidative stress response induced by DSS to a certain extent.

Inflammation plays a key role in the pathogenesis and the therapeutic target for UC.[46] Inflammatory cytokines including TNF-α, IL-1β and IL-6 were detected to evaluate the effects of DP on inflammatory response. As expected, the levels of TNF-α, IL-1β and IL-6 in DSS mice were notably increased than that of the NC group (Fig. 3E–G). Whereas, DP administration significantly inhibited the production of IL-1β, IL-6, and TNF-α in serum induced by DSS ($P < 0.05$). Therefore, it was demonstrated that DP intervention was able to attenuate inflammation in DSS-induced UC mice.

3.5. Composition and abundance of the gut microbiota

The regulation of DP on the gut microbiota was evaluated by 16S rDNA sequencing technology. As illustrated in Fig. 4A, the richness of gut microbes was remarkedly lowed in DSS group when compared to NC group in the microbial alpha diversity both in Chao1 and Shannon. However, there was no significantly difference in Chao1 and Shannon between DP group and NC group. In addition, the rank abundance curve of DSS group dropped faster than that in NC and DP treatment group (Fig. 4B), also indicating low species abundance in the DSS group. The results of this study are similar to those of a previous study, in which lower gut microbiome diversity has been found in the UC patients.[47] Whereas, the decreased species abundance in DSS mice was reversed after the DP administration. As PcoA analysis in Fig. 4C shown, based on

