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Changyanqing decoction ameliorates ulcerative colitis via PI3K-Akt pathway: Insights from network pharmacology and *in-vitro* validation

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Abstract: **Background**: Ulcerative colitis (UC) is a chronic inflammatory bowel disease with limited therapeutic options and notable side effects. Changyanqing (CYQHJ) Decoction, a traditional Chinese medicine formula, has shown therapeutic potential; however, its underlying mechanisms remain unclear. **Objectives**: This study aimed to elucidate the pharmacological mechanisms of CYQHJ in UC, with a focus on identifying key bioactive compounds and signaling pathways. **Methods**: Network pharmacology was leveraged to pinpoint potential targets, bioactive compounds and signaling pathways underlying CYQHJ's therapeutic effects. Protein–protein interaction and KEGG enrichment analyses highlighted PI3K-Akt as a central pathway. *In-vitro* assays using Caco-2 cells validated the protective and anti-inflammatory effects of quercetin, the major active compound, in a DSS-induced injury model. Cell viability (CCK-8), cytokines (ELISA) and pathway protein expression (Western blot) were evaluated. **Results**: Quercetin was identified as a key compound targeting PI3K-Akt signaling. It significantly improved cell viability and reduced TNF-α and IL-6 levels ($P<0.001$). Western blot confirmed quercetin inhibited PI3K and Akt phosphorylation, while the PI3K-Akt activator Recilisib reversed these effects ($P<0.001$). **Conclusion**: CYQHJ exerts anti-inflammatory effects against UC mainly through PI3K-Akt inhibition, with quercetin as the core bioactive compound, providing mechanistic insight into its therapeutic action.

Keywords: Changyanqing Decoction; *In-vitro* validation; Network pharmacology; PI3K-Akt pathway; Quercetin; Ulcerative colitis

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INTRODUCTION

Ulcerative colitis (UC) represents a long-standing, noninfectious inflammatory disease of the colon, primarily affecting the mucosal and submucosal layers. It is marked by recurrent episodes of abdominal pain, diarrhea and bloody stools, significantly impairing patients' quality of life (Muzammil, *et al*., 2023; Armuzzi and Liguori, 2021). The global burden of UC has been rising steadily, making it an important public health concern. Although its exact etiology remains unclear, inherited risk factors, aberrant immune responses, gut microbial dysbiosis, and environmental stimuli are thought to contribute to disease development (Alemany-Cosme. Current therapies, including aminosalicylates, corticosteroids, immunosuppressants and biologics such as anti-TNF-α agents, are often limited by adverse effects, resistance, high cost and poor patient compliance (Park and Cheon, 2022; Ferretti, *et al*., 2022). Therefore, identifying safer and more effective therapeutic strategies with well-defined mechanisms remains an urgent priority.

Traditional Chinese Medicine (TCM), characterized by its combination of numerous active constituents, broad therapeutic targets, and involvement in multiple signaling cascades, has shown notable clinical efficacy in inflammatory bowel diseases, including UC (Xu. Changyanqing (CYQHJ) Decoction, a classical TCM formula composed of herbs with heat-clearing, dampness-eliminating, and blood-activating properties, has been widely used in the clinical management of colitis and related conditions (Song *et al.,* 2009; Lu. However, the basis of its pharmacological action and the molecular pathways through which it exerts therapeutic effects remain largely undefined, hindering its modern pharmacological interpretation and global acceptance.

Network pharmacology has gained attention as a robust method for decoding the mechanistic intricacies of TCM by integrating data on herbal components, target genes and signaling pathways (Li, *et al*., 2025). When combined with *in vitro functional assays, this approach enables systematic identification and validation of key bioactive compounds and their* targets.

In this research, network pharmacology was employed to pinpoint active ingredients and potential targets of CYQHJ, highlighting the PI3K/Akt signaling cascade as a major therapeutic axis. Quercetin, a major bioactive compound, was selected for validation in a DSS-induced Caco-2 cell model of UC. Cell viability, inflammatory cytokine levels and protein expression related to the PI3K-Akt pathway were assessed and pathway specificity was further confirmed using the PI3K-Akt activator Recilisib. This work aims to elucidate the molecular

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mechanism of CYQHJ in UC treatment and offer a scientific rationale for its therapeutic use.

MATERIALS AND METHODS

Active compound and target identification

Every herbal constituent in CYQHJ was sourced from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database. Bioactive compounds were screened based on two key ADME parameters: an OB cutoff of ≥ 30% and a DL cutoff of ≥ 0.18. For each selected compound, the chemical structure was acquired using the CAS number via the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Afterward, SMILES structures were submitted to the SwissTargetPrediction platform to identify their potential protein targets. Targets obtained from both TCMSP and SwissTargetPrediction were assembled, with duplicate results eliminated. The resulting target proteins were then standardized and mapped to official gene symbols via the UniProt database (https://www.uniprot.org/).

UC-related target retrieval

UC-related targets were acquired by querying the keyword "ulcerative colitis" in GeneCards (https://www.genecards.org/) and OMIM (https://omim.org/). Targets identified in the two databases were integrated and de-duplicated to generate the UC-related target set.

Construction of compound-target-disease network

Genes shared by drug-related and UC-related targets were detected using a Venn diagram tool. The interaction network comprising herbs, bioactive compounds, and common targets was generated in Cytoscape (version 3.8.0). Network topology parameters, such as "degree," were calculated to identify key nodes.

Establishment of the protein–protein interaction (PPI) network

The common targets were submitted to the STRING platform (https://string-db.org/) for PPI network generation, with the organism restricted to Homo sapiens and the confidence threshold set to 0.900 (highest confidence). Targets lacking interaction links were removed. The PPI network was then displayed in Cytoscape to identify core targets based on topological parameters.

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) functional enrichment

GO annotation and KEGG pathway enrichment were conducted for the shared targets using DAVID (https://david.ncifcrf.gov/), to explore the biological functions and signaling pathways potentially involved. Terms with $p < 0.05$ were considered statistically significant. Bubble plots summarizing the enrichment patterns were generated using ImageGP (http://www.ehbio.com/ImageGP/).

