Journal of Clinical and Nursing Research, 2025, Volume 9, Issue 2
http://ojs.bbwpublisher.com/index.php/JCNR
Online ISSN: 2208-3693
Print ISSN: 2208-3685

The Molecular Mechanism of Weilingxian and Guizhi in the Treatment of Gout was Studied Based on Network Pharmacology

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Abstract: *Objective:* To explore the mechanism of action of "Weilingxian-Guizhi" two drugs in the treatment of gout. *Method:* First obtain the 15 chemical components contained in the "Weilingxian-Guizhi" drug, predict its target points through the database, and then construct the gout-related protein-protein interaction network (PPI), and then construct the "Weiling," the "Xian, Guizhi" drug pair "active ingredient-predicted target" network, the construction of the gout-related "Weilingxian-Guizhi" drug pair "active ingredient-potential target" network, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathway enrichment analysis and gene ontology (GO) functional enrichment analysis, to study the mechanism of action of the two drugs "Weilingxian-Guizhi" in the treatment of gout. *Results:* The gout-related "Weilingxian-Guizhi" drug pair "active ingredient-potential target" network contains 14 targets. KEGG pathway enrichment analysis yields 32 pathways, and GO functional enrichment analysis yields 517 GO entries. Among them, there are 468 items related to biological processes, 21 items related to molecular functions, and 28 items related to cell composition. *Conclusion:* The results of this study initially verified and predicted the mechanism of the "Weilingxian-Cinnamon Stick" medicine on the treatment of gout, and laid a good foundation for further revealing its mechanism of action.

Keywords: Weilingxian; Guizhi; Gout; Network pharmacology; Target; Signal pathway

Online publication: March 10, 2025

1. Introduction

If a doctor does not understand a disease, it is like a general not knowing his troops. To treat a disease, one must first understand it. The term "gout" in traditional Chinese medicine includes conditions such as gout, bi syndrome (a type of rheumatic disease), and Li Jie (a condition characterized by painful joints). These disease names have existed since ancient times, tracing back to the "Yellow Emperor's Inner Canon of Medicine." In the "Plain

Questions: Discussion on Bi Syndrome" chapter, it is stated, "When wind, cold, and dampness combine, they form bi syndrome" [1]. Clematis Root (Weilingxian) has a pungent and salty taste, warm properties, and slight toxicity. It is effective in dispelling wind and dampness, promoting the flow of Qi and blood, and softening bones. It is a commonly used herb in the clinical practice of traditional Chinese medicine [2]. Guizhi (Cassia Twig) has a pungent and sweet taste, and warm properties, and is effective in inducing sweating to relieve muscle tension, warming and unblocking meridians, assisting Yang to transform Qi, and calming the rebellion of Qi. Guizhi is often used in combination with other herbs to treat various diseases [3]. Weilingxian and Guizhi are frequently used in the treatment of gout and bi syndrome, and a typical example is Guizhi Shaoyao Zhimu Decoction [4]. This formula was devised by Zhang Zhongjing, and in the "Synopsis of Golden Chamber: Stroke and Li Jie" chapter, it is stated, "For pain in all limbs and joints, emaciation of the body, swelling of the feet as if they were about to fall off, dizziness, shortness of breath, and a feeling of warmth and nausea, Guizhi Shaoyao Zhimu Decoction is prescribed."

So far, countless doctors have used Weilingxian and Guizhi, with adjustments, to treat bi syndrome. Studies have also shown that Weilingxian and Guizhi have significant anti-gout effects [5]. With the modernization of traditional Chinese medicine, research on the mechanism of action of these two herbs in treating gout has gradually deepened, entering the stage of molecular biology. However, from a general perspective, there are few examples of using network pharmacology methods to reveal their mechanism of action. Unlike chemical drugs, which typically have a single component and target [6], traditional Chinese medicines are characterized by multiple components, targets, and pathways that work synergistically. Because of this, traditional pharmacological research has difficulty elucidating the material basis and related mechanisms of action of traditional Chinese medicines. Network pharmacology has emerged to fill this gap in traditional Chinese medicine research.

