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RESEARCH ARTICLE

Network Pharmacology-Based Study of Active Ingredients and Mechanisms of Compound Xuanju for Rheumatoid Arthritis

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Abstract

Objective: Compound Xuanju has good effects in treating rheumatoid arthritis (RA), but its composition is complex, and its active ingredients and mechanism have not been fully defined. In this study, the active ingredients and mechanism of compound Xuanju for the treatment of RA were explored through network pharmacological methods. Methods: TCMSP and TCMID, Pubmed, CNKI, Wanfang, and VIP databases were used to screen, select active pharmaceutical ingredients and targets; Drugbank disease target screening database, GeneCards database, Therapeutic The Target Database (TTD) database and DisGeNET database were used to collect RA targets, and OmicShare was used to screen compound Xuanju and RA for common targets and construct a Venn diagram. A protein target database String was used to construct a common target interaction network. OmicShare mapping software builds a ”drug-active ingredient-target” network and analyzes their associations. DAVID online software performs gene annotation (GO) and KEGG pathway enrichment analysis on key targets. Results: A total of 73 effective ingredients of compound Xuanju were obtained, and corresponding to 229 targets; 2337 targets for RA. 155 key targets for potential active ingredients of compound Xuanju predicted therapeutic effect of RA, the key targets map 55 active ingredients of compound Xuanju capsules. These targets mainly involve signaling pathways such as Toll-like receptor signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, and TNF signaling pathway acting on RA. Conclusion: Compound Xuanju may via its potential 55 active ingredients act on 155 targets to treat RA through Toll-like receptor signaling pathway, PI3K-Akt signaling pathway, NF-κB signaling pathway, HIF-1 signaling pathway and TNF signaling pathway. This study lays the theoretical basis for the widespread application of compound Xuanju in clinical practice.

Keywords: Network pharmacology, Compound Xuanju, Rheumatoid arthritis, Active ingredients; Traditional Chinese medicine

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease with pathological features characterized by excessive hyperplasia of synovial tissue, joint swelling, vascular formation, and cartilage erosion (1). The high disability rate, poor prognosis, recurrent and long treatment cycle which brings a heavy economic burden on patients' families and society. The incidence of RA worldwide amounts to 0.18–1.07%, and it is increasing year by year (2). Depending on the U.S. administrative health insurance claims database, the prevalence of RA from 2004 to 2014 was from 0.41% to 0.54% (3). The prevalence of Chinese patients is about 0.4%. For patients who have not been diagnosed and treated promptly, the disability rate is 50% after 2 years, and 70% of patients after 3 years have varying degrees of disability, and their quality of life is severely affected (4). Current research shows that the pathogenesis of RA is related to multiple mechanisms such as infection, genetics, immune system disorders, and metabolic abnormalities (5, 6), but it has not yet been conclusively determined, and effective preventive and therapeutic drugs are continue to be lacking.

Western medicines currently available include nonsteroidal anti-inflammatory drugs, glucocorticoids, anti-rheumatic drugs (DMARD) to relieve disease, and biological agents. However, its potential side effects may affect patient compliance with treatment (7). In addition, pathophysiological and genetic differences between patients also limit the therapeutic effectiveness of such drugs in RA treatment. Therefore, as an adjuvant treatment for patients with chronic diseases, complementary therapies have become more and more common (8). The most prevalent of these complementary therapies is traditional Chinese medicine (TCM). In China, a nationwide study of the incidence of TCM use in RA and non-RA populations has demonstrated that RA patients have a higher TCM use tendency compared to the general population, with a range of 30% (9).

Studies have shown (10) that compound Xuanju have significant anti-inflammatory and immune-regulating effects. Compound Xuanjuare mainly composed of Chinese herbal ingredients such as Xuanju (Polyrhachis vicine Roger), Epimrdii Herba, Cnidii Fructus, and Lycii Fructus. Xuanju is salty, sour, and mild in nature, which can make stronger, nourish blood, strengthen pain, and relieve pain and Quyu Tongluo. Lycii Fructus is sweet and flat, achieves strong bones, and nourishes liver and kidney; Epimrdii Herba can remove wind and dehumidification, kidney and aphrodisiac. Cnidii Fructus tastes bitter, mild, has warm kidney and impotence Cold effect. The compatibility of these drugs can achieve the effects of removing wind and dispel cold and warming kidney, and it can treat the RA from the two aspects of righting and eliminating evil. Although some studies have shown that compound Xuanjucan effectively treat RA (11, 12), there is still an urgent need to further clarify and describe its underlying material basis and mechanism of action. Therefore, this study uses network pharmacology to conduct a comprehensive network analysis of the effects of compound Xuanju for RA targets, and to construct a RA target network with hub genes, in order to explain the key active ingredients of compound Xuanju and their mechanism of action. The workflow is shown in Figure 1.

2 | MATERIALS AND METHODS

1. RA disease target acquisition and collection

Using "Rheumatoid arthritis" as a key topic word in Drugbank disease target screening database (https://www.drugbank.ca/), GeneCards database (https://www. genecards.org/), Therapeutic Target Database (TTD) database (http://db.idrblab. net/ttd/) and DisGeNET (http://www.disgenet.org/) database to search RA related targets. The Drugbank database contains detailed drug, drug target, drug action, and drug interaction information on FDA-approved

