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The mechanism of *Leonuri Herba* in improving polycystic ovary syndrome was analyzed based on network pharmacology and molecular docking

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Background: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting women. Chinese herbs have been considered as an alternative treatment for PCOS, and Yi-mu-cao (*Leonuri Herba*) is one of the most commonly used herbs to treat PCOS, which can relieve symptoms of PCOS patients. But the mechanism of its treatment remains unclear.

Method: The main active ingredients and potential targets of *Leonuri Herba* were obtained by TCMSP and Swiss Target Forecast, and the related targets of PCOS were obtained by searching DrugBank, GeneCard and DisGeNet databases. The Protein-Protein Interaction (PPI) network was constructed using STRING database. GO and KEGG were used to detect the enrichment pathways of key targets. Cytoscape software was used to construct the component-target-pathway network, analyze the PPI network core, and verify the reliability of target binding by molecular docking technology.

Result: 8 components and 116 targets of *Leonuri Herba* on PCOS were screened. Common targets mainly involve the Lipid and atherosclerosis, Endocrine resistance, AGE-RAGE signaling in diabetic complications and other signaling pathways. It is suggested that it can form multi-target and multi-pathway regulatory network through quercetin, kaempferol and other active substances to regulate endocrine disorders and reduce inflammatory response, so as to systematically improve PCOS. Molecular docking experiments showed that the active constituents of Leonurus had good binding activity with potential targets of PCOS.

Abbreviation: AR, androgen receptor; AKT1, RAC-alpha serine/threonine-protein kinase; E2, estradiol; EGFR, epidermal growth factor receptor; FSH, follicle stimulating hormone; GO, gene ontology; HIF1A, hypoxia-inducible factor 1-alpha; IL-6, interleukin-6; IL1B, interleukin-1 beta; JUN, transcription factor Jun; KEGG, kyoto encyclopedia of genes and genomes; LH, luteinizing hormone; MAPK, mitogen-activated protein kinase; MMP9, matrix metalloproteinase-9; MYC, Myc proto-oncogene protein; PCOS, polycystic ovary syndrome; PPI, protein-protein interaction; T, testosterone; TCM, Traditional Chinese medicine; TCMSP, Traditional Chinese Medicine System Pharmacology Database and Analysis Platform; VEGFA, vascular endothelial growth factor A.

Conclusion: In summary, this study elucidates the potential effect of *Leonuri Herba* on PCOS, which is helpful to provide reference for clinical practice. This is also conducive to the secondary development of motherwort and its monomer components, and precision medicine for PCOS.

KEYWORDS

***Leonuri Herba*, polycystic ovary syndrome, network pharmacology, molecular docking, endocrine disorder**

Introduction

Polycystic ovary syndrome (PCOS) can affect 5–18% of women (1). It is characterized by androgen excess, infertility, irregular menstrual cycle, and abnormal ovarian androgen production caused by PCOM (2). PCOS increases the risk of infertility, endometrial dysfunction, cardiovascular disease, diabetes, metabolic syndrome, and other diseases (3). It seriously affected the quality of life of patients. At present, PCOS treatment mainly relies on antiandrogen drugs, insulin sensitizers, ovulation promoting drugs, oral contraceptives and so on (4–6). However, the treatment of PCOS is still a difficult problem in obstetrics and gynecology. As PCOS is a multi-system disease with complex pathological mechanism, heterogeneous symptoms and numerous complications, sometimes western medicines cannot achieve good therapeutic effects, and more drug targets need to be explored.

Yi-mu-cao (*Leonuri Herba*) is naturally found in plants and has traditionally been used in China for thousands of years for uterine contractions, postpartum congestion, breast tenderness, and other gynecological disorders (7, 8). It has been reported as a prescription single herb with antioxidant activity that can treat dysmenorrhea by relieving uterine spasms, reducing inflammation, reducing concentrations of prostaglandin F2α and prostaglandin synthase-2 in uterine smooth muscle, increasing serum progesterone levels, and effectively relieving symptoms of PCOS (9–11). However, because *Leonuri Herba* is a kind of herbal medicine with diverse ingredients and targets, the therapeutic mechanism is not clear at present, and it is necessary to further explore its therapeutic mechanism.

In recent years, Chinese herbal medicine has become a new research hotspot because of its multi-component and multi-target characteristics. But the mechanism of treatment is complex and unclear.

In this study, we applied a network pharmacology approach to achieve a multilevel study to determine the interaction between motherwort and PCOS. Network pharmacology is a new strategy to study the interaction between drugs and diseases (12). This research method can bring a lot of benefits to traditional Chinese medicine (TCM), because the underlying mechanism of a large proportion of TCM has not been fully understood (13). After network pharmacological analysis, we further confirmed the potential pharmacological effects of *Leonuri Herba* components on PCOS by molecular docking of the analyzed core genes with the main effective drugs. The entire study can be seen in Figure 1.

Materials and methods

Screening and target analysis of active constituents from *Leonuri Herba*

The active components were found by TCMSP (http://tcmspw.com/tcmsp.php), which were screened according to the two ADME attribute values of oral availability (oral bioavailability, OB) ≥30% and drug-like drugs (drug-likeness, DL) ≥ 0.18 (14). A total of 8 active components were analyzed in Table 1. In addition, using the PubChem website (https://pubchem.ncbi.nlm.nih.gov/), get PubChem ID, smiles number, and 2D chemical structures. Then, SwissTargetPrediction (http://swisstargetprediction.ch/) was used to predict protein targets, the feasibility of value as the >0.5, get the target of 189. All target genes are converted into gene symbols using the UniProt knowledge base (http://www.UniProt.org).

