Using Network Pharmacology and Molecular Docking Technology to Explore the Mechanism of Modified Pulsatilla Decoction in the Treatment of Ulcerative Colitis

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Abstract

Objective: Using network pharmacology and molecular docking technology, our aim was to clarify the biological activity, key targets, and potential pharmacological mechanisms of modified Pulsatilla decoction (MPD) in the treatment of ulcerative colitis (UC).

Materials and Methods: The main active ingredients of MPD were screened using the traditional Chinese medicine systems pharmacology platform. UC targets were obtained from the GeneCard, OMIM, DisGeNET, PharmGkb, and DrugBank databases. The common genes of MPD in the treatment of UC were identified by Venn diagram. The visual interactive network diagram of “active ingredient-target-disease” was constructed using the software Cytoscape. We used the STRING database to construct a protein–protein interaction network and analyze the correlation in protein interaction. We conducted gene ontology enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis for common genes using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) database and R software. Subsequently, the molecular docking verification of ingredients and targets was conducted through Discovery Studio. Last, in vivo experiments were conducted to further verify the findings.

Results: A total of 51 active ingredients were screened, involving 141 common genes. The top 5 ingredients in MPD were quercetin, β-sitosterol, luteolin, kaempferol, and stigmasterol. Pathways involved in the treatment of UC include the advanced glycation end products-receptor for advanced glycation end products (AGE-RAGE) signaling pathway, the interleukin-17 (IL-17) signaling pathway, the tumor necrosis factor (TNF) signaling pathway, viral infection-related signaling pathways, and some cancer pathways. Molecular docking showed that the important ingredients of MPD were well docked with mitogen-activated protein kinase 1 (MAPK1), mitogen-activated protein kinase 8 (MAPK8), RAC-alpha serine (AKT1), vascular endothelial growth factor-A (VEGFA), transcription factor AP-1 (JUN), and interleukin-6 (IL-6). Animal experiments showed that MPD could ameliorate the injury and colitis in dextran sulfate sodium (DSS)-induced colitic rats. MPD inhibited the expression of p-p38A and p-MLC in UC rats.

Conclusions: MPD has the characteristics of a multisystem, multi-ingredient, and multitarget in the treatment of UC. The possible mechanisms include inhibition of inflammation, apoptosis, oxidation, and tumor gene transcription. MPD may have a protective effect in the treatment of UC.

Keywords

network pharmacology, mechanism prediction, modified Pulsatilla decoction, ulcerative colitis, quercetin

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Background

Ulcerative colitis (UC) is not a specific intestinal inflammatory disease. Its lesions involve the mucosa and submucosa of the colon and rectum, and it has been listed as one of the modern refractory diseases. In China, the incidence of UC is increasing yearly, and its symptoms include recurrent or continuous diarrhea, abdominal pain, mucous, pus, bloody stools, and tenesmus. The course of the disease is protracted, and it does not heal, which has an impact on patients’ quality of life. There are many types of drugs for the treatment of UC, including salicylic acid preparations, immunosuppressive agents, hormones, and biological agents. However, their curative effect is not satisfactory, and toxic side effects are prone to occur. Currently, there is no effective treatment for UC. The principle of treatment is to control the onset of the disease, stabilize the condition, reduce the recurrence rate, and reduce complications. Therefore, it is of great clinical significance to explore the pathogenesis of UC and find effective intervention methods.

Traditional Chinese medicine has attracted increasingly more attention in the treatment of UC, which belongs to the category of “dysentery,” “intestinal dysentery,” and “long dysentery” in Chinese medicine. Pulsatilla decoction (PD) is a classic prescription for the treatment of dysentery. Many studies have confirmed that this prescription has a definite effect in the treatment of UC. Professor Sun Hongwen, a well-known Chinese medical doctor in Jiangsu Province, supplemented PD with herbs for regulating qi and blood. She concluded that modified Pulsatilla decoction (MPD) can clear heat and dampness, remove blood stasis, protect membranes, cool blood, and stop dysentery. Clinically, MPD is often used to assist in the treatment of UC patients. Previous animal studies find that MPD can promote the colonization of intestinal dominant Bifidobacterium in intestinal epithelial cells and increase Bifidobacterium adhesion and adhesion receptors. MPD can also downregulate the level of interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), diamine oxidase, D-lactic acid, and deoxycholic acid in serum, and increase the serum level of ursodeoxycholic acid. This may be related to regulating the barrier of the intestinal mucosa and repairing the damage to it.

Traditional Chinese medicine is a multitargeted synergy with a strong system and integrity. Although previous studies have confirmed that MPD has good clinical efficacy in UC, there remains a lack of systematic research. Network pharmacology is a new subject integrating systems biology, multidirectional pharmacology, computational biology, and network analysis. It emphasizes the multichannel regulation of signal pathways to improve the therapeutic effect of drugs and reduce toxic side effects. Through the construction of a multilevel network, the relationship between drugs and diseases can be explored from a holistic perspective, and the mechanism of the action between drugs and the body can be analyzed.

Therefore, the aim of this study was to explore the target and signal pathways of MPD in the treatment of UC with the help of network pharmacology and molecular docking technology. In this way, the relationship between drugs and related genes can be evaluated, and new ideas and methods can be provided for follow-up research. The flowchart of this study is shown in Figure 1.

Materials and Methods

Screening the Active Ingredients of MPD

By searching the Traditional Chinese Medicine System Pharmacology Database (TCMSP https://tcmspw.com/tcmsp.php, Version 2.3), we gathered the main ingredients of Radix Pulsatilla, Rhizoma Coptidis, Phellodendri chinensis cortex, Fraxini cortex, hairyvein agrimony, Panax notoginseng, Rhizoma Bletillae, and Radix Arnebiae in the MPD. The screening conditions were set as oral BioAvailability (OB) ≥30% and druglikeness (DL) ≥0.18 to obtain active ingredients with better druggability.

