Using Network Pharmacology to Explore the Mechanism of Huanglian Jiedu Decoction in the Treatment of Ulcerative Colitis

YANG LIU, FANG SHI, PINGPING CHEN¹, JIANHUI SUN, BIN WANG AND QIQUAN LIU²*

Department of Gastroenterology, First Affiliated Hospital of Hebei University of Chinese Medicine, Shijiazhuang, Hebei 050011, ¹Department of Pharmacology, School of Basic Medical Sciences, Hebei University of Chinese Medicine, Shijiazhuang, Hebei 050200, ²Key Laboratory of Integrated Chinese and Western Medicine for Gastroenterology, Shijiazhuang, Hebei 050011, China

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The aim of this study was to identify the potential compounds, core targets and possible mechanism of Huanglian Jiedu decoction in treating ulcerative colitis based on network pharmacology and molecular docking. At first, potential compounds of Huanglian Jiedu decoction were retrieved from traditional Chinese medicine systems pharmacology. And then, the targets related to compounds and ulcerative colitis was obtained from traditional Chinese medicine systems pharmacology, Online Mendelian Inheritance in Man, GeneCards and DisGeNET. Next, Cytoscape was used to visualize drug-compound-common target-disease network and protein-protein interaction network. Moreover, gene ontology and Kyoto encyclopedia of genes and genomes enrichment analysis was performed by database for annotation, visualization and integrated discovery to investigate possible mechanism of Huanglian Jiedu decoction against ulcerative colitis. At last, molecular docking verified the reliability of the prediction results. 55 compounds and 84 targets of Huanglian Jiedu decoction were screened out as potential players on ulcerative colitis. After network analyses, 10 core compounds (quercetin, kaempferol, wogonin, baicalein, acacetin, 5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl) chromone, beta-sitosterol, moslosooflavone, 5,7,4′-trihydroxy-8-methoxyflavone, oroxylin A) and 10 core targets (interleukin-6, interleukin-1 beta, tumor necrosis factor-alpha, threonine-protein kinases, tumor antigen p53, prostaglandin-endoperoxide synthase 2, JUN, CXCL8, C-C motif chemokine 2, matrix metalloproteinase-9) were identified. Furthermore, the inflammatory response, tumor necrosis factor-alpha signaling pathway, pathways in cancer, T cell receptor signaling pathway, toll-like receptor signaling pathway and nuclear factor kappa B signaling pathway may be involved in the treatment of ulcerative colitis using Huanglian Jiedu decoction. This study reveals that Huanglian Jiedu decoction contains multiple ingredients, multiple targets and multiple pathways in treating ulcerative colitis, which provides a basis for further research.

Key words: Ulcerative colitis, inflammatory bowel disease, nuclear factor kappa B, matrix metalloproteinase-9, diarrhea

Ulcerative Colitis (UC), as a chronic idiopathic Inflammatory Bowel Disease (IBD), is characterized by long-lasting mucosal inflammation of the colon. The main symptoms of UC are abdominal pain, bloody diarrhea and tenesmus. With the increasing prevalence of IBD, UC evidently influences the health and life quality of patients all over the world. The incidence of UC is higher in developed countries than other regions of the world. However, the incidence and prevalence of UC in Asian countries is increasing year by year. In China, a previous meta-analysis of IBD patients based on population and hospital studies showed that the incidence rate of UC was 1.2/100 000 persons. At present, the pharmacological management of UC is focused on controlling symptoms with 5-Aminosalicylates (5-ASA), corticosteroids and thiopurines, which is still not ideal. Given this, discovering more effective and less toxic treatments for UC is urgently needed.