Collection of disease targets for PCOS

With "PCOS" and "Polycystic ovary syndrome" as key words, Mine Gene in GeneCard database (https://www. genecards.org) and Map database of OMIM (http://www.omim.org), DisGeNet database (http://bidd.nus.edu.sg/group/cjttd) the potential targets of PCOS, Enter DrugBank database (https://www.drugbank.ca) to search for first-line clinical western drug targets for the treatment of PCOS. In the GeneCards database, a higher score indicates that the target is closely related to the disease. According to experience, if there are too many targets, the target whose Score is greater than the median is set as the potential target of PCOS twice. After merging the four disease database targets together, there are 2,440 disease target intersection genes in the four databases in Figure 2A.

Construction and analysis of disease-medicine network

To explore active ingredients of *Leonuri Herba* targets with PCOS disease targets, the online mapping tool platform (http://www.bioinformatics.com.cn/) was used to draw the Venn diagram, get 116 intersection genes in Figure 2B. Cytoscape3. 7.2 software was used to map the TCM-active ingredients-disease target network.

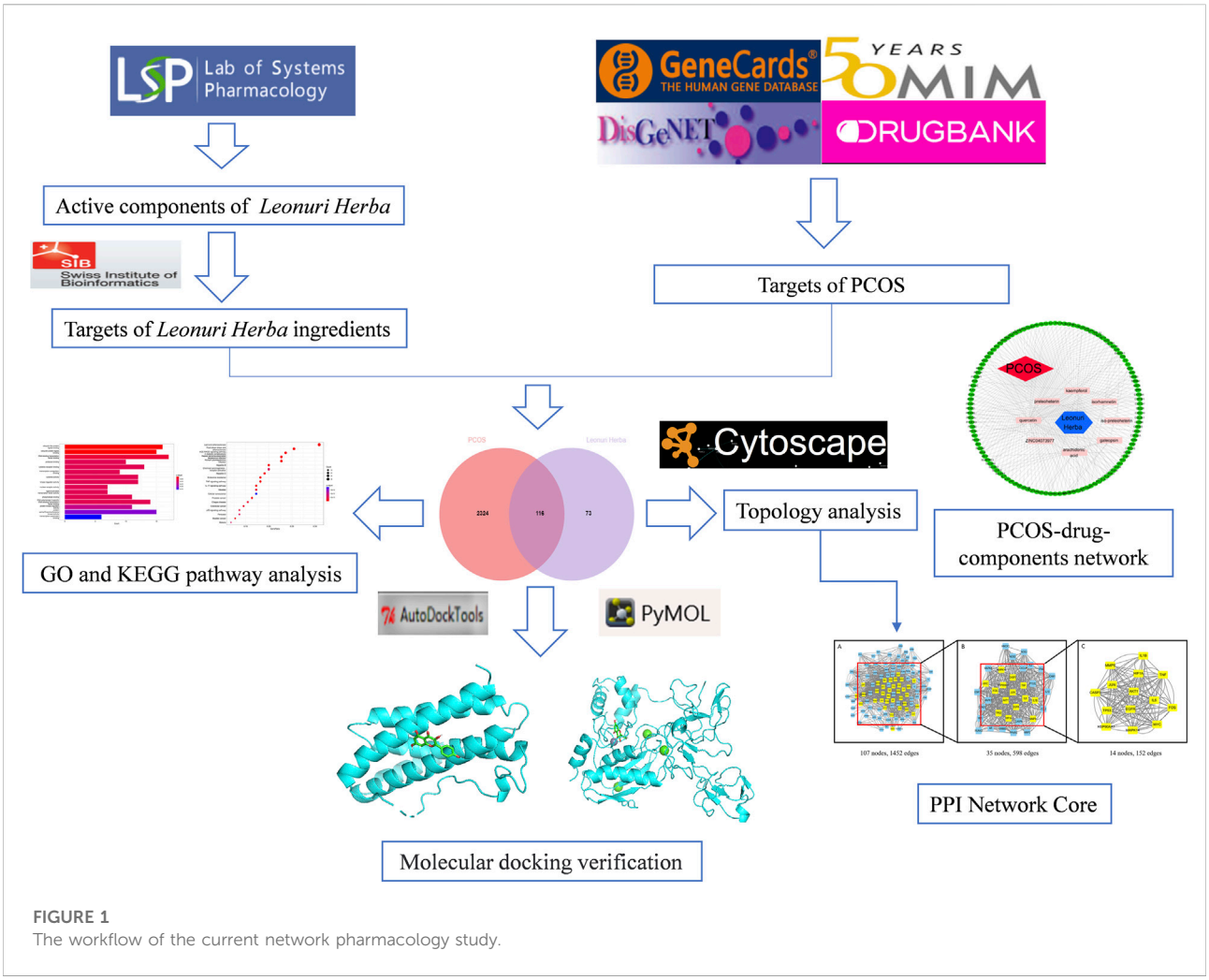

FIGURE 1
The workflow of the current network pharmacology study.

TABLE 1 A list of the active compounds in *Leonuri Herba*.

Mol ID	Molecule name	OB (%)	DL
MOL001418	galeopsin	61.01548	0.3753
MOL001420	ZINC04073977	37.99619	0.75755
MOL001421	preleoheterin	85.97259	0.33044
MOL001422	Iso-preleoheterin	66.28878	0.33032
MOL000098	quercetin	46.43335	0.27525
MOL001439	arachidonic acid	45.57325	0.20409
MOL000354	isorhamnetin	49.60438	0.306
MOL000422	kaempferol	41.88225	0.24066

Constructing PPI network of intersection targets between PCOS and *Leonuri Herba*

The STRING database (https://string-db.org) was used to construct the PPI network diagram of 116 related targets: Set the biological species as "*Homo sapiens*," and the minimum interaction threshold Highest confidence >0.7 as the screening condition. Each node represents a protein and its structure, and each edge represents the association between different proteins.

Screening key targets

The PPI results are exported as TSV files and then imported into Cytoscape3.7.2 for further network analysis using the CytoNCA plug-in (15). Each gene receives a score based on the six dimensions of the "Betweenness/Closeness/Degree/Eigenvector/LAC/Network." 14 key targets were screened out under the condition that the median score was more than 6 parameters.

