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The combination of molecular docking and network pharmacology reveals the molecular mechanism of Danggui Niantong decoction in treating gout

Yuan Liu, MM[a], Di Luo, MD[b], Bo Xu, MD[a,*]

Abstract

Background: Due to unhealthy diet and living habits, the incidence of gout is on the rise and has become a common disease with a high incidence. Danggui Niantong decoction (DGNTD), as a classic formula composed of 15 common herbs, has been widely used in clinical practice since ancient times to prevent and treat gout. However, the pharmacological mechanism and target of DGNTD are not clear.

Methods: The potential active compounds and targets of DGNTD were obtained by traditional Chinese medicine systems pharmacology (TCMSP) database, and the differential genes of gout patients and controls were analyzed in gene expression omnibus (GEO) database. GSEA analysis of differential genes with GSEA 4.1.0 software and then the differential genes were intersected with the gout-related disease targets searched by GeneCard, CTD and OMIM disease database to obtain the final disease target. The "Traditional Chinese medicine-Active compounds-Targets" network was constructed by Cytoscape3.7.2 software. The R packet is used for enrichment analysis. The molecular docking between the active compound of DGNTD and the core target was verified by AutoDockTools software.

Results: Two hundred eighty six and 244 targets of DGNTD-related active components and 652 targets of gout were obtained, of which 13 targets were potential targets of DGNTD in the treatment of gout. GSEA analysis showed that the differential genes were mainly involved in apoptosis, inflammatory reaction, and receptor metabolism and so on. Gene ontology (GO) functional enrichment analysis shows that DGNTD regulates many biological processes, such as the response to purine-containing compound and response to lipopolysaccharide, positive regulation of acute inflammatory response and other cellular components. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis shows that DGNTD treatment of gout is mainly related to interleukin-17 (IL-17), Toll-like receptor, rheumatoid arthritis, tumor necrosis factor (TNF) and so on. The results of molecular docking showed that the five active compounds in DGNTD had strong binding activity to core protein receptors.

Conclusions: The active compounds of DGNTD may achieve the purpose of treating gout by acting on the core target (CASP8, CXCL8, FOS, IL1B, IL6, JUN, PTGS2, STAT1, MMP1, TNF) to regulate cell metabolism, proliferation and apoptosis, and improve inflammatory response, which is the result of multi-component, multi-target and multi-pathway interaction. It provides an idea for the development of new combined drugs for gout.

Abbreviations: DGNTD = Danggui Niantong decoction, DL = drug-likeness, GEO = gene expression omnibus, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, OB = oral bioavailability, PPI = protein-protein interaction, TCM = traditional Chinese medicine, TCMSP = traditional Chinese medicine systems pharmacology.

Keywords: Danggui Niantong decoction, gout, molecular docking, network pharmacology, traditional Chinese medicine

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The [GSE datasets] data that support the findings of this study are available in the GEO database (https://www.ncbi.nlm.nih.gov/geo/) with the following data accession identifier(s):GSE160170. Other data used to support the results of this study can be obtained from the corresponding authors according to the requirements.

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1. Introduction

Gout is a metabolic disease caused by hyperuricemia, it is the most common chronic disease in the world. The improvement of modern living standards leads to changes in dietary structure and living habits, the incidence of gout is gradually increasing.[1–3] According to the 2014 New Zealand gout Epidemiological Survey, the incidence of gout in people over 20 years old is 5%, and the incidence rate of men is 3 times higher than that of women.[4] In the UK, the prevalence of gout in 2015 was 2.5%.[5] According to the World Health Organization (WHO), about 3.9% of people worldwide suffered from gout in 2015.[6] The main manifestation of gout is intermittent attacks of acute painful arthritis, which is caused by the deposition of monosodium urate crystals in joint and non-joint structures.[7] This acute pain seriously affects the quality of life of patients.[8] The treatment of gout mainly includes anti-inflammation and analgesia in acute phase and oral drug control of uric acid level in chronic phase, among which oral drugs mainly include non-steroidal anti-inflammatory drugs, colchicine, and biological agents and so on.[9] Chronic phase requires long-term medication to control uric acid, but long-term use of the above drugs is easy to produce hepatorenal toxicity and stimulate gastrointestinal adverse reactions.[10,11] Therefore, it is necessary to find safer and more effective alternative drugs to treat gout.

The development of Traditional Chinese medicine (TCM) has a history of nearly 5000 years. TCM believes that gout belongs to the category of arthralgia syndrome. TCM has been used to treat gout since ancient times. Danggui Niantong decoction (DGNTD) is a classic prescription for the treatment of gout in ancient China. This prescription originated from Medical Qiyuan in the Jin Dynasty (1186 A.D.). DGNTD is composed of Radix Notopterygii (Qianghuo), Licorice (Gancao), Herba Artemisiae (Yinchen), Radix Atractylodis (Cangzhu),Saposhnikoviae Radix (Fangfeng), Angelica sinensis (Danggui), Anemarrhena asphodeloides (Zhimu), Polyporus umbellatus (Zhuling), Alisma orientalis (Zexie), Cimicifuga (Shengma), Atractylodes macrocephala (Baizhu), Radix Scutellariae (Huangqin), Radix puerariae (Gegen), Ginseng (Renshen) and Sophora flavescens (Kushen). DGNTD has the effect of relieving dampness and clearing heat, soothing wind and relieving pain. DGNTD has been widely used in clinical treatment of gout in China and achieved satisfactory results.[12,13] Previous studies have shown that DGNTD can improve synovitis in arthritic rats by inhibiting synovial hyperplasia, infiltration of inflammatory cells, and destruction of cartilage and bone during inflammation.[14] Recently, DGNTD has been proved to effectively alleviate the degree of inflammatory swelling of paws in AGA mice, down-regulate the expression of inflammatory factors IL-1β, TNF-α and iNOSmRNA, and reduce the concentration of serum uric acid. It is an effective prescription to solve many core problems of AGA with high safety.[15,16] However, the specific mechanism and related targets of DGNTD in the treatment of gout have not been fully studied.

Compared with western medicine, the treatment of TCM has the characteristics of multi-components, multi-targets and

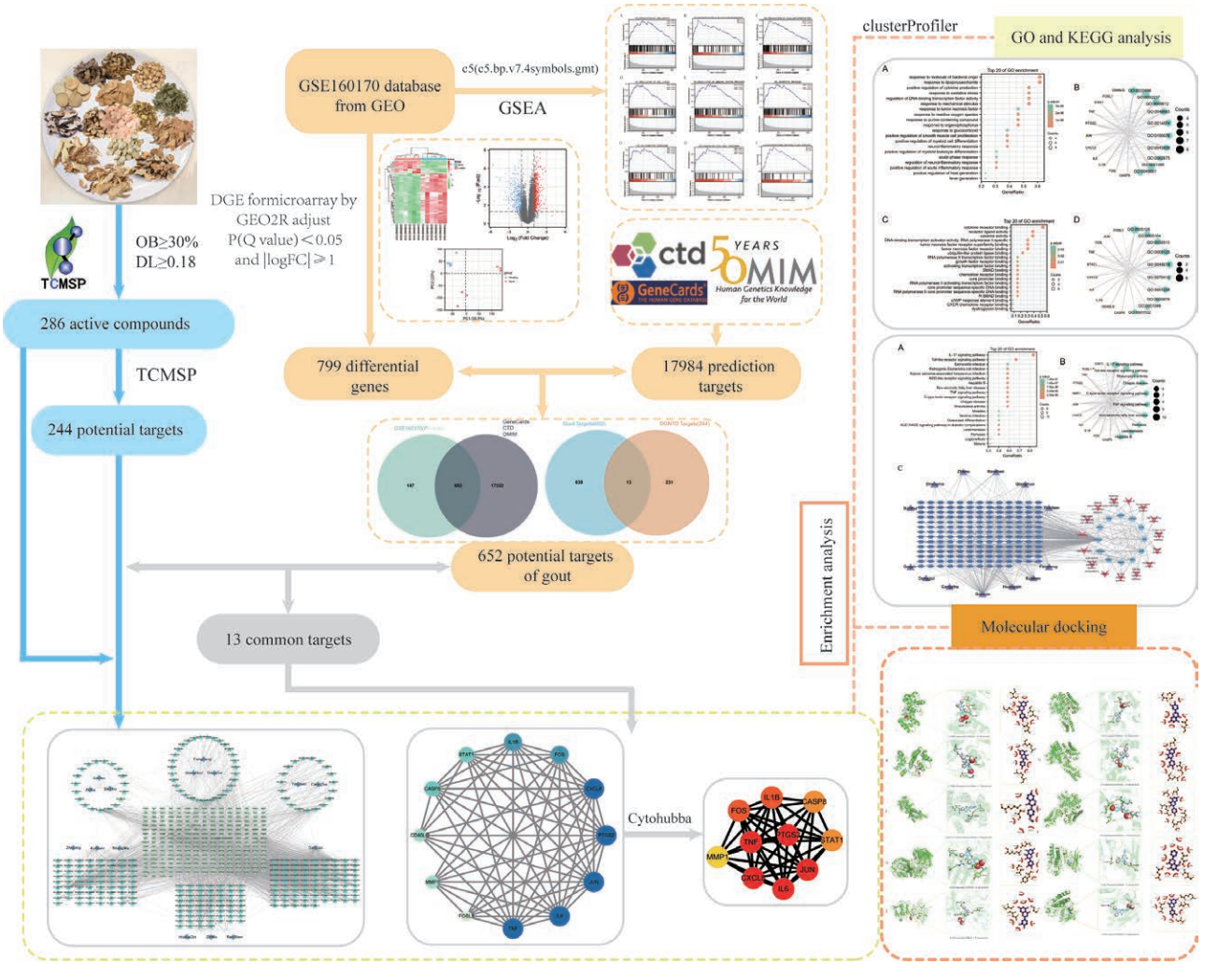

Figure 1. The flowchart of the analysis procedures of the study.

Table 1

Some active compounds of DGNTD.