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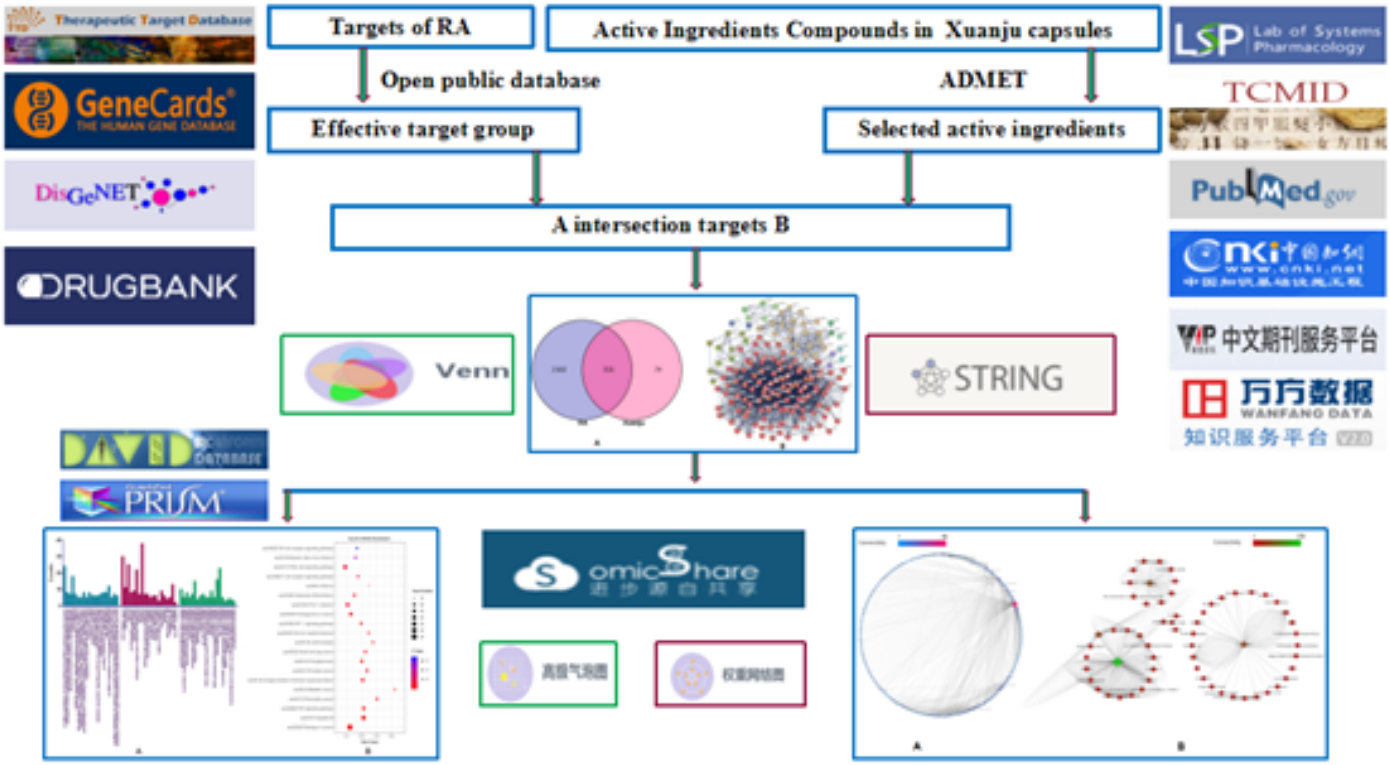

FIGURE 1: Workflow of network pharmacology analysis.

drugs and experimental drugs that have undergone FDA approval procedures (13). The TTD database provides information on known therapeutic protein and nucleic acid targets, targeted disease conditions, pathway information, and target drug/ligand counterparts (14) . DisGeNET (http://www.disgenet.org/) is used to retrieve the molecular basis of specific human diseases, analysis of disease gene characteristics, generation of hypothesis for drug treatment effects and adverse drug reactions, verification of predicted disease genes and evaluation of text mining the performance of the method etc (15) . The chemical composition and target data obtained from the three databases are combined, duplicate and invalid targets are deleted, and the full names of the selected targets are entered into DrugBank to obtain the gene abbreviation and Uniprot ID.

2. Active ingredients and action targets of compound Xuanju

Through the Traditional Chinese Medicine System Pharmacology database (TCMSP) (http://tcmspw.com/tcmsp.php), Traditional Chinese Medicine Integrated Database (TCMID) (http://119.3.41.228:8000/tcmid/search/) and Pubmed, CNKI, Wanfang and VIP databases, the active ingredients and active targets of the four main components of compound Xuanju, namely, Xuanju, Epimrdii Herba, Cnidii Fructus, and Lycii Fructus were searched and retrieved. The TCMSP database is a unique traditional Chinese medicine system pharmacological data analysis platform that can analyze chemical components, targets, and disease networks, including oral bioavailability (OB), drug similarity (DL) and other natural compounds. (16), the screening conditions in this study were OB≥30%, DL≥0.18, and HL≥4. It has priority over TCMSP database retrieval. If TCMSP is not included, the TCMID database is used. The TCMID database is a comprehensive database that provides information for TCM and modern life sciences and bridge the gap. It collects information including prescriptions, herbs and herbal ingredients, and modern pharmacological information (17). If the two databases are not included, the literature query is conducted through the Pubmed, CNKI, Wanfang, and VIP databases to obtain the active ingredients and the target of traditional Chinese medicine.

3. Target screening and protein interaction network construction

Map the matching target of the active ingredient of compound Xuanju and the target of RA in OmicShare (https://www.omicshare.com/tools/index. php/) , and construct a Venn diagram. The common target with the intersection module may be the key target for active ingredients of compound Xuanjuto treat RA. The common targets were used to build a protein-protein interaction (PPI) network model on the String database online platform (https://string-db.org/).

4. Construction and analysis of the active ingredient-target network

OmicShare were used to construct and visualize the collected traditional Chinese medicine ingredients and their target information. The nodes represent the active ingredient and the target, and the edges represent the interaction between them, and the connectivity analysis is performed. The more connected knots are, the more important the compound or target is. The key active components and central genes of compound Xuanju against RA were determined by analyzing the strength of network connectivity.

