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RESEARCH ARTICLE

Phyllanthus emblica Polysaccharide Ameliorates Ulcerative Colitis *via* Gut Microbiota Regulation

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ABSTRACT

Ulcerative colitis is one of the classical chronic inflammatory bowel diseases, has complex etiology involving immune dysfunction and gut microbiota dysbiosis. Traditional medicine polysaccharides have shown promising potential to alleviate intestinal inflammation associated with UC. However, there is still insufficient reporting on how the polysaccharides from *Phyllanthus emblica* can exert therapeutic and repairing effects by regulating gut microbiota. So, the current study was conducted to assess the therapeutic potential of *Phyllanthus emblica* fruit polysaccharide (PEP) on UC and its influence on the gut microbiota and metabolite profiles in a DSS-induced mice UC model. This study assessed the clinical, histopathological, inflammatory/oxidative stress indices, and analyzed gut microbiota by 16S rRNA sequencing and fecal metabolites by untargeted metabolomics in four mouse groups with oral PEP treatment in DSS-induced UC model. PEP treatment significantly alleviated clinical severity, evidenced by decreased level of disease activity index, restoring the length of colon, and improvement in histopathology. It strengthened intestinal barrier wholeness by upregulating tight junction proteins (ZO-1, Occludin) and suppressed colonic pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), Myeloperoxidase and malondialdehyde. In addition, multi-omics analysis revealed that PEP alleviated UC by remodeling the gut microbiota and its metabolic function. PEP selectively enriched beneficial commensals such as *Bacteroides acidifaciens* and *Paraprevotella clara*, and increasing the SCFA-producing, rhamnose, histidine metabolism, and peptidoglycan. PEP selectively enriched beneficial commensals such as *Bacteroides acidifaciens*, and *Paraprevotella clara*. Then, that supported the ecological stability of the consortium, which subsequently increased the production of SCFA, rhamnose, histidine metabolism, and peptidoglycan, contributing to the alleviation of intestinal inflammation. In summary, PEP alleviates UC by first strengthening the intestinal colonization of core beneficial bacteria, which in turn produce metabolites that directly repair the epithelial barrier and resolve inflammation.

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INTRODUCTION

The inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis (UC), is a long-term, relapsing condition of the gastrointestinal tract that occurs in humans as well as in various animal species (Kobayashi *et al*., 2020). The pathogenesis is complex, characterized by persistent intestinal mucosal inflammation as a core

pathological feature, and involves genetic susceptibility, immune dysregulation, and gut microbiota dysbiosis (Ordás I *et al*., 2012). IBD inflicts severe pathological damage in animals; for instance, in dogs, it triggers massive infiltration of PAS-positive macrophages in the colonic lamina propria and submucosa, accompanied by core clinical manifestations of hematochezia, diarrhea, and weight loss (Mansfield *et al*., 2009).

Epidemiologically, the global prevalence of human UC had reached approximately 5 million cases by 2023 (Le Berre C *et al*., 2023). Meanwhile, in the livestock and companion animal sectors, UC has led to substantial economic losses due to the impaired growth performance, elevated mortality rates, and reduced product quality. Currently, the mainstream therapeutic agents for the condition remain aminosalicylates, glucocorticoids, biologics, and antibiotics (Cottone *et al*., 2011; Berends *et al*., 2019). However, these therapeutic regimens are commonly linked to the adverse effects such as antibiotic resistance and immunosuppression, while their clinical efficacy remains markedly limited (Aggarwal *et al*., 2017). According to these limitations, the plant-derived polysaccharides have emerged as highly promising natural therapeutic candidates, due to the properties on the potent immunomodulatory, anti-inflammatory, and gut microbiota-regulating (Wang *et al*., 2022).

Polysaccharides, one important bio-active constituents in traditional Chinese medicine (TCM), have great research value based on their regulating intestinal health. Current research indicated that polysaccharides protect intestinal health through the following mechanisms: repairing colonic function, reducing inflammatory cell infiltration, modulating gut microbiota composition and diversity, reducing pro-inflammatory cytokine expression, and promoting the restoration of integrity of intestinal mucosal barrier (Jin *et al*., 2021; Wei *et al*., 2025).

Phyllanthus emblica fruits rich in various bio-active components, including polyphenols, polysaccharides, vitamins, amino acids, and trace elements (Gul *et al*., 2022). It is commonly used to alleviate symptoms arising from damp-heat stasis on the large intestine in TCM, such as dysentery and hematochezia. Within the theoretical framework of TCM, UC is categorized under this syndrome. *Phyllanthus emblica* is characterized by the TCM medicinal properties of cooling yet non-irritating, astringent yet non-stagnant, a trait that aligns with modern therapeutic principles of alleviating intestinal inflammation without impairing mucosal integrity (Chen *et al*., 2024; Huang *et al*., 2025). As a key active component of the fruit of *P. emblica*, polysaccharides have attracted considerable attention for their ability to modulate the gut microbiota and host metabolism (Song *et al*., 2020). While *P. emblica* polysaccharides have been reported to exert therapeutic effects on colitis, insufficient research exists to elucidate their therapeutic mechanisms for UC - an illness driven primarily by gut microbiota dysbiosis (den Besten *et al*., 2013) - *via* modulating gut microbiota and its metabolites. Although many studies link the UC pathogenesis to intestinal microbiota and metabolomic disturbances, the key microbial taxa and metabolites for UC treatment remain unclear. Especially, the mechanisms of PEP *via* microbial-metabolite networks are not well understood. Exploring these determinants could provide a stronger theoretical basis for UC therapy.

Therefore, this work targeted to investigate the therapeutic potential of *Phyllanthus emblica* polysaccharide (PEP) against UC, with a particular focus on the regulatory effects on the gut microbiota and host metabolism. PEP was isolated using water extraction and ethanol precipitation, and its efficacy was evaluated by considering murine colitis model. We hypothesized that PEP alleviates colitis not only through direct anti-inflammatory actions but also by restoring gut microbial homeostasis and reprogramming relevant metabolic pathways. The findings of this study would provide a multi-target therapeutic strategy for UC and offer a theoretical foundation for its prevention and treatment (Wirtz *et al*., 2007).