Molecular docking

Molecular docking was performed to assess the binding affinity between quercetin and core target proteins. The three-dimensional structures of the proteins were obtained from the Protein Data Bank (PDB, https://www.rcsb.org/) and the structure of quercetin was retrieved from PubChem. Protein structures were prepared by removing water molecules and adding hydrogen atoms, while the ligand was energy-minimized prior to docking. Docking simulations were performed using AutoDock, and the conformation with the lowest binding energy was selected for subsequent visualization and analysis.

Cell culture

Under humidified conditions at 37°C with 5% CO_2, human colorectal epithelial Caco-2 cells (Cat#TCHu157; Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were maintained in high-glucose DMEM (Cat#11995065) with 10% fetal bovine serum (FBS; Cat#10099141) and 1% penicillin–streptomycin (Cat#15140122) (all from Gibco, Thermo Fisher Scientific, USA). The medium was replaced every 2–3 days and cells were passaged at 70–80% confluence using 0.25% trypsin–EDTA (Cat#25200056; Gibco). Cells at the third passage were used for subsequent experiments.

Drug preparation

Under light-protected conditions, quercetin (≥98% purity; MedChemExpress, Cat#HY-N0005) was solubilized in DMSO (Sigma-Aldrich, Cat#D8418) to obtain a 100 mg/mL master stock, which was aliquoted and stored at −20 °C. Working solutions (0.1, 1, 10, and 100 μg/mL) were freshly prepared by diluting the stock in culture medium, with the final DMSO concentration maintained at <0.1% (v/v) in all groups.

Recilisib (≥98% purity; MedChemExpress, Cat#HY-101625) was dissolved in DMSO as a 10 mM stock, kept at −20°C and further diluted with culture medium to achieve a working concentration of 10 μM. Final DMSO content was maintained below 0.1% (v/v).

Experimental grouping

(1) Quercetin Dose Screening

Caco-2 cells were exposed to quercetin (0, 0.1, 1, 10 and 100 μg/mL) for 24 or 48 h and cell viability was assessed using the CCK-8 assay (Volstatova, *et al*., 2019).

(2) DSS-Induced UC Model with Quercetin Treatment

Cells were incubated with 2% dextran sulfate sodium (DSS; MP Biomedicals, Cat#0216011080) (Toutounji, *et al*., 2020) for 48 hours to induce epithelial injury and co-treated with quercetin in three doses: 0.1, 1 and 10 μg/mL.

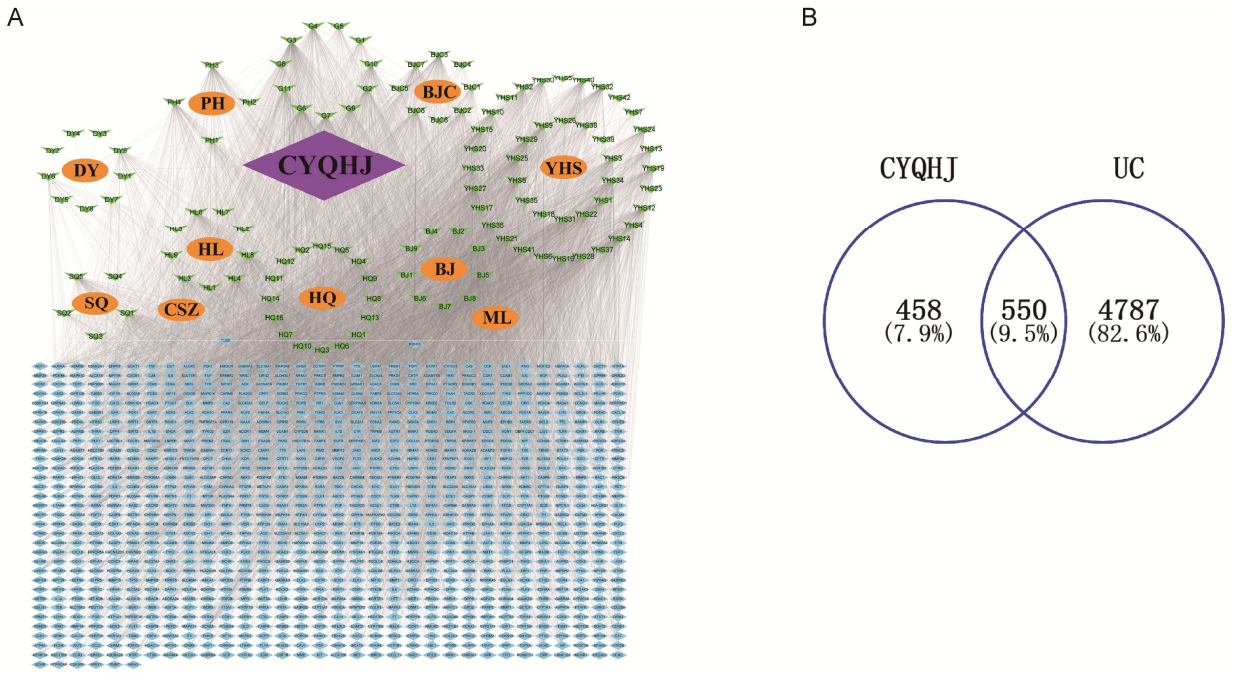

Fig. 1: Identification of Active Compounds and Potential Targets of CYQHJ. (A) Network of CYQHJ herbs, active compounds, and predicted targets, constructed using Cytoscape; (B) Venn diagram showing 550 shared targets between CYQHJ and UC.

(3) PI3K-Akt Pathway Verification with Recilisib

To clarify the functional role of the PI3K-Akt pathway, the cells were treated with 2% DSS in combination with quercetin (1 μg/mL) and/or Recilisib (10 μM) (Niu, *et al*., 2024) for 48 hours.

Cell counting kit-8 (CCK-8) assay

At a density of 5 × 10^4 cells/mL (100 μL/well), Caco-2 cells were dispensed into 96-well plates and subsequently processed according to the treatments described in Section 2.8. At 0, 24 and 48 h following treatment onset, 10 μL of CCK-8 reagent (Dojindo, Japan; Cat#CK04) was applied to the wells and incubated at 37°C for 2 h, after which the Absorbance at 450 nm (A450) was determined using a microplate reader (BioTek, USA).