Traditional Chinese Medicine (TCM), as a significant part of complementary and alternative medicine
system, has been used to treat and prevent UC for a long time. TCM can alleviate the symptoms of UC, heal the ulcers and improve the quality of life. Hence, it is well worth studying in TCM prescription against UC deeply. Huanglian Jiedu Decoction (HLJDD) originates from “The Handbook of Prescriptions for Emergencies”, is an important and classic TCM formula to clear heat and detoxify. HLJDD is composed of four herbs; *Coptidis rhizoma* (Huanglian (HL)), *Scutellariae radix* (Huangqin (HQ)), *Phellodendri chinensis* cortex (Huangbai (HB)) and *Gardeniae fructus* (Zhizi (ZZ)) at a ratio of 3:2:2:3, respectively. At present, HLJDD has been widely used in treating inflammatory gastrointestinal disease including UC. Based on the TCM theory, the pathogenesis of UC is closely associated with toxic heat, which could be solved by HLJDD. Modern pharmacological study has shown that the n-butanol fraction of HLJDD can significantly control the symptoms and pathological damage of UC mice and inhibit the inflammatory response. Chinese researchers have also reported that HLJDD could alleviate the colonic mucosal inflammation and damage in UC mice by reducing the levels of Tumor Necrosis Factor alpha (TNF-α), Interleukin (IL)-6 and IL-1 beta (β). However, the clear molecular mechanism of HLJDD against UC still lacks systematic understanding and enough relevant studies. Network pharmacology, first proposed by Andrew L Hopkins, has been a powerful tool to illuminate our understanding of drug action. Similar to TCM, network pharmacology is also holistic and systematic in methodology, which can be applied to TCM herbal studies. Based on network pharmacology, we can discover new bioactive ingredients and special biomarkers, elucidating possible mechanisms of interactive actions between multicomponents and multitargets of herb formula in TCM. In this study, we employed network pharmacology technology to predict the mechanism of HLJDD on UC and the prediction results were verified by molecular docking subsequently. The detailed workflow of our study was exhibited in fig. 1.

**MATERIALS AND METHODS**

**Collection and screening of potential bioactive compounds in HLJDD:**

The collection of four candidate herbs in HLJDD was retrieved by TCM Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmsp-e.com/), a TCM systems pharmacology platform which contains the relationship between drugs, targets and disorders. According to relevant literature report, the pharmacokinetic properties including Absorption, Distribution, Metabolism and Excretion (ADME) were generally employed to screened out potential bioactive compounds in herbs. As suggested by TCMSP, the compounds whose Oral Bioavailability (OB) ≥30 % and drug-likeness ≥0.18 at the same time could be identified as the potential bioactive compounds in HLJDD for further analysis.

**Target fishing for HLJDD:**

The compounds in HLJDD depend on their targets to exert their role. After searching in TCMSP with the name of potential compounds in HLJDD, the targets of compounds were obtained. Target names were then standardized through the UniProt database, an informative and resourceful protein knowledgebase and we selected “Homo sapiens” as the potential target species of the potential compounds of HLJDD.

**Search targets of UC:**

In this process, the key word “UC” was searched in Online Mendelian Inheritance in Man (OMIM), GeneCards and DisGeNET to collect the targets related to UC. UC targets were finally obtained after removing repetitive data. Common targets of both potential bioactive compounds and UC were considered as the related targets of HLJDD treatment for UC. The relationship between UC targets and HLJDD targets was shown by Venny 2.1.

**Protein-Protein Interaction (PPI) data:**

Based on the above research, intersective targets about HLJDD on UC were imported into the STRING platform (https://string-db.org, ver 11.0) to obtain the PPI data. The species was limited to “Homo sapiens”, the confidence score ≥0.4 as the threshold and the discrete targets were deleted.

**Network construction:**

Network construction was visualized by Cytoscape software (version 3.8.2) as follows; drug-
compound-common target-disease network; PPI
network was constructed by linking common targets
between UC and HLJDD. Via the network analyzer
tool in Cytoscape, the potential core targets of the
PPI network were obtained by the rank of degree
numbers.

Gene Ontology (GO) and pathway enrichment
analysis:
To figure out the possible mechanisms of HLJDD
on UC, intersective targets were imported into
the Database for Annotation, Visualization and
ncifcrf.gov/) with species limited to “Homo sapiens”.
DAVID is a functional annotation tool to predict
possible Cellular Component (CC), Molecular
Function (MF), Biological Process (BP) and signaling
pathway associated with specific genes. With p<0.01
to keep the reliability of the enrichment analyses and
the analytic results were visualized by R language.

Molecular docking:
We used molecular docking technology to verify the
reliability of the network pharmacology prediction
results. The structures of core compounds in HLJDD
were downloaded from the PubChem and the Three-
Dimensional (3D) structures of the core target
proteins were obtained from the Protein Data Bank
(PDB)[22] (https://www.rcsb.org/). The molecular
docking and conformation scoring were carried out
in the AutoDock Vina and the heat map was drawn by
the GraphPad Prism 8. The Pymol and Maestro 11.9
were used to draw the structure of the representative
docking results.

