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·营养与保健·

Craterellus cornucopioides（灰喇叭菌）水提取物减轻结肠炎并通过网络药理学和分子对接探索其潜在机制

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摘　要: 目的：本研究探讨 *Craterellus cornucopioides*（灰喇叭菌）水提取物（*Craterellus cornucopioides* water extracts，CCWE）对大鼠溃疡性结肠炎（ulcerative colitis，UC）模型的保护作用。方法：使用三硝基苯磺酸（trinitrobenzene sulfonic acid，TNBS）诱导大鼠溃疡性结肠炎（UC）模型。通过监测大鼠体重变化、疾病活动指数（disease activity index，DAI）评分和结肠组织苏木精-伊红（hematoxylin-eosin，HE）染色来评估病理损伤。检测结肠组织中髓过氧化物酶（myeloperoxidase，MPO）活性、一氧化氮（NO）、超氧化物歧化酶（superoxide dismutase，SOD）活性、丙二醛（malondialdehyde，MDA）和促炎细胞因子（IL-1β、TNF-α、IL-6）水平，评价 CCWE 对 UC 的治疗效果。采用网络药理学筛选 CCWE 针对 UC 的活性成分、靶点和通路，然后对关键相互作用进行分子对接验证，以阐明其物质基础和作用机制。结果：TNBS 组的 DAI 评分显著高于对照组（P<0.05）。与 TNBS 组相比，CCWE 显著降低 DAI 评分（P<0.05）并改善宏观和组织学损伤。此外，与 TNBS 组相比，CCWE 还显著降低了 MPO、NO、MDA、IL-1β、TNF-α 和 IL-6 水平（P<0.01），同时显著提高了 SOD 水平（P<0.01）。通过网络药理学预测 CCWE 治疗 UC 的核心靶点为 STAT3、SRC、MAPK3、PIK3R1 和 AKT1。作用机制包括 HIF-1、relaxin 和 PI3K/Akt 信号通路。CCWE 治疗 UC 的主要成分是 illudin T、槲皮素（quercetin）、4-羟基-4-异丙烯基环己烷甲醇乙酸酯（4-hydroxy-4-isopropenylcyclohexanemethanol acetate）、乙酸-4-氧代-5-己烯酯（4-oxo-hex-5-enyl acetate）、craterellins B、阿魏酸（ferulic acid）、craterellins A、咖啡酸（caffeic acid）、4-氧代己-1,6-二基二乙酸酯（4-oxohex-1,6-diyl diacetate）和隐陡头菌素 M（illudin M）。分子对接结果表明，CCWE 的活性成分与这五个核心靶点表现出良好的结合亲和力，其中槲皮素和乙酸-4-氧代-5-己烯酯显示出最显著的对接分数。结论：CCWE 的活性成分槲皮素和乙酸-4-氧代-5-己烯酯可调节包括 STAT3、SRC、MAPK3、PIK3R1 和 AKT1 等蛋白表达，最终激活 HIF-1、relaxin 和 PI3K/Akt 通路治疗溃疡性结肠炎（UC）。

关键词: *Craterellus cornucopioides*（灰喇叭菌）水提取物, 食用菌, 结肠炎, 网络药理学, 分子对接

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Craterellus cornucopioides Water Extracts Attenuate Colitis and Explore Underlying Mechanisms via Network Pharmacology and Molecular Docking

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Abstract: Objective: The protective effects of *Craterellus cornucopioides* water extracts (CCWE) on rat model of UC were investigated in this study. Methods: Trinitrobenzene sulfonic acid (TNBS) was used to induce an ulcerative colitis (UC) model in rats. Pathological injury was assessed by monitoring body weight changes, disease activity index (DAI) scores, and hematoxylin-eosin (HE) staining of colonic tissues. The levels of myeloperoxidase (MPO) activity, nitric oxide (NO), superoxide dismutase (SOD) activity, malondialdehyde (MDA), and pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) in colon tissues were examined to evaluate the therapeutic effects of CCWE on UC. Network pharmacology was employed to screen active ingredients, targets, and pathways of CCWE against UC, followed by molecular docking validation of key interactions to elucidate its material basis and mechanism of action. Results: The DAI score of the TNBS group was significantly higher than that of the control group (P<0.05). Compared with the TNBS group, CCWE significantly reduced the DAI score (P<0.05) and improved macroscopic and histological damage. In addition, compared with the TNBS group, CCWE also significantly decreased the levels of MPO, NO, MDA, IL-1β, TNF-α and IL-6 (P<0.01), while significantly increasing the level of SOD (P<0.01). The core targets of CCWE in treating UC were predicted to be STAT3, SRC, MAPK3, PIK3R1 and AKT1 by network pharmacology. The mechanisms of action encompass the HIF-1, relaxin, and PI3K/Akt signaling pathway. The main components of CCWE for treating UC might be illudin T, quercetin, 4-hydroxy-4-isopropenylcyclohexanemethanol acetate, 4-oxo-hex-5-enyl acetate, craterellins B, ferulic acid, craterellins A, caffeic acid, 4-oxohex-1,6-diyl diacetate, and illudin M. Molecular docking results indicate that the active components of CCWE exhibit good binding affinity with these five core targets, with quercetin and 4-oxo-hex-5-enyl acetate showing the most significant docking scores. Conclusion: The active ingredients in CCWE, quercetin and 4-oxo-5-hexenyl acetate, modulate the expression of proteins including STAT3, SRC, MAPK3, PIK3R1 and AKT1, ultimately activating the HIF-1, relaxin, and PI3K/Akt signaling pathways to treat ulcerative colitis.

Key words: *Craterellus cornucopioides* water extracts; edible fungus; colitis; network pharmacology; molecular docking

Inflammatory bowel disease（IBD）is primarily categorized into two types: Ulcerative colitis （UC）and Crohn's disease（CD）. The main characteristic of IBD is inflammation in the small intestine and colon[1−2]. Clinical data indicates that UC elevates the cumulative CRC risk by 18%~20%, while CD increases it by up to 8% following a 30-years duration of the disease[3]. Currently, the therapeutic options for UC include 5-ASA drugs, immunosuppressants, corticosteroids, cell therapy, and surgical intervention. However, therapeutic approaches often present significant adverse effects and limited tolerability. They are unsuitable for prolonged use[4−5]. Consequently, there is a pressing and urgent need to explore innovative adjunct therapies.

The pathogenesis of UC is considered to be caused by combination of factors, involving the intestinal epithelial barrier dysfunction, deregulated immune and inflammatory reactions to the gut microbiota and genetic susceptibility[6]. So far, increasing evidence suggests that imbalanced redox status and overproduction of proinflammatory cytokines play essential role in the pathogenesis and progression of UC[7−8]. During the initial stages of intestinal inflammation, excessive ROS production leads to oxidative stress and the release of excessive pro-inflammatory mediators, triggering an inflammatory response. Vice versa, the activation of inflammatory cells could accelerate oxidative stress, which induces intestinal epithelial barrier dysfunction. Therefore, the maintenance of redox homeostasis and regulation of inflammatory cytokines could be an effective approach against UC[9].

