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Potential Molecular Mechanisms of *Paederia Foetida* L. In Gout Treatment Through Network Pharmacology And Molecular Docking

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Abstract

The global incidence of gout has been steadily increasing. *Paederia foetida* L., a traditional medicine, is used to treat gout in Vietnam, though its active compounds' molecular mechanisms remain uncertain. This study used network pharmacology and molecular docking to predict the potential targets and pathways of *P. foetida* bioactive components in gout treatment, providing insights for clinical applications. Compounds and targets of *P. foetida* were identified using the TCMSP database, while targets associated with gout were obtained from GeneCards, TTD, and OMIM databases. A Venn diagram was employed to determine the common targets, and Cytoscape software was used to construct the compound-target-pathway interaction network. GO and KEGG enrichment analyses were performed to identify key biological processes and pathways. AutoDockTools was used to verify molecular docking between active compounds of *P. foetida* and core targets. Five active compounds and 49 common targets were identified. GO enrichment analysis revealed that *P. foetida* influenced multiple biological processes, cellular components, and molecular functions. KEGG analysis elucidated that the primary mechanism of *P. foetida* in gout treatment may be primarily related to the IL-17 signaling pathway and several other anti-inflammatories signaling pathways. Molecular docking confirmed strong binding (affinity < -5 kcal/mol) between five active compounds and core protein targets, including TP53, IL6, HSP90AA1, TNF, IL1B, BCL2, PTGS2, MAPK1, and MAPK8. Among the targets, the docking scores of MAPK8 (7.2-10.1 kcal/mol) were the best. Active compounds such as quercetin, beta-sitosterol, kaempferol, pelargonidin, and paederosidic acid methyl ester exhibited potential therapeutic effects on gout. Through *in silico* screening, the mechanism of action of *P. foetida* in treating gout can be determined to act on multiple targets through multiple pathways. This provides more ideas for *in vitro* and *in vivo* experiments to develop herbal medicines for gout treatment.

Keywords: *Paederia foetida*, gout, network pharmacology, molecular docking

Introduction

Gout is a form of microcrystalline arthritis resulting from the accumulation of monosodium urate crystals within joint spaces or adjacent tissues when the concentration of MSU exceeds its solubility in the blood [1]. Hyperuricemia is the basis of gout disease [2], which is defined according to the guidelines of the Ministry of Health Vietnam (Decision 361/QD-BYT dated January 25, 2014) when uric acid concentration in the blood is > 7 mg/dL (> 420 µmol/L) in men and > 6 mg/dL (> 360 µmol/L) in women. Globally, the incidence of gout has doubled in the past 20 years. In developed countries, the proportion of the population diagnosed with gout is increasing, especially in North America and Europe. Specifically, the incidence of gout in the US adult population in 2015-2016 was 3.9% [3]. A 2012 UK report found that 2.5% of the population had gout, a significant increase since 1997 [4]. In Vietnam, a report from 2023 indicated that the incidence of gout was approximately 1.5% of the population [5]. According to the World Health Organization, about 3.9% of the global population had gout in 2015 [6]. The incidence of gout worldwide has been increasing in recent years, mainly due to dietary habits that include frequent consumption of fast food, lack of exercise, rising rates of obesity, and metabolic syndrome [7]. This trend emphasizes the need for preventive measures and lifestyle changes. Modern

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medicine has made many advances in controlling gout, mainly focusing on addressing the problem of anti-inflammation, pain relief in the acute phase, and controlling serum uric acid levels in the chronic phase. However, there are still many concerns about the adverse reactions of pharmaceutical drugs when used for a long time [8]. Therefore, it is imperative to discover safer natural medications to treat gout.

Paederia foetida L. is a long-standing medicinal herb used in traditional Chinese, Vietnamese, Indian, and other traditional medicine systems to treat arthritis, asthma, dysentery, hemorrhoids, diarrhea, bladder stones, diabetes, and other ailments [9,10]. Extracts, essential oils, and purified compounds isolated from *P. foetida* exhibit a wide range of biological and pharmacological activities, including anti-hyperuricemic, antiarthritic, analgesic, antibacterial, antioxidant, antidiabetic, hepatoprotective, nephroprotective, wound healing, and anticonvulsant effects [10]. According to folk experience in Vietnam, *P. foetida* is used by traditional medicine practitioners to treat gout effectively. However, the mechanism of action of *P. foetida* in treating gout has not been comprehensively and systematically evaluated at the molecular level.

Understanding compounds' mechanisms and synergistic effects is crucial for effectively utilizing medicinal materials and traditional medicines in new drug research [11]. Network pharmacology and molecular docking have emerged as valuable tools for comprehensively assessing the relationships between pharmacological data and targets in multi-mechanism diseases by establishing a "compound-protein/gene-disease" network [12]. Therefore, applying these modern techniques, this study aims to explore the molecular mechanism of *P. foetida* in gout treatment through computer simulation. From there, build a network of active components of *P. foetida* and their therapeutic targets for gout. Molecular docking determines the binding ability of the compounds and potential targets. The research flowchart is shown in Figure 1.

Materials and Methods

2.1. Collection of compounds of *P. foetida*

The components in *P. foetida* (also called JiShiTeng in traditional Chinese medicines) were collected based on the TCMSP platform (https://www.tcmsp-e.com/#/home) [13] with the keyword "JiShiTeng". The potential active compounds in *P. foetida* were obtained with the criteria of OB ≥ 30% and DL ≥ 0.18 [6,14]. OB (oral bioavailability) measures the proportion of an orally administered drug entering the body's circulatory system, reflecting the efficiency of absorption, distribution, metabolism, and excretion [15]. DL (Druglikeness) evaluates the potential of a compound to act like a drug. The DL value of 0.18 is often used as a threshold for selecting drug-like

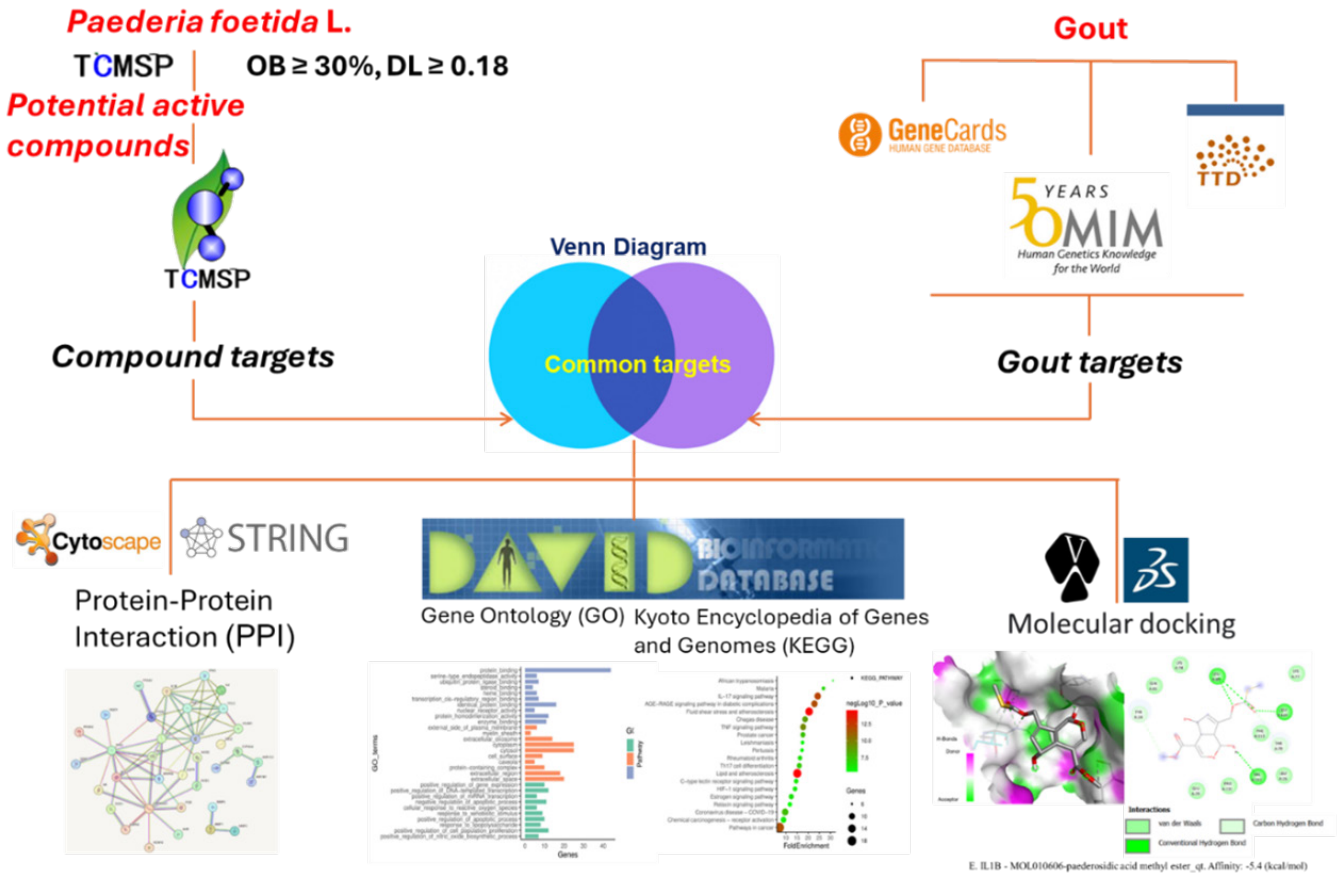


Figure 1. The research flowchart

compounds from traditional Chinese herbs [16].