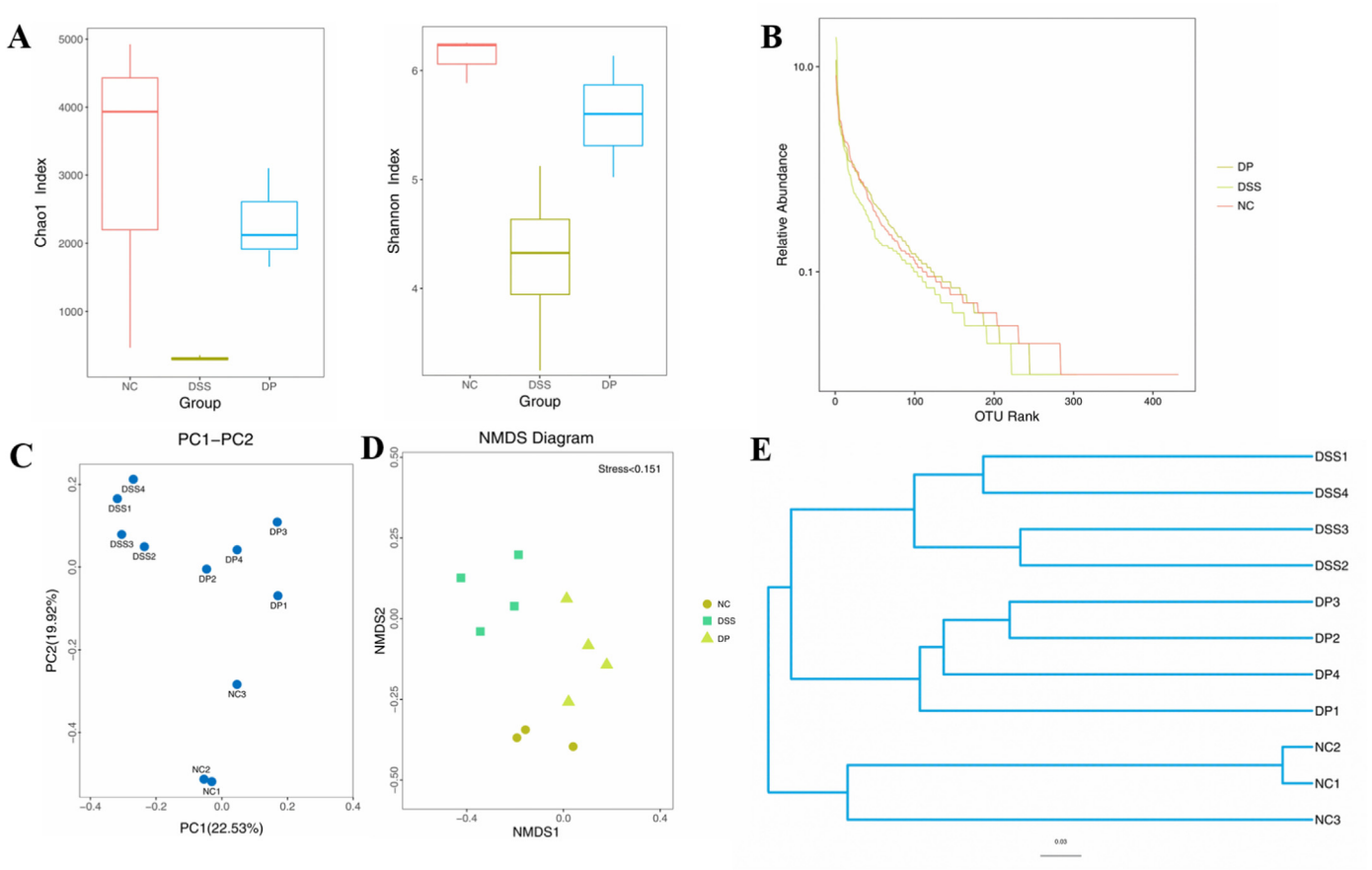

Fig. 4. The comparison of gut microbiota alpha diversity and beta diversity between each group. (A) Alpha diversity boxplot (Chao 1 and Shannon). (B) Rank abundance distribution curve (C) Beta diversity boxplot (PcoA). (D) Beta diversity boxplot (NMDS). (E) UPGMA clustering tree.

the Bray-Curtis distance at the OUT level, the DP group was well differentiated from the DSS group, and trend to the NC group. Similarly, as shown in Fig. 4D, NMDS analysis which also represented microbial beta diversity demonstrated that the NC, DSS and DP groups distinctly clustered with a stress than 0.151 (An NMDS value less than 0.2 indicates its validity). Additionally, the NC, DSS and DP groups showed distinct microbial landscapes in UPGMA clustering tree (Fig. 4E). Collectively, according to microbial alpha diversity and beta diversity results, DP administration restored gut microbiota diversity.

To reveal the composition of microbiota, microbial differences at different levels (phylum, genus) were evaluated. In terms of the phylum level, as can be seen from Fig. 5A, the major taxonomic composition were Firmicutes, Bacteroidetes and Proteobacteria. There was an increase in the relative abundance of Bacteroidetes and Proteobacteria, and a reduction in the relative abundance of Firmicutes caused by the induction of colitis, in comparison with the NC group. It is believed that Firmicutes and Bacteroidetes are the two most prevalent phyla in the gastrointestinal tract.[48] Additionally, the ratio of Firmicutes to Bacteroidetes (F/B ratio) is considered as an important indicator to dysbiosis. The decreased F/B ratio was observed in patients with colitis.[49] There is evidences supports the idea that enterotoxigenic Bacteroides fragilis is related to inflammatory bowel disease, whilst the abundance of *Firmicutes* is negatively associated with gastrointestinal inflammation.[50] In our study, the ratio of Firmicutes to Bacteroidetes was calculated. As shown in Fig. S2, the ratio of *Firmicutes* to *Bacteroidetes* was significantly reduced ($P < 0.001$) in the DSS group as compared to the NC group. On the contrary, compared with the DSS mice, the ratio of *Firmicutes* to Bacteroidetes was evidently elevated ($P < 0.05$). Proteobacteria is a lager phylum of pathogenic bacteria and often increased in inflammatory bowel disease.[51] Our study documented that the intervention of DP significantly increased the F/B ratio and decreased the relative abundance of Proteobacteria, which may be responsible for the protect effects of DP on UC mice.