Herb	Coding	Active compounds	OB (%)	DL
Radix Notopterygii	MOL001941	Ammidin	34.55	0.22
Radix Notopterygii	MOL011962	6'-Feruloylnodakenin	32.02	0.67
Radix Notopterygii	MOL011963	8-geranoxy-5-methoxypsoralen	40.97	0.5
Radix Notopterygii	MOL011968	Coumarin, glycoside	33.07	0.78
Radix Notopterygii	MOL011969	Demethylfuropinnarin	41.31	0.21
Licorice	MOL000098	Quercetin	46.43	0.28
Licorice	MOL000211	Mairin	55.38	0.78
Licorice	MOL000239	Jaranol	50.83	0.29
Licorice	MOL000354	Isorhamnetin	49.60	0.31
Licorice	MOL000359	Sitosterol	36.91	0.75
Herba Artemisiae	MOL004609	Areapillin	48.96	0.41
Herba Artemisiae	MOL005573	Genkwanin	37.13	0.24
Herba Artemisiae	MOL007274	Skrofulein	30.35	0.3
Herba Artemisiae	MOL008039	Isoarcapillin	57.4	0.41
Herba Artemisiae	MOL008040	Eupalitin	46.11	0.33
Radix Atractylodis	MOL000173	wogonin	30.68	0.23
Radix Atractylodis	MOL000179	2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydrobenzofuran-5-carboxylic	45.2	0.2
Radix Atractylodis	MOL000184	NSC63551	39.25	0.76
Radix Atractylodis	MOL000186	Stigmasterol 3-O-beta-D-glucopyranoside_qt	43.83	0.76
Radix Atractylodis	MOL000188	3β-Acetoxyatractylone	40.57	0.22
Saposhnikoviae Radix	MOL000011	(2R,3R)-3-(4-Hydroxy-3-methoxy-phenyl)-5-methoxy-2-methylol-2,3-dihydropyrano[5,6-h][1,4] benzodioxin-9-one	68.83	0.66
Saposhnikoviae Radix	MOL011730	11-Hydroxy-sec-o-beta-d-glucosylhamaudol_qt	50.24	0.27
Saposhnikoviae Radix	MOL011732	Anomalin	59.65	0.66
Saposhnikoviae Radix	MOL011737	Divaricatacid	87	0.32
Saposhnikoviae Radix	MOL011740	Divaricatol	31.65	0.38
Angelica sinensis	MOL000358	beta-Sitosterol	36.91	0.75
Angelica sinensis	MOL000449	Stigmasterol	43.83	0.76
Anemarrhena asphodeloides	MOL001677	Asperglaucide	58.02	0.52
Anemarrhena asphodeloides	MOL001944	Marmesin	50.28	0.18
Anemarrhena asphodeloides	MOL003773	Mangiferolic acid	36.16	0.84
Anemarrhena asphodeloides	MOL000422	Kaempferol	41.88	0.24
Anemarrhena asphodeloides	MOL004373	Anhydroicaritin	45.41	0.44
Polyporus umbellatus	MOL000279	Cerevisterol	37.96	0.77
Polyporus umbellatus	MOL000282	Ergosta-7,22E-dien-3beta-ol	43.51	0.72
Polyporus umbellatus	MOL000796	(22e,24r)-Ergosta-6-en-3beta,5alpha,6beta-triol	30.2	0.76
Polyporus umbellatus	MOL000797	(22e,24r)-Ergosta-7,22-dien-3-one	44.88	0.72
Polyporus umbellatus	MOL000798	Ergosta-7,22-diene-3β-ol	43.51	0.72
Alisma orientalis	MOL000830	Alisol B	34.47	0.82
Alisma orientalis	MOL000831	Alisol B monoacetate	35.58	0.81
Alisma orientalis	MOL000832	Alisol,b,23-acetate	32.52	0.82
Alisma orientalis	MOL000849	16β-Methoxyalisol B monoacetate	32.43	0.77
Alisma orientalis	MOL000853	Alisol B	36.76	0.82
Cimicifuga	MOL011991	23-Epi-26-deoxyactein_qt	47.64	0.35
Cimicifuga	MOL011999	24-Epi-acerinol	31.31	0.42
Cimicifuga	MOL012011	25-o-Acetylcimigenol-3-o-beta-d-glc(1-2)beta-d-xylopyranoside_qt	30.04	0.32
Cimicifuga	MOL012023	7,8-Didehydrocimigenol	36.79	0.4
Cimicifuga	MOL012038	Heracleifolinoside F	47.98	0.18
Atractylodes macrocephala	MOL000020	12-Senecioyl-2E,8E,10E-atractylentriol	62.4	0.22
Atractylodes macrocephala	MOL000021	14-Acetyl-12-senecioyl-2E,8E,10E-atractylentriol	60.31	0.31
Atractylodes macrocephala	MOL000022	14-Acetyl-12-senecioyl-2E,8Z,10E-atractylentriol	63.37	0.3
Atractylodes macrocephala	MOL000028	α-Amyrin	39.51	0.76
Atractylodes macrocephala	MOL000049	3β-Acetoxyatractylone	54.07	0.22
Radix Scutellariae	MOL008206	Moslosooflavone	44.09	0.25
Radix Scutellariae	MOL010415	11,13-Eicosadienoic acid, methyl ester	39.28	0.23
Radix Scutellariae	MOL012245	5,7,4'-Trihydroxy-6-methoxyflavanone	36.63	0.27
Radix Scutellariae	MOL012246	5,7,4'-Trihydroxy-8-methoxyflavanone	74.24	0.26
Radix Scutellariae	MOL012266	Rivularin	37.94	0.37
Ginseng	MOL005308	Aposiopolamine	66.65	0.22
Ginseng	MOL005314	Celabenzine	101.88	0.49
Ginseng	MOL005317	Deoxyharringtonine	39.27	0.81
Ginseng	MOL005318	Dianthramine	40.45	0.2
Ginseng	MOL005320	Arachidonate	45.57	0.2
Sophora flavescens	MOL006561	(+)-14alpha-Hydroxymatrine	35.73	0.29
Sophora flavescens	MOL006562	(+)-7,11-Dehydromatrine,(leontalbinine)	62.08	0.25
Sophora flavescens	MOL006563	(+)-9alpha-Hydroxymatrine	32.04	0.29
Sophora flavescens	MOL006564	(+)-Allomatrine	58.87	0.25
Sophora flavescens	MOL006565	AIDS211310	68.68	0.25

multi-approaches.[17] In recent years, network pharmacology is considered as a promising method to study TCM from a systematic point of view and molecular level.[18] Network pharmacology is a new subject of multi-target drug molecular design for specific signal nodes on the basis of system biology. It emphasizes the multi-molecular, multi-target and multi-pathway regulation of drugs to improve drug efficacy and reduce toxicity and side effects, so as to improve the efficiency of new drug research and development and save drug research and development costs.[19,20] Therefore, in this study, we use network pharmacology, bioinformatics and molecular docking techniques to explore the active components of DGNTD in the treatment of gout, predict the potential therapeutic targets and pathways of DGNTD, and explore the mechanism of DGNTD in the treatment of gout at the molecular level. To provide reference for the in-depth research, clinical application and new drug development of the prescription. The specific flow chart is shown in Figure 1.

2. Materials and methods

2.1. Collection and screening of herb compounds in DGNTD

The drugs contained in DGNTD were input into the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform[21] (TCMSP, http://tcmspw.com/tcmsp.php), and all the chemical compounds of the drugs were obtained. Oral bioavailability (OB) is one of the important pharmacokinetic parameters in Pharmacokinetics (ADME). It indicates the speed and degree at which the active compounds or active groups of oral drugs are absorbed to systemic circulation. The higher the OB value, the better the drug-likeness (DL) of the bioactive molecule of the drug.[22] Therefore, the active compounds in DGNTD were screened by OB ≥ 30% and DL ≥ 0.18.The study has been approved by the Ethics Committee of the affiliated Hospital of Shandong University of TCM.

2.2. Identification of targets of active compounds

The selected active compounds were input into the TCMSP platform for retrieval to obtain the corresponding targets. The corresponding gene names of the target were searched and standardized by Uniprot database[23] (https://www.uniprot.org/).

2.3. Construction of herb-active compound-target network

The active compounds of DGNTD and their corresponding targets are used to construct the "Herb-Active compound-Target" network. The network is built by the Cytoscape3.7.2 version and topologically analyzed. Cytoscape is an open source software platform for visualizing molecular interaction networks and biological pathways and integrating these networks with annotations, gene expression profiles, and other state data.[24]

2.4. Screening of genes related to gout

Download the data set related to gout from the gene expression omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database. The screening criteria include: the research object is "Homo sapiens," the information of the data set is complete and the sample size is not less than 4.By GEO2R analysis, the differential genes were obtained according to logFC (Fold change) > 1 or <-1, and adjust p (Q value) < .05 as screening criteria. In addition, we defined the species as "Homo Sapiens" with "gout" and "gouty arthritis" as keywords, and obtained disease-related targets by searching

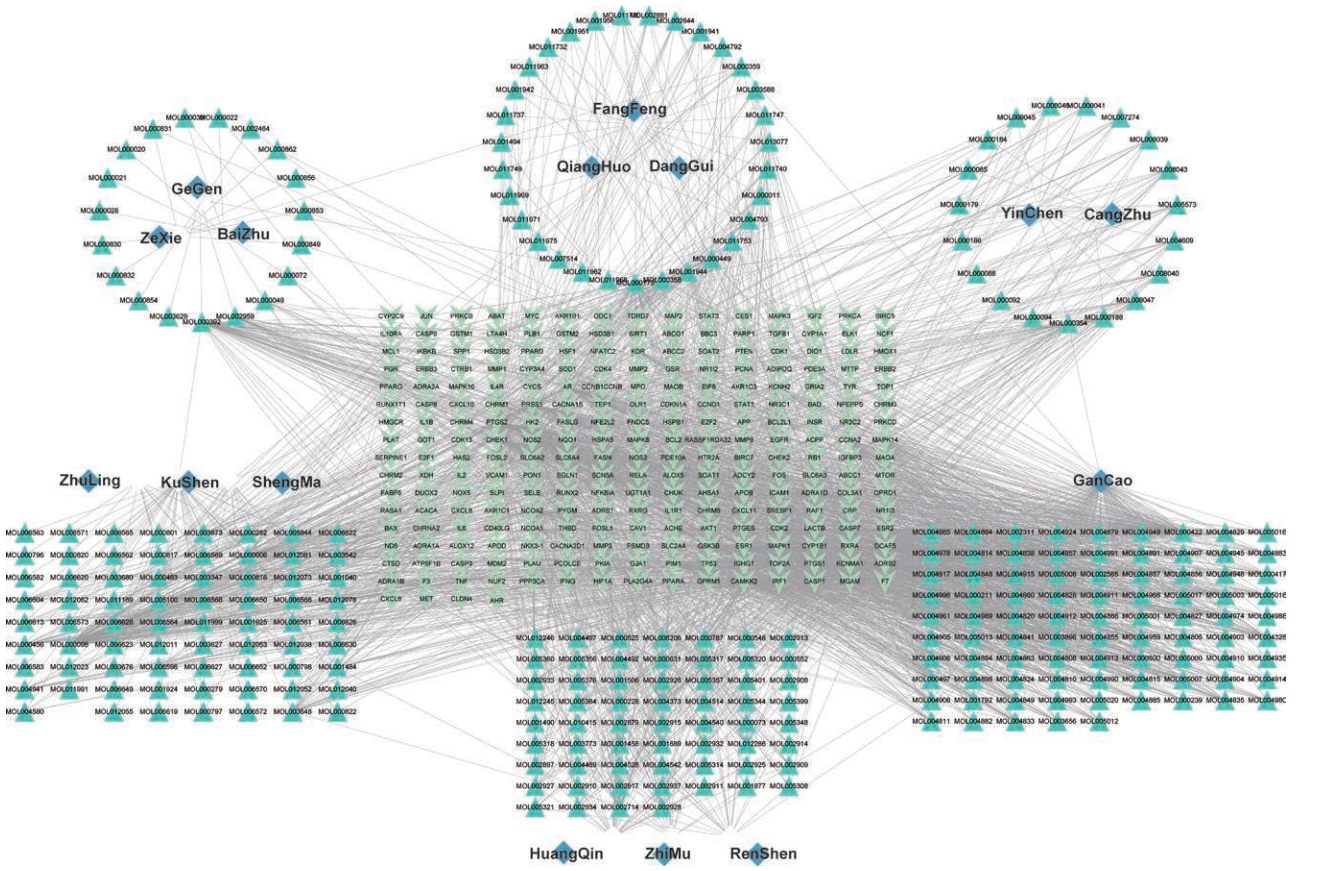

Figure 2. A network of the herb-active compound-target. The blue diamond represents the traditional Chinese medicine in DGNTD. The green triangle represents the active compound. The light cyan arrow represents the target.