5. Analysis of key target functions annotation and pathway enrichment

Through the DAVID database (https://david.ncifcrf.gov/), P <0.05 was used as the screening condition to obtain key target proteins for gene ontology (GO) functional enrichment analysis and Kyoto Gene and Genome Encyclopedia (KEGG) pathway enrichment analysis. Three modules including biological process, cellular component and molecular function were selected for mapping. Use GraphPad Prism 5.0 to visualize the top 20 entries of biological processes, cell composition, and molecular function entries in GO function enrichment analysis as a visual bar chart. Use the OmicShare to present the channel enrichment results in the form of a bubble chart for KEGG pathway analysis on key targets.

3 | RESULTS

1. Active ingredients of compound Xuanju and targets

Three components of compound Xuanju(Epimrdii Herba, Cnidii Fructus, and Lycii Fructus) were searched in the TCMSP and TCMID databases. Xuanju was searched through Pubmed, CNKI, Wanfang and VIP databases. 42 active ingredients of Lycii Fructus, 14 of Cnidii Fructus, 18 of Epimrdii Herba, and 4 of Xuanju were obtained. After removing 5 repetitive ingredients, a total of 73 single active ingredients were obtained from the compound Xuanju Table 1 . A total of 842 targets were acted on by the active ingredients, and 229 targets were obtained after de-duplication.

2. Screening of interaction targets of compound Xuanju and RA in addition construction of protein interaction network

A total of 2707 RA-related targets were collected through Drugbank, GeneCards, DisGeNET and TTD databases, 370 duplicate targets were deleted to obtain 2337 targets for RA. The RA targets were mapped and matched with 229 sole targets of compound Xuanju. The Venn diagram (Figure 2A) showed that the potential active ingredients of compound Xuanju intersect with RA. The module predicts a total of 155 targets Table 2. A protein-protein interaction (PPI) network model was constructed with 155 common targets in the String database (Figure 2B). These 155 common targets may be the key targets for the active ingredients of compound Xuanju to treat RA.

3. Analysis of key active ingredients and targets of compound Xuanju against RA

In order to further understand the mechanism of action of key genes, we identified 155 overlapping genes based on the predicted results and reversely searched for compounds that interacted with them. Finally, 55 compounds were identified from Xuanju, Epimrdii Herba, Cnidii Fructus, and Lycii Fructus. The compounds are shown in Table 3 . And visualize the traditional Chinese medicine-chemical composition-target network Figure 3. The results showed that the three components with the strongest connectivity were: Quercetin (96), Kaempferol (64), and Luteolin (46); the four targets with the strongest connectivity were: PGR (30), PTGS2 (28), PTGS1 (19) and DDP4 (17). It is suggested that compound Xuanju mainly treat targets such as PGR, PTGS2, PTGS1 and DDP through active ingredients such as quercetin, kaempferol and luteolin to treat RA.

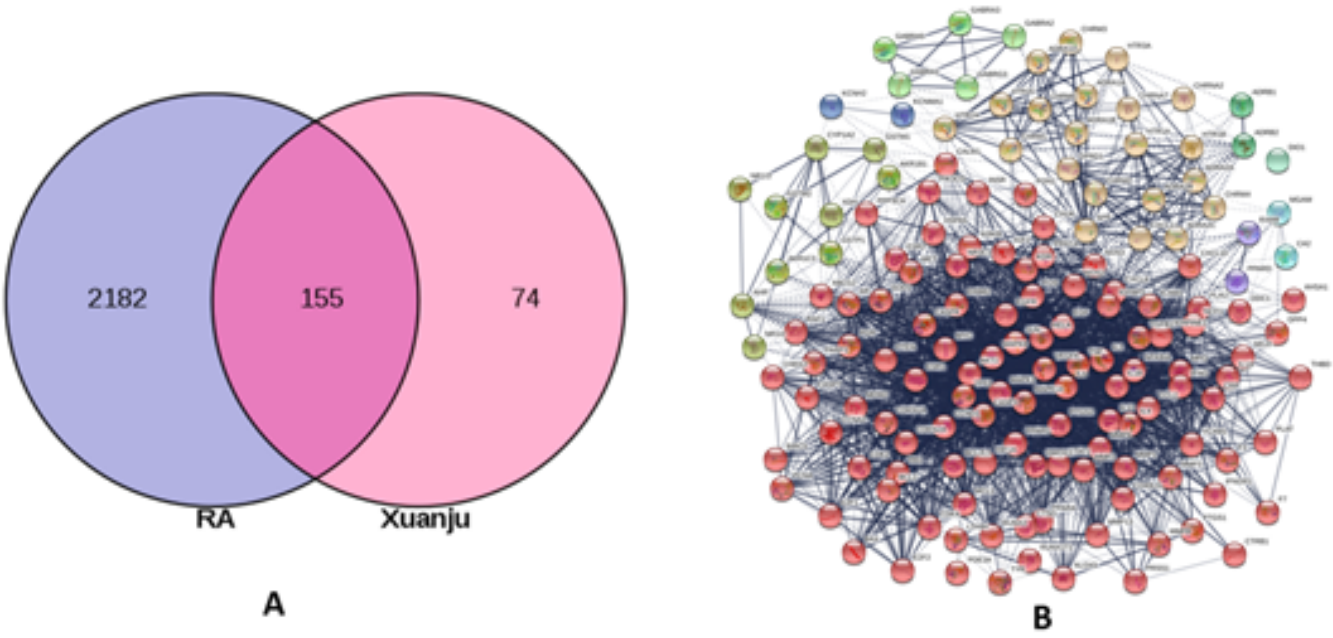

FIGURE 2: Common target andprotein-protein interaction network of compound Xuanju and RA. A, Common targets of compoundXuanju and RA are 155 in total; B, 155 common targets protein-proteininteraction network.