2.2. Collection of compound targets, gout targets, and common targets

Collection of compound targets: The targets related to the potential components in *P. foetida* are collected through the TCMSP platform, and the corresponding gene names of targets were standardized to official gene symbols in the Uniprot protein database (https://www.uniprot.org/) [17].

Collection of gout targets: The keyword "gout" was used. The databases to collect gout targets include GeneCard (https://www.genecards.org) [18], Therapeutic Target Database (https://db.idrblab.net/ttd/) [19], OMIM (https://www.omim.org) [20]. All target names were reviewed against the Uniprot database to avoid name bias, with the species limit criterion being "Homo sapiens."

Collection of common targets: After obtaining the targets of *P. foetida* and gout, use the Venn graph on the website https://bioinfogp.cnb.csic.es/tools/venny/ [21] to obtain the common target.

2.3. Construction of compound-common target network and protein-protein interaction network

Construction of compound-common target network: To visualize the complex connections between compounds and potential targets, Cytoscape software (version 3.10.1; http://cytoscape.org/) was used to construct a network, with nodes designating compounds or targets, while edges connecting them indicate their interactions.

Construction of Protein-Protein Interaction Network: The STRING dataset is one of the most notable protein-protein interaction (PPI) databases, which was used to help construct the PPI interaction network, and the common targets of *P. foetida* and gout were imported into STRING (https://string-db.org) [22]. The scoring threshold was set to > 0.90 to ensure high confidence in the data, and isolated proteins were excluded from visualization. In addition, the selected target proteins were restricted to "Homo sapiens". Then, Cytoscape software was used to visualize the results of the analysis. "Nodes" and "edges" are the main components of a network. Nodes interact with one another through connections edges. The degree of interaction for each node in a Protein-Protein Interaction (PPI) network is represented by various topological parameters, including degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC) [23]. Key nodes exhibit higher values for these network topological parameters than others, indicating their significance within the interaction network. The Network Analysis tool in Cytoscape will be used to calculate the values of these topological parameters [23,24].

2.4. GO enrichment analysis and KEGG pathway enrichment analysis

Biochemical pathway and function discovery is performed to elucidate the mechanism, and biochemical pathway-target network construction is performed. This stage is performed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis on important targets. GO annotates gene properties and gene products uniformly across species. GO includes three independent ontologies: biological process, molecular function, and cellular component [25]. KEGG is a knowledge base for systematically analyzing gene functions and linking genomic and high-level functional information. KEGG can find the pathways leading to diseases that are maximally correlated with important targets, thereby discovering the mechanism of medicinal herbs on pathology [26]. Finding GO ontology or KEGG pathways and their association with targets is called "enrichment" analysis. And one of the tools used for this is DAVID (https://david-d.ncifcrf.gov) [27].

Common targets will be analyzed using the DAVID tool. GO Enrichment and KEGG terms were filtered with a stringent p-value threshold of < 0.05 [27]; the smaller the p-value, the more biological processes, molecular functions, cellular components, or KEGG pathways are involved in the important nodes [28]. The 20 pathways with the most significant p-values were selected for further exploration. Among these, pathways with the most significant potential relevance to gout will be prioritized for detailed analysis. Subsequently, a table will be generated to summarize the biological processes associated with gout.

2.5. Molecular docking

Molecular docking is a method used to model the interaction between a small molecule (called ligand) and a macromolecular structure (protein or enzyme), thereby allowing the study of the interaction of the molecule in the binding site as well as clarifying the related biological mechanisms [29]. The molecular docking process goes through the following stages: protein preparation, ligand preparation, and molecular docking. The protein structures of the potential targets are obtained from the PDB (Protein Data Bank) (http://www.rcsb.org) in *.pdb format. Then, the co-crystallized molecules (water, solvent, co-crystallized ligand) will be removed from the protein using BIOVIA Discovery Studio Visualizer 2021 software. Hydrogen atoms were added to the proteins and exported to *.pdbqt format using AutoDockTools 1.5.7. Ligands were obtained from the TCM database and converted to *.pdbqt format with AutoDockTools 1.5.7. AutoDock Vina 1.1.2 software is used for molecular docking [30]. Molecular docking was performed at the following parameters: spacing is 1.00, exhaustiveness is 20, and energy_range is 4. The optimal docking results were visualized using BIOVIA Discovery Studio Visualizer. Binding energy less than -5 kcal/mol shows that the compound has a strong binding force with the potential target [24].

Results and Discussion

3.1.1. Collection compounds of *P. foetida*

With the keyword "Jishiteng", 119 components of *P. foetida* were found in the TCMSP database. Through the two parameters, OB and DL, 11 potential active compounds were finally

Table 1. Potential active compounds of *P. foetida* in gout treatment

No	MOL ID	Molecular name	Group	OB (%)	DL
1	MOL010606	Paederosidic acid methyl ester_qt	Iridoid	67.65	0.19
2	MOL010603	Paederoside	Iridoid	57.18	0.75
3	MOL000098	Quercetin	Flavonoid	46.43	0.27
4	MOL000422	Kaempferol	Flavonoid	41.88	0.24
5	MOL004798	Delphinidin	Flavonoid	40.63	0.27
6	MOL001790	Linarin	Flavonoid	39.84	0.70
7	MOL001004	Pelargonidin	Flavonoid	37.98	0.21
8	MOL001771	Poriferast-5-en-3beta-ol	Sterol	36.91	0.75
9	MOL000358	Beta-sitosterol	Sterol	36.91	0.75
10	MOL000359	Sitosterol	Sterol	36.91	0.75
11	MOL001689	Acacetin	Flavonoid	34.97	0.24

obtained. Among them, paederosidic acid methyl ester_qt is iridoid, quercetin, kaempferol, delphinidin, linarin, pelargonidin, and acacetin are flavonoids, while sitosterol and beta-sitosterol are steroids. Thus, the compounds that may be related to gout treatment belong to the groups of flavonoids, iridoids, and steroids (Table 1).

3.1.2. Collection of compound targets, gout targets, and common targets

Collection of compound targets: From eleven potential compounds of *P. foetida*, the corresponding target proteins were identified on the TCMSP platform, from which the corresponding gene names were identified on the Uniprot database. But only 10/11 compounds found related targets, which were: paederosidic acid methyl ester_qt, quercetin, kaempferol, delphinidin, linarin, pelargonidin, poriferast-5-en-3beta-ol, beta-sitosterol, sitosterol, acacetin corresponding to 98 potential targets after duplicate removal.

Collection of gout targets and common targets: We found 1687 targets in GeneCards databases, 36 in OMIN databases,

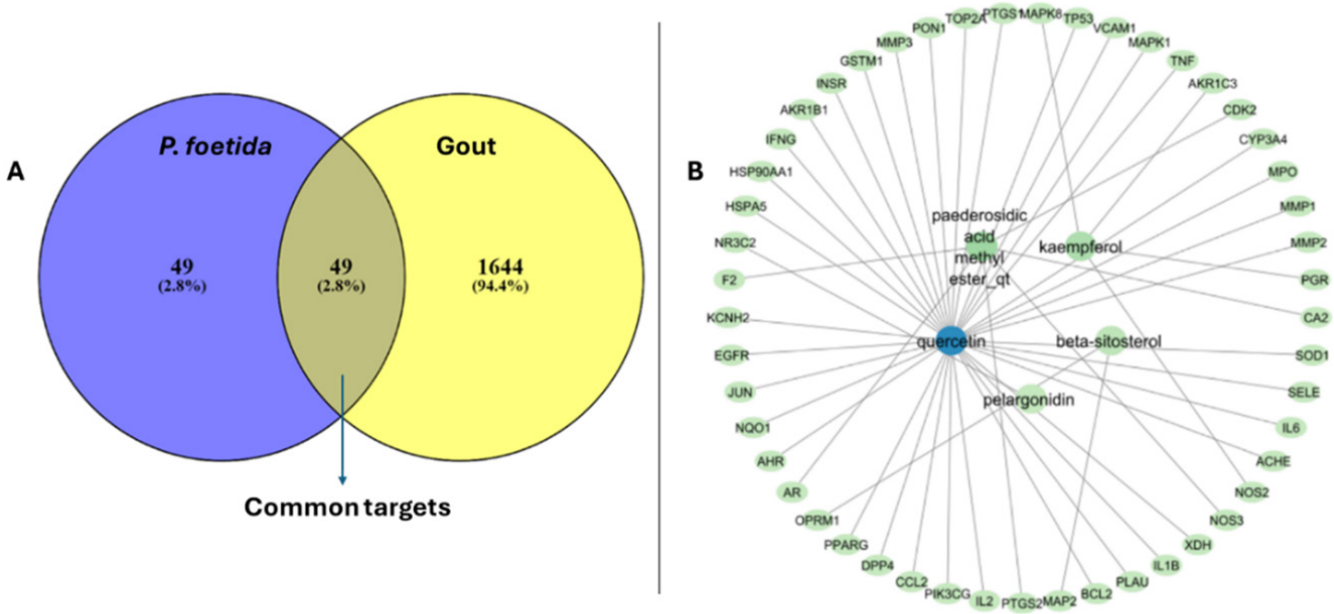


Figure 2. A: Common targets of *P. foetida* and gout. Note: Yellow symbolizes the integer of targets for gout, and blue symbolizes the integer of targets of active components in *P. foetida*. The intersection is the common targets. B: Network compound-common target. The darker the node's color, the more target it is associated with.

