Scholars Journal of Agriculture and Veterinary Sciences OPEN ACCESS
Abbreviated Key Title: Sch J Agric Vet Sci
ISSN 2348–8883 (Print) | ISSN 2348–1854 (Online)
Journal homepage: https://saspublishers.com

Exploring the Mechanism of Chen Pi in Treating Acute Ulcerative Colitis Based on Network Pharmacology and Molecular Docking

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DOI: https://doi.org/10.36347/sjavs.2024.v11i05.001 | **Received:** 29.06.2024 | **Accepted:** 03.08.2024 | **Published:** 06.08.2024

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Abstract **Original Research Article**

To investigate the mechanism of action of Chenpi in the treatment of acute ulcerative colitis, this experiment employed a combination of network pharmacology and molecular docking technology to make a preliminary prediction of its mechanism of action. The TCMSP and PubChem databases were employed to identify the active components of Chenpi and their respective targets. In contrast, the SwissTargetPrediction database was utilized to predict the potential targets of action of Chenpi. The relevant targets of acute ulcerative colitis were obtained from the GeneCards disease database and subsequently intersected with the targets of Chen Pi to identify the key targets of Chen Pi for the treatment of acute ulcerative colitis. The results of the network pharmacological analysis indicated that Chenpi may exert its effects through ESR1, potentially via the action of sitosterol, naringenin, 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl) chroman-4-one, Citromitin, and nobiletin, five active components, as well as PTGS2, PPARG, BCL2, SRC, and MMP9, and an additional 104 core targets. Following the enrichment analysis of the aforementioned 104 core targets, it was determined that pathways such as "Pathways in cancer," "Arachidonic acid metabolism," "Prostate cancer," "EGFR tyrosine kinase inhibitor resistance," and others were involved in the treatment of Chen Pi. The results of molecular docking also demonstrated that the active ingredients of Chen Pi exhibited favorable binding affinities with the key targets, thereby substantiating the precision of the network pharmacology-predicted outcomes. The results of this experiment suggest that Chenpi may exert its therapeutic effects on acute ulcerative colitis through a multi-component, multi-target, and multi-pathway mechanism.

Keywords: Acute Ulcerative Colitis, Network Pharmacology, Molecular Docking, Chenpi, Mechanism of Action.

INTRODUCTION

Ulcerative colitis (UC), a non-specific inflammatory disease of the intestinal tract, is characterized by diffuse inflammation of the colon and rectum, with prominent symptoms including abdominal pain, diarrhea, weight loss, and even bloody stools [1], and in extreme cases can lead to rectal prolapse or even colon cancer. The disease is not only common in humans but also in pets, especially dogs and cats, where it is often triggered by the ingestion of indigestible hard foreign bodies [2]. The incidence of UC is increasing year by year due to changes in the living environment, changing lifestyles, and increasing stress levels worldwide, posing a serious challenge to human health and quality of life. Currently, the main therapeutic strategies for UC include the use of corticosteroids, aminosalicylic acid azathioprine, etc. However, these drugs often have significant side effects and are prone to relapse after treatment. Given this, traditional Chinese medicine (TCM), with its unique advantages of multi-component, multi-target, and multi-pathways, has demonstrated a broad application prospect in the treatment of such complex diseases and is regarded as a potential therapeutic drug [3, 4].

Chenpi is the dried ripe fruit peel of mandarin in the family Rutaceae, with a pungent and bitter taste, and a warm nature; it belongs to the spleen and lung meridians and has the efficacy of regulating qi and strengthening the spleen, and it is recognized by the Ministry of Health as a wild herb with the same source of medicine and food, which is of high value for development and utilization. Modern studies have shown that the main components of Chenpi are flavonoids, essential oils, polysaccharides, and alkaloids [5-7], which have biological functions such as anti-inflammatory, anti-tumor, digestive, hepatoprotective, expectorant, antidepressant, and so on [8-11]. Yuan Li [12], *et al.*, investigated the ameliorative effect of chenpi on 3% dextran sodium sulfate (DSS)-induced acute ulcerative colitis and found that chenpi significantly

decreased the expression of colonic pro-inflammatory factors or proteins; while significantly increasing colonic anti-inflammatory factor (Il-10), tight junction protein [Zo-1 (zonula occludens-1), occludin, claudin-1], and mucin (mucin 2, Muc2), and Akkermansia abundance was significantly positively correlated with colonic anti-inflammatory factors and tight junction proteins, which led to the conclusion that Chenpi enzyme could ameliorate acute ulcerative colitis in mice by regulating the homeostasis of enterobacteria and repairing the intestinal mucosa. Although Chenpi has the effect of ameliorating acute ulcerative colitis in mice, the mechanism of action is not clear. Based on the above points, this experiment took Chenpi as the research object, explored the components, targets, and pathways of Chenpi based on network pharmacology and molecular docking, obtained the key genes of therapeutic effects through screening, and verified the binding degree of them with the active ingredients by molecular docking, to investigate the molecular mechanism of Chenpi in the treatment of acute ulcerative colitis and the accuracy of predicting the targets, to provide the corresponding theoretical basis for the research and development of new drugs and subsequent trials.