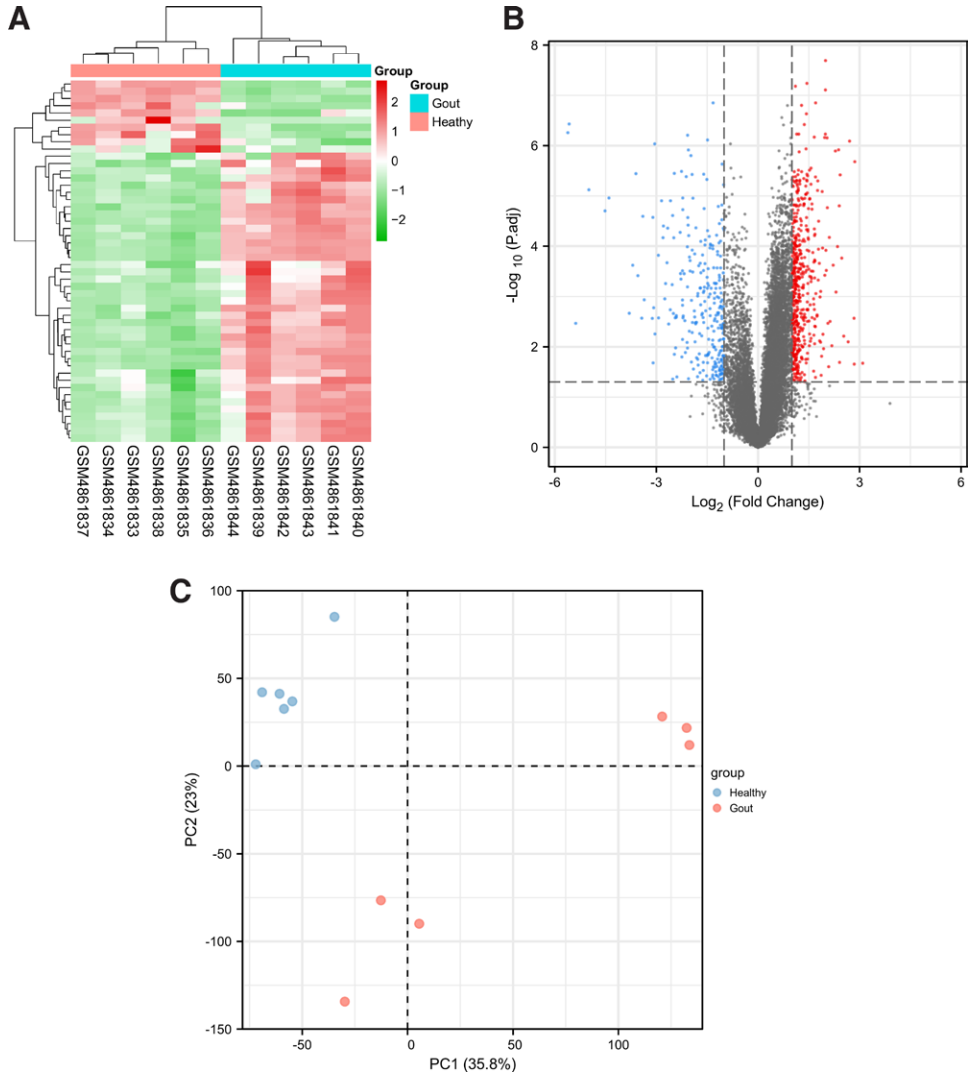

Figure 3. Analysis of differential genes related to gout. (A) Heat map of GSE160170, each grid represents each gene, and the color depth represents the expression of the gene. The higher the expression level, the darker the color (red up-regulated, green down-regulated). (B) Volcano diagram of differential genes. Blue dots represent down-regulated genes, red dots represent up-regulated genes, and gray dots represent genes with little difference. (C) PCA principal component analysis.

Human Gene Database (GeneCards, https://www.genecards.org/), Comparative Toxicogenomics Database (CTD, http://ctdbase.org/) and Online and Mendelian Inheritance in ManHuman (OMIM, https://www.omim.org/). These targets are intersected with differential genes to get the final target gene of gout.

2.5. GSEA analysis of differential genes

GSEA is a method for the analysis of genome-wide expression profile microarray data, which compares genes with predefined gene sets. Through the analysis of gene expression profile data, to understand their expression status in specific functional gene sets, and whether there is some statistical significance of this expression status.[25] GSEA 4.1.0 was used for functional enrichment analysis, and the background gene set was set to c5 (c5.bp.v7.4 symbols. gmt).

2.6. Prediction of potential targets of DGNTD in the treatment of gout

We intersect the target regulated by active compounds in DGNTD with the final target gene of gout disease. After that, the potential targets of DGNTD in the treatment of gout were obtained. Use the ggplot2 package in R4.0.5 software to generate Venn diagrams of potential targets.

2.7. Construction of protein–protein interaction (PPI) network

The potential targets of DGNTD in the treatment of gout were imported into STRING database[26] (https://string-db.org/). Select "Multiple Proteins" and define "Organism" as "Homo sapiens," set the lowest interaction score to "medium confidence (0.40)," obtain the protein interaction relationship, and save the data in TSV format. Visual processing is carried out by using Cytoscape3.7.2 software. The top 10 core targets are screened by plug-in cytoHubba with MNC, MCC and Degree as screening conditions.

2.8. Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The clusterProfiler package (version 3.14.3) in R4.0.5 software was used to analyze the potential targets of DGNTD in the treatment of gout by GO and KEGG, and the gene ID conversion

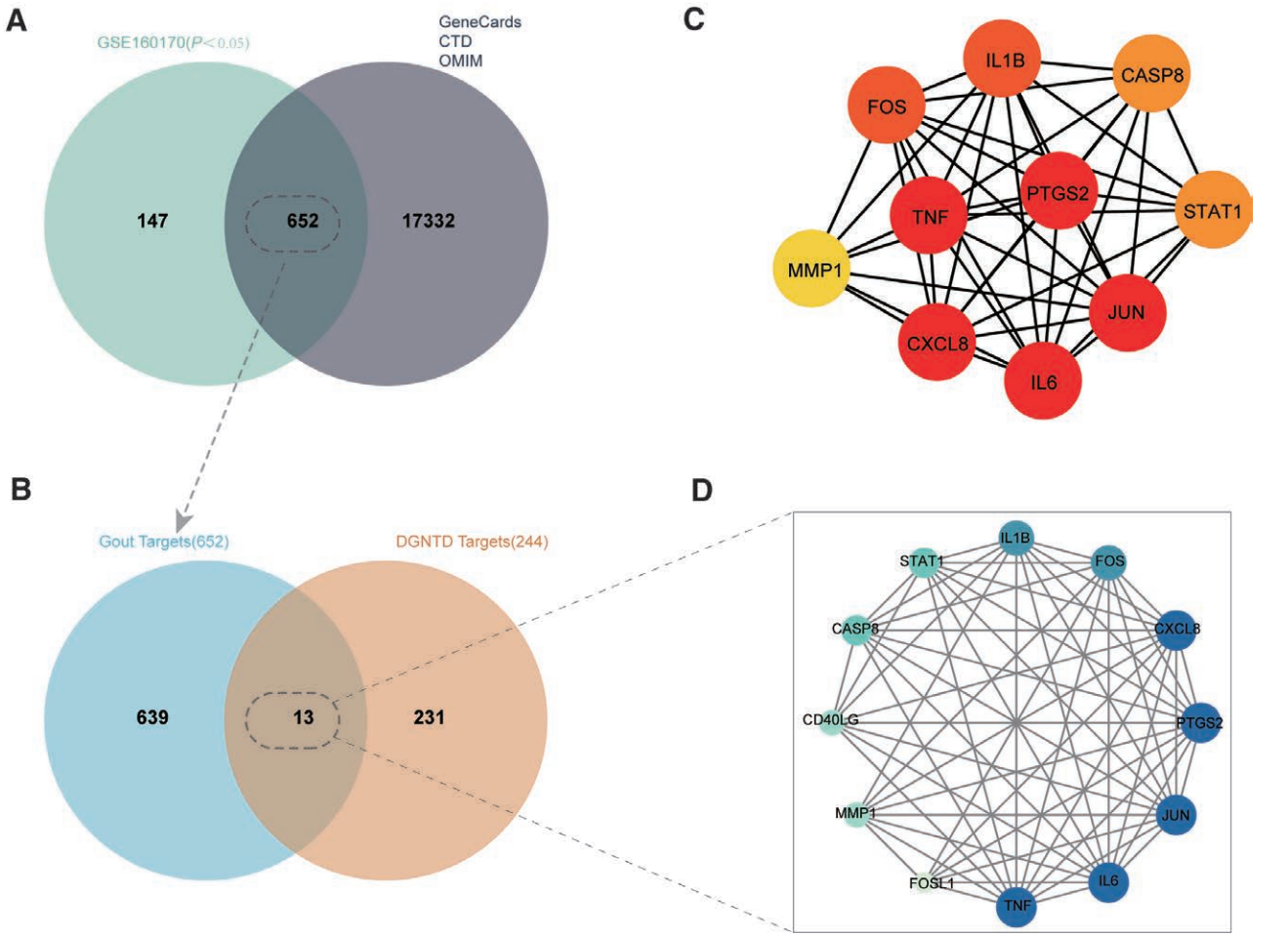

Figure 4. Potential target acquisition and PPI network analysis of DGNTD in the treatment of gout. (A) Gout final target acquisition. (B) Potential targets of DGNTD in the treatment of gout. (C) PPI network construction. (D) Screening of Hub gene (core target).

was completed by R package org.Hs.eg.db (version 3.10.0). With p.adj < 0.05 and q value < 0.2 as screening conditions, the first 20 biological processes (BP), molecular function (MF) and KEGG pathway were selected to construct bubble map. The information of the first 15 KEGG pathways was screened and the network diagram of KEGG "pathway-target-active ingredient-TCM" was drawn by Cytoscape 3.7.2 software. After that, we chose two signal pathways closely related to gout for visualization.