and 20 in TTD databases. After deleting the duplicate targets, 1697 gout-related targets were obtained. Get the Venn diagram based on targets of potential active components and disease targets. The result showed 49 common targets of *P. foetida* and gout.

Construction of compound-common target network: Notably, the only five components related to 49 common targets were quercetin, paederosidic acid methyl ester_qt, kaempferol, beta-sitosterol, and pelargonidin with corresponding 36, 6, 4, 2, and 1 targets, respectively. They were the active compounds of *P. foetida* in gout treatment (Figure 2).

3.1.3. Construction of protein-protein interaction network (PPI)

Forty-nine common targets were inputted using the STRING tool to construct the PPI network. After removing the disconnected nodes, the network obtained 35 nodes (Figure 3A). Using the Cluster function on the STRING tool to cluster the nodes that are related to each other, with the DBSCAN method, there were 21 impact targets concentrated in one significant cluster, and the IL-17 signaling pathway was most highly associated with this cluster of nodes (Figure 3B).

Use the Analyze network tool in Cytocape 3.10.1 software to calculate the parameters of nodes with DC, CC, and BC as screening criteria. The larger nodes of DC, CC, and BC (greater than or equal to the median value of all nodes) are important in the network. After screening, 14 targets were obtained (Table 3). Among them, the top ten-degree values include cellular tumor antigen p53 (*TP53*) (DC=11), interleukin-6 (*IL6*) (DC=11), heat shock protein HSP 90-alpha (*HSP90AA1*) (DC=10), transcription factor AP-1 (*JUN*) (DC=8), tumor necrosis factor (*TNF*) (DC=8), interleukin-1 beta (*IL1B*) (DC=7), apoptosis regulator Bcl-2 (*BCL2*) (DC=6), prostaglandin G/H synthase 2 (*PTGS2*) (DC=6), mitogen-activated protein kinase 1 (*MAPK1*) (DC=5), mitogen-activated protein kinase 8 (*MAPK8*) (DC=5) may be the core targets for *P. foetida* to play a critical function in the treatment of gout.

3.1.4. GO and KEGG enrichment analysis

Based on 49 common targets, 271 entries were acquired by GO enrichment analysis (p-value < 0.05), including 194 BP entries involving: positive regulation of nitric oxide biosynthetic process ($p = 9.90\times10^{-10}$), positive regulation of cell population proliferation ($p = 2.70\times10^{-8}$), response to lipopolysaccharide ($p = 4.30\times10^{-8}$), positive regulation of apoptotic process ($p = 7.00\times10^{-8}$), response to xenobiotic stimulus ($p = 1.60\times10^{-7}$), cellular response to reactive oxygen species ($p = 1.80\times10^{-7}$), negative regulation of apoptotic process ($p = 3.10\times10^{-7}$), positive regulation of miRNA transcription ($p = 3.40\times10^{-7}$), positive regulation of DNA-templated transcription ($p = 8.50\times10^{-7}$), positive regulation of gene expression ($p = 3.20\times10^{-6}$)…; 28 CC entries involving: extracellular space ($p = 4.00\times10^{-8}$), extracellular region ($p = 2.10\times10^{-6}$), protein-containing complex ($p = 2.20\times10^{-5}$), caveola ($p = 2.60\times10^{-5}$), cell surface ($p = 1.10\times10^{-4}$), cytosol ($p = 5.90\times10^{-4}$), cytoplasm ($p = 7.80\times10^{-4}$), extracellular exosome ($p = 1.40\times10^{-4}$), myelin sheath ($p = 2.60\times10^{-3}$), external side of plasma membrane ($p = 3.00\times10^{-3}$)…; 49 MF entries involving: enzyme binding ($p = 2.10\times10^{-8}$), protein homodimerization activity ($p = 1.70\times10^{-6}$), nuclear receptor activity ($p = 9.70\times10^{-6}$), identical protein binding ($p = 1.20\times10^{-}$

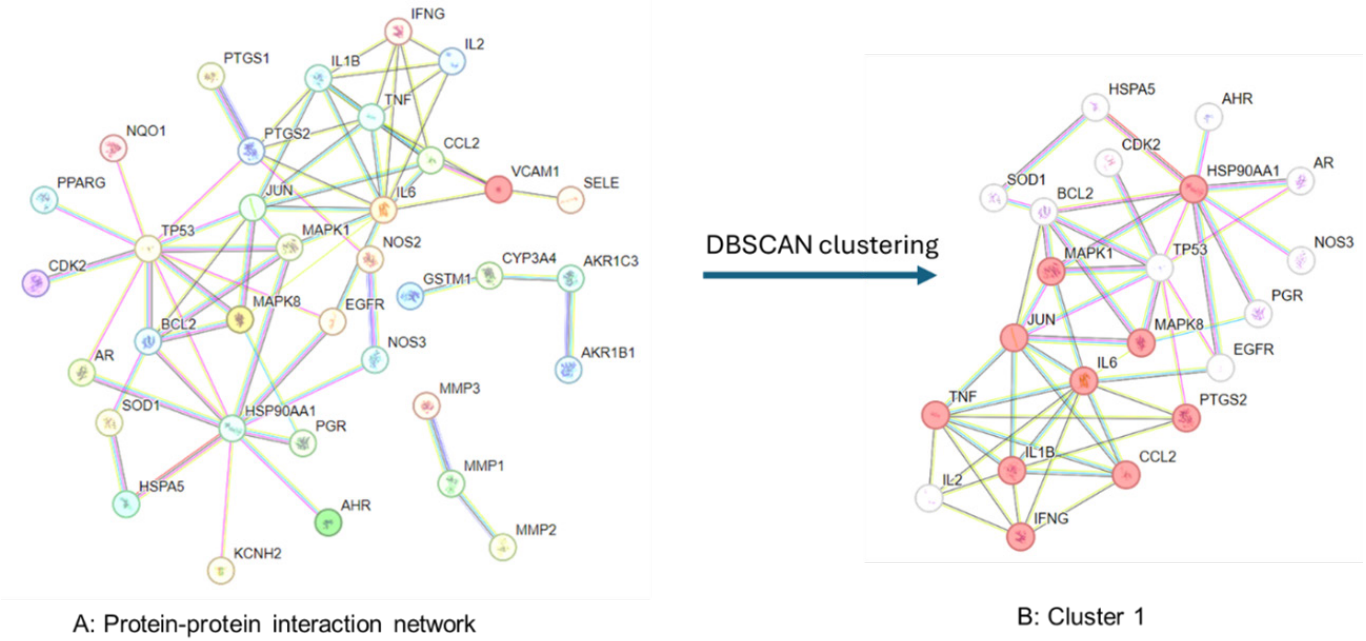


Figure 3. A: Protein-protein interaction network of *P. foetida* for gout. Note: The nodes illustrate the protein, and the edges are protein interactions in the network. B: Cluster 1, the red nodes in the cluster represent the targets involved in the IL-17 signaling pathway.

Table 2. The important targets in the PPI network

No	Gene Name	Degree centrality (DC)	Betweenness centrality (BC)	Closeness centrality (CC)
1	*TP53*	11	0.331706	0.5625
2	*IL6*	11	0.228267	0.519231
3	*HSP90AA1*	10	0.292042	0.509434
4	*JUN*	8	0.117997	0.509434
5	*TNF*	8	0.050083	0.450000
6	*IL1B*	7	0.022447	0.435484
7	*BCL2*	6	0.080379	0.490909
8	*PTGS2*	6	0.171680	0.500000
9	*MAPK1*	5	0.065670	0.519231
10	*MAPK8*	5	0.052787	0.482143
11	*EGFR*	3	0.048688	0.490909
12	*AKR1C3*	2	0.666667	0.750000
13	*CYP3A4*	2	0.666667	0.750000
14	*MMP1*	2	1.000000	1.000000

Table 3. 20 KEGG pathways associated with the mechanism of action of *P. foetida* in treating gout.