As presented in Fig. 5B, at the genus level, the distinct changes of gut microbes mainly included *f_Muribaculaceae_Unclassified*, *Bacteroides*, *Lactobacillus*, *Unclassified_f_Lachnospiraceae*, *Lachnospiraceae_NK4A136_group*, *Alloprevotella*, *Odoribacter* and *Ruminococcaceae_UCG−014*. Compared to the NC group, the relative abundances of *f_Muribaculaceae_Unclassified*, *Bacteroides*, *Odoribacter* and *Ruminococcaceae_UCG−014* were evidently increased, but the levels of *Lactobacillus*, *Unclassified_f_Lachnospiraceae* and *Lachnospiraceae_NK4A136_group* were significantly decreased in the DSS group. In the meantime, DP administration notably increased the levels of *Lactobacillus*, *Unclassified_f_Lachnospiraceae* and *Lachnospiraceae_NK4A136_group*, but significantly decreased the relative abundances of *f_Muribaculaceae_Unclassified*, *Bacteroides*, *Odoribacter* and *Ruminococcaceae_UCG−014* when compared to the DSS group. *Lactobacillus*, which is a well-known probiotic and can provide anti-inflammatory effects, was observed sharply elevated in the DSS group. After the intervention of DP, *Lactobacillus* displayed a relative enrichment, which might be associated with the DP-mediated alleviation of colitis. Similar findings of polysaccharides from plant or fungus increasing the abundance of *Lactobacillus* in inflammatory diseases were reported.[52] *Lachnospiraceae*, a family of digestive tract-associated bacteria, has been reported to possess potent capabilities of degrading the polysaccharides. The decreased abundance of *Lachnospiraceae* may in trigger the recurrence of UC.[53] Lower amounts of *Lachnospiraceae* were also previously reported in UC patients.[54] It's reported that the decreased abundance of family *Lachnospiraceae* bacteria was observed in the ulcerative colitis models compared to in the healthy subjects,[55] which was consistent with our study. The present results suggested that DP could significantly

