Explore the molecular mechanism of Moslae Herba’s anti-inflammatory based on network pharmacology

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Abstract. Moslae Herba has the function of inducing sweating to releasing exterior, removing dampness for regulating stomach. It is often used clinically in the treatment of summer heat dampness syndrome, fever in summer, acute enteritis, and herpes pharyngitis in children. There have been more studies on anti-inflammatory effects of Moslae Herba. However, research on the anti-inflammatory mechanism of Moslae Herba is rarely reported. Therefore, in this paper, a network pharmacologic approach was adopted to explore the potential molecular mechanism of the active ingredients in Moslae Herba for the anti-inflammatory. Firstly, all chemical components of Moslae Herba were searched through TCMSP, TDT, TCMID and other databases. According to ADME parameters (OB ≥ 30% and DL ≥ 0.18), the active ingredient in Moslae Herba was screened. Secondly, the targets of active chemical components were searched by traditional Chinese medicine target database, TCMSP database and BATMAN-TCM database, and the target data sets are established. PPI analysis and STRING database were used to construct a protein interaction network for the target of Moslae Herba active ingredients and inflammation. Finally, Cytoscape 3.6.1 software was used to construct a complex network diagram of “drug components-targets-disease”. GO functional and KEGG pathway enrichment analysis were performed by DAVID. As a result, 13 active compounds were screened out of 161 compounds. These active compounds were retrieved from 309 targets, and 143 potential targets were most closely related to the anti-inflammatory mechanism of action of Moslae Herba. Through GO biological function pathway enrichment analysis and KEGG pathway enrichment analysis, 20 biological processes and 20 signal pathways were screened. It is suggested that the active ingredients of Moslae Herba may regulate the inflammation mechanism through the main pathways such as Pathways in cancer, Tuberculosis, PI3K-Akt signaling pathway, HIF-1 signaling pathway, TNF signaling pathway.

1. Introduction
Moslae Herba is a Labiatae plant and is the aboveground part of Mosla chinensis Maxim or Mosla chinensis ‘Jiangxiangru’ [1]. It is a traditional medicine in releasing exterior with pungent-warm, with inducing sweating to releasing exterior, removing dampness for regulating stomach effect. Clinically, it is often used to treat summer heat dampness syndrome, fever in summer, acute enteritis, and herpes
pharyngitis in children [2]. Moslae Herba mainly contains volatile oils, flavonoids and coumarins, and has pharmacological effects of antibacterial, antipyretic, diuretic, analgesic and immune enhancement [3]. Moslae Herba had an inhibitory effect on the polysaccharide-induced inflammation of rat and mouse monocyte macrophages (RAW264.7) [4]. Moslae Herba’s water extract could effectively inhibit hepatitis B virus (HBV) and hepatitis B virus surface antigen (HBsAg) [5, 6]. Diketopiperazines extracted from Moslae Herba could inhibit inflammation in the lungs [7]. The above research shows that Moslae Herba has obvious anti-inflammatory effects, but it is rarely reported to comprehensively explain the anti-inflammatory mechanism of Moslae Herba at the molecular level.

Traditional Chinese medicine has a long history of treating diseases in China, and has the characteristics of good curative effect and smaller side effects. Due to the multi-component, multi-target, and multi-path characteristics of traditional Chinese medicine, its mechanism for treating diseases is very complicated and difficult to understand. However, the holistic and systemic characteristics of network pharmacology are consistent with the synergistic effect of traditional Chinese medicine with multi-component, multi-target, multi-path. This method provides new ideas for the study of complex systems of traditional Chinese medicine [8, 9]. Moslae Herba has a long history of medicinal and abundant resources in China [10]. The chemical composition of Moslae Herba is complex. It is difficult for the current studies to explain its medicinal substance basis and molecular mechanism, which hinders Moslae Herba's in-depth research. However, using the network pharmacology method to construct a complex network of “medicine ingredients - targets - disease” of Moslae Herba can more clearly study the molecular mechanism of active ingredients in Moslae Herba. Therefore, in this paper, a network pharmacology method was used to analyze the anti-inflammatory targets and signal pathways of the active ingredients in Moslae Herba. In order to explore the anti-inflammatory molecular mechanism of Moslae Herba.