1 MATERIAL AND METHODS

1.1 Screening of Chen Pi Compounds and Related Targets

The TCMSP database (https://www.tcmsp-e.com/) was searched by entering "Chenpi" and setting the screening values of oral bioavailability (OB) ≥30% and drug-like properties (DL) ≥0.18. The existing targets of Chenpi were collected from the PubChem database (https://www.tcmsp-e.com/), and the potential targets of Chenpi were collected from the SwissTargetPrediction database (https://www.tcmsp-e.com/). PubChem (https://pubchem.ncbi.nlm.nih.gov/) database to collect the existing targets of Chenpi, and SwissTargetPrediction (http://swisstargetprediction.ch/) database to predict the potential targets of Chenpi. The obtained components and compound targets were integrated to obtain the drug component and compound target data set. The obtained target results were de-emphasized to obtain the drug components and related targets of Chenpi.

1.2 Disease Target Collection and Obtaining Overlapping Targets and Drawing Venn Diagrams

The GeneCards database (http://www.genecards.org/) was searched with the keyword "acute ulcerative colitis" to obtain the relevant targets of acute ulcerative colitis. The obtained drug targets of Chenpi and disease targets of acute ulcerative colitis were imported into the online mapping tool of Weixin (https://www.bioinformatics.com.cn/) to obtain the intersecting targets, and the Venn diagrams of the intersection of drug targets of Chenpi and disease targets were drawn.

1.3 Protein Interaction Network Analysis and Network Visualization

The 104 overlapping targets were entered into the String database (https://string-db.org/cgi/input.pl), the species was limited to "Homo sapiens", the interaction threshold was selected as "highest confidence (0.400)", and the color shade of the edges was set to reflect the degree of relationship. The interaction threshold was selected as "highest confidence (0.400)", and the color shade of the edges was set to reflect the degree of relationship to obtain the PPI network relationship graph, and the data results were exported into TSV format. The output data were imported into Cytoscape 3.9.1 to visualize the PPI network, and the "network analyzer" function was used to analyze the topological parameters of the PPI network, and the area size, color and order of the nodes (target genes) in the network were adjusted according to the degree value. According to the degree value, the area size, color, and sorting of the nodes (target genes) in the network are adjusted, the higher the degree value, the larger the area of the nodes, the darker the color, and the deeper is red; the combined score size is expressed by the thickness of the connecting lines, so we can get the visualized network of the PPI relationship and obtain the main targets in the interaction network.

1.4 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

The DAVID database (https://david.ncifcrf.gov/) is a bioinformatics database that provides comprehensive biofunctional annotation information for a large number of gene proteins. Targets at the drug-disease interface were entered into the DAVID database, and the species was selected as "Homo sapiens" for pathway analysis. The key pathways were screened, and the bar graph of GO pathway enrichment analysis and the bubble graph of KEGG pathway enrichment analysis were output by the online mapping tool of Microbiology (http://www.bioinformatics.com.cn/).

1.5 Construction of "Component-Disease-Target" Network Diagram of Chenpi

According to the main active ingredients of Chenpi, the main targets regulated by Chenpi, the pathways associated with the targets, and the main targets of the disease, we constructed the "component-disease-target" network diagram by using Cytoscape 3.9.1, and the "component-disease-target" network diagram by using Cytoscape 3.9.1. Cytoscape 3.9.1 was used to construct the component-disease-target network diagram.

1.6 Molecular Docking

Molecular docking is a widely used computational tool in the field of new drug development because it can accurately predict the binding sites of small molecule ligands of known structures in the

corresponding protein ligands, and evaluate their binding modes and binding affinities [13]. In this study, the target with higher degree value in the PPI network was used as the receptor, and the human-derived protein structure with more recent year was selected from the RCSB-PDB database (https://www.rcsb.org/); the PDB 3D structure of each drug was used as the ligand, and its SDF 3D structure was obtained from the Pubchem database (https:// pubchem.ncbi.nlm.nih.gov/) and converted to PDB format by OpenBabel-3.1.1. All processed ligand small molecules and receptor proteins were converted to PDBQT format using AutoDockTools-1.5.7 for water removal and hydrogenation ionization of all receptor proteins and for ligand small molecules for water removal. Molecular docking was verified using AutoDockTools-1.5.7 software and default docking parameters were used for docking. The docked conformation with the highest output score was considered as the binding conformation and Pymol was used for 3D visualization of molecular docking results.