Screening Targets Common to MPD and UC

According to the TCMSP database, the targets involved in the active ingredients in MPD are sorted. We obtained the MPD gene targets using the UniProt database (https://www.uniprot.org/). Using UC as a keyword, we searched for the genes related to UC and screened them through GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), PharmGKB (https://www.pharmgkb.org/), DisGeNET (https://www.disgenet.org), and DrugBank databases (https://www.drugbank.ca). Finally, the genes corresponding to the active ingredients of MPD were mapped with UC-related target genes, and common genes were screened out. We used the Venny 2.1.0 tool to create the Venn diagram.

Constructing a Network of Active Drug Ingredients and Disease Targets

The active ingredients and common genes data of MPD were imported into Cytoscape version 3.7.1 software to construct the “active ingredients-target-disease” network. A node in the network represents a target or active ingredients, and an edge represents the interactions between nodes. The degree value of a node indicates the No. of routes connected to other nodes in the network. A node with a high degree value may play a key role in the network and can be used to evaluate the importance of the node to screen out the important ingredients in MPD.

Construction of the PPI Network and Screening of Core Genes

The protein-protein interaction (PPI) network is formed by the interaction between proteins and other protein molecules in the network. This interaction is the basis of cell metabolism and is essential for maintaining the function of life. The systematic
The analysis of protein interactions in biological systems is highly significant for understanding the changes in biological signals and the functional relationships between proteins under disease conditions. The STRING database (https://string-db.org/) collects many protein interactions and contains a series of data confidence (e.g., low confidence: < 0.4, medium confidence: 0.4-0.7, and high confidence: > 0.7), and so can be used to predict protein interactions. We imported the common genes into the STRING database, set the species search to *Homo sapiens* with a confidence of 0.7, and hid the isolated target to perform PPI network analysis on the common genes. The result was stored in Tab Separated Values format and imported into Cytoscape software. We used the CytoNCA app to evaluate the topological properties of nodes in the interactive network. The core targets were selected according to degree centrality (DC), betweenness centrality (BC), closeness centrality (CC), eigenvector centrality (EC), network centrality (NC), and local average connectivity (LAC). These six parameters measured the importance of nodes in the network and indicated the nature of the nodes in the network. High DC, BC, CC, EC, NC, and LAC values indicate that a node is vitally important to the network. According to the analysis results, a value greater than the median is selected as the condition for screening, and the core target is finally obtained after 2 screenings.

**GO and KEGG Enrichment Analysis**

First, we imported all common genes into the Database for Annotation, Visualization, and Integrated Discovery (DAVID, http://david.nicrf.gov/). Then, the data were analyzed for gene ontology (GO) function enrichment (molecular function, biological process, cellular ingredient) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. In the programming language, `pvalueCutoff = 0.05, qvalueCutoff = 0.05`, and the `emapplot` function were used to support hypergeometric tests and to obtain the results of the target enrichment analysis. The...
relationships among the overlapping targets based on the enriched GO terms were displayed. Differentially expressed genes that overlap 2 GO terms represent 2 nodes that have overlapping relationships. In the diagram, the overlapping targets are connected with lines, and the top 30 targets in the network are shown. In the KEGG enrichment analysis, the top 20 items, determined by the highest enrichment degrees, were selected and displayed in the form of bar graphs.

**Molecular Docking Steps and Results Evaluation**

We used Discovery Studio software to perform molecular docking and the Research Collaboration for Structural Bioinformatics Protein Data Bank Database (https://www.rcsb.org/) was used to obtain the core target structures. We obtained the pretreated protein structures using the Prepare Protein tool, and the parameters were set to the default values, including pretreatments such as dehydration, hydrogenation, and ring building. We used the Define and Edit Binding Site tool to define the active center of the target molecule and determined the location and size of the active center based on the positive ligand position for the protein as obtained from the Protein Data Bank and related crystal information in the literature.

In the Chemical Book database (https://www.chemicalbook.com/), the active compound structures were accessed in mol2 format. We generated different conformations of the active compounds using the Prepare Ligands tool to compile a small-molecule ligand library. The LibDock method in the Dock Ligands module was used for molecular docking. The docking preferences were set to high quality, the conformation method was fast, and the parallel processing was true. Other parameters were selected as the default values. The docking results were given by the scoring function LibDock score. The higher the LibDock score, the higher the binding activity of the predicted ingredients with the target.

**Animals**

A total of 50 male SD rats, SPF grade, weighing 180 to 220 g, were purchased from Suzhou Sino Biotechnology Co., Ltd. The rats were randomly assigned to 6 groups (n = 10): control group, model group, high-MPD (H-MPD) group, middle-MPD (M-MPD) group, and low-MPD (L-MPD) group. All animals were maintained under a 12 h light/dark cycle environment. The room temperature was controlled at 20 to 24 °C, with 45% to 70% humidity, and rats partook freely after disinfection of drinking water and feed. The study protocol was approved by the Ethics Committee of Suzhou Hospital of TCM (ECMUC2019005AO).