GO and KEGG pathway enrichment analyses

The results of pathway enrichment analysis from Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and

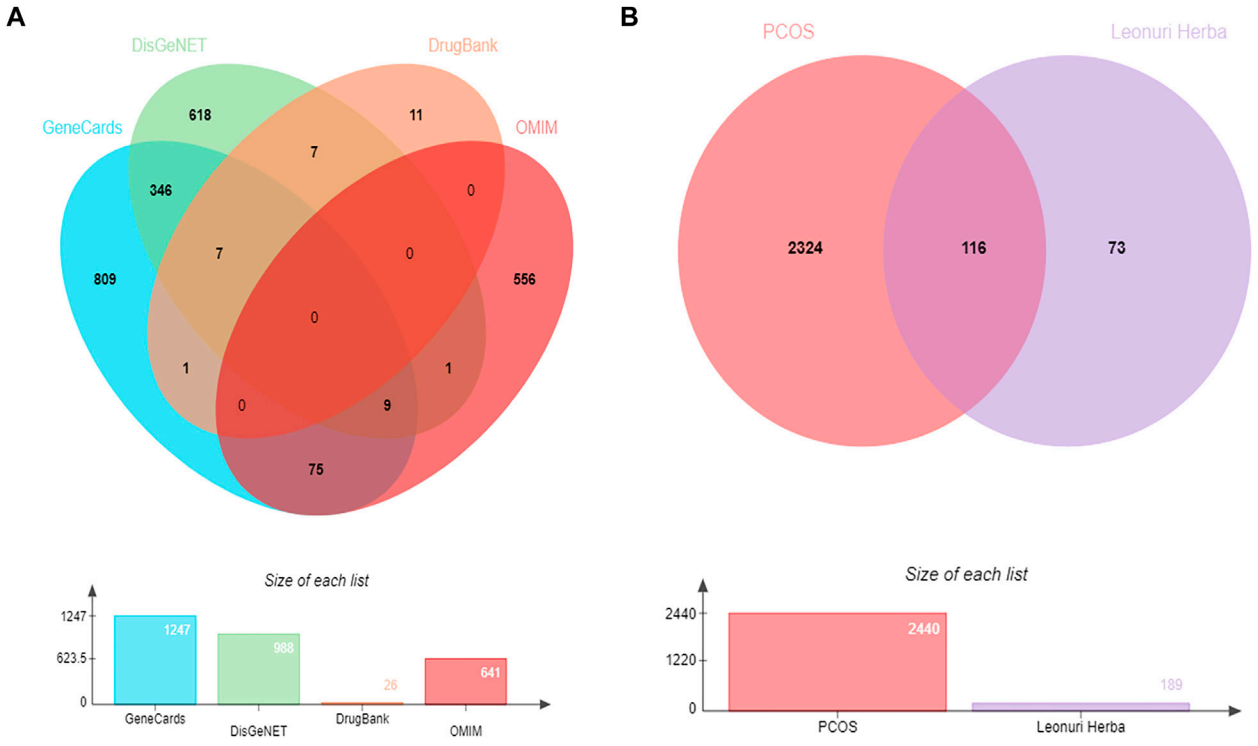

FIGURE 2
(A) Venn diagram of disease targets in four databases. **(B)** Venn diagram of active ingredients and disease targets. PCOS, polycystic ovarian syndrome.

Genomes (KEGG, https://www.kegg.jp/) were applied to the STRING online database (https://string-db.org/) to annotate and classify common targets (16). After setting an adjusted P value cutoff of 0.05, we collected and analyzed the data by Rstudio 3.6.3 (Bioconductor, clusterProfiler).

Molecular docking between *Leonuri Herba* and key targets

According to the enrichment results of compounds KEGG and GO and the comprehensive analysis of the current research status, we selected the two most critical molecules of this drug: quercetin (MOL000098) and kaempferol (MOL000422). TCMSP database (https://tcmspw.com/tcmsp.php) to download the molecular structures and transform them into mol2 formats. The structure of the receptor can be downloaded from the PDB Protein Database (http://www.rcsb.org). The docking simulation was performed with selected key proteins such as AKT1, IL6, EGFR, and MMP9 by AutoDock Vina 1.5.6. The binding affinity between molecules and proteins is predicted based on the docking minimum free energy. The lower the free energy, the higher the affinity. The results are saved in the pdbqt file. Finally, the results were analyzed and demonstrated by PyMOL.

Results

Identification of the ingredients of *Leonuri Herba* and predicted target genes of PCOS

There was a total of 8 active compounds of *Leonuri Herba*, as shown in Table 1. 189 potential targets could be obtained after prediction and deweighting of the active compounds with potential targets through screening in SwissTargetPrediction database. The PCOS related target genes were downloaded from four disease databases, and the genes obtained from GeneCards, DisGeNET, OMIM and DrugBank were screened and de-weighted to obtain 2,440 genes. They were then combined with 189 target genes from *Leonuri Herba* for analysis. Finally, 116 common target genes were extracted. Venn diagrams were plotted accordingly in Figure 2.

Construction and analysis of target PPI network

Target genes were uploaded to STRING online database to form PPI network. 116 nodes (genes) and 726 edges (interactions) were identified, representing the major genes corresponding to the active ingredient of *Leonuri Herba* in Figure 3. The more interacting target

