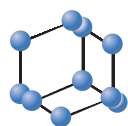
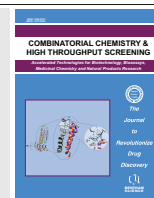


## RESEARCH ARTICLE

BENTHAM  
SCIENCE

# An Investigation of the Mechanism of Rapid Relief of Ulcerative Colitis Induced by Five-flavor Sophora Flavescens Enteric-coated Capsules Based on Network Pharmacology



Sizhen Gu<sup>1</sup>, Yan Xue<sup>2</sup>, Yuli Zhang<sup>1</sup>, Kanjun Chen<sup>1</sup>, Shigui Xue<sup>3</sup>, Ji Pan<sup>3</sup>, Yini Tang<sup>3</sup>, Hui Zhu<sup>4</sup>, Huan Wu<sup>1</sup> and Danbo Dou<sup>1,\*</sup>

<sup>1</sup>Department of Traditional Chinese Medicine, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China; <sup>2</sup>Shi's Center of Orthopedics and Traumatology, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China; <sup>3</sup>Digestive Endoscopy Center, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China; <sup>4</sup>Emergency Department, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China

**Abstract: Aim and Objective:** Five-Flavor Sophora flavescens Enteric-Coated Capsules (FSEC) are the only proprietary Chinese medicine approved for the treatment of ulcerative colitis (UC) in China. Phase II and III clinical trials have shown that the curative effect of FSEC in relieving UC was not inferior to that of mesalazine granules and enteric-coated tablets, but its pharmacological mechanism is unclear. Therefore, the network pharmacology is used to reveal the more comprehensive effective components and targets of FSEC in the treatment of UC.

**Methods:** We screened the components of FSEC based on the TCMSP database, determined the action targets of these compounds through target fishing, and integrated the UC disease targets of several disease gene databases. The FSEC-UC composite targets were obtained by matching the two results, and then a PPI network was constructed to analyze the relationship between these targets, and the core targets were selected by topological correlation parameters. Finally, GO-BP and KEGG enrichment analyses were carried out using the clusterProfiler software package.

**Results:** One hundred and sixty active components of FSEC were identified and 77 targets were obtained. Of these, 30 core targets were the main targets of FSEC in the treatment of UC. And quercetin, kaempferol, luteolin and mangiferin were regarded as the core active components of FSEC. The results screened by GO and KEGG enrichment analysis showed that FSEC played a comprehensive therapeutic role in immune recognition, anti-inflammation and antioxidation mainly through IL-17, TNF, Toll-like receptor, NF-kappa B, and Th17 cell differentiation.

**Conclusion:** The molecular mechanism of UC remission induced by FSEC was predicted by network pharmacology. These findings provide an important theoretical basis for further study of the effective substances and mechanism of FSEC in the treatment of UC.

**Keywords:** Five-flavor sophora flavescens enteric-coated capsules (FSEC), network pharmacology, ulcerative colitis, chinese medicine, active compounds, UC treatment.

## 1. INTRODUCTION

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD), which mainly involves the mucosa and submucosa layer of the rectum and colon. Diarrhea,

abdominal pain, purulent stool and other symptoms are the main clinical manifestations. The etiology involves psychological, environmental, genetic, infection and immunity factors [1], in which the inflammatory response mediated by abnormal immune response is directly related to the pathological injury of intestinal mucosa [2-4]. The regimens used to treat UC mainly include aminosalicic acid, steroids, immunosuppressants and biological agents; however, many adverse reactions occur and drug withdrawal leads to relapse. Traditional Chinese Medicine (TCM) has

\*Address correspondence to this author at the Department of Traditional Chinese Medicine, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China;  
E-mail: [doudanbo@126.com](mailto:doudanbo@126.com)

been widely used in the prevention and treatment of UC in China for a long time. A large number of studies have proved that TCM can control the symptoms of UC in patients, accelerate the healing of ulcers and improve the quality of life. Many researchers have devoted themselves to clinical and basic research on TCM for the prevention and treatment of UC intending to repair intestinal mucosa, regulating immunity, and maintaining treatment to prevent recurrence.

UC belongs to the category of "Chronic Dysentery" and so on. TCM believes that the pathogenesis of UC is mostly due to damp-hot and hot-toxin which accumulates in the intestine over a long time, resulting in intestinal mucosal injury, abdominal pain, diarrhea, purulent stool and blood symptoms. Treatment should clear heat and dampness detoxification, heal ulcers and promote mucosal repair. Five-Flavor Sophora flavescens Enteric-Coated Capsules (FSEC) is a TCM prescription based on this treatment principle. It is derived from the enema prescription of the 301 Hospital of the Chinese Liberation Army. It is composed of five TCMs, namely Sophora flavescens, Indigo Naturalis, Radix Sanguisorbae, Bletilla striata and licorice. The early clinical effects of FSEC are worth affirming. Following the improvement of UC with FSEC, an orally administered colon-specific released drug has been developed, which is the only proprietary Chinese medicine approved for the treatment of UC in China. FSEC can quickly induce remission of mild to moderate UC. The repair of intestinal mucosa is especially suitable for TCM syndrome differentiation belonging to the damp-heat syndrome of UC. Before it was placed on the market, FSEC was called Fufangkushen colon-coated capsule. Multicenter, randomized, controlled, double-blind and double-simulant studies have been completed in China, and the efficacy and safety of Fufangkushen colon-coated capsule were evaluated. In a comparative study of FSEC and mesalazine sustained release granules (trade name: Aidisha) [5], it was found that after 8 weeks of treatment, the total effective rate of FSEC, the total effective rate of enteroscopy lesion improvement, histological lesion improvement rate and DAI (Disease Activity Index) score were not significantly different to those in the control group ( $P > 0.05$ ). There were no serious adverse events in two groups during the trial. In a controlled study with mesalazine enteric-coated tablets (trade name: Huidi), it was found that after 8 weeks of treatment, the clinical remission rate between the two groups was similar, and there were no significant differences in mucosal healing rate, Mayo score and safety between the two groups. These findings suggested that FSEC was not inferior to Aidisha in the treatment of damp-heat intrinsic active UC, and tended to be superior to Aidisha, and can be used as a substitute for the treatment of Aidisha and other chemotherapy drugs, and compared with Huidi, it was equally effective and safe. FSEC was more suitable for patients with left colon involvement [6].

Network pharmacology is a new subject based on system biology, disease and drug network databases, high throughput groups and so on [7-9]. It systematically and comprehensively reveals the mechanism of drug action on disease. As TCM and TCM compound prescriptions often involve multi-components, multi-targets and multi-pathways, we constructed a "drug-component-target" network based on

known chemical components and disease targets in TCM. This is similar to the extension and refinement of the compatibility of TCM, which can intuitively reveal the mechanism of action [10]. As FSEC has a remarkable clinical effect and no obvious side effects, it is expected to become a breakthrough in the treatment of UC with TCM. In order to further examine the mechanism behind this curative effect, we analyzed the prescription using network pharmacology in order to obtain the main functional components, targets and pathways.

This study aimed to screen out the components of FSEC using the TCM database, obtain the action targets by target fishing, and then search and integrate the multi-source database to screen the relevant targets of UC. The FSEC targets were matched with UC targets, and the composite targets were established by the protein-protein interaction (PPI) network to analyze the interaction between these targets, and the core targets were selected by topological structure. Finally, GO-BP and KEGG enrichment analysis of the composite target was carried out, and the main process is shown in Fig. (1).

