Anti-Inflammatory Effect and Pharmacological Study of Polygonum Capitatum Based on Network Pharmacology

Rongze Fang1, Xiaofen Li2, Juan Kong1, Xiaosong Yang1, Linlin Zhang1, Deguang Wan2, Xiangpei Wang1, Hongmei Wu1, *

1Department of Pharmacognosy, Guizhou University of Traditional Chinese Medicine, 50, Nanning District, Guiyang City, Guizhou Province, Guiyang 550002, PR China
2Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, Chengdu 611137, PR China

*Corresponding author: hongmeiwu@gzy.edu.cn

Abstract. To observe the inhibitory effect of Polygonum capitatum extract on auricle swelling induced by xylene in mice, and explore the mechanism of anti-inflammatory action with the method of network pharmacology. Extract the total flavonoids, sugars and tannins from Polygonum capitatum, and act on xylene induced auricular inflammation in mice. Use 8mm hole punch to lay down the ear piece and measure the quality of the ear piece. Through consulting CNKI, Wanfang, Vip and other databases, the chemical constituents related to Polygonum capitatum were searched.Screening the active chemical constituents of Polygonum capitatum by ADME parameters. Find a target with the active chemical composition and establish a target data set; Protein interaction network (PPI) was used to construct the protein interaction network of target and inflammatory target of Polygonum capitatum. Cytoscape software was used to construct the complex network diagram of “component-target-pathway-disease”; DAVID was used for GO functional enrichment analysis and KEGG pathway enrichment analysis. Result: The experimental results of auricle inflammation induced by xylene in mice showed that the high dose group of total flavones, the low dose group of total flavones and the low dose group of tannin had inhibitory effects on auricle inflammation induced by minor xylene in mice. There were 37 reported compounds, 6 active compounds and 2 flavonoids compounds. A total of 268 targets were retrieved from 6 active compounds, and 41 potential targets were most closely related to the anti-inflammatory mechanism of the Polygonum capitatum. Through the biological function pathway enrichment and KEGG pathway enrichment analysis, 20 biological processes and 76 signal pathways were screened. Conclusion: The anti-inflammatory components of the Polygonum capitatum predicted by the network pharmacology and the experimental results showed that the anti-inflammatory effect of the total flavonoids in the high-dose group was basically the same as that in the low-dose group. It is suggested that Quercetin and Kaempferol may be the anti-inflammatory flavonoids in Polygonum capitatum.
Keywords: Polygonum capitatum; Active ingredient; Inflammation; Network pharmacology; Pharmacological studies.

1. Introduction

Polygonum capitatum is the dry whole grass or aboveground part of Polygonum capitatum Buch.-ham.ex D.Don. Polygonum capitatum is mainly used to treatment of dysentery, urocystitis, eczema and pyelonephritis [1-4]. It has the functions of clearing heat and removing dampness, activating blood analgesia and stranguria-relieving. It has been included in the 2003 edition of “Quality Standards for Chinese Medicinal Materials and National Medicinal Materials of Guizhou Province” and is one of the commonly used seedling medicines in Guizhou [5].

There are traditional Chinese medicines made from Polygonum capitatum, such as Relinqing capsules, Relinqing granules, etc. Now widely used clinically for nephritis and unfavorable urination. Modern research shows that the Polygonum capitatum contains flavonoids, phenolic acids, organic acids, alcohols, esters, aldehydes, lignans, triterpenes, etc. These ingredients have antibacterial, anti-inflammatory, antioxidant, antipyretic and analgesic properties, etc. Pharmacological action [6]. Which is rich in compounds such as quercetin, gallic acid, kaempferol, and a variety of phenolic flavonoids have anti-inflammatory effects [7-8].

In recent years, there have been many reports on the anti-inflammation of Polygonum capitatum. However, it is rarely reported from the cellular and molecular level to comprehensively reveal the ways in which the Polygonum capitatum exerts its anti-inflammatory action mechanism, which needs to be further strengthened. Chinese medicine is a complex system with multiple components, multiple action targets, and multiple action pathways. Network pharmacology is an important part of systems biology, and its holistic, systematic, and drug-focused characteristics are consistent with the basic characteristics of traditional Chinese medicine [9]. Network pharmacology constructs complex network graph of “component-target-pathway-disease” by mining a large amount of data. Combined with the study of pharmacodynamics, it laid a foundation for the foundation of the pharmacodynamics of traditional Chinese medicine, and provided new ideas and methods for the study of the mechanism of action. It further promoted the rapid development of traditional Chinese medicine [10]. Therefore, it is necessary to explore the mechanism of action of Polygonum capitatum by means of network pharmacology.