Network pharmacology is a new method and model for drug design and development based on modern pharmacology. It analyzes the overall level of disease pathogenesis from a systems perspective and observes the complex network relationships between "drug-target-disease," further guiding new drug development and research on pharmacological effects [7]. Network pharmacology upgrades the previous drug research model of "one component, one target" to a new model of "multiple targets, multiple components," thus revealing the complex relationships between multiple components and targets of traditional Chinese medicines more deeply. Due to the complex mechanism of action of traditional Chinese medicines, further research has encountered significant obstacles. However, network pharmacology provides new ideas and entry points for the study of complex traditional Chinese medicine systems [8]. Network pharmacology can be used to discover active components in complex traditional Chinese medicines, identify targets, and predict indications [9]. This article analyzes the targets of the active components of Weilingxian and Guizhi in the treatment of gout based on network pharmacology, explores their mechanism of action and material basis, and hopes to provide references for further basic experimental research and clinical rational application of "Weilingxian and Guizhi" in the treatment of gout, as well as open up new ideas.

2. Materials and methods

2.1. Collection of active ingredients and corresponding targets of the "Weilingxian and Guizhi" herbal pair

Using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://

lsp.nwu.edu.cn/tcmsp.php) [10], this study searched for "Weilingxian" and "Guizhi" to identify active ingredients. The study screened for a subset of highly relevant ingredients based on an OB greater than 30% and a DL greater than 0.18. The corresponding targets for these active ingredients were then obtained through TCMSP and verified using the UniProt database to determine the abbreviated human gene names for each target, ultimately acquiring the target proteins.

2.2. Prediction of gout disease-related targets

Using "gout" as the keyword, the study searched the GeneCards database (http://www.genecards.org/) and the Online Mendelian Inheritance in Man (OMIM) database (http://www.omim.org/) to obtain gout-related targets. After removing duplicate gene targets, the final set of targets was determined.

2.3. Intersection of drug targets and disease targets

The study created a Venn diagram online using the website (http://bioinformatics.psb.ugent.be/webtools/Venn/) to identify the intersection of target proteins and potential targets obtained from steps 2.1 and 1.2. This allowed us to acquire the targets related to both "Weilingxian, Guizhi" and gout.

2.4. Construction of Protein-protein Interaction Network (PPI)

The common targets obtained from step 2.3 were imported into the STRING website (https://string-db.org/), selecting "*homo sapiens*" as the organism type to generate a protein-protein interaction information graph.

2.5. GO functional enrichment analysis and KEGG pathway enrichment analysis

DAVID website (https://david.ncifcrf.gov/gene2gene.jsp) was logged in and DAVID 6.8 (Functional Annotation Clustering) was used to perform GO gene functional analysis of the target proteins related to the treatment of gout with "Weilingxian and Guizhi." This analysis covered three aspects: Molecular Function (MF), Cellular Component (CC), and Biological Process (BP). To elucidate the role of these therapeutic targets in signaling pathways, the study conducted a KEGG pathway enrichment analysis. The study selected GO functional terms and KEGG pathway terms as the main gene functional biological processes and signaling pathways involved in gout treatment, predicting the mechanism of action of the two herbs in treating gout.

3. Results

3.1. Screening of active ingredients from "Weilingxian and Guizhi"

Using the keywords "Weilingxian" and "Guizhi" in the TCMSP database, a total of 277 known active ingredients were obtained, among which 13 met the criteria (OB > 30%, DL > 0.18). See **Table 1**.

Table 1. Active ingredients of "Weilingxian and Guizhi"

Serial Number	Mol ID	Compound	OB value	DL value
1	MOL001736	(-)-taxifolin	60.51	0.27
2	MOL000358	beta-sitosterol	36.91	0.75
3	MOL000359	sitosterol	36.91	0.75
4	MOL000492	(+)-catechin	54.83	0.24

Table 1 (Continued)

Serial Number	Mol ID	Compound	OB value	DL value
5	MOL000073	ent-Epicatechin	48.96	0.24
6	MOL004576	Taxifolin	57.84	0.27
7	MOL011169	Peroxyergosterol	44.39	0.82
8	MOL001663	(4aS,6aR,6aS,6bR,8aR,10R,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-Tetradecahydropicene-4a-carboxylic acid	32.03	0.76
9	MOL002372	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	33.55	0.42
10	MOL000449	Stigmasterol	43.83	0.76
11	MOL005594	ClematosideA'_qt	37.51	0.76
12	MOL005598	Embinin	33.91	0.73
13	MOL005603	Heptyl phthalate	42.26	0.31