4. Analysis of key target functions annotation and pathway enrichment

To further understand the function and enrichment of key targets, GO and KEGG analyses were performed. The results showed that the highest scores in the biological process, cellular component, and molecular function of GO functional enrichment analysis were positive regulation of transcription from RNA polymerase II promoter (GO: 0045944), extracellular space (GO: 000561), and protein homodimerization activity (GO : 0042803). KEGG pathway enrichment analysis showed that compound Xuanju mainly passed Toll-like receptor signaling pathway (ssc04620), PI3K-Akt signaling pathway (ssc04151), NF-κB signaling pathway (ssc04064), HIF-1 signaling pathway (ssc04066), TNF Signaling pathways (ssc04668) and other signaling pathways act on RA. Figure 4

4 | DISCUSSION

The ingredient of traditional Chinese medicine is a complex chemical system, and the biological body is also a complex system. The interaction between the chemical substance entity of the traditional Chinese medicine product and the biological activity of the body has become an important entry point for modern Chinese medicine research. Network pharmacology researches the pharmacology of relating drugs through big data analysis. It takes herbs, chemical components, targets, diseases and other perspectives as the starting point to achieve a comprehensive and systematic comprehensive network analysis of traditional Chinese medicine research. It has the unique advantage and great potential to predicts and identifies active ingredients of traditional Chinese medicine, groups and targets, clarify the mechanism of action, reveal the formulas, discover new indications and new active compounds. Moreover, network pharmacology emphasizing on ”one target, one

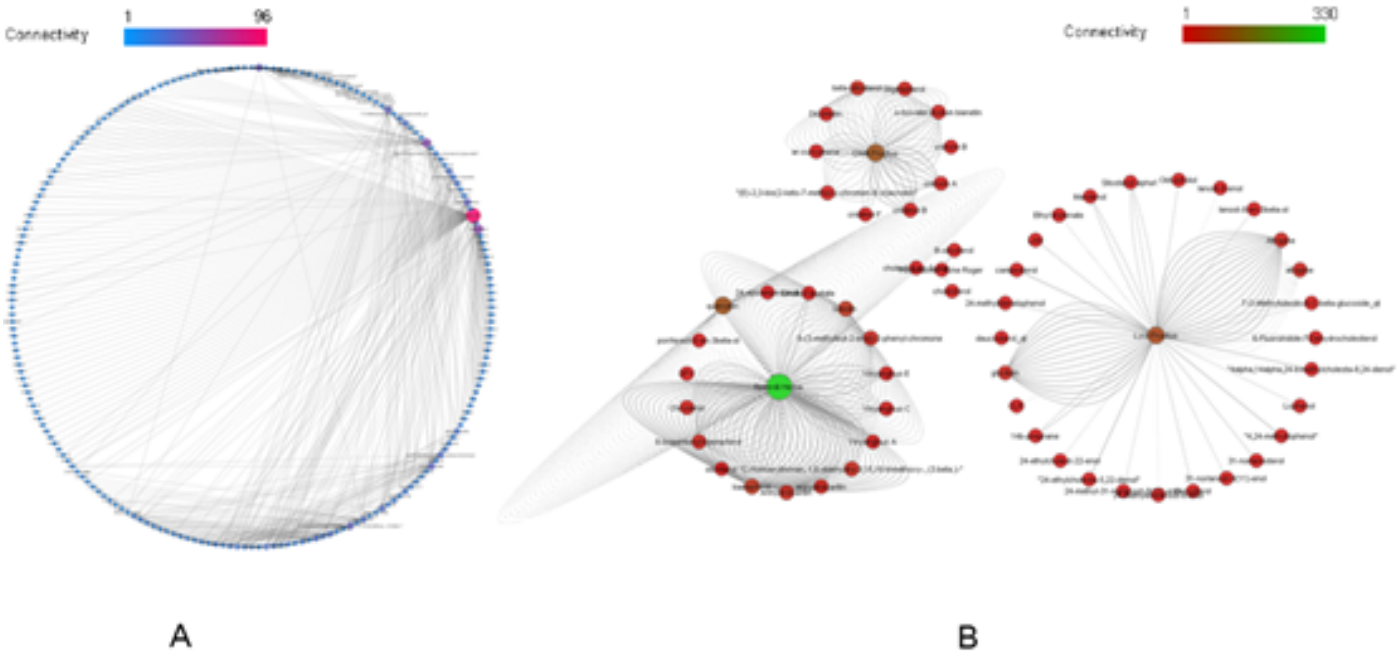

FIGURE 3: Key active ingredients-targets networks of compound Xuanju against RA.A, keyactive ingredients-targets network, red indicates the strongest connectivity ofactive ingredients (Quercetin 96), Kaempferol (64) and Luteolin (46) are next, strongestconnectivity of four targets are: PGR (30), PTGS2 (28), PTGS1 (19) and DDP4(17). B, Active ingredients-herbs network, green indicates the strongestconnectivity (Epimrdii Herba 330), followed by Cnidii Fructus (98), LyciiFructus (80), and Xuanju (3).