To date, abundant reports have revealed that natural edible products serving as complementary and alternative medicine (CAM) play an important role in maintaining health and preventing diseases, particularly for diseases caused by oxidative stress and inflammation[10−12]. *Craterellus cornucopioides*, a member of the Cantharellaceae family, is a globally distributed edible fungus that is high in protein, essential amino acids, dietary fiber, and vitamins, and low in fat and cholesterol. It is a functional food with higher nutritional value than other plants. Because of its natural antioxidant properties, it has excellent effects in preventing cardiovascular disease and also possesses anti-tumor, anti-inflammatory and anti-bacterial properties[13]. Previous literatures have indicated that both the ethanolic and aqueous extracts of *Craterellus cornucopioides* exhibit anti-inflammatory and antioxidant activities[14−15]. Meanwhile, a polysaccharide isolated from *Craterellus cornucopioides* was obtained which exhibited strong anti-oxidant and anti-inflamma-

tory activities[16]. Therefore, it is worthy to study whether the crude water extract of *Craterellus cornucopioides*（CCWE）have protective effect on UC. Network pharmacology is a research paradigm integrating systems biology, bioinformatics, and polypharmacology, which predicts the targets of active ingredients and their regulatory pathways by constructing a "drug-component-target-disease" multi-level interaction network. Unlike the traditional single-target research model, this method comprehensively analyzes the synergistic effects of multiple components, targets, and pathways in natural products, and can more comprehensively reveal the complex regulatory mechanisms of drugs[17−18]. This study explored the therapeutic effect of orally administered CCWE on UC with a TNBS-induced colitis model. Furthermore, the underlying mechanism of CCWE in treating UC were investigated using network pharmacology and molecular docking.

1 Materials and Methods

1.1 Materials and instrument

The dried fruiting bodies of *Craterellus cornucopioides* were sourced from Yunnan Province in China. 2,4,6-trinitrobenzene sulfonic acid (TNBS) and all other chemical regents were of analysis grade and purchased from Sigma-Aldrich Company (Germany).To obtain subjects for the study, male Sprague Dawley rats (200~220 g, approval number: SCXK2013-0004) were purchased from Hunan Silaikejingda Experimental Animal Co., Ltd. All animals were housed in a controlled environment (temperature range: 22~24 ℃; humidity: 40%~55%; 12-hour light/dark cycle). The experimental procedures were approved by the Jiangxi University of Chinese Medicine.

UV-1800 ultraviolet-visible spectropho-tometer was obtained from Japan Shimadzu; Varioskan LUX multimode microplate reader, ULT-7150-9-V ultra-low temperature freezer were purchased from Thermo Fisher Scientific, USA.

1.2 Experimental method

1.2.1 Preparation process of aqueous extract of *Craterellus cornucopioides* To eliminate lipids and pigments, *Craterellus cornucopioides* powder was refluxed in ethanol at 80 ℃ for 4 hours. Following this, the residue was extracted three times with hot water (at 90 ℃), each extraction lasting for 2 hours. The obtained supernatants were mixed and concentrated by rotary evaporation to yield CCWE.

1.2.2 Animals experiments After adaptation, the rats were randomly assigned to five groups. The groups were categorized as the control group, the TNBS-induced model group, the CCWE-treated groups (50 and 100 mg/kg), and the positive drug group receiving SASP at a dose of 500 mg/kg. Each group contained 8 rats. The colitis induction model was established using a previously reported method. Briefly, TNBS was dissolved in 50% ethanol (v/v) to prepare a solution. The prepared TNBS solution was then injected into the colon through a silicone tube (2 mm outer diameter) inserted 8 cm deep, and the administered dose was 0.5 mL/kg[19]. Rats were administered saline using the same method in the control group. To ensure that TNBS did not leak from the colon, all rats were maintained in a head-down position for five minutes. And then they were returned to their cages. Once the model was successfully established, the rats were gavaged once daily for 7 consecutive days (1.0 mL per 100 g of body weight). The animals were euthanized and the colon was collected on the 8th day. All colons were sectioned proportionally and stored at -80 ℃ for subsequent analysis.

1.2.3 Assessment of disease activity index (DAI) The body weight of the rats was measured daily to calculate the body weight change rate, while simultaneously monitoring the consistency of their feces and the presence of rectal bleeding. DAI of the rats was assessed based on criteria previously established in literature. DAI score=（A+B+C）/3[20]. A represented the body weight change score, B represented the fecal consistency score, and C represented the bloody stool score.

1.2.4 Macroscopic and histological evaluation of colitis Macroscopic changes in the colon are one of the main indicators for assessing the severity of colitis. The macroscopic score of the colon was determined based on criteria described in previous studies[21]. Histologic evaluation of the colon was also performed by fixing approximately 0.5×0.5 cm^2 of colon tissue in 4% formalin solution, which was then embedded in paraffin and stained with hematoxylin-eosin (H&E) before light microscopy. Sections were scored for the degree, extent, and depth of inflammation and the amount of glandular and epithelial damage as previously reported in the literature[22].

1.2.5 Measurement of the levels of inflammatory cytokines and MPO, SOD, MDA, NO in colon tissues After collecting the colonic tissues, they were washed and then homogenized using pre-cooled PBS. The ho-

mogenate was centrifuged at 4 ℃ for 10 minutes. The supernatant was collected, and the total protein concentration was determined using the BCA method. The concentrations of TNF-α, IL-6, and IL-1β were measured using their respective ELISA kits, while the levels of MPO, SOD, MDA, and NO were assayed using corresponding kits. The entire experimental procedure was carried out according to the instructions.

1.2.6 Network pharmacology study

1.2.6.1 Prediction of targets and screening of potential targets in CCWE The chemical composition of CCWE reported in the literature was searched and obtained[23−27]. The SMILES codes for these compounds were obtained from the PubChem database. These SMILES codes were then input into the Swiss Target Prediction and TCMSP databases to predict the potential targets of the active ingredients in *Craterellus cornucopioides*. The keyword "ulcerative colitis" was used as a search term to obtain the relevant targets for this disease from OMIM, Gene Card, Drug Bank, and TTD databases, respectively. Subsequently, a Venn diagram was drawn to obtain the potential intervention targets of CCWE for the treatment of colitis.