**Drugs and Reagents**

The composition of the MPD was 30 g of Fraxini cortex, 10 g of Phellodendri chinensis cortex, 30 g of Radix Pulsatillae, 10 g of Rhizoma Coptidis, 30 g of hairy vein agrimony, 6 g of Panax notoginseng, 30 g of Radix Arnebiae, and 20 g of Rhizoma Bletillae. All the traditional Chinese medicine decoction samples were provided by the pharmacy department of Suzhou Traditional Chinese Medicine Hospital and were identified as qualified traditional Chinese medicine decoction samples. We took one dose of MPD, soaked it for 30 min, decocted it twice according to the conventional decocting method, and filtered and merged the extracts, which were concentrated in a rotary evaporator under vacuum (temperature 80 °C) to the equivalent of 2 g/mL of crude drug. Finally, we stored the liquid medicine in a 4 °C refrigerator for subsequent use. Dextran sulfate sodium (DSS) was obtained from MP Biomedicals (Santa Ana, CA, USA), rabbit anti-human antibodies against p-p38MAPK, p-MLC, β-actin, and secondary antibodies from Abcam, and pentobarbital sodium from Sigma (Sigma-Aldrich).

**Treatment Protocol for DSS-Induced Colitic Rats**

After 1 week of adaptive feeding, the drinking water of the remaining 40 rats (except the rats in the blank group) was replaced with DSS solution for 6 days. When the model rats displayed effects such as weight loss, mental lethargy, loose stools, and mucus purulent bloody stools, we deemed the modeling successful. The rats were dosed on the third day after establishing a model according to the conversion of body surface area between people and rats. The H-MPD, M-MPD, and L-MPD groups were given 17.28 g/kg, 8.64 g/kg, and 4.32 g/kg MPD, respectively, and the control group and model group were given the same volume of saline enema. The dosage volume of each group was 3 mL once a day for 14 consecutive days. Rats in each group fasted for 24 h after the last administration. After weighing, the rats were anesthetized with pentobarbital sodium (50 mg/kg) and then euthanized. Then, 4 to 8 cm of colon tissue was removed from the anal margin upward and a cut was made along the longitudinal axis of the mesentery. The tissue was rinsed with distilled water, and then the intestinal mucosal tissue morphology was observed before the tissue was refrigerated at −80 °C for later use.

**Histomorphology of Colon Mucosa**

The colon tissues were fixed, embedded, sectioned, and stained with uranium acetate and lead citrate before the ultrastructure of the colon under an electron microscope was observed. The degree of colonic mucosal injury was observed macroscopically, and the colonic mucosal damage index (CMDI) was evaluated. The scoring standard was as follows: no damage to the colonic mucosa was scored zero; local congestion and edema of the mucosa without thickening of the intestinal wall or ulcer was scored one; mucosal hyperemia, edema, and thickening of the intestinal wall without ulcer formation was scored 2; a single ulcer formation was scored 3; ulcers or local
inflammation in multiple locations scored 4; and an ulcer along the long axis of the colon >1cm was scored 5.

Western Blotting Analysis

Radioimmunoprecipitation lysis buffer with the protease inhibitor cocktail (Beyotime Biotechnology, China) was used to extract the total protein from the colon tissue. The sample protein content was determined according to the BCA method and the loading amount was calculated. The separation gel and concentrated gel were configured according to the molecular weight of the target protein, the sample was loaded according to the calculated loading amount, and electrophoresis began. When the dye reached the bottom of the gel, the electrophoresis was stopped. To the transferred membrane was added the primary antibody and the secondary antibody, and Tanon 5200 gel image processing system was used to analyze the expression of the target band after being exposed to film.

Statistical Processing

SPSS 23.0 statistical software (IBM Corp.) was used to analyze the data. GraphPad Prism 7.0 software (GraphPad Software Inc.) was used for the calculations. All results are expressed as means ± SD. Student’s t-test was used to evaluate the paired data, and ANOVA was used to make multiple comparisons. A P value < .05 was considered statistically significant.

Results

Screening of Active Ingredients in MPD

According to the conditions of OB ≥ 30% and DL ≥ 0.18, 82 active ingredients were found in MPD. After deleting 31 that were not associated with UC, 51 active ingredients were ultimately included. See Supplementary Table 1 for details.

Target Acquisition for Active Ingredients and Disease-Related Effects

The targets involved in the active ingredients of MPD were retrieved from the TCMSP database. The UniProt database was used to obtain the gene name of the target, and 204 active ingredient targets were obtained after correction and deduplication. As shown in Supplementary Table 2, 4622 UC-related gene targets were obtained after searching in multiple databases with “ulcerative colitis” as the search term. We mapped 204 active ingredient targets with 4622 UC-related targets and obtained 141 common genes. See Supplementary Table 3 for details. The Venn diagram is shown in Figure 2.

"Active Ingredients-Target-Disease" Network of MPD

We matched 141 common genes with 51 active ingredients and imported them into Cytoscape software to construct an active ingredients-target-disease network. The detailed information is shown in Supplementary Table 4. The network has 192 nodes (51 active ingredients, 141 common genes) and 884 edges, as shown in Figure 3. The quadrilateral represents the active ingredients and the ellipse represents the common genes. Figure 3 shows that one target can correspond to one or more active ingredients, and multiple targets can correspond to the same active ingredient, which indicates that MPD has the characteristics of a multi-ingredient and multi-target treatment of UC.

PPI Network Construction and Core Gene Screening

We uploaded common genes to the STRING database to construct a protein interaction network. After that, we imported the file into Cytoscape for visualization. We used CytoNCA to calculate the topological parameters of the network nodes to get the DC, BC, and CC parameters. The first screening threshold was DC ≥ 16, BC ≥ 0.065, LAC ≥ 7.124, BC ≥ 173.773, CC ≥ 0.443, and NC ≥ 10.113. The results show that there are 26 nodes and 230 edges in total. The second screening threshold was DC ≥ 37.767, BC ≥ 0.158, LAC ≥ 14.110, BC ≥ 590.397, CC ≥ 0.546, and NC ≥ 24.624. The second screening results were 6 nodes and 12 edges, including mitogen-activated protein kinase 1 (MAPK1), mitogen-activated protein kinase 8 (MAPK8), RAC-alpha serine (AKT1), vascular endothelial growth factor-A (VEGFA), transcription factor AP-1 (JUN), and interleukin-6 (IL-6). The core target screening process is shown in Figure 4.