RESULTS AND DISCUSSION
A total of 80 compounds were screened out and
collected from TCMSP based on the screening criteria;
25 in HB, 11 in HL, 32 in HQ and 12 in ZZ, respectively;
among them, 14 compounds were duplicated and
removed. For example, beta-sitosterol existed in HB,
HQ and ZZ. By using TCMSP, 228 targets of potential
bioactive compounds in HLJDD were obtained after
deleting duplicates.

With “UC” as the key word, a combined total of 928
disease targets were collected from OMIM, GeneCards
and DisGeNET databases after removing duplicates. As
a result, the intersections of predicted targets of HLJDD
and disease targets resulted in 84 common targets for
UC (fig. 2). At last, 55 potential compounds were
identified from HLJDD and some bioactive compounds
were deleted in lack of common targets (Table 1).

Fig. 1: Workflow of the pharmacology-based study of HLJDD used in treating UC
Fig. 2: Venn diagram of HLJDD and UC-related targets
Note: The blue part represented the targets of HLJDD, the yellow part represented the genes associated with UC, and the intersection represented the potential targets for the ingredients of HLJDD in the treatment of UC

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Fig. 3: Drug-compound-common target-disease network
Note: Pink hexagons represented drugs of HLJDD, green triangles represented compounds of HLJDD, blue nodes represented common targets of HLJDD on UC and red rhomb represented UC
PPI data were obtained from STRING by uploading 84 common targets and PPI network was mapped by Cytoscape, consisting of 84 nodes and 1568 interaction edges. In the network, the greater the degree, the larger and darker color the node was (fig. 4). 10 targets with the largest degree value (degree ≥62) were selected as core targets for UC. The core targets, which may play a significant role against UC, were (IL-6, degree=75), (IL-1β, degree=73), (TNF, degree=73), (RAC-alpha serine/Threonine-Protein Kinase (AKT1), degree=72), cellular (Tumor Antigen p53 (TP53, degree=69), Prostaglandin G/H Synthase 2 (PTGS2), degree=67), Transcription Factor AP-1 (JUN, degree=66), IL-8 (CXCL8, degree=62), C-C Motif Chemokine 2 (CCL2, degree=62) and Matrix Metalloproteinase-9 (MMP-9, degree=62).

GO term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment results were acquired by DAVID. Top 10 enriched conditions in BP, CC and MF were exhibited in fig. 5. 255 BPs, 21 CCs and 42 MFs enriched for common targets have a p<0.01. In GO term enrichment, the BP of HLJDD against UC may relate to response to drug, positive regulation of transcription from Ribonucleic Acid (RNA) polymerase II promoter, inflammatory response and so on. Top 3 MF were enzyme binding, identical protein binding and cytokine activity. Cell compound analysis showed that extracellular space, extracellular region, cytosol, perinuclear region of cytoplasm and external side of plasma membrane were ranked as the top 5 CCs. Besides, 77 KEGG pathways were recognized as p<0.01 and top 20 KEGG pathway’s enrichment analysis was shown in fig. 6. The KEGG analysis results showed that the mechanism of HLJDD against UC might focus on multiple signaling pathways such as TNF signaling pathway, pathways in cancer, T cell receptor signaling pathway, Toll-Like Receptor (TLR4) signaling pathway and Nuclear Factor Kappa B (NF-κB) signaling pathway.

The core targets IL-6, IL-1β, TNF, AKT1, TP53, PTGS2, JUN, CXCL8, CCL2 and MMP-9 were used for molecular docking with the top 10 crucial compounds in HLJDD (fig. 7). Most of the compounds of HLJDD had good binding with core targets and markers, which means that HLJDD has a strong potential used to treat UC. Results showed that baicalein has a strong binding ability with PTGS2 (Vina score=−9.4 kcal/mol). Quercetin has a strong binding ability with TNF (Vina score=−7.9 kcal/mol). Wogonin binds with AKT1 (Vina score=−7.4 kcal/mol), kaempferol binds with IL-6 (Vina score=−6.9 kcal/mol) and acacetin binds with IL-1β (Vina score=−6.9 kcal/mol). Further molecular docking revealed that baicalein binds with PTGS2 through hydrophobic interaction at sites LEU152, VAL46, CYS47, PRO153 and ARG469 and has hydrogen bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR130 and CYS47. Quercetin binds with TNF through hydrogen interaction at sites including CYS98, ARG104 and TYR103 and has hydrophobic bonding action with HIS39, TYR103 and has hydrophobic bonding action with GLN130 and GLU131. Wogonin has hydrogen bonding action with PHE88 and GLY16 by binding with AKT1. Kaempferol binds with IL-6 through hydrogen interaction at ARG30 and has hydrophobic bonding action with LEU33 and LEU178. Acacetin binds with IL-1ß through hydrogen interaction at sites including ASN7, PRO87 and ASN66 and has hydrophobic bonding action with PRO91 and LYS63. Fig. 8 illustrates these local structures of molecular docking in detail.