2. Materials and Methods

2.1 Establishment of chemical composition

Find the most recently reported compounds in Polygonum capitatum by consulting related literature, and through the database of traditional Chinese medicine in Taiwan.(TDT, http://tcn.cmu.edu.tw/), Comprehensive database of traditional Chinese Medicine (TCMID, http://www.megabionet.org/tcmpid/search/) and Pharmacology analysis platform of traditional Chinese medicine system (TCMSP, http://lsp.nwu.edu.cn/TCMSP.php) retrieving all the chemical components of Polygonum capitatum [11].

2.2 Screening of active ingredients

According to the TCMSP database [12], ADME parameters (OB ≥ 30% and DL ≥ 0.18) were used as standards to screen the active chemical constituents of Polygonum capitatum [13-16]. We also used PubChem database to find the chemical structure formula corresponding to the active chemical ingredients.

2.3 Target selection and establishment

Through the comprehensive analysis of the TCMSP database, the target database of traditional Chinese medicine and the BATMAN-TCM database [17], the target targets of the active chemical constituents of Polygonum capitatum were screened and a component target data set was established.
The spot potential targets of active readings and screens through the database of active readings of Chinese medicine (HIT, http://lifecenter.sgst.cn/hit/) and database of traditional Chinese medicine in Taiwan (TDT, http://tcm.cm.edu.tw/), and the data set of potential target targets of active chemical products Polygonum capitatum was established. Furthermore, inflammatory-related genes targets and protein targets and were screened through a company comprehensive database of the human gene (www.omim.org/) Establishing an inflammatory target data set [18]. Human target target connexins are observed from the interactive protein database (Http://dip.doe-mbi.ucla.edu). At last, all the targets are transformed into Gene name format by UniProt database query.

2.4. PPI network construction
Through PPI (http://www.genome.jp/kegg/) analysis, the interaction relationship between the structure and functional genes of related proteins in biological systems is obtained [19]. The results of the PPI analysis will be exported in TSV format. Then obtain 41 proteins in the PPI network through the Network Analyzer plug-in of Cytoscape software to obtain the three topology parameters of Degree, Betweenness centrality and Closeness centrality of each node [20]. We select the above 3 topological parameter values to satisfy targets that are larger than all the median values, respectively, and visualize the top 10 key targets.

2.5. GO and kegg enrichment analysis
Screening of drug-disease targets using DAVID (https://david.ncifcrf.gov/) database for KEGG pathway analysis and GO (Gene Ontology) biological process analysis [21].

2.6. Anti-inflammatory pathway of Polygonum capitatum
The KEGG Mapper function in the KEGG signaling pathway database was used to mark the target on the signaling pathway most closely related to inflammation. It is verified that Polygonum capitatum exerts anti-inflammatory effects through multiple targets and multiple channels.

2.7. Experimental materials
Experimental animal: Kunming mice (18 g ~ 22 g), Provided by experimental animal center of Chengdu University of Traditional Chinese Medicine.

Reagents and Instruments: Gj-8402 hot plate measuring instrument (Electronic Medical Instrument Factory, Zhejiang Ninghai Whitehead). Acetic acid, Xylene, Ethanol, Acetone, Ether, Ethyl Acetate, Petroleum Ether, Polyamide, 8 mm punch.

Medicinal Materials: Polygonum capitatum Buch. - ham.ex D.Don, collected to Mount Emei, Sichuan. The variety was identified and approved by Professor Deguang Wan of Chengdu University of Traditional Chinese Medicine.