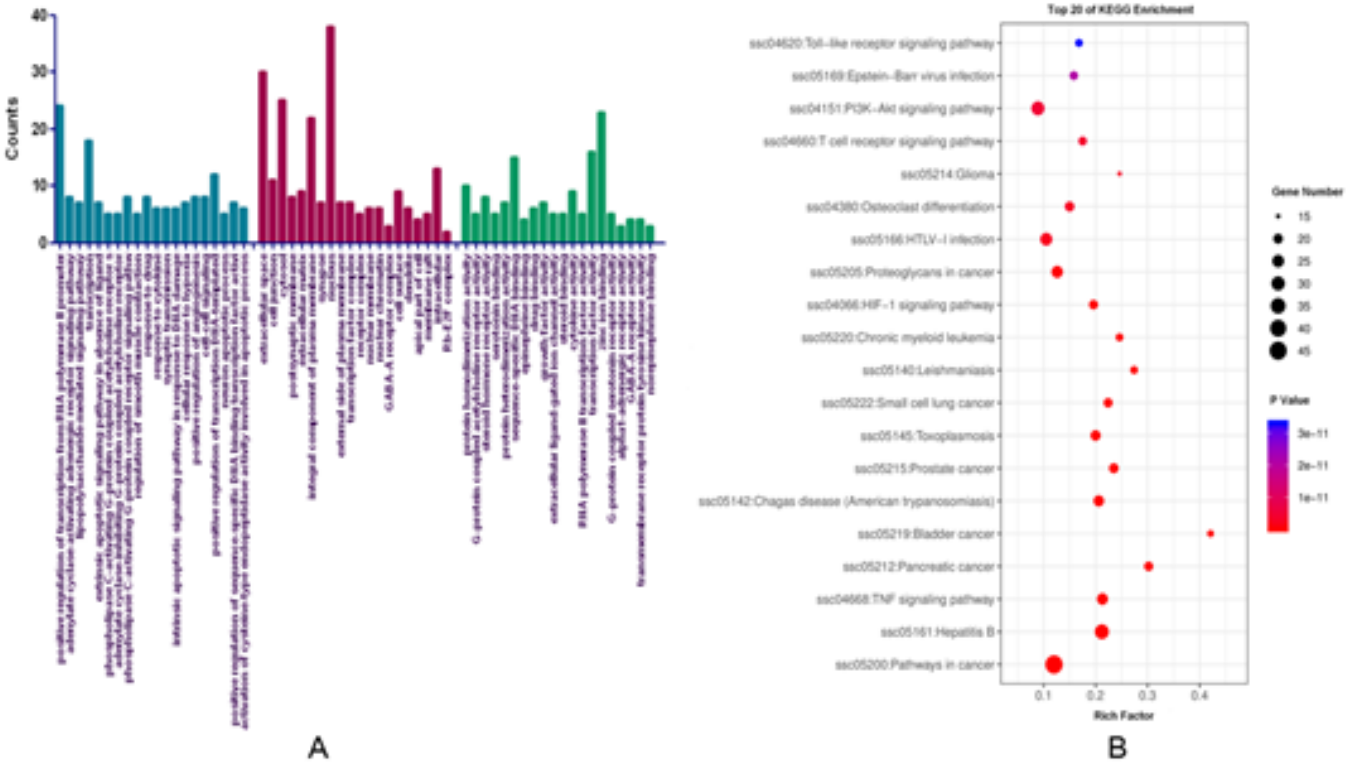

FIGURE 4: The analysis of keytarget function annotation and pathway enrichment. A, Top 20 of GO functionannotation; B, The analysis of top 20 of KEGG enrichment.

drug" to "network target, multiple groups paradigm shift. This gives us a better understanding of the mechanism of action of drugs, then it is not difficult to understand that network pharmacology is widely used in drug research (18) In the treatment of RA,compound Xuanju have received more attention due to its obvious effectiveness and fewer side effects. Therefore, in this study, we utilized network pharmacology to study the potential targets of compound Xuanju for the treatment of RA. Construct and analyze target networks, perform enrichment analysis on hub genes, and uncover their potential mechanism.

In this study, we first excavated the targets of RA, the active ingredients and targets of compound Xuanju capsules. By mapping and matching the targets, we obtained the key targets of compound Xuanju for RA; Point the corresponding active ingredients; construct the protein-protein interaction network of key targets, and then perform GO analysis and KEGG analysis to clarify the functional annotation and enrichment of key targets.

The results of this study show that the four most active ingredients are: quercetin in Lycii Fructus and Epimrdii Herba, kaempferol in Epimrdii Herba, luteolin in Lycii Fructus and Epimrdii Herba, β-sitosterol in Xuanju and Cnidii Fructus. This component group may play a therapeutic role in RA through highly connected targets such as PGR, PTGS2, PTGS1, and DDP4. Among them, quercetin has the effects of anti-oxidation and scavenging of free radicals, and also has anti-inflammatory, immunomodulatory, anti-cancer, antibacterial, antiviral, hypoglycemic and antihypertensive effects, and cardiovascular protection (19, 20) Jayanti et al (21) reported that quercetin gel preparation can effectively treat rheumatoid arthritis. Kaempferol has anti-inflammatory, antibacterial, anti-cancer and other physiological activities (22, 23). β-sitosterol (beta-sitosterol) inhibit tumor cell proliferation and inhibit blood angiogenesis (24). PTGS1 is thought to play a key role in the pathophysiology of inflammation, arthritic disease, and cancer (25) Clinical studies have shown that as a specific inhibitor of PTGS, low-dose aspirin can effectively exert antipyretic, analgesic and anti-inflammatory effects (26). Importantly, PTGS1 inhibitors or silencing of PTGS1 expression through negative regulation greatly reduces the inflammatory response caused by LPS (27). Wang et al (28) showed that knockout of PTGS1 promotes osteogenic differentiation of ASCs in vitro and in vivo by targeting p65 cytoplasm/nuclear translocation, and that during bone remodeling, PTGS1 silencing may provide regulation of the inflammatory microenvironment a potential means. Wei et al (29) reported that PTGS2 plays an important role in inflammation and fibroblast-like synoviocytes in RA. MiR-101-3p reduced joint swelling and arthritis index in RA rats by down-regulating PTGS2. The negative expression of miR-101-3p and PTGS2 was found in the synovial tissues of RA patients and RA rats, overexpressed MiR-101-3p and silenced PTGS2. RA-FLS was observed to have reduced cell proliferation, migration and invasion, promote cells apoptosis.