1.2.6.2 Construction of protein–protein interaction (PPI) network The above intersection targets were input into a STRING database to construct a PPI network. During this process, "Homo sapiens" was set as the species, and a "highest confidence" threshold of 0.9 was established for minimum interactions to guarantee the PPI network's clarity. The relevant data files were integrated into Cytoscape 3.9.1 for visualization and analytical. In order to estimate the topological characteristics of the network nodes, three parameters including degree, betweenness centrality, and closeness centrality were used to predict protein-protein interactions in this study.

1.2.6.3 GO and KEGG pathway enrichment analysis GO and KEGG pathway enrichment analyses were conducted using the DAVID platform (https://david.ncifcrf.gov/). The top 10 enriched GO terms and the top 20 enriched KEGG pathways were obtained and presented as bubble and bar graphs, respectively. These results explore the potential biological functions and major signaling pathways of *Craterellus cornucopioides* in the treatment of UC.

1.2.6.4 Active ingredient-target-disease pathway network construction of CCWE The chemical compositions of the screened active ingredients in CCWE, the intersection target genes and the top 20 major pathways from the KEGG enrichment analysis were loaded into Cytoscape 3.9.1 software for visualization, resulting in the construction of the ingredient-target-disease network.

1.2.7 Molecular docking The MOE software was utilized to dock the active components screened from the "active ingredient-target-pathway" network with the core target proteins. The 3D structures of the target proteins were downloaded from the RCSB PDB database（http://www.rcsb.org/pdb）, while the 3D chemical structures of the active ingredient were obtained from the PubChem database or previous literature. The MOE software was employed to perform molecular docking between the compounds and targets, and the binding energies were calculated. Among them, a lower binding energy of the docking conformation indicates a more stable docking.

1.3 Statistical analysis

The data are shown as means±standard deviation (SD). For statistical analysis, one-way analysis of variance was performed using SPSS version 21.0. A statistical significance level of $P<0.05$ was set.

2 Results and analysis

2.1 Effect of CCWE on body weight and DAI score in rats with colitis

The body weight was also significantly altered in

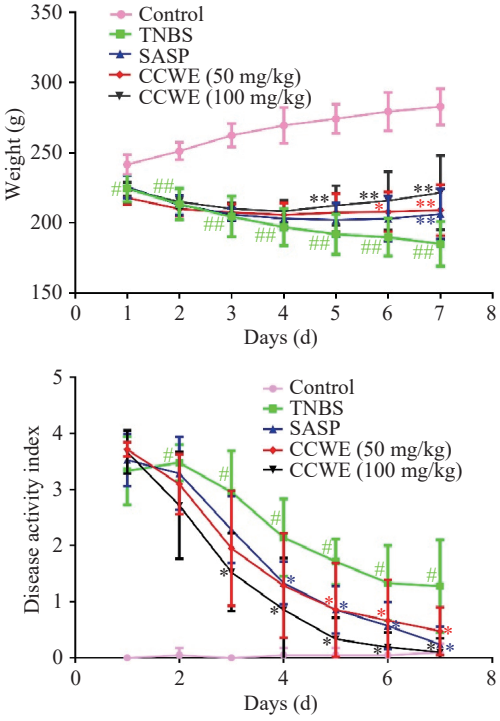


Fig.1 Effect of SASP (500 mg/kg) and different doses of CCWE (50, 100 mg/kg) on body weight (A) and DAI scores (B) of rats with colitis

Note: Data are showed as the mean±SD. #$P<0.05$ vs. control group, ##$P<0.01$ vs. control group, *$P<0.05$ vs. TNBS group, **$P<0.01$ vs. TNBS group.

the UC rat model. As shown in Fig.1A, the body weight of the TNBS group was significantly lower than the control group (P<0.01). Rats treated with SASP and CCWE (50 mg/kg and 100 mg/kg) showed significantly higher body weights compared to the TNBS group (P<0.01). Thus, CCWE could alleviate weight loss in rats with colitis. DAI scores reflected the clinical manifestation of UC. As illustrated in Fig.1B, the TNBS group exhibited a significantly higher DAI score than the control group (P<0.05). In contrast, rats treated with SASP and CCWE at doses of 50 mg/kg and 100 mg/kg showed significant reduction in DAI scores compared to the TNBS group (P<0.05). Therefore, CCWE could ameliorate DAI scores in colitis rats.

2.2 Effect of CCWE on macroscopic and histological alterations in rats with colitis

The macroscopic changes were observed in Fig.2A. The TNBS group displayed sever edematous changes, hemorrhagic, ulcerated and necrotic in the colonic mucosa. And the SASP and CCWE (50, 100 mg/kg) groups relieved the symptoms in the colonic tissue. The macroscopic scores were also shown in Fig.2B. The TNBS group exhibited a significantly higher macroscopic score than the control group (3.43±0.53 vs. 0.14±0.38, P<0.01). In addition, administration of SASP and CCWE at doses of 50 mg/kg and 100 mg/kg respectively, resulted in a notable reduction in the scores compared to the TNBS group (P<0.01).

The same phenomenon was found in the histological changes. According to Fig.3A, the colonic tissue of TNBS group showed the goblet cells and epithelium disappeared, deformed or disappeared crypt and diffuse inflammatory cell infiltration. However, treatment with SASP and CCWE (50, 100 mg/kg) groups partially recovered the crypt architecture and reduced inflammatory cell infiltration in colonic tissue. The histological scores for both SASP and CCWE treatments at doses of 50 mg/kg and 100 mg/kg were notably lower than those of the TNBS group (Fig.3B, P<0.01).

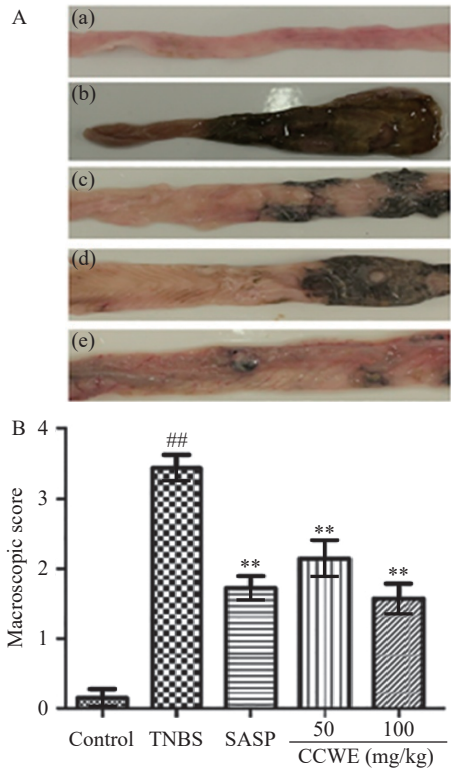


Fig.2　Effect of SASP (500 mg/kg) and different doses of CCWE (50, 100 mg/kg) on modifications in colonic macroscopic morphology (A) and macroscopic score (B)

Note: (a) control group; (b) TNBS group; (c) SASP group; (d) CCWE treatment group, 50 mg/kg; (e) CCWE treatment group, 100 mg/kg. Data are showed as the mean±SD. ##P<0.01 vs. control group, **P<0.01 vs. TNBS group.