2.8. Experimental methods

2.8.1. Preparation of tannin solution. 500 g crude medicinal powder was soaked in 60% acetone at room temperature for 7 days and filtered. The drug residue was soaked for 3 days with 8 times 60% acetone at room temperature, filtered and combined with filtrate twice. The acetone is recovered by decompression at 45℃, and it is allowed to stand overnight and filtered. The filtrate was extracted 3 times with 1/3 volume of ether. The water layer was extracted 3 times with 1/3 volume of ethyl acetate. Reduce pressure to recover ethyl acetate and dry it in vacuum dryer. The extract was dissolved in appropriate amount of water and prepared into the equivalent raw material concentration of 3.6 g/mL. Extract is given in distilled water diluted to the required concentration.
2.8.2. Preparation of carbohydrate and flavonoids. 500 g crude powder was extracted with 10 times 75% ethanol reflux for 2 h and filtered. The residue was refluxed and extracted with 8 times 75% ethanol for 1 h, filtered and combined with two filtrates. The ethanol was recovered by decompression at 45°C, left overnight, and filtered to obtain filtrate and precipitation. Dry the precipitation, and reflux extraction with proper amount of petroleum ether (1.5 h, 1 h). Filter out the petroleum ether, volatilize the petroleum ether from the residue on the water bath, then reflux extraction with appropriate amount of ethyl acetate (1.5 h, 1 h), and filter it. The ethyl acetate is recovered under reduced pressure, and the extract is dissolved with appropriate amount of water, which is combined with the previous filtrate. The pretreated polyamide column was successively eluted with water, and the aqueous solution was collected. Then washing liquid with 95% ethanol, collection of 95% ethanol eluent. Water washing liquid and 95% ethanol washing liquid were respectively decompressed and recycled, respectively dried in a vacuum dryer, and the extracts were respectively dissolved with appropriate amount of water and mixed into the corresponding raw medicine concentration of 3.6 g/mL. Extract is given in distilled water diluted to the required concentration.

2.8.3. Grouping and Drug administration. There were 70 mice, male and female in half (18 g-22 g), randomly divided into 7 groups. The extracts of Polygonum capitatum were respectively given by gavage for 3 consecutive days, 3.6 g/kg (high), 0.9g/kg (low) and normal saline (0.1 mL/10 g). 40 minutes after the last administration, 0.03 mL xylene was dripped into the right auricle of mice with a microliteter. After 30 min, the mice were killed by necking off.

2.8.4. Measurement index. The borer was punched down with a hole punch with a diameter of 8 mm at the same part of the left and right ears, and weighed. The weight difference between the right ear slice and the left ear slice was used as the index of inflammation and swelling. The results were processed statistically and the percentage of inflammation inhibition was calculated as follows: Inhibition Rate of the Inflammatory (%) = (Average swelling in the blank group - Average swelling in the administration group)/Average swelling in the blank group × 100%.

3. Results

3.1. Screening of active ingredients
Through TDT database, TCMSP database and TCMID database and related literature, 37 chemical components of Polygonum capitatum were found. With ADME parameters (OB ≥ 30% and DL ≥ 0.18) as the standard, 6 active compounds and structural formula were screened out, and the results are shown in Table 1.
Table 1 Active compounds and target numbers of *Polygonum capitatum*

| Mol ID     | Chemical components | Targets | OB (%) | DL | Chemical Structure |
|------------|---------------------|---------|--------|----|-------------------|
| MOL001002  | Ellagic acid [22]   | 19      | 43.06  | 0.43 | ![Chemical Structure](image) |
| MOL008487  | Hirsutine [23]     | 23      | 34.44  | 0.43 | ![Chemical Structure](image) |
| MOL000098  | Quercetin [24]     | 147     | 46.43  | 0.28 | ![Chemical Structure](image) |
| MOL000422  | Kaempferol [25]    | 20      | 41.88  | 0.24 | ![Chemical Structure](image) |
| MOL000263  | Oleanic acid [26]  | 6       | 29.02  | 0.76 | ![Chemical Structure](image) |
| MOL000511  | Ursolic Acid [27]  | 53      | 16.77  | 0.75 | ![Chemical Structure](image) |

3.2. Screening of disease and drug related targets

Using “inflammation” as a keyword in Genecards, retrieved related target genes with an inflammation-related score greater than 5 and searched OMIM for a total of 1093 disease-related genes. The related targets with the active ingredients of *Polygonum capitatum* were converted into the UniProt ID format by querying the UniProt database. As a result, 84 related targets had the UniProt ID format. The disease genes were matched with related targets of *Polygonum capitatum*, and Venn diagram was drawn to obtain 41 common targets, as shown in Figure 1.
3.3. Analysis of topological parameters of direct-acting targets of Anti-inflammatory

The interaction diagram between key targets is constructed with String database, as shown in Figure 3. It can be seen that there are interactions among 41 targets. It indicates that these targets are interrelated and play an anti-inflammatory role through multi-channel and multi-aspect coordination. By counting the number of times each target appears, that is, the number of nodes connected to the gene, the more the number of connected nodes, the more important the role of the target in the treatment of inflammation. The results are exported in TSV format, and the screening method is to obtain the top 10 key proteins in the PPI network through Cytoscape3.6.1 on this basis. Computing the Degree of each protein, Betweenness centrality and Closeness centrality of the median, the three topological parameters are: 16, 0.008921 and 0.6142, the Avg. The number of neighbors = 16.5, as shown in Table 2. The values of the three selected topological parameters were all greater than the mean median, and the targets ranked in the top ten were the key targets of *Polygonum capitatum* for anti-inflammation, as shown in figure 4. The darker the color, the higher the score. According to the results, the top 10 target genes are IL6, MAPK8, VEGFA, CASP3, EGFR, MYC, CCND1, ESR1, ERBB2, and PPARG. IL6 and other proteins have the highest degree of correlation with each other, revealing that their possible pharmacological effects are most obvious. These target genes are of great significance in the treatment of inflammation in *Polygonum capitatum*.
Table 2 Analysis of gene topology parameters

