Hedysarum multijugum Maxim treats ulcerative colitis through the PI3K-AKT and TNF signaling pathway according to network pharmacology and molecular docking

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Background: Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that prevails mainly in western countries. Due to the unknown etiology of UC, the purpose of treatments has predominantly comprised symptomatic and pain relief. With extensive research focusing on the pathogenesis of UC, various novel treatments have emerged, although their efficiency has remained unsatisfactory. *Hedysarum multijugum Maxim* (HMM), a crucial constituent of traditional Chinese medicine, has a broad application in many diseases and has been found beneficial for UC patients.

Methods: In this study, network pharmacology and molecular docking analyses were applied to explore the potential mechanism of HMM treating UC. Active ingredients of HMM and target genes were acquired from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). UC-related genes were obtained from three disease databases. Common genes were selected from these two gene sets, and a compound-genes network was drawn by Cytoscape. Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO) enrichment, and protein-protein interaction (PPI) analyses were performed to identify the essential pathways and proteins in UC.

Results: A total of 121 genes were found related to UC and targeted by HMM. The GO and KEGG analyses showed that these genes were associated with inflammation and immune signaling pathways and inflammation-related biological processes (BP) such as the tumor necrosis factor (TNF) and PI3K-AKT signaling pathways. Four active ingredients (quercetin, kaempferol, formononetin, and isorhamnetin) and five genes (*RELA*, *MAPK14*, *MAPK1*, *JUN*, *AKT1*) were reserved after screening. Molecular docking further showed that the receptor had a high binding affinity with HMM active ingredients.

Conclusions: This study revealed that HMM treats UC through four active ingredients (quercetin, kaempferol, formononetin, and isorhamnetin) targeting five hub genes (*RELA*, *MAPK14*, *MAPK1*, *JUN*, *AKT1*) by regulating the PI3K-AKT1 and TNF signaling pathways.

Keywords: Traditional Chinese medicine (TCM); *Hedysarum multijugum Maxim* (HMM); ulcerative colitis (UC); network pharmacology; molecular docking

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that impairs the colon and rectal mucosae; to date, no recognized cause has been confirmed (1). Based on recent studies, patients with UC probably have genetic and environmental predispositions, intestinal microenvironment dysregulation, and immunity disorder. An inability to distinguish pathogens and commensal microbes, overactivated innate and adaptive immune responses, and weak inhibitory immune regulation are the potential pathophysiology of UC (2,3). Pathological changes are limited to the mucosal membrane and submucosal layer of the colon and rectum, which are distributed continuously and diffusely. Bloody diarrhea is the major symptom of UC, and abdominal pain is experienced by UC patients with mild symptoms. The aim of treatment is to maintain symptom relief and mucosal healing, prevent complications, and improve the quality of life of patients. Mesalazine, corticosteroids, and immunosuppressive drugs are usually applied to control the inflammatory response. Symptomatic treatment is used to maintain the patient's water and electrolyte balance. Surgery is required for patients with complications or severe symptoms. The currently available treatments either have limited effects or safety problems (4).

Traditional Chinese medicine (TCM) treatment has been shown to have incredible effects on UC (5-7). *Hedysarum multijugum Maxim* (HMM), a type of traditional Chinese herb which is mainly distributed in central China, has long been used to treat UC. In general, HMM is used for ulceration and to improve immunity. Previous research has revealed that HMM has good effects on treating UC (8,9). However, the mechanism of HMM treating UC needs further exploration. Therefore, in the present research, network pharmacology and molecular docking were applied to explore the effect target of UC and active ingredients of HMM, not only explaining the underlying mechanism but providing a direction for drug innovation in the future.

The HMM targeting genes were collected according to HMM's active ingredients and intersected with UC-related genes. Enriched functions and signaling pathways were explored by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. Besides, a network comprising significant genes, active compounds, and corresponding signaling pathways was visualized by software such as Cytoscape (https://cytoscape.org/) or R package (https://www.r-project.org/). This technology helps researchers to study the effect of multiple compounds on different genes at one time, providing a comprehensive understanding of the mechanism of disease treatment (10). Additionally, molecular docking was first used to discover molecular interactions and now is applied to drug discovery (11). In this study, molecular docking was executed to predict the binding region of ingredients and receptors and to assess their binding affinity. In recent years, there has been plenty of network pharmacology research conducted focusing on the treatment of chronic diseases with TCM (12). According to the findings of this study, HMM treats UC by modulating immune response and inflammation via the PI3K-AKT and tumor necrosis factor (TNF) signaling pathways. This research might establish a solid foundation for future research and therapeutic innovation. We present the following article in accordance with the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4815/rc).

Methods

Acquisition of the active ingredients and target genes of HMM

Ingredients of HMM were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (13) (TCMSP; http://tcmspw.com/). The parameters of oral bioavailability (OB) greater than 30%, and drug-likeness (DL) greater than 0.18 were applied to screen active ingredients (14). The OB reflects the proportion of the amount of drug absorbed by blood circulation (15). The DL refers to the similarity between ingredients and known drugs. Target genes related to active ingredients were selected from the TCMSP database (13). Target gene symbols were annotated by gene information from Uniport (16) (http://www.uniprot.org/). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Obtainment of a UC-related gene set

Three databases were used to search genes related to UC: Genecards database (17) (https://www.genecards.org/), PharmGkb database (18) (https://www.pharmgkb.org/), and Therapeutic Target Database (TTD) database (19) (http://db.idrblab.net/ttd/). Genes acquired from the Genecards database were filtered by relevance score. Genes with relevance scores greater than 1 were retained. The common genes of these three databases comprised the UC-related gene set.

Analysis of overlapping genes between HMM and UC

Candidate genes screening

The candidate genes were the intersection of HMM target genes and UC-related genes. They were screened through R software (version 4.0.4) and a Venn diagram was drawn using the VennDiagram package in R.

Protein-protein interaction (PPI) network and compound-candidate gene network

PPI networks were created by the Search Tool for the Retrieval of Interacting Genes/genomes (STRING); (https://string-db.org) database based on candidate genes data, with the highest confidence selected and disconnected nodes hidden (20). A bar plot exhibiting the most frequently interacted proteins among candidate genes was created by R software (version 4.0.4). Cytoscape 3.8.2, a platform for integrating biomolecular interaction networks, was used to construct a compound candidate genes network (21).

GO and KEGG enrichment analysis

We performed GO and KEGG enrichment analyses to evaluate the biological processes (BP), cellular components (CC), molecular functions (MF), and signaling pathways that candidate genes potentially regulated. For these two enrichment analyses, the thresholds of the P value and q-value were both 0.05. Enrichment analyses were carried out by the Cluster profile package in R version 4.0.4 (22).

Hub genes screening and compound-hub genes-signaling pathway construction

Betweenness, Closeness, Degree, Eigenvector, LAC, and Network of each gene were calculated via the CytoNca plugin in Cytoscape (23,24). The primary subnetwork comprised candidate genes with all parameters greater than the median value. Hub genes were selected from genes screened from the primary subnetwork under the same screening parameters. The top 10 signaling pathways, hub genes, and corresponding compounds were connected, and the averages of the respective degrees were set as the threshold values to explore the significant compounds, candidate genes, and signaling pathways that comprised the critical network.

Molecular docking simulation

Ligand preparation

Two-dimensional (2D) structures of active ingredients were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and were transformed into three-dimensional (3D) structures by ChemBio 3D (25). The energy of the 3D structure was minimized for optimization.