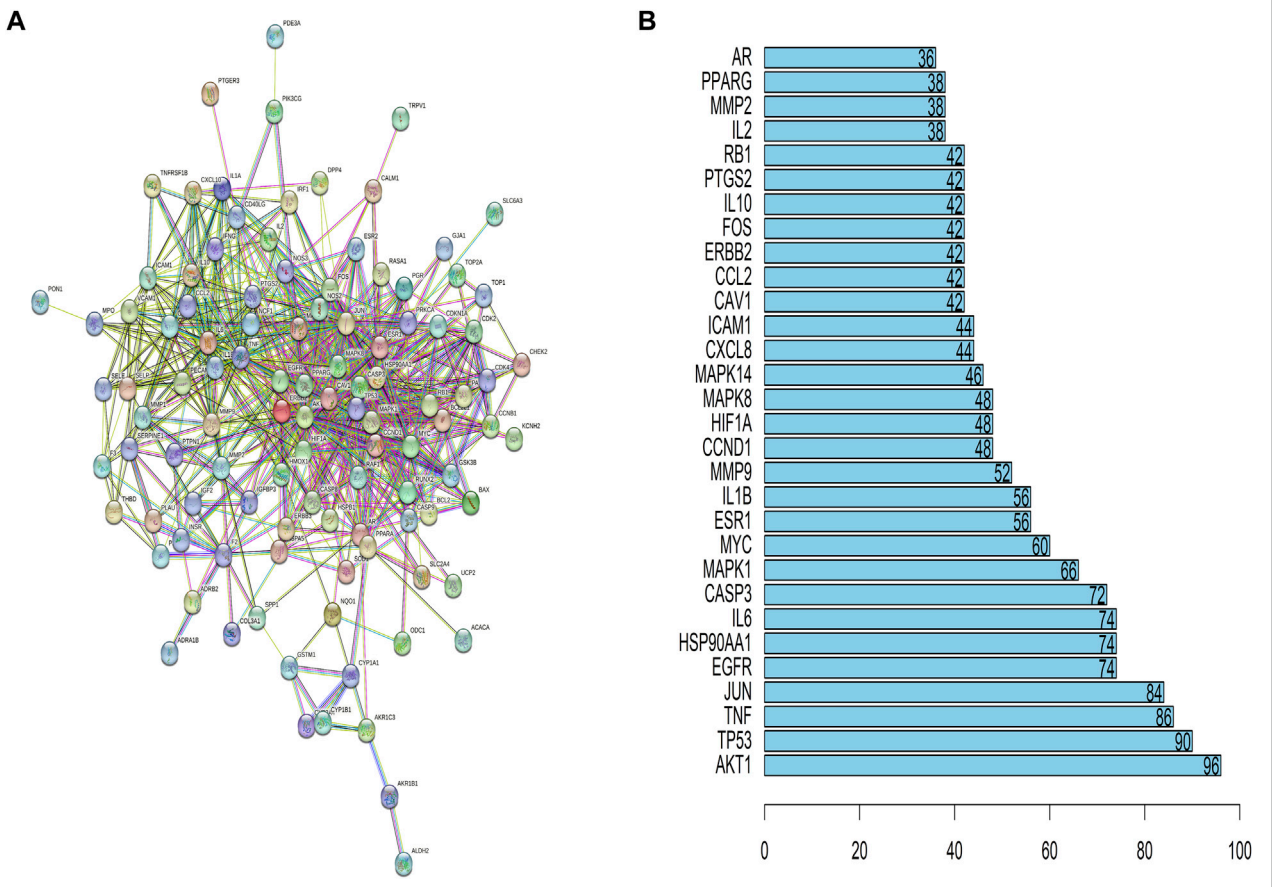

FIGURE 3
(A) Protein–protein interaction network of the common active ingredients and disease targets. 122 nodes (target genes) and 726 edges (associations between proteins) are presented. **(B)** Top 30 PPI network core gene visualization.

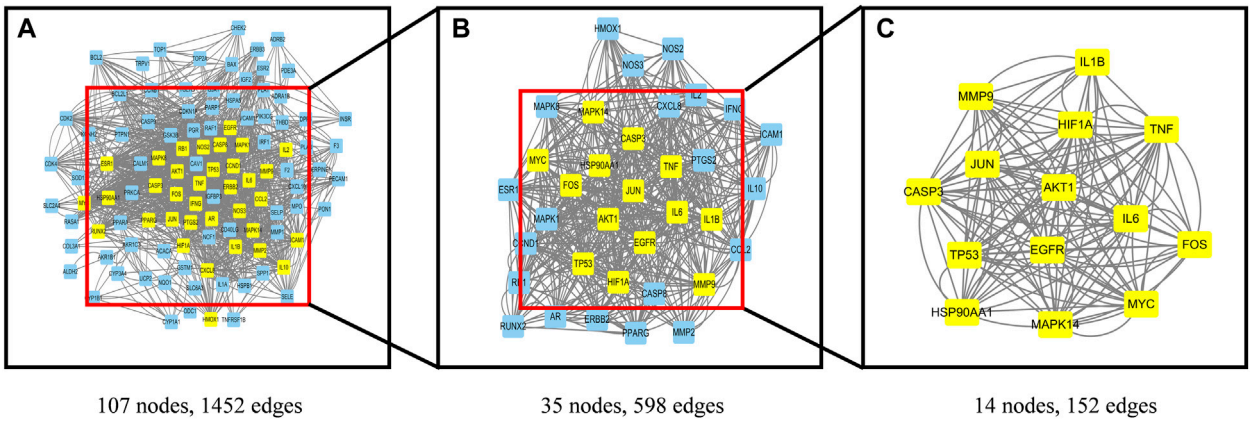

FIGURE 4
Topological analysis of the PPI network. CytoNCA score greater than median. The yellow squares represent the core genes for screening. The blue squares represent unscreened genes. **(A)** The PPI network of PCOS-Leonuri Herba target genes. **(B)** The PPI network of significant proteins extracted from **(A)**. **(C)** The PPI network of crucial Leonuri Herba targets for PCOS treatment extracted from **(B)**.

