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Research article

Therapeutic effects of Zhuling Jianpi capsule on experimental ulcerative colitis and characterization of its chemical constituents and metabolomics using UHPLC-Q-TOF-MS

Jian Li [b,c,1], Ziqi Zhou [a,1], Dan Liu [a], Haijuan Dong [d], Jianping Zhou [b,**], Jie Wu [a,*]

[a] Department of Life Science and Technology, China Pharmaceutical University, Nanjing, China
[b] Department of Pharmaceutics, China Pharmaceutical University, Nanjing, China
[c] Jinling Pharmaceutical Co., Ltd., Nanjing, China
[d] The Public Laboratory Platform, China Pharmaceutical University, Nanjing, China

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ABSTRACT

Zhuling Jianpi Capsule (Zhuling) is a traditional Chinese medicinal formula used to treat symptoms such as abdominal pain, bloating and diarrhea associated with inflammatory bowel disease (IBD). However, the protective effects of Zhuling on experimental ulcerative colitis (UC) and the effective substance responsible for its efficacy have rarely been reported. In this study, we evaluated the therapeutic effects of orally administrated Zhuling on DSS-induced UC in mice. The chemical constituents and metabolomics of Zhuling were qualitatively analyzed by ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS). The results showed that Zhuling treatment markedly alleviated DSS-induced clinical symptoms, restrained the secretion of pro-inflammatory cytokines, and improved intestinal epithelial barrier function. Furthermore, a total of 167 compounds have been identified or characterized, and 120 prototype components were detected in the urine, plasma, bile and feces of mice. Among them, altogether 26 representative prototypes were associated with 139 metabolites via the corresponding biotransformation pathways, and both of them mainly contained flavonoids, alkaloids, organic acids, monoterpenes, phenylpropanoids, triterpenoids, sesquiterpenoids and anthraquinones. Finally, 12 potent compounds mainly containing flavonoids, terpenoids and phenylpropanoids were screened out as potential quality control index components and might be the main substances that exert a pharmacological effect. Our data indicated that Zhuling administration prominently alleviates DSS-induced colitis in mice. Additionally, the chemical and metabolic profiling provided helpful information on the potential pharmacodynamic substances of Zhuling, which can be further investigated in the future.

Abbreviations: UC, ulcerative colitis; IBD, inflammatory bowel disease; CFDA, China Food and Drug Administration; UHPLC-Q-TOF-MS, Ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry; TCM, traditional Chinese medicine; DSS, dextran sulfate sodium; DAI, disease activity index; HE, hematoxylin and eosin; MPO, myeloperoxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

* Corresponding author.
** Corresponding author.
E-mail addresses: zhoujianping@cpu.edu.cn (J. Zhou), wujie@cpu.edu.cn (J. Wu).
[1] Contributed equally to this work.

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1. Introduction

Traditional Chinese medicine (TCM) prescriptions has been considered to perform therapeutic effects through a multiple-target and multiple-component model, and has relatively mild adverse reactions and toxicity [1]. Zhuling Jianpi Capsule (Zhuling), also known as Chang Dean Capsule, was authorized by China Food and Drug Administration (CFDA) in 2014. Though Zhuling has been applied in invigorating spleen and kidney and treating gastrointestinal diseases such as abdominal pain and diarrhea in hospitals and clinics in China, few clinical trials have been reported. A randomized, double-blind, placebo-controlled trial (No. CTR20171305) of Zhuling treatment has now been performed on 2000 patients with IBS and the results will be available soon. In animal experiments, Zhuling has been proven to have an anti-colitis effect in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced ulcerative colitis in guinea pigs [2]. More studies are needed to further elucidate the protective effect of Zhuling on gastrointestinal diseases.

Zhuling is made of shenling Baizhu Powder and pain laxative prescription with modern technology, composed of 18 Chinese herbs, including Drynaria roosii Nakaike (Polypodiaceae), Atractylodes Macocephala Koidz.(Asteraceae), *Codonopsis pilosula* subsp. tangshen (Oliv.) D.Y.Hong (Campanulaceae), Smilax glabra Roxb. (Smilacaceae), Coix lacryma-jobi var. ma-yuen (Rom.Caill.) Stapf (Poaceae)., Citrus × aurantium L. (Rutaceae), Phellodendron Amurense Rupr.(Rutaceae), Solidago Gigantea Aiton. (Asteraceae), Acalypha Australis L. (Euphorbiaceae), Alisma Plantago-aquatica L.(Alismataceae), Rheum palmatum L.(Polygonaceae), Areca catechu L. (Arecaceae), *Paeonia lactiflora Pall.*(Paeoniaceae), Lindera Aggregate (Sims) Kosterm. (Lauraceae), Mangnolia Officinalis Rehder & E. H.Wilson (Magnoliaceae), Saposhnikoviae Divaricatae (Turcz. ex Ledeb.) Schischk (Apiaceae), Neolitsea cassia (L.) Kosterm.(Lauraceae) *and* Glycyrrhiza glabra L (Fabaceae). (Note: All the medicinal materials in the formula have been certified with http://mpns.kew.org). Among the formulations, most of them have been proven anti-inflammatory, gastrointestinal mucosal protection effects, such as *Atractylodes Macocephala Koidz.* [3] *Paeonia lactiflora Pall.* [4] and *Smilax glabra* [5]. The components of single prescription such as *Atractylodes Macocephala Koidz* [6] and *Coicis Semen* [7] have been reported, however, the chemicalome and typical metabolome in Zhuling have not been systematically investigated, which will be meaningful to reveal the material basis of its medicinal effect.

Recently, an ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) method was employed to identify constituents from TCM prescriptions due to its high resolution, excellent sensitivity, and precise mass value of both precursor and product ions [8]. In this experiment, we used 2.5% dextran sulfate sodium (DSS) to construct a mouse model of chronic ulcerative colitis (UC), preliminarily explored the therapeutic effect of Zhuling on UC, and used UHPLC-Q-TOF-MS to characterize the chemical components and metabolic profile of Zhuling to provide chemical foundation for further research.

2. Material and methods

2.1. Animals and test drugs

Male eight-weeks-old C57BL/6J mice weighing 22–24 g were obtained from the Qinglong shan Animal breeding Farm (Nanjing, China). Male Wistar rats (260–280 g) were supplied by Experimental animal center of Yangzhou University (Yangzhou, China). All animals were maintained with standard rodent chow and water ad libitum in a standard SPF class facility at ambient temperature (22 °C) and a 12-h light-dark cycle. All mice were maintained under SPF conditions in Animal Experimental Center of China Pharmaceutical University (Nanjing, China). Animal care and all animal experiments were approved by university animal ethics committees (ethical approval number: 2021-04-006).