Target protein preparation

The 3D structure of receptor proteins encoded by candidate genes was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (http://www.pdb.org/) (26). Water molecules and organic ligands of receptor proteins were removed by PyMol2.4.2. Hydrogenation and charge calculation on receptor proteins were performed by Autodock Tools (27). Active pocket sites of receptor proteins were searched based on settings.

Molecular docking

Based on active pocket sites, molecular docking of compound-target was performed by Autodock Vina. Compound-target binding models were then visualized by PyMol2.4.2.

Statistical analysis

All statistical analyses were conducted with R software (Version 3.6.1). A P value of less than 0.05 was regarded as statistically significant unless otherwise stated, and all P values were two tailed.

Results

Active ingredients and target genes screening

The flowchart of this study is exhibited in *Figure 1*. 20 active ingredients and 180 target genes of HMM were obtained from the TCMSP database (Table S1 and https://cdn.amegroups.cn/static/public/atm-22-4815-1.xlsx). Then, 2,691, 46, and 14 genes related to UC were obtained from the Genecards database, TTD database, and PharmGKB database, respectively (*Figure 2A*). There were 2,725 UC-related genes after screening (https://cdn.amegroups.cn/static/public/atm-22-4815-2.xlsx). The intersecting parts of compound target genes and UC-related genes were taken (https://cdn.amegroups.cn/static/public/atm-22-4815-3.xlsx), and in the end, 121 candidate genes were preserved (*Figure 2B*).

PPI and candidate genes-compound network

Processed by the STRING database, the PPI network showed complicated interactions between candidate

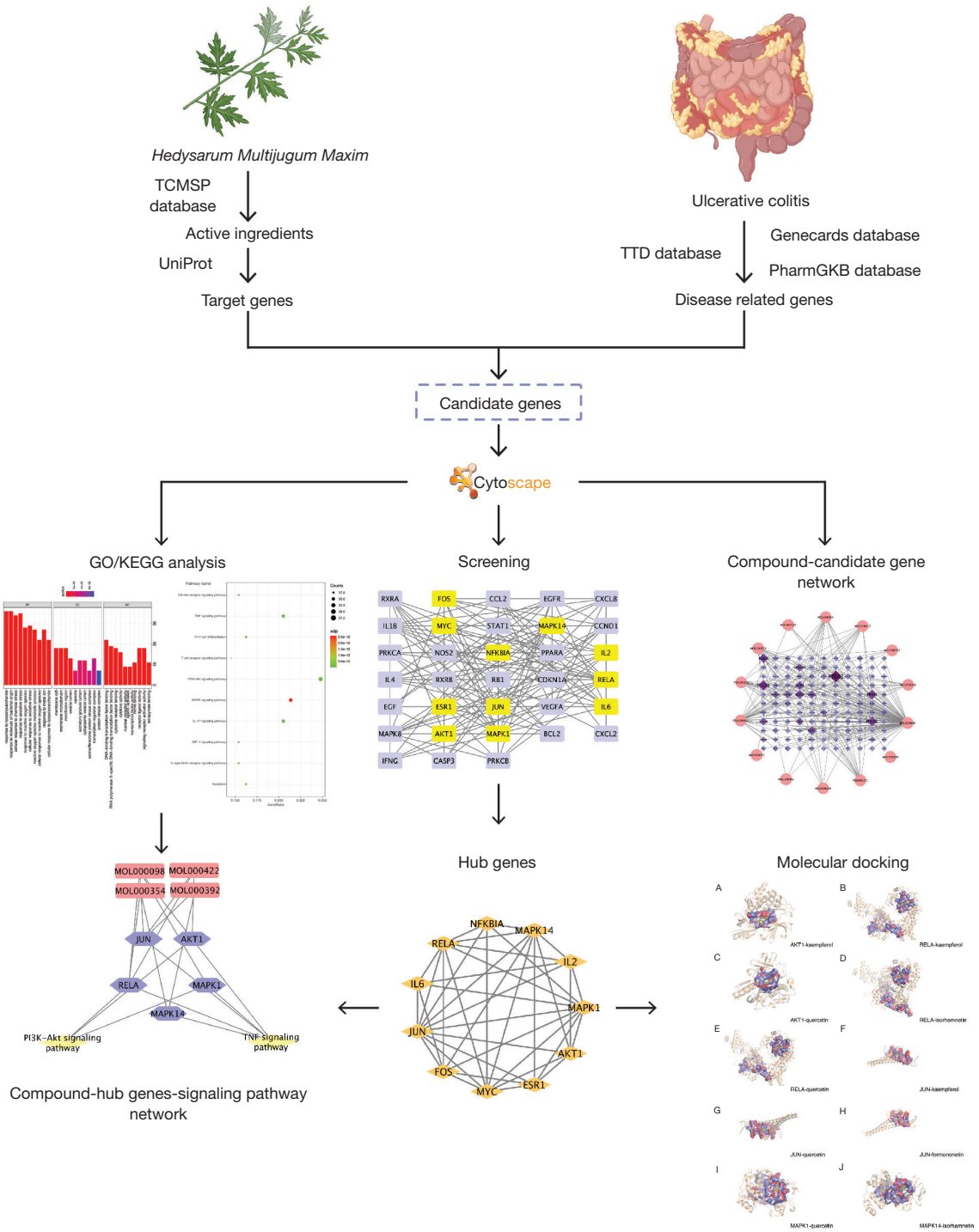

Figure 1 Flowchart of investigating the mechanism of HMM in UC treatment. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HMM, *Hedysarum multijugum Maxim*; UC, ulcerative colitis.

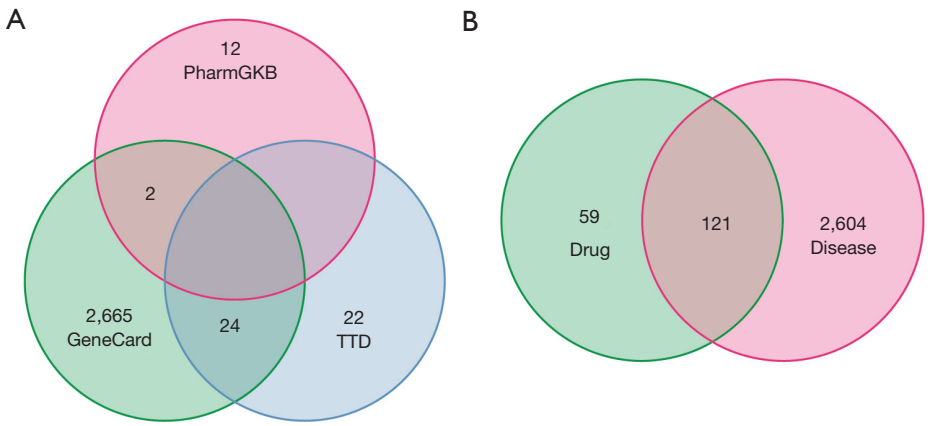

Figure 2 Venn diagram. (A) Collect UC-related genes in PharmGKB, GeneCard, and Therapeutic Target Database. (B) Screen candidate genes through UC related genes and HMM targeted genes. HMM, *Hedysarum multijugum Maxim*; UC, ulcerative colitis.