No	Pathway	*p*-value	Related Target
1	Lipid and atherosclerosis	3.19×10^{-15}	*JUN, HSP90AA1, VCAM1, HSPA5, NOS3, MMP1, MMP3, SELE, TNF, IL6, MAPK8, IL1B, BCL2, CCL2, MAPK1, PPARG, TP53*
2	Fluid shear stress and atherosclerosis	3.92×10^{-15}	*NQO1, JUN, HSP90AA1, GSTM1, VCAM1, NOS3, MMP2, SELE, TNF, MAPK8, IFNG, IL1B, BCL2, CCL2, TP53*
3	IL-17 signaling pathway	9.17×10^{-13}	*IL6, HSP90AA1, JUN, MAPK8, IFNG, MMP1, IL1B, MMP3, MAPK1, CCL2, PTGS2, TNF*
4	AGE-RAGE signaling pathway in diabetic complications	1.83×10^{-12}	*IL6, JUN, MAPK8, VCAM1, NOS3, IL1B, MMP2, BCL2, MAPK1, CCL2, SELE, TNF*
5	Pathways in cancer	2.74×10^{-12}	*NQO1, JUN, HSP90AA1, GSTM1, NOS2, MMP1, MMP2, F2, PTGS2, EGFR, IL2, AR, IL6, MAPK8, IFNG, CDK2, BCL2, MAPK1, PPARG, TP53*
6	TNF signaling pathway	2.86×10^{-10}	*IL6, JUN, MAPK8, VCAM1, IL1B, MMP3, MAPK1, CCL2, PTGS2, SELE, TNF*
7	Chagas disease	1.74×10^{-9}	*IL6, JUN, MAPK8, IFNG, NOS2, IL1B, MAPK1, CCL2, TNF, IL2*
8	Coronavirus disease - COVID-19	1.94×10^{-8}	*IL6, JUN, MAPK8, MMP1, IL1B, MMP3, MAPK1, CCL2, F2, TNF, EGFR, IL2*
9	Prostate cancer	2.63×10^{-8}	*AR, HSP90AA1, PLAU, CDK2, MMP3, BCL2, MAPK1, TP53, EGFR*
10	Th17 cell differentiation	5.66×10^{-8}	*IL6, HSP90AA1, JUN, MAPK8, IFNG, IL1B, MAPK1, AHR, IL2*
11	Malaria	1.66×10^{-7}	*IL6, VCAM1, IFNG, IL1B, CCL2, SELE, TNF*
12	Rheumatoid arthritis	4.00×10^{-7}	*IL6, JUN, IFNG, MMP1, IL1B, MMP3, CCL2, TNF*
13	Estrogen signaling pathway	4.03×10^{-7}	*HSP90AA1, JUN, NOS3, MMP2, BCL2, MAPK1, PGR, OPRM1, EGFR*
14	C-type lectin receptor signaling pathway	8.54×10^{-7}	*IL6, JUN, MAPK8, IL1B, MAPK1, PTGS2, TNF, IL2*
15	Chemical carcinogenesis -receptor activation	1.04×10^{-6}	*AR, HSP90AA1, JUN, GSTM1, BCL2, MAPK1, PGR, AHR, CYP3A4, EGFR*
16	African trypanosomiasis	1.05×10^{-6}	*IL6, VCAM1, IFNG, IL1B, SELE, TNF*
17	HIF-1 signaling pathway	1.10×10^{-6}	*IL6, IFNG, NOS2, NOS3, INSR, BCL2, MAPK1, EGFR*
18	Leishmaniasis	2.23×10^{-6}	*JUN, IFNG, NOS2, IL1B, MAPK1, PTGS2, TNF*
19	Pertussis	2.41×10^{-6}	*IL6, JUN, MAPK8, NOS2, IL1B, MAPK1, TNF*
20	Relaxin signaling pathway	3.61×10^{-6}	*JUN, MAPK8, NOS2, MMP1, NOS3, MMP2, MAPK1, EGFR*

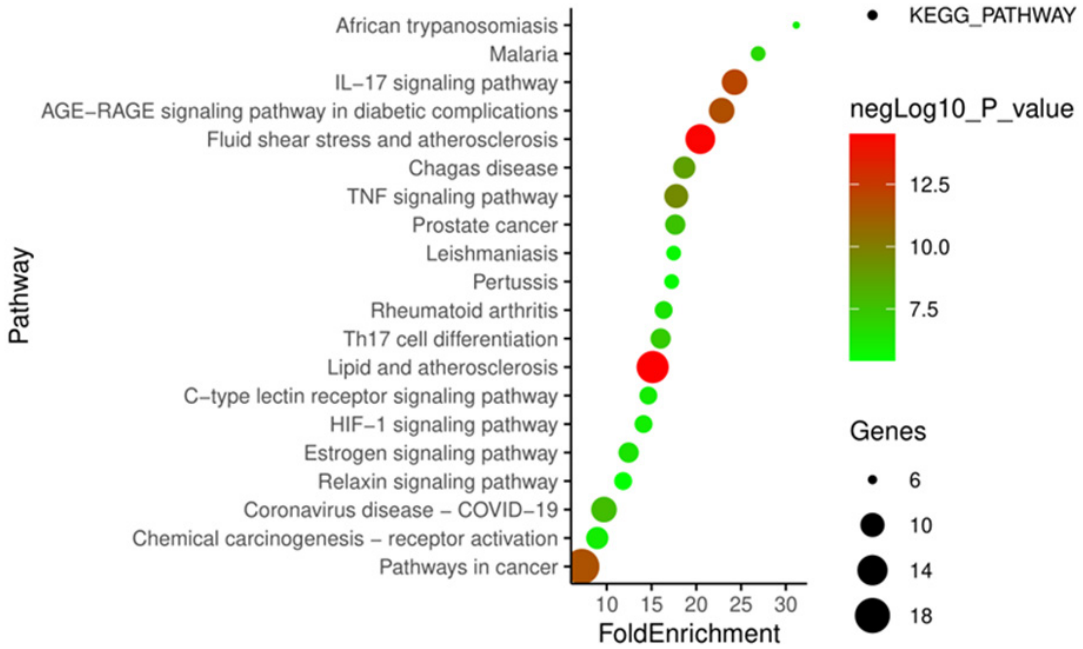


Figure 4. KEGG pathway enrichment analysis. The top twenty pathways were displayed on the Y-axis Fold Enrichment in the X-axis ($p < 0.05$). The coloring symbolizes the different p-value ranges; the shadier the color, the more important the enrichment. The dark circles symbolize the number of genes related to the pathway; the larger the size, the more genes are involved.