MATERIALS AND METHODS

Material: DSS was purchased from MP Biomedicals (USA). Primary antibodies including anti-NF-κB p65 (Affinity Biosciences) and anti-Occludin (Servicebio) were used. Fresh *Phyllanthus emblica* fruits were sourced from Yunnan Tianqi Biotechnology Co., Ltd. *Phyllanthus emblica* fruit polysaccharide (PEP) was extracted by alcohol precipitation method, using a modified water extraction. The 1500g dried seedless pulp was degreased with 95% ethanol. Then, the sample was extracted with double-distilled water at 100℃ at (1:8) solid-liquid ratio, collecting the supernatant. The fourfold volume of 95% ethanol was added to the supernatant to precipitate polysaccharides at room temperature, and the polysaccharide precipitate was harvested by centrifugation. The precipitate was redissolved and deproteinized using Sevag reagent composing chloroform and n-butanol (4:1). Sample was then transferred into 3500 Da MWCO dialysis bag and dialyzed against water for 48h. Following concentration, the sample was freeze-dried to obtain 46.23g of *Phyllanthus emblica* polysaccharides.

Animal experiments and design: The animal experiments had been approved by IACUC of Yunnan Agricultural University (No. APYNAU-2025-03089). The female mice (6-8 weeks old with weight ranging from 70-80g, obtained from Beijing SPF were acclimatized for 7 days and randomly divided into 4 groups (n = 8): the control group received saline for 14 days; the UC model group (Model group) received 3% DSS for 14 days; the APS (Astragalus polysaccharides) treatment group (APS group) and the PEP treatment group (PEP group) received 3% DSS for 14 days, supplemented with 50mg/kg APS or PEP, respectively, during the last 7 days. The body weight and disease activity index (DAI) were monitored everyday. On day 14, mice were euthanized for sample collection.

Histopathological and Immunohistochemical Assessment: Colon length was measured immediately after euthanasia. Tissues were processed by H&E and AB-PAS staining. Histopathological scoring was performed to evaluate inflammatory infiltration, crypt architecture damage, and goblet cell depletion, according to a 0-4 semi-quantitative scale as reported by (Xiong *et al.*, 2022). Immunohistochemistry was performed to assess the expression of tight junction protein (ZO-1 and Occludin).

Biochemical and Molecular Analyses: The pro-inflammatory cytokine levels (IL-1β, TNF-α, IL-10 and IL-6) in mouse colon tissue were assayed using commercial ELISA kits (Elabscience, China, E-MSEL-M0003, E-MSEL-M0002, E-MSEL-M0001). Oxidative stress was analysed by measuring malondialdehyde (MDA) via MDA Content Assay Kit (Boxbio Techsupport, Beijing) content and activity of total superoxide dismutase (T-SOD) using T-SOD Content Assay Kit (Boxbio Techsupport, Beijing). Total RNA was extracted from colon tissues for assay the IL-6, TNF-α, and Il-1β mRNA expression by quantitative real-time PCR, referenced to β-actin.

16S rRNA Sequencing: The colonic contents were used to extract genomic DNA via OMEGA DNA Kit (Bio-Tek, Norcross, GA, USA). The forward and reverse primers 338F/806R F: ACTCCTACGGGAGGCAGCA and R: GGACTACHVGGGTWTCTAAT were employed to amplify the region of bacterial 16S rRNA gene (V3-V4). The instrument used for sequencing was Illumina MiSeq platform. Raw sequences were processed using QIIME2, including quality filtering, denoising, chimera removal, and amplicon sequence variant (ASV) clustering.

The SILVA database reference dataset was considered for Taxonomic classification. Alpha diversity (Shannon, Chao1) and beta diversity analyses were done.

Untargeted Metabolomic Analysis: To characterize metabolites in fecal samples, non-targeted metabolomics was performed using UHPLC-Orbitrap LC-MS. Fecal samples were extracted with pre-chilled 80% methanol (incubating on ice), then centrifuged at 15,000 g and 4℃. The supernatant was diluted to 53% methanol with LC-MS grade water and got sediment again. The final supernatant was used for LC-MS/MS analysis. Then chromatography was separated by Hypersil Gold C18 column (100 × 2.1mm, 1.9μm) at 40℃ with a 5μL injection volume, 0.2mL/min rate of flow, and 12-min linear gradient. In positive mode, moving phases were 0.1% formic acid in water (A) and methanol (B); in negative mode, were 5 mM ammonium acetate (pH 9.0, A) and methanol (B). The mass spectrometry was performed by Orbitrap Exploris 120 system for both of positive and negative ESI modes with data-dependent acquisition, and QC samples were analyzed to ensure stability. Raw data were qualitative and quantitative analyzed using Compound Discoverer 3.3 and annotated against mzCloud and MassList databases. Multivariate statistical analysis was performed in R, and different metabolites were selected with VIP>1, $P<0.05$, and FC≥2 or ≤0.5, followed by annotation and pathway enrichment using KEGG and HMDB databases.

Statistical analysis*:* Data were presented as mean ± standard deviation (mean ± SD). Statistical analysis among different groups were conducted using one-way ANOVA followed by Tukey's post-hoc test. Significance was set at $P<0.05$. Statistical analysis was done via GraphPad Prism 8.0 and R software.

RESULTS

The PEP protective affects on DSS-induced UC mice: After 7 days of free access to 3% DSS (Fig. 1A), mice developed typical colitis symptoms, including weight loss, loose stools, and hematochezia, confirming successful model establishment. As shown in (Fig. 1B), mice in the Model group exhibited the most pronounced body weight loss contrasted to Control group. Oral administration of APS or PEP from day 7 onward attenuated this weight loss and reduced the incidence of bloody stools in the respective treatment groups. Consistently, Disease Activity Index (DAI) scores were lower in both the PEP- group and APS-treated groups as compared to the Model group (Fig. 1C). Furthermore, PEP treatment ameliorated DSS-induced colon shortening, with effects comparable to those of APS group (Fig. 1D, E). Collectively, these results illustrated that PEP exercises a significant protective effect in DSS-induced colitis.

Histopathological changes in colon tissues: To evaluate the effect of PEP on colon pathology, tissue was stained by H&E and AB-PAS. As shown in (Fig. 2A and B), contrasted to Control group, colon tissues from the Model group displayed severe inflammatory lesions, including inflammatory cell infiltration, mucosal damage, and altered crypt architecture. In contrast, both APS and PEP treatments markedly alleviated these pathological features. The integrity of colon tissues was improved. The inflammatory cell infiltration was reduced in the APS and PEP groups relative to the Model group. Histological scoring based on H&E staining indicated that the Model group had significantly greater inflammatory scores than the control group, whereas both APS group and PEP treatment group significantly lowered these scores ($P<0.05$).