2 RESULTS

2.1 Active Components of Chenpi and Their Predicted Targets

A total of 63 components of Chenpi were screened in the TCMSP database, and five active components were found to meet both OB≥30% and DL≥0.18 (Table 1). The potential action targets of Chenpi were predicted using the SwissTargetPrediction database, and the obtained target results were de-emphasized by removing 85 duplicates to obtain 163 Chenpi-related targets.

Table 1: Chenpi active ingredient information

Mol ID	Molecule Name	OB(%)	DL
MOL000359	Sitosterol	36.91	0.75
MOL004328	Naringenin	59.29	0.21
MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl) chroman-4-one	47.73	0.27
MOL005815	Citromitin	86.90	0.51
MOL005828	Nobiletin	61.66	0.51

2.2 Disease Targets and Target Intersection Venn Diagrams

By searching the GeneCards database for human acute ulcerative colitis disease targets, a total of 3961 relevant targets were obtained; intersection with 163 drug targets of Chenpi, 104 key targets were obtained, including ESR1, PTGS2, PPARG, BCL2, SRC, MMP9, etc., which are the potential anti-acute ulcerative colitis targets of Chenpi. Ulcerative colitis. To better demonstrate the logical connection between drug targets and disease targets, this experiment used the online mapping tool of Weishengxin to make Venn diagrams of the intersection of Chenpi's drug targets and disease targets to visualize the characteristic relationship between each set (Figure 1).

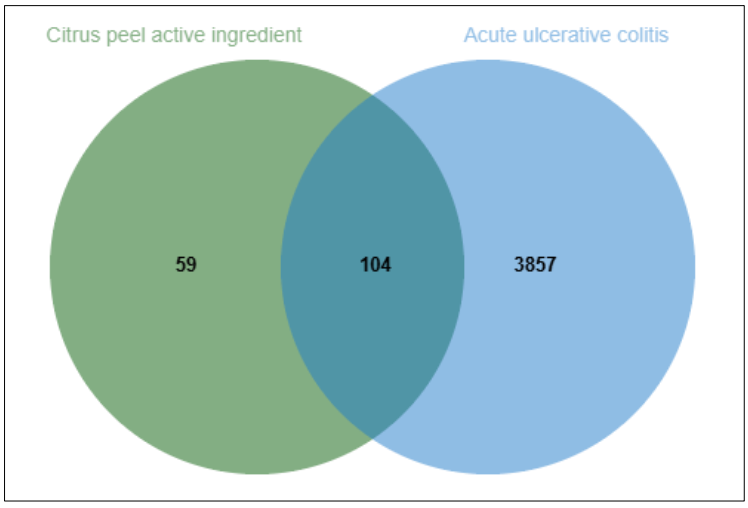

Figure 1: Intersecting Targets Venn diagram

2.3 Results of PPI Network Analysis

The above 104 target genes were uploaded into the String database, and the PPI data were obtained according to the set conditions, and then imported into Cytoscape software to draw the PPI network (Figure 2). There were 104 nodes (target genes) in the PPI network, as shown in the figure, and the targets closest to the central region of the network were associated with more and more targets. The size and color of the nodes are set according to the degree value, the larger the degree value, the larger the node and the darker the color; the strength of the relationship between the nodes is indicated by the

thickness of the edges, and the thicker the lines of the edges, the higher the value of the combined score. After collation, 15 overlapping targets such as ESR1, PTGS2, PPARG, BCL2, SRC, MMP9, and so on have a degree value greater than 30 (Table 2), and it can be assumed that these targets play an important role in the treatment of UC by Chen Pi.