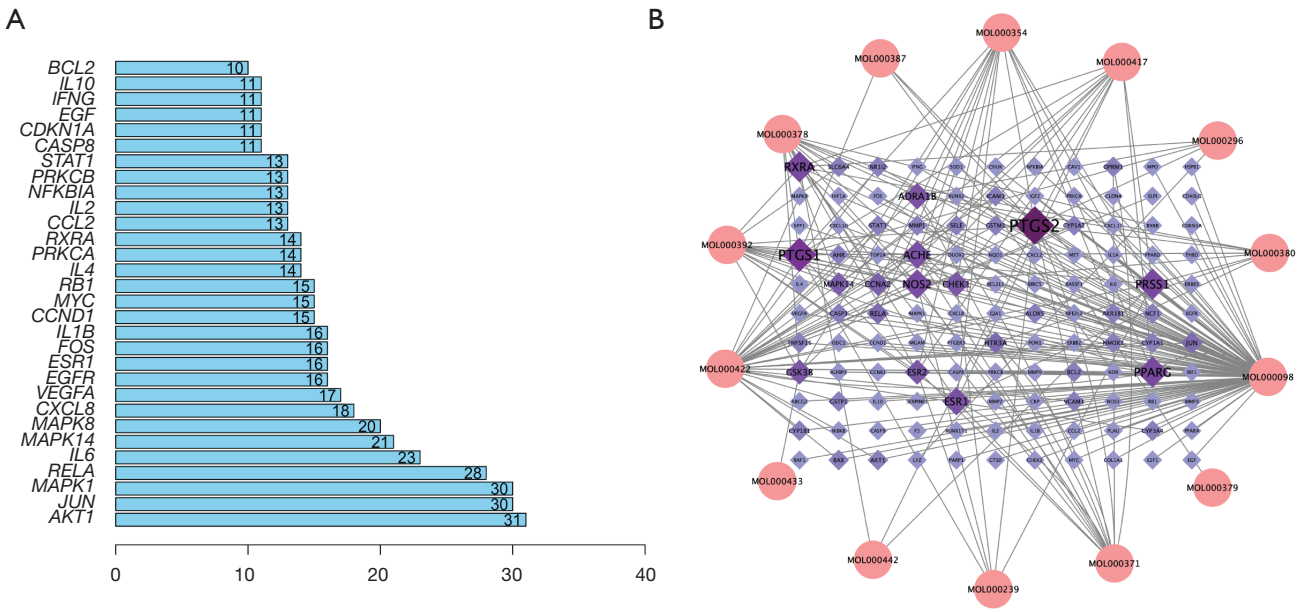

Figure 3 Candidate genes and connection with active ingredients. (A) Bar plot of connections between proteins encoded by candidate genes. The x-axis represents the number of neighboring proteins of the target protein. The y-axis represents the target protein. (B) Active compound and candidate gene network. The sizes and colors of the nodes are illustrated from big to small and dark purple to light purple in descending order of degree values.

genes at the protein level (https://cdn.amegroups.cn/static/public/atm-22-4815-4.xlsx). The PPI network was used for further analysis in Cytoscape (Figure S1). The number of interactions proteins was calculated and sorted (*Figure 3A*). As shown in the bar plot, AKT1 was the most frequent protein that other proteins interacted with. Meanwhile, the compound-candidate genes network was visualized by Cytoscape. The more interactions between candidate genes and active ingredients, the darker and larger nodes would be. The network indicated that each active ingredient targeted multiple candidate genes, with *PTGS1* and *PTGS2*, also known as cyclooxygenase 1 and 2 (COX-1, COX2), being the most frequently targeted genes (*Figure 3B*).

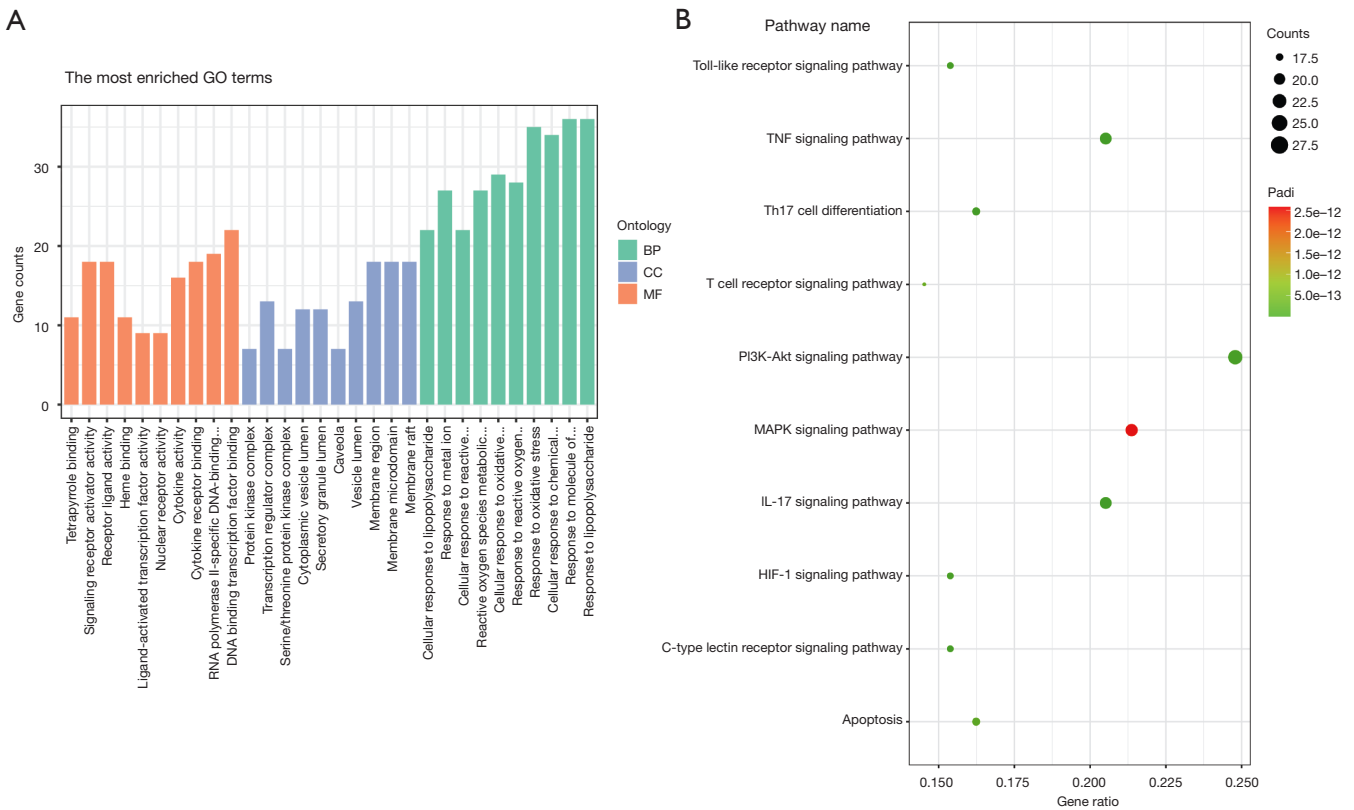

Figure 4 Enrichment analysis of candidate genes. (A) GO enrichment analysis. Top 10 terms of biological processes, cell components, and molecular function. (B) KEGG pathway analysis. Top 10 signaling pathways. The size of bubbles grows as 'Counts' increase. The bubble color represents the adjusted P value of the signaling pathway. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function.

GO enrichment and KEGG enrichment analysis

Totals of 2,270 BP GO terms, 48 CC GO terms, and 143 MF GO terms were identified by the R package cluster profiler. The top 10 GO terms of each aspect were exhibited (*Figure 4A*). In terms of BP, the results showed that candidate genes play an important role in response to external stress and macromolecules. As for CC, candidate genes mainly affected proteins located on the cell membrane. The MF analysis indicated that protein binding capacity and enzyme receptor activity were regulated. The KEGG analysis showed that candidate genes participated in 167 KEGG signaling pathways. The top 10 KEGG pathways are listed in *Table 1*. In general, KEGG enrichment analysis indicated that candidate genes were involved in signaling pathways that pertained to immune functions such as the IL-17 signaling pathway (has04657), TNF signaling pathway (hsa04668), Th17 cell differentiation (hsa04659), and the T cell receptor signaling pathway (hsa04660). Besides, the PI3K-AKT signaling pathway (hsa04151) and MAPK signaling pathway (hsa04010) were two prevailing signaling pathways that candidate genes enriched (*Figure 4B*).