In GO analysis, the biological process mainly involves positive regulation of transcription from RNA polymerase II promoter, adenylate cyclase-activating adrenergic receptor signaling pathway, and lipopolysaccharide-mediated signaling pathway; Molecular function mainly involves protein homodimerization activity, G-protein coupled acetylcholine receptor activity, and steroid hormone receptor activity. The KEGG analysis mainly involved TNF signaling pathway, HIF-1 signaling pathway, T cell receptor signaling pathway, PI3K-Akt signaling pathway, Toll-like receptor signaling pathway, NF-κB signaling pathway and p53 signaling pathway. The NF-κB signaling pathway plays an important role in the inflammatory process of rheumatoid arthritis. Its transcription factor NF-κB is involved in regulating the expression of multiple cytokines and cell adhesion factors during the inflammation process (30). Activation can lead to T cell activation and thereby mediate the inflammatory response. It can also induce abnormal proliferation of RA-FLS and stimulate the proliferation and activation of osteoclasts. As a result, joint deformities and bone erosion can be caused, which can aggravate the condition of RA. In addition, after activation of NF-κB, cytokines and acute response proteins are produced to activate the immune response and increase the expression of adhesion molecules. These immune molecules exacerbate the inflammation level of RA (31) Toll-

like receptors (TLRs) in the toll-like receptor signaling pathway are type I transmembrane protein receptors, which are widely distributed on the surface of immune cells and epithelial cells. TLRs/NF-κB in signal transduction pathways that mediate immunity signaling pathways play an extremely important role in immune regulation (32, 33) TNF-α in the TNF signaling pathway can induce the expression of multiple inflammatory cytokines (such as IL-6‘IL-8) and matrix metal protein (MMP) in synovial cells, which is a persistent and prolonged progression of rheumatoid arthritis key factors (34, 35). Study found that P53 is mutated in synovial tissue of RA patients. Mutated P53 can cause cells to over express IL-6. It can be seen that abnormal proliferation T cell differentiation, and various inflammations of RA synovial tissue is regulated by the P53 signal pathway Factor production (36).

In summary, we utilized network pharmacological methods to discover 73 ingredients and 229 potential targets in compound Xuanju, and 2337 targets related to RA disease, of which compound Xuanju-RA common targets 155 points, and through the GO biological process and KEGG signal pathway enrichment analysis of these targets, it is predicted that compound Xuanju may regulate the TNF signal pathway, toll-like receptors signal pathway, NF-κB signal pathway, P53 signal pathway to act on PTGS2, PTGS1, DDP4, and PGR targets. Thereby inhibiting inflammatory response, regulating immune function and regulating apoptosis to treat RA. But it’s worth noting that network pharmacology develops rapidly. Our research present certain limitations. First of all, this study is based on a network pharmacological approach to study the potential targets of compound Xuanju for RA, but the specific mechanism of action remains to be confirmed by cell and animal experiments. Second, due to the difficulty of obtaining data, the database may not include all known or unknown active ingredients, targets, and protein-protein interactions. However, it is undeniable that the results of this study can provide ideas and reference for experimental research, and provide a theoretical basis for the wide application of compound Xuanju in clinical practice.

Conflicts of Interests : The authors have no conflicts of interests.

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Table 1: Effective chemical composition information of Compound Xuanju

Molecule Name	MW	AlogP	OB (%)	Caco -2	BBB	DL	HL
Sitosterol alpha1	426.8	8.15	43.28	1.41	0.97	0.78	5.64
Cycloartenol	426.8	7.55	38.69	1.53	1.33	0.78	5
Mandenol	308.56	6.99	42	1.46	1.14	0.19	5.39
Ethyl linolenate	306.54	6.55	46.1	1.54	1.12	0.2	6.2
LAN	426.8	8.12	42.12	1.52	1.18	0.75	5.84
Stigmasterol	412.77	7.64	43.83	1.44	1	0.76	5.57
beta-sitosterol	414.79	8.08	36.91	1.32	0.99	0.75	5.36
campesterol	400.76	7.63	37.58	1.34	0.95	0.71	4.83
24-methylidenelophenol	412.77	7.75	44.19	1.41	1	0.75	5.1
daucosterol_qt	414.79	8.08	36.91	1.33	0.85	0.75	5.6
glycitein	284.28	2.32	50.48	0.56	-0.29	0.24	16.32
CLR	386.73	7.38	37.87	1.43	1.13	0.68	4.52
14b-pregnane	288.57	6.54	34.78	1.8	1.95	0.34	4.57
(24R)-4alpha-Methyl-24-ethylcholesta-7,25-dien-3beta-ylacetate	482.87	8.79	46.36	1.39	1	0.84	8.25
24-Methylenecycloartan-3beta,21-diol	456.83	6.7	37.32	0.95	0.3	0.8	5.67
24-ethylcholest-22-enol	414.79	7.89	37.09	1.33	0.99	0.75	5.32
24-ethylcholesta-5,22-dienol	412.77	7.64	43.83	1.31	0.84	0.76	5.76
24-methyl-31-norlanost-9(11)-enol	428.82	8.15	38	1.33	0.93	0.75	5.49
24-methylenelanost-8-enol	440.83	8.43	42.37	1.44	1.18	0.77	5.43