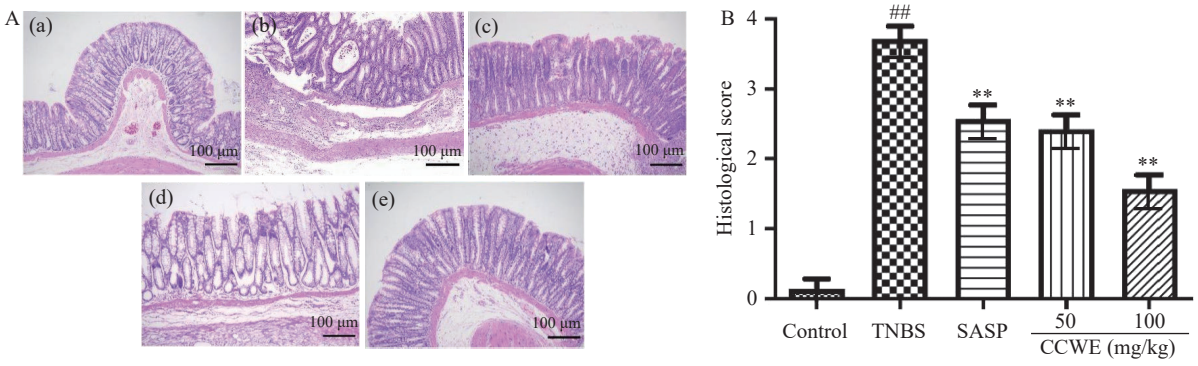


Fig.3　Effect of SASP (500 mg/kg) and different doses of CCWE (50, 100 mg/kg) on colonic histological changes (A) and histological score (B)

Note: (a) control group, (b) TNBS group, (c) SASP group, (d) CCWE treatment group, 50 mg/kg, (e) CCWE treatment group, 100 mg/kg. Data are showed as the mean±SD. ##P<0.01 vs. control group, **P<0.01 vs. TNBS group. H&E straining, magnification 100×.

2.3 Effect of CCWE on the MPO, SOD, MDA and NO levels in rats with colitis

Meanwhile, the levels of MPO, SOD, MDA, and NO were measured to evaluate the degree of oxidative stress. MPO is a major marker of the degree of neutrophil infiltration in tissues. TNBS group caused significantly increased in the colonic MPO (P<0.01) compared to the control group. Administration of both SASP and CCWE at dosages of 50 mg/kg and 100 mg/kg markedly suppressed MPO activity in colon (P<0.01, Fig.4A). The data showed that colonic SOD activity in the TNBS group was significantly reduced compared to the control group (P<0.01). The colonic SOD activity was increased to 42.71±7.94 U/mg, 33.34±6.54 U/mg and 50.30±9.42 U/mg (P<0.01, Fig.4B) with SASP and CCWE at different dose (50 mg/kg and 100 mg/kg).

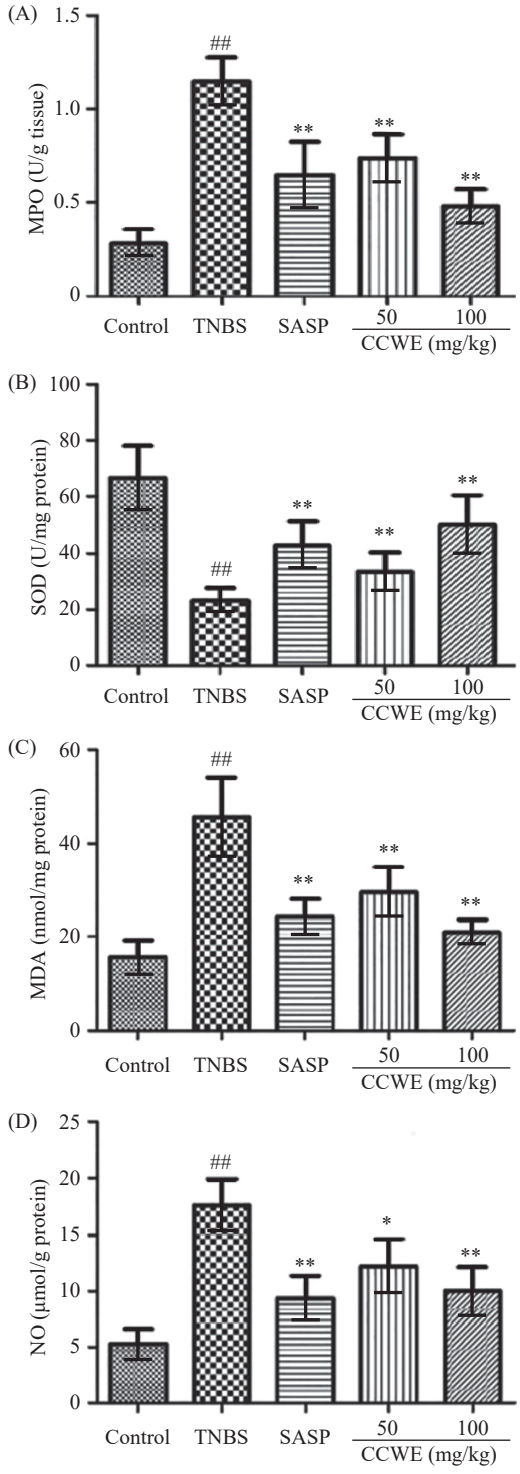


Fig.4 Effect of SASP (500 mg/kg) and different doses of CCWE (50, 100 mg/kg) on antioxidant index

Note: (A) MPO; (B) SOD; (C) MDA; (D) NO. Data are showed as the mean±SD. ##P<0.01 vs. control group, *P<0.05 vs. TNBS group, **P <0.01 vs. TNBS group.

Higher tissue levels of MDA, NO were also found in TNBS group compared to the control group (P<0.01). The MDA and NO levels was decreased to 24.47±3.61 nmol/mg, 29.76±4.84 nmol/mg, 21.29±2.37 nmol/mg and 9.38±1.79 μmol/g, 12.25±2.18 μmol/g, 10.04±1.97 μmol/g after treatment with SASP and CCWE (50 mg/kg and 100 mg/kg) (Fig.4C~Fig.4D), respectively. These results indicated that CCWE could reduce the oxidative stress in TNBS-induced colitis of rats.

2.4 Effect of CCWE on the pro-inflammatory cytokines in rats with colitis

According to Fig.5, the TNBS group exhibited a significant elevation in the levels of TNF-α, IL-1β and IL-6 in comparison to the control group (P<0.01). SASP and CCWE (50 mg/kg and 100 mg/kg) significantly decreased the levels of TNF-α, IL-1β and IL-6 (P<0.01). These results indicated that CCWE suppressed the production of inflammatory cytokines in rats with colitis.