## 2. MATERIAL AND METHODS

### 2.1. Constructing the FSEC Components Database

The active components of five Chinese herbs in FSEC were searched and collected using the Pharmacological Database of traditional Chinese Medicine system (<http://tcmsp.com/tcmssp.php>) [11]. As the TCMS platform has its virtual screening function of drug ADME (absorption, distribution, metabolism and excretion), it is more comprehensive and professional for the analysis of network pharmacology. ADME refers to toxic pharmacokinetics, and includes the absorption of (absorption), distribution of (distribution), metabolic (metabolism) and excretion of (excretion). The two most commonly used pharmacokinetic parameters of candidate drugs are oral bioavailability (OB) and drug similarity (Drug-Likeness, DL) [12, 13]. OB refers to the absorption of oral drugs by the gastrointestinal tract. The percentage of drugs that pass through the liver to the circulating blood as a percentage of the oral dose. DL refers to the similarity of the compound to a known drug and is used to predict the likelihood of the compound becoming a drug. According to the relevant literature, it was proved that a better pharmacological performance can be obtained using  $OB \geq 30\%$  and  $DL \geq 0.18$ . We also used these values to screen the compounds in FSEC [14].

### 2.2. Target Fishing

The effective compounds in drugs depend on their targets to exert their role. On the basis of the active compounds of the TCM obtained above, we further obtained their related targets directly from the TCMS database. In addition, these compounds were also used to supplement the targets by means of target fishing. After obtaining the Canonical SMILES from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) [15], a large number of targets were screened using the similarity integration method database (<http://sea.bkslab.org/>) [16], and duplicates were eliminated.

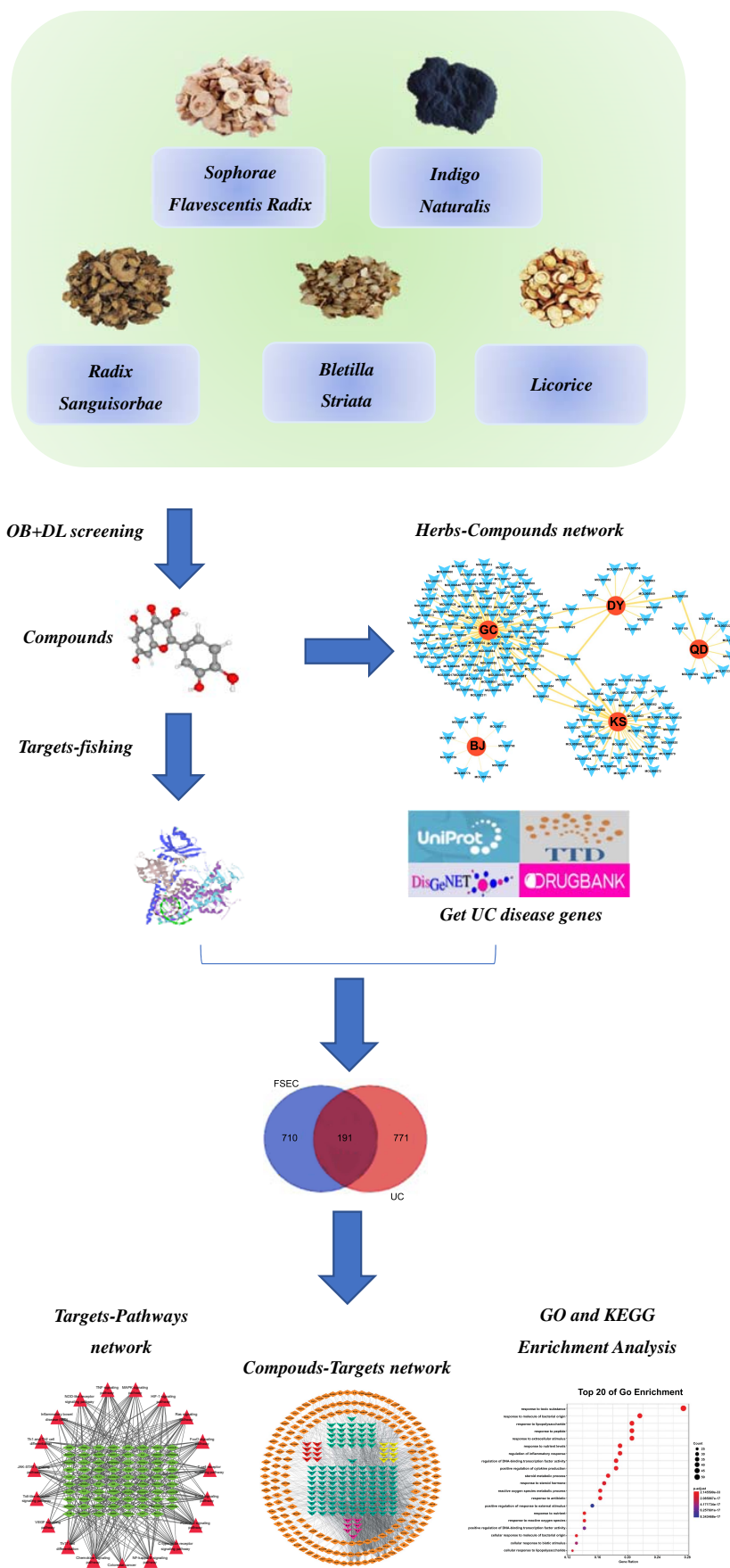


Fig. (1). The whole framework based on an integration strategy of network pharmacology.

### 2.3. Building the Disease Targets Database

In this process, we integrated three disease databases to obtain disease-related targets for UC, including a therapeutic target database (<https://db.idrblab.org/ttd/>) [17], Drugbank database (<https://www.drugbank.ca/>) [18], and DisGeNET database (<http://www.disgenet.org/web/DisGeNET/menu/home>) [19], and using "Ulcerative Colitis" as the keyword, combined with the Uniprot database (<http://www.uniprot.org/>) [20], repetitive disease targets were eliminated and a database of UC disease targets was established.

### 2.4. Network Construction

#### 2.4.1. PPI Network Construction

Based on the above research, we matched the targets of the TCM composition of FSEC with the disease targets of UC (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). The repeated targets were selected and the String online tool (<https://string-db.org/>) was used to construct the PPI network [21].

#### 2.4.2. Gene Ontology (GO) and Pathway Enrichment Analysis

After obtaining the above drug-disease compound target, we used the R3.6.1 cluster Profiler GO and cluster Profiler KEGG software package for GO and KEGG enrichment analysis of the compound targets [22]. The installation of these two software packages can be obtained via the (<http://www.bioconductor.org/>) website. GO study mainly analyzes the function of genes. KEGG enrichment can obtain the potential biological functions of genes and the biological pathways involved.

#### 2.4.3. Building a Variety of Composite Networks

Integrating all the above information, we built the following networks, including 10 The FSEC Herbs-Compounds network (H-C network), 20 Active Compounds-Targets network (C-T network), 30 PPI core target network, 40 Core targets-Compounds reverse mapping network (C-C network), 50 Targets-pathways network (T-P network). All visualizations were completed by Cytoscape3.6.1 (<http://www.cytoscape.org/>) [23].