2. Methods

2.1. Screening of active ingredients and chemical structure

All chemical ingredients of Moslae Herba were searched through CNKI database, PubMed database and TCMSP database. The chemical composition was input into the TCMSP pharmacological analysis platform (TCMSP, http://tcmspw.com/tcmsp.php), and the active chemical composition of Moslae Herba was selected based on ADME parameters (OB ≥ 30% and DL ≥ 0.18). The chemical structural formula was obtained from the TCMSP database.

2.2. Target selection and establishment

The targets of active chemical components in Moslae Herba were screened through the TCMSP database, the comprehensive analysis of the target database of traditional Chinese medicine and the BATMAN-TCM database, and the target database of components was established. These targets were imported into the Chinese Herbal Active Ingredient Database (HIT, http://lifecenter.sgst.cn/hit/) and Therapeutic Target Database (TTD, http://bidd.nus.edu.sg/group/cjttd/) to screen potential targets for active ingredients. The data set of potential targets was established. A comprehensive database of human genes and gene phenotypes (OMIM, http://www.omim.org/) was used to screen for inflammation-related genes and protein targets to establish an inflammatory target dataset. Then, human target connexins were obtained through an interactive protein database (http://dip.doe-mbi.ucla.edu). Finally, all potential targets were transformed into the gene name format by the UniProt database.

2.3. Network construction of key targets

The target of the active ingredient of Moslae Herba, the target of inflammation, and the target of the interacting protein were connected into a “component - target - disease” network by PPI (http://www.genome.jp/kegg/) analysis. The Degree, Betweenness centrality and Closeness centrality of each node of the PPI network protein were obtained through the Network Analyzer plug-in in Cytoscape.
3.6.1 software. Targets with topological parameter values greater than the median value of the three nodes were selected, and key targets with a ranking of 10 were visualized.

2.4. Biological process and pathway analysis
The selected targets were analyzed by DAVID (https://david.ncifcrf.gov/) database for KEGG pathway analysis and GO (Gene Ontology) biological process analysis. Then, the selected targets were analyzed for protein interaction through STRING (https://string-db.org/). And the above data screening was based on P value (P ≤ 0.001). Finally, the KEGG Mapper in the KEGG (https://www.genome.jp/kegg/) signaling pathway database was used to mark the most closely related signaling pathways between targets and inflammation to verify that Moslae Herba passed multiple targets and Pathways play an anti-inflammatory effect.

3. Results

3.1. Active ingredients and chemical structure
161 chemical components in Moslae Herba were found by databases such as CNKI, PubMed and TCMSP. 13 active compounds were selected and their structural formulas were shown in Table 1.

Table 1. Active chemical components and targets of Moslae Herba

| Mol ID     | Chemical ingredients | Targets | OB (%) | DL | Chemical Structure |
|------------|----------------------|---------|--------|----|--------------------|
| MOL012101  | Mosloflavone         | 19      | 34.04  | 0.26 | ![Chemical Structure](image1) |
| MOL012108  | Negletein            | 15      | 41.16  | 0.23 | ![Chemical Structure](image2) |
| MOL001689  | acacetin             | 19      | 34.97  | 0.24 | ![Chemical Structure](image3) |
| MOL000239  | Jaranol              | 13      | 50.83  | 0.29 | ![Chemical Structure](image4) |
| MOL002937  | Dihydrooroxylin      | 11      | 66.06  | 0.23 | ![Chemical Structure](image5) |
| MOL003044  | Chryseriol  | 18  | 35.85 | 0.27 |
|-------------|-------------|-----|-------|------|
| MOL000358  | beta-sitosterol | 32  | 36.91 | 0.75 |
| MOL000422  | kaempferol   | 44  | 41.88 | 0.24 |
| MOL000492  | (+)-catechin | 10  | 54.83 | 0.24 |
| MOL005911  | 5-Hydroxy-7,4'-dimethoxyflavanon | 11  | 51.54 | 0.27 |
| MOL000006  | luteolin     | 30  | 36.16 | 0.25 |
| MOL000737  | morin        | 12  | 46.23 | 0.27 |
| MOL000098  | quercetin    | 75  | 46.43 | 0.28 |
3.2. Drug and disease-related target screening