Whereas, the AB-PAS staining (Fig. 2C and D) demonstrated that the dextran sulfate sodium (DSS) exposure reduced the goblet cells in the Model group. While both APS and PEP treatments effectively were increased their abundance. The protective effects of PEP on colon tissue integrity and goblet cell content were comparable to those of APS. Above all, PEP supplementation significantly ameliorated colon tissue damage in DSS-induced UC mice.

PEP strengthened the intestinal barrier integrity: Occludin and ZO-1, the intestinal epithelial tight junction complex, play as the indispensable role in maintaining paracellular barrier integrity. In the dextran sulfate sodium -induced UC mice, a marked downregulation in expression of ZO-1 and Occludin protein was observed in the tissues of colon as compared with the control group (Fig. 3A and B). Moreover, the disruption of the intestinal mechanical barrier during active inflammation was observed. Notably, intervention with PEP effectively counteracted this DSS-induced damage. PEP treatment significantly increased the expression of proteins such as Occludin and ZO-1, reached to the protein expression level in Control group. PEP's regulatory effects on tight junction proteins were strongly correlated with the observed improvements in colonic histology and clinical symptoms. In summary, these results illustrated that PEP exerts a therapeutic effect against UC by enhancing epithelial tight junction integrity and improving intestinal barrier function.

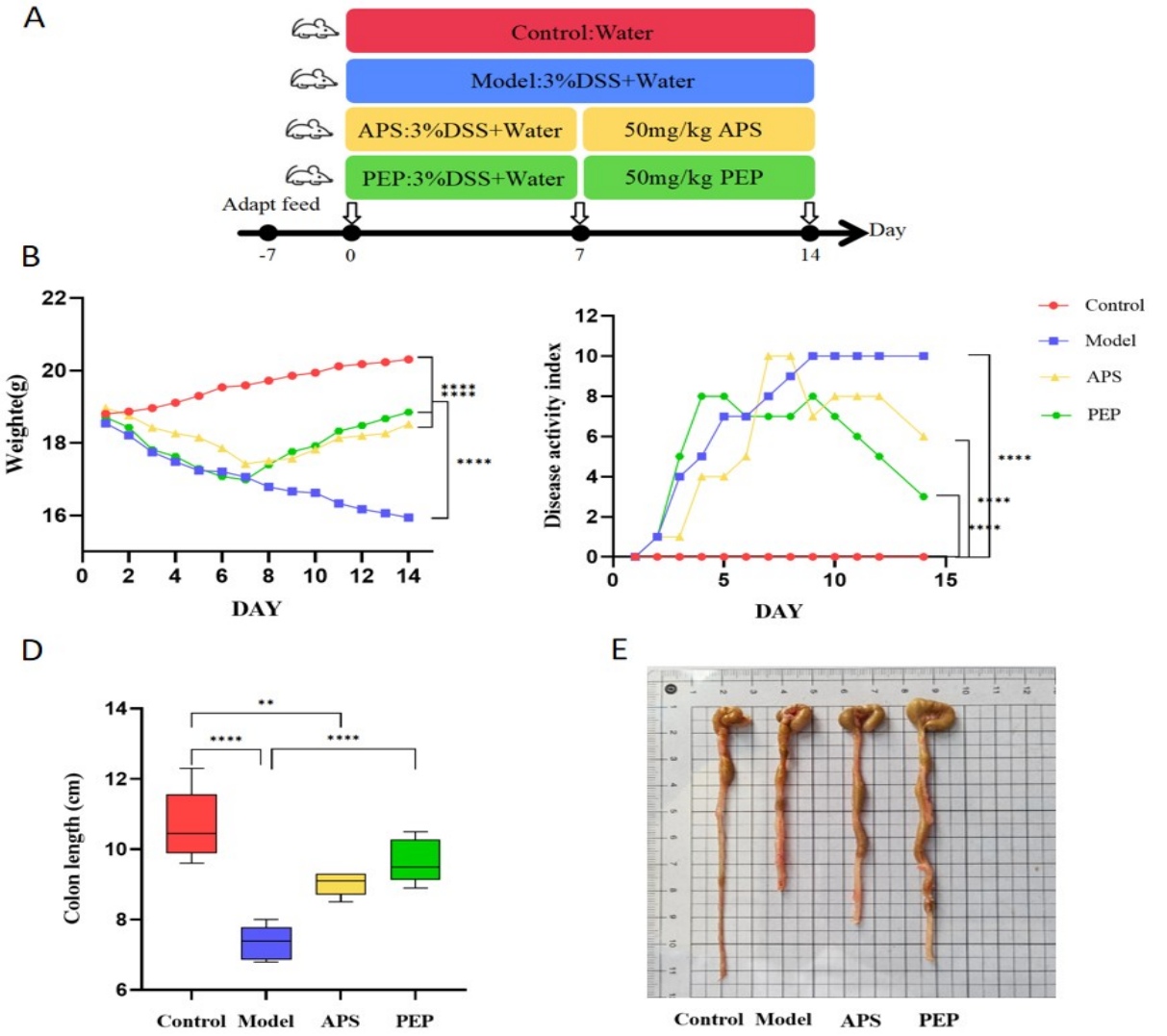


Fig. 1: PEP markedly alleviated disease symptoms in UC mice. (A) Experimental design; (B) Changes in body weight; (C) Disease activity index (DAI) scores; (D, E) Colon length measurements. Data are presented as mean ± SD (n=6).

PEP reduced the oxidative stress and alleviated the inflammatory damage: To assess the anti-inflammatory effect of PEP, we measured the levels of key pro-inflammatory cytokines in colonic tissue. As shown in (Fig. 4A and C), DSS induction significantly enhanced the levels of IL-6, TNF-α, and IL-1β in the Model group to Control group. The PEP treatment significantly reduced the pro-inflammatory cytokines as compared to the Model group, with effects comparable to those APS group. Oxidative stress parameters were also evaluated. The malondialdehyde (MDA) content and Myeloperoxidase (MPO) activity were markedly enhanced in Model group, additionally the activity of total superoxide dismutase (T-SOD) was decreased, indicating severe oxidative damage (Fig. 4D and E). PEP intervention effectively reversed these changes, significantly lowering MPO and MDA levels and enhancing T-SOD activity. Notably, PEP exhibited a stronger regulatory effect on these oxidative markers than APS.