## 3. RESULTS

### 3.1. Active Compounds of FSEC

A total of 499 compounds of FSEC were retrieved from the TCMSP database, of which KS (*Radix Sophorae Flavescentis*) was found in 113 (22.6%), QD (*Indigo Naturalis*) in 29 (5.8%), BJ (*Bletilla Striata*) in 36 (7.2%), DY (*Radix Sanguisorbae*) in 41 (8.2%), and GC (*licorice*) in 280 (56.1%). When the ADME parameter was set to  $OB \geq 30\%$  and  $DL \geq 0.18$ , 168 active compounds were obtained, including KS in 45 (26.8%), QD in 9 (5.4%), BJ in 9 (5.4%), DY in 13 (7.7%), and GC in 92 (54.8%), after removing the repeated 8 active components, we finally got 160 active components (Table S1), and an H-C network was built which had 165 nodes and 168 edges as shown in Fig. (2). After constructing the medicinal material-compound network and

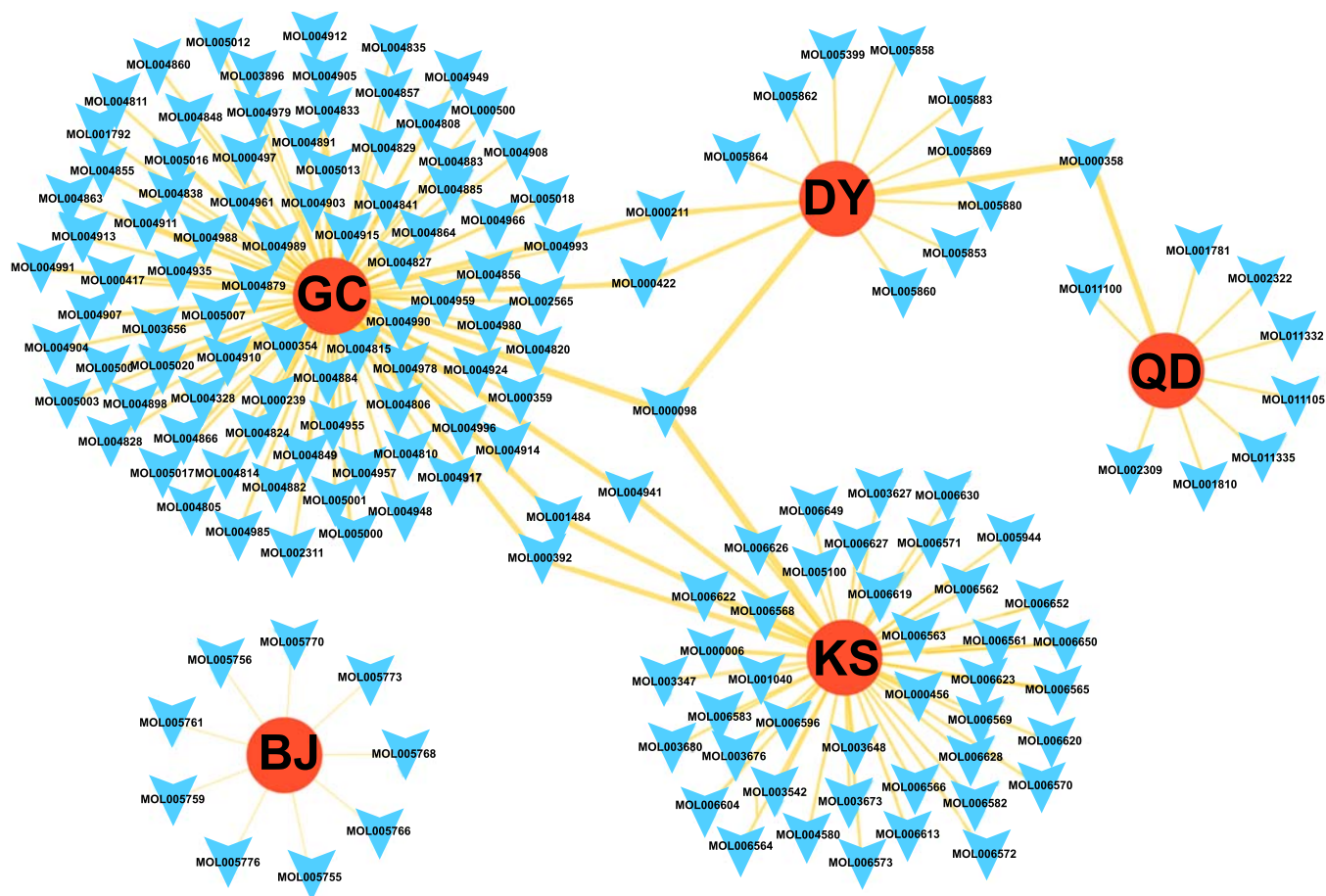
analyzing 160 active components, according to the descending order of edge betweenness, the first seven compounds were quercetin (MOL000098,  $OB=46.43$ ,  $DL=0.28$ , found in DY, GC, KS), inermine (MOL001484,  $OB=75.18$ ,  $DL=0.54$ , found in KS, GC), (2R)-7-hydroxy-2-(4-hydroxyphenyl) chroman-4-one (MOL004941,  $OB=71.12$ ,  $DL=0.18$ , found in KS, GC), formononetin (MOL000392,  $OB=69.67$ ,  $DL=0.21$ , found in KS, GC), beta-sitosterol (MOL000358,  $OB=36.91$ ,  $DL=0.75$ , found in DY, QD), kaempferol (MOL000422,  $OB=55.38$ ,  $DL=0.78$ , found in DY, GC), and mairin (MOL000211,  $OB=41.88$ ,  $DL=0.24$ , found in DY, GC) (Fig. 2).

### 3.2. Targets Prediction and Analysis

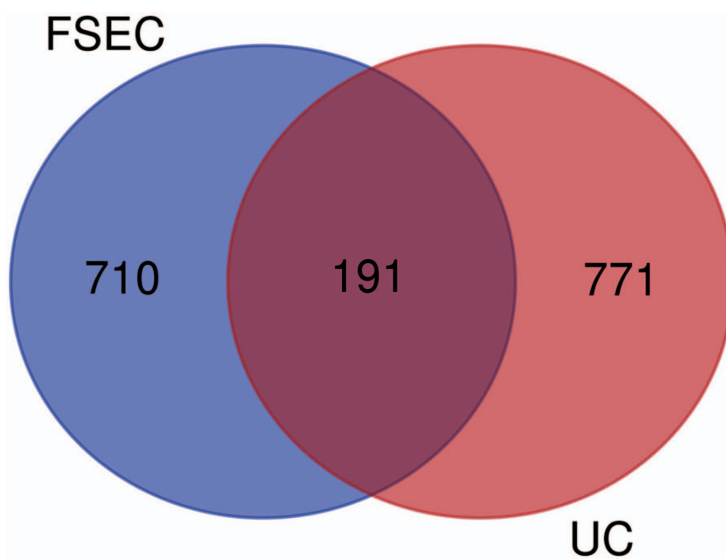
In order to further obtain the targets of these 160 active components of traditional Chinese medicine, on one hand, we obtained them directly from TCMSP database. The results are as follows: KS 376, QD 71, BJ 53, DY 155, GC 1257. On the other hand, through PubChem query and similarity integration analysis, the results were as follows: KS 1027, QD 365, BJ 110, DY 458, GC 4424. Through the integration of multiple disease gene databases of TTD, Drugbank and DisGeNet, we obtained 962 disease targets for ulcerative colitis. Then, the collected traditional Chinese medicine targets of FSEC were matched with the related targets of UC, and a total of 191 FSEC-UC targets were obtained (Fig. 3).