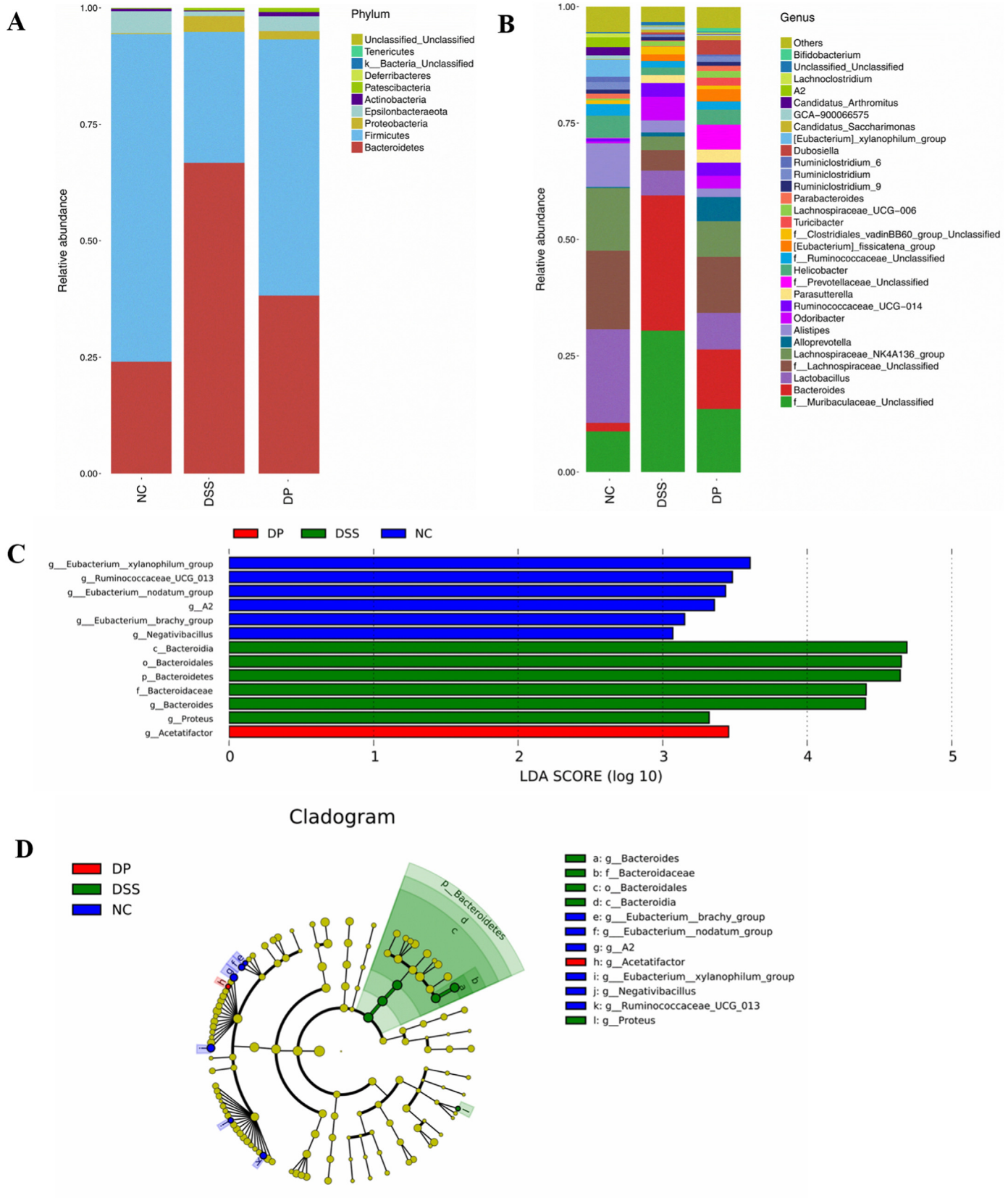

Fig. 5. Comparisons of gut microbiota community in each group. The relative abundances of the major bacteria at (A) phylum, (B) genus levels. LEfSe analysis of DP intervention on gut microbiota. (C) LDA score computed from features differentially abundant between NC, DSS and DP groups. (D) Taxonomic cladogram from LEfSe analysis.

enhance the levels of *Lactobacillus* and *Lachnospiraceae* and reduce the relative abundance of *Bacteroides*, especially for *Muribaculaceae* and *Odoribacter*, which is also thought to be associated with the DP-mediated alleviation of colitis.

To identify the dominant bacterial communities after DP administration, LEfSe analysis was conducted. OTUs with a LDA value of 3.0 or higher were gathered. As shown in Fig. 5C, for the DSS group, *Bacteroides* were dominant OTUs with LDA values higher than 3.0. Meanwhile, values greater than 3.0 was *Acetatifactor* in the DP group. The graph of evolutionary branches showed *Bacteroidetes* was the most abundant bacterial community in DSS group, while *Acetatifactor* was dominant taxa in DP administration group, which displayed a consistent tendency with the above results. In the cladogram (Fig. 5D), each node which represented a species taxonomic type. Red nodes denote the taxonomic types with more abundance in DP administrated group, the blue nodes represent the taxonomic types more abundant in NC group, whilst the green nodes represent the taxonomic types more abundant in DSS group. And the yellow nodes denote the species that are not significantly differentiate with groups. *Acetatifactor*, a butyrate bacterium, has been demonstrated to decrease in DSS-induced UC mice, which was in agreement with previous study.[56] Moreover, Butyric acid was reported to relieve UC effectively.[57] However, the effects of DP on short-chain fatty acids (SCFAs), especially butyrate are yet to be elucidated, further experiments are need to be undertaken.