ELISA assay

According to the manufacturer's instructions, TNF-α and IL-6 levels in culture supernatants were measured using human ELISA kits (Elabscience, Wuhan, China). After treatment, the supernatants were collected and centrifuged (1000 × g, 10 min) at 4℃ to pellet debris, before being transferred to fresh tubes. Next, standards and samples were dispensed into 96-well plates and incubated with the corresponding detection antibodies. Optical density (OD) at 450 nm was read with a microplate reader (BioTek). Cytokine levels were quantified based on the standard curve.

Western blotting

Following exposure, Caco-2 cells were homogenized in RIPA buffer containing protease/phosphatase inhibitors (Beyotime, China), and protein concentration was quantified using the BCA assay (Beyotime). Proteins (20–30 μg) were resolved by 10% SDS-PAGE and electrotransferred to PVDF membranes (Millipore, USA).

After blocking with 5% non-fat milk in TBST for 1 h at room temperature, membranes were probed overnight at 4°C with primary antibodies (Abcam) against phosphorylated PI3K (p-PI3K; Tyr458, Cat#ab191606, 1:1000), phosphorylated Akt (p-Akt; Ser473, Cat#ab81283, 1:1000) and GAPDH (Cat#ab181602, 1:2000). After being washed, membranes were exposed to HRP-linked goat anti-rabbit IgG (Abcam, Cat#ab205718, 1:5000) for 1 h at room temperature and protein signals were visualized using an ECL chemiluminescent kit (Millipore, Cat#WBKLS0500) and captured on a ChemiDoc XRS+ system (Bio-Rad). Band intensities were quantified using ImageJ software (NIH, USA) and normalized to GAPDH. Quantitative data were obtained from at least three independent experiments.

Statistical analysis

Results are expressed as mean ± SD based on at least three independent experiments. All statistical procedures were carried out in SPSS 23.0 (IBM Corp., Armonk, NY, USA). One-way ANOVA was used to test for overall differences among groups and Tukey's test was subsequently employed to identify differences between individual groups. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Screening of bioactive compounds and putative targets of CYQHJ

To explore the potential molecular basis of CYQHJ for treating UC, a network pharmacology approach was employed to identify bioactive constituents and their corresponding targets. Based on the TCMSP database and using screening criteria of OB ≥ 30% and DL ≥ 0.18, 134 active compounds were screened from the 11 herbal components of CYQHJ. These included 14 compounds from Coptis chinensis (HL), 20 from Astragalus membranaceus (HQ), 8 from Panax notoginseng (SQ), 13 from Patrinia scabiosaefolia (BJC), 13 from Sanguisorba officinalis (DY), 8 from Typha angustifolia (PH), 49 from Corydalis yanhusuo (YHS) and 9 from Bletilla striata (BJ). Notably, Corydalis yanhusuo and Astragalus membranaceus contained the highest number of active compounds, indicating their potentially major roles in CYQHJ's pharmacological activity.

The SMILES formats for these compounds were sourced via the PubChem platform and submitted to SwissTargetPrediction to identify potential protein targets, which were then standardized using the UniProt database. In total, 1008 non-redundant targets were identified. Cytoscape was applied to build the compound–target network (Fig. 1A). The network confirmed the multi-constituent, multi-target nature of CYQHJ. Some compounds, such as quercetin (HQ15) and BJ3, showed high degree values, indicating their potential central roles.

To identify disease-related targets, 5337 UC-linked genes were sourced from GeneCards and OMIM. Venn diagram analysis revealed 550 overlapping targets between CYQHJ and UC (Fig. 1B), representing candidate therapeutic targets for subsequent enrichment analysis and experimental validation.

These results imply that CYQHJ may deliver therapeutic benefits for UC through multi-component and multi-target synergy, laying a foundation for further mechanistic exploration.

GO and KEGG enrichment analysis reveals involvement of inflammation and PI3K-Akt pathway

To examine the biological implications of the 550 overlapping targets between CYQHJ and UC, GO term and KEGG pathway enrichment analyses were performed using DAVID. The findings were presented as bubble plots (Figs. 2A–D). In the BP category (Fig. 2A), enriched terms primarily included signal transduction, protein phosphorylation, the inflammatory response, activation of the RNA polymerase II promoter, and control of cellular proliferation and apoptosis. These processes are tightly linked to UC pathogenesis, especially the inflammation-driven epithelial injury and immune dysregulation.

In the CC category (Fig. 2B), the targets were predominantly located in the plasma membrane, cytoplasm, cytosol and nucleus, indicating that CYQHJ may regulate both membrane-associated receptors and intracellular signaling molecules.

In the MF category (Fig. 2C), the top enriched terms included binding to proteins, binding to ATP, kinase activity and binding to metal ions, further suggesting that CYQHJ targets are involved in signal transduction cascades and enzymatic regulation.

Importantly, KEGG pathway enrichment (Fig. 2D) showed that the PI3K–Akt axis ranked among the most enriched pathways, along with pathways related to cancer, MAPK signaling, lipid metabolism, and atherosclerosis. These results highlight the PI3K-Akt cascade as a potential critical mechanism mediating CYQHJ's therapeutic effects in UC.

Overall, GO and KEGG analyses indicate that CYQHJ may alleviate UC by modulating inflammation, cell proliferation and apoptosis, with the PI3K-Akt pathway emerging as a central regulatory axis.

PPI network analysis of CYQHJ–UC overlapping targets

To further identify the key therapeutic targets of CYQHJ in UC, 550 common genes were submitted to the STRING platform (organism: Homo sapiens; confidence score ≥ 0.900). Isolated nodes were hidden and the resulting PPI network was analyzed and visualized via Cytoscape.

The final network consisted of 455 nodes and 2207 edges (Fig. 3). Topological analysis based on degree value identified several core targets, with TP53 exhibiting the highest degree (80), followed by AKT1 (60), SRC (60), STAT3 (54) and PIK3R1 (52). These proteins are known to participate in inflammatory responses, regulation of apoptosis, and intracellular signaling. Their central location in the network suggests they may serve as major mediators through which CYQHJ exerts therapeutic effects on UC.

The size and color intensity of nodes in the visualization reflect node degree centrality, with core targets clustered toward the center. The network topology highlights PI3K-Akt-related proteins as prominent hubs, supporting the hypothesis of pathway involvement.

In summary, the PPI network analysis identified TP53, AKT1 and PI3K-related proteins as key regulatory nodes, providing further evidence that CYQHJ may act through modulation of the PI3K-Akt signaling pathway.