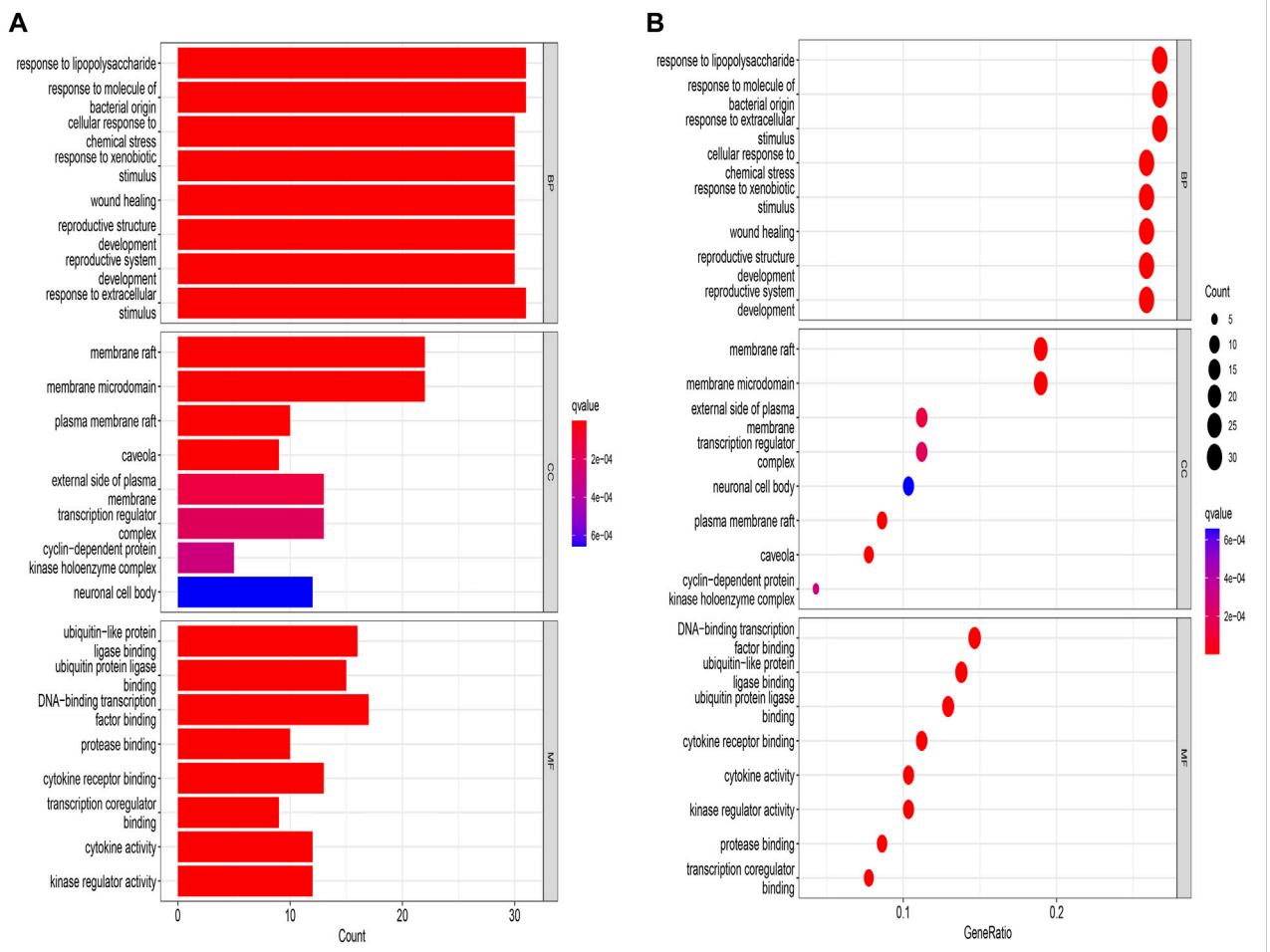

FIGURE 5
GO Pathway Enrichment Analysis. **(A)** The horizontal axis of BP, CC, and MF bar represents the number of genes enriched in each, while the color visualizes the significance based on the corrected P value. **(B)** The bubble diagram demonstrates the gene proportion enriched in each subset. BP, biological process; MF, molecular function; CC, cell component.

genes are located in the central region of the network. RAC-alpha serine/threonine-protein kinase (AKT1), interleukin-6 (IL6), epidermal growth factor receptor (EGFR), vascular endothelial growth factor A (VEGFA), matrix metalloproteinase-9 (MMP9), transcription factor Jun (JUN), myc proto-oncogene protein (MYC), interleukin-1 beta (IL1B), and hypoxia-inducible factor 1-alpha (HIF1A) are most important genes in *Leonuri Herba* 's pharmacological effects on PCOS according to their degree.

The PPI network core of *Leonuri Herba* and PCOS cross targets

The PPI results are exported as TSV files and then imported into Cytoscape3.7.2 for further network analysis using the CytoNCA plug-in. Through 6 parameters in CytoNCA "Betweenness/Closeness/Degree/Eigenvector/LAC/Network" to filter 116 genes. First filter condition, "Betweenness: 49.38088469/Closeness: 0.436213992/Degree: 24/Eigenvector: 0.063172609/LAC: 11.38461538/Network: 12.8". Getting 35 nodes, 598 edges; Second filter condition, "Betweenness: 11.15689699/Closeness: 0.653846154/Degree: 32/Eigenvector: 0.156082243/LAC: 20.66666667/Network: 23.41193154". Get 14 nodes (core genes), 152 edges, specific genes such as JUN, AKT1, HSP90AA1, CASP3, FOS, MYC, EGFR, HIF1A, TP53, TNF, IL6, IL1B, MMP9, MAPK14 in Figure 4. Betweenness/Closeness/Degree/Eigenvector/LAC/Network of the 14 core genes in Supplementary Figure S1.