The Genecards database was searched for inflammation-related target genes with scores greater than 5 points, and 1014 genes were searched for disease in the OMIM database. The relevant targets of Milaee Herba’s active ingredients were transformed into UniProt ID format. The disease genes were matched with Milaee Herba-related targets, and 45 targets were obtained to participate in the anti-inflammatory system of the active ingredients of Milaee Herba. The Venn diagram was drawn. The result was shown in Figure 1.

![Venn diagram of Milaee Herba’s active compounds and disease target genes](image)

**Figure 1.** Venn diagram of Milaee Herba’s active compounds and disease target genes

3.3. Topological Parameters Analysis of Milaee Herba’s Direct Anti-inflammatory Targets

The String database was used to build the interaction between 45 key targets. It was obvious from the figure that these targets were interconnected. It shows that it is anti-inflammatory through multiple ways. The results were shown in Figure 2. Key proteins with topological parameters of the top 10 in PPI network were obtained by Cytoscape3.6.1 software. The results were shown in Table 2. The top 10 targets were selected as the key anti-inflammatory targets of Milaee Herba in degree. The 10 target genes were IL6, ESR1, VEGFA, EGFR, MAPK8, PARG, AKT1, PTGS2, AR, and HSP90AA1. The darker the color, the higher the score. The results were shown in Figure 3.
Figure 2. Protein-protein interactions of potential targets of Moslae Herba’s anti-inflammatory

Table 2. Topological parameters related to the direct anti-inflammatory target of Moslae Herba

| Uniprot ID | Protein names                          | Gene name | Degree | Closeness Centrality | Betweenness Centrality |
|-----------|----------------------------------------|-----------|--------|----------------------|------------------------|
| P05231    | Interleukin-6                           | IL6       | 35     | 0.8302               | 0.175                  |
| Q96B36    | Proline-rich AKT1 substrate 1           | AKT1      | 32     | 0.7857               | 0.122                  |
| P15692    | Vascular endothelial growth factor A    | VEGFA     | 28     | 0.7333               | 0.0566                 |
| P00533    | Epidermal growth factor receptor        | EGFR      | 26     | 0.7097               | 0.0709                 |
| P35354    | Prostaglandin G/H synthase 2            | PTGS2     | 25     | 0.6984               | 0.0353                 |
| P03372    | Estrogen receptor                       | ESR1      | 22     | 0.6667               | 0.029                  |
| P45983    | Mitogen-activated protein kinase 8      | MAPK8     | 22     | 0.6667               | 0.0278                 |
| P37231    | Peroxisome proliferator-activated receptor gamma | PPARG | 21     | 0.6567               | 0.0351                 |
| P07900    | Heat shock protein HSP 90-alpha         | HSP90A A1 | 20     | 0.6377               | 0.0184                 |
| P10275    | Androgen receptor                       | AR        | 19     | 0.6197               | 0.0134                 |
3.4. Biological function analysis of GO

The 45 potential targets were mapped into the DAVID database for GO biological function enrichment analysis, a total of 188 biological processes were obtained. 20 biological processes were screened with P value (P ≤ 0.001) as the standard, and it was made into the form of scatter plot. The results were shown in Table 3 and Figure 4.