In the process of target salvage and integration of FSEC active compounds, we found that not every active component screened from TCM had a corresponding target and did not intersect with FSEC-UC composite targets. Therefore, we constructed a C-T network of FSEC, which had 331 nodes (FSEC, including 26 compounds of KS, eight compounds of QD, seven compounds of BJ, eight compounds of DY, 91 compounds of GC and 191 FSEC-UC composite targets) and 2227 edges (Fig. 4). The network shows that many targets can be regulated by multiple compounds, and the relationships between the active compounds and the targets can be observed.

Then, 191 FSEC-UC targets were introduced into the String database, and a PPI network was established, the unconnected nodes in the network were removed, and the network had a total of 190 nodes and 2882 edges (Fig. 5). The results were then introduced into Cytoscape3.6.1 to obtain the network, and the PPI core target network was selected according to three parameters of network topology Degree [24], Closeness Centrality [25], and Betweenness Centrality [26]. In addition, the number of gene adjacent nodes was calculated by R3.6.1 for correction (Fig. 6). The top 30 targets were selected, and the final screening parameters were Degree  $\geq 60$ , Closeness Centrality  $\geq 0.56$  and a Betweenness Centrality score  $\geq 0$ , and 30 nodes and 407 edges were obtained. The order of core targets according to Degree was as follows: IL6, VEGFA, PTGS2, STAT3, EGFR, CXCL8, CASP3, MAPK1, EGF, IL10, TLR4, IL1B, MMP9, CCL2, CCND1, ESR1, IL4, FOS, IL2, MAPK14, ICAM1, HRAS, CD44, STAT1, TLR2, and STAT1 (Table 1). The 191 nodes were arranged according to the descending order of Degree to form the PPI network, and the first 30 large hub nodes are the core targets of FSEC-UC (Fig. 7).



**Fig. (2).** Herbs-Compounds network. The red nodes represent herbs in FSEC, and the blue nodes represent active compounds. The edges represent the relationship between them, and the length, width and color of the edges is proportional to the edge.



**Fig. (3).** The venn diagram of the targets both in UC targets and FSEC.



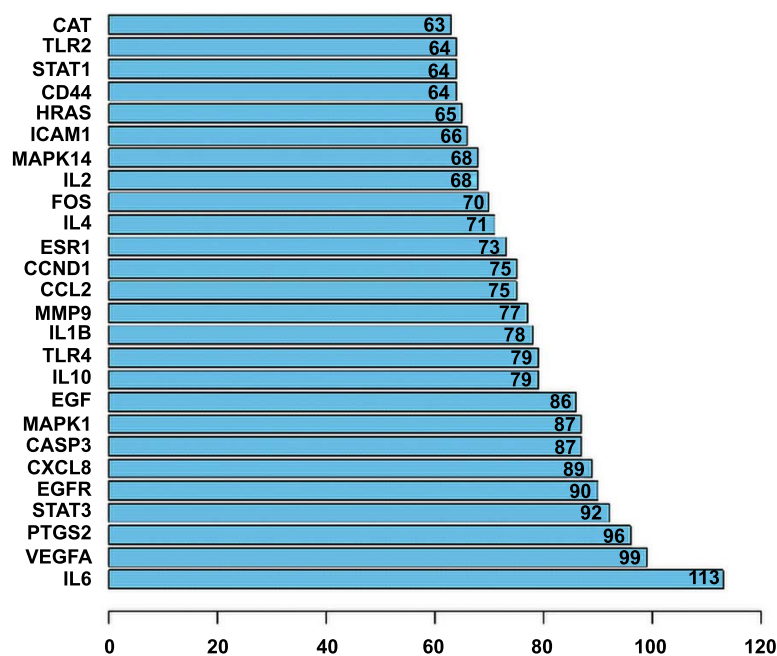
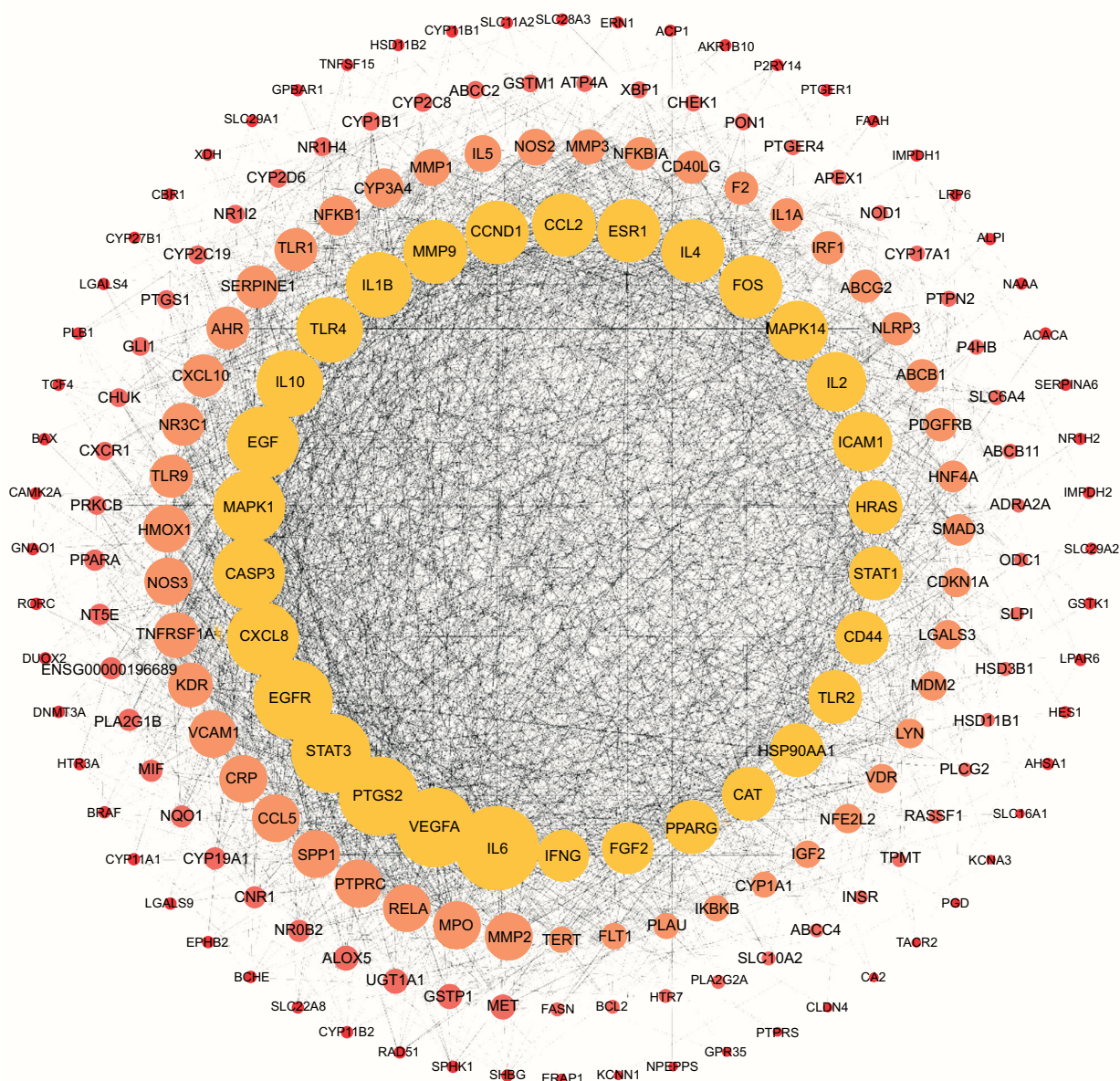


Fig. (6). The number of gene adjacent nodes is calculated by R3.6.1.