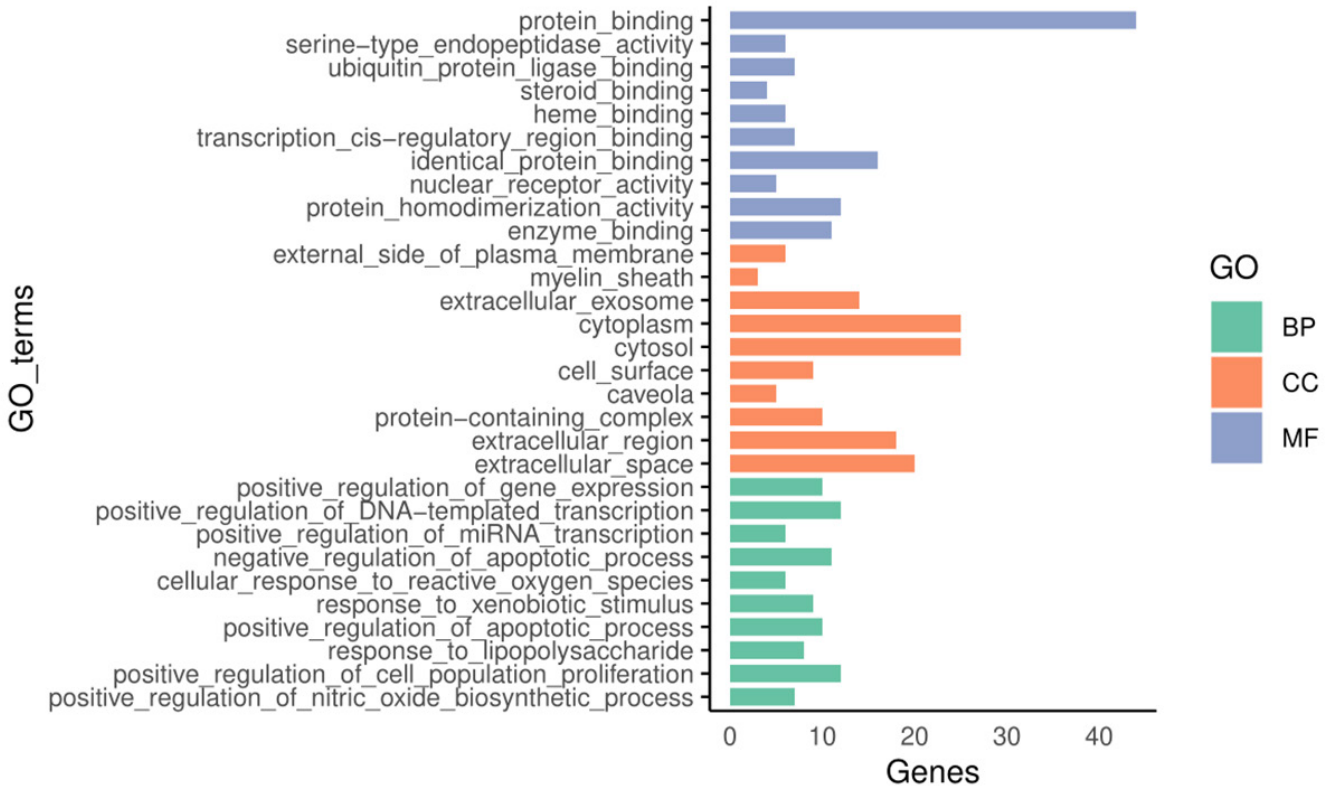


Figure 5. GO functional enrichment analysis. The Y-axis symbolizes BP (green), CC (orange), and MF (blue) names, and the X-axis represents the number of genes.

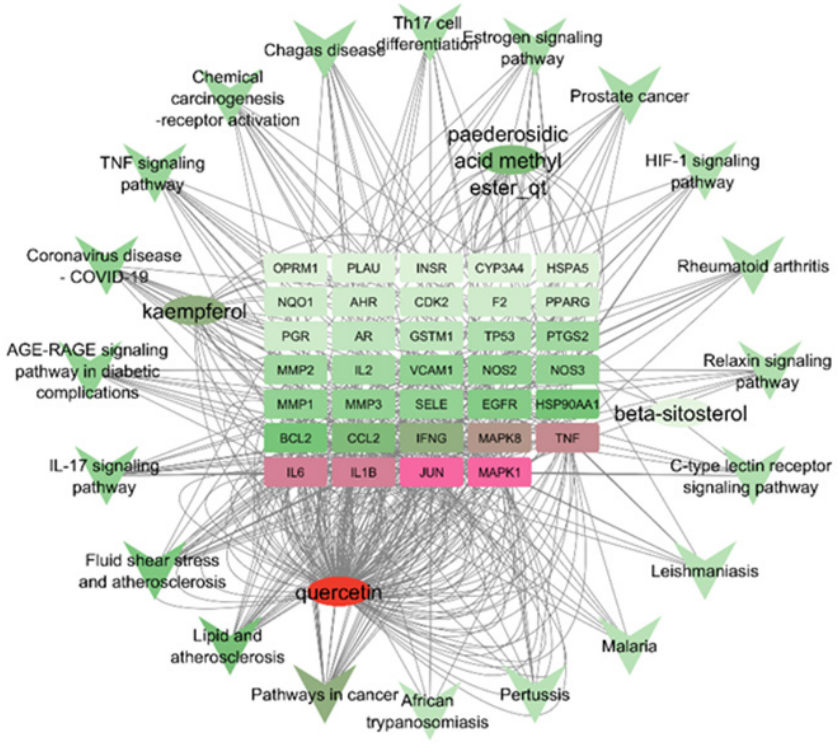


Figure 6. The network graph of the functional compound-target-KEGG pathway. The triangles represent KEGG pathways, the ellipses represent targets, and the rectangles represent active compounds. The darker the color, the more important the node.

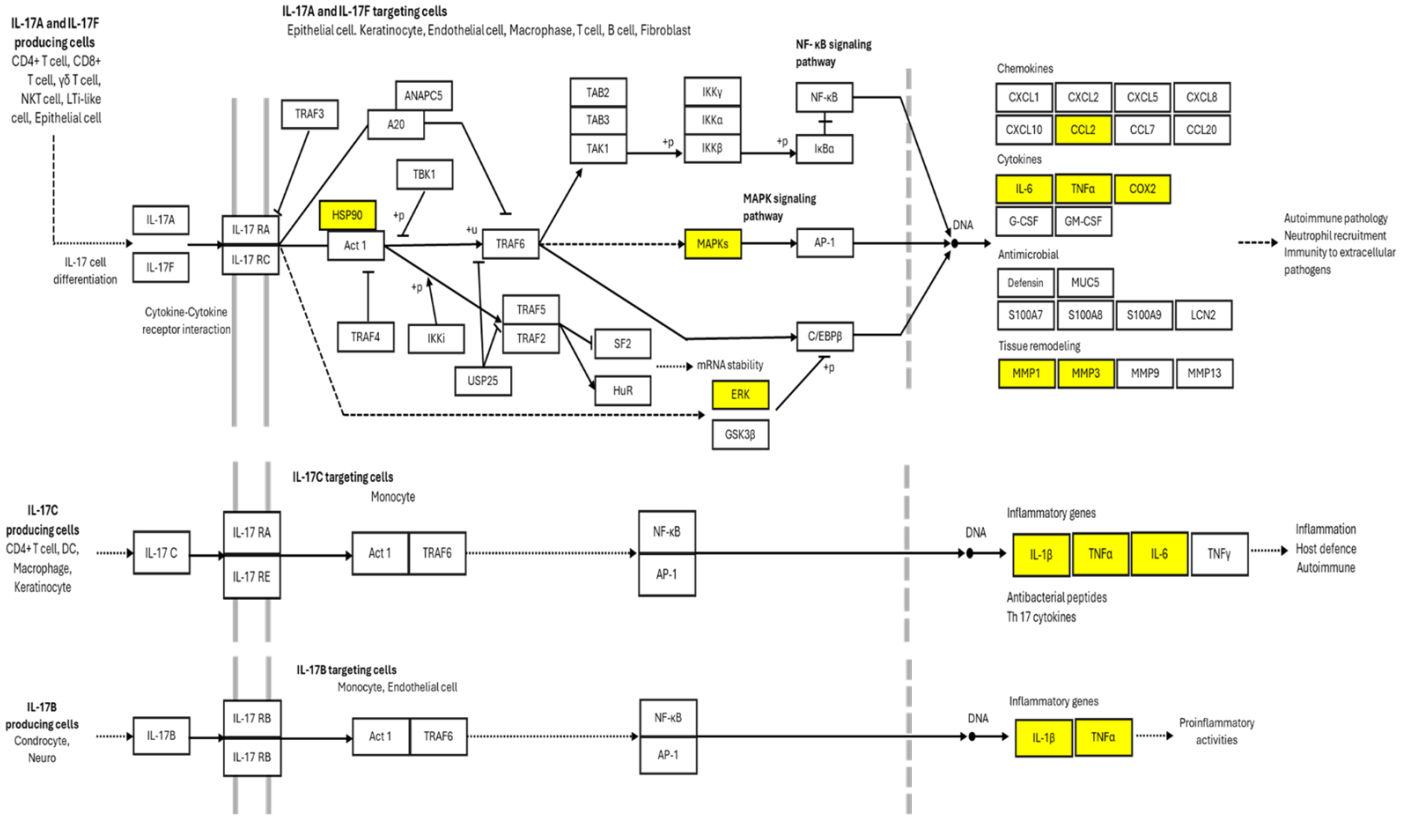


Figure 7. IL-17 signaling pathway. The nodes in the yellow box in the roadmap represent the related target.

[5]), transcription cis-regulatory region binding ($p = 3.00\times10^{-5}$), heme binding ($p = 4.10\times10^{-5}$), steroid binding ($p = 6.20\times10^{-5}$), ubiquitin protein ligase binding ($p = 1.20\times10^{-4}$), serine-type endopeptidase activity ($p = 1.30\times10^{-4}$), protein binding ($p = 1.70\times10^{-4}$)…

A total of 88 KEGG pathways were identified through KEGG pathway enrichment analysis, using a significance threshold of p-value < 0.05. The 20 pathways with the lowest p-values are presented in Table 3. Notably, the lipid and atherosclerosis pathways exhibited the smallest p-values ($p = 3.19 \times 10^{-15}$). Additionally, several significant inflammatory pathways were also listed in Table 3, including the IL-17 signaling pathway, AGE-RAGE signaling pathway in diabetic complications, TNF signaling pathway, rheumatoid arthritis pathway, HIF-1 signaling pathway, and relaxin signaling pathway.