In summary, this study suggests that DP may have therapeutic effects on UC due to its ability to both reduce inflammation and modulate the diversity and composition of gut microbiota. Specifically, DP was found to recover the abundance of beneficial bacteria such as *Acetatifactor*, *Lachnospiraceae*, *Lactobacillus* and Proteobacteria, while also improving the ratio of Firmicutes to Bacteroidetes. These findings suggest that DP could be a potential treatment option for UC.

4. Conclusion

In this study, we utilized the TPP method to isolate the refined *D. persimilis* polysaccharide (DP) with an average molecular weight of 96.44 kDa. DP is composed of glucose and galactose. We investigated the effect of DP on DSS-induced ulcerative colitis in C57BL/6 mice. Our results showed that DP administration significantly ameliorated DSS-induced colitis by reducing weight loss, rescuing colon length, improving colonic mucosal damage, suppressing crypt loss, and inhibiting inflammatory cell infiltrates. DP treatment was found to suppress DSS-stimulated production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, while also reducing oxidative stress in the colonic mucosa. This suggests that DP may have a remedial effect against UC. Additionally, 16S rDNA sequencing of gut microbiota showed that DP administration could restore gut microbiota diversity, regulate the composition of the gut microbiota, and prevent the imbalance of intestinal flora in DSS-induced colitis in mice. Dietary supplementation with DP has been shown to increase the relative abundances of beneficial bacteria such as *Acetatifactor*, *Lachnospiraceae* and *Lactobacillus*, while decreasing the relative abundance of Proteobacteria. Additionally, DP has been found to increase the ratio of Firmicutes to Bacteroidetes, resulting in functional changes in gut microbiota. Our study suggests that DP has the potential to be used as a nutritional supplement to treat experimentally induced acute UC in mice and may have promising benefits in ameliorating colitis.