Hub gene screening

To screen the core genes among 121 candidate genes, the primary subnetwork and secondary subnetwork were screened based on the following parameters: Betweenness, Closeness, Degree, Eigenvector, LAC, and Network, and genes with values of all parameters that were higher than average were highlighted (*Figure 5A*,*5B*). In the end, 11 hub genes, *NFKBIA*, *MAPK14*, *IL2*, *IL6*, *MAPK1*, *AKT1*, *ESR1*, *MYC*, *FOS*, *JUN*, and *RELA*, were preserved after screening (*Figure 5C*). Function analyses were performed on the hub genes. GO analysis confirmed that the hub genes were closely related to inflammation and immune

Table 1 Detailed information for top 10 pathway

Pathway	Hub genes in pathway	Count	Adjp
IL-17 signaling pathway	*MAPK14, RELA, JUN, FOS, MAPK1, IL6, NFKBIA*	24	2.89E-22
TNF signaling pathway	*MAPK14, RELA, JUN, AKT1, FOS, MAPK1, IL6, NFKBIA*	24	1.6E-20
Th17 cell differentiation	*MAPK14, RELA, JUN, FOS, MAPK1, IL6, NFKBIA, IL2*	19	5.93E-15
Toll-like receptor signaling pathway	*MAPK14, RELA, JUN, AKT1, MAPK1, IL6, NFKBIA*	18	4.51E-14
C-type lectin receptor signaling pathway	*MAPK14, RELA, JUN, AKT1, MAPK1, IL6, NFKBIA, L2*	18	4.51E-14
PI3K-Akt signaling pathway	*RELA, AKT1, MAPK1, IL6, IL2*	29	7.58E-14
HIF-1 signaling pathway	*RELA, AKT1, MAPK1, IL6*	18	9.16E-14
T cell receptor signaling pathway	*MAPK14, RELA, JUN, AKT1, FOS, MAPK1, NFKBIA*	17	5.73E-13
Apoptosis	*RELA, JUN, AKT1, FOS, MAPK1, NFKBIA*	19	3.54E-13
MAPK signaling pathway	*MAPK14, RELA, JUN, AKT1, FOS, MAPK1, MYC*	25	2.58E-12

Adjp, adjust P value. IL-17, interleukin 17; TNF, tumor necrosis factor; HIF, Hypoxia-Inducible Factor; MAPK, mitogen-activated protein kinase.

function (Figure S2A). Besides, KEGG analysis revealed that, through PI3K-AKT signaling pathway, the hub genes worked (Figure S2B).

Molecular docking simulation

The potential interaction activity between 11 hub genes and their related HMM compounds was explored in this study using molecular docking analysis. Meanwhile, the docking affinity values supplied by AutoDock Vina were used to further select the obtained targets and active molecules. In total, 23 pairs were delivered to the docking simulation (*Table 2*). The stronger the binding ability between the compounds and the active sites of the targets, the higher the absolute value of the docking affinity. Most binding complexes had a high binding affinity, with an average of –8.73 kcal/mol, according to the docking studies. Among the complexes composed by active ingredients and hub genes, the top 10 complexes were selected based on binding affinity which were FOS-quercetin complex (–10.4 kcal/mol), AKT-kaempferol complex (–9.6 kcal/mol), RELA-kaempferol complex (–9.5 kcal/mol), AKT-quercetin complex (–9.4 kcal/mol), RELA-isorhamnetin complex (–9.2 kcal/mol), RELA-quercetin complex (–9.2 kcal/mol), JUN-kaempferol complex (–9.2 kcal/mol), JUN-quercetin complex (–8.8 kcal/mol), JUN-formononetin complex (–8.4 kcal/mol), and MAPK1-quercetin complex (–8.4 kcal/mol) (*Figure 6*).

Compound-hub genes-signaling pathway network

After two rounds of screening, MOL000098 (quercetin, degree =9), MOL000422 (kaempferol, degree =3), MOL000392 (formononetin, degree =3), and MOL000354 (isorhamnetin, degree =3), were identified as the ingredients which had frequent interactions with hub genes, *RELA* (degree =12), *MAPK14* (degree =11), *MAPK1* (degree =10), *JUN* (degree =10), and *AKT1* (degree =9) (*Figure 7A*). According to KEGG analysis, the PI3K-AKT and TNF signaling pathways were the signaling pathways with the highest involvement with these five hub genes. A critical network was then established based on these selected active ingredients, hub genes, and signaling pathways (*Figure 7B*). Therefore, the underlying mechanism by which HMM treats UC was shown intuitively. Additionally, the PI3K-AKT signaling pathway and TNF signaling pathway with enriched candidate genes were highlighted.

Discussion

Originally characterized in 1859, UC is one of two primary types of IBD (28). The incidence of UC is rising worldwide, with that of developed countries staying stable and developing countries increasing, especially India (29). The etiology of UC is not completely understood. Genetic background, environmental and internal factors, and immune dysregulation are regarded as possible risk factors (30). Currently, UC is diagnosed using a