To visualize the results and the multi-compound, multi-target, and multi-pathway mechanism of action of *P. foetida* on gout. Twenty pathways with the smallest p-value ($3.19\times10^{-15} \leq p \leq 3.61\times10^{-6}$) were selected to draw the bubble map (Figure 4), IL-17 signaling pathway was selected for visualization (Figure 6), the first ten significantly enriched BP, CC, and MF categories in GO functional enrichment analysis were selected for drawing the Bar chart map (Figure 5). The relationships between the first twenty KEGG pathways and their corresponding targets and ingredients were analyzed by assembling the network graph of the active ingredient-KEGG pathway-core target (Figure 7). The network graph has 58 nodes (four active components, 20 pathways, and 34 core targets) and 410 edges. According to the network graph of the active component-KEGG pathway-key target, the most significant five core targets were MAPK1, JUN, IL6, IL1B, TNF, and only 4/5 components were quercetin, kaempferol, paederosidic acid methyl ester_qt, beta-sitosterol appeared in the network.

3.1.5 Molecular Docking Analysis

Using Autodock Vina software, five active compounds (quercetin, paederosidic acid methyl ester_qt, kaempferol, beta-sitosterol, pelargonidin) in *P. foetida* were docked with nine core target proteins (*TP53, IL6, HSP90AA1, TNF, IL1B, BCL2, PTGS2, MAPK1, MAPK8*) in the PPI network. Except for *JUN*, it is not suitable without a PDB ID. The lower the binding energy, the better the binding ability of the compound and the protein receptor. The results demonstrated that docking five active compounds with nine primary targets was less than -5.0 kcal/mol (Table 4 and Figure 8). Among the targets, the docking scores of *MAPK8* to quercetin (-9.0), beta-sitosterol (-10.1), kaempferol (-9.0), pelargonidin (-8.9), paederosidic acid methyl ester_qt (7.2) were the best.

3.2. Discussion

Gout is a disease caused by purine metabolism disorder. In people with gout, uric acid in the blood accumulates over time. When this concentration is too high, MSU crystals are formed. These crystals accumulate in the joints and cause inflammation, swelling, and pain for the patient [1]. The treatment and control strategy for gout is to use colchicine, NSAIDs, or corticosteroids to treat acute gout attacks. IL-1 inhibitors are used instead if the above drugs are not effective. After resolving the acute gout attack, measures to lower serum uric acid are applied, including appropriate diet and lifestyle adjustments, using uric acid synthesis inhibitors such as allopurinol and uric acid excretion drugs such as probenecid and sulfonamides [31]. Although effective in treating gout, chemical drugs often cause many adverse reactions [32]. Herbal medicine could be used as an essential complementary or alternative therapy for gout because of fewer adverse reactions and lower prices. In this research, we explore that the active compounds of *P. foetida* in the treatment of gout include quercetin, beta-sitosterol, kaempferol, pelargonidin, and paederosidic acid methyl ester_qt. Quercetin is a flavonoid that can inhibit enzymes such as adenosine deaminase, ketohexokinase, and xanthine oxidase, reducing uric acid levels in the blood. In addition, it also helps increase urate excretion through the kidneys by inhibiting the activity of urate reabsorption transporters and enhancing the activity of urate excretion transporters. Finally, quercetin also participates in anti-inflammatory activity through the TLR and NLRP3 inflammasome-IL-1β signaling pathways [33]. Kaempferol is a flavonoid that effectively reduces gouty arthritis by modulating the Th17/Treg balance. In addition, it inhibits the expression of IL-1β, IL-6, TNF-α, and TGF-β1. Kaempferol also affects RORγt and Foxp3 through the IL-17 pathway [34]. Besides, Yajie Wang at el. (2014) [35] showed that the primary mechanism by which kaempferol inhibits xanthine oxidase (XO) activity is likely attributable to its insertion into the enzyme's active site, which occupies the catalytic center. This interaction prevents the binding of the substrate and induces conformational alterations in the structure of XO, thereby impeding its enzymatic function. Beta-sitosterol is also a vital compound in gout treatment [36]. Beta-sitosterol is a type of phytosterol that shares a chemical structure closely resembling cholesterol. Previous studies have reported that beta-sitosterol possesses anti-inflammatory effects and rheumatoid inflammation [37]. Although paederoside has no corresponding target, asperuloside and scandoside, which did not satisfy the criteria of OB and DL values but were reported s that extract including three compounds possess antiinflammatory effects by modulating pro-inflammatory mediators' production in synovial tissue and inactivating NF-κB pathway transmembrane signal transduction which plays a crucial role in the pathogenesis of acute gouty arthritis, in addition, they had high contents in *P. foetida* [38].

PPI network analysis shows that the core targets of *P. foetida* in the treatment of gout include TP53, IL6, HSP90AA1, TNF, IL1B, BCL2, PTGS2, MAPK1, and MAPK8. The TP53 protein, known as p53, is pivotal in genomic stability, apoptosis regulation, and inflammatory responses. Under cellular stress conditions, TP53 is activated and can modulate cytokine and chemokine expression in the inflammatory process. Many studies have shown that TP53 can repress inflammation by inhibiting NF-κB, an essential factor in promoting inflammatory

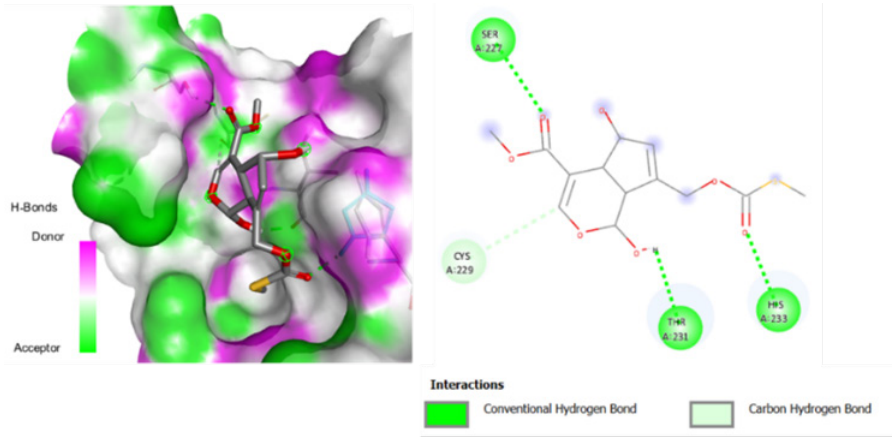
Table 4. The affinity of essential targets and active compounds

Gene name	PDB ID	Affinity (kcal/mol)				
		quercetin	beta-sitosterol	kaempferol	pelargonidin	paederosidic acid methyl ester_qt
TP53	5AB9	-6.9	-6.3	-6.9	-6.6	-5.4
IL6	4ZS7	-5.8	-6	-5.7	-5.5	-5.2
HSP90AA1	1UY6	-8.9	-9.5	-8.9	-8.8	-7.0
TNF	7JRA	-8.3	-8.8	-8.6	-8.3	-7.4
IL1B	5R85	-7.2	-7.5	-7.1	-6.9	-5.4
BCL2	8HTS	-6.8	-7.7	-6.7	-6.4	-5.5
PTGS2	5IKR	-7.5	-5.9	-7.1	-7.2	-7.0
MAPK1	6SLG	-7.9	-9.1	-7.9	-7.7	-6.4
MAPK8	4L7F	-9.0	-10.1	-9.0	-8.9	-7.2

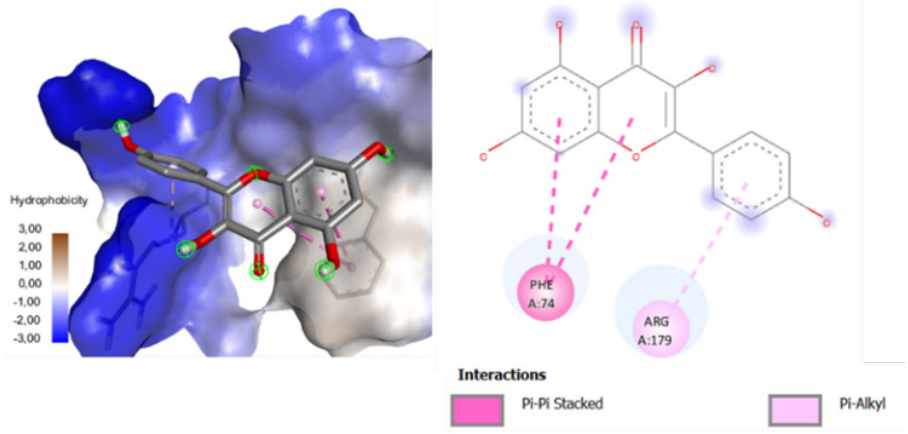
responses. TP53 is activated and found to downregulate NF-κB activity; this reduces excessive inflammation and protects tissues from inflammatory damage [39,40]. The HSP90AA1 gene encodes the human heat shock protein 90-kDa alpha (HSP90α), a member of the HSP90 protein family. HSP90α is a vital protein for the activation and stabilization of a numerous range of client proteins, including essential signaling molecules like nuclear transcription factors (e.g., p53, NF-κB, and STATs) and kinases (PI3K/AKT, p38/MAPK, and Raf/MEK/ERK). Dysregulation of HSP90α has been associated with numerous pathological conditions, including cancer and autoimmune or inflammatory diseases. Given its vital role in cellular processes, HSP90 has emerged as a promising therapeutic target. Indeed, studies have demonstrated that inhibition of HSP90 can ameliorate autoimmune conditions such as encephalomyelitis, rheumatoid arthritis, systemic lupus erythematosus, and epidermolysis bullosa acquisita [41]. Soluble mediators, including pro-inflammatory cytokines, lipid mediators, and complement, have been implicated in initiating and amplifying gout flares. Among these, IL-1β plays a pivotal role. Monosodium urate (MSU) crystals interact with resident macrophages to activate the NLRP3 inflammasome, releasing bioactive IL-1β. IL-1β signaling subsequently triggers the production and release of pro-inflammatory mediators, including IL-8 and IL-6, leading to neutrophil recruitment into joint cavities and the intensification of the gout flare. Clinical trials have shown the efficacy of biological agents targeting IL-1β in mitigating gout flares. These agents have been shown to prevent the development of initial flares, reduce the recurrence of flares in patients with a history of gout, and effectively treat established flares [1,8,42]. Furthermore, studies have demonstrated the critical role of tumor necrosis factor-alpha (TNF-α), a prototypical pro-inflammatory cytokine, in inflammation and pain within an experimental gout model in mice. TNF drives the production of pro-IL-1β mRNA and subsequent IL-1β generation. Consequently, agents that inhibit TNF production or function may represent promising therapeutic targets for gout treatment [43,44].