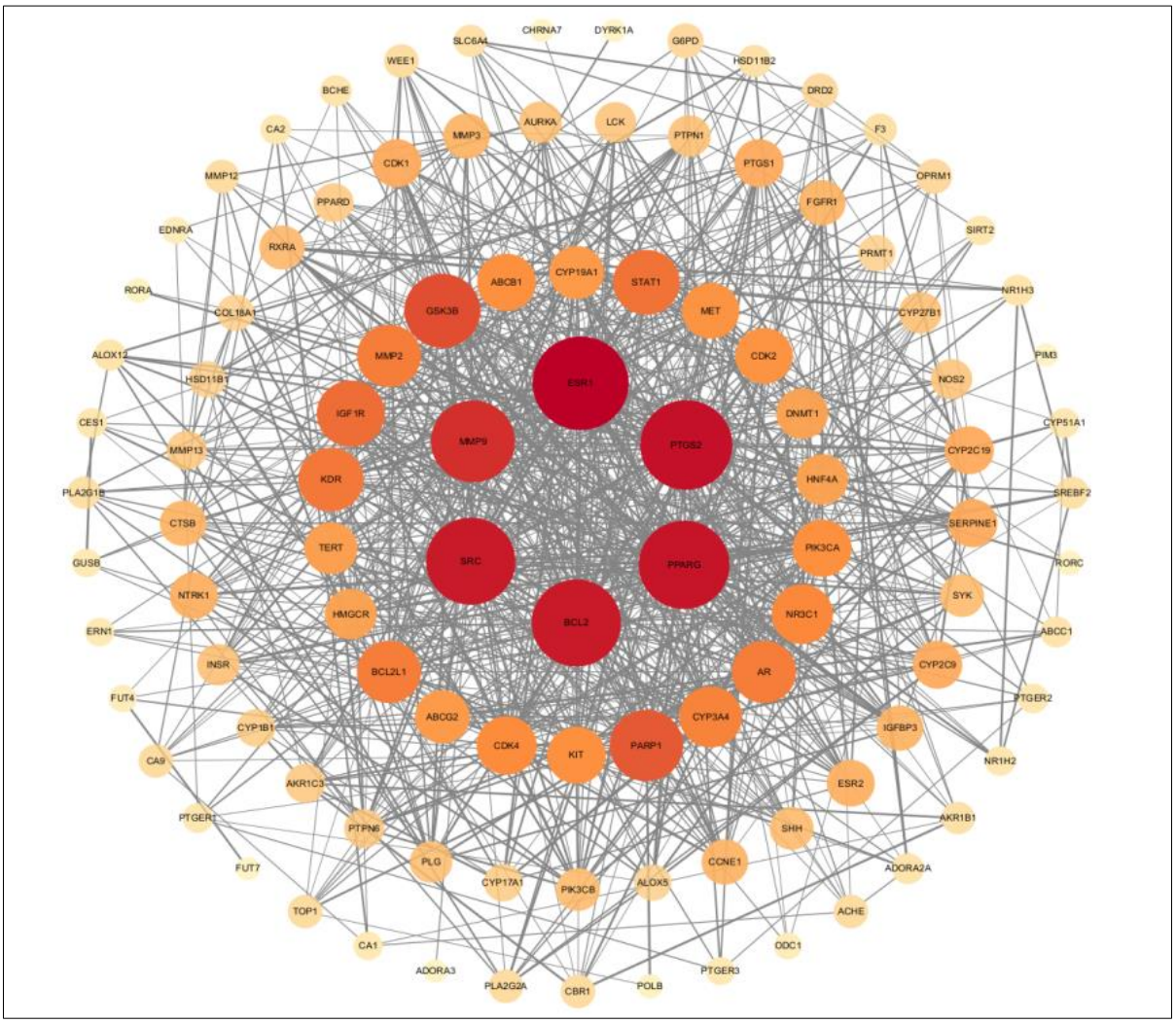

Figure 2: PPI Network Diagram of Chen Pi for UC

Table 2: Topological parameter analysis of potential targets in the core network of Chen Pi for UC treatment

Target	Degree	Betweenness Centrality	Closeness Centrality
ESR1	55	0.096725205	0.673203
PTGS2	52	0.116787831	0.664516
PPARG	51	0.082178226	0.660256
BCL2	50	0.090397503	0.651899
SRC	50	0.065352303	0.660256
MMP9	46	0.040477291	0.635802
GSK3B	40	0.044903333	0.616766
PARP1	38	0.044789272	0.605882
IGF1R	34	0.009122025	0.588571
STAT1	33	0.033875030	0.578652
KDR	32	0.024664838	0.575419
AR	31	0.015717342	0.572222
BCL2L1	31	0.025475208	0.575419
MMP2	31	0.009913732	0.578652
CYP3A4	30	0.030014924	0.550802

2.4 Results of Pathway Enrichment Analysis

The potential targets of Chenpi for UC treatment were analyzed by GO and KEGG pathway enrichment in the DAVID database. Using P≤0.01 as the cutoff point, the target genes in the GO analysis involved 165 biological processes (BP) entries, 24 cellular components (CC) entries, and 57 molecular functions (MF) entries. Biological processes included response to xenobiotic stimulus, phosphorylation, protein phosphorylation, peptidyl tyrosine phosphorylation, protein autophosphorylation, etc.; cellular components included receptor complex, cytoplasm, endoplasmic reticulum membrane, nucleoplasm, intracellular membrane-bound organelle, etc.; molecular function included nuclear receptor activity, zinc ion binding, protein tyrosine kinase activity, enzyme binding, etc. The bar graph of the GO enrichment analysis is shown in Figure 3.