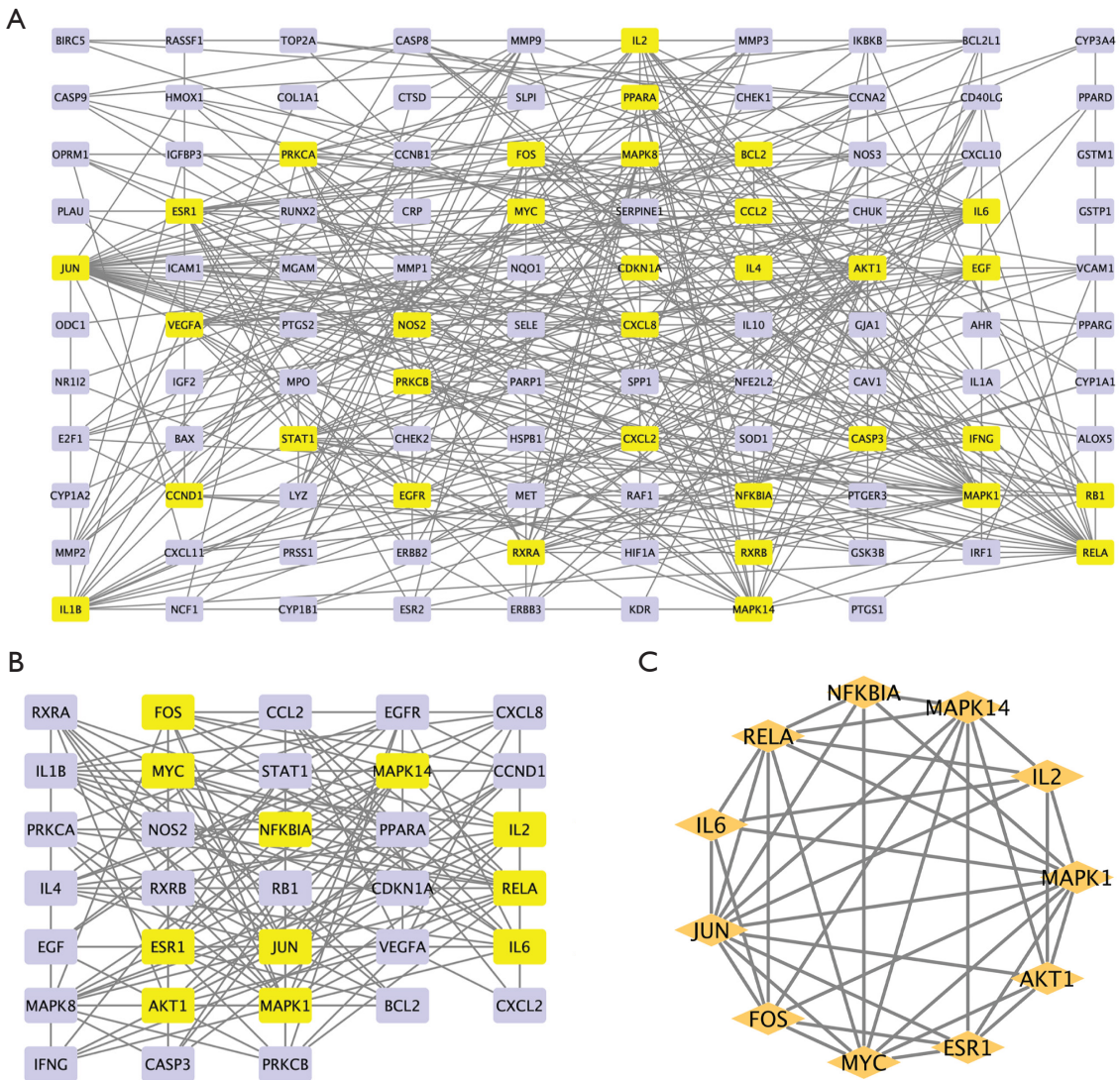

Figure 5 Screening for hub genes. (A) Primary subnetwork. Genes in yellow nodes are used for following screening. (B) Secondary subnetwork. Genes in yellow nodes are regarded as hub genes (C) interactions between 11 hub genes.

combination of clinical symptoms, endoscopic appearance, and histological outcomes. Bloody diarrhea is the most common symptom of UC. Endoscopic results usually show that the mucosa is consistently irritated and extending proximally from the anorectal verge, with the extent deteriorating from mild (only granular appearance) to severe UC (shallow ulcerations). Histological examination suggests that the inflammation is generally confined to the mucosal layer (1). In patients with UC, the therapeutic strategy is mostly determined by the severity of the illness. For acute severe UC patients, hospitalization is required, and intravenous corticosteroids and anticoagulants are applied (31). Medically refractory UC, poor medication tolerance, and UC-associated colorectal cancer are the most common reasons for surgery in UC patients (32). 5-aminosalicylic acid (5-ASA), oral corticosteroids, and thiopurines are the first-line treatment for mild-to-moderate patients. There is about a 50% clinical remission rate reported for 5-ASA treatments after two weeks. Thus, oral corticosteroids are the next step. However, remission and side effects cannot be prevented after oral corticosteroids. Besides, the use of thiopurines is limited because of the slow onset and severe adverse effects (33). Therefore, the efficiency of first-line treatment

Table 2 Molecular docking for hub genes and compound of HMM

Number	Hub genes	Compound	Docking affinity (kcal/mol)
1	*AKT1*	Kaempferol	–9.6
2	*AKT1*	Quercetin	–9.4
3	*ESR1*	3,9-di-O-methylnissolin	–6.9
4	*ESR1*	7-O-methylisomucronulatol	–6.8
5	*ESR1*	Calycosin	–7.8
6	*ESR1*	Formononetin	–8.4
7	*ESR1*	Isorhamnetin	–8.0
8	*FOS*	Quercetin	–10.4
9	*IL2*	Quercetin	–7.9
10	*IL6*	Quercetin	–7.8
11	*JUN*	Formononetin	–8.4
12	*JUN*	Kaempferol	–9.2
13	*JUN*	Quercetin	–8.8
14	*MAPK1*	Quercetin	–8.4
15	*MAPK14*	7-O-methylisomucronulatol	–7.0
16	*MAPK14*	Calycosin	–8.2
17	*MAPK14*	Formononetin	–7.8
18	*MAPK14*	Isorhamnetin	–8.5
19	*MYC*	Quercetin	–7.6
20	*NFKBIA*	Quercetin	–7.7
21	*RELA*	Isorhamnetin	–9.2
22	*RELA*	Kaempferol	–9.5
23	*RELA*	Quercetin	–9.2

HMM, *Hedysarum multijugum Maxim*.

is unsatisfactory as expected. Nowadays, many hospitals regard TCM as a primary treatment, as the effect is surprisingly positive, and research focusing on TCM is mounting (5-7). In the present study, a TCM named Huangqi, also known as *Hedysarum multijugum Maxim* (HMM), is a common ingredient in plenty of decoctions, piqued our attention owing to the favorable outcomes in treating UC patients.

According to TCMSP, HMM is the root of the leguminous plant safflower astragalus. Based on previous studies, HMM has been shown to take effect on many diseases. Yang *et al.* indicated that HMM treated ischemic stroke and protected patients from cerebral ischemia-reperfusion injury by regulating inflammation, oxidative stress, endoplasmic reticulum stress, and angiogenesis through signaling pathways, such as the PI3K-AKT, FoxO, TNF, HIF-1, Rap1, and VEGF signaling pathways (34,35). Zhang *et al.* showed that HMM improved the general condition of diabetic cardiomyopathy in a rat model (36). Even for coronavirus disease of 2019 (COVID-19), HMM made its contribution to treating COVID-19 pneumonia by targeting PTGS2, TNF, and IL-6 and regulating the TNF and IL-17 signaling pathways (37). The important role HMM plays in treating UC has drawn much attention and has been researched in a UC rat model, yet no exact conclusion has been reached (8,9). Exploring the pathways