Author contribution statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Qian Zhang, Guorong Wu and Chong Li. The first draft of the manuscript was written by Qian Zhang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgments

This work was supported by Guangzhou Basic and Applied Basic Research Foundation (Grant No. 2023A04J1154); and College Students' Innovation and Entrepreneurship Training Program (Grant No. S202110573035).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhip.2023.09.004.

References

1. Liso M, Verna G, Cavalcanti E, et al. Interleukin 1 beta blockade reduces intestinal inflammation in a murine model of tumor necrosis factor-independent ulcerative colitis. *Cell Mol Gastroenterol Hepatol*. 2022;14:151–171.
2. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. *World J Gastroenterol*. 2014;20:91–99.
3. Hyams JS, Turner D, Cohen SA, et al. Pharmacokinetics, safety and efficacy of intravenous vedolizumab in paediatric patients with ulcerative colitis or crohn's disease: results from the phase 2 hubble study. *J Crohns Colitis*. 2022;16:1243–1254.
4. Feuerstein JD, Isaacs KL, Schneider Y, et al. Aga clinical practice guidelines on the management of moderate to severe ulcerative colitis. *Gastroenterology*. 2020;158:1450–1461.
5. Ngan NTT, Hoang NH, Hien NT, et al. Secondary metabolites from the tubers of *Dioscorea persimilis*. *Vietnam J Chem*. 2021;59:684–688.
6. Ngan NTT, Hoang NH, Hien NT, et al. Cytotoxic phenanthrenes and phenolic constituents from the tubers of *Dioscorea persimilis*. *Phytochem Lett*. 2020;40:139–143.
7. Liu Y, Li H, Fan Y, et al. Antioxidant and antitumor activities of the extracts from Chinese yam (*Dioscorea opposite* thunb.) flesh and peel and the effective compounds. *J Food Sci*. 2016;81:H1553–H1564.
8. Zeng M, Zhang L, Li M, et al. Estrogenic effects of the extracts from the Chinese yam (*Dioscorea opposite* thunb.) and its effective compounds in vitro and in vivo. *Molecules*. 2018;23:3–13.
9. Zeng M, Zhang L, Zhang B, et al. Chinese yam extract and adenosine attenuated LPS-induced cardiac dysfunction by inhibiting ras and apoptosis via the er-mediated activation of shc/ras/raf1 pathway. *Phytomedicine*. 2019;61:152857.
10. Jeon JR, Lee JS, Lee CH, et al. Effect of ethanol extract of dried Chinese yam (*Dioscorea batatas*) flour containing dioscin on gastrointestinal function in rat model. *Arch Pharm Res (Seoul)*. 2006;29:348–353.
11. Hao L-X, Zhao X-H. Immunomodulatory potentials of the water-soluble yam (*Dioscorea opposita* thunb) polysaccharides for the normal and cyclophosphamide-suppressed mice. *Food Agric Immunol*. 2016;27:667–677.
12. Fan Y, He Q, Luo A, et al. Characterization and antihyperglycemic activity of a polysaccharide from *Dioscorea opposita* thunb roots. *Int J Mol Sci*. 2015;16:6391–6401.
13. Li Q, Li X, Ren Z, et al. Physicochemical properties and antioxidant activity of maillard reaction products derived from *Dioscorea opposita* polysaccharides. *Lwt*. 2021:149.
14. Zhang CQ, Chen X, Ding K. Structural characterization of a galactan from *Dioscorea opposita* thunb. And its bioactivity on selected bacteroides strains from human gut microbiota. *Carbohydr Polym*. 2019;218:299–306.
15. Ouyang J, Wang F, Li W, et al. Structure characterization of polysaccharide from Chinese yam (*Dioscorea opposite* thunb.) and its growth-promoting effects on streptococcus thermophilus. *Foods*. 2021;10:2–14.
16. Li P, Xiao N, Zeng L, et al. Structural characteristics of a mannoglucan isolated from Chinese yam and its treatment effects against gut microbiota dysbiosis and DSS-induced colitis in mice. *Carbohydr Polym*. 2020;250:116958.
17. Jiang Q, Gao W, Shi Y, et al. Physicochemical properties and in vitro digestion of starches from *Different dioscorea* plants. *Food Hydrocolloids*. 2013;32:432–439.
18. Gao H, Tian Hui, Zhu H, et al. General situation of modern research on *Dioscorea persimilis*. *Pharm J Chin PLA*. 2017;33:72–74.
19. Guo XY, Liu XJ, Hao JY. Gut microbiota in ulcerative colitis: insights on pathogenesis and treatment. *J Dig Dis*. 2020;21:147–159.
20. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol*. 2021;19:55–71.
21. Zhang Y, Si X, Yang L, et al. Association between intestinal microbiota and inflammatory bowel disease. *Animal Model Exp Med*. 2022;5(4):311–322.
22. Feng J, Geng J, Wu J, et al. A potential role of plant/macrofungi/algae-derived non-starch polysaccharide in colitis curing: review of possible mechanisms of action. *Molecules*. 2022;27:1–12.
23. Guo C, Guo D, Fang L, et al. *Ganoderma lucidum* polysaccharide modulates gut microbiota and immune cell function to inhibit inflammation and tumorigenesis in colon. *Carbohydr Polym*. 2021;267:118231.