The KEGG pathway enrichment analysis showed that 64 pathways were involved in the UC target genes of Chen Pi treatment at the cutoff point of P≤0. 01, pathways in cancer, arachidonic acid metabolism, prostate cancer, EGFR tyrosine kinase inhibitor resistance, PI3K-Akt signaling pathway, small cell lung cancer, ovarian steroidogenesis, endocrine resistance, proteoglycans in cancer, chemical carcinogenesis - receptor activation and other signaling pathways play a key role in the treatment of Chen Pi, KEGG enrichment analysis bubble map is shown in Figure 4.

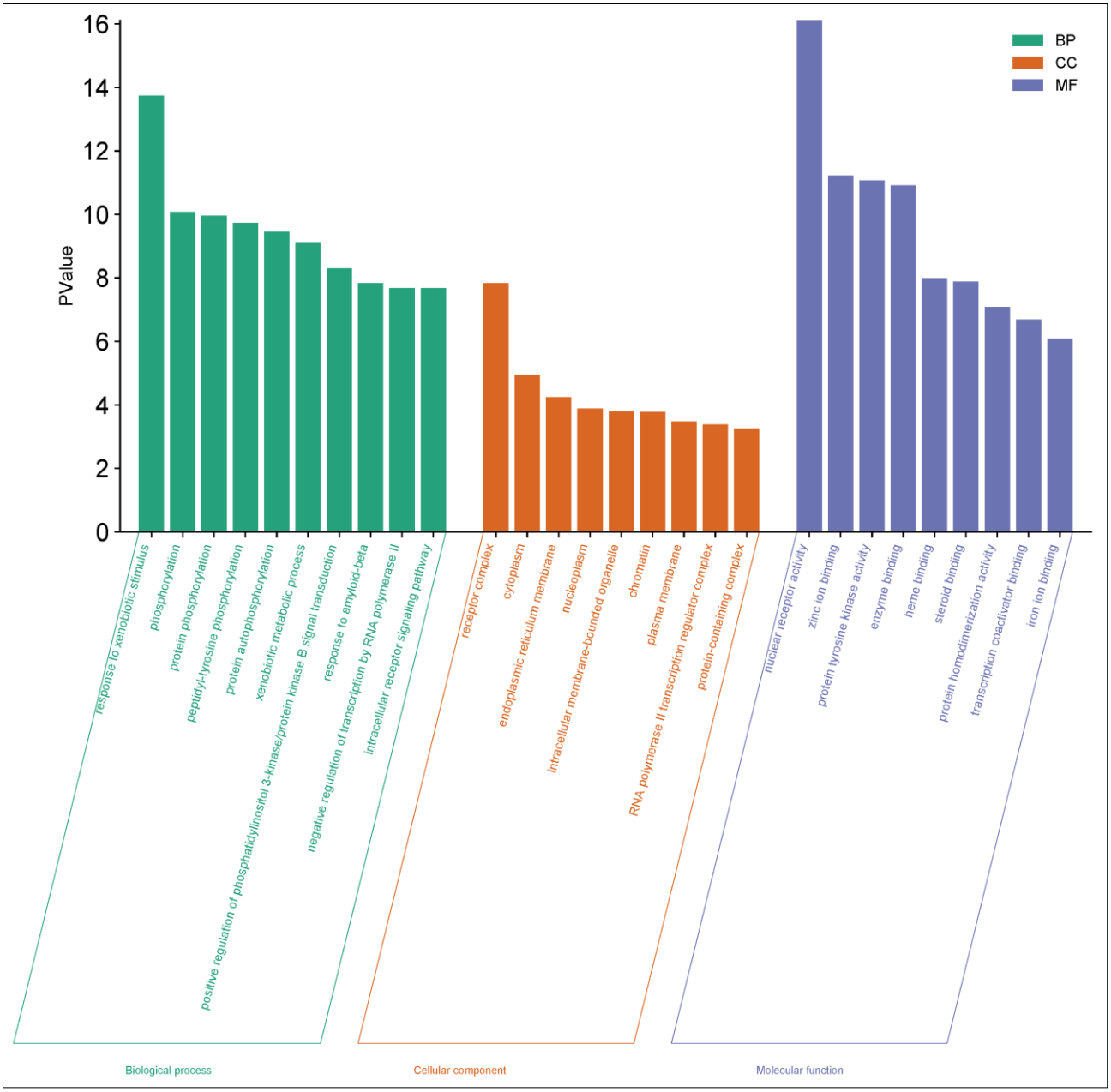

Figure 3: GO enrichment analysis bar graph

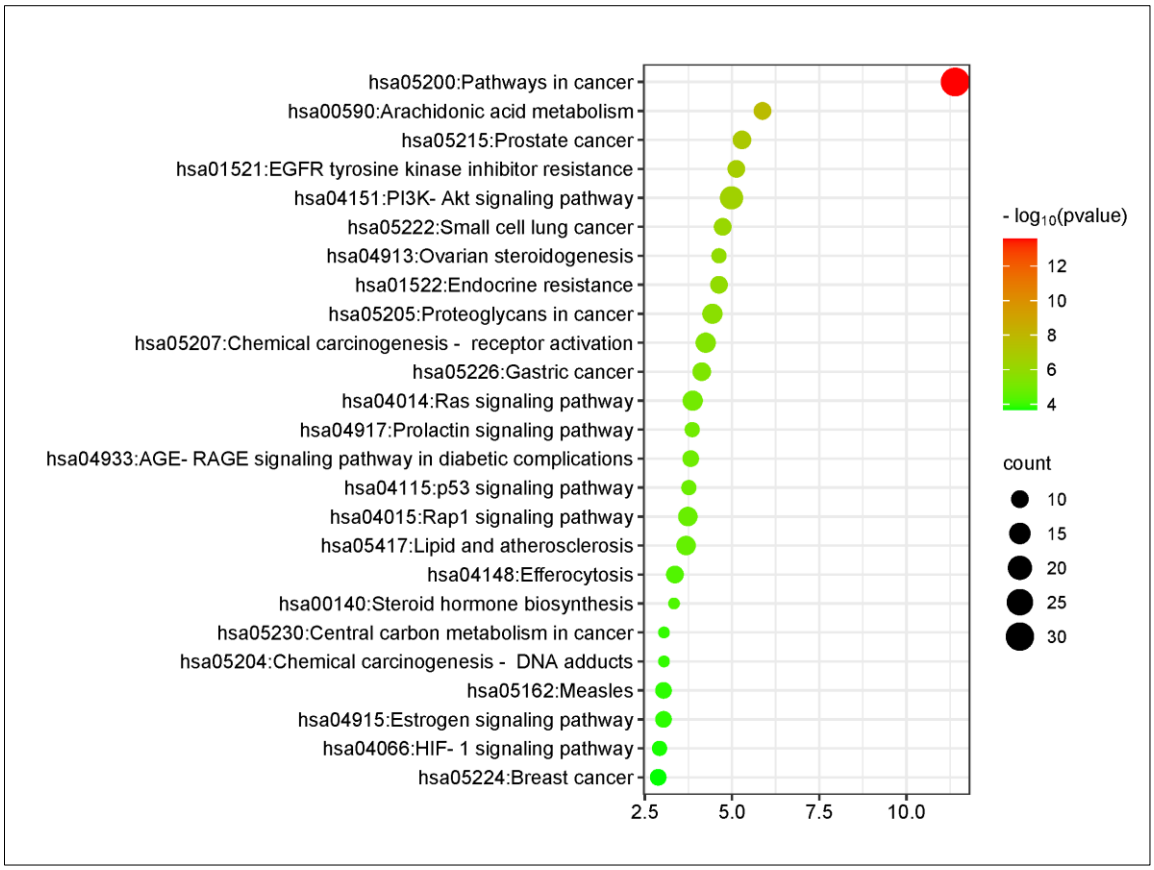

Figure 4: Bubble diagram for KEGG enrichment analysis

2.5 Analysis of "Component-Disease-Target" Network Diagram Construction

Cytoscape software was used to construct a network model of Chenpi's main drug-disease-target (see Figure 5). There are 110 nodes in the network, of which 5 nodes of Chenpi's main active ingredient (circles), 104 nodes of target genes at the intersection of acute ulcerative colitis and Chenpi's active ingredient (diamonds), and quadrilateral nodes represent the acute ulcerative colitis disease. The edges from nodes to nodes represent the links between active ingredients, diseases, and target genes where interactions occur. Among them, sitosterol, naringenin, 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl) chroman-4-one, nobiletin are the Chenpi play a key component in the treatment of UC, and ESR1, PTGS2, PPARG, BCL2, SRC, MMP9 are the key targets of Chenpi in the treatment of UC.