A comprehensive analysis of KEGG signaling pathways elucidated that the primary mechanism of *P. foetida* in gout treatment may be primarily related to its anti-inflammatory activities. Indeed, key signaling pathways implicated in this process include IL-17, AGE-RAGE, TNF, rheumatoid arthritis, HIF-1, and relaxin signaling pathway. These pathways collectively contribute to the anti-inflammatory effects of *P. foetida*, suggesting its potential therapeutic efficacy in managing gout-related inflammation. Gout patients exhibited markedly elevated levels of various cytokines, including IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ, and TNF-α, compared to healthy controls [45]. Th17 cells were significantly upregulated in early-onset gout and cases without tophus formation. The activation of Th17 cells is linked to the secretion of IL-17, a potent cytokine that can rapidly induce the accumulation of neutrophils, leading to a cascade of inflammatory cytokine release and exacerbated joint inflammation. Furthermore, inhibiting IL-17 has been demonstrated to attenuate inflammation and is employed in treating rheumatic diseases [46]. In addition, IL-17 also activates the mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK1/2), Jun N-terminal kinase (JNK), and P38 [47]. This pathway is closely associated with renal tubular injury and inflammation in the setting of hyperuricemia [48] and plays an important role in initiating gouty arthritis attacks [49]. Inhibition of the MAPK signaling pathway effectively reduces inflammation in gouty arthritis [50]. Besides, AGE-RAGE, TNF, rheumatoid arthritis, HIF-1, and relaxin signaling pathways have also been shown to be closely related to inflammatory responses in the body and are considered important pathways in the pathogenesis of gout [24,51].

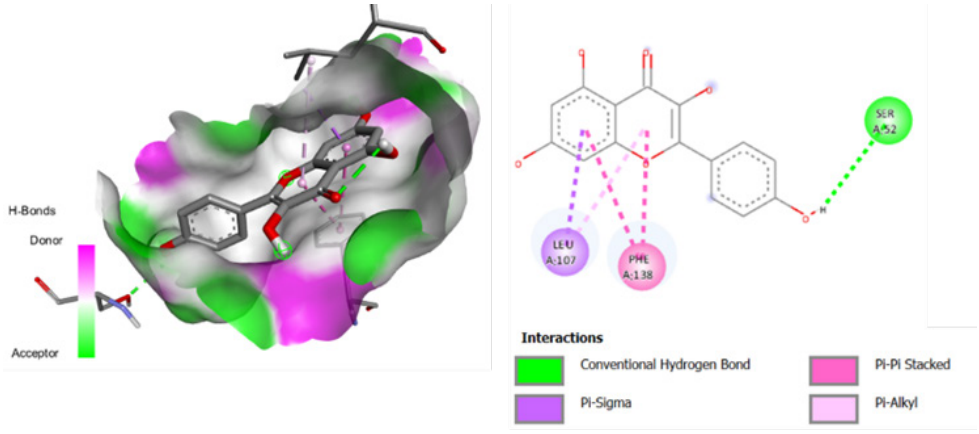
Molecular docking simulations elucidated interactions be-



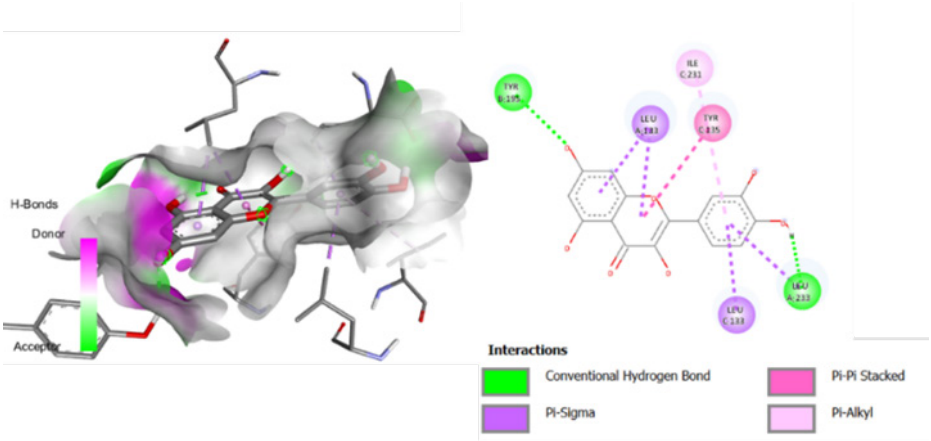
A. TP53 - MOL010606-paederosidic acid methyl ester_qt. Affinity: -5.4 (kcal/mol)



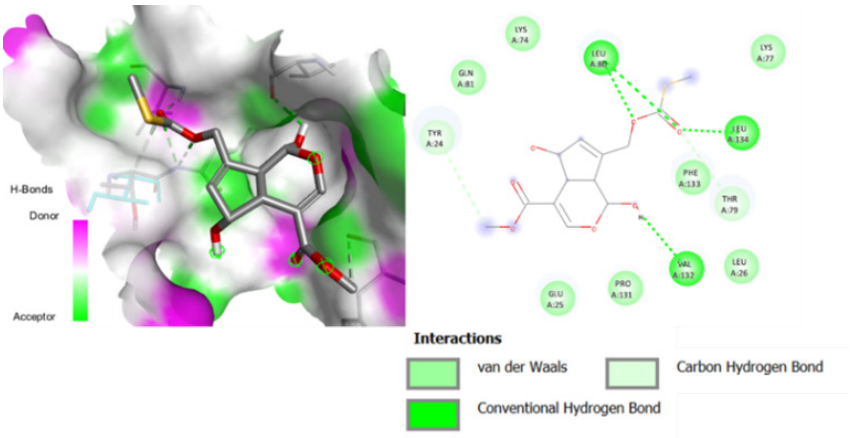
B. IL6 - MOL000422-kaempferol. Affinity: -5.7 (kcal/mol)



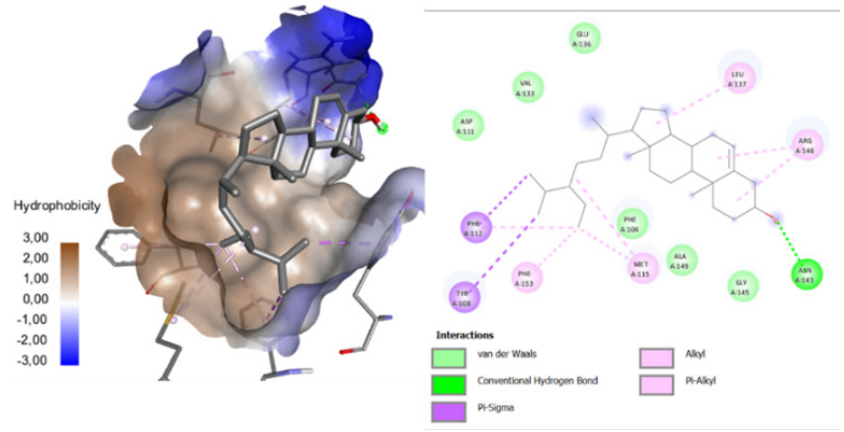
C. HSP90AA1 - MOL000422-kaempferol. Affinity: -8.9 (kcal/mol)