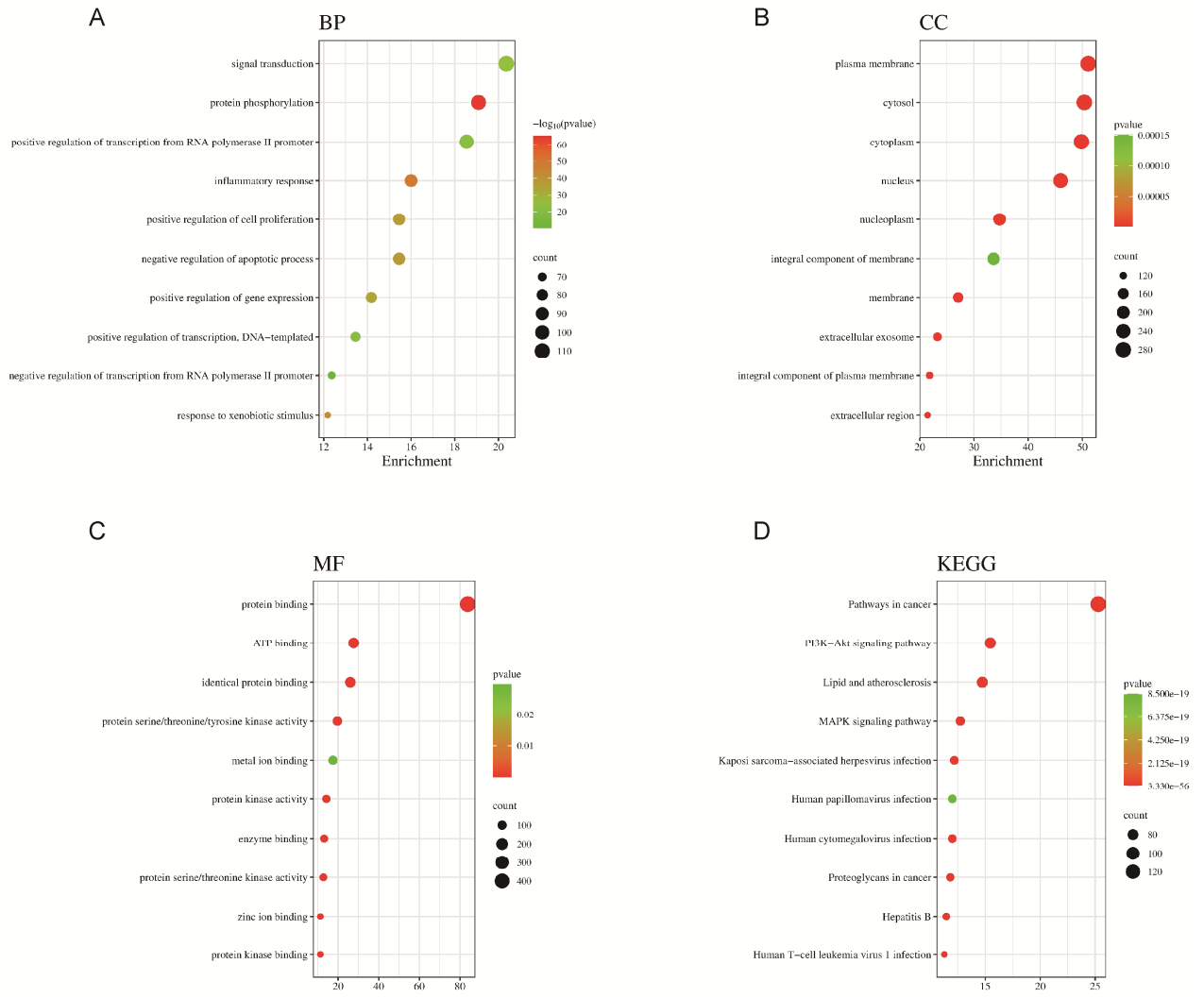

Fig. 2: GO and KEGG Enrichment Analysis Reveals Involvement of Inflammation and PI3K-Akt Signaling. (A) GO BP enrichment; (B) GO CC enrichment; (C) GO MF enrichment; (D) KEGG pathway enrichment.

Compound–target–disease network reveals multi-component regulation of UC by CYQHJ

To further elucidate the systemic therapeutic mechanisms of CYQHJ against UC, a compound–target–disease network was constructed using the active components and overlapping targets identified above (Fig. 4). The network integrates 11 herbs, 134 active compounds, 550 overlapping targets and UC-related disease nodes, illustrating the multi-level relationships among herbs, active components, targets and disease.

The topology shows that several core herbs in CYQHJ—such as Astragalus membranaceus, Coptis chinensis and Corydalis yanhusuo—exert effects through multiple active ingredients that interact with a wide range of UC-related targets. As summarized in table S1, several representative compounds, including quercetin, kaempferol, luteolin, isorhamnetin, acacetin and cryptotanshinone, exhibited relatively high degree values and were linked to multiple UC-related hub targets, such as TP53, AKT1, SRC, STAT3 and PIK3R1, highlighting their potential importance in the therapeutic network of CYQHJ.

In summary, the network analysis suggests that CYQHJ may produce therapeutic benefits against UC via multi-herb synergy and broad target regulation, embodying the multi-constituent, multi-target and multi-pathway features typical of traditional Chinese medicine.

Molecular docking analysis

Molecular docking analysis was conducted to further validate the interactions between quercetin and the identified hub targets. As shown in the fig. 5, quercetin exhibited stable binding with AKT1, PIK3R1, SRC, STAT3 and TP53. The docking results revealed that quercetin could fit well into the binding pockets of these proteins and form multiple interactions with surrounding residues.