2.9. Molecular docking verification

According to the "Herb-Active compound-Target" network, the top five active compounds were selected as docking small molecules. Download the 3D structure of five small molecules from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and use Chem3D (version 19.0) software to optimize the mechanics of small molecules. Then import the small molecular compound into AutoDockTools (version 1.5.6) to determine the rotatable bond and save it as "PDBQT" format file. We downloaded the 3D structure of the core target protein from RCSB PDB database (http://www.rcsb.org). The receptor protein was introduced into PyMOL (version 2.3.0) to remove water and excess small molecular ligands. The processed receptor protein was imported into AutoDockTools (version 1.5.6) for hydrogenation, charge calculation, atomic type addition, and saved as a "PDBQT" file. AutoDockVina is used for molecular docking and PyMOL (version 2.3.0) is used to visualize the optimal docking results. LigPlot + (version 2.2.5) is used to visualize 2D structures. The binding energy < -5kcal/mol indicates that the compound has a strong binding force.

3. Results

3.1. Active compounds of DGNTD

A total of 1729 compounds of DGNTD were retrieved from TCMSP database. Including 185 from Radix Notopterygii (Qianghuo), 280 from Licorice (Gancao), 53 from Herba Artemisiae (Yinchen), 49 from Radix Atractylodis (Cangzhu), 173 from Saposhnikoviae Radix (Fangfeng), 125 from Angelica sinensis (Danggui), 81 from Anemarrhena asphodeloides (Zhimu), 31 from Polyporus umbellatus (Zhuling), 46 from Alisma orientalis (Zexie), 187 from Cimicifuga (Shengma), 55 from Atractylodes macrocephala (Baizhu), 143 from Radix Scutellariae (Huangqin) and 18 from Radix puerariae (Gegen). One hundred ninety were from Ginseng (Renshen) and 113 from Sophora flavescens (Kushen). According to OB ≥ 30% and DL ≥ 0.18, 322 active compounds were screened. Including 17 from Radix Notopterygii (Qianghuo), 92 from Licorice (Gancao), 13 from Herba Artemisiae (Yinchen), 9 from Radix Atractylodis (Cangzhu), 20 from Saposhnikoviae Radix (Fangfeng), 2 from Angelica sinensis (Danggui), 16 from Anemarrhena asphodeloides (Zhimu), 11 from Polyporus umbellatus (Zhuling), 10 from Alisma orientalis (Zexie), 18 from Cimicifuga (Shengma), 7 from Atractylodes macrocephala (Baizhu), 36 from Radix Scutellariae (Huangqin) and 4 from Radix puerariae (Gegen). 22 from Ginseng (Renshen) and and 45 from Sophora flavescens (Kushen). After deleting the repeat value, a total of 286 active compounds were obtained. Some of the active compounds in DGNTD are shown in Table 1.

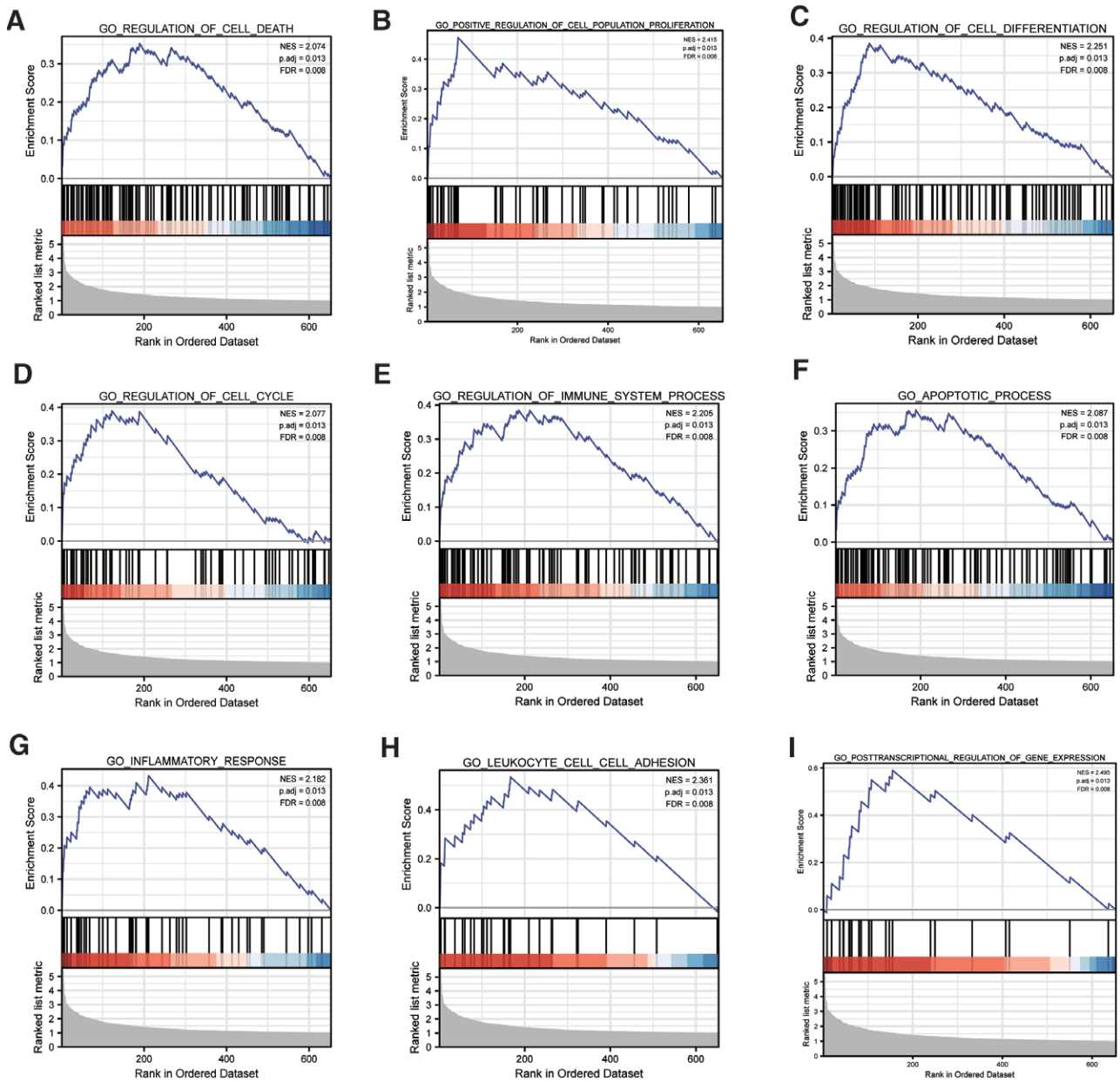

Figure 5. GSEA analysis. (A) Regulation of cell death (NES = 2.074,$P < .05$). (B) Positive regulation of cell population proliferation (NES = 2.415, $P < .05$). (C) Regulation of cell differentiation (NES = 2.251, $P < .05$). (D) Regulation of cell cycle (NES = 2.077, $P < .05$). (E) Regulation of immune system process (NES = 2.205, $P < .05$). (F) Apoptotic process (NES = 2.067, $P < .05$). (G) Inflammatory response (NES = 2.182, $P < .05$). (H) Leukocyte cell adhesion (NES = 2.361, $P < .05$). (I) Posttranscriptional regulation of cell expression (NES = 2.495, $P < .05$).

3.2. Acquisition of potential targets for DGNTD

Through the target prediction system in the TCMSP database, 283 potentially active compounds of DGNTD were input into the database, and a total of 244 potential targets were obtained after the repeated values were deleted. The corresponding standard gene name of each target was obtained by Uniprot database.

3.3. Construction and analysis of herb-active compound-target network

The Herb-Active compound-Target network was constructed using Cytoscape3.7.2 (Fig. 2). The network consists of 545 nodes (including 15 TCM nodes, 286 active compound nodes and 244 target nodes) and 2957 edges. Each edge in the network represents the active components contained in the drug and the interaction between the active components and the target genes, and the value of a node represents the number of routes connected to the nodes in the network. Carries on the topology analysis to the network through the "Network analyze" function. The larger nodes of Degree and Betweenness Centrality (BC) are selected for analysis. These nodes play a pivotal role and play a more critical role in the network. The average value of active components in the network is 10.34. 39.16% of the active components in the network had degree greater than 10. The top five active compounds in the network were MOL000098-quercetin, MOL000422-kaempferol, MOL000392-formononetin, MOL000006-luteolin and MOL000173-wogonin, with degree of 136, 105, 71, 52 and 52 respectively.

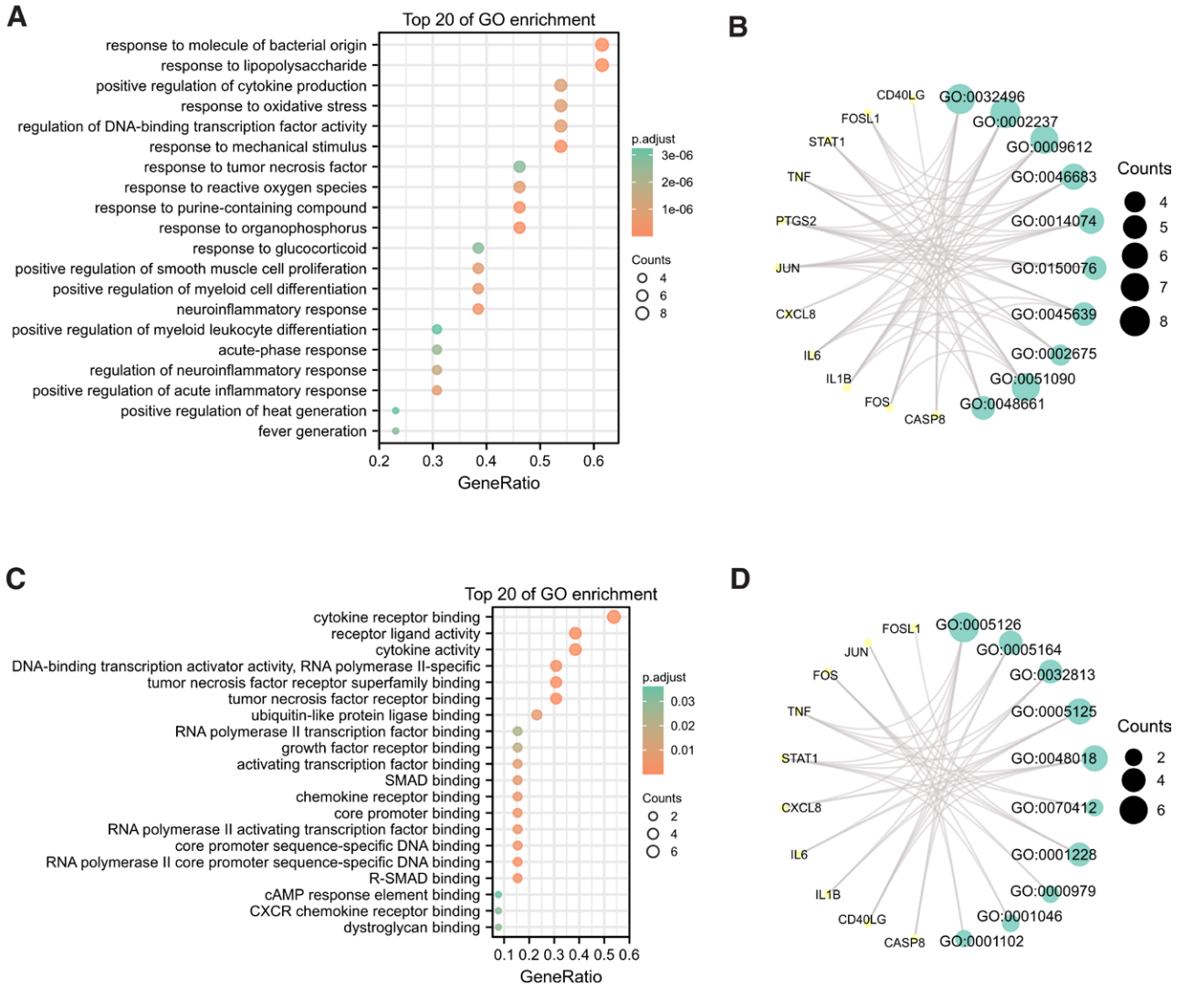

Figure 6. GO enrichment analysis. Y axis represents BP or MF name, X axis represents GeneRatio, bubble area represents the number of enrichment genes, the larger the bubble, the more the number of enrichment genes, the color of bubbles represents the size of *P* value, and the darker the color is, the more significant the enrichment degree is (p.adj < 0.05 and q value < 0.2).