2.5 Potential active ingredients and targets of CCWE

According to the previous literature reports, a total of 29 chemical components of CCWE were retrieved from the literature. The target information was obtained through Swiss Target Prediction and TCMSP databases, and a total of 397 target genes were obtained after removing duplicates. The "active ingredient-target" network of CCWE was built using Cytoscape 3.9.1 (Fig. 6).

2.6 Colitis-related targets

Disease targets were searched using the keyword "ulcerative colitis" in OMIM, GeneCard, DrugBank and TTD databases, and the disease genes collected from each database were summarized. A total of 1268 colitis targets were obtained. The potential targets of CCWE were intersected with the targets of ulcerative colitis, yielding 150 intersection targets, and a Venn diagram was subsequently constructed to visually represent this overlap (Fig.7).

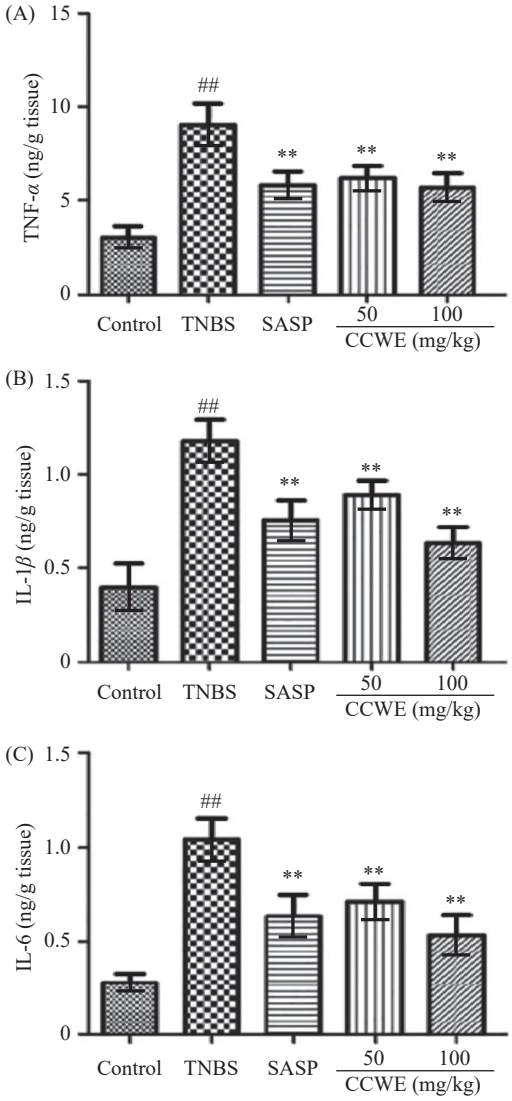


Fig.5　Effect of SASP (500 mg/kg) and different doses of CCWE (50, 100 mg/kg) on pro-inflammatory cytokines

Note: (A) TNF-α, (B) IL-1β, (C) IL-6. Data are showed as the mean±SD. ##P<0.01 vs. control group, **P<0.01 vs. TNBS group.

2.7　PPI network

The drug and disease intersection targets were imported into the STRING database to construct a PPI network, as shown in Fig.8. This network comprises 150 nodes and 648 edges. The between centrality, closeness centrality, and degree values for these targets were obtained by utilizing the Cytoscape function within the software. The top 5 targets were selected as key targets for further research, namely STAT3, SRC, MAPK3, PIK3R1 and AKT1. The main parameters of these targets were presented in Table 1.

Table 1　Key targets

No.	Target	degree	Betweenness centrality	Closeness centrality
1	STAT3	92	2297.604	0.53586495
2	SRC	78	1255.6414	0.50396824
3	MAPK3	74	1255.6116	0.48288974
4	PIK3R1	70	434.24405	0.4738806
5	AKT1	66	1313.3086	0.48473284
6	PTPN11	60	234.87926	0.45519713
7	PIK3CA	60	196.53142	0.45519713
8	HSP90AA1	60	1089.8737	0.4774436
9	RELA	58	647.37024	0.4810606
10	CTNNB1	52	692.9213	0.4618182

2.8　Results of GO enrichment and KEGG pathway enrichment analysis

The common targets between drugs and diseases were analyzed through GO and KEGG pathway enrichment analyses. The GO enrichment results are categorized into three parts: Biological process (BP), cellular component (CC), and molecular function (MF). This included 345 biological processes, 40 cellular components, and 90 molecular functions were identified based on P<0.01. The top 10 items from each category were visualized and presented in Fig.9(A). The primary BP involved negative regulation of apoptotic process, protein phosphorylation, and positive regulation of gene expression. CC were primarily associated with the cytoplasm, plasma membrane and MF