Table 1. Information on 30 core targets.

Uniprot ID	Gene Symbol	Protein Name	Degree
P05231	IL6	Interleukin-6	113
P15692	VEGFA	Vascular endothelial growth factor A	99
P35354	PTGS2	Prostaglandin G/H synthase 2	96
P40763	STAT3	Signal transducer and activator of transcription 3	92
P00533	EGFR	Epidermal growth factor receptor	90
P10145	CXCL8	Interleukin-8	89
P42574	CASP3	Caspase-3	87
P28482	MAPK1	Mitogen-activated protein kinase 1	87
P01133	EGF	Pro-epidermal growth factor	86
P22301	IL10	Interleukin-10	79
O00206	TLR4	Toll-like receptor 4	79
P01584	IL1B	Interleukin-1 beta	78
P14780	MMP9	Matrix metalloproteinase-9	77
P13500	CCL2	C-C motif chemokine 2	75
P24385	CCND1	G1/S-specific cyclin-D1	75
P03372	ESR1	Estrogen receptor	73
P05112	IL4	Interleukin-4	71
P01100	FOS	Proto-oncogene c-Fos	70
P60568	IL2	Interleukin-2	68
Q16539	MAPK14	Mitogen-activated protein kinase 14	68
P05362	ICAM1	Intercellular adhesion molecule 1	66
P01112	HRAS	GTPase HRas	65
P16070	CD44	CD44 antigen	64
P42224	STAT1	Signal transducer and activator of transcription 1-alpha/beta	64
O60603	TLR2	Toll-like receptor 2	64
P04040	CAT	Catalase	63
P07900	HSP90AA1	Heat shock protein HSP 90-alpha	63
P09038	FGF2	Fibroblast growth factor 2	61
P37231	PPARG	Peroxisome proliferator-activated receptor gamma	61
P01579	IFNG	Interferon gamma	60



**Fig. (7).** The PPI network of 191 nodes. The node size is proportional to the target degree in the network. The innermost 30 big hub nodes are the core targets of FSEC-UC.

On the basis of 30 core targets, we further constructed a large hub node-component composite network (Fig. 8) according to the relevant active components. In the network, there were 132 active components of TCM, including quercetin (MOL000098) associated with 20 key targets, kaempferol (MOL000422) linked to 16 key targets, luteolin (MOL000006) linked to 15 key targets and formononetin (MOL010586) associated with 15 key targets (four targets had a degree  $\geq 10$ ).

### 3.3. GO Biological Process and KEGG Pathway Enrichment Analysis

GO and KEGG enrichment analysis was carried out on 191 FSEC-UC composite targets using the R3.6.1 cluster Profiler GO and cluster Profiler KEGG software package. The first 20 items (Biological Process) in the biological

process of GO enrichment and 20 KEGG pathways related to UC were selected for analysis.

### 3.4. GO Biological Process Enrichment Analysis

After GO biological process enrichment analysis, the first 20 biological process items (Fig. 9) were mainly involved in inflammation regulation, immune recognition, antioxidation, etc. The regulation of inflammation included regulation of the inflammatory response (GO:0050727), positive regulation of cytokine production (GO:0001819), response to antibiotics (GO:0046677), response to steroids (GO:0048545), and steroid metabolism (GO:0008202), etc. Immune recognition included a response to bacterial molecules (GO:0002237), response to endotoxin (GO:0071219), response to toxic substances (GO:0009636), positive regulation of external stimulus response (GO:0032103), etc. Others, such as reactive



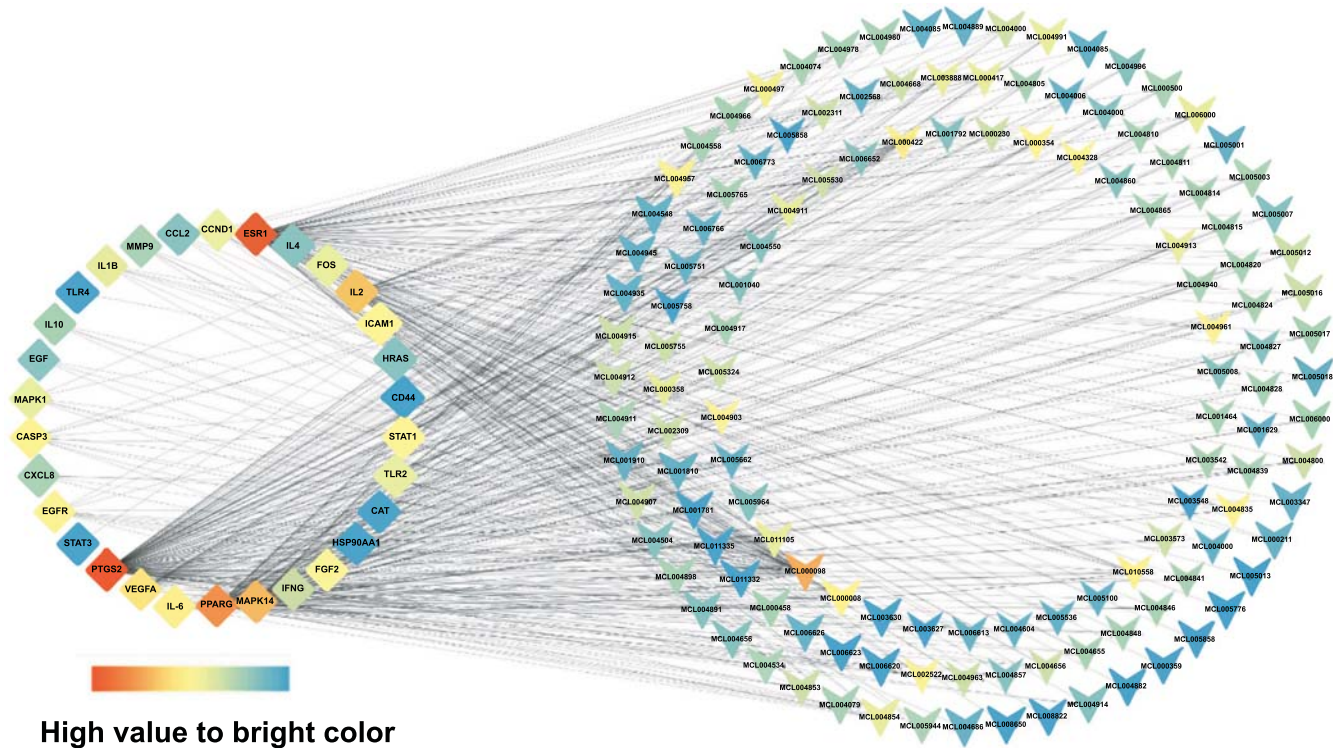


Fig. (8). Core targets -Compounds Network (C-C Network) . The rhombus nodes represent the big hub nodes, the triangle nodes represent the compounds. The node color changes from orange to blue reflect the degree value changes from high to low in the network.