3.4. Acquisition of gout-related targets

Finally, a gout related data set is obtained in the GEO database, and the data set is GSE160170 (GPL21827 platform, Agilent-079487Arraystar HumanLncRNAmicroarrayV4). GSE160170 included 6 peripheral blood samples from gout patients and 6 healthy controls. Using GEO2R to analyze the normalized principal component analysis of the original data set is shown in Figure 3C. In patients with gout, 534 genes were up-regulated and 278 genes were down-regulated. After deleting the repeat value, a total of 799 gout differential genes were obtained. Visualization is performed using R package ggplot 2 (version 3.3.3) and ComplexHeatmap package (version 2.2.0) (Fig. 3A and B). In addition, a total of 19,697 gout related targets were collected from three open databases, including 18004 in CTD database, 1642 in GeneCards database and 51 in OMIM database. After deleting the duplicate value, a total of 17,984 related targets were obtained. 652 gout final targets were obtained by intersecting the differential genes with the related targets obtained from the database. A total of 13 potential targets of DGNTD for treatment of gout were obtained by intersecting the potential targets of DGNTD with the final targets of gout (Fig. 4A and B).

3.5. GSEA analysis of differential genes in gout

GSEA4.1.0 was used to analyze the function of differential genes in GSE:160170 dataset. The background gene was set to c5 (c5.bp.v7.4symbols.gmt). The results of GSEA analysis showed that the differential genes in patients with gout were mainly involved in apoptosis, inflammation, receptor metabolism, immune response and so on (Fig. 5).

3.6. PPI network construction and core target screening

Use Cytoscape 3.7.2 software to build PPI network. The network has 12 nodes and 58 edges (Fig. 4D). Each point in the PPI network represents a protein, and each edge represents a PPI. Using cytoHubba plug-in to obtain the top ten core targets with MNC and MCC and Degree as screening criteria (Fig. 4C). The ten core targets include CASP8, CXCL8, FOS, IL1B, IL6, JUN, PTGS2, STAT1, MMP1 and TNF. These targets may be the key targets for DGNTD to play a role in the treatment of gout.

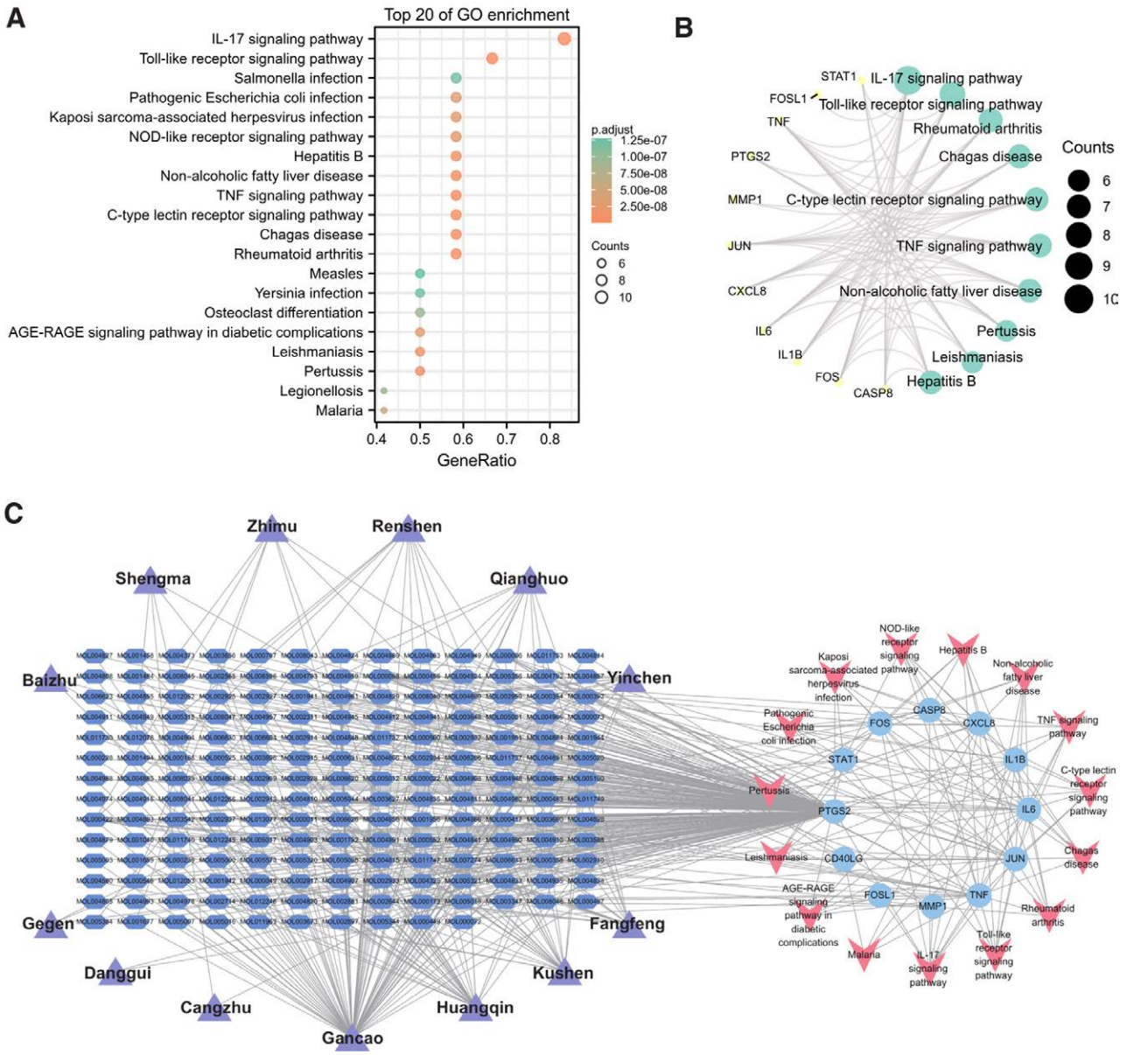

Figure 7. KEGG pathway enrichment analysis. The first 20 KEGG pathways were shown in Y axis and GeneRatio (p.adj < 0.05 and q value < 0.2) in X axis. The color represents the range of *P* value, and the darker the color, the more significant the enrichment. Purple triangles represent herb, blue hexagons represent active compounds, red arrows represent pathways, and sky blue circles represent core targets.

3.7. Go and KEGG enrichment analysis

A total of 949 GO items (p.adj < 0.05 and q value < 0.2) were obtained by GO enrichment analysis. These include 921 BP entries (involving the response to purine-containing compound and response to lipopolysaccharide, positive regulation of acute inflammatory response, regulation of DNA-binding transcription factor activity, positive regulation of myeloid cell differentiation), 24 MF entries (involving cytokine receptor binding, tumor necrosis factor receptor binding, cytokine activity, etc), and 4 CC entries (involving membrane raft, membrane microdomain, membrane region, etc.). We select the first 20 of BP and MF to draw the bubble chart (Fig. 6). A total of 77 KEGG pathways were obtained by KEGG enrichment analysis (p.adj < 0.05 and q value < 0.2). The potential targets of DGNTD in the treatment of gout are mainly concentrated in interleukin-17 (IL-17), toll-like receptor, rheumatoid arthritis, tumor necrosis factor (TNF), NOD-like receptor, osteoclast differentiation and other pathways. We selected the first 20 pathways to draw the bubble map and selected two pathways closely related to gout for visualization (Figs. 7A, B and 8). The details of the first 10 items of BP and MF in GO enrichment analysis and the first 10 items of KEGG enrichment analysis are shown in Table 2. We also analyzed the relationship between the first 15 pathways and their corresponding targets and compounds by constructing the network diagram of Herb-Active components-KEGG pathway-Core target (Fig. 7C). The network has 219 nodes (including 13 herb, 12 core targets, 15 pathways and 179 active compounds) and 541 edges. According to the number of regulatory pathways, the top five core targets were PTGS2, TNF, JUN, IL6 and CXCL8, with 189, 23, 22, 21 and 15 regulatory pathways, respectively.