RT-PCR analysis revealed that (Fig. 5A and C), the mRNA expression levels of IL-1β and IL-6 in the colon tissues of model mice were extremely significantly increased than control level, confirming the successful establishment of the inflammatory model, whereas no significant difference was observed in TNF-α expression. Following treatment with APS and PEP, the expression of IL-1β and IL-6 in both groups were extremely significantly downregulated, returning to levels comparable to the control group. Notably, TNF-α expression at both the protein and mRNA levels was significantly reduced by PEP, whereas no statistically significant effect on TNF-α mRNA was exhibited by APS. A key observation was the differential impact of PEP and APS on TNF-α transcriptional activity, with a more robust inhibitory capacity demonstrated by PEP. Although DSS treatment failed to induce a significant increase in mRNA and TNF-α level in model group, its expression was effectively downregulated to sub-basal levels following PEP treatment. The potent regulatory influence of PEP on TNF-α signaling is underscored by this high-intensity modulation. Collectively, these results demonstrate that DSS-induced UC is alleviated by PEP through the suppression of pro-inflammatory cytokine production and the attenuation of oxidative stress, with its multi-targeted anti-inflammatory and antioxidant properties highlighted.

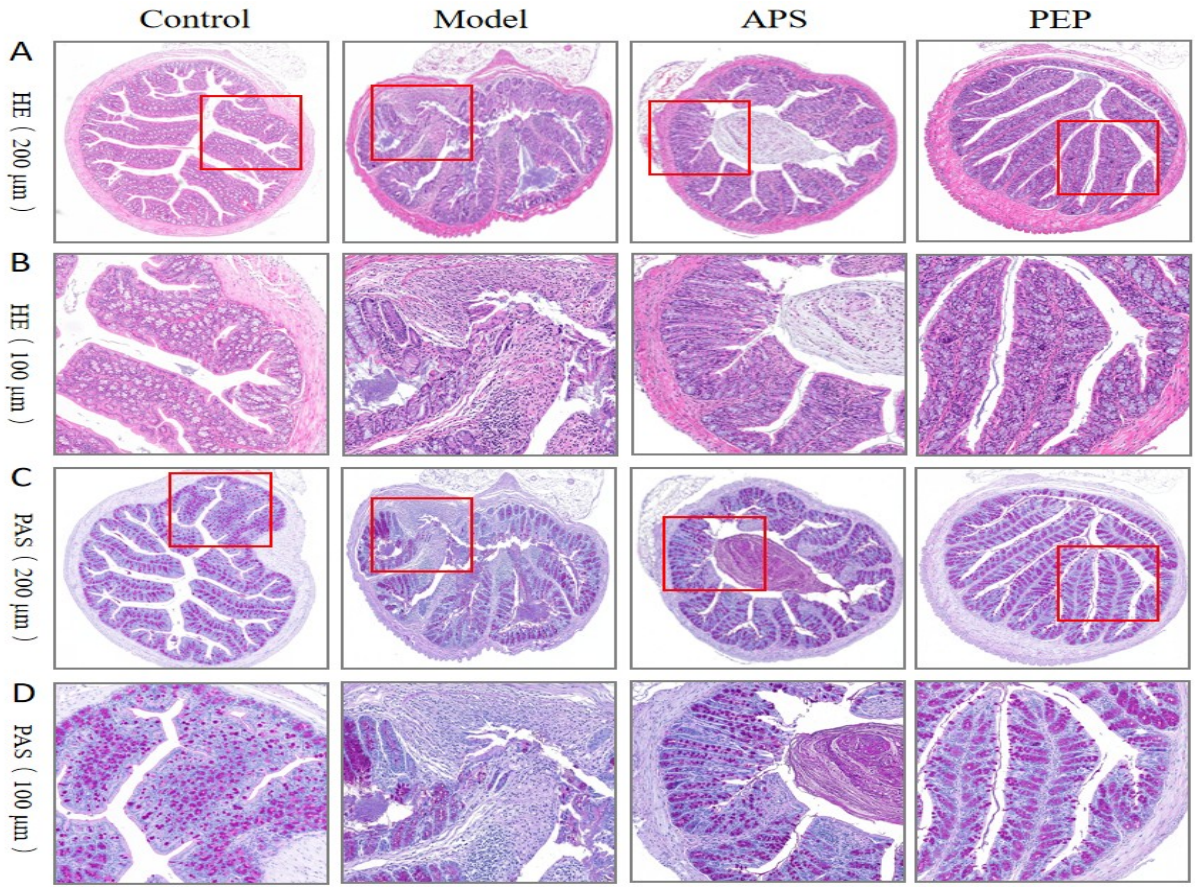


Fig. 2: PEP restored pathological damage in colon. (A, B) H&E-stained sections of the distal colon of different treatment; (C, D) AB-PAS staining and statistical analysis.