Zhuling was provided by Jinling Pharmaceutical Co., Ltd. The reference drug used in this experiment was Changyanning capsule, a compound herbal medicine often used to treat acute and chronic gastroenteritis, diarrhea, bacterial dysentery, and children's dyspepsia. The compound medicine primarily comprises five kinds of herbs: Brocade herb, Hedyotis flavescens, Camphor roots, Chinese elsholtzia and Maple leaves.

2.2. Induction of UC and Zhuling administration

As previously described [9], chronic colitis was induced by 2.5% (wt/vol) DSS (36–40 kDa, MP Biomedicals) in drinking water for two cycles of 5 days at interval of 7 days, and mice were sacrificed 3 days after the end of DSS-treated. All mice were divided into 5 groups andomly: Control: water + saline; Model: DSS + saline; Zhuling (L): DSS + Zhuling (40 mg/kg BW); Zhuling (H): DSS + Zhuling (120 mg/kg BW); Positive: DSS + Changyanning (180 mg/kg BW).

The Changyanning and Zhuling were intragastric administration daily from day 6 to day 20. All the drugs were administered at the same time. Meanwhile, the Model group mice were gavaged with equal amounts of saline. Daily weight change, stool consistency and rectal bleeding for scoring of disease activity index (DAI) were calculated as previously described [10]. Mice were executed on day 20 and the blood serum was collected.

2.3. Histopathological analysis

The fecal-free colonic tissues were fixed in 4% paraformaldehyde solution for 48 h, embedded and sliced by a microtome with a thickness of 4–5 μm. The section was stained with hematoxylin and eosin (H&E), and the histological score was assessed by four

individual inflammatory parameter scores: severity of inflammation, ulceration, area of inflammation involved, and hyperplasia and atypical hyperplasia, as previously described [11].

2.4. Western blot

Mice colon samples were snap frozen in liquid nitrogen and ground into homogenates in ice-cold RIPA lysate (Beyotime, Shanghai, China) containing 2 mM Protease inhibitor mixture (Glpbio, UCA). The lysates were centrifuge at 4 °C and 12,000 g for 15 min, and separated by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The blots were transferred to PVDF membranes, incubated with primary antibody against occludin (1:1000, Proteintech, Wuhan, China), and finally incubated with enzyme-labeled secondary antibody (1:5000, Beyotime, Shanghai, China). The final samples were exposed by development with the Enhanced Chemiluminescence Advanced Kit (Fudebiotechnology, Hangzhou, China) and blots were quantified with Image J.

2.5. Myeloperoxidase (MPO) enzyme activity

The colon tissues of mice was homogenized in pre-cooled saline and centrifuged (4000 g, 5 min) to obtain the supernatant. TMB solution (100 μL) (Beyotime, Shanghai, China) and colonic homogenate supernatant (10 μL) were added respectively in well plate, reacted at 37 °C for 5 min. MPO enzyme activity was measured by determining the absorbance at 450 nm at room temperature.

2.6. ELISA

The serum centrifuged (4000 g, 4 °C, 5min), and the supernatant was carefully collected, stored at −80 °C. The levels of TNF-α, IL-1β and IL-6 were measured by ultrasensitive ELISA Kit (Yutong, Jiangsu, China) according to the instructions of protocols.

2.7. Malondialdehyde (MDA) and superoxide dismutase (SOD) activity

SOD and MDA were detected in colon homogenate tissues by commercial kits (Nanjing Jiancheng Bioengineering Research Institute Co. Ltd, Nanjing, China) respectively, according to the method of kit instructions.

2.8. Quantitative reverse transcription PCR (qRT-PCR)

Total colonic RNA was extracted in low-temperature environment using AG RNAex ProReagent reagent (Accurate, Hunan, China). SYBR Green Premix Pro Taq HSqPCR Kit (Accurate, Hunan, China) was used toperform qRT-PCR experiments. qRT-PCR reaction conditions: 95 °C (30s) after pre-denaturation, denaturation 95 °C (7s), annealing 55 °C (30s), 72 °C (15s), 40 cycles. Finally, the relative expression of mRNA was calculated based on the amplification results according to the $2^{-\Delta\Delta Ct}$ method. Primer sequences: IL-1β-Fw, 5′-TGCCACCTTTTGACAGTGATG-3′, IL-1β-Rv, 5′-AAGGTCCACGGGAAAGACAC-3′; IL-6-Fw, 5′-GCCTTCTTGGGACTGATGCT-3′, IL-6-Rv, 5′-TGTGACTCCAGCTTATCTCTTGG-3′; TNF-α-Fw, 5′-ACCCTCACACTCACAAACCA-3′, TNF-α-Rv, 5′-ACCCTGAGCCA-TAATCCCCT-3′; GAPDH-Fw, 5′-AAGATGACCCAGATCATGTTTG-3′, GAPDH-Rv, 5′-AGCCAGTCCAGACGCAGGAT-3′.

2.9. Samples preparation of UHPLC-Q-TOF-MS

Subject drugs (Zhuling), rat plasma, urine, feces, bile and tissue sample preparation and further experimental details are available in supplementary material.

2.10. Material of UHPLC-Q-TOF-MS

A total of 20 reference standards (Chengdu Alpha Biotechnology Co., Ltd) were used in this experiment. The distilled water was filtered using Millipore's milliq system to obtain ultrapure water. Acetonitrile for LC-MS grade was purchased from Fisher Scientific (Fair Lawn, New Jersey, USA), and formic acid for LC-MS grade was purchased from Sigma-Aldrich (ST. Missouri, USA). The ultrapure water was obtained by filtration of distilled water using Millipore's Milli-Q system.

2.11. Instruments and condition of UHPLC-Q-TOF-MS

Chromatographic separation was performed using an ExonLC system (AB Sciex, Foster City, CA, USA). The chromatographic column was a Waters Acquity UHPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) with an operating temperature of 35 °C. The experiment was performed with 0.1% formic acid aqueous solution (v/v) as mobile phase A and acetonitrile solution (without formic acid) as mobile phase B. The following gradient program was used: 0–5min 3%–8% B, 5–11min 8%–30% B, 11–20min 30%–80% B, 20–21min 80%–95% B, 21–27min 95% B. 27–27.5min 95%–3% B, 27.5–32min 3% B. The sample injection volumes were all 2 μL. A 5600 Q-TOF mass spectrometer equipped with an electrospray ionization source (Turbo Ionspray) (AB Sciex, Foster City, CA, USA) was used to provide high-resolution detection in this experiment. MS detection work in positive and negative ion modes. Summary of mass spectrometer parameters: Gas 1 and Gas 2, 45 psi; Curtain gas, 35 lbs. Heat block temperature:550; ion spray voltage negative −4.5 kV and negative 5.5 kV; declustering potential,50 v; collision energy,35 v; collision energy spread (CES) of 15 V.