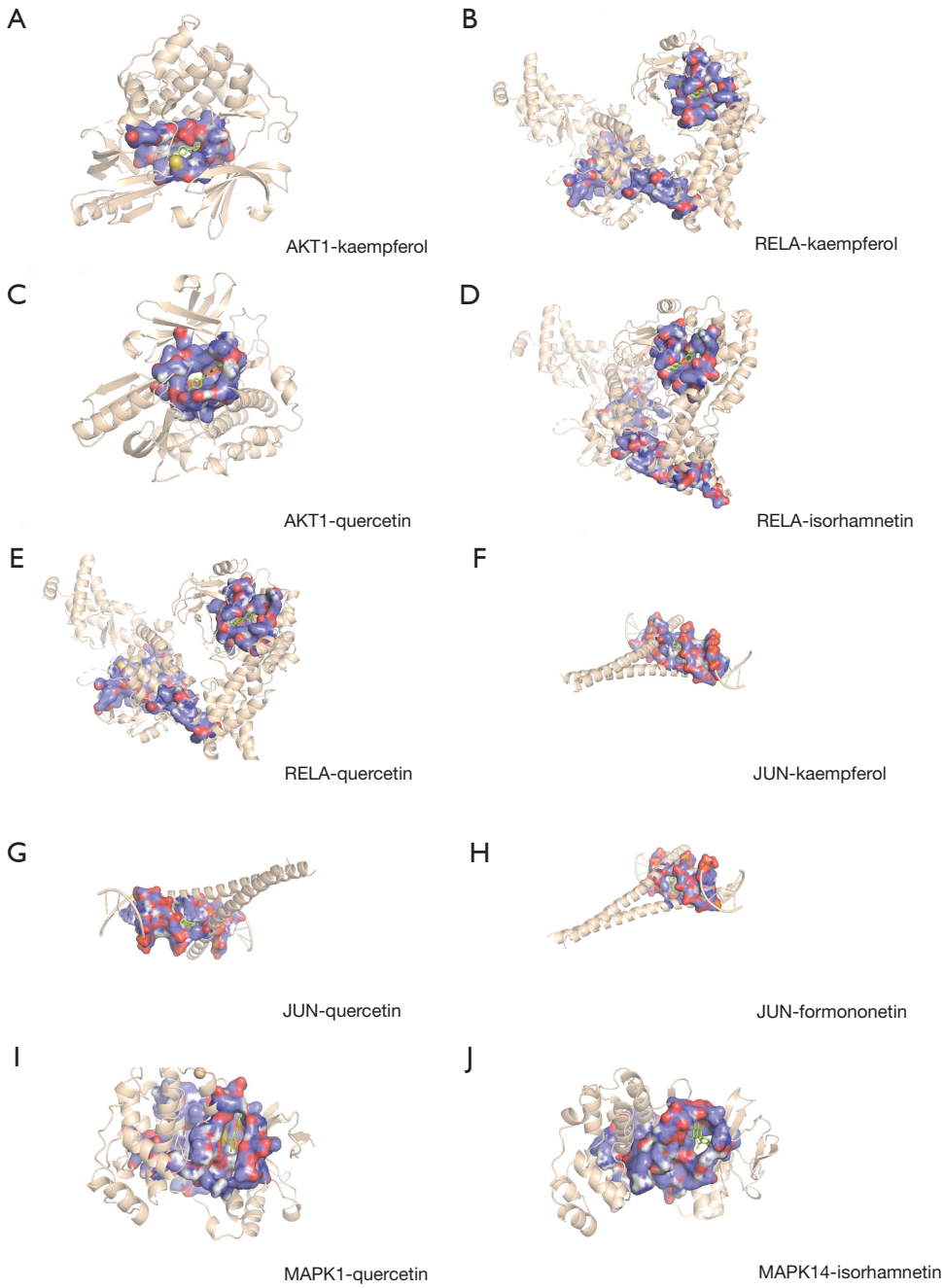

Figure 6 Molecular docking simulation of active compounds with ligands. The top 10 pairs of molecular docking simulations are exhibited. Ligands are displayed in green 3D structures. Receptors are displayed in a 3D white helix structure. Binding areas are covered by the surface. 3D, three-dimensional.

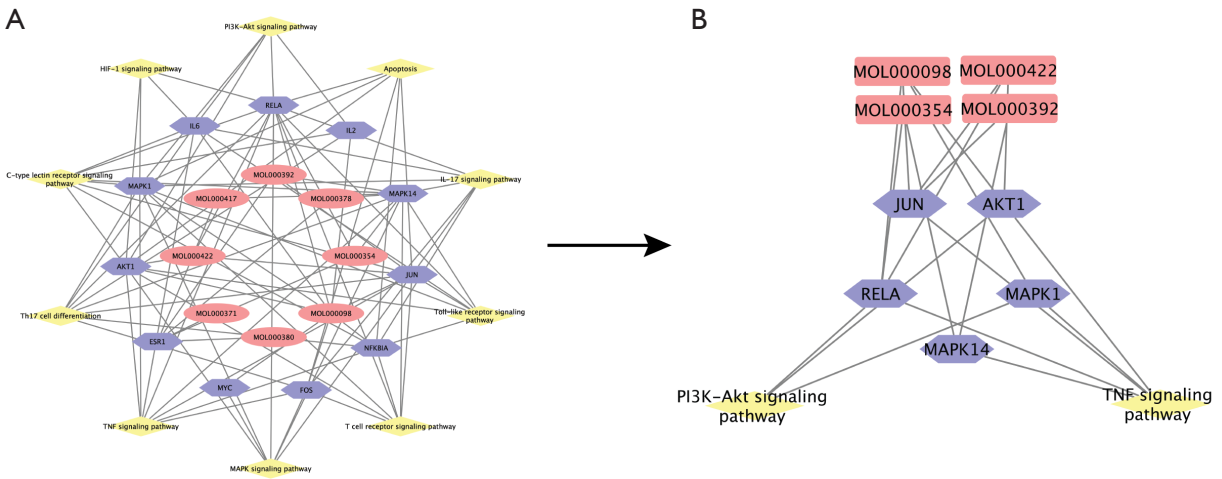

Figure 7 Compound-target-signaling pathway network. (A) Compound-target-signaling pathway network. Pink circle nodes represent active compounds of HMM, purple hexagon nodes represent hub genes and yellow diamond nodes represent signaling pathways. (B) The critical network comprising four active ingredients, five hub genes, and two signaling pathways. The core component of (a.), constructed after analysis. HMM, *Hedysarum multijugum Maxim*.

of compounds, proteins, or genes is the goal of network pharmacology, which can help explain the complexity of biological systems, drugs, and diseases. Besides, the docking sites for active ingredients and key targets of drugs can be predicted by molecular docking. Thereby, novel drugs developed with specific ingredients greatly improve clinical efficacy and reduce toxicity and side effect. For example, the underlying mechanism of Liu Jun An Wei formula in treating gastrointestinal reactions caused by chemotherapy for colorectal cancer is explored by network pharmacology (38). Besides, He *et al.* revealed that Danshen promoted JAL-STAT signaling pathway to treat anemia according to network pharmacology (39). In addition, it is reported that Moluodan treats chronic atrophic gastritis by regulating NF-kB signaling pathway (40). Therefore, in this study, network pharmacology and molecular docking were applied to explore the possible mechanism behind HMM treating UC.

As shown above, 20 active ingredients and 121 candidate genes were screened. The gene *PTGS2* is the most frequent target of active ingredients, followed by *PTGS1* and other genes. Both of *PTGS2* and *PTGS1* encode COX2 and COX1 to form prostanoids which are essential molecules in the inflammatory response and pain (41). Singer *et al.* indicated that upregulated COX2 was detected in UC patients compared to healthy controls, providing a possible pathogenesis for UC (42). Therefore, by targeting COX2, HMM not only inhibits the inflammatory response but also restricts the development of UC in the first place. Whereas, as shown in PPI analysis, AKT1 had the most connections with other protein genes encoded by candidate genes. AKT1 is a serine/threonine-specific protein kinase activated by phosphoinositide 3-kinases (PI3K) which regulate a majority of biological processes, such as proliferation, migration, angiogenesis, metabolism, and inflammation (43). The detailed function of AKT1 in treating UC would be discussed in the next part. An abundance of research has already shown that AKT1 and COX2 cooperate to fulfill complicated missions. St-Germain *et al.* had proved that AKT regulated COX2 expression through the NF-kB pathway (44,45). Besides, according to Leng *et al.*, COX2 positively regulates AKT phosphorylation (46). Therefore, it is reasonable to infer that HMM regulates top genes in bar plots, such as AKT and JUN, to inhibit the inflammatory response through COX2 and other frequent target candidate genes. Relying on research in recent years, the understanding of UC's pathogenesis has improved. Impaired epithelial barrier and intestinal microbiota dysfunction initiate neutrophil extracellular traps (NET) and immune responses which produce various cytokines in lamina propria of intestine, which, in the end, evolve into inflammatory responses. Cytokines such as TNF, IL-9, IL-13, IL-23, and IL-36 are important immune mediators in UC pathogenesis (28). Therefore, UC can also be defined as an immune disease. The results of GO and KEGG analysis were consistent with the studies mentioned above and matched novel findings. In

summary, HMM improves intestinal responses to external stress, such as nutrients, bacteria, and oxidative stress, which probably protects the intestine from epithelial damage and microbiota dysfunction, through the PI3K-AKT signaling pathway and some immune-related signaling pathways. To gain further understanding of HMM treating UC, 11 hub genes, all of which belong to the top 30 interacted genes, were preserved among candidate gene lists. Based on the compound-candidate gene network, eight active ingredients targeting hub genes showed up. A network comprising 11 hub genes, eight active ingredients, and the top 10 enriched signaling pathways was drawn, and a critical network was established after eliminating trivial elements, leaving two signaling pathways, four active ingredients, and five hub genes.