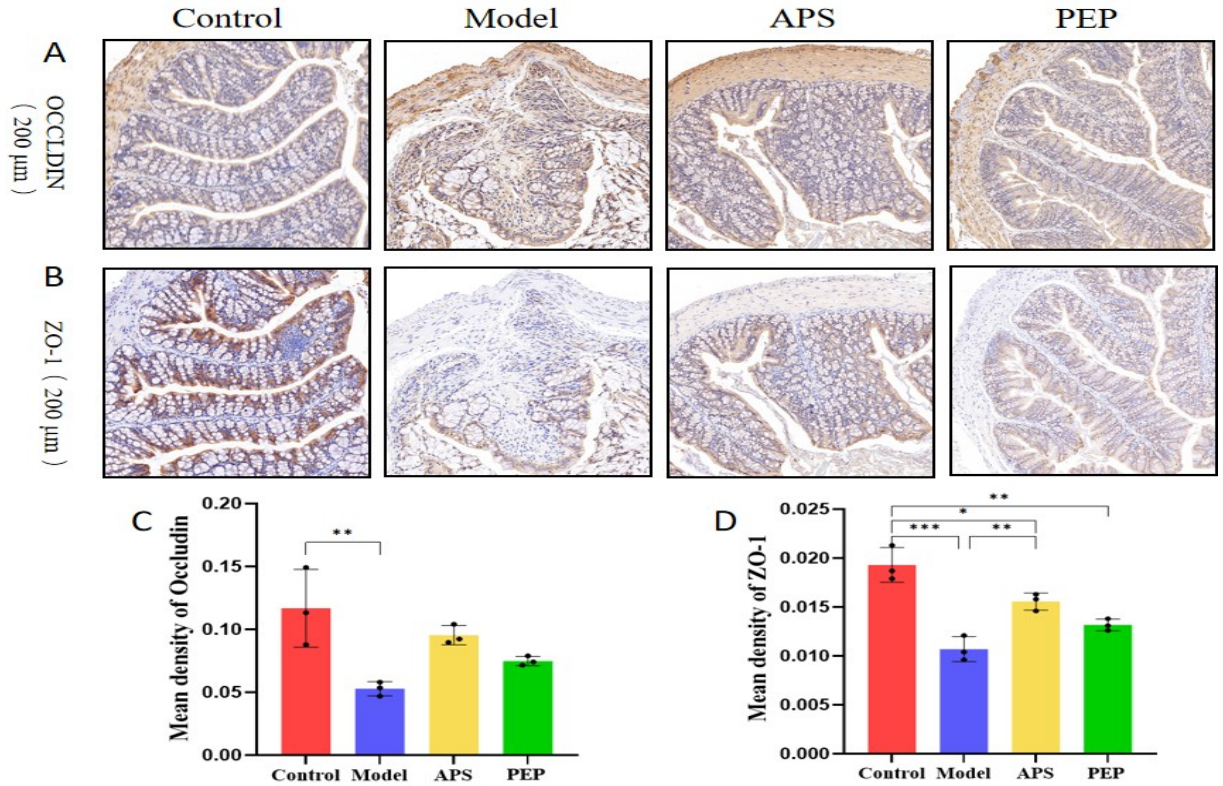


Fig.3: Effect of PEP on intestinal barrier integrity. (A, C) IHC staining of colon ZO-1 andoccludin-1 of mice in each group (X 200 magnification) (B, D).

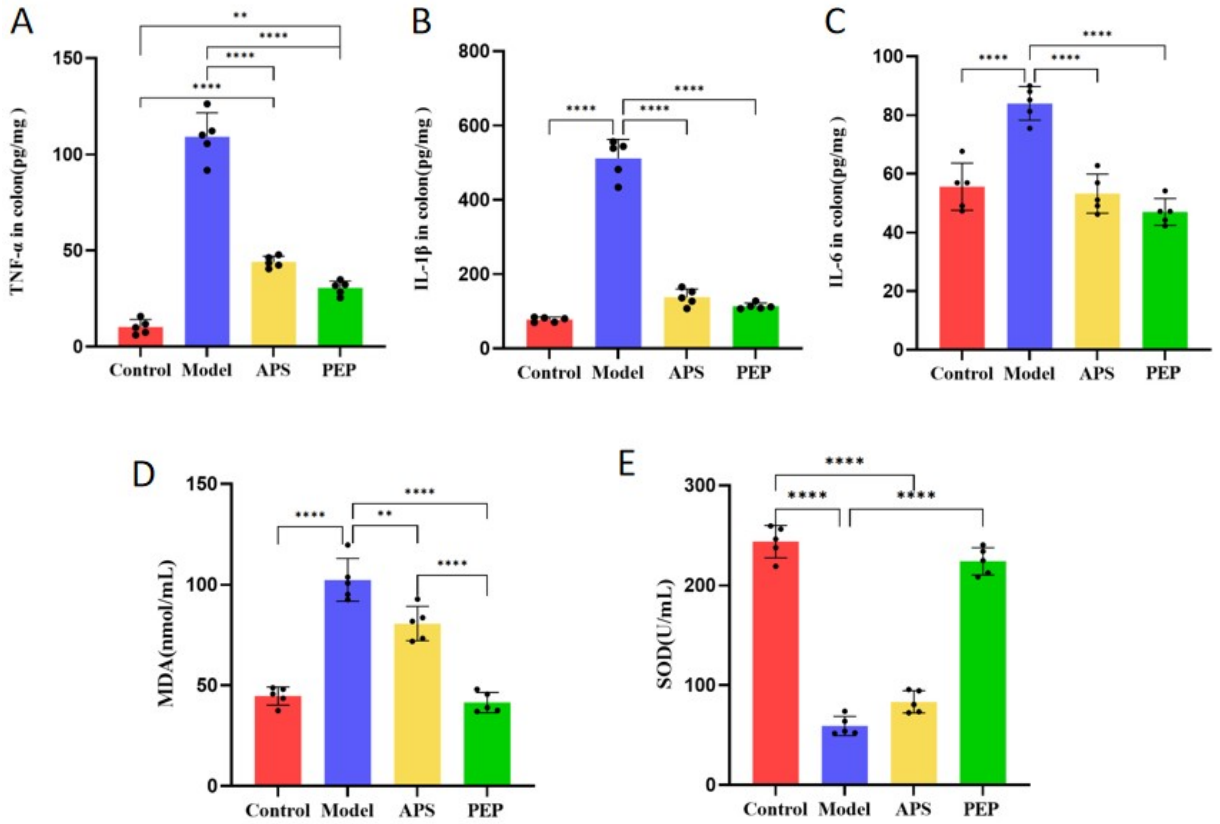


Fig. 4: Effects of PEP on inflammation and oxidative stress. PEP treatment reduced levels of TNF-α (A), IL-1β (B), IL-6 (C), MDA (D), and SOD (E) in colon tissues. Data are presented as mean ± SD (n=5).

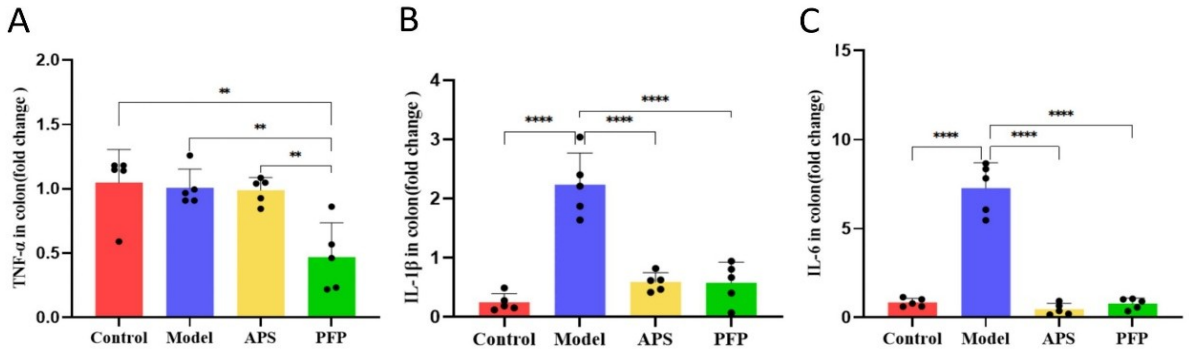


Fig. 5: mRNA expression levels of TNF-α (A), IL-1β (B), and IL-6 (C) in mouse colon tissue were measured by RT-PCR. Data are presented as mean ± SD (n=5 per group).