GO and KEGG Pathway Enrichment Analysis

GO enrichment analysis was performed on 141 common genes. The results showed that the key targets of MPD in the treatment of UC were highly enriched in 132 GO terms. The top 30 GO analysis results were screened with P < .05 as the threshold. The biological functions and processes included BH domain binding, oxidoreductase activity, ubiquitin-protein ligase binding, DNA-binding transcription activator activity, RNA polymerase II-specific, phosphatase binding, peroxidase activity, kinase regulator activity, cysteine-type endopeptidase activity involved in the apoptotic process, RNA polymerase II transcription factor binding, antioxidant activity, receptor–ligand activity, tetrapyrrole binding, heme binding, cytokine

Figure 2. Common genes of modified Pulsatilla decoction (MPD) and ulcerative colitis (UC).
activity, cytokine receptor binding, steroid hormone receptor,
transcription factor activity, and nuclear receptor activity. The
results are shown in Figure 5. A cluster profiler was used for
KEGG pathway enrichment analysis, and 158 pathways were
enriched. The first 20 pathways with more enriched genes
were screened with $P < .05$ as the threshold. They include
interleukin-17 (IL-17) signaling pathway, tumor necrosis
factor (TNF) signaling pathway, advanced glycation end
products-receptor for advanced glycation end products (AGE-RAGE) signaling pathway in diabetic complications,
C-type lectin receptor signaling pathway, viral infection-related
signaling pathways, and some cancer pathways. See Figure 6
for details.

**Target Path Analysis**

We used a KEGG mapper tool to obtain the pathway map of
MPD in the treatment of UC, and the AGE-RAGE signaling
pathway is shown in Figure 7. The target of the pathway is
marked in white, and the marker of MPD in the treatment of
UC in red. As shown in the figure, the effect of MPD in the
treatment of UC includes the MAPK signaling pathway, the
phosphatidylinositol 3-kinase (PI3K)-AKT signaling pathway,
the NF-$\kappa$B signaling pathway, the JNK/c-Jun signaling
pathway, the Jak-STAT signaling pathway, the TGF-ß signaling
pathway, and the ERK signaling pathway, involving 31 effective
targets of MPD in the treatment of UC. It was suggested that
MPD may play a role in the treatment of UC by regulating
several aspects, and its target may be located in these pathways.

**Molecular Docking Analysis**

The results of molecular docking indicate that the active ingre-
dients of MPD may act on the target of UC and are potentially
effective ingredients with certain pharmacodynamics. The
results of the ligand–receptor protein molecular docking are
shown in Supplementary Table 5. Figure 8 shows the effect of quercetin docking with JUN (Figure 8a), AKT1 (Figure 8b), MAPK8 (Figure 8c), IL-6 (Figure 8d), and MAPK1 (Figure 8e). The results show that the results of molecular docking are consistent with the screening results of network pharmacology, and the reliability of network pharmacology is verified by molecular docking.

**Morphology of Rat Colonic Mucosa**

The sliced specimens were observed under the electron microscope. In the control group, the microvilli were arranged neatly and tightly. No inflammatory cells were seen. In the model group, the microvilli were uneven, the gap was widened, and a large number of inflammatory cells were infiltrated. Compared with the model group, the morphology of rat colonic mucosa in the MPD groups was improved, accompanied by epithelial repair and glandular hyperplasia (Figure 9A). Compared with the control group, the CMDI score of the model group was significantly higher ($P < .01$). Compared with the model group, the CMDI scores of the H-MPD group and M-MPD group were significantly decreased ($P < .01$), as shown in Figure 9B.

**Protein Expression of p-p38MAPK and p-MLC in Colon Tissue of Rats**

The protein expression of p-p38MAPK and p-MLC in the model group was significantly higher than that in the control group ($P < .01$). Compared with the model group, the protein expressions of the H-MPD and M-MPD groups were significantly decreased ($P < .01$), as shown in Figure 10A and B.

**Discussion**

Experts in Chinese medicine believe that the pathogenesis of UC is the accumulation of damp and heat. The evil of dampness and heat invades the colon, blood stasis blocks the colon, and then flesh rots into pus. The evil persists for a long time, and the intestinal conduction is abnormal. In “Treatise on Febrile and Miscellaneous Diseases” Zhang Zhongjing wrote that patients with febrile diarrhea (dysentery) accompanied by tenesmus should be treated with PD.25 Professor Sun Hongwen, a well-known Chinese medicine practitioner in Jiangsu Province, is the inheritor of Wumen Chinese Medicine. She believes that repeated attacks and the inability to cure UC are consistent with the “long illness enters the collaterals” theory, which was created by Ye Tianshi (a representative of Wumen Chinese Medicine). Regarding PD, Professor Sun added traditional Chinese medicine for regulating qi and blood and recommended MPD to treat UC patients. The decoction is composed of Radix Pulsatilla, Rhizoma Coptidis, Phellodendri chinensis cortex, Fraxini cortex, hairyvein agrimony, Panax notoginseng, Rhizoma Bletillae, and Radix Arnebiae. It has the effects of clearing heat and dampness, removing blood stasis, protecting membranes, cooling blood, and stopping dysentery. This study scientifically investigated the pharmacological mechanism of MPD in the treatment of UC through network pharmacology, docking analysis, Western blotting, and in vivo animal study. Network pharmacology was used to screen and predict the active ingredients, core targets, and signal pathways of MPD in the treatment of UC. Then we used molecular docking analysis to simulate the binding of the active ingredients and the core target. The results of network pharmacology were verified by Western blot technology.