Biological functional analysis

Subsequently, GO enrichment analysis was performed. The top 8 enrichment results for BP, MFs and CCs are listed in Figure 5. The results suggest that biological processes include

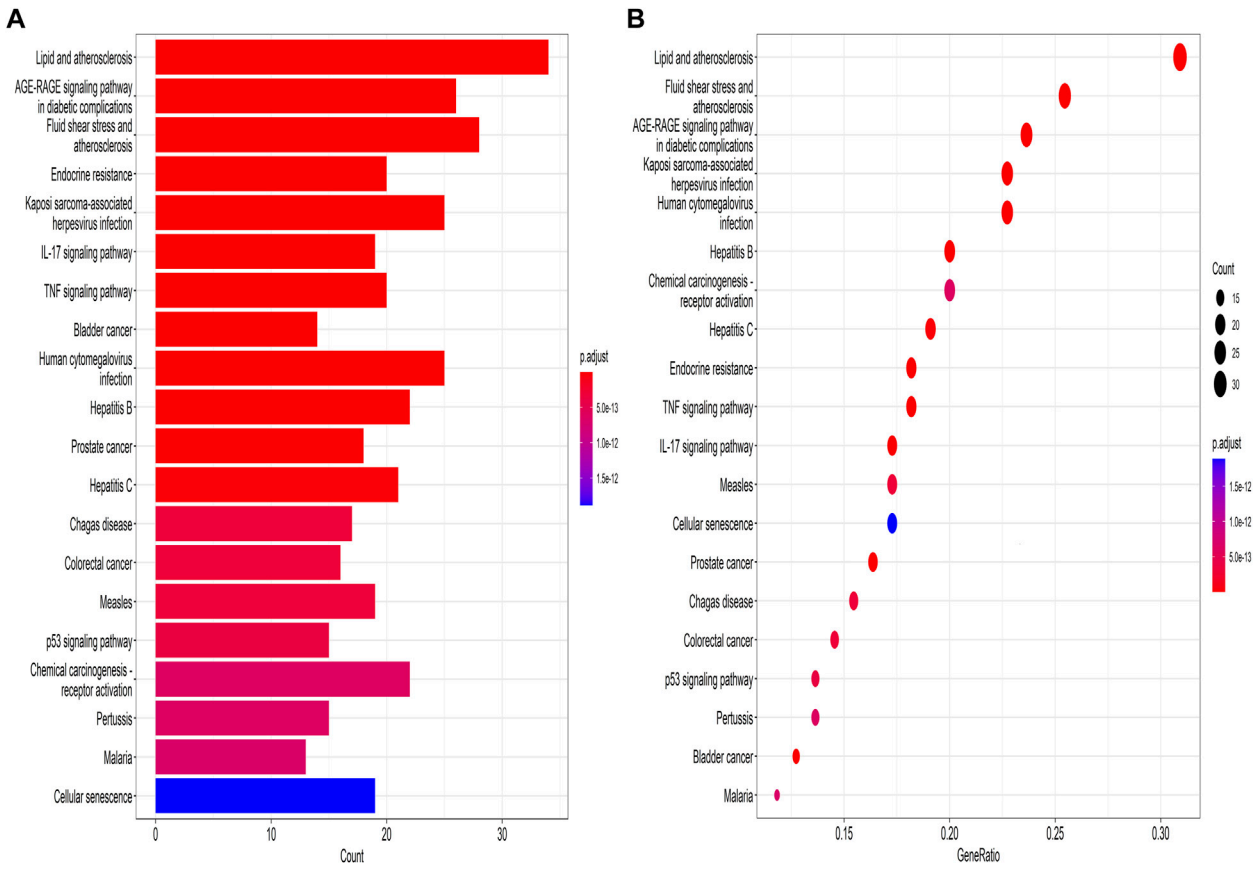

FIGURE 6
KEGG pathway enrichment analysis. **(A)** The red color in the upper part represents greater significance, while the blue represents less significance according to corrected P value. **(B)** The bubble diagram demonstrates the gene proportion enriched in each entry.

cellular responses to lipopolysaccharide, reproductive phylogeny, oxidative stress, and reactive oxygen species. In the drug-disease interaction, the molecular function is manifested by high level of nuclear steroid receptor activity, protein serine/threonine/tyrosine kinase activity, DNA binding and transcription factor binding, and the interaction was mainly enriched in membrane raft and membrane microdomain. The related pathway of *Leonuri Herba* was obtained through KEGG enrichment analysis. 166 signaling pathways were discovered, and the top 20 were shown in Figure 6. Lipid and atherosclerosis (has05417), AGE-RAGE signaling pathway (hsa04933) and fluid shear stress and atherosclerhass (hsa05418) are most prominent in the bar graph of Figure 6A.

Construction of compound-target-disease pathway

Plot the composition-target-pathway network between PCOS and *Leonuri Herba* (Figure 7). The analysis of Figure 7 shows that *Leonuri Herba* ort may have a therapeutic effect on PCOS through multiple active components, targets, and pathways. Among them, quercetin, isorhamnetin, and kaempferol are important components, and the main targets are AKT1, EGFR, IL6, MMP9 and so on.

Molecular docking of the compounds and the core targets

Molecular docking verification was carried out by AutoDock Vina. The results showed that the minimum free energy of quercetin (MOL000098), kaempferol (MOL000422) and key targets AKT1, IL6, EGFR and MMP9 were shown in Table 2. Especially among all the possible binding structures, kaempferol has the best affinity with MMP9 (−9.6 kcal/mol). Other detailed results are shown in Figures 8, 9.