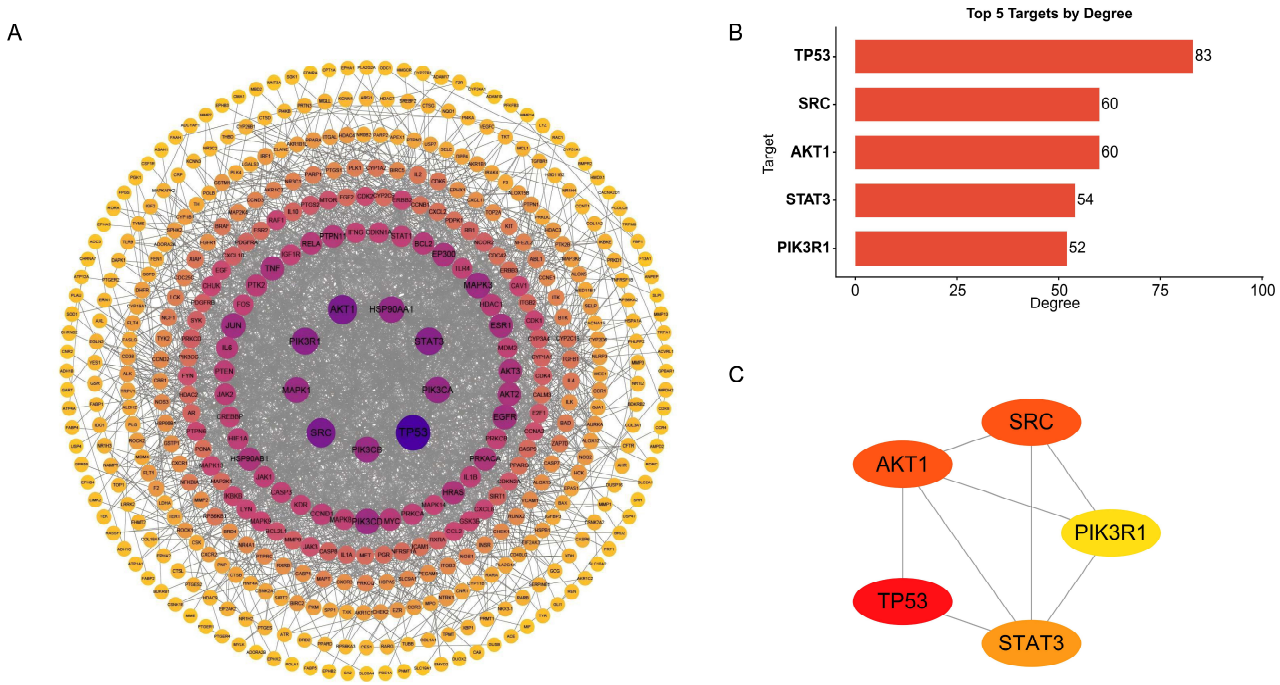

Fig. 3: Protein–protein interaction (PPI) network of overlapping targets between CYQHJ and UC. (A) The full PPI network of the overlapping targets, constructed using the STRING database and visualized in Cytoscape. Node size and color intensity reflect the degree value; (B) Top 5 hub targets ranked by degree value, including TP53, SRC, AKT1, STAT3, and PIK3R1; (C) Core PPI subnetwork extracted from the full network, illustrating the interactions among key hub targets.

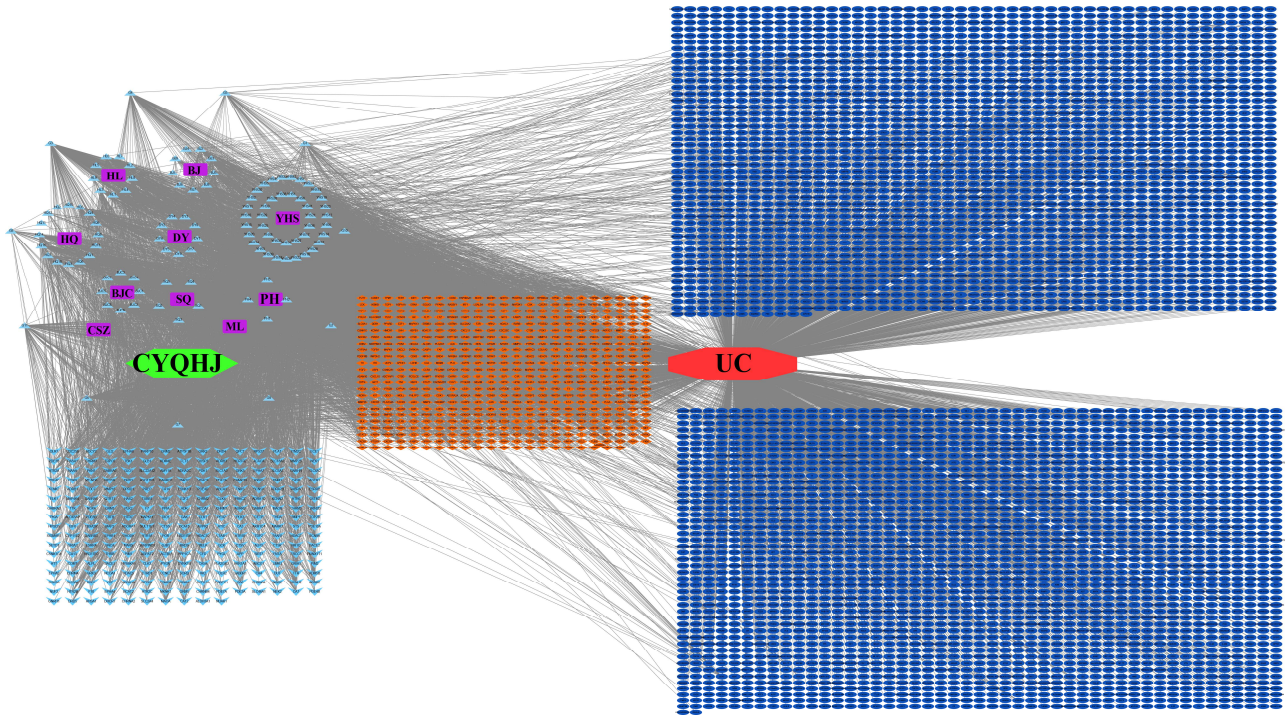

Fig. 4: Compound–target–disease network showing relationships among CYQHJ herbs, active ingredients, shared targets, and UC. Node types are color- and shape-coded; edges represent regulatory interactions. Node types are color- and shape-coded; edges represent compound–target–disease associations.