| Uniprot ID | Protein names                                      | name            | Degree | Closeness Centrality | Betweenness Centrality |
|------------|----------------------------------------------------|-----------------|--------|----------------------|------------------------|
| O15519     | CASP8 and FADD-like apoptosis regulator            | CASP8           | 18     | 0.6393               | 0.0083                 |
| P42574     | Caspase-3                                          | CASP3           | 31     | 0.8298               | 0.0498                 |
| P9WHG9     | Bifunctional ppGpp synthase                       | RELA            | 23     | 0.6842               | 0.0203                 |
| P25963     | NF-kappa-B inhibitor alpha                         | NFKBIA          | 15     | 0.6000               | 0.0027                 |
| Q92934     | Bcl2-associated agonist of cell death              | BCL2            | 10     | 0.5571               | 0.0028                 |
| P15692     | Vascular endothelial growth factor A               | VEGFA           | 32     | 0.8478               | 0.0572                 |
| Q16665     | Hypoxia-inducible factor 1-alpha                   | HIF1A           | 19     | 0.6500               | 0.0094                 |
| Q03135     | Caveolin-1                                          | CAV1            | 19     | 0.6393               | 0.0102                 |
| P00749     | Urokinase-type plasminogen activator               | NOS3            | 19     | 0.6500               | 0.0184                 |
| P00533     | Epidermal growth factor receptor                   | EGFR            | 31     | 0.8298               | 0.0567                 |
| Q9SAD4     | Ethylene-responsive transcription factor ESR1      | ESR1            | 25     | 0.7222               | 0.0272                 |
| P24385     | G1/S-specific cyclin-D1                            | CCND1           | 25     | 0.7091               | 0.0200                 |
| P45983     | Mitogen-activated protein kinase 8                 | MAPK8           | 32     | 0.8478               | 0.0690                 |
| P01100     | Proto-oncogene c-Fos                               | FOS             | 22     | 0.6842               | 0.0090                 |
| P09874     | Poly [ADP-ribose] polymerase 1                     | PARP1           | 16     | 0.6190               | 0.0037                 |
| P21730     | C5a anaphylatoxin chemotactic receptor 1           | AR              | 21     | 0.6724               | 0.0118                 |
| P01106     | Myc proto-oncogene protein                         | MYC             | 26     | 0.7358               | 0.0278                 |
| P08887     | Interleukin-6 receptor subunit alpha               | IL6             | 33     | 0.8667               | 0.0777                 |
| P04626     | Receptor tyrosine-protein kinase erbB-2            | ERBB2           | 24     | 0.7222               | 0.0269                 |
| P09917     | Arachidonate 5-lipoxygenase                        | ALOX5           | 8      | 0.5417               | 0.0011                 |
| P23219     | Prostaglandin G/H synthase 1                       | PTGS1           | 6      | 0.5000               | 3.61E-04               |
| P50238     | Cysteine-rich protein 1                            | CRP             | 13     | 0.5909               | 0.0072                 |
| P37231     | Peroxisome proliferator-activated receptor gamma   | PPARG           | 23     | 0.6964               | 0.0193                 |
| Q8H112     | PGR5-like protein 1A, chloroplastic                | PGR             | 16     | 0.6190               | 0.0033                 |
| P05362     | Intercellular adhesion molecule 1, ICAM-1          | PRKCA           | 11     | 0.5735               | 0.0028                 |
| P16581     | E-selectin                                         | SELE            | 10     | 0.5571               | 4.52E-04               |
| P05362     | Intercellular adhesion molecule 1, ICAM-1          | ICAM1           | 20     | 0.6500               | 0.0088                 |
| P15473     | Insulin-like growth factor-binding protein 3       | IGFBP3          | 14     | 0.5910               | 0.0019                 |
| P00749     | Urokinase-type plasminogen activator               | PLAU            | 15     | 0.6094               | 0.0044                 |
| Q16236     | Nuclear factor erythroid 2-related factor 2        | NFE2L2          | 14     | 0.6000               | 0.0286                 |
| P19320     | Vascular cell adhesion protein 1                   | VCAM1           | 16     | 0.6093               | 0.0046                 |
| GSTP1      | Glutathione S-transferase P                        | GSTP1           | 9      | 0.5493               | 0.0114                 |
| P08684     | Cytochrome P450 3A4                                | CYP3A4          | 9      | 0.5571               | 0.0144                 |
| P09488     | Glutathione S-transferase                          | GSTM1           | 3      | 0.4063               | 2.70E-04               |
| Q13950     | Runt-related transcription factor 2                | RUNX2           | 13     | 0.5821               | 7.33E-04               |
| Q15392     | Baculoviral IAP repeat-containing protein 5        | BIRC5           | 7      | 0.5200               | 0                      |
| P07858     | Cathepsin B                                       | CTSB            | 12     | 0.5821               | 0.0538                 |
| Q9NRD8     | Dual oxidase 2                                     | DUOX2           | 3      | 0.4937               | 0                      |
| Q9H3D4     | Tumor protein 63                                   | TP63            | 6      | 0.5065               | 7.94E-05               |
| P07477     | Trypsin-1                                         | PRSS1           | 1      | 0.3714               | 0                      |
3.4. GO biological function enrichment analysis