These four active ingredients all belong to the flavonols family which endows them with similar properties; they are known for their anti-inflammation and antioxidant effects (47-50), yet differences remain between them. Consistent with the former conclusion, it was reported that quercetin restricted COX2 expression by inhibiting PI3K phosphorylation, which consequently inhibited AKT1 phosphorylation and downstream gene expression (51,52). Apart from the PI3K signaling pathway, studies have shown that other pro-inflammation signaling pathways, MAPK, and JAK/STAT3, were also inhibited by kaempferol (53,54). According to recent research, quercetin attenuates intestinal damage and senescence by regulating increasing antioxidant capacity (55,56). Another important function of kaempferol is improving intestinal barrier function and integrity. By suppressing the activation of the NF-kB signaling pathway, kaempferol improved barrier function in a lipopolysaccharide (LPS)-induced epithelial-endothelial coculture model (57). Besides, quercetin has been widely applied in wound management owing to its migration and proliferation enhancing abilities (58). Thus, intestinal epithelial repair could be accelerated in UC patients treated with quercetin and kaempferol. As for formononetin, its primary function is antibacterial and antiviral (49). Research indicated that formononetin reshapes the intestinal microbiota by increasing maximum bacteria genera (59). Progestational hormone X receptor (PXR)-mediated up-regulation of xenobiotic metabolism and down-regulation of NF-kB signaling ameliorates the effects of isorhamnetin on experimental IBD. The findings might help to improve the use of isorhamnetin or its derivatives as a PXR ligand in the treatment of human IBD (60). Altogether, HMM treats UC through three aspects: diminishing inflammation, restoring intestinal homeostasis, and accelerating healing. Besides, studies have reported that quercetin and kaempferol inhibits TNF, IL9, and IL13 production (61,62). Thus, regulating immune responses is another pathway through which HMM treats UC.

Active ingredients function through regulating the hub genes mentioned above. According to Zhang *et al.*, AKT was activated by fibrinogen and caused colitis by increasing vascular permeability in the mice model (63). However, post-translational modification, like SUMOylation, was inactivated in UC patients (64). Therefore, the form of AKT1 determines its role in UC: pAKT promotes UC development, and SUMOylated-AKT1 protects the intestine from colitis. Besides, degradation of AKT2 via ubiquitination reduced colonic damage in the TNBS-induced UC mice model (65). MAPK14, an isoform of serine/threonine-specific kinase (SAPKs) is a major subfamily of mitogen-activated protein kinase, which plays an important role in TNF-alpha production (66). Patients with mutated MAPK14 showed adverse therapeutic effects when treated with glucocorticoid, suggesting that MAPK14 is a potential biomarker to predict therapeutic responses (67). Research has found that UC patients with high MAPK14 expression experience more severe abdominal pain than patients with low MAPK14 expression (68). JUN, also known as a subunit of the AP-1 transcription factor, decreased as dextran sulfate sodium (DSS)-induced colitis mice condition improved (69). RELA encodes transcription factor p65 which is a subunit of nuclear factor NF-kB p65 subunit. Many researchers have studied the function of NF-kB in UC. In general, these two transcription factors are used to activate signaling pathways that HMM regulated. As for MAPK1, no study has focused on its relationship with UC. The results of molecular docking showed that active ingredients have a good binding affinity with receptor proteins encoded by these hub genes, especially AKT-kaempferol complex (–9.6 kcal/mol), RELA-kaempferol complex (–9.5 kcal/mol), and AKT-quercetin complex (–9.4 kcal/mol). Kaempferol and quercetin targeted hub genes with the highest efficiency, which indicated that derivatives or synthetic drugs processed from kaempferol and quercetin could be an advanced solution for UC.

Concerning signaling pathways, PI3K-AKT and TNF were selected as most hub genes enriched in these signaling pathways. The PI3K-AKT signaling pathway participates in various processes and dysregulation of this pathway leads to multiple diseases, such as cancer, inflammation, and immune diseases (70,71). Research has shown that inhibition of the

PI3K-AKT signaling pathway relieves UC symptoms and histological damage in mice models owing to the reduction of pro-inflammatory cytokines such as TNF (72). The TNF-alpha signaling pathway is activated when TNF binds to its receptor on the cell membrane. The immune system is activated, either innate immune or adaptive immune, when TNF binds to TNF receptor 2 on immune cells like T cells, macrophages, and dendritic cells (73). According to studies, TNF is increased and related to intestinal barrier defects in UC patients (74). As pathophysiological mechanisms are explored, the emerging treatments vary. In recent years, anti-TNF therapies are becoming increasingly essential in UC treatment, such as infliximab, adalimumab, and golimumab (75). Hence, HMM inhibits PI3K-AKT signaling to reduce TNF production and inhibit the TNF signaling pathway, which functions similarly to anti-TNF therapy.

In summary, this study provides a theory that in the treatment of UC, the active ingredients of HMM, namely, quercetin, kaempferol, formononetin, and isorhamnetin regulate the PI3K-AKT and TNF signaling pathways by targeting essential genes, *AKT1*, *JUN*, *MAPK14*, *RELA*, and *MAPK1*. Still, the results need to be validated *in vivo* or *in vitro*. To a certain extent, the reliability of experimental results is affected by the inadequacies of the network pharmacology database. Hence, there is an urgent need to complete the construction of the database.

Conclusions

Overall, TCM provides novel UC treatments with high efficiency and security. In this study, five active ingredients and five hub genes were selected as key molecules in the treatment of UC by HMM through PPI analysis, GO and KEGG analyses, and molecular docking simulation. The PI3K-AKT and TNF signaling pathways were found to be the most targeted signaling pathways, which pointed out that the active ingredients control inflammation in the intestines through immune regulation and suppression. This study suggested that innovative drugs based on these active ingredients could be an efficient and safe way of treating UC. However, further experiments are needed validate our findings and promote the feasibility of this approach.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 Active ingredients of HMM

Mol ID	Molecule Name	OB(%)	DL
MOL000211	Mairin	55.38	0.78
MOL000239	Jaranol	50.83	0.29
MOL000296	hederagenin	36.91	0.75
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
MOL000354	isorhamnetin	49.6	0.31
MOL000371	3,9-di-O-methylnissolin	53.74	0.48
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69
MOL000378	7-O-methylisomucronulatol	74.69	0.3
MOL000379	9,10-dimethoxypterocarpan-3-O-Œ≤-D-glucoside	36.74	0.92
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42
MOL000387	Bifendate	31.1	0.67
MOL000392	formononetin	69.67	0.21
MOL000398	isoflavanone	109.99	0.3
MOL000417	Calycosin	47.75	0.24
MOL000422	kaempferol	41.88	0.24
MOL000433	FA	68.96	0.71
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26
MOL000439	isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48
MOL000098	quercetin	46.43	0.28