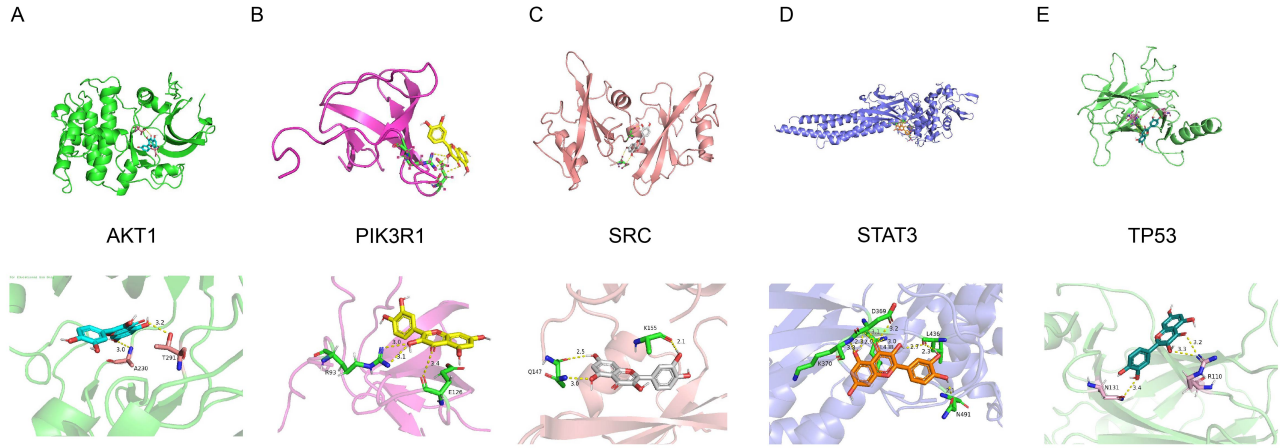

Fig. 5: Molecular docking analysis of quercetin with core target proteins.
(A) AKT1; (B) PIK3R1; (C) SRC; (D) STAT3; (E) TP53. For each target, the upper panel shows the overall binding conformation of quercetin within the protein structure, while the lower panel presents a close-up view of the binding pocket, highlighting interactions with key amino acid residues, including hydrogen bonds.

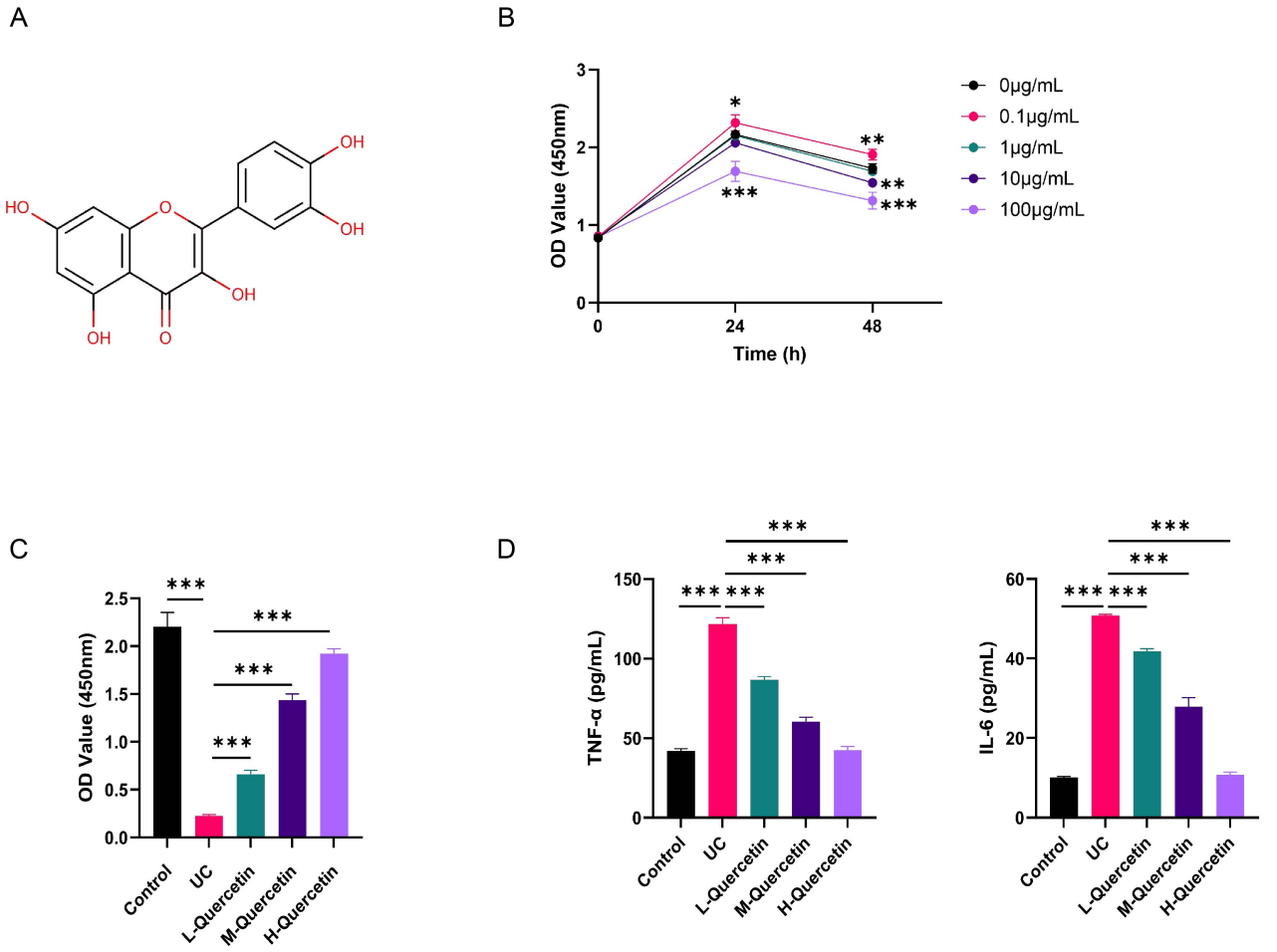

Fig. 6: Quercetin alleviates DSS-induced Caco-2 cell injury and inflammation. (A) Chemical structure of quercetin; (B) CCK-8 assay evaluating the effects of different concentrations of quercetin on Caco-2 cell viability under normal conditions. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$vs. 0 μg/mL; (C) CCK-8 assay assessing the effects of quercetin on Caco-2 cell viability in DSS-induced injury; (D) ELISA quantification of TNF-α and IL-6 levels in culture supernatants. $^{***}P < 0.001$ vs. Control group; $^{\#\#\#}P < 0.001$ vs. UC group.