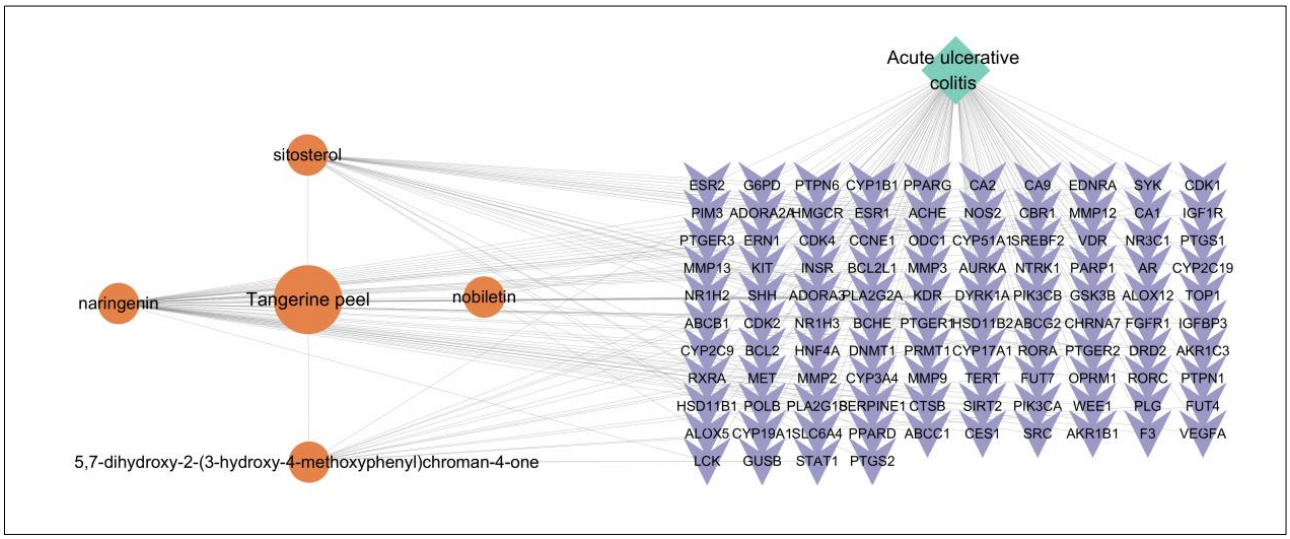

Figure 5: Visualization of "Chen Pi Active Ingredient-Disease-Target" Network Diagram

2.6 Molecular Docking Validation

Molecular docking validation of the key targets with large DEGREE values from the PPI network screening with the corresponding drug compounds was performed by obtaining the necessary files from the PDB database: PPARG (PDBID: 8WFE), ESR1 (PDBID: 6V8T), MMP9 (PDBID: 8K5V), BCL2 (PDBID: 6B4L), SRC (6E6E), all receptor files were treated with deletion of organic matter, water molecules and hydrogenation to assign charges. The 3D structures of sitosterol and naringenin were obtained from PubChem.The docking results were visualized in 3D using Pymol. The highest binding energy groups were shown by molecular docking (see Figure 6). pPARG formed hydrogen bonds with four amino acid residues in naringenin; ESR1 formed hydrogen bonds with one amino acid residue in sitosterol; MMP9 formed hydrogen bonds with three amino acid residues in naringenin; BCL2 formed hydrogen bonds with two amino acid residues in naringenin; SRC formed hydrogen bonds with three amino acid residues in naringenin. The results showed that sitosterol, naringenin, and other compounds have good affinity with PPARG, ESR1, and other key targets and can be used to treat or ameliorate acute ulcerative colitis by acting on the relevant targets.