Through the ingredient-target-disease network, we can draw several conclusions. First, the treatment of UC by MPD is achieved using multiple ingredients. Second, there are many target genes involved in this process, and the relationship between genes is complicated. This shows that the pathogenesis of UC is complex, and MPD has a multitarget effect on UC which reflects the characteristics and advantages of traditional Chinese medicine. The compounds reported to be most active were quercetin, β-sitosterol, luteolin, kaempferol, and stigmasterol. Quercetin, a bioflavonoid, has anti-inflammatory effects in various diseases. Quercetin, luteolin, and kaempferol are compounds with anti-inflammatory activities and are considered important candidate drugs for IBD treatment.26 Quercetin can inhibit the intestinal inflammatory response in...
UC mice, improve the cell structure of colon tissue and maintain the integrity of the intestinal epithelial barrier. It can also block MAPK and NF-κB signaling pathways to alleviate inflammatory response. Luteolin is a flavonoid that exists in many plants and has anti-inflammatory and anti-allergenic activities. Studies have shown that luteolin can reduce the inflammatory phenotype and oxidative stress of tissue cells by inhibiting the NF-κB pathway. It has also been reported that luteolin’s anti-inflammatory mechanism may be related to changing the diversity and composition of intestinal microbiota in UC rats. Kaempferol, a natural flavonoid, is believed to have anti-inflammatory activity and a potential immunomodulatory effect. It is confirmed that kaempferol plays a protective role in colitis mice by regulating intestinal microbiota and toll-like receptor-related signaling pathways. Stigmasterol has been shown to be an immunomodulator with great therapeutic potential. Stigmasterol plays an important anti-inflammatory role and can reduce oxidative stress in a variety of diseases. Stigmasterol can also reduce the score of colitis, the expression of Cyclooxygenase-2 (COX-2), and colony-stimulating factor-1, which indicates that stigmasterol can improve colitis.

Through PPI network analysis, 6 core targets (e.g., MAPK1, MAPK8, AKT1, VEGFA, JUN, and IL-6) were obtained. Inflammatory factors play an important role in the development of UC. The abnormal activation of the PI3K/AKT signaling pathway in UC has been demonstrated to enhance the expression and secretion of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6. TNF plays a vital role in the typical immune response through the regulation of a No. of pathways. IL-6 is significantly activated in the body’s response to injury and plays important role in inflammation. IL-6 can activate the NF-κB and IL-6/STAT3 pathways to induce intestinal epithelial cell barrier injury and regulate barrier function. It is one of the pathogeneses of intestinal inflammation such as UC and bacillary dysentery. MAPK1 and MAPK8 exist in most cells. They belong to the MAPK signaling pathway and are closely related to the proliferation and apoptosis of intestinal epithelial cells. After inflammatory factors activate the MAPK signaling pathway, the transcription and expression of inflammatory genes related to inflammatory bowel disease (IBD) are changed, which intensifies the development of inflammation. In IBD model mice, the expression of MAPK1/3 increased significantly. However, its expression decreased after drug treatment. AKT1 is a core factor in the PI3K/AKT signaling pathway. AKT1 can regulate cell function by phosphorylation of downstream target proteins. It plays an important role in cell survival and apoptosis. The PI3K/Akt pathway, the MAPK/ERK pathway, and the JNK/c-Jun pathways are responsible for regulating a variety of cellular processes including cell growth, migration, invasion, and apoptosis. The 3

Figure 6. KEGG pathway enrichment analysis of common genes. The smaller the P value, the greater the correlation.
pathways are essential to the progression of chronic inflammation and tumors.47-49 Multiple studies have shown that inflammation is closely related to the occurrence and development of UC.50,51 Colorectal cancer is a recognized complication of UC. Inflammation predisposes one to the development of cancer and promotes all stages of tumorigenesis.52,53 In inflamed tissue, the blocked VEGF signaling pathway exacerbates inflammation in a variety of disease models, including IBD.54 In addition, a retrospective cohort study showed that the expression of VEGF in the intestinal mucosa of UC patients is increased and is related to the development and recurrence of the patient’s disease. Therefore, angiogenesis is not only a passive process driven by inflammation but also a hypothesis of active participants in mucosal lesions of UC.55 These core targets are related to inflammation, angiogenesis, cell proliferation, apoptosis, and carcinogenesis, and may be potential targets for the treatment of UC.

GO function enrichment analysis shows that the molecular functions involved in these genes are complex and diverse. They are involved in multiple processes such as receptor binding, transcription regulation, and post-translational modification, which indicate that MPD can intervene in the occurrence and development of UC in multiple ways from different levels. Through KEGG enrichment analysis, we found that the signaling pathway of MPD in the treatment of UC is mainly related to cell proliferation, apoptosis, and carcinogenesis. The involved pathways include the IL-17 signaling pathway, the TNF signaling pathway, the AGE-RAGE signaling pathway in diabetic complications, viral infection-related signaling pathways, and some cancer pathways. AGEs are formed as a result of nonenzymatic reaction between the free reducing sugars and proteins, lipids, or nucleic acids. AGEs are predominantly synthesized during chronic hyperglycemic conditions or aging. AGEs interact with their receptor RAGE to induce inflammation and immunosuppression by producing proinflammatory cytokines, reactive oxygen species, and reactive nitrogen intermediates. The accumulation of AGEs and the upregulated expression of RAGE are related to various pathological processes. They lead to the injury of vascular endothelial cells, increase the stiffness and fragility of the tissue, and reduce the ability of cells to rebuild the extracellular matrix so that tissues are more vulnerable to mechanical damage. AGEs

Figure 7. The AGE-RAGE signaling pathway of MPD in the treatment of UC.