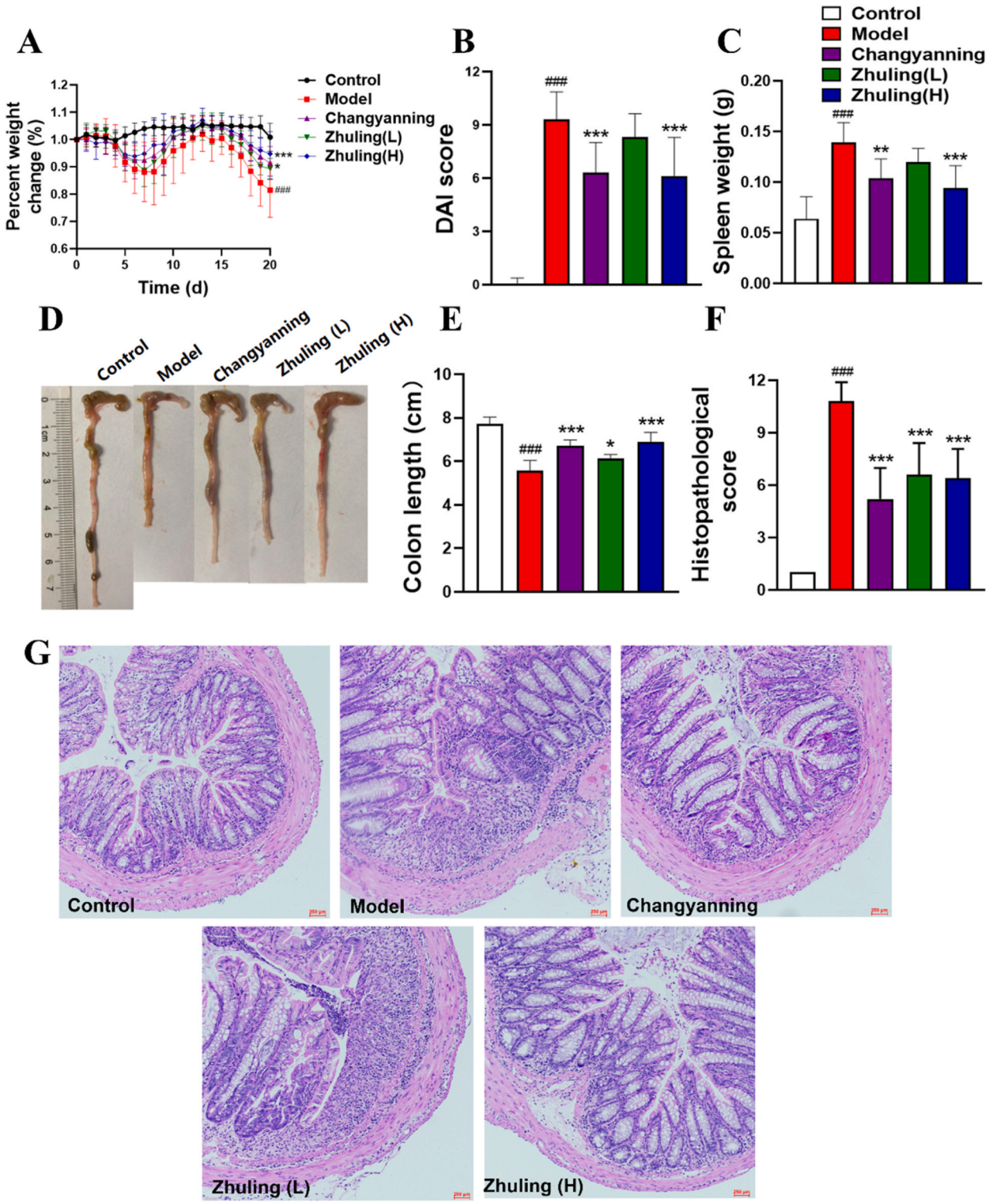


Fig. 1. Zhuling administration significantly alleviates DSS-induced colitis. A. Mean daily body weight change of mice in each group, n = 10. B. DAI score of mice in each group, n = 10. C. The spleen weight was recorded when mice were executed on day 22, n = 10. D-E. The entire colon of mice was isolated, the length was measured and recorded, n = 10. F-G. H&E staining was performed to examine histopathological changes in colonic tissues, Scale bar, 250 μm, n = 6. ###P < 0.001, compared with the Control group; *P < 0.05, **P < 0.01, ***P < 0.001, compared with the Model group.

2.12. Statistical analysis

GraphPad prism 5.0 software was used for data analysis. One-way analysis of variance (ANOVA) was used for statistical comparisons, and $P < 0.05$ was considered a statistically significant difference.

3. Result

3.1. Zhuling improved UC symptoms in mice

To evaluate the therapeutical effect of Zhuling on UC, mice were induced with 2.5% DSS and subsequently administered intragastrically with Zhuling, as well as the positive control drug Changyanning. Compared with the Control group, mice in the Model group had exhibited appetite, abnormally disheveled hair, poorer mental status, and persistent blood in the stool, accompanied by decreased body mass(Fig. 1A), which were consistent with the previous study [12]. Zhuling and Changyanning administration significantly improved DSS-induced clinical index of mice, such as body weight DAI score, spleen weight and colon length, especially in the Zhuling (H) group. (Fig. 1B–E). H&E histopathological examinations revealed that DSS-induced mice exhibited reduced goblet cells, severe mucosal injury and obvious inflammatory cell infiltration in the colon, while the structure of lesions showed less inflammatory cell infiltration and epithelial loss, and a lower histological score after administration of Zhuling and Changyanning (Fig. 1F and G).