41 potential targets were mapped into the DAVID database for GO biological function enrichment analysis, and a total of 216 biological processes were obtained. By screening out 20 biological processes with a P.adjust ≤ 0.0001, the top 20 processes with significant enrichment are displayed in the form of a bar graph. The results are shown in Table 3.

The results show that the anti-inflammatory effect of Calyx chinensis is related to the regulation of multiple biological processes, as shown in Figure 5, which mainly involves negative regulation of apoptotic processes (12 targets) and positive regulation of gene expression (10 targets), positive regulation of transcription from RNA polymerase II promoter (15 targets), and response to drug (9 targets), positive regulation of transcription, DNA-templated (10 targets), etc. It reflects that the anti-inflammatory mechanism of Calyx chinensis involves abnormalities in multiple biological processes in the body, and also indicates that the active ingredients of Polygonum capitatum may play an anti-inflammatory role by regulating these biological processes.

| Category               | Term                                      | Count | Count% | P-Value     |
|------------------------|-------------------------------------------|-------|--------|-------------|
| GOTERM_BP_DIRECT       | negative regulation of apoptotic process | 12    | 29.3   | 5.80E-09    |
| GOTERM_BP_DIRECT       | positive regulation of gene expression    | 10    | 24.4   | 8.60E-09    |
| GOTERM_BP_DIRECT       | positive regulation of transcription from RNA polymerase II promoter | 15    | 36.6   | 2.70E-08    |
| GOTERM_BP_DIRECT       | response to drug                          | 9     | 22     | 4.90E-07    |
| GOTERM_BP_DIRECT       | response to amino acid                    | 5     | 12.2   | 8.30E-07    |
| GOTERM_BP_DIRECT       | response to estradiol                     | 6     | 14.6   | 2.40E-06    |
3.5. **KEGG pathway enrichment analysis**

41 potential targets were mapped into the database for KEGG pathway enrichment analysis, and a total of 76 signal pathways were obtained. Through screening KEGG enrichment analysis $P \leq 0.001$, a total of 29 signal pathways were selected for the major enrichment of key targets. The results are shown in Table 4. The first 10 paths with significant differences are output in the form of a bar graph, and the results are shown in Figure 6.
These pathways are closely related to the anti-inflammatory mechanism of calyx, including Pathways in cancer, Hepatitis B, TNF signaling pathway and HIF-1 signaling pathway, etc. At the same time, the KEGG Mapper function in the KEGG signal pathway database was used to label the 41 target proteins associated with inflammation with the most closely related signaling pathways. The results show that there are 19 target proteins involved in the regulation of the Pathways in cancer signaling pathway, as shown in Figure 7.