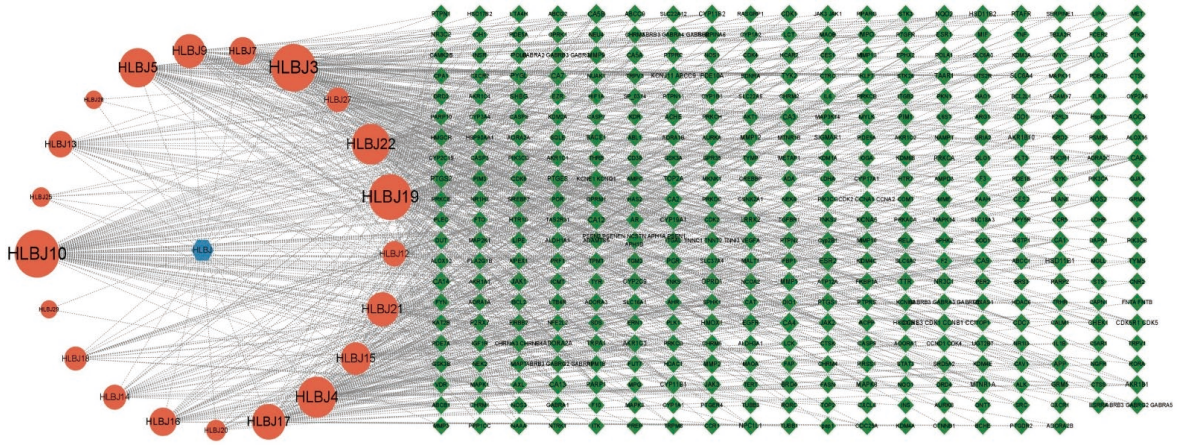


Fig.6　Active component-target network

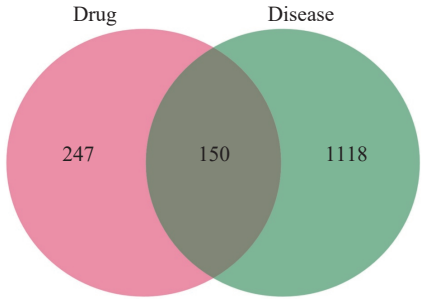


Fig.7 Active component-target network

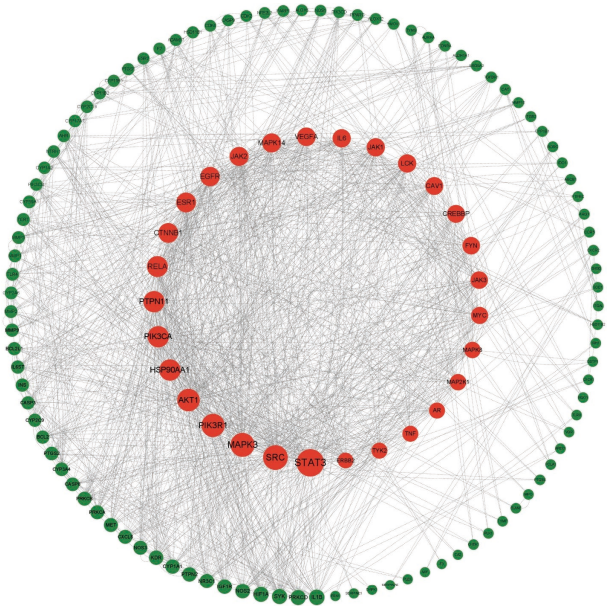


Fig.8 PPI network

were implicated in protein binding, ATP binding, and protein serine/threonine/tyrosine kinase activity. The KEGG pathway enrichment analysis yielded 152 pathways at P<0.01. The top 20 pathways, including the HIF-1 signaling pathway, relaxin signaling pathway, PI3K-Akt signaling pathway, etc., were presented in Fig.9 (B).

2.9 Active ingredient-target-disease pathway network construction of CCWE

The information on drugs, active components, possible targets, and pathways was integrated into Cytoscape 3.9.1 to build a network linking CCWE's active ingredients to their targets and disease pathways (Fig.10). This network comprises 44 nodes and 150 interaction edges, indicating that CCWE exerts therapeutic effects on UC through multi-component, multi-target, and multi-site approaches. The top 10 active ingredients screened according to the degree value were illudin T, quercetin, 4-hydroxy-4-isopropenylcyclohexanemethanol acetate, 4-oxo-hex-5-enyl acetate, craterellins B, ferulic acid, craterellins A, caffeic acid, 4-oxohex-1,6-diyl diacetate and illudin M. It is hypothesized that these components are the core components

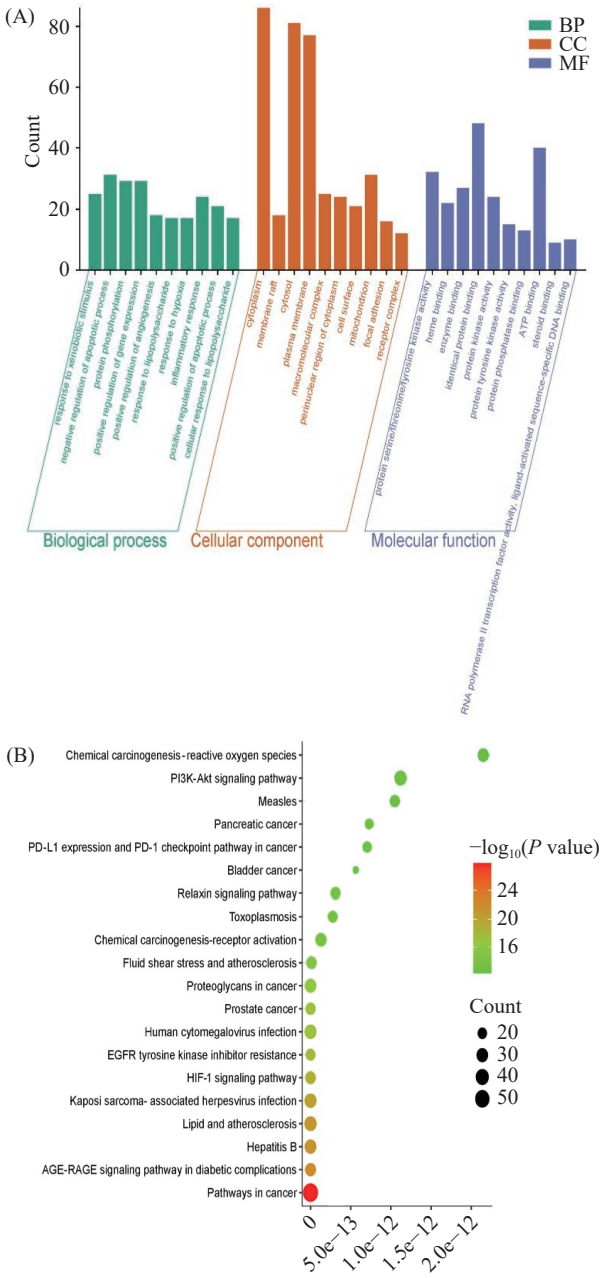


Fig.9 Active component-target network

of *Craterellus cornucopioides* for the treatment of UC.

2.10 Molecular docking

As shown in Table 2, based on the findings from network pharmacology, the 10 selected core components were subjected to docking validation with the top 5 corresponding targets (STAT3, SRC, MAPK3, PIK3R1 and AKT1) in the PPI network. Among these, a binding energy of <−5 kcal/mol indicated a strong binding affinity. The results indicated that quercetin exhibits good binding capability with STAT3, SRC, MAPK3 and AKT1, while 4-oxohex-1,6-diyl diacetate displays good binding ability with PIK3R1. Fig.11 illustrated the optimal schematic diagrams representing the best docking outcomes for