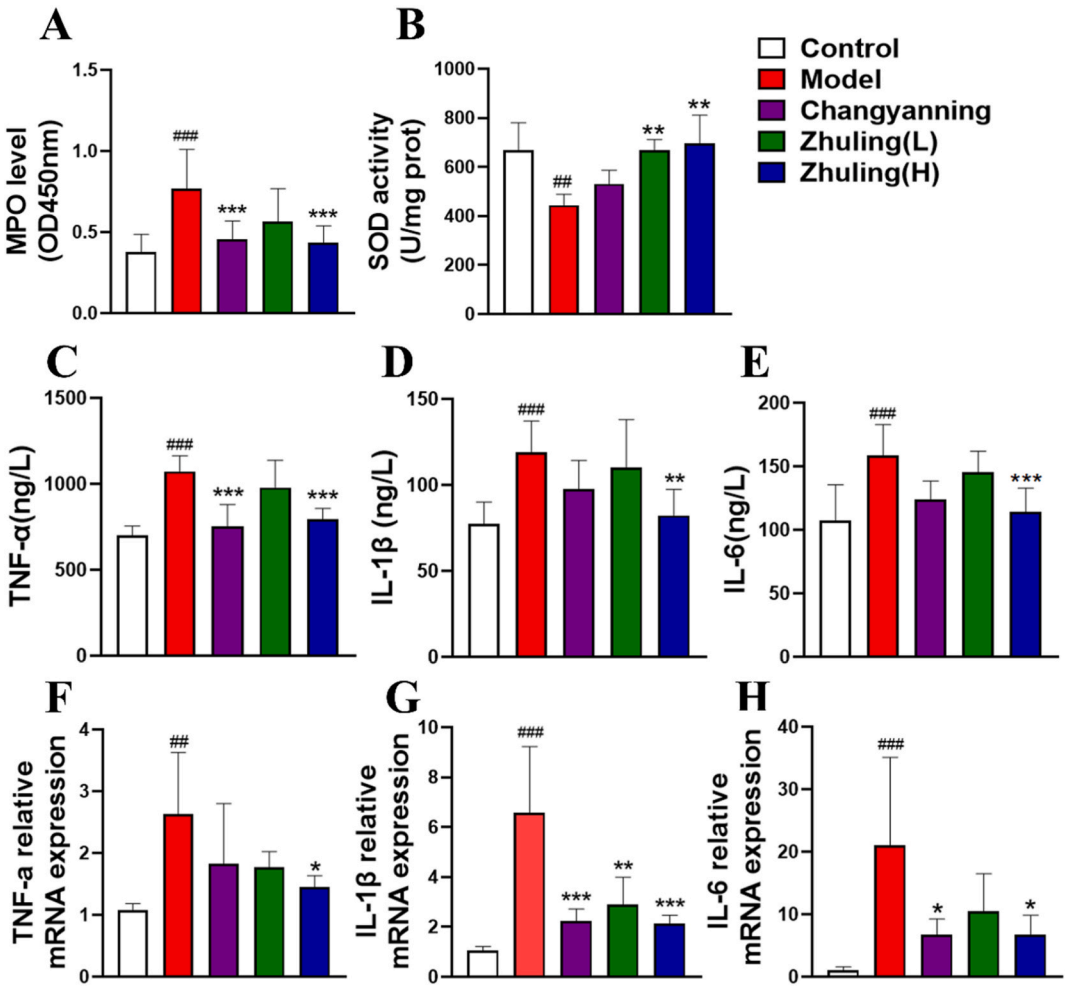


Fig. 2. Zhuling diminished proinflammatory cytokine production and oxidative stress in DSS-induced colitis mice. A. MPO activity was determined by the absorbance at a wavelength of 450 nm, n = 8. B. SOD activity of colon tissues, n = 8. C-E. The inflammatory cytokines levels in serum determined by ELISA, n = 8. F–H. The relative mRNA expression levels of the inflammatory cytokines in colon tissues, n = 8. ##P < 0.01, ###P < 0.001 compared with the control group *P < 0.05, **P < 0.01, ***P < 0.001 compared with the model group.

3.2. Zhuling diminished oxidative stress and proinflammatory cytokine production in mice with DSS-induced colitis

In order to evaluate the effects of Zhuling on oxidative stress in UC experimental mice, we measured the levels of MPO and SOD activity in colon tissues. In Model group, mice showed significantly higher MPO levels, but lower SOD activity compared to the Control group. As expected, Zhuling and Changyanning treatment resulted in decreased MPO levels and increased SOD activity (Fig. 2A–B). Excessive and uncontrolled inflammation is a key component of many epidemic diseases and can result in treatment failure. The ELISA and qRT-PCR results revealed that the pro-inflammatory cytokine levels in Model group were significantly higher compared to Control group, while Zhuling decreased the levels of all proinflammatory cytokines in colon in a dose-dependent manner (Fig. C–H). Equivalent anti-inflammatory effects were observed in Changyanning groups.

3.3. Zhuling treatment ameliorates intestinal permeability

UC isusually associated with an increased intestinal permeability [13]. We found that the expressions of tight junction proteins including occludin, ZO-1 and TJP-1, detected by qRT-PCR, were clearly increased in the Model group, while the increase was restored after treatment with the medicines, especially at the Zhuling (H) and Changyanning group (Fig. 3 A-C). Furthermore, consistent with the Changyanning group, the protein levels of occludin measured with Western blot were increased in the Zhuling groups (Fig. 3 D-E).

3.4. Chemical profiling of Zhuling by UHPLC-Q-TOF-MS

To profile the chemical ingredients of Zhuling decoction, UHPL-Q-TOF-MS was used to carry out a systematic study as an integrated strategy. The Zhuling Jianpi capsule samples were prepared as described in Supplementary Methods 1.2. Positive and negative ion modes were used to screen as many potential components as possible. The base peak chromatogram (BPC) of the Zhuling in positive and negative ions is shown in Fig. 4. A total of 167 components, including 56 flavonoids, 37 alkaloids, 21 organic acids, 13 monoterpene glycosides, 8 sesquiterpenoids, 6 triterpenoids, 9 phenylpropanoids, 9 anthraquinones and 7 other type components were identified or tentatively characterized, and the representative structures of each medicine are shown in Fig. 5. Detailed chemical composition characterization information for all component sources is listed in Supplementary Tab. 1.

3.5. Metabolites analysis of Zhuling in rats

3.5.1. Prototypes analysis of Zhuling in vivo

A total of 12 rats were randomly divided into three groups, three for blank biospecimens, three for urine, plasma, feces and other tissues, and six for bile. Except for the blank group, rats in each group were orally administrated with Zhuling (29.28 mg/kg BW) after 16 h of fasting. The sample collection and preparation of urine, plasma, feces, bile, and other organ tissues were shown in Supplementary Methods 1.3–1.6. SCIEX OS software enables rapid data acquisition and data processing, and was engaged to compare ion chromatograms of prototypes and metabolites in the sample and blank groups (plasma, urine, bile, feces) as well as in the Zhuling group (in vitro). The validation criteria for samples in biological fractions were based on retention time, mass spectra and mass

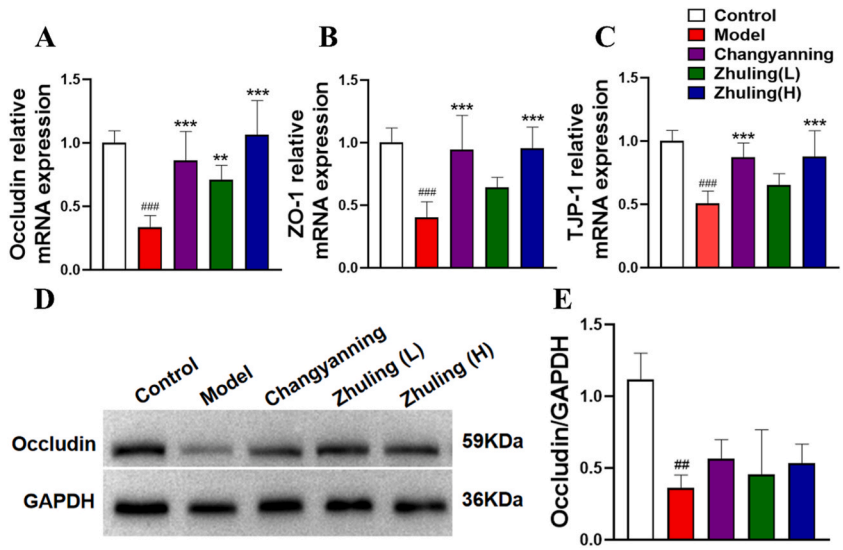


Fig. 3. Zhuling ameliorates intestinal permeability. A-C. Occludin, ZO-1 and TJP-1 relative mRNA expression in colon tissues, n = 8. D-E. Occludin relative protein expression in colon tissues, n = 3. ##P < 0.01, ###P < 0.001 compared with the control group; **P < 0.01, ***P < 0.001 compared with the model group.