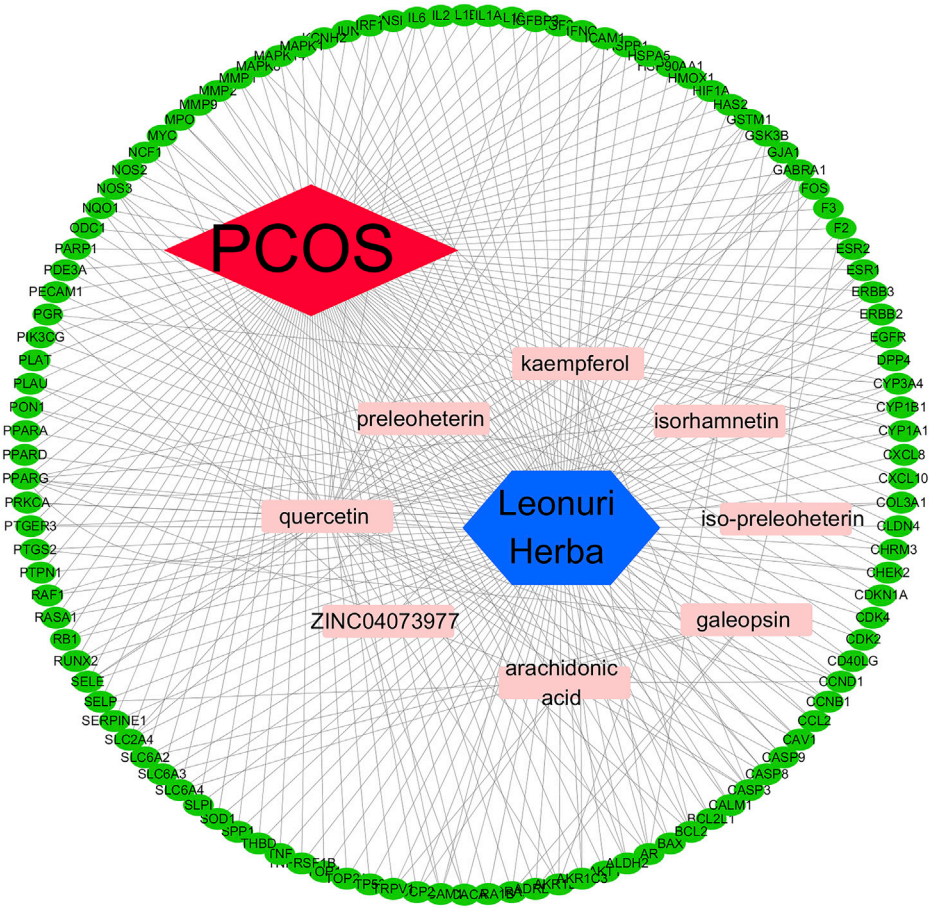

FIGURE 7
Chinese medicine-active ingredient-disease target network. PCOS, polycystic ovarian syndrome.

TABLE 2 Affinity of active chemicals to key targets.

Receptor name	Ligand name	Affinity(kcal/mol)
AKT1	quercetin	−6.1
IL6	quercetin	−6.9
EGFR	quercetin	−8.6
MMP9	quercetin	−8.1
AKT1	kaempferol	−6.1
IL6	kaempferol	−6.7
EGFR	kaempferol	−8.5
MMP9	kaempferol	−9.6

Discussion

PCOS is one of the most common endocrine disorders among women of reproductive age and is heterogeneous in that women may develop reproductive, endocrine, and/or metabolic symptoms that vary throughout their lives (17, 18). Due to its wide range of causes, it can lead to a range of disease symptoms. Such as low fertility, sparse menstruation, hirsutism, and insulin resistance pose a serious threat to women's reproductive health and daily life

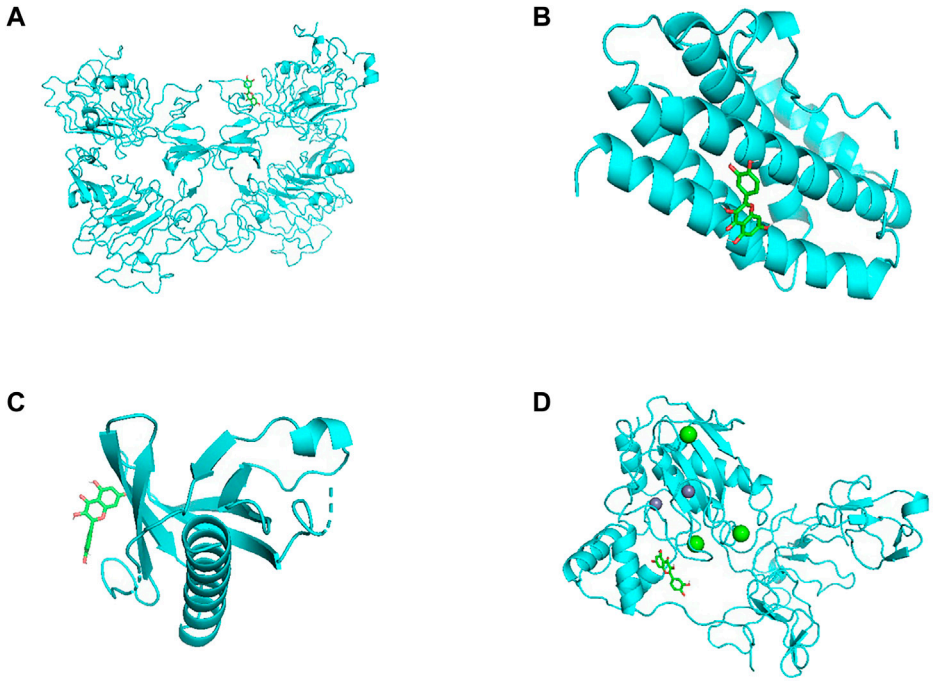

FIGURE 8
(A) Quercetin docked with EGFR. **(B)** Quercetin docked with IL6. **(C)** Quercetin docked with AKT1. **(D)** Quercetin docked with MMP9.