### Top 20 of Go Enrichment

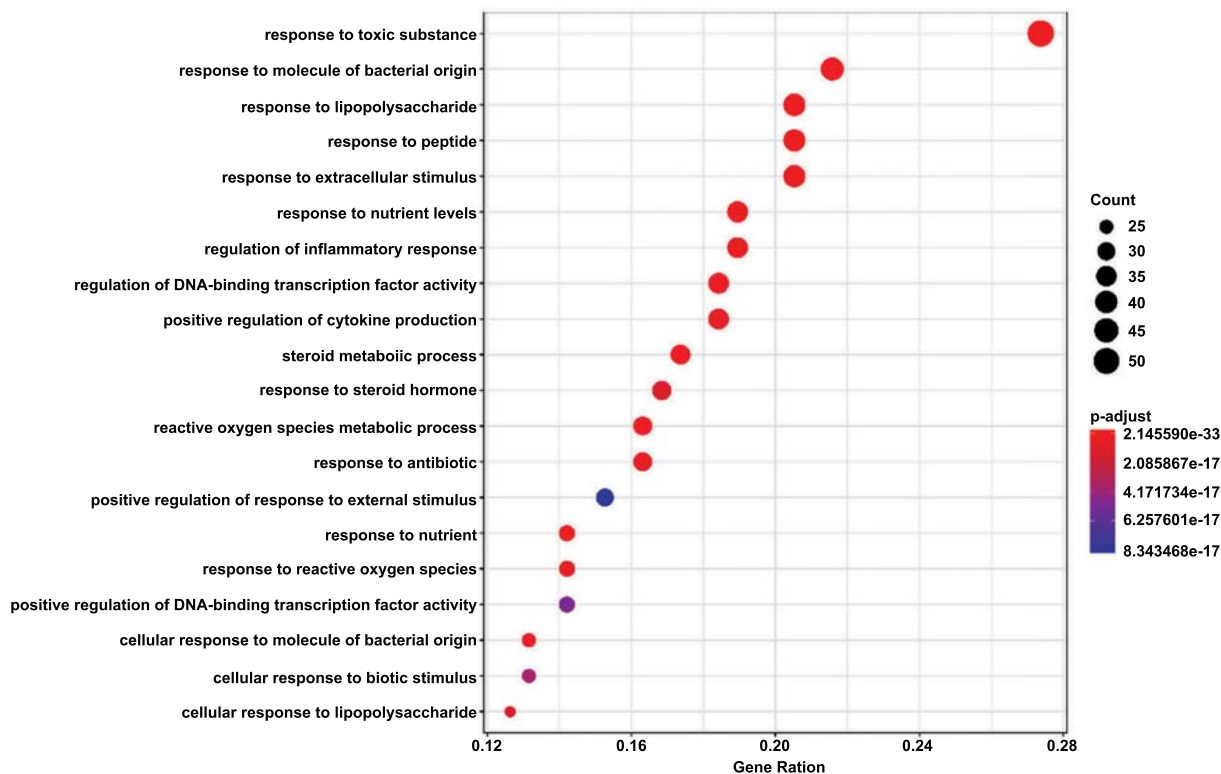
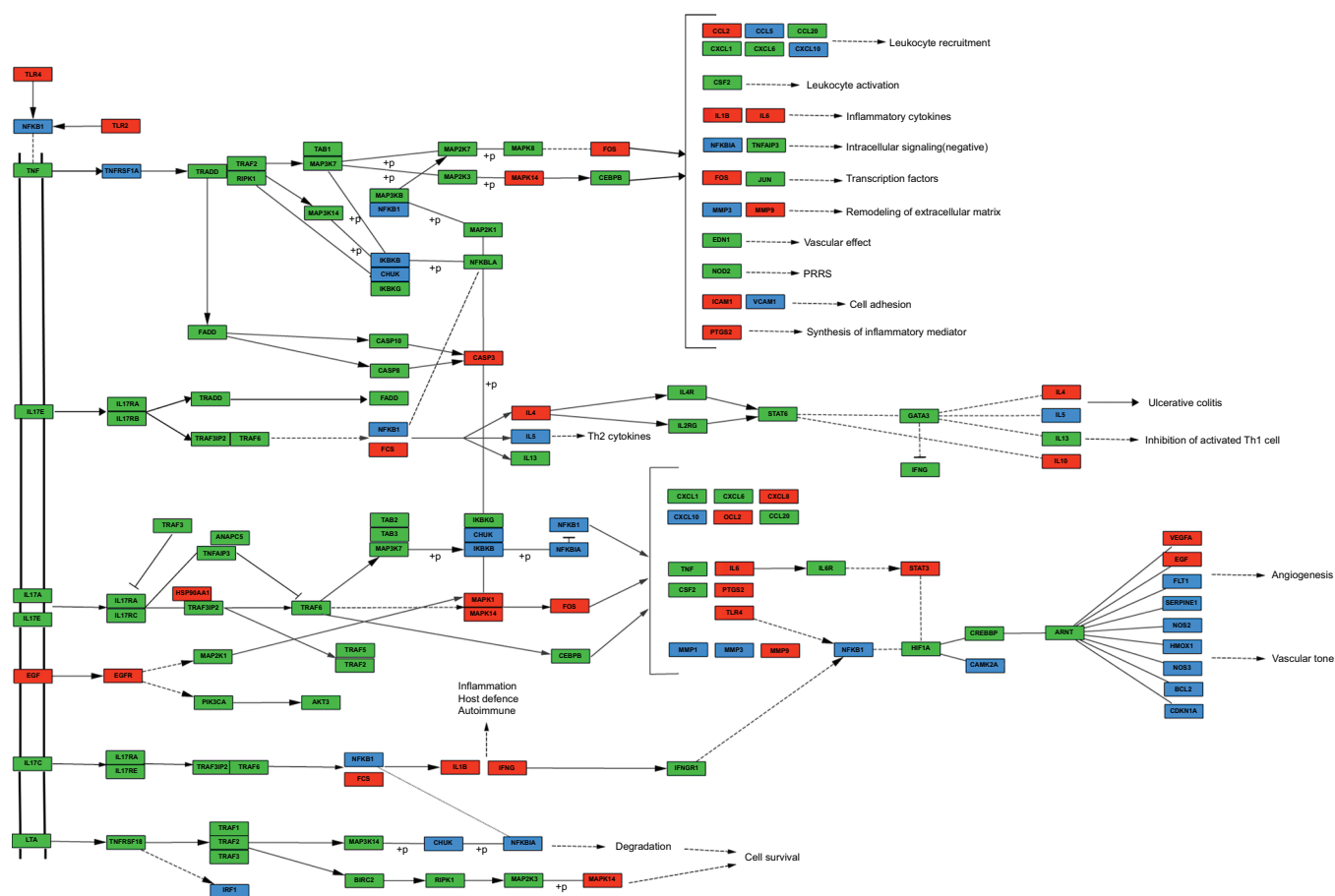