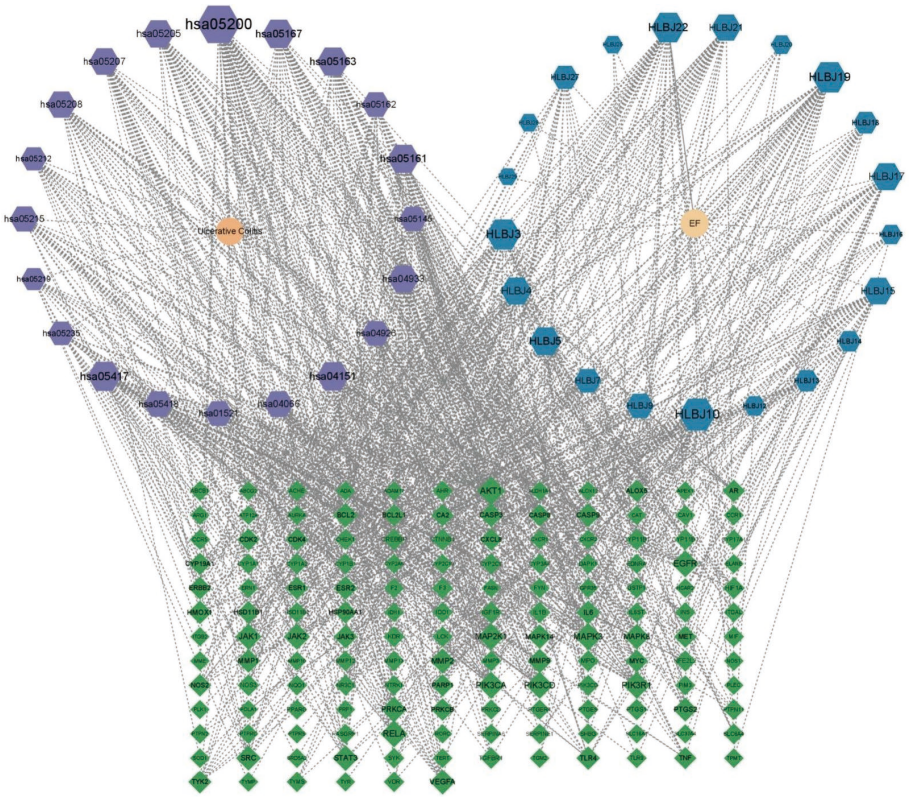


Fig.10　Active component-target network

Table 2　Details on the molecules and their respective targets for molecular docking

Key target	PDB ID	Docking score（kcal/mol）									
		illudin T	quercetin	4-hydroxy-4-isopropenylcyclohe-xanemethanol acetate	4-oxo-hex-5-enyl acetate	craterellins B	ferulic acid	craterellins A	caffeic acid	4-oxohex-1,6-diyl diacetate	illudin M
STAT3	6NJS	−4.61	−5.42	−5.01	−4.91	−4.47	−4.99	−4.39	−4.34	−5.01	−4.42
SRC	1FMK	−4.79	−6.14	−5.48	−4.87	−4.99	−5.47	−4.99	−4.97	−4.78	−4.70
MAPK3	3FHR	−5.41	−6.82	−5.51	−5.44	−4.57	−5.82	−5.42	−5.49	−6.59	−5.41
PIK3R1	2XS6	−5.29	−5.33	−5.59	−5.04	−5.19	−4.83	−5.34	−5.09	−5.41	−5.13
AKT1	1UNQ	−4.95	−5.08	−4.96	−4.61	−5.03	−4.66	−4.97	−4.75	−5.00	−4.91

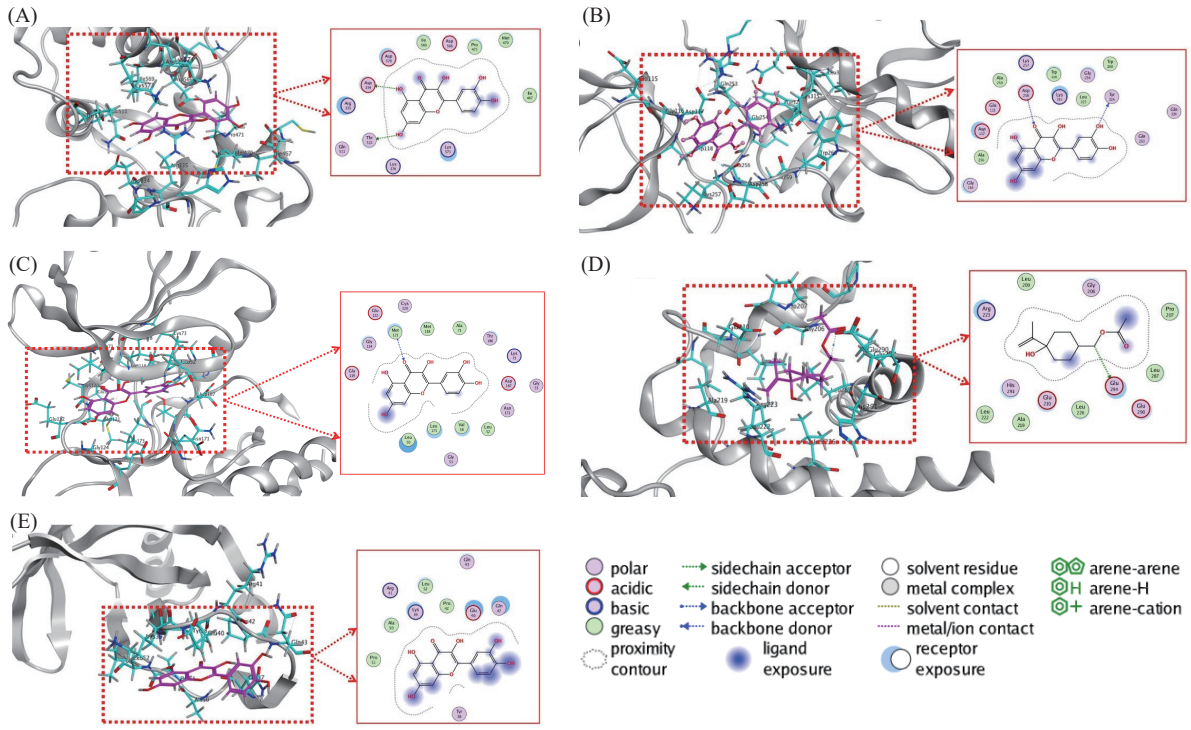


Fig.11　Results of docking experiments between key targets and specific active compounds

Note: (A) STAT3-quercetin; (B) SRC-quercetin; (C) MAPK3-quercetin; (D) PIK3R1-quercetin; (E) AKT1-quercetin.

each of the targeted proteins. As visualized in the results, quercetin forms two hydrogen bonds with STAT3 via the amino acid residues Asp-334 and Thr-515, exhibiting a binding energy of −5.42 kcal/mol. Quercetin established two hydrogen bonds with SRC mediated by Asp-258 and Tyr-326, demonstrating a binding energy of −6.14 kcal/mol. Quercetin formed a single hydrogen bond with MAPK3 at Met-121, yielding a binding energy of −6.82 kcal/mol. Quercetin interacted with PIK3R1 through a hydrogen bond at Glu-294, with a binding energy of −5.33 kcal/mol. Additionally, quercetin engaged with AKT1 via van der Waals forces, showing a binding energy of −5.08 kcal/mol.

3 Discussion

In this study, we investigated the effect of CCWE on TNBS-induced colitis in rats. The TNBS-induced colitis model was selected due to its similarities to human colitis as observed in prior research[28]. In this model, TNBS could elicit changes in DAI, and damage the colonic tissue, which was proven by macroscopic and histological indices. CCWE at different doses significantly reduced the DAI and attenuated the macroscopic and histological damage induced by TNBS. These findings indicated that CCWE exhibited a protective effect in the TNBS-induced colitis model.