Fig. 4: Network diagram of the PPI network
Fig. 5: GO function enrichment analysis of potential targets from active compounds in UC
Note: ( ) : BP; ( ) : CC and ( ) : MF

Fig. 6: The top 20 results of KEGG pathway enrichment analysis

Fig. 7: Heat maps of the docking scores of core targets combining with crucial compounds in HLJDD
UC is a type of IBD and it is a great threat to human health. However, there is in lack of relatively effective and safe way to treat this disease. TCM has accumulated rich clinical experience for many years in treating complex disease such as UC. In TCM, UC belongs to the category of “dysentery” and so on. HLJDD is widely used in China to treat UC, but its mechanism of action is still not fully understood. It has been revealed that HLJDD and its effective fraction can alleviate UC by inhibiting Cyclooxygenase-2 (COX-2) protein expression and PLA2, 5-LOX activity to regulate arachidonic acid metabolism and glycerophospholipid metabolism. In this study, a network pharmacological prediction was conducted on the bioactive compounds of the four TCMs (Coptidis rhizoma, Scutellariae radix, Phellodendri chinensis cortex, and Gardeniae fructus) in HLJDD and UC targets. This research could lay the foundation for further experimental verification and provide effective therapeutic strategies for better treatment of UC in the future.

Based on the results of network pharmacology, we speculated that core compounds in HLJDD play significant roles in UC treatment because of their high degree values. Quercetin is known to be a natural dietary flavonoid which has several biological effects including anti-inflammatory, anti-tumor, antioxidant and gastrointestinal cytoprotective effects. Modern research has found that quercetin administration could suppress inflammation in UC-organoids from mice. Researchers also found quercetin-loaded microcapsules could ameliorate the inflammation in colons. Kaempferol, considered being a natural flavonol and its anti-inflammatory property has been confirmed. The plasma levels of Nitric Oxide (NO), Prostaglandin E2 (PGE2) and Leukotriene B4 (LTB4) in Dextran Sodium Sulfate (DSS) induced colitis in C57BL/6J mice can be significantly reduced by oral kaempferol, indicating that kaempferol can be an effective anti-inflammatory agent in protecting colonic mucosa from UC. Wogonin is also a kind of flavonoid showing anti-inflammatory potential inhibiting UC. By up-regulating the IL-10 production, wogonin could enhance the therapeutic effects of mesenchymal stem cells in DSS induced colitis. By improving intestinal epithelial barrier via AHR/IL-22 pathway, baicalein can ameliorate UC. Baicalein can regulate Treg cell differentiation and maintain immune homeostasis in DSS induced colitis mice, which might be a potential drug for UC. Acacetin is an O-methylated flavone which possesses anti-inflammatory, anticancer and anti-oxidative activities. Research has shown that acacetin could inhibit the macrophage inflammatory response and adjust intestinal microbiota to alleviate DSS-induced colitis in mice. The results of molecular docking also verified that the core compounds have good binding properties with most core targets. These results could provide novel thought on studying the mechanism of specific compounds in HLJDD against UC. However, some of the compounds are in lack of ample evidences related to UC to support our study’s prediction results, which needs further study to verify.
In order to further explore the possible mechanism of HLJDD on UC, GO and KEGG pathway analysis is indispensable. According to CC analysis results, HLJDD mainly took action in the region of intracellular and extracellular. So far, the pathogenesis of UC has not been elucidated. From top 10 enriched conditions in BP, we could speculate that response to drug, positive regulation of transcription from RNA polymerase II promoter, inflammatory response and negative regulation of apoptotic process were mainly involved in the BP of HLJDD in treating UC. These BP are mainly associated with the process of occurrence and development of UC. KEGG enrichment analysis suggested that HLJDD targeted genes were mainly associated with inflammation related diseases. Furthermore, we found that HLJDD employed a therapeutic effect on UC through multiple pathways. Among them, TNF signaling pathway, pathways in cancer, T cell receptor signaling pathway, TLR4 signaling pathway and NF-κB signaling pathway were the key points and principal signaling pathways that might be related to the mechanism of HLJDD against UC.