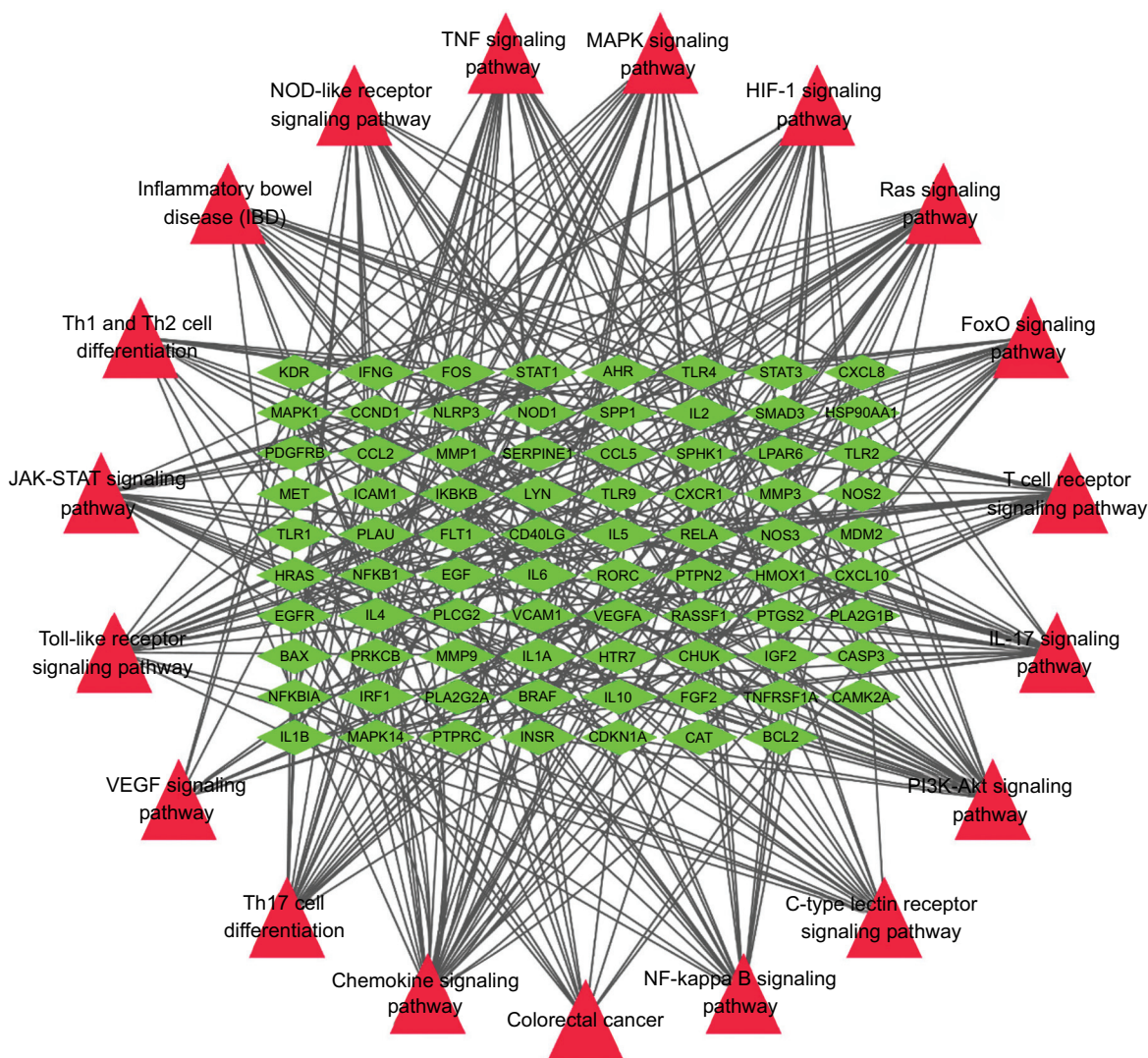
Fig. (9). The GOBP enrichment analysis of 191 nodes.

**Table 2. Information on the 20 pathways related to UC.**

Term ID	Pathway Name	Count	FDR
hsa04657	IL-17 signaling pathway	22	7.51E-16
hsa04066	HIF-1 signaling pathway	21	2.06E-13
hsa04668	TNF signaling pathway	21	2.75E-13
hsa05321	Inflammatory bowel disease (IBD)	16	2.84E-12
hsa04620	Toll-like receptor signaling pathway	19	4.33E-12
hsa04659	Th17 cell differentiation	19	6.92E-12
hsa04064	NF-kappa B signaling pathway	18	2.28E-11
hsa04625	C-type lectin receptor signaling pathway	18	2.93E-11
hsa01521	EGFR tyrosine kinase inhibitor resistance	16	3.64E-11
hsa04151	PI3K-Akt signaling pathway	29	1.39E-09
hsa04660	T cell receptor signaling pathway	16	1.76E-09
hsa04068	FoxO signaling pathway	17	8.22E-09
hsa04014	Ras signaling pathway	22	1.32E-08
hsa04010	MAPK signaling pathway	24	4.42E-08
hsa04621	NOD-like receptor signaling pathway	18	1.37E-07
hsa04658	Th1 and Th2 cell differentiation	13	1.66E-07
hsa04630	JAK-STAT signaling pathway	16	7.25E-07
hsa04370	VEGF signaling pathway	10	8.45E-07
hsa04062	Chemokine signaling pathway	17	1.10E-06
hsa05210	Colorectal cancer	12	0.00000557



**Fig. (10).** The anti-UC pathways of FSEC. The red nodes represent the Core targets, the blue nodes represent the other nodes, and the green nodes represent the targets in the pathway.



**Fig. (11).** Targets-Pathways Network (T-P Network). The green round nodes represent the targets of FSEC-UC. The red triangles represent the related pathways.

oxygen species response (GO:0000302), reactive oxygen metabolism (GO:0072593), and DNA binding transcription factor activity regulation (GO:0051090) were also observed in the above analysis. The rapid induction of UC relief by FSEC may be based on these complex synergistic effects.

### 3.5. KEGG Pathway Enrichment Analysis

KEGG pathway enrichment analysis was performed in order to further reveal the mechanism of UC remission induced by FSEC. We analyzed the enrichment of the KEGG pathway in 191 targets, and selected the first 20 pathways related to the pathogenesis of UC according to the threshold of FDR (adjusted p value) < 0.01. These pathways included the IL-17 signaling pathway (hsa04657), HIF-1 signaling pathway (hsa04066), TNF signaling pathway (hsa04668), IBD (hsa05321), Toll-like receptor signaling pathway (hsa04620), Th17 cell differentiation (hsa04659), NF-kappaB signaling pathway (hsa04064) and so on (Table 2). A T-P network was also established according to the target of FSEC on each path (Fig. 10 and 11).

## 4. DISCUSSION

UC is a type of IBD. The incidence of UC in Asian countries is increasing year by year. Research on TCM has resulted in vital evidence for the prevention and treatment of UC. FSEC is one of the most representative prescriptions for the treatment of UC and is the only patent proprietary Chinese medicine specially approved for the treatment of UC in China. Its efficacy has been proved in phase II and III clinical trials. At present, phase IV clinical trials are being carried out. Relevant basic experiments have also been carried out and are very valuable in the in-depth evaluation of the effects of FSEC in UC. Network pharmacology has been an important method in studying the complex action of TCM in recent years [27-29]. This tool is suitable for research on the prescription of TCM, which has a solid clinical foundation and the relevant mechanism is not comprehensive or is incomplete, and can allow researchers to predict its action targets and effective components in a more comprehensive way. It also lays the foundation for further experimental verification and new drug development.