PEP effects on intestinal flora diversity: The 16S rRNA gene sequencing was performed to assess the modulatory effects of PEP on gut microbiota in ulcerative colitis (UC). Venn diagram analysis identified a total of 1,986 operational taxonomic units (OTUs) across all groups, with 554, 477, 480, and 475 unique OTUs detected in the Control, Model, APS, and PEP groups, respectively (Fig. 6A).

A significant reduction in the Chao1 index was confirmed in the animals of Model group as compared to the Control group's animals, reflecting diminished microbial richness. Notably, both PEP and APS treatments significantly increased the Chao1 index compared with untreated UC mice ($P<0.05$; Fig. 6B), suggesting a restorative effect on microbial diversity. On the other hand, the rank-abundance curves of PEP groups distributed broader than the other groups, suggesting an enhanced microbial evenness and richness (Fig. 6C). The PCoA results showed the clear separation among the Model, PEP, APS, and Control groups. While, the PCoA of PEP covered the PCoA of APS group (Fig. 6D). At the phylum level, the intervention of PEP group and APS group made Firmicutes A flora significantly lower than UC mice, and Firmicutes D flora were increased. Notably, PEP exerted a more robust regulatory effect than APS. It was characterized by specifically elevating the relative abundances of *Verrucomicrobiota* and *Bacteroidota*, and the Firmicutes reducing. This indicated that PEP primarily acts by selectively inhibiting dysbiosis-associated taxa (Fig. 6E). At the species level, further analyses identified distinct modes of action between PEP and APS. PEP selectively enriched

the *Bacteroides acidifaciens, Dubosiella sp. 000403415* and *Schaedlerella* sp. *000403295* among others (Fig. 6F). Based on the functional prediction results, PEP may potentially restored microbial function and host health by enhancing the formation of short-chain fatty acids and polysaccharide metabolism.

The 16S rRNA and Untargeted Metabolomics Correlation Analysis Reveals Key Metabolic Pathways Regulated by PEP: In the 16S rRNA dataset, comparing to UC mice, PEP selectively enriched the abundance of commensal bacteria, *Bacteroides acidifaciens* and *Paraprevotella clara*. Based on the prediction of metabolic functions from the 16S rRNA dataset, that exhibited significant increase, four metabolic pathways, namely the L-lysine fermentation to acetate and butanoate pathway, the dTDP-L-rhamnose biosynthesis I pathway, the L-histidine biosynthesis pathway, and the peptidoglycan biosynthesis I pathway, might be crucial pathways (Fig. 7A-D).

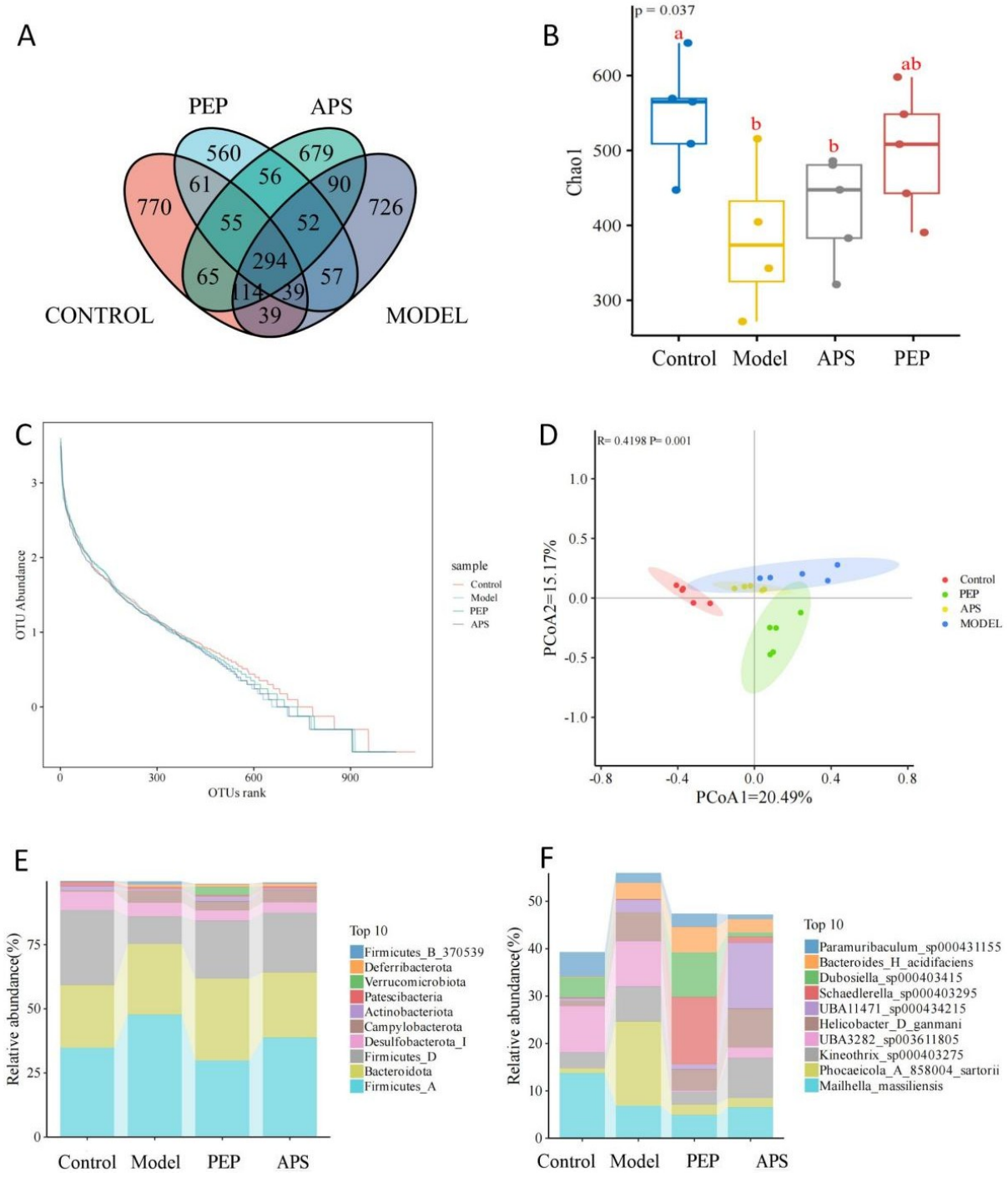
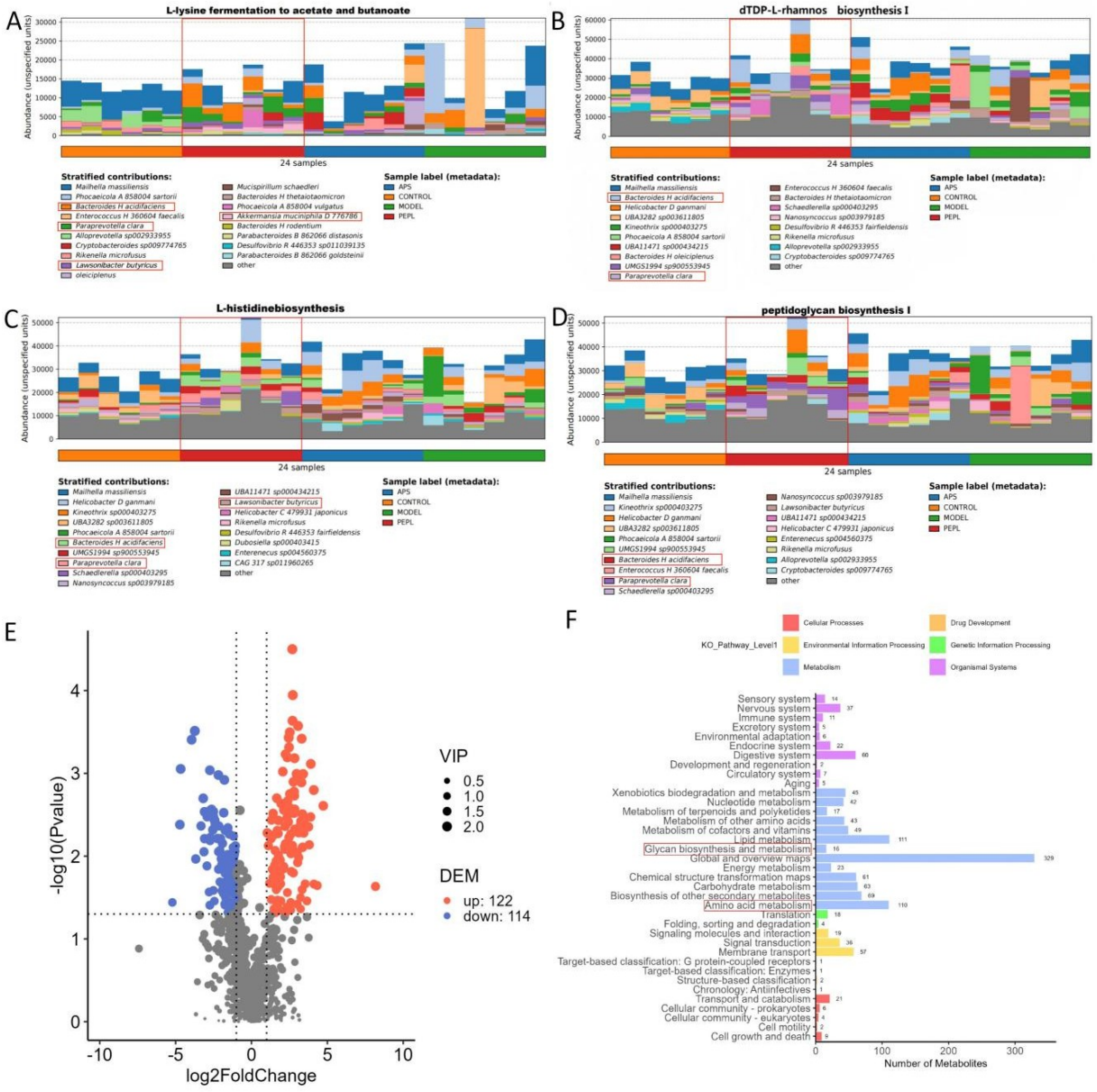