Fucosterol	412.77	7.83	43.78	1.34	1.01	0.76	5.44
31-Norcyclolaudenol	440.83	8.04	38.68	1.35	0.94	0.81	4.88
31-norlanost-9(11)-enol	414.79	7.9	38.35	1.34	1.03	0.72	5.37
31-norlanosterol	412.77	7.85	42.2	1.4	1.03	0.73	5.27
4,24-methyllophenol	414.79	7.95	37.83	1.43	1.1	0.75	4.9
Lophenol	400.76	7.7	38.13	1.36	1.1	0.71	4.76
4alpha,14alpha,24-trimethylcholesta-8,24-dienol	426.8	8.29	38.91	1.4	1.06	0.76	6.67
4alpha,24-dimethylcholesta-7,24-dienol	412.77	7.89	42.65	1.43	0.98	0.75	5.44
4alpha-methyl-24-ethylcholesta-7,24-dienol	426.8	8.35	42.3	1.45	1.08	0.78	5.83
6-Fluoroindole-7-Dehydrocholesterol	402.7	7.04	43.73	1.03	0.54	0.72	5.1
7-O-Methylluteolin-6-C-beta-glucoside_qt	318.3	2.01	40.77	0.33	-0.47	0.3	14.1
Atropine	289.41	1.72	42.16	0.57	0.39	0.19	5.27
Cryptoxanthin monoepoxide	568.96	9.5	46.95	1.49	-0.03	0.56	4.4
Cycloeucalenol	426.8	7.59	39.73	1.42	1.04	0.79	5.01
(E,E)-1-ethyl octadeca-3,13-dienoate	308.56	6.99	42	1.46	1.11	0.19	5.47
methyl(1R,4aS,7R,7aS)-4a,7-dihydroxy-7-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-1,5,6,7a-tetrahydrocyclopenta[d]pyran-4-carboxylate	406.43	-3.18	39.43	-1.43	-2.06	0.47	7.64
Lantadene A	552.87	7.21	38.68	0.32	-0.3	0.57	5.44
Physalin A	526.58	0.5	91.71	-0.78	-1.11	0.27	13.21
Physcion-8-O-beta-D-gentiobioside	608.6	-0.91	43.9	-2.68	-3.52	0.62	28.46

lanost-8-en-3beta-ol	428.82	8.37	34.23	1.48	1.25	0.74	5.48
lanost-8-enol	428.82	8.37	34.23	1.37	1.12	0.74	6.41
Obtusifoliol	426.8	8.15	42.55	1.47	1.25	0.76	5.91
quercetin	302.25	1.5	46.43	0.05	-0.77	0.28	14.4
Methyl oleate	296.55	7.09	31.9	1.42	1.13	0.17	5.37
cholesterol	386.73	7.38	37.87	1.43	1.13	0.68	4.52
cholest-4-en-3-one	412.77	8.18	36.08	1.46	1.22	0.76	5.49
β-sitosterol	546.57	1.55	33.94	-0.44	-1.37	0.7	13.56
24-epicampesterol	400.76	7.63	37.58	1.43	1.15	0.71	4.5
poriferast-5-en-3beta-ol	414.79	8.08	36.91	1.45	1.14	0.75	5.07
Diosmetin	300.28	2.32	31.14	0.46	-0.66	0.27	16.34
Ethyl oleate (NF)	310.58	7.44	32.4	1.4	1.1	0.19	4.85
ar-curcumene	426.5	5.05	52.34	0.59	0.17	0.65	9.88
cnidimol B	292.31	0.88	68.66	-0.06	-0.84	0.26	16.79
cnidimol F	290.29	0.65	54.43	-0.32	-1.39	0.28	14.68
(E)-2,3-bis(2-keto-7-methoxy-chromen-8-yl)acrolein	404.39	3.54	56.38	0.26	-0.22	0.71	6.98
cniforin A	374.42	3.09	55.89	0.37	-0.13	0.47	6.91
cniforin B	414.49	4.63	36.7	0.6	-0.05	0.6	6.65
o-Isovalerylcolum bianetin	330.41	3.78	64.03	0.73	0.38	0.36	5.79

Ostruthin	298.41	5.32	30.65	1.2	0.79	0.23	4.09
Linoleyl acetate	308.56	6.85	42.1	1.36	1.08	0.2	7.48
DFV	256.27	2.57	32.76	0.51	-0.29	0.18	17.89
Chryseriol	300.28	2.32	35.85	0.39	-0.53	0.27	16.31
8-Isopentenyl-kaempferol	354.38	3.63	38.04	0.53	-0.49	0.39	15.37
sitosterol	414.79	8.08	36.91	1.32	0.87	0.75	5.37
kaempferol	286.25	1.77	41.88	0.26	-0.55	0.24	14.74
Anhydroicaritin	368.41	3.88	45.41	0.72	0.01	0.44	15.01
C-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)-	329.48	2.89	39.14	1.02	0.68	0.49	6.58
Yinyanghuo A	420.49	4.2	56.96	0.38	-0.49	0.77	14.44
Yinyanghuo C	336.36	3.39	45.67	0.75	-0.11	0.5	15.74
Yinyanghuo E	352.36	3.12	51.63	0.51	-0.54	0.55	15.47
8-(3-methylbut-2-enyl)-2-phenyl-chromone	290.38	4.99	48.54	1.53	0.99	0.25	18.73
Anhydroicaritin-3-O-alpha-L-rhamnoside	676.73	0.77	41.58	-1.59	-2.89	0.61	16.23
Icariin	676.73	0.77	41.58	-1.82	-3	0.61	19.93
luteolin	286.25	2.07	36.16	0.19	-0.84	0.25	15.94