Using network pharmacological analysis, according to the H-C network, FSEC had a total of 160 effective chemical constituents of TCM, including quercetin, inermine, (2R)-7-hydroxy-2-(4-hydroxy phenyl) chroman-4-one, formononetin, beta-sitosterol, kaempferol and mairin, which are common components in 2 or 3 Chinese herbs. The effective components mentioned above were then obtained and matched with the collected UC targets, and a total of 191 FSEC-UC composite targets were obtained, and the C-T network was constructed. Excluding the components in each drug that did not act on UC targets (140 compounds remaining), the C-T network illustrated the interaction between multiple compounds and multiple targets, of which 84.3% of the targets were connected to more than one compound. 92.9% of the compounds were linked to more than two targets. Through String and calculated by R, 30 core targets were identified, which were the most important targets for FSEC to induce rapid remission of UC, and the PPI target network was then constructed according to Degree. In addition, we also matched the 30 core targets to the active components. The Core targets-Compounds network was constructed, and the four central compounds were quercetin, kaempferol, luteolin and formononetin, all of which are flavonoids. These four compounds were the core active compounds of FSEC, three of which belong to the common compounds mentioned above. Finally, we used GO and KEGG enrichment to analyze 191 targets, analyzed the results of the first 20 enriched biological processes and the first 20 signals related to UC, and constructed their T-P network. It can be seen that these biological functions and signaling pathways are also the main mechanisms of FSEC.

Quercetin is known to be the most abundant flavonol compound in the plant kingdom. Quercetin is widely found in vegetables, fruits and Chinese herbs. A large number of studies have confirmed the effectiveness of quercetin in the treatment of UC. The mechanisms of quercetin in UC, which include anti-inflammation, regulation of immunity, hemostasis and antioxidation, result in activation of the NF-kappaB/STAT1 signaling pathway being blocked and inhibition of the activity of p38MAPK/JNK/1. Regulation of the TLR4/NF-kappaB pathway resulted in downregulation of the protein expression of iNOS, COX-2, IL-1 $\beta$  and other inflammatory mediators [30, 31]. In addition, quercetin can also cooperate to improve the oral availability of curcumin, another common monomer of TCM. Due to its poor water solubility and stability, curcumin is difficult to absorb in vivo and is easily absorbed by intestinal and liver enzyme systems. In particular, the metabolism and elimination of glucuronyltransferase (uridine diphosphate glucuronosyl transferase, UGT) leads to its low blood concentration and poor bioavailability in vivo, which greatly limit its clinical application. Moreover, high expression and increased activity of UGT at the inflammatory site of UC, further accelerates the metabolic inactivation of curcumin [32, 33]. Reports have shown that quercetin has a significant inhibitory effect on the activities of curcumin phase II metabolic enzymes UGT1A1, 1A8, 1A9 and 1A10 [34], which requires further study.

Kaempferol is considered a natural steroid, and its anti-inflammatory effect has been confirmed. Oral kaempferol can significantly reduce the levels of plasma NO and PGE-2 induced by DSS (Dextran sulfate sodium) in C57BL/6J UC mice and inhibit the activity of MPO in colonic mucosa.

The level of TFF3 mRNA as a functional marker of goblet cells was also up-regulated [35]. Similarly, the intestinal inflammatory cell model induced by LPS also showed that kaempferol significantly reduced the excessive secretion of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and ICAM-1, indicating the negative regulation of TLR4, NF-kappaB and STAT signal transduction pathways [36].

Luteolin has a strong function in scavenging free radicals, and oxidative stress plays an important role in the pathogenesis of IBD. In the inflammatory cell model of HT-29 induced by cytokines, it was confirmed that luteolin can significantly inhibit the expression of IL-8, COX-2 and INOS, and the key mechanism of its anti-inflammatory effect may be due to inhibition of the JAK/STAT pathway [37]. Luteolin also effectively reduced the expression of proinflammatory factors such as TNF- $\alpha$  and IL-6 in the colon of 3% DSS C57BL/6J mice (20 and 50 mg/kg), and increased the activity of antioxidant molecules such as catalase (CAT) [38].

Formononetin decreased the level of NLRP3 pathway proteins (NLRP3, AsC, IL-1 $\beta$ ) at 25, 50 mg/kg in vivo and in vitro, and significantly decreased the levels of DSS-induced TNF- $\alpha$ , IL-6 and COX-2, and increased the expression of Nrf2. Nrf2 knockout mice showed no therapeutic response. Formononetin prevented the occurrence and development of UC by activating the Nrf2 axis, and its protective effect was Nrf2-dependent [39].

Basic studies on FSEC were previously carried out to determine its mechanism. For example, in the acute UC model induced by 3% DSS in BALB/C mice, 7 days after FSEC enema treatment, colon pathology was observed by HE staining. It was found that compared with the model group, the inflammatory injury treated with FSEC showed varying degrees of improvement. FSEC reduced the levels of ROR  $\gamma$  t, IL-17, IL-6 protein and MPO activity in the colon tissue of UC mice induced by DSS, and the difference compared with the model group was statistically significant ( $P < 0.05$ ,  $P < 0.01$ ) [40]. In another study using the same strain of mice and the same model, it was found that in addition to improving pathological injury in the colon, FSEC also reduced the protein contents of Caspase-1 and IL-1 $\beta$  in the colon tissue of mice, compared with the model group. The difference was statistically significant ( $P < 0.05$ ) [41]. In a previous clinical study, it was also found that after eight weeks of treatment, compared with SASP (Sulfasalazine) the expression of NF-kappaB, p65 and STAT6 in colonic mucosa of patients treated with FSEC was lower than that in the Western medicine group ( $P < 0.05$ ) [42].

The above core active components and previous studies of FSEC illustrate the mechanism of its protective and repairing effects on intestinal injury both in vivo and in vitro, respectively. Toll-like receptor, NF-kappaB, MAPK, JAK-STAT, NOD-like receptor, Th17 cell differentiation, EGFR tyrosine kinase inhibitor resistance and other signaling pathways play a role in immune recognition, anti-inflammation and antioxidation. These results are also consistent with the results of GO enrichment and KEGG enrichment analysis.

## CONCLUSION

In this study, the mechanism of rapid induction of UC remission and repair of intestinal mucosa by FSEC was

demonstrated for the first time using network pharmacology. The results predicted the possible effective components and targets of FSEC in the treatment of UC. The mechanism of FSEC involves the direct or indirect synergistic effect of multi-components, multi-targets and multi-pathways, and provides an important theoretical basis for further study of the effective substances and mechanism of FSEC in the treatment of UC.

Network pharmacology has some limitations. Due to the timeliness of network pharmacology methods based on data research, the target research in many diseases and chemical components is inadequate. For example, at present, only TCMSP in the TCM database has the component screening criteria of ADME, and the components not included may also be effective chemical components. For example, some of the effective components of TCM in FSEC are not found in the database, and these components may also be involved in the mechanism of FSEC. In addition, this study is based on the chemical composition and targets of the FSEC prescription to determine the mechanism of action of FSEC, which do not fully reflect the effect of the metabolites of FSEC on the mechanism of action. Therefore, it is necessary to further verify the results of network pharmacological prediction analysis and improve the results by mass spectrometry and pharmacokinetics of TCM.

#### AUTHORS' CONTRIBUTIONS

GSZ wrote the manuscript, XY and ZH edited and improved the manuscript, ZYL compiled the TCM database and target capture, XSG, PJ and TYN collated the disease target database, CKJ and WH performed GO and KEGG analysis. DDB directed the research and proposed changes to the manuscript, and all authors reviewed the manuscript and approved the final version of the manuscript.

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

#### HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

#### CONSENT FOR PUBLICATION

Not applicable.

#### AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available within the article.

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#### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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#### SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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