Fig. 6: Effects of PEP on gut microbiota diversity in UC mice. (A) Venn diagram; (B) Chao1 index; (C) Rank-abundance curve; (D) Principal coordinate analysis (PCoA) based on Bray-Curtis distance; (E, F) Relative abundance of bacterial taxa at the phylum (E) and species (F) levels in each group.



G Table 1

Name	Up	Down	KEGG pathway annotation
L-lysine fermentation to acetate and butanoate	Pipecolic acid、2-Ketobutyric acid、Indoleacetic acid、3-Methoxyphenylacetic acid		Metabolism → Amino acid metabolism
dTDP-L-rhamnose biosynthesis I	Rhamnose		Metabolism → Glycan biosynthesis and metabolism
L-histidine biosynthesisL	N-Acetyl-beta-alanine	prolyl-phenyl-alanine	Metabolism → Amino acid metabolism
peptidoglycan biosynthesis I	N-Acetyl-beta-alanine	prolyl-phenyl-alanine	Metabolism → Glycan biosynthesis and metabolism

Fig. 7: Taxonomic composition of differential pathways. L-lysine fermentation to acetate and butanoate (A); dTDP-L-rhamnose biosynthesis I (B); L-histidine biosynthesis (C); peptidoglycan biosynthesis I(D); Volcano plot of differential metabolites between PEP VS Model groups(E); KEGG pathway annotation(F); the differential metabolites that are associated with four target metabolic pathways(G).

According to untargeted metabolomics data, compared with the Model group, PEP groups were significantly different in glycan biosynthesis metabolism and amino acid metabolism (Fig. 7E). The volcano plot (KEGG annotated) revealed that, compared to the Model group, PEP significantly upregulated 122 metabolites and downregulated 114 metabolites (Fig. 7F).

Combining the 16S rRNA metabolic function prediction data with metabolomics data (Fig. 7G), it was observed that the L-lysine fermentation to acetate and butanoate pathway in the PEP group exhibited an upregulation trend. This aligns with the high expression results of acetic acid and butanoic acid-related products (such as pipecolic acid, 2-ketobutyric acid, indoleacetic acid, 3-methoxyphenylacetic acid) within short-chain fatty acids (SCFA). The dTDP-L-rhamnose biosynthesis I pathway in the PEP group also showed upregulation, consistent with the high expression results of rhamnose products in metabolomics. Similarly, the L-histidine biosynthesis pathway and the peptidoglycan biosynthesis I pathway in the PEP group were upregulated, matching the high expression results of N-Acetyl-beta-alanine products.