3.8. Molecular docking analysis

The selected active compounds (including quercetin, kaempferol, formononetin, luteolin and wogonin) were docked with the core target (CASP8, CXCL8, FOS, IL1B, IL6, JUN, PTGS2, STAT1, MMP1 and TNF) by AutodockVina software. The

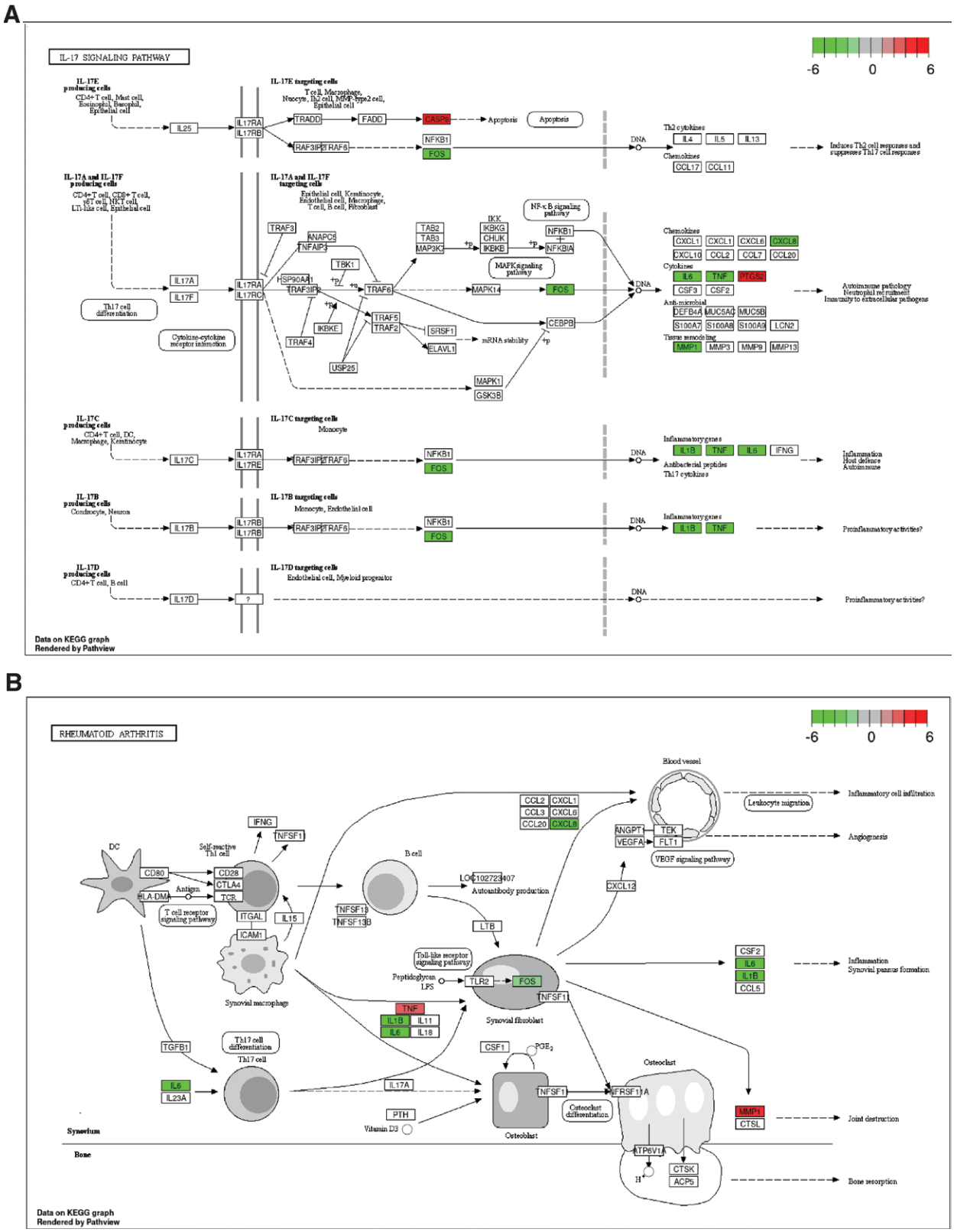

Figure 8. KEGG signal pathway. (A) IL-17 signal pathway. (B) Rheumatoid arthritis pathway. The green and red nodes in the roadmap represent the core targets that exist in our DGNTD.

higher the binding activity of small molecular compounds to the core target protein receptor is, the lower the binding energy is. The docking results showed that the binding energies of 5 small molecular compounds to 9 core target protein receptors were all less than -5 kcal/mol (Table 3). Among them, MMP1 could not be docked because there was no suitable 3D structure. The results of molecular docking are shown in 2D images and 3D structures (Fig. 9).

Table 2

Details of the top 10 entries for 2BP, MF, and KEGG pathways.

Ontology	ID	Description	GeneRatio	BgRatio	*P* value	*P* adjust	*q* value
BP	GO:0032496	Response to lipopolysaccharide	8/13	330/18670	1.04e–11	1.05e–08	3.11e–09
BP	GO:0002237	Response to molecule of bacterial origin	8/13	343/18670	1.42e–11	1.05e–08	3.11e–09
BP	GO:0009612	Response to mechanical stimulus	7/13	210/18670	3.34e–11	1.65e–08	4.86e–09
BP	GO:0046683	Response to organophosphorus	6/13	134/18670	2.01e–10	7.45e–08	2.20e–08
BP	GO:0014074	Response to purine-containing compound	6/13	149/18670	3.83e–10	1.13e–07	3.34e–08
BP	GO:0150076	Neuroinflammatory response	5/13	75/18670	1.15e–09	2.83e–07	8.35e–08
BP	GO:0045639	Positive regulation of myeloid cell differentiation	5/13	91/18670	3.07e–09	6.50e–07	1.92e–07
BP	GO:0002675	Positive regulation of acute inflammatory response	4/13	31/18670	4.40e–09	7.42e–07	2.19e–07
BP	GO:0051090	Regulation of DNA-binding transcription factor activity	7/13	432/18670	5.15e–09	7.42e–07	2.19e–07
BP	GO:0048661	Positive regulation of smooth muscle cell proliferation	5/13	101/18670	5.21e–09	7.42e–07	2.19e–07
MF	GO:0005126	Cytokine receptor binding	7/13	286/17697	4.23e–10	3.00e–08	1.47e–08
MF	GO:0005164	Tumor necrosis factor receptor binding	4/13	31/17697	5.45e–09	1.93e–07	9.46e–08
MF	GO:0032813	Tumor necrosis factor receptor superfamily binding	4/13	46/17697	2.81e–08	6.64e–07	3.25e–07
MF	GO:0005125	Cytokine activity	5/13	220/17697	3.37e–07	5.98e–06	2.92e–06
MF	GO:0048018	Receptor ligand activity	5/13	482/17697	1.58e–05	2.24e–04	1.10e–04
MF	GO:0070412	R-SMAD binding	2/13	23/17697	1.25e–04	0.001	7.23e–04
MF	GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	4/13	439/17697	2.24e–04	0.002	0.001
MF	GO:0000979	RNA polymerase II core promoter sequence-specific DNA binding	2/13	34/17697	2.76e–04	0.002	0.001
MF	GO:0001046	Core promoter sequence-specific DNA binding	2/13	45/17697	4.84e–04	0.004	0.002
MF	GO:0001102	RNA polymerase II activating transcription factor binding	2/13	53/17697	6.72e–04	0.005	0.002
KEGG	hsa04657	IL-17 signaling pathway	10/12	94/8076	1.81e–18	2.34e–16	8.96e–17
KEGG	hsa04620	Toll-like receptor signaling pathway	8/12	104/8076	2.73e–13	1.76e–11	6.76e–12
KEGG	hsa05323	Rheumatoid arthritis	7/12	93/8076	1.61e–11	6.94e–10	2.66e–10
KEGG	hsa05142	Chagas disease	7/12	102/8076	3.13e–11	9.29e–10	3.56e–10
KEGG	hsa04625	C-type lectin receptor signaling pathway	7/12	104/8076	3.60e–11	9.29e–10	3.56e–10
KEGG	hsa04668	TNF signaling pathway	7/12	112/8076	6.11e–11	1.31e–09	5.04e–10
KEGG	hsa04932	Nonalcoholic fatty liver disease	7/12	150/8076	4.86e–10	7.80e–09	2.99e–09
KEGG	hsa05133	Pertussis	6/12	76/8076	5.02e–10	7.80e–09	2.99e–09
KEGG	hsa05140	Leishmaniasis	6/12	77/8076	5.44e–10	7.80e–09	2.99e–09
KEGG	hsa05161	Hepatitis B	7/12	162/8076	8.36e–10	1.08e–08	4.14e–09

Table 3

Docking binding energy of core target proteins with small molecular compounds.

Gene ID	PDB ID	Affinity-Quercetin (kcal/mol)	Affinity-Kaempferol (kcal/mol)	Affinity-Wogonin (kcal/mol)	Affinity-Formononetin (kcal/mol)	Affinity-Luteolin (kcal/mol)
CASP8	7lvm	–7.9	–7.9	–7.7	–7.4	–7.8
CXCL8	6lfl	–7.0	–7.3	–6.0	–7.4	–7.3
FOS	6s32	–8.3	–7.7	–7.6	–7.6	–8.2
IL1B	4gai	–8.3	–8.2	–7.6	–7.6	–8.4
IL6	5gw9	–10.6	–10.3	–9.1	–9.1	–10.5
JUN	6yoj	–9.1	–8.9	–9.2	–9.0	–9.1
PTGS2	3nt1	–7.8	–7.5	–7.5	–7.1	–7.5
STAT1	5y4z	–7.2	–7.1	–6.5	–6.2	–6.7
TNF	1vyr	–8.7	–8.7	–8.9	–8.5	–8.8

4. Discussion

The most common initial symptom of gout is gouty arthritis. The course of the disease is slow, repeated and difficult to cure, and it has become the "second largest" metabolic disease after diabetes.[27] In the 2018 update of evidence-based recommendations for gout diagnosis, European League Against Rheumatism recommends the use of NSAIDs, colchicine and glucocorticoids to control symptoms in the acute attack of gout.[28] Chemical drugs are effective for gout, but the side effects are serious and the compliance of patients is poor.[11] TCM is unique in the diagnosis and treatment of gout. Adopting the method of combining disease differentiation with syndrome differentiation and syndrome differentiation by stages, and using the methods of clearing dampness-heat, promoting blood circulation and removing blood stasis, dredging collaterals and relieving pain will become a new breakthrough to solve the bottleneck of the research and development of new anti-gout drugs. Gout belongs to "arthralgia syndrome" in the category of TCM, most of which are caused by lack of congenital endowment, or old visceral qi declining day by day, coupled with fat, sweet and thick taste, which leads to spleen and kidney dysfunction, dampness and turbid inward, phlegm turbid mutual knot, damp-heat and phlegm turbid erosion joint causing redness, swelling and heat pain, as arthralgia syndrome.[29] Therefore, the key to treatment lies in clearing heat and detoxification, promoting blood circulation and removing blood stasis, dredging collaterals and relieving pain, dispelling dampness and relieving turbid, invigorating the spleen and kidney.[30]

DGNTD has the effect of relieving dampness and clearing heat, soothing wind and relieving pain. it is a classic prescription for the treatment of gout.[13] In this study, we found that the core active components of DGNTD in the treatment of gout include quercetin, kaempferol, formononetin, luteolin and wogonin. Quercetin is the active compound of Licorice (Gancao), Herba Artemisiae (Yinchen) and Sophora flavescens