3.2. Prediction of targets for "Weilingxian and Guizhi" drugs and gout disease

Using the target prediction function in TCMSP, target prediction was performed on the 13 active ingredients identified in 3.1. This resulted in 71 target proteins for "Weilingxian" and 51 target proteins for "Guizhi." After removing duplicates, 64 target proteins remained. Using "GOUT" as the keyword, a total of 1062 targets related to gout were identified in the OMIM and GeneCards databases. After removing duplicates, 1057 targets were obtained. By creating a venn diagram to find the intersection, 14 targets associated with gout in "Weilingxian and Guizhi" were identified. See **Figure 1**. The venn diagram was created using the online tool at (http://bioinformatics.psb.ugent.be/webtools/Venn/).

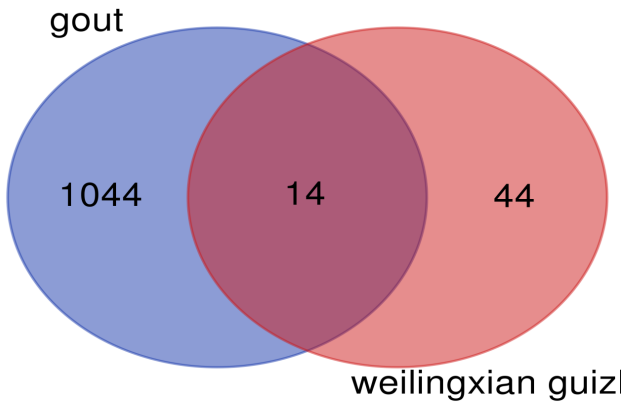

Figure 1. Venn diagram of drug targets and predicted disease targets.

3.3. PPI network of "Weilingxian and Guizhi" targets for gout treatment

The 14 targets of "Weilingxian and Guizhi" for gout treatment were input into the STRING database (https://string-db.org). Based on preset conditions, a PPI network diagram was constructed, as shown in **Figure 2**. It can be observed that PTGS2 has the most connections with other targets, followed by TGFB1, ICAM1, PLAU, and JUN.

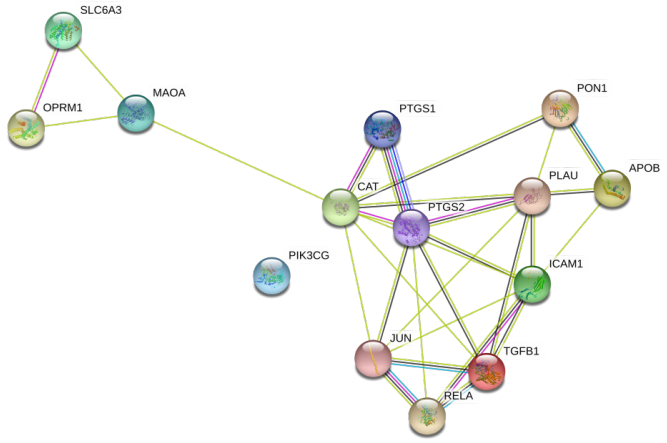

Figure 2. PPI network diagram of "Weilingxian and Guizhi" targets for gout treatment.

3.4. GO functional analysis and KEGG pathway enrichment analysis

GO functional enrichment analysis was performed using the DAVID platform (https://david.ncifcrf.gov/gene2gene.jsp). The 14 targets obtained in 3.2 were input, resulting in 517 GO terms. Based on the *P*-value, these terms were arranged in ascending order, and the top ten terms from each category (Biological Process, Molecular Function, and Cellular Component) were selected, totaling 30 terms (**Figure 3**). Among them, there were 468 terms related to Biological Process (BP), 21 terms related to Molecular Function (MF), and 28 terms related to Cellular Component (CC). Furthermore, using the KEGG pathway enrichment analysis function of the DAVID platform, the roles of the 14 proteins in the signal pathways of the "Weilingxian and Guizhi" active ingredient-potential target network related to gout were marked. This resulted in 37 signaling pathways, and 32 were selected based on $P < 0.05$ (**Figure 4**).