| Category           | Term                                           | Count | Count% | P-Value     |
|--------------------|------------------------------------------------|-------|--------|-------------|
| KEGG_PATHWAY       | Pathways in cancer                             | 19    | 46.3   | 3.36E-13    |
| KEGG_PATHWAY       | Hepatitis B                                     | 12    | 29.3   | 1.84E-10    |
| KEGG_PATHWAY       | TNF signaling pathway                           | 10    | 24.4   | 4.25E-09    |
| KEGG_PATHWAY       | HIF-1 signaling pathway                         | 9     | 22.0   | 3.71E-08    |
| KEGG_PATHWAY       | Proteoglycans in cancer                         | 11    | 26.8   | 8.01E-08    |
| KEGG_PATHWAY       | Colorectal cancer                               | 7     | 17.1   | 9.24E-07    |
| KEGG_PATHWAY       | Prostate cancer                                 | 7     | 17.1   | 7.34E-06    |
| KEGG_PATHWAY       | MicroRNAs in cancer                             | 10    | 24.4   | 1.84E-05    |
| KEGG_PATHWAY       | Pancreatic cancer                               | 6     | 14.6   | 2.54E-05    |
| KEGG_PATHWAY       | Toxoplasmosis                                   | 7     | 17.1   | 2.65E-05    |
| KEGG_PATHWAY       | African trypanosomiasis                         | 5     | 12.2   | 2.89E-05    |
| KEGG_PATHWAY       | Bladder cancer                                  | 5     | 12.2   | 6.93E-05    |
| KEGG_PATHWAY       | NF-kappa B signaling pathway                    | 6     | 14.6   | 1.04E-04    |
| KEGG_PATHWAY       | Focal adhesion                                  | 8     | 19.5   | 1.12E-04    |
| KEGG_PATHWAY       | Legionellosis                                   | 5     | 12.2   | 2.05E-04    |
| KEGG_PATHWAY       | NOD-like receptor signaling pathway             | 5     | 12.2   | 2.37E-04    |
| KEGG_PATHWAY       | Chagas disease (American trypanosomiasis)      | 6     | 14.6   | 2.42E-04    |
| KEGG_PATHWAY       | Viral myocarditis                               | 5     | 12.2   | 2.54E-04    |
| KEGG_PATHWAY       | Toll-like receptor signaling pathway            | 6     | 14.6   | 2.65E-04    |
| KEGG_PATHWAY       | Transcriptional misregulation in cancer         | 7     | 17.1   | 2.71E-04    |
| KEGG_PATHWAY       | Influenza A                                     | 7     | 17.1   | 3.39E-04    |
| KEGG_PATHWAY       | Apoptosis                                       | 5     | 12.2   | 3.51E-04    |
| KEGG_PATHWAY       | HTLV-1 infection                                | 8     | 19.5   | 4.07E-04    |
| KEGG_PATHWAY       | Herpes simplex infection                        | 7     | 17.1   | 4.44E-04    |
| KEGG_PATHWAY       | Epithelial cell signaling in Helicobacter pylori infection | 5     | 12.2   | 4.73E-04    |
| KEGG_PATHWAY       | PI3K-Akt signaling pathway                      | 9     | 22.0   | 4.80E-04    |
| KEGG_PATHWAY       | Epstein-Barr virus infection                    | 6     | 14.6   | 5.08E-04    |
| KEGG_PATHWAY       | Prolactin signaling pathway                     | 5     | 12.2   | 5.90E-04    |
| KEGG_PATHWAY       | Pertussis                                       | 5     | 12.2   | 7.27E-04    |
Fig 5 Bar graph of KEGG pathway enrichment analysis

KEGG mapper was used to obtain the anti-inflammatory pathway map of *Polygonum capitatum*, and one pathway was selected as the final mapping. The arrows in the figure represent the promotion, the T arrow represents the inhibition, and the red box represents the pathway targets. The figure shows a total of 19 related targets (46.3% of the anti-inflammatory targets in *Polygonum capitatum*), including BCL2, FOS, NFKBIA, RELA, AR and IL6, indicating the distribution of the promotion and inhibition of the anti-inflammatory targets in *Polygonum capitatum*.

Fig 6 Labeling of potential targets of the active ingredients of *Polygonum capitatum* on the Pathways in cancer signaling pathway

In order to more clearly show the relationship between active ingredients, core targets and pathways. The above 41 common genes, diseases and components were visualized and analyzed by Cytoscape 3.6.1 software. Through network pharmacology, the interactive network for the control of inflammation of *Polygonum capitatum* was constructed, and corresponding interactive proteins were screened out. After visualizing it with different colors and shapes, you can intuitively see the network relationship between the active chemical component and the target [27]. The results are shown in Figure 8. Among them, there are 6 in purple, representing the active components of the drug, 41 in green, representing the active targets, the triangle representing the pathway, red for the disease, blue for the drug, the larger the connectivity, the larger the shape.
3.6. Experimental results

Compared with the model group, the high and low flavonoid total and tannin high doses of *Polygonum capitatum* can inhibit the swelling and inflammation of mice caused by xylenes (p < 0.01). The flavonoid high-dose group performed better.