PPI analysis revealed that the top 10-ranking genes, IL-6, IL-1β, TNF, AKT1, TP53, PTGS2, JUN, CXCL8, CCL2 and MMP9 might be the crucial targets regulated by HLJDD for treating UC. Most of them have been documented associated with the above signaling pathways, which confirms the reliability of the KEGG analysis in the other aspect. IL-6, a key proinflammatory cytokine, exists in the pathogenesis of UC and the level of IL-6 is linked to the disease severity[34]. As a central element in the regulation of inflammation at the gastrointestinal mucosal level, IL-1β can be served as a key target for IBD prevention and treatment[35]. TP53, a tumor suppressor gene, its mutations is the first step in the evolution from inflamed colonic epithelium to Colorectal Cancer (CRC), demonstrating the close connection between TP53 and UC[36]. According to research data, haplotype of PTGS2 contributes to the susceptibility of IBD, including UC[37]. As an immediate-early gene, proto-oncogene JUN (c-Jun) plays a significant role in inflammatory responses. The expression of c-Jun increases rapidly in cells, enabling cells to adapt to environmental changes when faced with external stimuli[38]. CXCL8 is acting as a proinflammatory chemokine. Compared with healthy volunteers, patients with UC have elevated levels of CXCL8 and CCL2 in the colonic mucosa[39].

Although the pathogenesis of UC has not yet been clearly elucidated, scientists commonly believe that immune system and inflammation bears on it. It has been universally acknowledged that cancer is closely related to inflammation[40]. Longstanding chronic inflammation mainly contributes to the occurrence of CRC[41]. Researchers believe that increased duration and extent of UC increases the risk of CRC[42]. A meta-analysis concerning about 116 studies found that the incidence of CRC positively correlated with the duration of UC[43]. According to the prediction results, HLJDD possibly could treat UC by inhibiting the process from inflammation to cancer, which needs further studies. TNF is a pleiotropic cytokine with important functions in inflammatory disease pathogenesis, such as inducing inflammation, orchestrating the tissue recruitment of immune cells and promoting tissue destruction[44]. Anti-TNF therapy has been proven to be an effective means to induce clinical and endoscopic remission in UC patients; however, its relevant risks require deep concern[45]. T-Cell Receptor (TCR) play significant role in function of T cells and formation of the immunological synapse[46]. TCR pathway was reported to be significant in regulation of UC. Research has found that the increased levels of TCR core fucosylation are required for activating TCR signaling and inducing UC in mice, which might be a therapeutic strategy to block the process of UC formation[47]. By using transcriptional profiling of circulating T cells isolated from patients with UC, scientists considered that the prognosis in patients with UC could be predicted by gene expression profiling of Cluster of Differentiation 8 (CD8+) T cells[48]. As a pattern recognition receptors TLRs are crucial in immune response. TLRs playing a key role in identifying invading pathogens and upregulating signals related to inflammatory cytokines and costimulatory molecules[49]. An experimental study found that TLR4 deficiency aggravated intestinal injury in UC by down-regulating IL-6, CCL2 and CSF3[50]. NF-κB induces cytokine expression and neutrophil aggregation. NF-κB signaling pathway is a complex network which is involved in the processes of immune and inflammatory responses[51]. It is well accepted that activating NF-κB signaling pathway plays a key role in the development of UC and NF-κB regulates the expression of multiple pro-inflammation genes and maintains immune system homeostasis[52]. By suppressing NF-κB signaling pathway, the colonic glandular structure destruction and inflammatory cell infiltration of UC could be reduced, thus protecting epithelial barrier integrity[53].

In summary, the prediction results uncover that
HLJDD has multiple ingredients, multiple targets and multiple approaches in treating UC, which enhance our understanding of the potential anti-inflammatory mechanisms of HLJDD. However, this study also has some limitations on account of insufficient retrieval data and lacking in relevant experiments to validate the prediction results. Therefore, there is a lot of unfinished work needed for researchers to improve and accomplish to clarify the mechanism of HLJDD against UC.

From the network pharmacology prediction, we could conclude the active compounds, potential targets and possible mechanisms of HLJDD treating UC. 55 constituent compounds of HLJDD were summarized and filtered from TCMSP. 84 targets were identified as the common targets of UC and compounds. By network construction and topological calculation, 10 core active compounds and 10 core targets of HLJDD against UC were identified. Furthermore, possible MF, BP and pathways regulated by HLJDD against UC were systematically interpreted, which could provide a basis for its clinical application and further research.

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**Conflict of interests:**

The authors declared no conflict of interests.

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