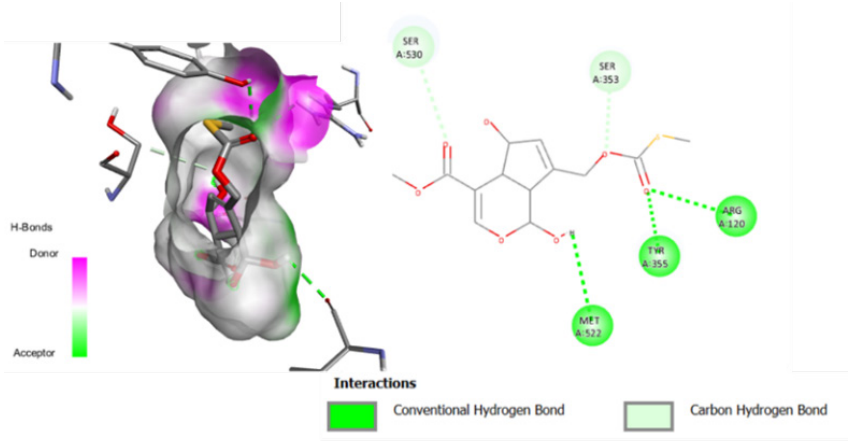
D. TNF - MOL000098-quercetin. Affinity: -8.3 (kcal/mol)



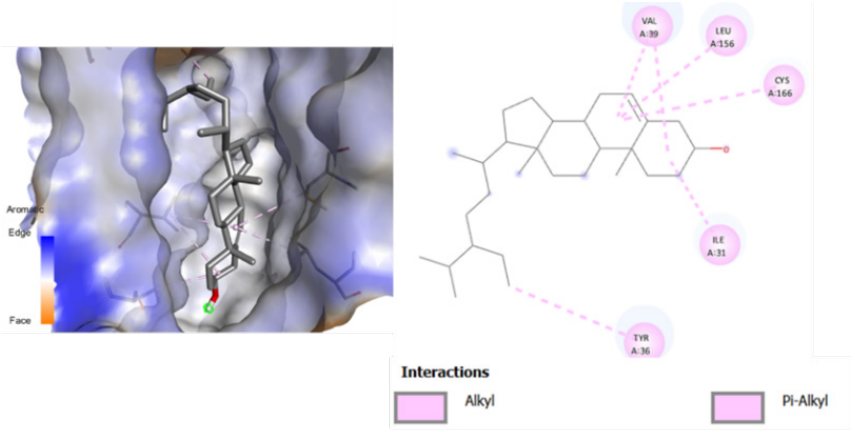
E. IL1B - MOL010606-paederosidic acid methyl ester_qt. Affinity: -5.4 (kcal/mol)



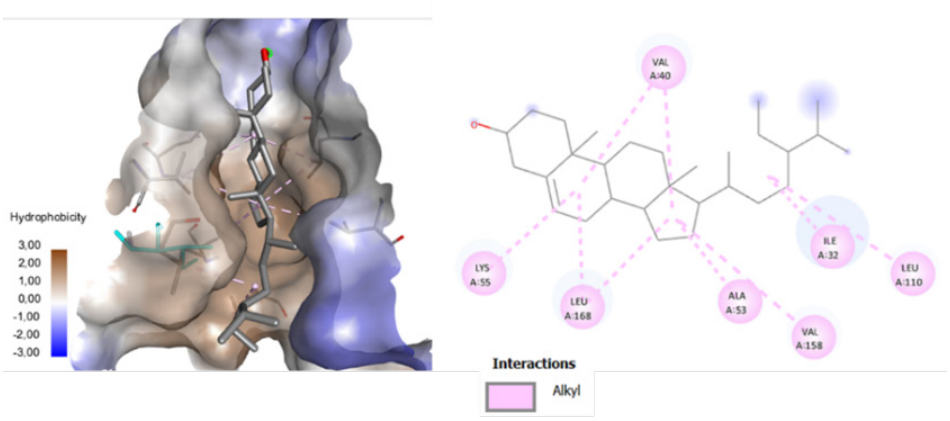
F. BCL2 - MOL000358-beta-sitosterol. Affinity: -7.7 (kcal/mol)



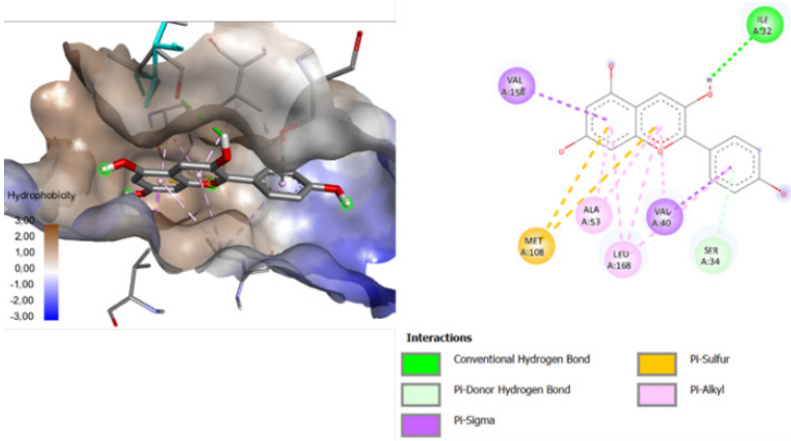
G. PTGS2 - MOL010606-paederosidic acid methyl ester_qt. Affinity -7.0 (kcal/mol)



H. MAPK1 - MOL000358-beta-sitosterol. Affinity -9.1 (kcal/mol)



I. MAPK8 - MOL000358-beta-sitosterol. Affinity -10.1 (kcal/mol)



K. MAPK8 - MOL001004-pelargonidin. Affinity -8.9 (kcal/mol)

Figure 8. The five active compounds of *P. foetida* in the cure of gout are docked with essential target proteins.

tween network components, enhancing their predictive accuracy. Core target proteins associated with gout were docked with active compounds from *P. foetida*. The results indicated that TP53, IL6, HSP90AA1, TNF, IL1B, BCL2, PTGS2, MAPK1, and MAPK8 exhibited relatively low binding energies with the compounds quercetin, beta-sitosterol, kaempferol, pelargonidin, and paederosidic acid methyl ester_qt. Notably, molecular docking showed that MAPK8 has a higher binding affinity than other core targets (from -7.2 to -10.1 kcal/mol). Studies have also confirmed that MAPK8 is required for macrophage IL-1β production through transcriptional regulation and inflammasome activity [52]. Thus, increased MAPK8 expression leads to increased IL-1β production in macrophages, manifesting as clinical gout [53]. *In vivo* testing also confirmed that restoration of mRNA and protein overexpression of the MAPK8 gene can treat gout or hyperuricemia [54]. These results suggest that targeting MAPK8 with active components from *P. foetida* may be an effective therapeutic strategy for gout. However, further studies are needed to confirm these results.

Through network pharmacology and molecular docking methods, this study shows the complex mechanism of action of P. foetida on gout through its effects on many substances, targets, and pathways. However, studying still has many shortcomings that need to be overcome. Firstly, the results of network pharmacology depend significantly on the quality of the databases and related prediction algorithms. Suppose the data are incomplete, inaccurate, or out of date. In that case, the analysis results will be distorted, leading to incorrect predictions about the mechanism of action of herbs or the relationship between herbs and diseases. Secondly, although this method is modern, it lacks experimental verification, so the results are only theoretical. Therefore, network pharmacology and molecular docking methods are only for research orientation; further research is needed to confirm the results. Thirdly, several signaling pathways emerged during our analysis, complicating the identification of the primary mechanisms of action related to *P. foetida* and gout. In some cases, pathways exhibited small p-values yet lacked sufficient data for further investigation; a case in point is the lipid and atherosclerosis pathway. We found a correlation between hyperuricemia, increased arterial stiffness, inflammation, and decreased uric acid concentration inhibiting atherosclerosis [55]. However, we did not find studies demonstrating the opposite relationship, so further studies are needed to explore whether lowering lipids and atherosclerosis can lead to lower uric acid levels and aid in gout treatment. Finally, it is not easy to assess the safety of herbs because the analysis mainly focuses on the primary mechanism of action of the drug. In future studies, we plan to seek evidence through

in vitro and *in vivo* experiments to precisely identify the active compounds, targets, and signaling pathways involved in the anti-gout mechanism of *P. foetida*.

Conclusions

This study contributed to the systematic description of the active compounds of *P. foetida* to support gout treatment and elucidate its underlying molecular mechanisms. Network topology analysis identified quercetin, beta-sitosterol, kaempferol, pelargonidin, and paederosidic acid methyl ester_qt as the primary active compounds. Protein-protein interaction (PPI) screening revealed TP53, IL6, JUN, HSP90AA1, TNF, IL1B, BCL2, PTGS2, MAPK1, and MAPK8 as core targets for gout treatment. Except for JUN, these primary targets exhibited good binding affinity to the active compounds; among the targets, MAPK8 was identified as a potential target for further research. Furthermore, *P. foetida* was found to modulate gout-related signaling pathways, especially the IL-17 signaling pathway.

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Conflict of Interest Statement

There is no conflict of interest regarding the publication of this paper.

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