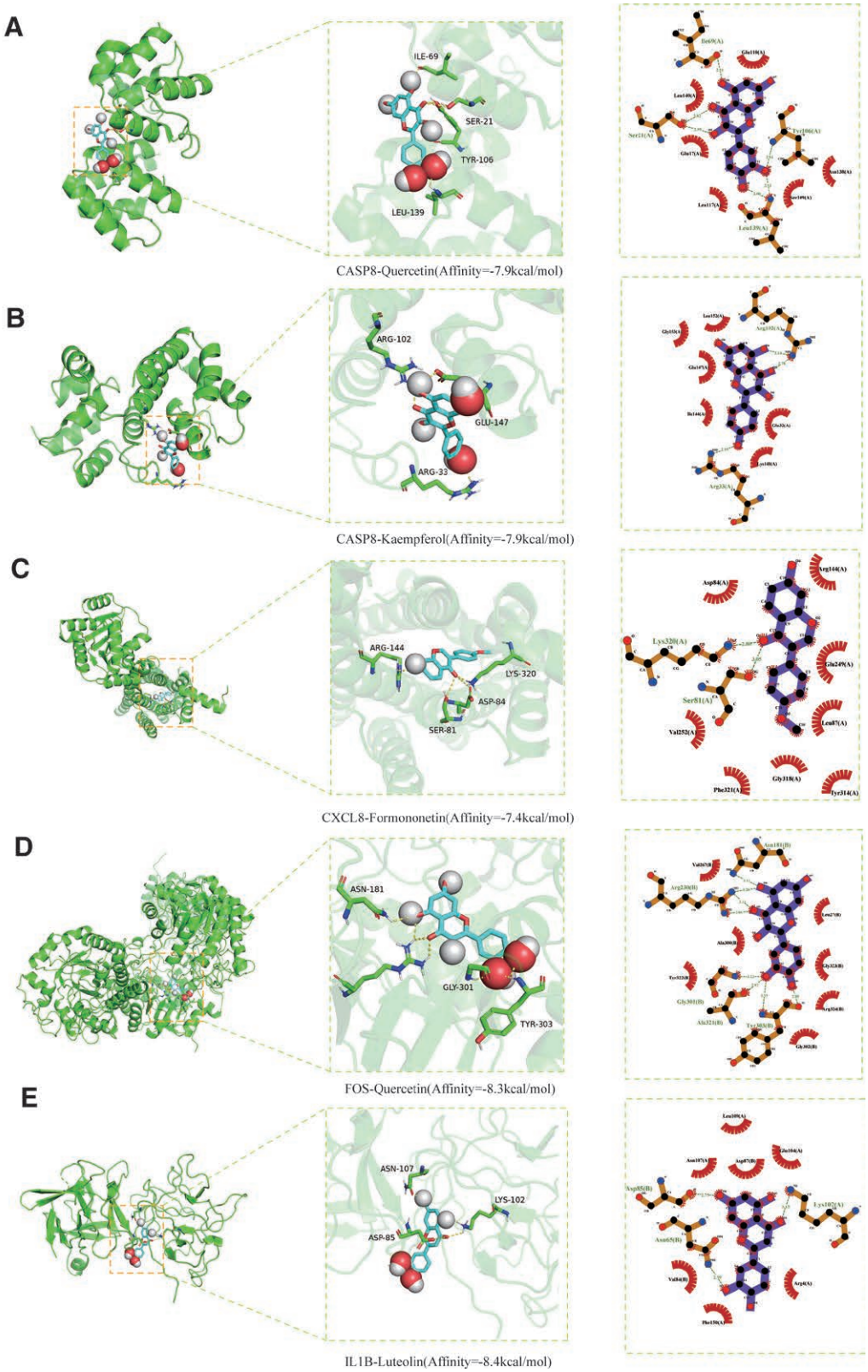

Figure 9. The nine core targets of DGNTD in the treatment of gout are docked with five active compounds.

(Kushen), kaempferol is the active compound of Licorice (Gancao), Ginseng (Renshen) and Sophora flavescens (Kushen), formononetin is the active compound of Licorice (Gancao), Radix puerariae (Gegen) and Sophora flavescens (Kushen), luteolin is the active compound of Radix Atractylodis (Cangzhu), and baicalein is the active compound of Saposhnikoviae Radix (Fangfeng), Radix Atractylodis (Cangzhu) and Radix Scutellariae (Huangqin). Quercetin, kaempferol, formononetin and wogonin are the common active components of two kinds of TCM, which are similar to the synergistic effect of TCM compound prescription.Quercetin is a natural flavonoid. Many studies have shown that quercetin can reduce fructose-induced

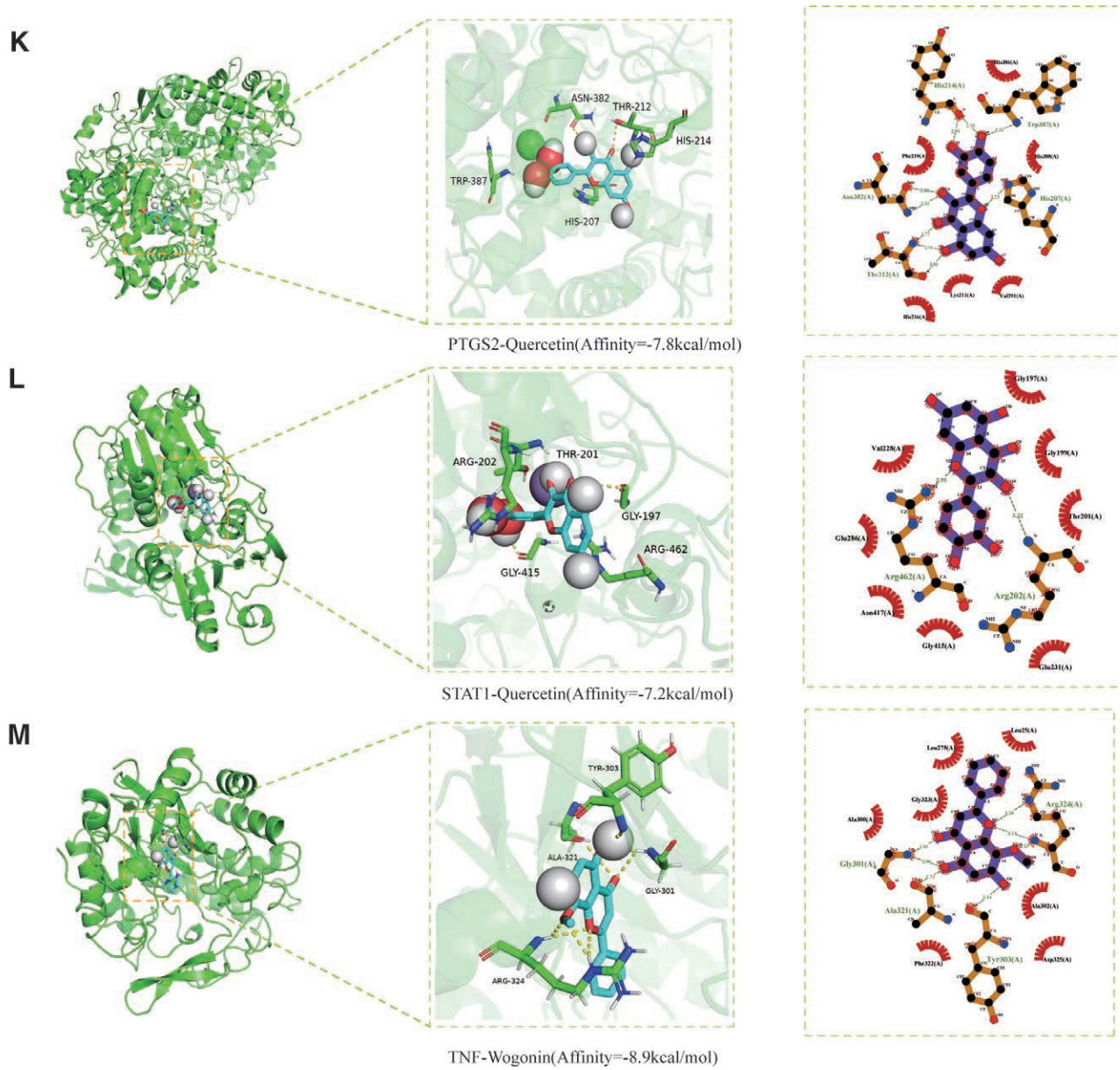

Figure 9. Continued

hyperuricemia and inflammation, and reduce uric acid levels by inhibiting the activity of xanthine oxidoreductase (XO).[31,32] A randomized, double-blind, placebo-controlled, crossover trial showed that quercetin in diet can help maintain healthy serum uric acid levels and prevent the formation of uric acid crystals (gouty arthritis).[33] In addition, Ruiz-Miyazawa KW et al found that quercetin can inhibit gouty arthritis induced by monosodiumurate (MSU), reduce mechanical hyperalgesia and leukocyte recruitment, thus play an anti-inflammatory and analgesic effect.[34] Kaempferol is a flavonoid widely found in diet and Chinese herbal medicine, accounting for 22% of total flavonoid intake 29%.[35] Recent results show that kaempferol can be used as a potential XO inhibitor. Kaempferol blocks the entry of xanthine by inserting the hydrophobic active site of XO, resulting in conformational changes in XO and competitive inhibition of XO activity.[36,37] Previous studies have also shown that kaempferol can reduce serum uric acid levels, serum XO activity and liver XO activity in hyperuricemia mice.[38] Formononetin is a bioactive isoflavone. A number of studies have shown that formononetin has significant anti-inflammatory, anti-allergic, antioxidant, anti-hepatic steatosis and anti-apoptosis effects in vitro and animal models of many diseases.[39,40] The anti-inflammatory effects of formononetin are mainly as follows: reducing inflammatory cell infiltration, goblet cell proliferation and collagen deposition, reducing the expression of inflammatory cytokines, and inhibiting inflammatory signals mediated by NF-kB and JNK.[41] In addition, formononetin can regulate lipid metabolism, improve metabolic microenvironment and inhibit the oxidation activity of XO.[42] Luteolin is also a flavonoid. Studies have shown that luteolin has a strong uric acid-lowering effect on hyperuricemia mice, mainly by reducing uric acid transporter 1 (MURAT1) and inhibiting XO in the kidney. The type of inhibition is competitive inhibition.[43,44] Luteolin is expected to be a promising XO inhibitor in clinical application. Eating foods rich in luteolin, such as celery and green peppers, may be useful for the treatment of hyperuricemia.[44] In addition, it has been proved that the combination of quercetin and luteolin can increase the effect of reducing uric acid.[45] Wogonin can regulate the activity of Th1 and Th2 immune cells, thus reducing inflammation. Huynh DL et al found that lutein