**Table 3.** Enrichment analysis of GO biological function of anti-inflammatory of Moslae Herba

| Category | Term                                                                 | Count | Count % | P-Value   |
|----------|----------------------------------------------------------------------|-------|---------|-----------|
| GOTERM_BP_DIREC | positive regulation of transcription from RNA polymerase II promoter | 17    | 37.78   | 9.70E-10  |
| GOTERM_BP_DIREC | transcription initiation from RNA polymerase II promoter         | 9     | 20.00   | 4.15E-09  |
| GOTERM_BP_DIREC | positive regulation of nitric oxide biosynthetic process         | 6     | 13.33   | 7.76E-08  |
| GOTERM_BP_DIREC | steroid hormone mediated signaling pathway                        | 6     | 13.33   | 3.29E-07  |
| GOTERM_BP_DIREC | peptidyl-serine phosphorylation                                   | 7     | 15.56   | 7.34E-07  |
| GOTERM_BP_DIREC | peptidyl-threonine phosphorylation                                | 5     | 11.11   | 2.58E-06  |
| GOTERM_BP_DIREC | negative regulation of apoptotic process                         | 9     | 20.00   | 1.72E-05  |
| GOTERM_BP_DIREC | protein phosphorylation                                           | 9     | 20.00   | 1.75E-05  |
| GOTERM_BP_DIREC | signal transduction                                               | 13    | 28.89   | 2.32E-05  |
| GOTERM_BP_DIREC | positive regulation of peptidyl-serine phosphorylation            | 5     | 11.11   | 3.02E-05  |
| GOTERM_BP_DIREC | inflammatory response                                             | 8     | 17.78   | 4.51E-05  |
| GOTERM_BP_DIREC | positive regulation of gene expression                            | 7     | 15.56   | 5.12E-05  |
| GOTERM_BP_DIREC | response to hypoxia                                               | 6     | 13.33   | 7.47E-05  |

**Figure 3.** The top ten most relevant proteins in Moslae Herba
3.5. Enrichment analysis of KEGG pathway

45 potential targets were mapped into the DAVID database for KEGG pathway enrichment analysis and 88 signal pathways were obtained. According to the P value (P ≤ 0.001) as a standard, 20 signal pathways that were mainly enriched in key targets were screened. The 20 paths with significant differences were output in the form of a scatter plot. The results were shown in Table 4 and Figure 5.
### Table 4. KEGG pathway enrichment analysis of anti-inflammatory effect of Moslae Herba

| Category       | Term                                      | Count | Count%   | P-Value      |
|----------------|-------------------------------------------|-------|----------|--------------|
| KEGG_PATHWAY   | Pathways in cancer                        | 17    | 37.78    | 2.63E-10     |
| KEGG_PATHWAY   | Tuberculosis                              | 10    | 22.22    | 6.95E-07     |
| KEGG_PATHWAY   | HIF-1 signaling pathway                   | 8     | 17.78    | 1.26E-06     |
| KEGG_PATHWAY   | TNF signaling pathway                     | 8     | 17.78    | 2.63E-06     |
| KEGG_PATHWAY   | Prostate cancer                           | 7     | 15.56    | 1.16E-05     |
| KEGG_PATHWAY   | Regulation of lipolysis in adipocytes      | 6     | 13.33    | 1.79E-05     |
| KEGG_PATHWAY   | Estrogen signaling pathway                | 7     | 15.56    | 2.28E-05     |
| KEGG_PATHWAY   | PI3K-Akt signaling pathway                | 11    | 24.44    | 2.46E-05     |
| KEGG_PATHWAY   | Pancreatic cancer                         | 6     | 13.33    | 3.71E-05     |
| KEGG_PATHWAY   | Prolactin signaling pathway               | 6     | 13.33    | 5.70E-05     |
| KEGG_PATHWAY   | Influenza A                               | 8     | 17.78    | 6.38E-05     |
| KEGG_PATHWAY   | Hepatitis C                               | 7     | 15.56    | 0.00012      |
| KEGG_PATHWAY   | ErbB signaling pathway                    | 6     | 13.33    | 0.00015      |
| KEGG_PATHWAY   | Progesterone-mediated oocyte maturation   | 6     | 13.33    | 0.00015      |
| KEGG_PATHWAY   | Proteoglycans in cancer                   | 8     | 17.78    | 0.00015      |
| KEGG_PATHWAY   | Focal adhesion                            | 8     | 17.78    | 0.00018      |
| KEGG_PATHWAY   | Hepatitis B                               | 7     | 15.56    | 0.00019      |
| KEGG_PATHWAY   | Ras signaling pathway                     | 8     | 17.78    | 0.00033      |
| KEGG_PATHWAY   | Insulin resistance                        | 6     | 13.33    | 0.00042      |
| KEGG_PATHWAY   | VEGF signaling pathway                    | 5     | 11.11    | 0.00044      |