Abbreviations: AGE-RAGE, advanced glycation end products-receptor for advanced glycation end products; MPD, modified Pulsatilla decoction; UC, ulcerative colitis.
may also enhance the inflammatory response of cells by inducing the MAPK pathway. RAGE and s-RAGE may be a useful biomarkers of ligand-RAGE pathway activation and cancer.\textsuperscript{56-58} KEGG enrichment analysis showed that the MAPK pathway directly or indirectly participates in the AGE-RAGE signaling pathway in diabetic complications. Therefore, this pathway may be an important target for MPD to treat UC. The relationship between the TNF-\(\alpha\) pathway and inflammation has been confirmed. TNF-\(\alpha\) can activate the TNF signaling pathway by binding to the receptors TNFR1 and TNFR2 and ultimately lead to the occurrence of intestinal mucosal inflammation.\textsuperscript{39} TNF-\(\alpha\) induces the expression of cell adhesion molecules in vascular endothelial cells. It can also be a stimulating factor for the local release of angiogenic substances, which can stimulate the release of VEGF and promote the proliferation and migration of endothelial cells.\textsuperscript{59} Interleukin-17 (IL-17) is a pro-inflammatory cytokine that can exert its effects through a variety of cytokines (eg, IL-1, IL-8, IL-6, and macrophage inflammatory proteins) and pathways (eg, NF-\(\kappa\)B, MAPK, and JNK/c-jun). IL-17 can simultaneously activate and increase the expression of VEGF and then aggravate inflammation response.\textsuperscript{60} The IL-17 signaling pathway is related to intestinal flora and plays a key role in regulating intestinal and autoimmune diseases.\textsuperscript{61} In addition, multiple cancer pathways ranked high in the KEGG enrichment analysis. This may be related to the susceptibility to inflammation-cancer transformation of UC. Chronic inflammation is closely related to the occurrence of tumors. In recent years, numerous studies have shown that PD also has antitumor effects. The above results reveal the potential mechanism of MPD in the treatment of UC. 

Subsequently, we conducted a molecular-docking simulation of the active ingredient and the core target. The results showed that quercetin, \(\beta\)-sitosterol, luteolin, kaempferol, and stigmasterol all docked well with core targets. These core targets are related to inflammation, angiogenesis, cell proliferation, apoptosis, and carcinogenesis. The above active ingredients are expected to become potential drugs for the treatment of UC and will become the focus of future research.

Figure 8. Molecular docking of compounds with core targets. The left side is the 3D docking diagram and the right side is the 2D docking diagram. (a) Docking process of quercetin with JUN. (b) Docking process of quercetin with AKT1. (c) Docking process of quercetin with MAPK8. (d). Docking process of quercetin with IL-6. (e) Docking process of quercetin with MAPK1.

Abbreviations: AKT1, RAC-alpha serine; IL-6, interleukin-6; JUN, transcription factor AP-1; MAPK1, mitogen-activated protein kinase 1; MAPK8, mitogen-activated protein kinase 8.
To validate the prediction results by network pharmacology analysis and molecular docking analysis, we established a UC model induced by DSS. We observed colon inflammation and injury by electron microscopy and conducted Western blot to detect the role of the MAPK signaling pathway in UC. The rats in the model group exhibited more severe injury and colitis, but these abnormalities were ameliorated by MPD administration. We found that the expression levels of p-p38MAPK and p-MLC in the colon tissue of the model group were significantly increased. However, they decreased after being treated with MPD. The results indicated that the p38 MAPK/MLCK signaling pathway was activated abnormally in the model group and that MPD had a protective effect in the treatment of UC. These results partly support our network pharmacology analysis results of MPD.

Conclusions
This study investigated the effective active ingredients and molecular mechanisms of MPD in the treatment of UC from the perspective of network pharmacology. The active ingredients of MPD in UC treatment comprise of 51 compounds: Quercetin, β-sitosterol, luteolin, kaempferol, and stigmasterol.
are the important active ingredients. There are 141 target genes involved in the treatment of UC by MPD, among which MAPK1, MAPK8, AKT1, VEGFA, JUN, and IL-6 are the key target genes. The signaling pathways of MPD in the treatment of UC primarily include the AGE-RAGE signaling pathway, the IL-17 signaling pathway, the MAPK signaling pathway, and the TNF signaling pathway. In vivo experiment indicated MPD could effectively ameliorate DSS-induced colitis, and the effect of MPD was associated with the MAPK signaling pathway.

This study has some limitations. We can conduct relevant clinical and basic experiments to verify further the accuracy of the results.

Author Contributions
Huiping Zhu, Jinwei Guo, and Hongwen Sun designed the study. Tingting Wu and Bo Xu wrote the manuscript. Xin Yang and Guoqiang Liang performed the experiments. Yu Zhou analyzed the data. All authors have read and approved the final manuscript.

Declaration of Competing Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval
The study received ethical approval from the Ethics Committee of Suzhou Hospital of TCM (ECMUC2019005AO).

Informed Consent
Not applicable.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

Statement of Animal Rights
We confirm that guidelines on animal rights and treatment have been met and any details of approval obtained are indicated within the text of the submitted manuscript.

Availability of Data and Materials
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Supplemental Material
Supplemental material for this article is available online.