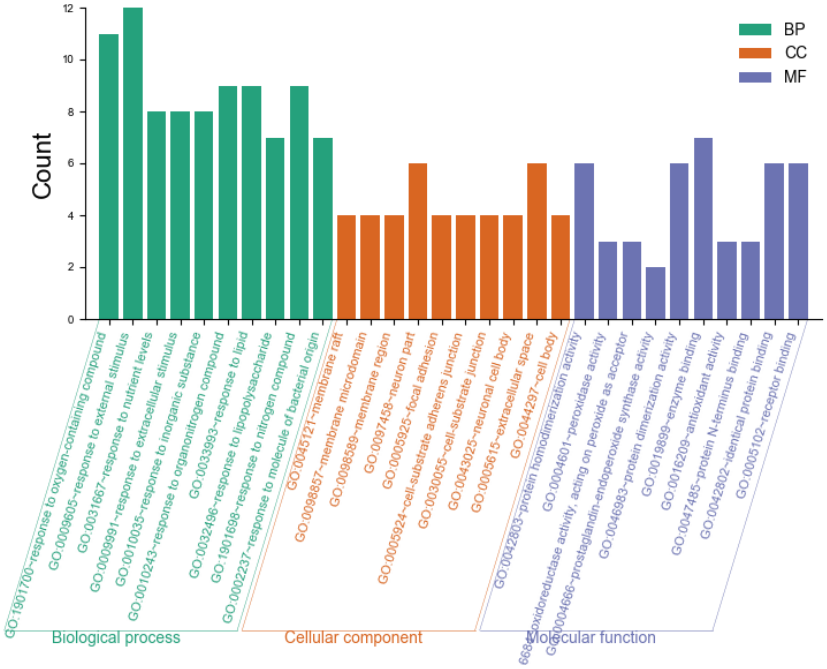

Figure 3. GO functional enrichment analysis of potential target effects.

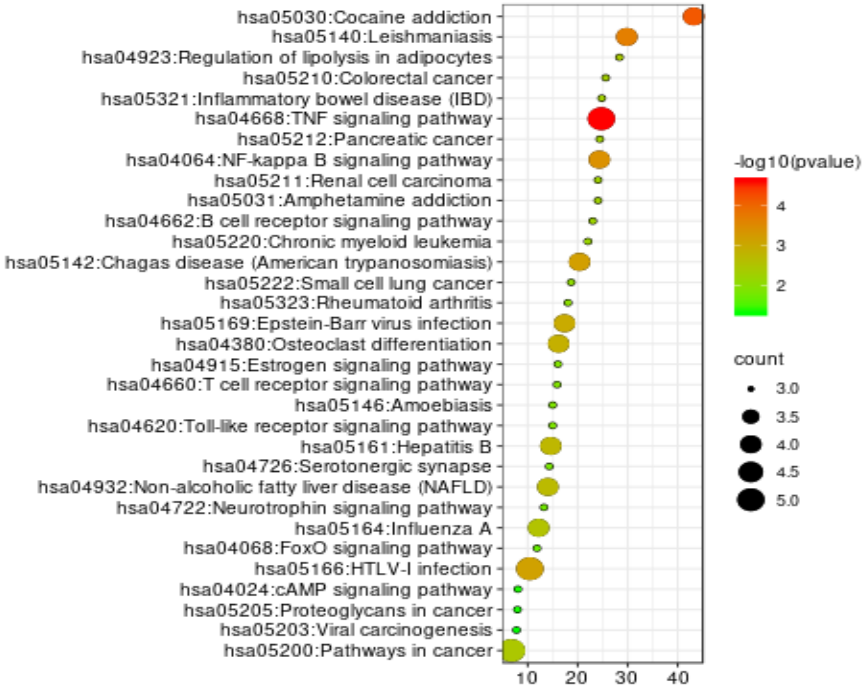

Figure 4. KEGG pathway enrichment analysis of potential target effects.

4. Discussion

Gout has been known by many names in ancient times, such as “Bi syndrome” and “Li Jie” [11]. Weilingxian and Guizhi are commonly used clinical medications for the treatment of gout, with significant effects [12]. With the development of society and the continuous improvement of people’s living standards, the incidence of gout has shown a continuously increasing trend. In China, it is about 0.34–2.84%, and the average age of the affected population is getting younger [13]. Modern pathophysiological studies have found that gouty arthritis can be divided into primary and secondary gouty arthritis, which is related to the phagocytosis of autoimmune cells in the body’s immune system and the negative regulation of inflammatory mediators such as NLRP3 inflammatory corpuscles and interleukin [14].