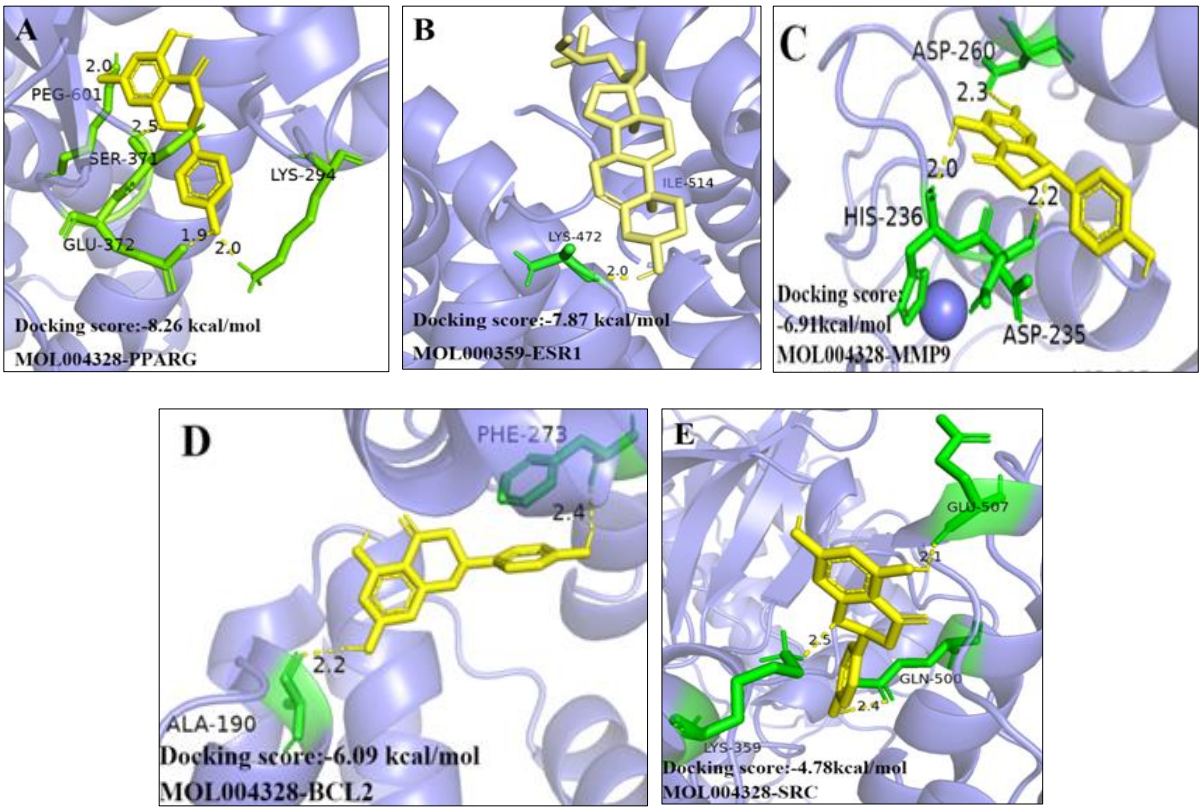

Figure 6: Stereoscopic molecular docking conformation

3. DISCUSSION

Acute ulcerative colitis is an important disease that causes diarrhea in the organism and may be accompanied by symptoms associated with megacolon, inflammation of the eye and joint areas, and colon cancer. The etiology of acute ulcerative colitis is still unclear, and autoimmune diseases, genetics, changes in intestinal flora, and environmental factors may lead to acute ulcerative colitis [13]. In recent years, Traditional Chinese Medicine (TCM) has gained widespread attention as a valuable resource base for drug development. Its uniqueness lies in its ability to demonstrate therapeutic efficacy through multi-component, multi-target, and multi-dimensional mechanisms of action, which highlights the advantages of TCM but also poses a challenge for in-depth pharmacological analysis and quality assessment. Network pharmacology, as a new strategy, integrates the essence of systems biology, bioinformatics, pharmacology, and TCM, and analyzes the complex relationships among components, targets, and disease states of TCM by drawing on the vast biological and pharmaceutical data resources, which is in line with the holistic therapeutic concept of TCM, and contributes to the research of molecular mechanisms of drugs, quality markers, clinical applications, and new drug development. This approach is in line with the holistic treatment concept of Chinese medicine, which is helpful for the research of molecular mechanisms, quality markers, clinical application, and new drug development. Chenpi has a long history of use in the treatment of epigastric distention and fullness, vomiting, and diarrhea with little food.

In this experiment, network pharmacology was combined with molecular docking technology to construct a network diagram for the treatment of acute ulcerative colitis by Chen Pi, and the active ingredients and key targets predicted by network pharmacology were validated, which preliminarily elucidated the potential mechanism of action of Chen Pi in the treatment of acute ulcerative colitis. Relying on the TCMSP database, a total of five active compounds were obtained by screening based on OB and DL values, and the combination of the PPI action network revealed that the targets of ESR1, PTGS2, PPARG, BCL2, SRC, MMP play important roles in the treatment. One of the active components of Chenpi, sitosterol, is an important phytosterol [15], which has been shown to have anti-inflammatory and antioxidant activities in several studies [16]. Naringenin and nobiletin have been shown to have good anti-inflammatory activities [17, 18]. The above studies suggest that sitosterol, naringenin, and nobiletin, the main active ingredients of Chenpi, are likely to play a role in the treatment of colitis by reducing inflammation, and the KEGG pathway is involved in pathways in cancer, arachidonic acid metabolism, and prostate cancer. Prostate cancer, ulcerative colitis is very likely to develop into colon cancer if left untreated in the course of its development, and the network pharmacology results predicted that the therapeutic targets of chenpi are more enriched in the cancer pathway, suggesting that chenpi may prevent colon cancer by inhibiting the cancer pathway. Molecular docking revealed that all the active ingredients of Chenpi had good docking scores with the target proteins, indicating stable binding between the active ingredients and the targets to exert a therapeutic effect on colitis.

4. CONCLUSION

In conclusion, this experiment took Chenpi as the research object and first revealed that Chenpi played a therapeutic role in acute ulcerative colitis through multiple active ingredients acting on multiple targets and multiple pathways by network pharmacological methods, which provided corresponding references for the development of the subsequent products, as well as ideas for the further development of Chenpi.

Acknowledgement: This work was supported by Heilongjiang BaYi Agricultural University's innovative training project for college students.

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