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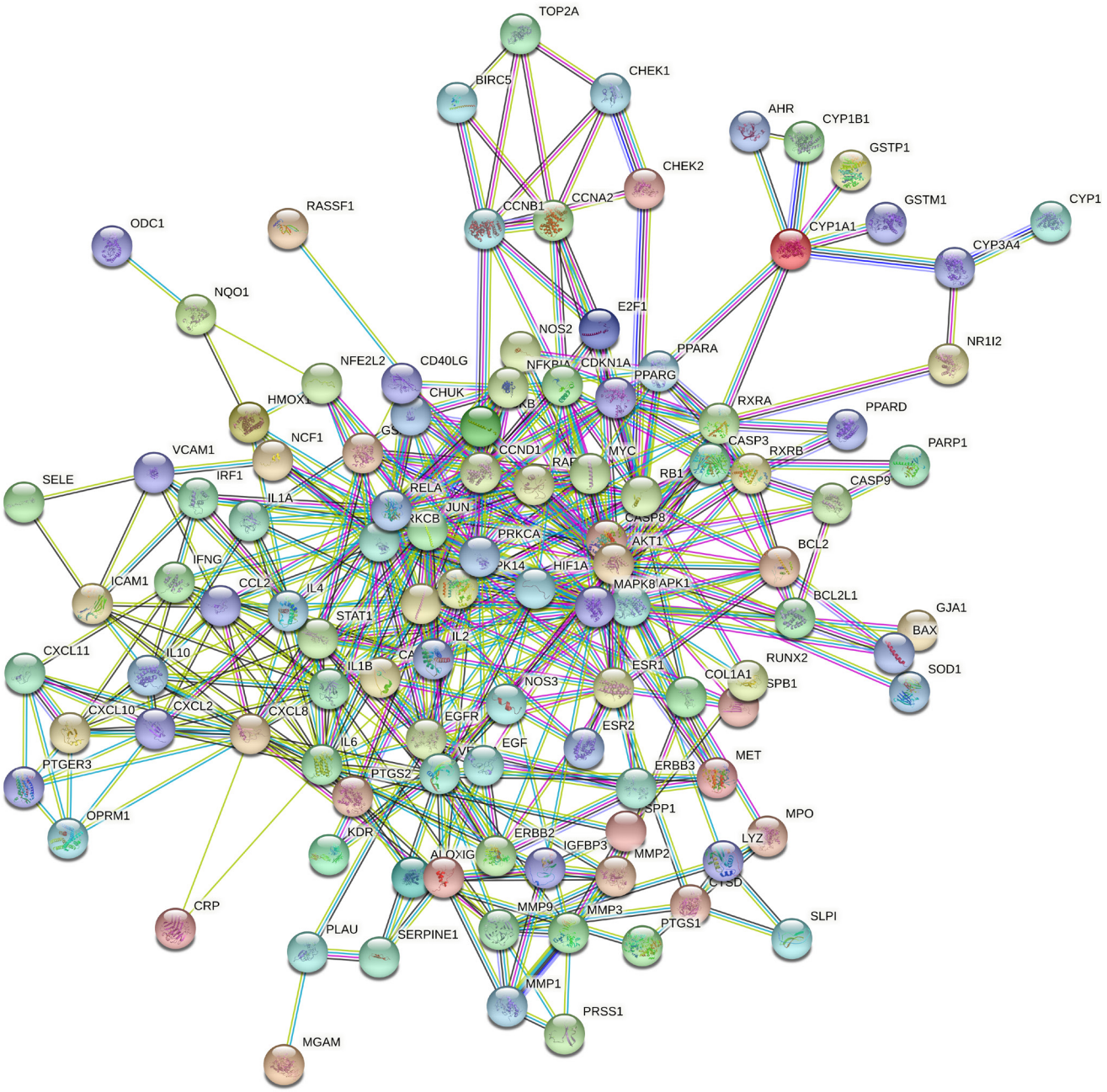

Figure S1 PPI analysis for candidate genes. PPI, protein-protein interaction.

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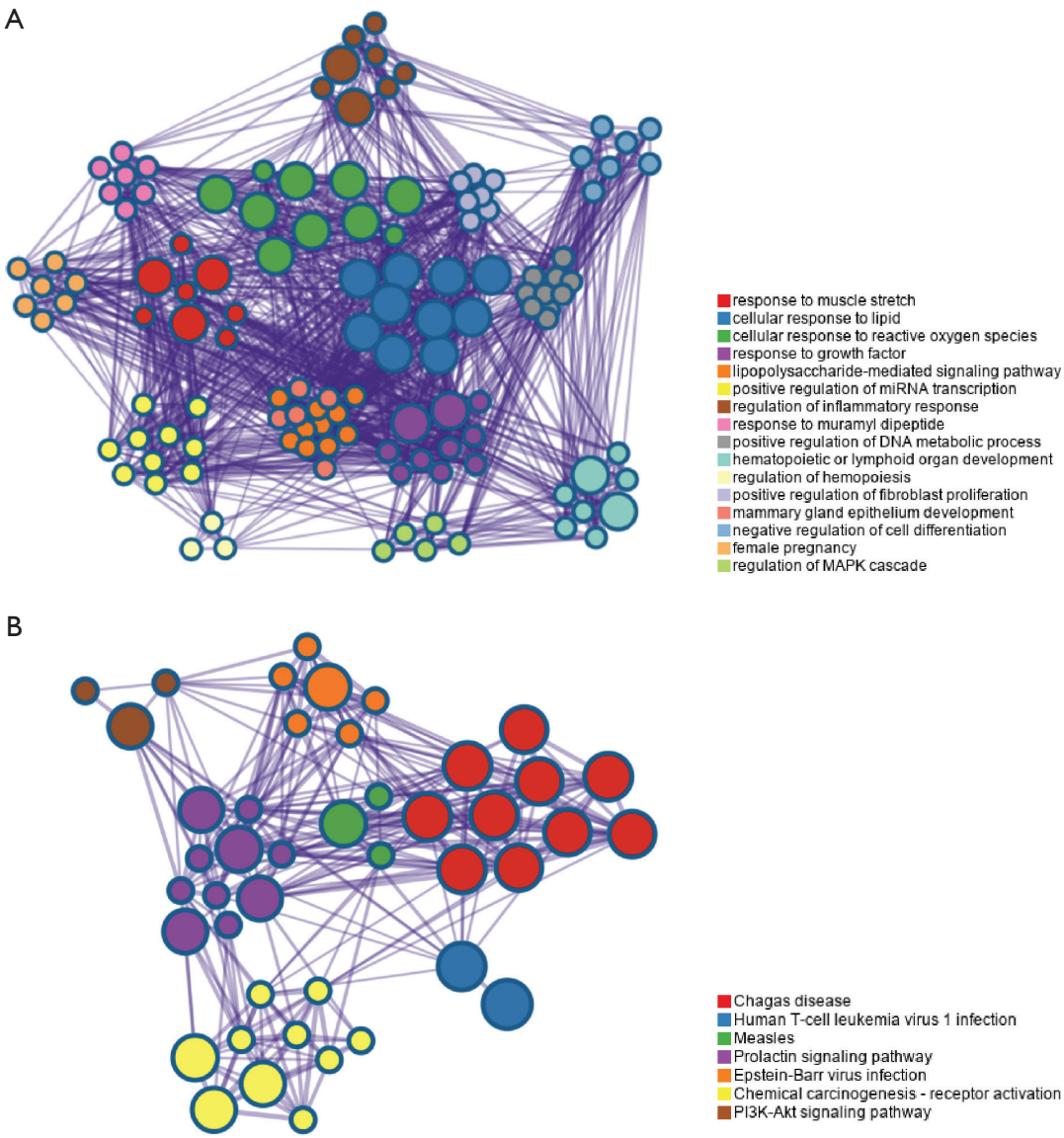

Figure S2 Functional analysis for hub genes. (A) GO analysis for hub genes, (B) KEGG analysis for hub genes, and different color was used to represent cluster annotations.

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