This study found that the main active ingredients of Weilingxian and Guizhi for the treatment of gout include dihydroquercetin, β-sitosterol, catechin, epicatechin, ergosterol peroxide, taxifolin, and stigmasterol. Studies have confirmed that dihydroquercetin and β-sitosterol have the effect of inhibiting inflammatory responses [15,16]. Gabay *et al.* found that stigmasterol also has a certain inhibitory effect on inflammatory responses, possibly by blocking the NF-kB pathway activated by IL-1β [17]. However, catechin is currently mainly used for anti-radiation [18], and whether it has an anti-inflammatory effect and whether it promotes the treatment of gout requires further research and confirmation.

The pathogenesis of gout is related to immune regulation and inflammatory responses. Uric acid sodium, which is recognized by immune cells and inflammatory factors, activates the NF-kB transcription factor pathway, releasing inflammatory mediators such as TNF and IL-1β. These inflammatory mediators cascade to produce inflammatory responses, joint spasms, swelling, fever, and pain [19,20]. The results of the KEGG pathway enrichment analysis in this study showed that the pathways regulated by Weilingxian and Guizhi, which are related

to immune regulation and inflammation, mainly include the tumor necrosis factor signaling pathway, nuclear transcription factor-kB signaling pathway, B-cell receptor signaling pathway, and cAMP signaling pathway. At the same time, some disease pathways are also enriched, such as rheumatoid arthritis, hepatitis B, small cell lung cancer, and colorectal cancer. Studies have shown that these diseases are closely related to inflammatory pathways, and Weilingxian and Guizhi have good therapeutic effects on them [21–23]. It can be inferred that the most critical pathway for the treatment of gout with Weilingxian and Guizhi is the inflammatory signaling pathway.

5. Conclusion

Network pharmacology is a new discipline that has emerged in recent years. Based on the theory of systems biology, it analyzes biological systems networks and selects specific signal nodes (Nodes) for multi-target drug molecule design. Network pharmacology emphasizes multi-pathway regulation of signaling pathways to improve the therapeutic effect of drugs, and reduce toxic and side effects, thereby increasing the success rate of clinical trials of new drugs and saving drug development costs [24]. The "single drug-single gene-single disease" approach has been the long-standing method for developing new drugs and is one of the important reasons for the failure of 70% of new drugs in clinical trials. In fact, many chronic diseases in clinical practice, such as tumors, cardiovascular and cerebrovascular diseases, and diabetes, are multi-gene and multi-factor diseases. It is difficult to achieve a good therapeutic effect by relying on a single target [25]. Of course, network pharmacology also has its limitations. For example, the efficacy of the macromolecular substance Polysaccharide from Clematis root (Weilingxian polysaccharide) has been confirmed, but it is not included in TCMSP, BATMAN, etc. Another example is that the KEGG pathway analysis results show a correlation with HTLV-I infection, EB virus infection, and amoebiasis pathways, but there is not enough evidence to confirm this, and further investigation is needed.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Li G, 2017, Experience in the Treatment of Gout. Modern Chinese Medicine, 37(4): 5–7.

[2] Du X, Sun Z, Gong S, et al., 2020, Research on the Historical Evolution of the Medicinal Use of Weilingxian. Shandong Journal of Traditional Chinese Medicine, 39(8): 874–878.

[3] Zhu H, Qin L, Du P, et al., 2017, Research Progress on Pharmacological Activity and Clinical Application of Guizhi. Chinese Ethnic Medicine and Ethnopharmacology, 26(22): 61–65.

[4] Wu S, 2014, Study on the Mechanism of Action of Guizhi Shaoyao Zhimu Decoction in the Treatment of Gout, thesis, Shanxi Academy of Traditional Chinese Medicine.

[5] Zhang L, 2019, Research on the Clinical Medication Rules and Mechanism of Action of Traditional Chinese Medicine in the Treatment of Rheumatic Diseases Based on Data Mining, thesis, Beijing University of Chinese Medicine.

[6] Jiang N, Zhang Y, Du G, 2016, New Ideas and New Methods - Research on Pharmacology of Traditional Chinese Medicine and Development of New Traditional Chinese Medicine. Chinese Journal of Pharmacology and Toxicology, 30(9): 893–909.