24. Guo C, Wang Y, Zhang S, et al. *Crataegus pinnatifida* polysaccharide alleviates colitis via modulation of gut microbiota and scfas metabolism. *Int J Biol Macromol*. 2021; 181:357–368.
25. Pan X, Yin M, Guo M, et al. The latest progress of natural food polysaccharides preventing ulcerative colitis by regulating intestinal microbiota. *J Funct Foods*. 2022; 96:105201.
26. Sang T, Guo C, Guo D, et al. Suppression of obesity and inflammation by polysaccharide from sporoderm-broken spore of *Ganoderma lucidum* via gut microbiota regulation. *Carbohydr Polym*. 2021;256:117594.
27. Cui L, Guan X, Ding W, et al. *Scutellaria baicalensis* georgi polysaccharide ameliorates dss-induced ulcerative colitis by improving intestinal barrier function and modulating gut microbiota. *Int J Biol Macromol*. 2021;166:1035–1045.
28. Fu YP, Li CY, Peng X, et al. Pectic polysaccharides from aconitum carmichaelii leaves protects against DSS-induced ulcerative colitis in mice through modulations of metabolism and microbiota composition. *Biomed Pharmacother*. 2022;155:113767.
29. Gudi R, Suber J, Brown R, et al. Pretreatment with yeast-derived complex dietary polysaccharides suppresses gut inflammation, alters the microbiota composition, and increases immune regulatory short-chain fatty acid production in C57bl/6 mice. *J Nutr*. 2020;150:1291–1302.
30. Liu C, Hu B, Cheng Y, et al. In-depth analysis of the mechanisms of aloe polysaccharides on mitigating subacute colitis in mice via microbiota informatics. *Carbohydr Polym*. 2021;265:118041.
31. Yan JK, Yu YB, Wang C, et al. Production, physicochemical characteristics, and in vitro biological activities of polysaccharides obtained from fresh bitter gourd (momordica charantia l.) via room temperature extraction techniques. *Food Chem*. 2021;337:127798.
32. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Calorimetric method for determination of sugars and related substances. *Anal Chem*. 1956;28:350–366.
33. Yi P, Li N, Wan JB, et al. Structural characterization and antioxidant activity of a heteropolysaccharide from *Ganoderma capense*. *Carbohydr Polym*. 2015;121:183–189.
34. Zhou S, Huang G. Extraction, purification and antioxidant activity of *Juglans regia* shell polysaccharide. *Chem Biol Technol Ag*. 2023;10(1):1–14.
35. Zhang S, Zhang Q, Zhang D, et al. Anti-osteoporosis activity of a novel *Achyranthes bidentata* polysaccharide via stimulating bone formation. *Carbohydr Polym*. 2018;184: 288–298.
36. Chassaing B, Aitken JD, Malleshappa M, et al. Dextran sulfate sodium (Dss)-induced colitis in mice. *Curr Protoc Im*. 2014;104:15–25.
37. Yu W, Kang C, Zhang Y, et al. The san-qi-xue-shang-ning formula protects against ulcerative colitis by restoring the homeostasis of gut immunity and microbiota. *J Ethnopharmacol*. 2023;305:116125.
38. Liu L, Lu Y, Li X, et al. A novel process for isolation and purification of the bioactive polysaccharide tlh-3′ from tricholoma lobayense. *Process Biochem*. 2015;50: 1146–1151.
39. Yu X, Wang Z, Shu Z, et al. Effect and mechanism of sorbus pohuashanensis (hante) hedl. Flavonoids protect against arsenic trioxide-induced cardiotoxicity. *Biomed Pharmacother*. 2017;88:1–10.
40. Azmi AF, Mustafa S, Hashim DM, et al. Prebiotic activity of polysaccharides extracted from *Gigantochloa levis* (buluh beting) shoots. *Molecules*. 2012;17:1635–1651.
41. Chen Q, Zhang W, Huang G. Preparation and antioxidant activity of acetylated mung bean peel polysaccharides. *Chem Biodivers*. 2023;20(8):106507.
42. Lin B, Wang S, Zhou A, et al. Ultrasound-assisted enzyme extraction and properties of *Shatian pomelo* peel polysaccharide. *Ultrason Sonochem*. 2023;98.
43. Zhu K, Zhang Y, Nie S, et al. Physicochemical properties and in vitro antioxidant activities of polysaccharide from *Artocarpus heterophyllus* lam. Pulp. *Carbohydr Polym*. 2017;155:354–361.
44. Wang W, Zou Y, Li Q, et al. Immunomodulatory effects of a polysaccharide purified from *Lepidium meyenii* walp. On macrophages. *Process Biochem*. 2016;51:542–553.
45. Aratani Y. Myeloperoxidase: its role for host defense, inflammation, and neutrophil function. *Arch Biochem Biophys*. 2018;640:47–52.
46. Sun J, Chen H, Kan J, et al. Anti-inflammatory properties and gut microbiota modulation of an alkali-soluble polysaccharide from purple sweet potato in DSS-induced colitis mice. *Int J Biol Macromol*. 2020;153:708–722.
47. Xenoulis PG, Palculict B, Allenspach K, et al. Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. *FEMS Microbiol Ecol*. 2008;66:579–589.
48. Kaoutari AE, Armougom F, Gordon JI, et al. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol*. 2013; 11:497–504.
49. Fabrega MJ, Rodriguez-Nogales A, Garrido-Mesa J, et al. Intestinal anti-inflammatory effects of outer membrane vesicles from escherichia coli nissle 1917 in dss-experimental colitis in mice. *Front Microbiol*. 2017;8:1274.
50. Stojanov S, Berlec A, Strukelj B. The influence of probiotics on the Firmicutes/ Bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. *Microorganisms*. 2020;8:1–16.
51. Powell N, Walker AW, Stolarczyk E, et al. The transcription factor t-bet regulates intestinal inflammation mediated by interleukin-7 receptor+ innate lymphoid cells. *Immunity*. 2012;37:674–684.
52. Liu L, Li M, Yu M, et al. Natural polysaccharides exhibit anti-tumor activity by targeting gut microbiota. *Int J Biol Macromol*. 2019;121:743–751.
53. Vacca M, Celano G, Calabrese FM, et al. The controversial role of human gut lachnospiraceae. *Microorganisms*. 2020;8:1–25.
54. Frank DNA ALS, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA*. 2007;104:13780–13785.
55. Sasaki K, Inoue J, Sasaki D, et al. Construction of a model culture system of human colonic microbiota to detect decreased lachnospiraceae abundance and butyrogenesis in the feces of ulcerative colitis patients. *Biotechnol J*. 2019;14: e1800555.
56. Kim J, Choi JH, Ko G, et al. Anti-inflammatory properties and gut microbiota modulation of porphyra tenera extracts in dextran sodium sulfate-induced colitis in mice. *Antioxidants*. 2020;9:1–15.
57. Parada Venegas D, De la Fuente MK, Landskron G, et al. Short chain fatty acids (SAFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol*. 2019;10:277.