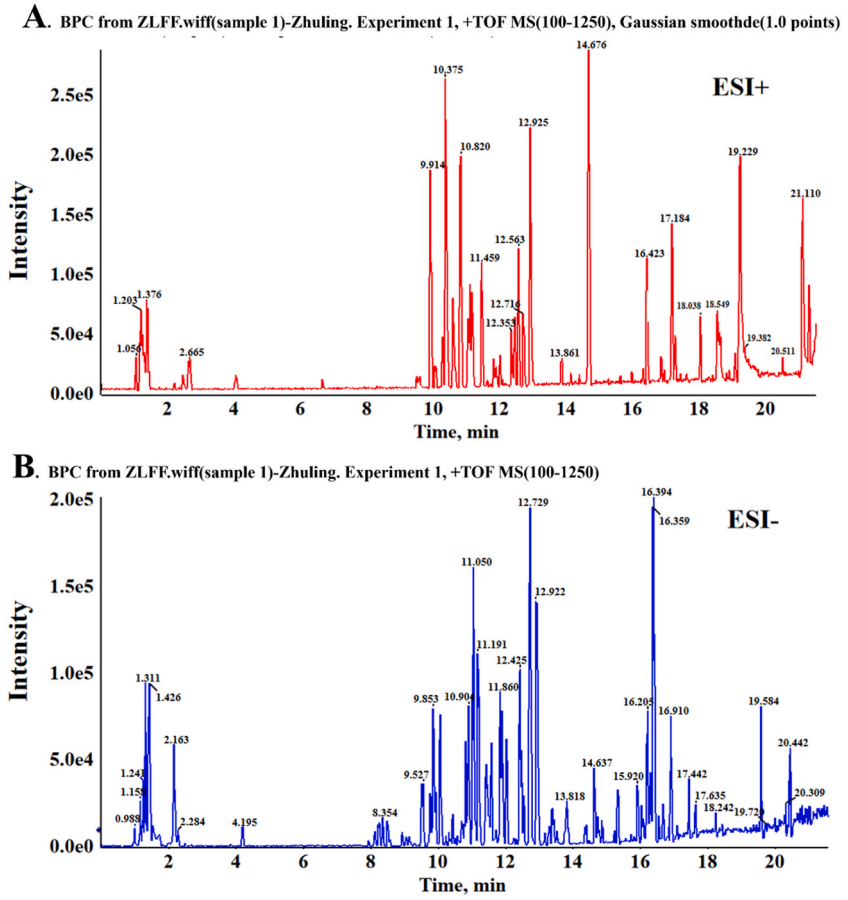


Fig. 4. The BPC of Zhuling capsule. (A) Negative mode. (B) Positive mode.

spectra/mass spectra similarity of the components characterized by Zhuling. According to the results of the chemical composition spectrum analysis of Zhuling, total of 120 prototypes were detected. They were distributed in urine, plasma, bile or feces, including 48 flavonoids, 30 alkaloids, 8 monoterpenes, 13 organic acids, 5 phenylpropanoids, 4 triterpenoids, 4 sesquiterpenoids, 4 anthraquinones and 4 others. The BPC of bile, feces, plasma and urine is shown in Supplementary F. 2.

3.5.2. Metabolites analysis of Zhuling

According to the chemical composition results of Zhuling, 26 representative structural components were screened for process chemicalome-metabolome matching, including 5-*O*-Methylvisammioside, Cimifugin, Prim-*O*-glucosylcimifugin, Poncirin, Apigenin, Isoliquiritigenin, Isoliquiritin, Narirutin, Sec-*O*-glucosylhamaudol, Liquiritin, Neoeriocitrin, Arginine, Boldine, N-Methyllauroteatanine, Ethyl gallate, Albiflorin, Paeoniflorin, Atractylenolide III, Atractylenolide II, Licoricesaponin A3, Alisol C, Ferulic acid, Neoeriocitrin, Altechromone A, Randainal, Rhein.

MS information was extracted using XIC with MDF, and possible metabolites were screened from it using mass loss analysis, peak area analysis, retention time analysis, etc. Finally, the auxiliary tools PIF and NLF are used to match their fragmentation pathways or signature ions to the prototypes. Metabolites are associated with the prototype through the corresponding biotransformation pathways (phase I versus phase II metabolic patterns). The general biotransformations were loss of O, loss of GlcA, methylation, ketone production, sulfate conjugation, Glc conjugation, GlcA conjugation and so on (Supplementary Tab. 2, Supplementary F. 3). In summary, metabolite screening underwent two steps: Software screening and false positive elimination. False positives are defined as those screened prototype metabolites that also existed in the control group. Notably, endogenous metabolites produced by the organism's metabolism could affect the identification results. Hence, these screened false positives will be eliminated in subsequent analysis. As a result, a total of 139 metabolites that connected with 26 representative compounds were screened in plasma, urine, bile or feces, including 56 from flavonoids, 19 from alkaloids, 4 from organic acids, 6 from sesquiterpenoids, 15 from monoterpenes, 20 from triterpenoids, 17 from phenylpropanoids, 1 from anthraquinones and 1 from others (Fig. 7, Supplementary Tab. 3). The prototype-metabolite (chemicalome-metabolome) matching network was summarized in Fig. 6A. Detailed distribution of each compound is shown in Fig. 6B. Herein, P87 was used as the template to elucidate the prototype-metabolite-tissue distribution. P87 is distributed in the liver, and its metabolites M25, 35, M37 and M31 are distributed in the heart, kidney and liver respectively. P81 is both the