Quercetin attenuates DSS-induced injury and inflammation in Caco-2 cells

As identified in the previous network analysis, quercetin is one of the core active compounds in CYQHJ, with high target connectivity and significant enrichment of key pathways, including PI3K-Akt signaling. As illustrated in Fig. 6A, the structural formula of quercetin reveals that it is a natural flavone derivative with a tricyclic core and several hydroxyl groups, which contribute to its strong bioactivity and anti-inflammatory properties (Ramavath et al., 2023; Erdogan.

To assess its therapeutic potential in ulcerative colitis, the effect of quercetin on Caco-2 cell viability was first evaluated using the CCK-8 assay. As illustrated in fig. 6B, treatment with 0.1 μg/mL quercetin significantly enhanced cell viability at 24 h ($P < 0.05$), while higher concentrations (10 and 100 μg/mL) resulted in reduced viability, showing a biphasic response under normal conditions.

When Caco-2 cells were treated with DSS to mimic UC conditions, cell viability was substantially reduced compared with control cells ($P < 0.001$). However, treatment with quercetin at different concentrations (0.1, 1, and 10 μg/mL) gradually restored cell viability in a concentration-dependent manner, with the 10 μg/mL group approaching normal levels (Fig. 6C; $P < 0.001$), suggesting a protective effect.

Furthermore, ELISA results revealed that DSS stimulation substantially enhanced TNF-α and IL-6 secretion, whereas quercetin treatment significantly reduced their levels, particularly at high doses (Fig. 6D, $P < 0.001$).

In summary, quercetin exhibits structural and functional anti-inflammatory properties and effectively mitigates DSS-induced epithelial cell injury and inflammation *in-vitro*, providing robust experimental evidence for its role in the therapeutic mechanism of CYQHJ.

Quercetin exerts protective effects by inhibiting the PI3K-Akt signaling pathway

Given the observed anti-inflammatory and protective effects of quercetin *in-vitro* and the pathway enrichment results implicating PI3K-Akt signaling, further validation was performed via Western blot analysis to explore its underlying mechanism.

As illustrated in fig. 7A, p-PI3K and p-Akt levels were markedly increased in DSS-induced Caco-2 cells ($P < 0.001$), indicating pathway activation. Treatment with quercetin significantly decreased p-PI3K and p-Akt levels in a dose-dependent manner, suggesting inhibition of PI3K-Akt signaling by quercetin.

To assess whether this pathway is functionally involved in quercetin's protective effect, the PI3K-Akt agonist Recilisib was used. As shown in the fig. 7B, Recilisib attenuated the quercetin-induced suppression of p-PI3K and p-Akt levels ($P < 0.001$). Furthermore, quercetin's ability to restore cell viability was markedly diminished in the presence of Recilisib (Fig. 7C, $P < 0.001$). Similarly, the inhibitory effects of quercetin on TNF-α and IL-6 secretion were also attenuated when PI3K-Akt signaling was reactivated (Fig. 7D, $P < 0.001$).

Together, these findings confirm that quercetin alleviates DSS-induced epithelial injury and inflammation in Caco-2 cells, predominantly by inhibiting the PI3K-Akt pathway.

DISCUSSION

This study systematically explored the potential therapeutic mechanism of CYQHJ for UC from three interconnected levels: multi-herb synergy, core compound identification and pathway-specific validation. Unlike conventional single-target research strategies, network pharmacology was employed to identify the PI3K-Akt signaling axis as a central regulatory pathway. Quercetin was subsequently selected as a representative compound for functional verification and its pathway specificity was further confirmed through pharmacological intervention.

Intestinal epithelial injury, immune dysregulation and excessive inflammatory response are widely recognized as critical pathological bases for UC chronicity and relapse. Although many therapies target key pro-inflammatory mediators (e.g., TNF-α and IL-6), a fundamental gap remains between symptomatic relief and mechanistic restoration (Bu, *et al*., 2025; Tang, *et al*., 2025). The PI3K-Akt pathway is widely recognized a central hub connecting upstream inflammatory triggers with downstream cellular fate, including proliferation, apoptosis and metabolic activity (Niu and Zhang, 2025). In this study, significant activation of p-PI3K and p-Akt was observed in DSS-triggered Caco-2 cells, which was dose-dependently suppressed by quercetin, suggesting its integrative regulatory potential.

To further assess functional dependence on this pathway, the PI3K-Akt agonist Recilisib was introduced. The reversal of quercetin's effects—both in restoring cell viability and reducing cytokine release—reinforced the role of PI3K-Akt as a necessary mediator. Similar regulatory patterns have been reported in previous studies, in which PI3K-Akt inhibitors alleviated colonic inflammation and epithelial damage in DSS-induced murine models (Rahmani et al., 2020; Zhang. This finding fills a methodological gap in natural compound studies, which often describe phenotypic effects without direct validation of pathways. It provides experimental evidence supporting PI3K-Akt as a therapeutic target in UC.