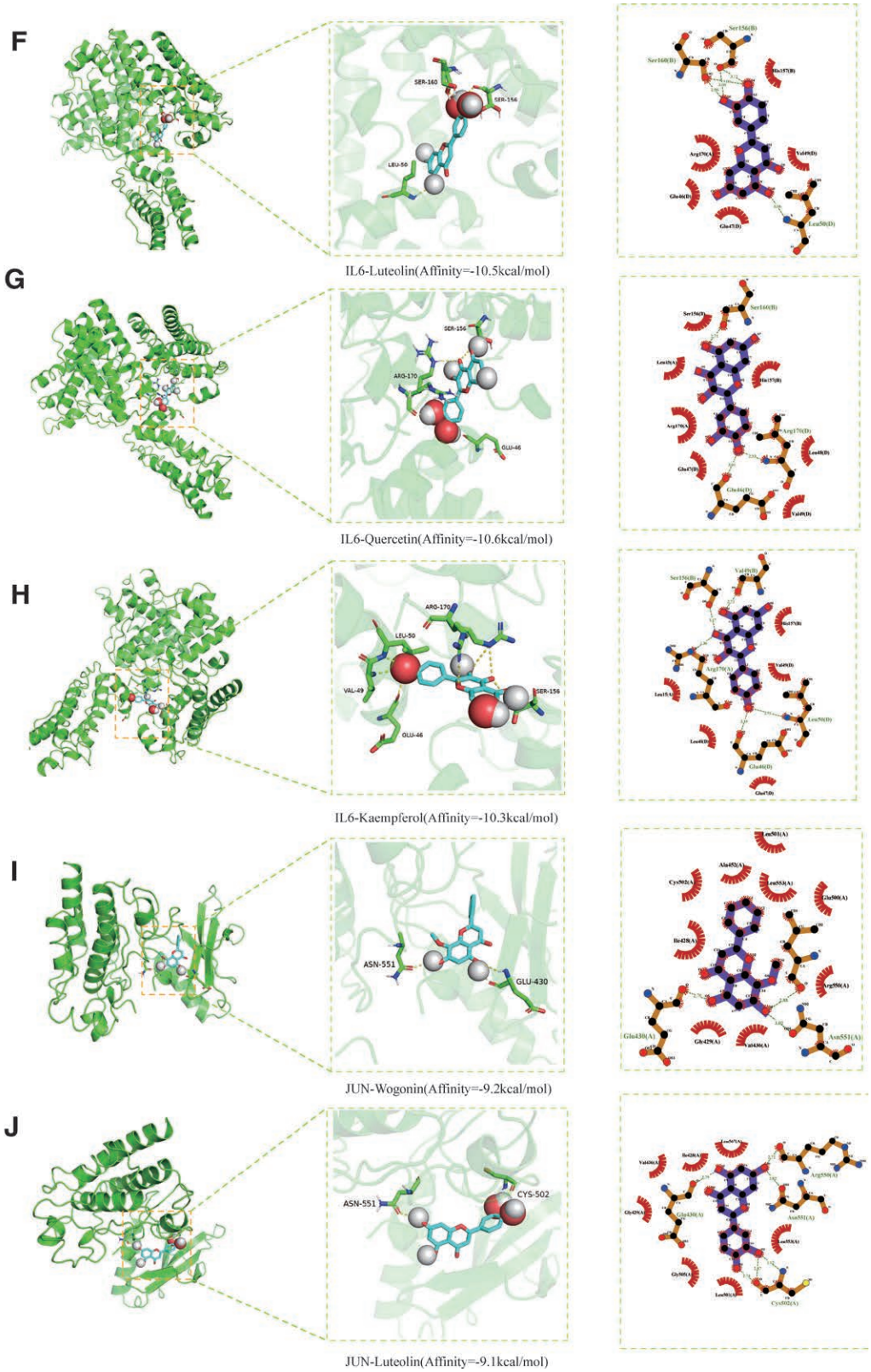

Figure 9. Continued

can significantly down-regulate the expression of biomarkers of osteoarthritis, such as transforming growth factor-β-1, matrix metalloproteinase-13, nuclear factor-β B.[46] Local application of wogonin can reduce the severity of osteoarthritis and relieve the pain of osteoarthritis surgical mouse model.[47] In short, the main active components of DGNTD can regulate a variety of biological mechanisms, thereby inhibiting the biological activity of XO, reducing the level of uric acid and regulating uric acid metabolism, reducing inflammatory response, regulating apoptosis, and preventing or alleviating the development of gout.

PPI network analysis and cytoHubba plug-in screening show that the key targets of DGBTD in the treatment of gout include

CASP8, CXCL8, FOS, IL1B, IL6, JUN, PTGS2, STAT1 and TNF. Caspase-8 is a cysteine-aspartate protease, which is mainly involved in apoptosis and necrotizing ptosis and determines the fate of cells.[48] Studies have shown that Caspase-8 can affect the pathogenesis of rheumatoid arthritis by regulating RIPK signal pathway.[49] CXCL8 (interleukin-8) is a neutrophil chemotactic factor and pro-inflammatory cytokine. Studies have shown that CXCL8 levels in synovial fluid and blood of patients with gout are generally increased.[50] CXCL8 is involved in arthritis and plays a vital role in the occurrence and development of gout.[51] The expression of FOS protein is up-regulated in synovium and chondrocytes of arthritis patients.[52] In the analysis of ceRNA network construction, it was found that FOS protein may be a biomarker of gout.[53] In addition, the activation of FOS signal plays an important role in arthritic joint destruction.[52]IL-1B, IL-6 and TNF are typical proinflammatory cytokines. IL-1B is produced under many inflammatory conditions, but what is unique is that in gout, it is mainly due to the production and drive of IL-1B, which promotes the pathogenesis of gout.[54] Studies have found that gout is caused by the precipitation of uric acid in the blood into insoluble monosodium urate crystals, which can accumulate in the joints, activate NOD-likere ceptorfamilypyrindomaincontaining-3 (NLRP3) inflammatory bodies, and lead to the secretion of IL-1β.[54] The proportion of elevated plasma IL-6 level in patients with gout was significantly higher than that in the control group.[55] In addition, the level of IL-6 in synovial fluid of patients with chronic arthritis is also higher, and the increase of IL-6 secretion may be related to the local and systemic symptoms and signs of patients with chronic arthritis, suggesting that IL-6 may be involved in the pathophysiological process of inflammatory arthritis.[55] Pathology shows that TNF is not only the main mediator in the pathogenesis of osteoarthritis, but also an important regulator of inflammatory response and the initiating factor of inflammation, so it can be used as a target for the treatment of gout.[56] STAT1 is an inflammatory transcription factor, which can inhibit the expression of inflammatory genes by targeting the activation of STAT1.[57] In general, the results show that DGNTD has multi-target characteristics in the treatment of gout.

GO enrichment analysis showed that the therapeutic effects of DGNTD on gout were mainly the response to purine compounds and lipopolysaccharide, the positive regulation of acute inflammation, the regulation of DNA binding transcription factor activity, the positive regulation of myeloid cell differentiation and so on. Through the analysis of KEGG signal pathway, the main mechanism of DGNTD in the treatment of gout is to reduce inflammation and regulate uric acid metabolism. Key signal pathways include IL-17, NOD-like receptor, Toll-like receptor, Rheumatoid arthritis, C-type lectin receptor, TNF and Osteoclast differentiation.IL-17 is an important pro-inflammatory cytokine produced by T helper 17 (Th17) cells, GDT cells and natural killer (NK) cells. Some studies have shown that IL-17 exists in the pain site of inflammatory arthritis, and its synergistic effect can amplify the inflammatory response caused by IL-1, IL-6, IL-8 and TNF-a.[58] Liu et al it was found that IL-17 was involved in the progression of gout inflammation. The level of serum IL-17 in patients with gout increased 8 hours after the acute attack of gout. With the relief of gout symptoms, the expression of IL-17 decreased gradually.[59] In addition, inhibition of IL-17 has been found to slow inflammation and joint erosion in other arthritis animal models.[60] IL-17 has been confirmed by many studies as a biomarker for the diagnosis of gout.[53,61] NOD-like receptor signaling pathway has long been considered to be associated with immunity, inflammation and autophagy.[62] NOD-likereceptor (NLR) is a protein family in which NLRP3 inflammatory bodies can be activated by a variety of stimuli, resulting in aseptic inflammation. One of the main characteristics of gout is the existence of inflammation and specific NLRP3-containing inflammatory domain, which leads to the production and release of inflammatory cytokine IL-1β.[63] Recent studies have confirmed that MSU crystals in the joints of patients with gouty arthritis are effective activators of NLRP3 in vitro.[64] In the pathogenesis of gout, soluble uric acid may cause innate immune damage through the activation of Toll-like receptor signaling pathway, thus promoting the formation of NALP3 inflammatory bodies, the activation of caspase-1, the expression of IL-1β and the excessive production of downstream inflammatory cytokines.[65]C-type lectin receptor signaling pathway is one of the few leukocyte inhibitory receptor pathways associated with disease. The neutrophil response of CLEC12A in its pathway is enhanced due to the decreased expression of CLEC12A plasma membrane after exposure to MSU crystals, which is the evidence that C-type lectin receptor is involved in the pathogenesis of inflammatory diseases.[66] Other research data have shown that neutrophil CLEC12A plays an important role in regulating the severity of chronic inflammatory arthritis.[67] TNF signal pathway has also been proved to be an important inflammatory signal pathway. Studies have shown that gouty arthritis can effectively inhibit local inflammation and relieve pain by inhibiting the expression of TNF-α in synovial cell during acute attack of gouty arthritis.[68]

Molecular docking reveals the interaction between the components of the network, thus improving the accuracy of the network. The core target protein of DGNTD for gout was docked with active compounds. The results showed that the docking binding energies of IL-6, JUN and TNF with quercetin, kaempferol, formononetin, luteolin and wogonin were relatively low in each group. It shows that IL-6 has higher binding affinity than other core targets. Among the docking results, the binding energy of quercetin and IL-6 is the lowest, which is -10.6 kcal/mol. According to the above analysis, quercetin may target IL-6 to treat gout and relieve gout inflammatory symptoms. In addition, the main characteristic chemical components of DGNTD (quercetin, kaempferol, formononetin, luteolin, wogonin) have good binding ability with the core target (CASP8, CXCL8, FOS, IL1B, JUN, PTGS2, STAT1, TNF), indicating that the combination of the active components of DGNTD with the core target can effectively treat gout. This is consistent with the results of network pharmacological analysis. These results may be of great significance to further study and determine the molecular targets of DGNTD in the treatment of gout and the application of therapeutic mechanism in the development of new gout drugs. However, there are still some shortcomings in this study. In the later stage, in vivo and in vitro experiments, metabonomics and pathway verification are needed to further analyze the mechanism of DGNTD in the treatment of gout.

5. Conclusion

In this study, we systematically described the active compounds of DGNTD in the treatment of gout and its molecular mechanism. The main active compounds of DGNTD were screened by interaction network topology analysis, including quercetin, kaempferol, formononetin, luteolin and wogonin; the core target of DGNTD for gout treatment was CASP8, CXCL8, FOS, IL1B, IL6, JUN, PTGS2, STAT1, MMP1 and TNF by PPI and Hub gene screening. These core targets have stable binding activity to the main active compounds (except MMP1). DGNTD can also regulate gout-related signaling pathways, such as IL-17, NOD-like receptor, Toll-like receptor, Rheumatoid arthritis, C-type lectin receptor and TNF. It is suggested that DGNTD has potential synergistic effect in the treatment of gout through multi-component, multi-target and multi-signal pathway. However, due to the limitations of network pharmacology and bioinformatics, further animal experiments and clinical trials are needed to verify the relevant conclusions in the future.

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None.

Author contributions

Conception and design of the research: YL; acquisition of data: DL; analysis and interpretation of data: BX; statistical analysis: DL; drafting the manuscript: YL; revision of manuscript for important intellectual content: BX, YL. All authors read and approved the final manuscript.
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Investigation: Yuan Liu.
Methodology: Yuan Liu.
Software: Yuan Liu.
Supervision: Bo Xu.
Project administration: Bo Xu.
Resources: Bo Xu, Di Luo.
Visualization: Yuan Liu.
Writing – original draft: Yuan Liu.

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