Table 5 Experimental results of auricle inflammation induced by xylenes in mice

| Group                   | n  | Degree of auricle swelling | Inhibition rate |
|-------------------------|----|----------------------------|-----------------|
| Normal saline           | 10 | 20.3±3.5                   | -               |
| High dose of tannins    | 10 | 16.2±4.6                   | 20.20*          |
| LOW dose of tannins     | 10 | 21.2±3.5                   | -               |
| High dose of carbohydrate| 10 | 20.5±3.8                   | -               |
| Low dose of carbohydrate| 10 | 18.7±5.7                   | 7.88            |
| High dose of total flavonoids | 10 | 10.4±4.5                   | 48.77**         |
| Low dose of total flavonoids | 10 | 16.1±4.4                   | 20.69*          |

4. Discussion

The anti-inflammatory experiment of *Polygonum capitatum* extract was the result of screening the anti-inflammatory compounds of *Polygonum capitatum* based on network pharmacology. The results showed that flavonoids had an inflammatory effect on auricle inflammation in mice. The results showed that Quercetin and Kaempferol had inhibitory effect on inflammation. Quercetin compounds play a major role in many chemical components. Among the top ten related proteins, 9 targets are derived from Quercetin compounds, indicating that Quercetin compounds may play an important role in the anti-inflammatory of total flavonoids in *Polygonum capitatum*. It was found that different administration methods (gavage and intravenous injection) of *Polygonum capitatum* extract had different inhibitory effects on inflammation in rats, among which intravenous injection had the best effect [28]. Zhang Jun found that the oil metabolites of A. terreus, have anti-multidrug resistance and anti-inflammatory effects [29]. Quercetin can inhibit LPS-induced inflammation in mouse RAW264.7 cells, and its mechanism may be related to the regulation of TLR4 / NF-κB signaling pathway [30]. In the KEGG prediction of target NF-κB signaling pathway, 6 corresponding targets are related to this
pathway. $P < 0.01$ indicates significant differences. However, the Pathways in cancer signaling pathway, which is the target of the active components of *Polygonum capitatum*, has not been reported. Therefore, this article conducts preliminary screening and analysis of its target and mechanism, with a view to providing scientific basis for the follow-up anti-inflammatory mechanism and pathway research of *Polygonum capitatum*, and also to provide reference for in-depth research and development of *Polygonum capitatum*.

Acknowledgements

This work was supported by the Guizhou Domestic First-Class Construction Project [(Chinese Materia Medica) (GNYL [2017] 008)]. The authors thank the government of China for their financial support.

References

[1] Yang Y, Wang X P, Wu H M, et al. Study on the plant resource distribution and variety of medicinal materials of Miao Yaotou [J]. Chinese Journal of National Medical Medicine, 2012, (7): 33.
[2] Chinese Botany Editorial Board, Chinese Academy of Sciences. Chinese Botany [M]. Beijing: Science Press, 1998, 25.
[3] Guizhou Provincial Drug Administration. Quality Standards for Traditional Chinese Medicine and National Medicine in Guizhou Province [S]. Guiyang: Guizhou Science and Technology Press, 2003.147.
[4] Qiu D W, Du J. Chinese Herbal Medicine, Miao Medicine Volume [M]. Guiyang: Guizhou Science and Technology Press, 2005.
[5] Guizhou Provincial Drug Administration. Guizhou Provincial Traditional Chinese Medicine and National Medicine Quality Standard. Guiyang. Guizhou Science and Technology Press [S]. 2003.147
[6] Yang J Y. Research progress on chemical constituents and detection methods of Miao Yaotou Calyx [J]. Agricultural Technology Services, 2019,36 (9): 46-48.
[7] Lv Y X, Wang L Sh, Cheng D Y, et al. A review of the chemical constituents and pharmacological effects of the head of Chinese herbal medicine, Calyx annua [J]. China Pharmacist, 2017,20(10):1849-1853.
[8] Liao S G, Zhang L J, Sun F, et al. Identification and characterisation of phenolics in Polygonum capitatum by ultrahigh-performance liquid chromatography with photodiode array detection and tandem mass spectrometry [J]. Phytochem Anal, 2013,24(6):556-568.
[9] jie J, Gao Sh, Li L, et al. Research progress and application strategies of network pharmacology in the field of Chinese medicine [J]. Chinese Traditional and Herbal Medicine, 2019,50 (10): 2257-2265.
[10] Yong Ch, Lu L, Wang Y. Study on the active ingredients and pharmacological mechanism of Poria cocos based on network pharmacology [J]. Liaoning Journal of Traditional Chinese Medicine, 2019,46 (9): 1926-1930.
[11] Ru J L. Construction and Application of Database and Analysis Platform of Traditional Chinese Medicine System Pharmacology [D]. Xianyang: Master Degree Thesis of Northwest A & F University, 2015.
[12] Ru J L. Construction and application of pharmacological database and analysis platform of traditional Chinese medicine system [D]. Yang Ling: Northwest A & F University, 2015.
[13] Shi H L, Zhao Y F, Chen Zh. Computer Virtual Screening of HIV-1 Protease Inhibitors in Traditional Chinese Medicine Databases [J]. Journal of Liaoning University of Traditional Chinese Medicine, 2016,18 (10): 73-77.
[14] Li J, Zhao P, Li Y, et al. Systems pharmacology based dissection of mechanisms of Chinese medicinal formula Bufei Yishen as an effective treatment for chronic obstructive pulmonary disease [J].Sci Rep,2015,15:15290.
[15] Miao Zh W, Xu Y, Ning L Q, et al. Network pharmacological analysis and preliminary verification of the molecular mechanism of Baitoweng Decoction in treating ulcerative colitis [J]. China Journal of Chinese Materia Medica, 2019, 11 (22): 1-10.