**Figure 5.** Scatter diagram of KEGG pathway enrichment analysis
3.6. Analysis of KEGG pathway
Moslae Herba’s anti-inflammatory action pathway map was obtained using KEGG Mapper. The arrows in the figure indicate the promoting action, the T arrows represent the inhibitory action, and the red boxes represent the key targets of the pathway. A signal pathway most closely related to inflammation was selected and marked out. The results showed 21 related targets. The Pathways in cancer signaling pathway was most closely related to inflammation. The results were shown in Figure 6.

![Figure 6. Signal pathways of potential targets of Moslae Herba’s active ingredients in Pathways in cancer](image)

3.7. Visual analysis of active ingredients, diseases and genes
Cytoscape 3.6.1 software was used for network visual analysis of 45 genes, diseases and components. In order to more clearly shown the relationship between effective components, core targets and pathways. Different colors and shapes were used to represent the network relationship between the active ingredient and the target. There were 13 purple, which represent the active ingredients of the Moslae Herba. There were 45 green, which represents the target of the active ingredient. The triangle was the pathway, the dark blue represented the disease, and the cyan was the drug. The results were shown in Figure 7.
Figure 7. “Composition-target-disease-pathway” interactive network of anti-inflammatory effects of Moslae Herba

4. Discuss

Traditional Chinese medicine is the product of long-term clinical practice and has the characteristics of multi-component, multi-link and multi-target overall effects. Its complexity limits the development of traditional Chinese medicine modernization [11]. Network pharmacology is an important part of systems biology. Its holistic, systematic, and drug-focused characteristics are consistent with the characteristics of traditional Chinese medicine. It is an emerging discipline that systematically explains the role of Chinese medicine on the body’s regulatory network [12]. At present, it is a research hotspot to explore the pharmacological substance basis and molecular mechanism of traditional Chinese medicine for treating diseases by using network pharmacology methods. And many modern studies have found that Moslae Herba has obvious anti-inflammatory effects. Therefore, this paper studies the molecular mechanism of Moslae Herba’s anti-inflammatory through a network pharmacological approach. 13 active ingredients were selected from Moslae Herba, and a “component-target-disease” interactive network diagram was constructed to obtain 45 potential anti-inflammatory targets. Through KEGG enrichment analysis, 20 pathways were found to be closely related to the treatment of inflammation with Moslae Herba. According to the literature, the effect of Moslae Herba on Hepatitis B and TNF signaling pathways has been experimentally verified [4,5]. This article also predicts that the anti-inflammatory pathways of Moslae Herba were the TNF signaling pathway and Hepatitis B. In addition, the pathways closely related to the anti-inflammatory mechanisms of Moslae Herba also include Pathways in cancer, Tuberculosis, PI3K-Akt signaling pathway, HIF-1 signaling pathway. The results of this study indicate that Moslae Herba’s anti-inflammatory has multi-component, multi-target and multi-path action characteristics, and provides new ideas for clarifying the anti-inflammatory mechanism of Moslae Herba. It is beneficial to the in-depth research on anti-inflammatory of Moslae Herba.

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