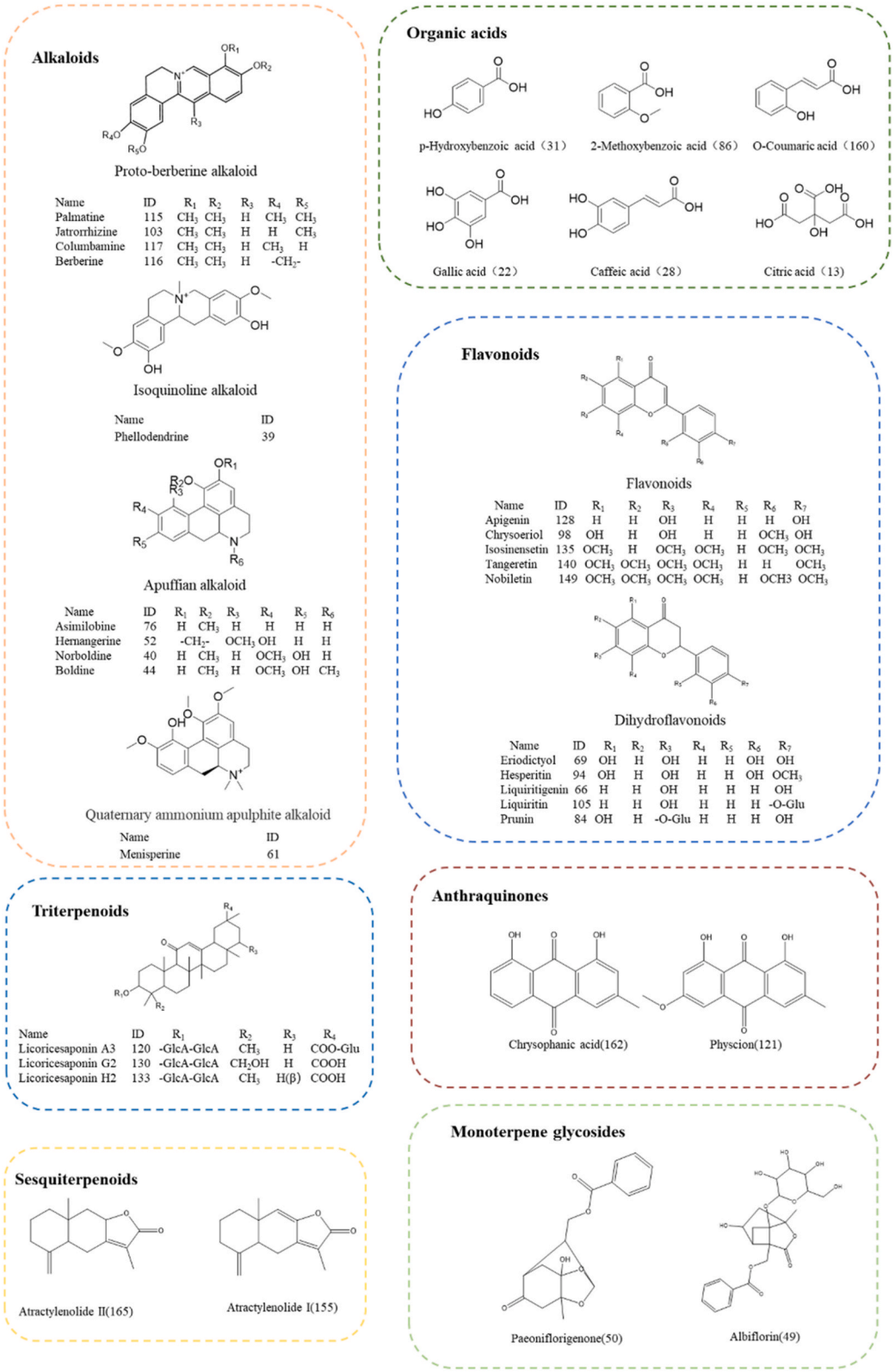


Fig. 5. Representative structures of components in Zhuling capsule.

metabolite of p87 and the prototype of other metabolites.

The biotransformation patterns of representative structural components and the tissue distribution of prototypes and metabolites are shown in Supplementary Tab. 3 and 4 and Fig. 7. Generally, metabolites in feces are metabolized and utilized by intestinal flora or drainage by the bile. In the present study, they were all distributed to intestinal bacterial metabolism. In addition, metabolites detected in urine, bile or plasma generally pass through the liver and are therefore classified as metabolic phase I and metabolic phase II (Fig. 7).

3.5.3. *Analysis of metabolome distribution of Zhuling*

To further explore the in vivo distribution of Zhuling, a total of 120 prototype compounds and 26 representative prototype compound components and their matching 139 metabolites were detected from the tissue samples. As shown in Figs. 7 and 10 prototypes and 41 metabolites were detected in brain tissue, 9 prototypes and 33 metabolites were detected in the heart, 7 prototypes and 29 metabolites were detected in the kidney, 11 prototypes and 50 metabolites were detected in the liver, and no prototypes and 50 metabolites were detected in the lung and spleen.

3.6. *Recommendation of quality control indicators for Zhuling*

This subsection integrates the results derived from in vivo distribution and metabolism (removing common components of living organisms, such as some amino acids, fatty acids, etc.), and performs the screening of quality control indicators, details of which are