The commensal bacteria metabolic function prediction results and untargeted metabolomics results indicated that PEP significantly enhanced the *Bacteroides acidifaciens* abundance and *Paraprevotella clar*a and improved levels of metabolic pathways. Their products are related to SCFA, rhamnose, histidine metabolism, and peptidoglycan. The data showed this process is a key mechanism through which PEP modulates microbiota and metabolism.

DISCUSSION

UC is a chronic inflammatory bowel disease affecting the colonic mucosa, clinically characterized by persistent diarrhea, mucopurulent bloody stools, and abdominal pain (Ng SC *et al*., 2017). Its pathogenesis is complex and involves the interplay of multiple factors, including genetic susceptibility, intestinal immune dysregulation, and gut microbiota dysbiosis (Zhang *et al.*, 2021). As key bio-active constituents of TCM, plant polysaccharides exert dual effects in immunomodulation and gut microbiota regulation. The therapeutic potential of various plant-derived polysaccharides in experimental UC models has been widely documented. For instance, Crataegus pinnatifida polysaccharide (CPP) ameliorates UC by modulating the gut microbiota to enhance arginine biosynthesis via ASS1 upregulation, thereby counteracting inflammation, oxidative stress, and intestinal barrier dysfunction; alginate oligosaccharide alleviates *Clostridium perfringens* induced intestinal mucosal damage in weaned pigs by downregulating the apoptotic signaling pathways (Donohoe DR *et al*., 2021; Wei *et al*., 2025). Therefore, this work investigated the therapeutic potential of *Phyllanthus emblica polysaccharide* against UC, with a particular focus on the regulatory effects on the gut microbiota and host metabolism.

The DSS-induced UC mice model exhibited the classical UC symptoms, including weight loss, bloody diarrhea, and colon shortening. PEP administration effectively reversed these clinical symptoms: improved DAI, reduced colon shortening, and promoted weight recovery. Histopathology results revealed that PEP treatment reduced the inflammatory cell infiltration, with the restoration of intestinal villus structure and goblet cell morphology. PEP upregulated the protein expression of Occludin and ZO-1 (tight junction proteins) in the tissues of colone, thereby enhanced the barrier integrity. Simultaneously, PEP suppressed the pro-inflammatory cytokines, including the IL-1β, TNF-α and IL-6, and also reduced the level of oxidative stress markers (MPO, MDA). These results were consistent with the bio-active polysaccharides, such as those from Grifola (Liu *et al.*, 2024).

The 16S rRNA sequencing revealed that PEP selectively enriched beneficial commensals such as *Bacteroides acidifaciens* and *Paraprevotella clara*. The *Bacteroides acidifaciens*, a key producer of acetate and propionate, played as a central role in complex polysaccharide degradation (Blandford *et al*., 2019; Wu *et al.*, 2024). The enrichment of *Paraprevotella clara* suggested its potential role in acetate and propionate generation (Morotomi *et al*., 2009; Wang *et al*., 2022); and *Lawsonibacter* species are recognized as butyrate-synthesizing bacteria (Sakamoto *et al*., 2018; Manghi *et al*., 2024). The coordinated enrichment of these taxa suggests that PEP may exert its effects by regulating a microbial community with complementary functions in SCFA generation.

Functional prediction analysis further supports this finding. After PEP intervention, the aforementioned bacteria showed significantly increased functional contribution to the L-lysine fermentation to acetate/butanoate pathway, directly linked to SCFA biosynthesis (Parada *et al.*, 2019; Dias *et al*., 2025). Concurrently, their enhanced contribution to the peptidoglycan biosynthesis pathway suggested a potential strengthening of cellular growth and colonization capacity (Wheeler *et al.*, 2024). These predictions based on 16S data suggest that PEP may directly promote SCFA synthesis by enhancing the specific metabolic functions of bacteria such as *Bacteroides acidifaciens*, *Paraprevotella clara*. This prediction found crucial resonance in the host metabolomic data (Zhang *et al.*, 2022; Yang *et al.*, 2025).

The Untargeted metabolomics results corroborated the functional prediction of 16S rRNA results. The L-lysine fermentation to acetate and butanoate pathway in the PEP group showed an upregulation trend, which aligns with the high expression of acetic acid and butanoic acid-related SCFA products such as: pipecolic acid, 2-ketobutyric acid, indoleacetic acid, 3-methoxyphenylacetic acid. Also, the dTDP-L-rhamnose biosynthesis I pathway in the PEP group was upregulated, consistent with the high expression of rhamnose products in metabolomics. Similarly, the L-histidine biosynthesis and peptidoglycan biosynthesis I pathways in the PEP group were upregulated, matching the high expression of N-Acetyl-beta-alanine products. The SCFAs were particularly efficiently utilized by host enterocytes for membrane synthesis and repair (Donohoe *et al.*, 2011; den Besten *et al.*, 2013). Established research clearly demonstrates that SCFAs can directly promote tight junction protein expression, suppress inflammatory responses, and support epithelial repair through the mechanisms including the activation of G-protein-coupled receptors and histone deacetylase inhibition (Chakraborty *et*

al., 2025).

This study has several limitations. Firstly, findings from the acute DSS-induced murine colitis model may not be directly translatable to human UC, due to the inherent interspecies physiological and genetic differences. Clinical studies are therefore essential to validate the treatment effects and safety profile of PEP for patients. Secondly, the chronic and relapsing nature of UC requires assessment of long-term treatment outcomes, which was not addressed in our acute intervention setting. Furthermore, our integrated multi-omics approach revealed associated changes in gut microbiota function and host metabolism, but the upstream signaling mechanisms through which PEP initiates these changes remain uncharacterized. Future investigations should employ molecular biology techniques-such as targeted protein assays and pathway modulation experiments-to directly validate candidate signaling axes (e.g., NF-κB, MAPK, Nrf2) in cellular and animal models. Complementary approaches, including germ-free animal models and bacterial co-culture systems, would help establish causal relationships between specific bacterial taxa, their metabolic outputs, and the observed therapeutic effects.

Conclusions: This study demonstrated PEP alleviates ulcerative colitis through targeted gut microbiota restructuring. PEP treatment specifically enriched beneficial bacteria (e.g. *Bacteroides acidifaciens, Paraprevotella clara*) and boosted their production (SCFAs, rhamnose, histidine metabolism, and peptidoglycan.) on host-repairing. This triggered a reparative shift in the host metabolome, which enhanced barrier function, reduced inflammation, and ameliorated disease symptoms. Our results highlight PEP's potential as a microbiota-targeted therapeutic strategy for ulcerative colitis.

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