[7] Gao N, Li T, Cheng Y, et al., 2017, Network Pharmacology Study on the Mechanism of Action of Effective

Components of Iridoid Glycosides in Gentiana. Chemical Engineer, 31(1): 14–16 + 26.

[8] Liu Z, Wang B, 2019, Research Progress on Screening of Pharmacological Components and Target Prediction of Traditional Chinese Medicine Network Pharmacology. Chinese Traditional Patent Medicine, 41(1): 171–178.

[9] Wu C, Lu L, Liang S, et al., 2016, Application of Drug Target Prediction Technology in Traditional Chinese Medicine Network Pharmacology. China Journal of Chinese Materia Medica, 41(3): 377–382.

[10] Zhao M, Li F, Shi B, et al., 2020, Systems Pharmacology (VI) - TCMSP Database and Its Application. Information on Traditional Chinese Medicine, 37(3): 22–29.

[11] Lu Y, Meng F, Liu H, 2015, The Evolution and Development of Disease Names Related to Gout in Traditional Chinese Medicine. World Chinese Medicine, 10(4): 609–612.

[12] Liang H, Zhang Y, Xie J, et al., 2019, Progress in the Treatment of Acute Gouty Arthritis with Traditional Chinese Medicine. Chinese Journal of Emergency in Traditional Chinese Medicine, 28(3): 553–556.

[13] Yang Q, Mao Y, Chen Y, et al., 2020, Clinical Observation of 60 Cases of Gout Treated with Gout Prescription Combined with Indomethacin. Hunan Journal of Traditional Chinese Medicine, 36(9): 9–11 + 23.

[14] Mangan MSJ, Olhava EJ, Roush WR, et al., 2018, Targeting the NL-RP3 Inflammasome in Inflammatory Diseases. National Reviews of Drug Discovery, 17(8): 588–606.

[15] Wang J, Chen K, Wang Y, et al., 2016, Comparative Study on Anti-inflammatory Activity of Dihydroquercetin and Dihydromyricetin. Chinese Journal of Veterinary Drug, 50(7): 46–52.

[16] Xiao Z, Liu X, Cheng R, et al., 2015, Experimental Study on the Effect of β-Sitosterol on Resistance to Aspirin Side Effects and Anti-inflammatory Effects. Journal of Inner Mongolia Medical University, 37(4): 350–354.

[17] Gabay O, Sanchez C, Salvat C, et al., 2010, Stigmasterol: A Phytosterol with Potential Anti-osteoarthritic Properties. Osteoarthritis Cartilage, 18(1): 106–116.

[18] Tan X, Dong X, Guo D, et al., 2016, Study on the Anti-radiation Effect and Mechanism of Ethanol Extract of Jixueteng and Its Active Ingredient Catechin. China Journal of Chinese Materia Medica, 41(9): 1718–1724.

[19] Jia L, Wang R, He D, 2018, Understanding of the Pathogenesis of Gout from the Perspective of Traditional Chinese and Western Medicine. Rheumatism and Arthritis, 7(2): 61–64.

[20] Liao G, 2017, Research Progress on the Pathogenesis and Treatment of Gout. Chinese and Foreign Medical Research, 15(8): 161–163.

[21] Li T, Li Y, Liu L, et al., 2009, Mechanism of Total Saponins from Weilingxian Against Rheumatoid Arthritis. Journal of China Pharmaceutical University, 40(2): 157–160.

[22] Guo Q, 2016, Prediction of Network Targets and Mechanism of Action of Guizhi Shaoyao Zhimu Decoction Against Rheumatoid Arthritis, thesis, China Academy of Chinese Medical Sciences.

[23] Gu Z, 2015, Study on the Molecular Signaling Pathway Mechanism of TLRs/MyD88 Intervention in Acute Gout Inflammation by Cold and Warm Compatibility of Huzhang-Guizhi Drug Pair, thesis, Guangdong Pharmaceutical University.

[24] Ren Y, Deng Y, Ma H, et al., 2020, Research Progress and Challenges of Network Pharmacology in the Field of Traditional Chinese Medicine. Chinese Herbal Medicine, 51(18): 4789–4797.

[25] Dong P, Li H, Han H, 2020, Application and Thinking of Traditional Chinese Medicine Network Pharmacology. Chinese Journal of Experimental Traditional Medical Formula, 26(17): 204–211.