Several studies confirmed that oxidative stress was associated with the pathogenesis of UC. Under normal circumstances, reactive oxygen species (ROS) were controlled by antioxidant systems. Nevertheless, excessive ROS production was due to oxidative stress which aggravated the inflammatory response in the progression of UC[29−30]. Hence, several typical indexes associated with oxidative stress were measured in this study. SOD as a crucial antioxidant enzyme reduced the oxidative damage. MDA is the producer of lipid peroxidation which is an indicator of oxidative stress. Previous literature indicated that colonic MDA levels were increased and SOD activity was decreased in both human and animal model, which was also be observed in our study[31−33]. Administering CCWE at different doses (50 mg/kg and 100 mg/kg) notably reduced MDA levels and enhanced SOD activity. The results indicated that CCWE provided protective effects in UC probably linked to decreased oxidative stress.

The MPO level in the colon serves as an indicator of neutrophil infiltration in colonic tissue, enabling assessment of the colonic inflammation's severity. Meanwhile, NO was involved in the pathogenesis of inflammation process which was synthesized by iNOS. It has been documented that excessive production of NO could damage the colonic mucosa[34−35]. Therefore, NO serves as a significant indicator of inflammatory bowel disease as well. In our study, the levels of MPO and NO in colon tissue were increased in TNBS group consistent with previous literature. The CCWE groups remarkably decreased colonic MPO and NO levels, thereby suggesting an anti-inflammatory effect of the treatment. Many studies have demonstrated the pathogenesis of UC was related to the cytokine release imbalance. Excessive levels of pro-inflammatory cytokines have been found in UC patients and experimental animal models[36]. Therefore, targeting pro-inflammatory cytokines has been used as a treatment for UC patients[37]. TNF-α, IL-1β and IL-6 were the critical indicators in the pathological development of UC among the pro-inflammatory cytokines. TNF-α was a pivotal target molecule in UC, mediating diverse biological effects such as neutrophil recruitment to inflammatory sites and causing edema[38−39].

IL-1β could provoke neutrophil migration to inflamed colonic tissue[40], while IL-6 played a crucial role in promoting T-cell survival and resistance to apoptosis in the lamina propria of inflamed areas[41]. IL-6 production returns to normal levels when intestinal inflammation resolves. Our study showed that CCWE significantly reduced the levels of TNF-α, IL-1β and IL-6 in colon, suggesting a mechanism which CCWE affects cytokine production and inhibit immune inflammation.

In order to further investigate the mechanism of CCWE efficacy in the treatment of UC, this study used network pharmacology to explore the mechanism and active ingredients of CCWE. According to KEGG pathway analysis, the main pathways enriched of CCWE in treating UC include the HIF-1 signaling pathway, relaxin signaling pathway, PI3K-Akt signaling pathway, etc. Studies showed that patients with UC often suffer from hypercoagulation of blood, leading to thrombosis of local blood vessels in the intestinal tract, which further exacerbates intestinal mucosal ischemia and hypoxia[42]. HIF-1, a transcription factor that is widely present in the body under hypoxic conditions, plays a key role in maintaining homeostasis of the internal environment. It has been recognized as playing an important role in improving hypoxic conditions. It can induce the expression of VEGF

and EPO genes, thereby promoting angiogenesis and erythropoiesis while inhibiting cell apoptosis. Therefore, the HIF-1 signaling pathway is believed to participate in the inflammatory regulation process in UC[43]. The relaxin signaling pathway is currently believed to be related to tissue dysfunction caused by tissue fibrosis. Studies have shown that intestinal fibrosis occurs in patients with UC. Recent studies have found that the drug treats UC by interfering with the relaxin signaling pathway in the intestinal epithelium, thereby exerting an antifibrotic effect[44]. The PI3K-Akt signaling pathway is also an important intracellular signal transduction pathway that participates in regulating various biological processes such as cell growth, metabolism, survival, and proliferation. As one of the major upstream pathways of NF-κB, which is closely related to inflammation[45], inhibiting the PI3K/Akt signaling pathway may also contribute to alleviating UC[46]. Therefore, it can be hypothesized that the therapeutic effect of CCWE on UC may be achieved through multiple targets and pathways.

This study also predicted the core components of CCWE for the treatment of UC. The top ten compounds based on the degree value of CCWE were illudin T, quercetin, 4-hydroxy-4-isopropenylcyclohexanemethanol acetate, 4-oxo-hex-5-enyl acetate, craterellins B, ferulic acid, craterellins A, caffeic acid, 4-oxohex-1, 6-diyl diacetate, and illudin M. Previous literature studies have shown that quercetin can protect and improve intestinal mucosal barrier function by regulating the composition of intestinal flora, mucosal layer thickness, intestinal epithelial cells, and the tight junctions (TJs) proteins[47]. Additionally, it inhibits the expression of pro-inflammatory factors such as IL-17, TNF-α, and IL-6, while increasing the expression of IL-10, which exhibits therapeutic effects on UC[48]. Additionally, research has also found that ferulic acid can inhibit inflammatory damage to endothelial cells in ulcerative colitis or alleviate TNF-α-induced damage to human intestinal microvascular endothelial cells by inhibiting the TXNIP/NLRP3 signaling pathway[49]. Ferulic acid can also inhibit inflammatory damage to endothelial cells in UC by inhibiting the TXNIP/NLRP3 signaling pathway. Furthermore, caffeic acid has also been found to effectively alleviate DSS-induced ulcerative colitis by enhancing the body's defenses against oxidative stress and inflammatory responses[50]. To explore the regulatory mechanisms of these components, molecular docking was utilized in this study to dock ten main components with the top five targets. It was surprising that quercetin exhibited strong binding capabilities with the core targets STAT3, SRC, MAPK3 and AKT1, while 4-oxo-hex-5-enyl acetate showed good binding ability with PIK3R1. This finding indicated that quercetin and 4-oxo-hex-5-enyl acetate are likely the primary bioactive compounds in CCWE for treating UC. They may regulate the HIF-1 signaling pathway, relaxin signaling pathway, and PI3K-Akt by binding to these key targets, ultimately achieving the therapeutic effect on UC. However, further experimental validation could be required to elucidate the specific regulatory mechanisms.

4 Conclusion

In summary, the results indicated that CCWE had an ameliorative effect on UC. Additionally, the network pharmacology method and molecular docking method were used to elucidate the mechanism and material basis of CCWE for the treatment of UC, which provided a basis for further exploration of the therapeutic mechanism of CCWE for the treatment of UC. However, the therapeutic mechanisms underlying CCWE's efficacy against UC remain to be elucidated.

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