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References
1. Tatiya-Aphiradee N, Chatuphonprasert W, Jarukamjorn K. Immune response and inflammatory pathway of ulcerative colitis. J Basic Clin Physiol Pharmacol. 2018;30(1):1-10.
2. Kucharzik T, Koletzko S, Kannengiesser K, Dignass A. Ulcerative colitis-diagnostic and therapeutic algorithms. Dtsch Arztebl Int. 2020;117(33-34):564-574.
3. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389(10080):1756-1770.
4. Argollo MC, Kotze PG, Spinelli A, Gomes T, Danese S. The impact of biologics in surgical outcomes in ulcerative colitis. Best Pract Res Clin Gastroenterol. 2018;32-33(02-04):79-87.
5. Liu X, He S, Li Q, et al. Comparison of the gut microbiota between Pulsatilla decoction and levofloxacin hydrochloride therapy on Escherichia coli infection. Front Cell Infect Microbiol. 2020;10(319):1-11.
6. Hua YL, Ma Q, Li W, et al. Metabolomics analysis of Pulsatilla decoction on treatment of wetness-heat-induced diarrhea in rats based on UPLC-Q/TOF-MS/MS. Biomed Chromatogr. 2019;33(11):1-33.
7. Yun HF, Liu R, Han D, et al. Pingkui enema alleviates TNBS-induced ulcerative colitis by regulation of inflammatory factors, gut Bifidobacterium, and intestinal mucosal barrier in rats. Evid Based Complement Alternat Med. 2020;2020(3896948):1-10.
8. Kibble M, Saarinen N, Tang J, et al. Network pharmacology applications to map the unexplored target space and therapeutic potential of natural products. Nat Prod Rep. 2015;32(8):1249-1266.
9. Zhang R, Zhu X, Bai H, Ning K. Network pharmacology databases for traditional Chinese medicine: review and assessment. Front Pharmacol. 2019;10(123):1-14.
10. Luo TT, Lu Y, Yan SK, et al. Network pharmacology in research of Chinese medicine formula: methodology, application and prospective. Chin J Integr Med. 2020;26(1):72-80.
11. Ru J, Li P, Wang J, et al. TC MSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform. 2014;6(13):1-6.
12. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019;47(D1):506-515.
13. Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics. 2016;54(6):1.30.1-1.30.33.
14. Amberger JS, Bocchini CA, Scott AF, Hamosh A. OMIM Org: leveraging knowledge across phenotype-gene relationships. Nucleic Acids Res. 2019;47(D1):1038-1043.
15. Huddart R, Hicks JK, Ramsey LB, et al. PharmGKB summary: sertraline pathway, pharmacokinetics. Pharmgenet Genomics. 2020;30(2):26-33.
16. Pinero J, Sauch J, Sanz F, Furlong LI. The DisGeNET cytoscape app: exploring and visualizing disease genomics data. *Comput Struct Biotechnol J*. 2021;19(5):2960-2967.
17. Wishart DS, Feunang YD, Guo AC, et al. Drugbank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res*. 2018;46(D1):1074-1082.
18. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*. 2021;49(D1):605-612.
19. Huang DW, Sherman BT, Tan Q, et al. DAVID Bioinformatics resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res*. 2007;35(Web Server issue):169-175.
20. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4(1):44-57.
21. Goodsell DS, Zardecki C, Di Costanzo L, et al. RCSB Protein data bank: enabling biomedical research and drug discovery. *Protein Sci*. 2020;29(1):52-65.
22. Ondas I, Eckmann I, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet*. 2012;380(9853):1606-1619.
23. Adams SM, Bornemann PH. Ulcerative colitis. *Am Fam Physician*. 2013;87(10):699-705.
24. Sanchez DFM, Romero-Calvo I, Mascarache C, Martinez-Augustino O. Intestinal inflammation and mucosal barrier function. *Inflamm Bowel Dis*. 2014;20(12):2394-2404.
25. Hua YL, Ma Q, Zhang XS, et al. Pulsatilla decoction can treat the inflammatory barrier function. *Int Immunopharmacol*. 2017;53(12):105-113.
26. Duan L, Cheng S, Li L, et al. Natural anti-inflammatory compounds as drug candidates for inflammatory bowel disease. *Front Pharmacol*. 2021;12:684486:1-22.
27. Dicarlo M, Teti G, Verna G, et al. Quercetin exposure suppresses the inflammatory pathway in intestinal organoids from Winnie mice. *Int J Mol Sci*. 2019;20(22):5771-5785.
28. Andrade A, Guerra G, de Souza AD, et al. Anti-inflammatory and chemopreventive effects of *Bryophyllum pinnatum* (Lamarck) leaf extract in experimental colitis models in rodents. *Front Pharmacol*. 2020;11(979):1-18.
29. Cheng SC, Huang WC SPJ, Wu YH, Cheng CY. Quercetin inhibits the production of IL-1beta-induced inflammatory cytokines and chemokines in ARPE-19 cells via the MAPK and NF-kappaB signaling pathways. *Int J Mol Sci*. 2019;20(12):2957-2980.
30. Kim WS, Song HY, Han JM, Byun EB. GLM, a novel luteolin derivative, attenuates inflammatory responses in dendritic cells: therapeutic potential against ulcerative colitis. *Biochem Biophys Res Commun*. 2019;518(1):87-93.
31. Li B, Du P, Du Y, et al. Luteolin alleviates inflammation and modulates gut microbiota in ulcerative colitis rats. *Life Sci*. 2021;269-(119008):1-9.
32. Park MY, Ji GE, Sung MK. Dietary kaempferol suppresses inflammation of dextran sulfate sodium-induced colitis in mice. *Dig Dis Sci*. 2012;57(2):355-363.
33. Qu Y, Li X, Xu F, et al. Kaempferol alleviates murine experimental colitis by restoring gut microbiota and inhibiting the LPS-TLR4-NF-kappaB axis. *Front Immunol*. 2021;12:679897:1-15.
34. Anrwi AO, Obiri DD, Osaro N, Forkuo AD, Essel I.B. Stigmasterol inhibits lipopolysaccharide-induced innate immune responses in murine models. *Int Immunopharmacol*. 2017;35(12):105-113.
35. Liang Q, Yang J, He J, et al. Stigmasteryl alleviates cerebral ischaemia/reperfusion injury by attenuating inflammation and improving antioxidant defenses in rats. *Brain Res*. 2020;40(4):1-12.
36. Anrwi AO, Obiri DD, Osaro N. Stigmasterol modulates allergic airway inflammation in Guinea pig model of ovalbumin-induced asthma. *Mediators Inflamm*. 2017;2017:2953930:1-11.
37. Feng S, Dai Z, Liu A, et al. beta-Sitosterol and stigmasterol ameliorate dextran sulfate sodium-induced colitis in mice fed a high fat Western-style diet. *Food Funct*. 2017;8(11):4179-4186.
38. Jiang W, Han YP, Hu M, et al. A study on regulatory mechanism of miR-223 in ulcerative colitis through PI3K/Akt-mTOR signaling pathway. *Eur Rev Med Pharmacol Sci*. 2019;23(11):4865-4872.
39. Holbrook J, Lara-Reyna S, Jarosz-Griffiths H, McDermott M. Tumour necrosis factor signalling in health and disease. *F1000Res*. 2019;8(1):1-12.
40. Kasembeli MM, Bharadwaj U, Robinson P, Tweardy DJ. Contribution of STAT3 to inflammatory and fibrotic diseases and prospects for its targeting for treatment. *Int J Mol Med*. 2018;19(8):1-30.
41. Hirano T. IL-6 in inflammation, autoimmunity and cancer. *Int Immunol*. 2021;33(3):127-148.
42. Li Y, Jia Y, Cui T, Zhang J. IL-6/STAT3 signaling pathway regulates the proliferation and damage of intestinal epithelial cells in patients with ulcerative colitis via H3K27ac. *Exp Ther Med*. 2021;22(2):890-898.
43. Unver N, McAllister F. IL-6 family cytokines: key inflammatory mediators as biomarkers and potential therapeutic targets. *Cytokine Growth Factor Rev*. 2018;41(6):10-17.
44. Zoborzi M, Momtaz S, Parvizi F, et al. Targeting mitogen-activated protein kinases by natural products: a novel therapeutic approach for inflammatory bowel diseases. *Curr Pharm Biotechnol*. 2020;21(13):1342-1353.
45. Quaglio AE, Castilho AC, Di Stasi LC. Experimental evidence of MAP kinase gene expression on the response of intestinal anti-inflammatory drugs. *Life Sci*. 2015;136(9):60-66.
46. Balasuriya N, McKenna M, Liu X, Li S, O’Donoghue P. Phosphorylation-dependent inhibition of Akt1. *Genes (Basel)*. 2018;9(4):450-465.
47. Setia S, Nehru B, Sanyal SN. Upregulation of MAPK/erk and PI3K/Akt pathways in ulcerative colitis-associated colon cancer. *Biomed Pharmacother*. 2014;68(8):1023-1029.
48. Zhao HF, Wang J, Tony TS. The phosphatidylinositol 3-kinase/Akt and c-Jun N-terminal kinase signaling in cancer: alliance or contradiction? (review). *Int J Oncol*. 2015;47(2):429-436.
49. Liu Y, Chen X, Cheng R, et al. The Jun/miR-22/HuR regulatory axis contributes to tumourigenesis in colorectal cancer. *Mol Cancer*. 2018;17(1):11-25.
50. Fritsch J, Garces L, Quintero MA, et al. Low-fat, high-fiber diet reduces markers of inflammation and dysbiosis and improves...
quality of life in patients with ulcerative colitis. Clin Gastroenterol Hepatol. 2021;19(6):1189-1199.