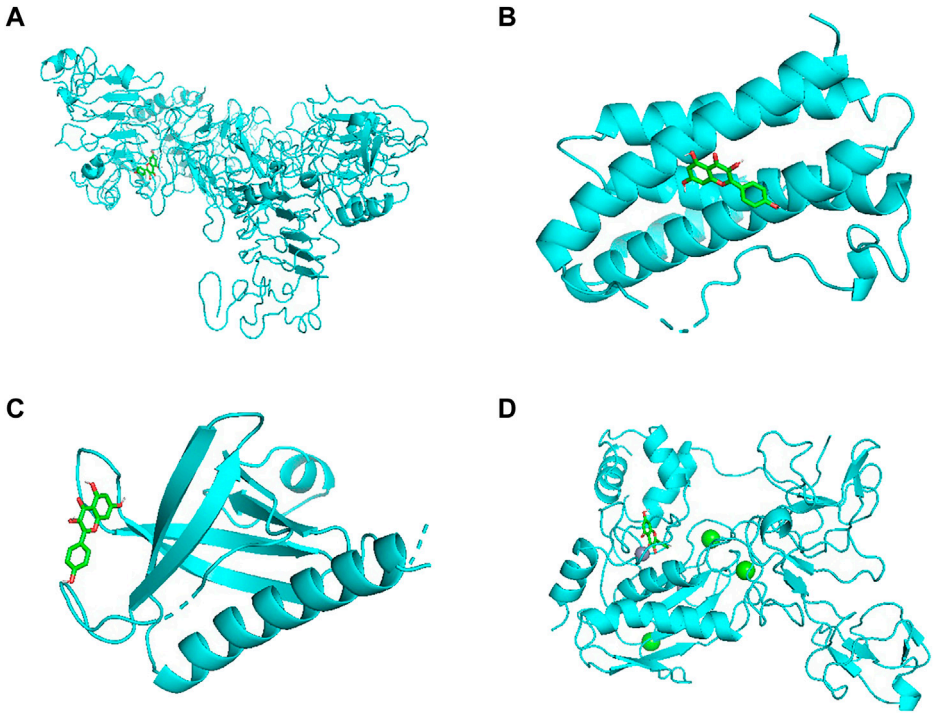

FIGURE 9
(A) Kaempferol docked with EGFR. **(B)** Kaempferol docked with IL6. **(C)** Kaempferol docked with AKT1. **(D)** Kaempferol docked with MMP9.

(19). However, the current western medicine treatment may be unable to solve parts of the symptoms of PCOS. The Chinese medicine and TCM may make up for the shortcomings of Western medicine.

In recent years, more and more Chinese medicines and their preparations have come into the world's attention. Of course, these TCMs can also be effective in treating PCOS, and many studies on the treatment of PCOS with TCMs have been reported (20). For example, TCM can be used to treat PCOS with oligomenorrhea and amenorrhea, relieve ovulation dysfunction, obesity, insulin resistance, and improve ovulation and pregnancy rates (21–24).

Leonuri Herba in this study is one of the traditional Chinese herbs, which has been widely used to treat gynecological and obstetric diseases for thousands of years. Its main ingredients are reported to include leonurine, 4′,5-dihydroxy-7-methoxyflavone, rutin, hyperoside, apigenin, quercetin, kaempferol and salicylic acid (25). So far, its main components have been found to have antioxidant stress, ROS reduction levels, anti-inflammatory, treatment of infertility or menstrual disorders, treatment of cardiovascular and cerebrovascular diseases (9, 26, 27). However, due to the complex chemical composition and unclear pharmacological mechanism of TCM, it faces great obstacles in pharmacological research, quality control and supervision (28). At present, TCM database and target prediction technology have brought new ideas and strategies for the research on the basis and mechanism of TCM pharmacodynamic substances and helped to identify the advantages of TCM such as good efficacy, high safety, multi-component, and multi-target (29). Network pharmacology allows researchers to study the interaction between the chemical composition of *Leonuri Herba* and PCOS-related genes.

In this study, we used the newly developed bioinformatics technology to explore the possible interaction between *Leonuri Herba* and PCOS in the network. We found that quercetin and kaempferol are the main active components of this drug, which can play an important role in anti-inflammation and antioxidation, which has been effectively verified in molecular docking research. In addition, quercetin, the main ingredient, has been shown to be effective in treating PCOS. It can significantly reduce the expression of testosterone (T), estradiol (E2), luteinizing Hormone (LH), Bax, IL-1β, IL-6 and TNF-α, increase the expression of FSH and Bcl-2, and inhibit the expression of androgen receptor (AR), thus restoring the maturation and ovulation of oocytes (30, 31). Moreover, it can also be anti-inflammatory to improve insulin resistance and relieve PCOS endocrine disruption (32, 33). It has been reported that kaempferol-7-O-methylether, another major component, may increase the activity of PPAR-γ and inhibit the TGF-β pathway, thus improving the metabolic disorder and ovarian fibrosis in PCOS rats (34).

In addition, we screened out 14 core genes (JUN, AKT1, HSP90AA1, CASP3, FOS, MYC, EGFR, HIF1A, TP53, TNF, IL6, IL1B, MMP9, MAPK1). Among them, High levels of AKT1 is associated with Granulosa cells (GC) dysfunction (35). MMP9 has been confirmed to be associated with atherosclerotic thrombosis, endothelial dysfunction, and non-alcoholic fatty liver disease in PCOS patients (36–38). IL1B, IL6 and TNF are associated with PCOS inflammation, endoplasmic reticulum stress and recurrent abortion, and can be regulated by the active components of *Leonuri Herba* (39–44). *Leonuri Herba* regulates the expression of these genes through AGE-RAGE, PI3K-Akt and MAPK signaling pathways.

To the best of our knowledge, this is the first time to reveal the active ingredients of *Leonuri Herba* and their pharmacological effects on PCOS. This helps researchers and pharmacologists understand the mechanisms of motherwort. However, further *in vitro* experiments are needed to verify the predicted process.

Conclusion

Through the analysis of network pharmacology, the step-by-step mining of data and the analysis of multi-target and multi-way methods, we can more clearly understand the important role of *Leonuri Herba* in PCOS. Our current research is only from the simple prediction of drug components on the target nuclear pathway of network pharmacology. However, further experiments are needed to confirm the specific therapeutic mechanism of *Leonuri Herba* in the treatment of PCOS. Look forward to the accurate treatment of PCOS with active ingredients of TCM in the future.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

MW and HL designed the study. MW and JZ analyzed the data. MW, JZ, and FD wrote the article. HL gave key advice and embellished the article during the process of revising the manuscript. YG and YC are in charge of the project and guides the writing of the thesis. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/jpps.2023.11234/full#supplementary-material

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