Table 2: Key targets of compound Xuanju against RA

NO.	Targets	Uniprot ID	NO.	Targets	Uniprot ID	NO.	Targets	Uniprot ID
1	MMP2	P08253	42	GABRA1	P14867	83	CHRNA7	P36544
2	KCNMA1	Q12791	43	PPARD	Q03181	84	ADRA2C	P18825
3	XDH	P47989	44	HMOX1	P09601	85	PLAU	P00749
4	NOS2	P35228	45	HTR1A	P08908	86	PTEN	P60484
5	CHRM5	P08912	46	MMP3	P08254	87	MMP13	P45452
6	MET	P08581	47	MMP8	P22894	88	ALOX5	P09917
7	BIRC5	O15392	48	GSTM1	P09488	89	HTR2C	P28335
8	PPP3CA	Q08209	49	IL10	P22301	90	DPP4	P27487
9	BCL2	P10415	50	MAPK1	P28482	91	PON1	P27169
10	ADRA2A	P08913	51	PLAT	P00750	92	KCNH2	Q12809
11	F7	P08709	52	MDM2	Q00987	93	E2F1	Q01094
12	HTR3A	P46098	53	EGFR	P00533	94	CASP8	Q14790
13	CHRM1	P11229	54	IKBKB	O14920	95	PPARG	P37231
14	CRP	P02741	55	SOD1	P00441	96	SELE	P16581
15	GSTP1	P09211	56	ERBB2	P04626	97	NR3C1	P04150
16	ADRA1A	P35348	57	IL4	P05112	98	THBD	P07204
17	AHR	P35869	58	MAPK8	P45983	99	MAPK14	Q16539
18	CHRNA2	Q15822	59	AKR1B1	P15121	100	RAF1	P04049
19	NR1I3	Q14994	60	CHEK2	O96017	101	MPO	P05164
20	CHRM4	P08173	61	RB1	P06400	102	ADRA1B	P35368
21	ADRB1	P08588	62	CDK2	P24941	103	PCNA	P12004
22	TNF	P01375	63	RUNX2	Q13950	104	CHUK	O15111

23	EGF	P01133	64	PGR	P06401	105	GABRA2	P47869
24	IL1A	P01583	65	ODC1	P11926	106	CASP7	P55210
25	IL8	P10145	66	E2F2	Q14209	107	ADRB2	P07550
26	PTGS2	P35354	67	CXCL10	P02778	108	CTSD	P07339
27	NCF1	P14598	68	TYR	P14679	109	MCL1	Q07820
28	RUNX1T1	Q06455	69	TP53	P04637	110	CCL2	P13500
29	CCND1	P24385	70	CDKN1A	P38936	111	IL6	P05231
30	ESR1	P03372	71	DIO1	P49895	112	CASP3	P42574
31	GABRG3	Q99928	72	GABRA5	P31644	113	PARP1	P09874
32	CDK4	P11802	73	IL1B	P01584	114	MGAM	O43451
33	PDE3A	Q14432	74	CHRM3	P20309	115	KDR	P35968
34	VEGFA	P15692	75	NFKBIA	P25963	116	ABCG2	Q9UNQ0
35	PIK3CD	O00329	76	IGFBP3	P17936	117	PPARA	Q07869
36	TGFB1	P01137	77	HTR2A	P28223	118	CYP1A2	P05177
37	MYC	P01106	78	GSTM2	P28161	119	AHSA1	O95433
38	ADRA1D	P25100	79	CTRB1	P17538	120	IGF2	P01344
39	CCNA2	P20248	80	CALM1	P0DP23	121	HTR1B	P28222
40	MMP1	P03956	81	ESR2	Q92731	122	ADRA2B	P18089
41	STAT1	P40763	82	LTA4H	P09960	123	IL2	P60568
124	FOS	P01100	135	CHRM2	P08172	146	JUN	P05412
125	IFNG	P01579	136	SERPINE1	P05121	147	AR	P10275
126	HSPA5	P11021	137	VCAM1	P19320	148	GABRA3	P34903
127	OPRD1	P41143	138	IFNGR1	P15260	149	BAX	Q07812

128	ICAM1	P05362	139	INSR	P06213	150	PRKCA	P17252
129	CxCL11	O14625	140	AKT1	P31749	151	CD40LG	P29965
130	BCL2L1	Q07817	141	AKR1C3	P42330	152	APP	P05067
131	CA2	P00918	142	PTGS1	P23219	153	PRSS1	P07477
132	RELA	Q04206	143	F3	P13726	154	MMP9	P14780
133	HIF1A	Q16665	144	NR1I2	O75469	155	RXRB	P28702
134	NOS3	P29474	145	CDKN2A	Q8N726			

TABLE 3: Table of activeingredients corresponding to key targets

NO.	Compound	NO.	Compound
1	24-epicampesterol	29	Ethyl linolenate
2	Linoleyl acetate	30	LAN
3	poriferast-5-en-3beta-ol	31	campesterol
4	DFV	32	24-methylidenelophenol
5	Chryseriol	33	daucosterol_qt
6	8-Isopentenyl-kaempferol	34	glycitein
7	sitosterol	35	CLR
8	kaempferol	36	14b-pregnane
9	Anhydroicaritin	37	24-ethylcholest-22-enol
10	C-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)-	38	24-ethylcholesta-5,22-dienol
11	Yinyanghuo A	39	24-methyl-31-norlanost-9(11)-enol
12	Yinyanghuo C	40	24-methylenelanost-8-enol
13	Yinyanghuo E	41	Fucosterol
14	8-(3-methylbut-2-enyl)-2-phenyl-chromone	42	31-norlanost-9(11)-enol
15	luteolin	43	31-norlanosterol
16	quercetin	44	4,24-methyllophenol
17	Diosmetin	45	Lophenol
18	beta-sitosterol	46	4alpha,14alpha,24-trimethylcholesta-8,24-dienol
19	ar-curcumene	47	6-Fluoroindole-7-Dehydrocholesterol
20	cnidimol B	48	7-O-Methylluteolin-6-C-beta-glucoside_qt
21	cnidimol F	49	atropine
22	(E)-2,3-bis(2-keto-7-methoxy-chromen-8-yl)acrolein	50	lanost-8-en-3beta-ol
23	cniforin A	51	lanost-8-enol
24	cniforin B	52	Obtusifoliol
25	o-Isovalerylcolum bianetin	53	cholesterol
26	Stigmasterol	54	cholest-4-en-3-one
27	Sitosterol alpha1	55	β-sitosterol
28	Mandenol		