[16] Kong Q Y. Study on the properties of drug-like drugs: Statistics and analysis of key physical and chemical properties and structures of drugs [D]. Shanghai: East China University of Science and Technology, 2015.

[17] Liu Z, Guo F, Wang Y, et al. BATMAN-TCM: a bioinformatics analysis tool for molecular mechanism of traditional Chinese Medicine [J]. Sci Rep, 2016, 6: 21146.

[18] Safran M, Dalah I, Alexander J, et al. Gene Cards Version3: the human gene integrator [J]. Database, 2010, 8 (5).

[19] Fang H Y, Zeng H W, Lin L M, et al. A network-based method for mechanistic investigation of Shexiang Baoxin Pill’s treatment of cardiovascular diseases [J]. Scientific Reports, 2017, 7: 43632.

[20] Zhang Chi L, Ni X J, Gu J Y, et al. Exploring the effect mechanism of Pueraria in the treatment of ischemic stroke based on network pharmacology [J]. New Journal of Traditional Chinese Medicine and Clinical Pharmacology, 2019, 4: 443-451.

[21] Su G, Morris J H, Demchak B, et al. Biological network exploration with cytoscape 3 [J]. Curr Protoc Bioinformatics, 2014, 47: 8.13.1-8.13.24.

[22] Wang H P, Cao F, Yang X W. Study on the Chemical Constituents of the Above-ground Part of the Flower Head [J]. Chinese Traditional and Herbal Drugs, 2013, 01: 24-30.

[23] Zhang L J, Wang Y L, Wang Zh, et al. Study on the chemical constituents of the active components of Capparis lobata [J]. Chinese Traditional Medical Materials, 2012, 35 (9): 1425-1428.

[24] Li Y J, Luo H F, Wang Y L, et al. Studies on the chemical constituents of flavonoids in Corydalis chinensis [J]. China Pharmaceutical Journal, 2000, 35 (5): 300-302.

[25] Yang Y, Cai F, Yang Q, et al. Studies on the chemical constituents of Capparis spinosa [J]. Journal of Second Military Medical University, 2009, 30 (8): 937-940.

[26] Liu Zhijun, Qi Jin, Zhu Danni, et al. Studies on the chemical constituents and antioxidant activity of Capparis chinensis [J]. Chinese Medicinal Materials, 2008, 31 (7): 995-998.

[27] Huang D W, Sherman B T, Lempicki R A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources [J]. Nat Protoc, 2009, 4(1): 44-57.

[28] Cao F, Duan P, Wang H P, et al. Effects of different administration methods of cephalosporin extract on inflammatory rats [J]. China Journal of Gerontology, 2016, 36 (24): 6065-6067.

[29] Liu J, Zhang Q Y, Yang X, et al. Identification of lipid metabolites of Aspergillus terreus endophytic fungus Aspergillus terreus and its anti-multidrug resistant bacteria and anti-inflammatory effects [J]. China Pharmacy, 2018, 29 (11): 1483-1487.

[30] Ren G Y, Zhang B Y, Huang J L. The protective effect of quercetin on LPS-induced inflammation of RAW264.7 cells in mice [J]. Chinese Traditional Patent Medicine, 2019, 41 (8): 1795-1799.