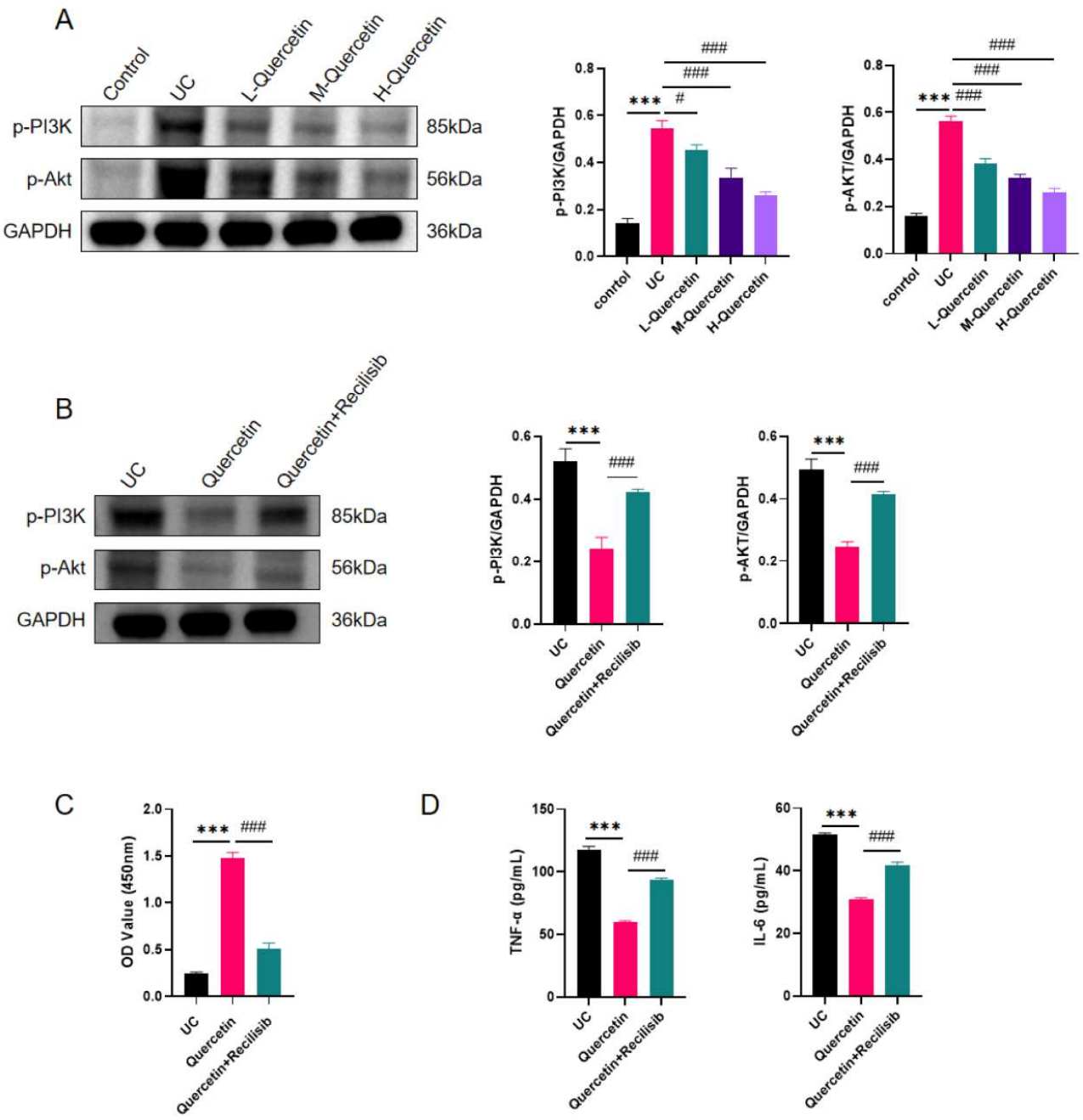

Fig. 7: Quercetin attenuates DSS-induced inflammation by inhibiting the PI3K-Akt signaling pathway. (A–B) Western blot detection of p-PI3K and p-Akt in Caco-2 cells treated with quercetin and/or Recilisib. $^{***}P < 0.001$ vs. Control group; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ vs. UC group; (C) CCK-8 assay measuring cell viability; (D) ELISA detection of TNF-α and IL-6 levels. $^{***}P < 0.001$ vs. UC group; $^{\#\#\#}P < 0.001$ vs. Quercetin group.

Notably, CYQHJ, as a multi-herbal traditional Chinese medicine formula, has long been applied in clinical settings under the principles of "heat-clearing, detoxifying and activating blood circulation." However, its molecular mechanisms have remained largely undefined. It was shown by network analysis that the multiple components in CYQHJ not only interact with a broad range of UC-related targets but also converge upon critical biological processes, including apoptosis, oxidative stress and immune modulation. Compared with previous studies, which reported quercetin's effects via TGF-β1 or NF-κB signaling (Zheng et al., 2025), a direct regulatory link between quercetin and the PI3K - Akt pathway is established, for the first time, through integrated network analysis and experimental validation. This represents a more complete research chain—from formula-level prediction to compound-level validation and mechanism-level confirmation.

Nevertheless, limitations remain. A single DSS-induced Caco-2 cell model was used in this study, which, while informative, cannot fully capture the complex intestinal microenvironment involving immune cells, the microbiota, and stromal interactions. Additionally, although the PI3K-Akt pathway was identified as a key axis, other enriched pathways, such as MAPK, HIF-1, and TNF signaling, may also contribute and warrant further investigation. Moreover, while quercetin emerged as a core compound, other highly connected constituents, such as tetrahydropalmatine and berberine in CYQHJ, also merit attention for their possible synergistic effects.

In summary, this research shows that quercetin, a key bioactive compound in CYQHJ, alleviates DSS-induced epithelial injury and inflammation by suppressing the PI3K-Akt signaling axis. These observations provide not only mechanistic support for the clinical use of CYQHJ but also conceptual guidance for future research into multi-component, multi-target strategies for inflammatory bowel disease.

CONCLUSION

This study leveraged network pharmacology and in vitro validation to decode the molecular mechanism of action of CYQHJ in ulcerative colitis. The results identified the PI3K-Akt pathway as a key regulatory axis and quercetin as a key bioactive compound. Functional assays demonstrated that quercetin improved Caco-2 cell viability and reduced TNF-α and IL-6 secretion under DSS-induced injury. Mechanistically, quercetin inhibited the phosphorylation of PI3K and Akt, an effect reversed by the PI3K-Akt activator Recilisib, confirming pathway dependence.

In summary, CYQHJ exerts anti-inflammatory and epithelial-protective effects primarily by modulating the PI3K-Akt pathway through compounds such as quercetin. These findings provide mechanistic support for its clinical application and underscore its promise as a multi-target treatment approach for inflammatory bowel disease.

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ChatGPT (version 5.2) was employed solely to assist with language editing and grammatical refinement during manuscript preparation. All scientific content, interpretations and conclusions are the sole responsibility of the authors.

Authors' contributions
All authors contributed significantly to the work reported. All authors were involved in the conception and design of the study. Xuechuan Wang, Wei He and Zhiheng Chen: Performed the experiments and data analysis; Longling Cong and Yujin Wu: Participated in data interpretation and manuscript drafting. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Data availability statement
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval
Not applicable.

Conflict of interests
The authors declare no conflicts of interest.

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