51. Yao D, Dong M, Dai C, Wu S. Inflammation and inflammatory cytokine contribute to the initiation and development of ulcerative colitis and its associated cancer. Inflamm Bowel Dis. 2019;25(10):1595-1602.

52. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. Immunity. 2019;51(1):27-41.

53. Galdiero MR, Marone G, Mantovani A. Cancer inflammation and cytokines. Cold Spring Harb Perspect Biol. 2018;10(8):1-18.

54. Schwager S, Detmar M. Inflammation and lymphatic function. Front Immunol. 2019;10(2):308-318.

55. Mateescu RB, Bastian AE, Nichita L, et al. Vascular endothelial growth factor - key mediator of angiogenesis and promising therapeutic target in ulcerative colitis. Rom J Morphol Embryol. 2017;58(4):1339-1345.

56. Shen CY, Lu CH, Wu CH, et al. The development of maillard reaction, and advanced glycation end product (AGE)-receptor for AGE (RAGE) signaling inhibitors as novel therapeutic strategies for patients with AGE-related diseases. Molecules. 2020;25(23):5591-5620.

57. Waghela BN, Vaidya FU, Ranjan K, et al. AGE-RAGE synergy influences programmed cell death signaling to promote cancer. Mol Cell Biochem. 2021;476(2):585-598.

58. Ahmad S, Khan H, Siddiqui Z, et al. AGEs, RAGEs and s-RAGE; friend or foe for cancer. Semin Cancer Biol. 2018;49(4):44-55.

59. Beguin EP, van den Eshof BL, Hoogendijk AJ, et al. Integrated proteomic analysis of tumor necrosis factor alpha and interleukin 1beta-induced endothelial inflammation. J Proteomics. 2019;192(2):89-101.

60. You T, Bi Y, Li J, et al. IL-17 induces reactive astrocytes and up-regulation of vascular endothelial growth factor (VEGF) through JAK/STAT signaling. Sci Rep. 2017;7(41779):1-15.

61. Kumar P, Monin I, Castillo P, et al. Intestinal interleukin-17 receptor signaling mediates reciprocal control of the gut microbiota and autoimmune inflammation. Immunity. 2016;44(3):659-671.