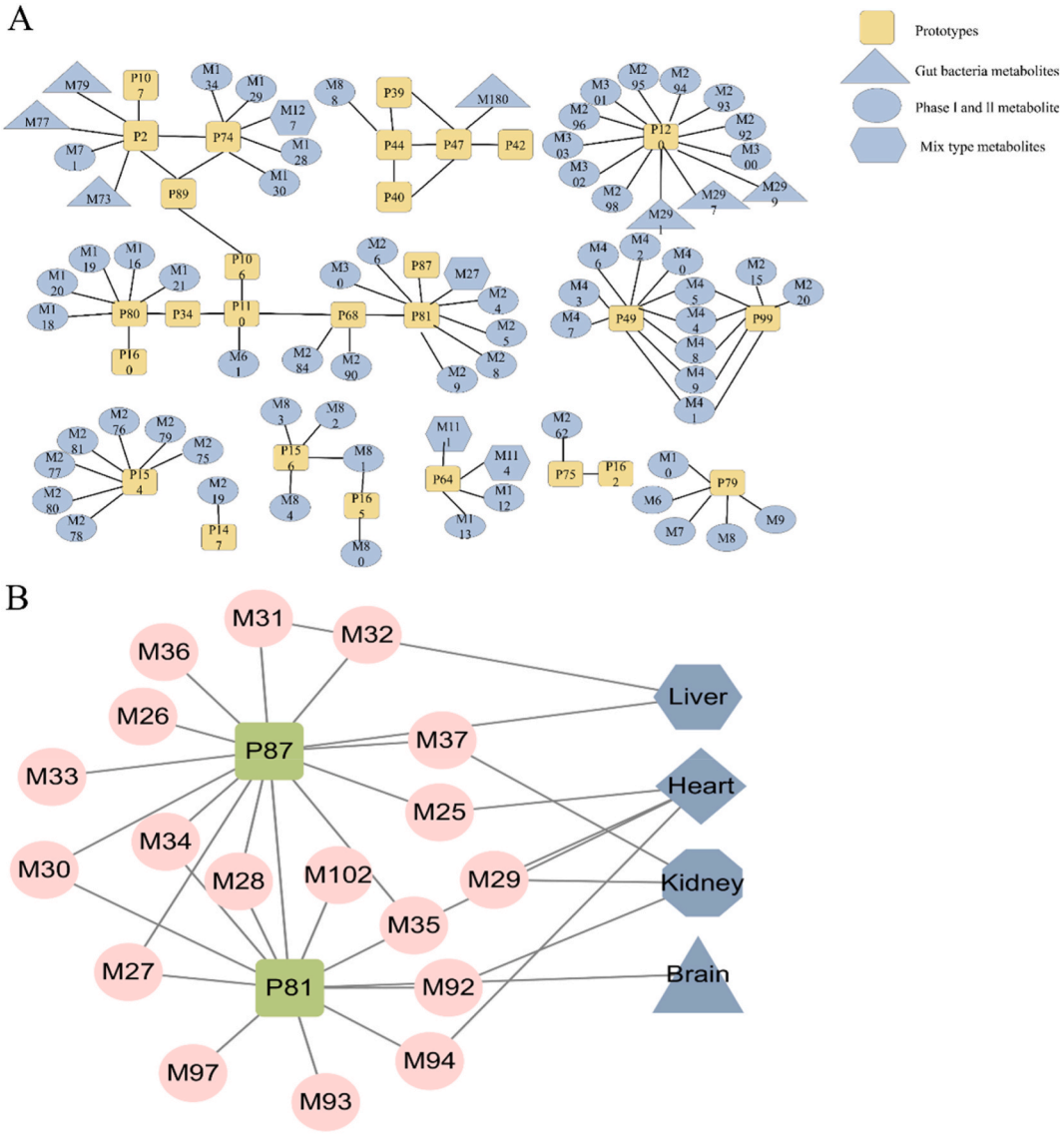
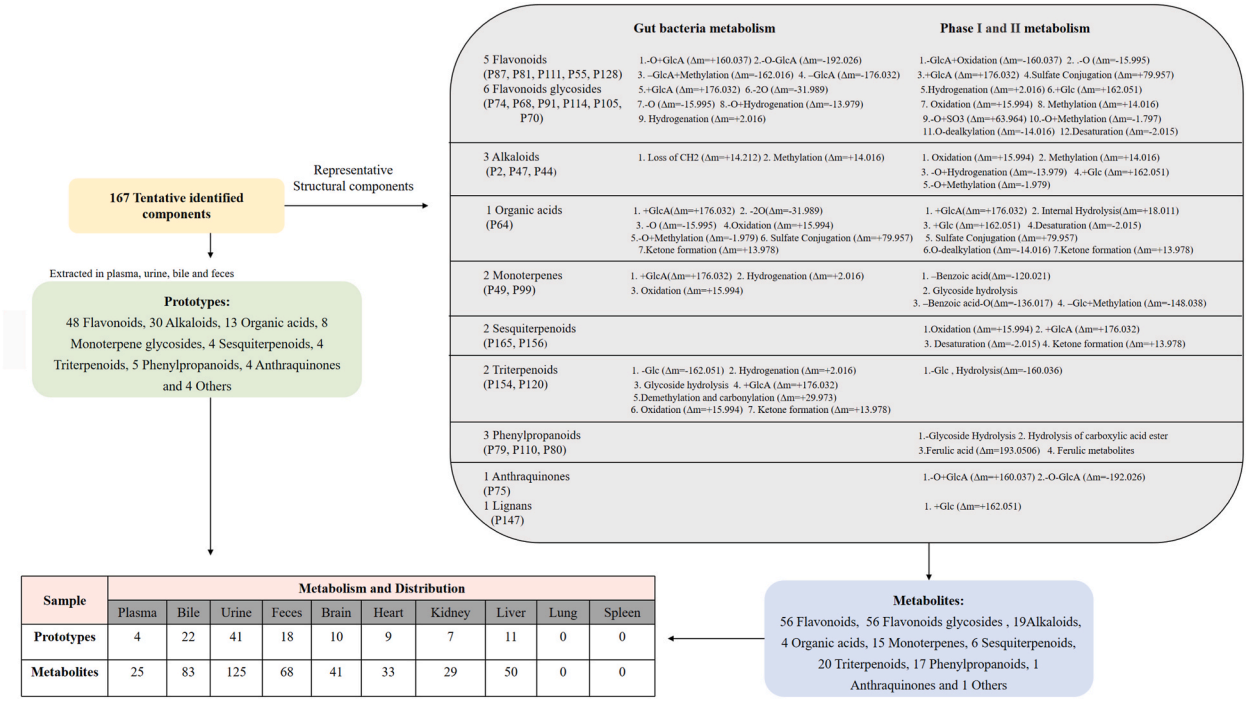


Fig. 6. **A**: Prototype-metabolotype component correlation; **B**: Prototype-metabolite-tissue distribution association network.



Sample	Metabolism and Distribution									
	Plasma	Bile	Urine	Feces	Brain	Heart	Kidney	Liver	Lung	Spleen
Prototypes	4	22	41	18	10	9	7	11	0	0
Metabolites	25	83	125	68	41	33	29	50	0	0

Fig. 7. Tissue distribution of prototypes and metabolites and biotransformation pathways of typical structural components.

shown in Table 1. The analysis of their in vivo distribution finally screened out 12 potentially potent compounds, which may enter the blood (plasma, urine, bile), exist in tissue distribution, or regulate the organism by acting on intestinal flora (bile, feces), and the 12 compounds were initially considered as candidate quality control indicator components (Table 1), which are mainly flavonoids, terpenoids and phenylpropanoids. The above compounds can be used as potential quality control index components for the quality control of the compound, and may exert more extensive pharmacological effects, which have more important research significance in the subsequent experiments.

4. Discussion

UC is characterized by chronic inflammation of the intestine, mucosal immune disorders, and invasion by intestinal microbes [14]. In this study, we successfully constructed a chronic colitis mice model induced by 2.5% DSS. The clinical parameters such as DAI score, spleen weight, body weight and colon length were remarkably improved after Zhuling treatment. The pathological manifestations of UC are increased inflammatory cell infiltration, damaged intestinal epithelial cells, and loss of crypt structures. Our data showed that Zhuling significantly relieved the pathological symptoms of UC and reduced the pathological score compared to the Model group.

As a leucocyte-derived protagonist of inflammation, MPO is strongly correlated with the severity of IBD [15]. SOD is an important component of the endogenous antioxidant defense system [16]. Our results showed that the administration of Zhuling significantly restrained the elevation of colonic MPO activity and increased colonic SOD activities in DSS-treated mice. The intestinal damage in UC

Table 1
Candidate quality control index component information.

ID	Name	Distribution	Compound Class	CAS	Compound Source	Metabolite
P70	Neoeriocitrin	U	Flavonoids glycosides	13241-32-2	CR, DF	M190-M202
P99	Paeoniflorin	U, B	Monoterpene glycosides	23180-57-6	PRA	M212-M220
P149	Nobiletin	U, B	Flavonoids	478-01-3	CS, CR	–
P156	Atractylenolide III	U, F, H, Li	Sesquiterpenoids	73030-71-4	AMR	M82-M85
P165	Atractylenolide II	U	Sesquiterpenoids	73069-14-4	AMR	M80-M81
P77	Phloretin	U, H	Flavonoids	60-82-2	CS	–
P79	3-(3, 4-dihydroxyphenyl)Propionic acid	U, B	Phenylpropanoids	5631-68-5	DF	–
P80	Ferulic acid	U, F, B, Li	Phenylpropanoids	1135-24-6	CP	M115-M121
P91	Narirutin	F, B, P	Flavonoids glycosides	14259-46-2	DF	M304-M310
P110	Coumarin	U, F	Phenylpropanoids	38215-36-0	DF,CR	–
P146	Bavachinin	B	Flavonoids	19879-30-2	DF	–
P49	Albiflorin	U, B, K	Monoterpene glycosides	39011-90-0	PRA	M40-M49

U: urine F: feces B: bile P: plasma H: heart S: spleen L: lung K: kidney Li: liver.

is supposed to be mediated by many inflammatory cytokines [17]. In our study, Zhuling significantly suppressed the levels of inflammatory cytokines, such as TNF-α, IL-1β and IL-6 both in colon tissues and serum. Tight junctions, represented by claudins, occludin, ZO-1 and TJP-1, are chiefly responsible for the formation of the constitutive barrier function of epithelial cells [18]. We have verified that Zhuling can increase the levels of occludin, ZO-1 and TJP-1. Briefly, these results indicated that Zhuling administration can alleviate DSS-induced colitis by boosting the antioxidant capacity, regulating the production of pro-inflammatory factors and improving the intestinal barrier.

Zhuling is a Chinese medicinal preparation composed of 18 Chinese herbs, which is based on the modified "Shen Ling Bai zhu San" decoction in the prescription book "Taiping Huimin He Ping Bureau Formula" of government in the Song dynasty, with the effect of strengthening the spleen, benefiting the kidney, resolving dampness, clearing heat and detoxifying. In general, Chinese medicine exerts therapeutic effects based on the interaction of multiple targets and components. Although we have discovered the therapeutic effect on UC, the actual bioactive components of Zhuling are still not well understood. In this study, UHPLC-Q-TOF-MS method was used to identify the components of Zhuling and illustrate the chemical and metabolic profile of Zhuling. In the result, a total of 167 compounds has been identified or characterized, mainly including flavonoids, alkaloids, organic acids, monoterpene glycosides, sesquiterpenoids, triterpenoids, phenylpropanoids, anthraquinones and other type components. Since most effective constituents need to be absorbed into the blood to elicit activities after administration of TCM [19], we performed metabolic component profiling of Zhuling in rats. In plasma, urine, bile or feces of rats, the results showed that a total of 120 prototypical compounds were detected, including 48 flavonoids, 30 alkaloids, 13 organic acids, 8 monoterpenes, 5 phenylpropanoids, 4 triterpenes, 4 sesquiterpenes, 4 anthraquinones and 4 others. Moreover, through corresponding biotransformation pathways, 26 prototypes were screened for process chemicalome-metabolome matching and found to be associated with 139 metabolites, which were distributed in urine, plasma, bile, feces, heart, kidney, lung and spleen through MetabolitePilot. As expected, one prototype component can be metabolized into multiple components by absorption into the blood and thus distributed in body fluids. However, what is worth noticing is that in both the prototype and metabolites, the components of the distribution are flavonoids, flavonoids glycosides, alkaloids, organic acids, monoterpenes, phenylpropanoids, triterpenoids, sesquiterpenoids and anthraquinones, suggesting that these categories were probably the main bioactive ingredients of Zhuling.

According to the theory of serum pharmacochemistry, only the constituents absorbed into the bloodstream have the chance to show effect and may be considered as potential bioactive ingredients and usually used as quality control index components [20]. In our study, Zhuling was discovered to have a rich chemical composition, among which we screened and identified 12 categories of key indicators as shown in Table 1: Neoeriocitrin, Paeoniflorin, Nobiletin, Atractylenolide III, Atractylenolide II, Phloretin, 3-(3, 4-dihydroxyphenyl)Propionic acid, Ferulic acid, Narirutin, Coumarin, Bavachinin, and Albiflorin, which mainly categorized as flavonoids, terpenoids and phenylpropanoids. We suggested these 12 compounds as potential quality control index components and might be the main components that exert a pharmacological effect.

According to previous reports, flavonoids and flavonoid glycosides have been demonstrated several beneficial health effects such as anti-inflammatory, anti-viral, anti-oxidative, and anti-carcinogenic properties [21,22]. Several scientific investigations have also confirmed the above beneficial biological properties of phenylpropanoids [23]. Terpenoids (such as monoterpenes, sesquiterpenes, triterpenes and paeoniflorin) are also endowed with a wide range of biological activities, such as such as regulating intestinal flora, promoting tissue repair and inhibiting oxidative stress [24]. Overall, the probable active ingredients consist of flavonoids, terpenoids and phenylpropanoids and the corresponding representative compounds could provide a more scientific approach to the quality control of Zhuling. However, it is worth noting that the above ingredients tend to function in multiple forms, and the remission of disease is commonly caused by the combined action of various bioactive components.

5. Conclusion

In this study, we demonstrated the protective effect on DSS-induced colitis in mice and explore the effective substance of Zhuling. As a result, a total of 167 components were identified in Zhuling and 120 prototypes were detected in plasma, urine, bile or feces. Among them, 26 prototypes were found to be related to 139 metabolites, which were produced through various biotransformation modes in vivo. Moreover, 12 categories of key indicators were identified, which might be more meaningful and scientific in the quality control of Zhuling. In sum, our results provided the chemical foundation for further study on effective substances and quality control of Zhuling.

Author contribution statement

Jian Li, Ziqi Zhou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dan Liu, Haijuan Dong: Performed the experiments; Analyzed and interpreted the data.

Jianping Zhou, Jie Wu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

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Declaration of competing interest

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

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Appendix B. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e16553.

Abbreviations

Ulcerative colitis (UC); Inflammatory bowel disease (IBD); China Food and Drug Administration (CFDA); Ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS); Traditional Chinese medicine (TCM); Dextran sulfate sodium (DSS); Disease activity index (DAI); Hematoxylin and eosin (H&E); Myeloperoxidase (MPO); Malondialdehyde (MDA); Superoxide dismutase (SOD).

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