Network pharmacology-based prediction of potential targets of ethnic medicine Blumea balsamifera (L.) DC acting on anti-inflammatory effect

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Abstract. Blumea balsamifera (L.) DC is an ethnic medicine with a significant anti-inflammatory effect. At present, there were many reports on the anti-inflammatory efficacy of B. balsamifera, but the mechanism of its is rarely reported. Therefore, the method of network pharmacology has been adopted in this paper to predict the molecular mechanism of the anti-inflammatory effect of B. balsamifera. The active chemical constituents of B. balsamifera were screened by reference to the literature, TCMSP and TCMID. A target data set of active chemical components was established by traditional chinese medicine target, TCMSP and BATMAN-TCM database. The target of the active ingredients were introduced into the HIT and TTD to establish a potential target data set of the B. balsamifera active ingredients. A OMIM was used to screen for inflammation-related genes and protein targets to establish an inflammatory target dataset. The complex network map of “active ingredient-target-disease” was constructed using Cytoscape3.6.1 software. A PPI analysis database was used to construct a protein interaction network of B. balsamifera component targets and inflammatory targets. GO functional and KEGG pathway enrichment analysis were performed by the biological information annotation database. As a result, 12 active chemical components in B. balsamifera were screened. The corresponding target of 724 active compounds were retrieved. There are 33 signaling pathways and 28 biological processes that were directly or indirectly related to the anti-inflammatory effects of B. balsamifera. Through enrichment analysis, the main signaling pathways of B. balsamifera include TNF signaling pathway, Hepatitis B, Toll-like receptor signaling pathway, NF-kappa B signaling pathway, etc. Finally, Network pharmacology provides new ideas and methods for the study of the anti-inflammatory mechanism of B. balsamifera.

1. Introduction
Blumea balsamifera (L.) DC belongs to the compositae family and is one of the ten famous miao national herbs in Guizhou Province, China. It has the functions of eliminating wind and dampness, detumescence and analgesic, antibacterial and insecticidal. Clinically, it is mainly used to treat rheumatoid arthritis, eczema dermatitis and pruritus [1-3]. B.alsamifera has a long medicinal history, which has been recorded in the “supplement to medicina” ancient books [4]. At present, B.alsamifera is also the only source of raw medicine for L-borneolum in pharmacopoeia of the People’s Republic of China in 2015. It contains flavonoids, phenolic acids, volatile oils and alkaloids, these chemical...
components were screened for activity [5-7]. Modern research shows the total flavonoids in *B. balsamifera* have obvious therapeutic effects on skin injury. *B. balsamifera* oil 1/5 and 1/10 reduced the number of inflammatory cells, increased wound-healing rates, and significantly increased the hydroxyproline content [8,9]. In addition, studies have shown that *B. balsamifera* has anti-acute and chronic inflammation effects [10]. Although most studies have shown that *B. balsamifera* has anti-inflammatory activity, no one has studied a complex interrelationship between its anti-inflammatory effects and cellular proteins.

The core idea of network pharmacology has a lot in common with the concept of holism and syndrome differentiation of TCM, and the characteristics of TCM with “multi-component, multi-channel, multi-target”. Network pharmacology provides a new method for the study of complex systems of TCM [11]. Therefore, in this paper, network pharmacology was adopted to collect the active components of *B. balsamifera* in the literature, find the target of active chemical components, establish a target dataset and a complex network diagram of “active component-target-disease”. Pathway enrichment analysis was performed based on GO conditions and KEGG. Finally, the anti-inflammatory mechanism of *B. balsamifera* was predicted. In order to provide reference for further study on anti-inflammatory effect of *B. balsamifera*.

2. Methods

2.1 The chemical structures and target screening

All the chemical components of *B. balsamifera* were collected from (1) literature (likely CNKI and PubMed database), (2) PubChem database (https://pubchem.ncbi.nlm.nih.gov), (3) Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php), Screening the active chemical constituents of *B. balsamifera* by ADME parameters (OB ≥ 30% and DL ≥ 0.15) or pharmacodynamic activity. In this paper, the target data set of *B. balsamifera* active chemical constituents was established by TCMSP database, comprehensive analysis of traditional chinese medicine target database, BATMAN-TCM and SwissADME databases. The target of the active ingredients were introduced into the Chinese herbal active ingredient database (HIT, http://lifecenter.sgst.cn/hit/) and the therapeutic target database (TTD, http://bidd.nus.edu.sg/group/cjttd/) to screen the potential targets of the active ingredients, to establish a potential target data set of the *B. balsamifera* active ingredients. 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, the human target connexins were obtained from an interactive protein database (http://dip.doe-mbi.ucla.edu). Finally, all potential targets were transformed into the UniProt ID format by the UniProt database.

2.2 Network construction

The target of the active ingredient of *B. balsamifera*, the target of inflammation, and the target of the interacting protein were linked by PPI (http://www.genome.jp/kegg/) analysis into a “component-target-disease” network. Cytoscape 3.6.1 software was used to visually analyze this network to obtain three topological parameters of Betweenness Centrality, Closeness Centrality and Degree for each node, and select the target of the median larger than the three topological parameters as the target of anti-inflammatory effects of *B. balsamifera*.

2.3 Biological process analysis

The target was introduced into the DAVID (https://david.ncifcrf.gov/) database for KEGG pathway analysis and GO (Gene Ontology) biological process analysis. Protein interaction relationship analysis was performed on the final selected target points by using STRING (https://string-db.org/).

3. Result
3.1 Screening of active ingredients
Quercetin, Taxifolin, Stigmasterol, Catechin, Luteolin, Tamarixetin, and β-sitosterol were screened for 7 active compounds based on ADME parameters (OB ≥ 30% and DL ≥ 0.15). By consulting related literature, five active compounds of Bloomatin, Grasshopper ketone, Daucosterol, 7-hydroxycoumarin and sterubin were obtained on account of the pharmacodynamic activity of B. balsamifera. In the end, a total of 12 active compounds were selected.

3.2 Network construction of component-target-disease
A total of 683 target sites belonging to B. balsamifera active chemistry were screened by TCMSP, BATMAN-TCM and SwissADME databases and comprehensive analysis of traditional chinese medicine target database. Through the OMIM database, 778 inflammation-related targets were obtained. An interactive network of B. balsamifera to prevent inflammation was constructed by network pharmacology to obtain 724 interacting proteins. Through Cytoscape 3.6.1 software, it performed interactive network analysis and visual analysis on B. balsamifera. Different color and shape graphics were used to visualize it, and the network relationship between active chemical components and disease targets could be visually seen. The results were shown in figure 1. The yellow squares represented the target of drugs and diseases. It had 87 targets and were the most important target protein for anti-inflammatory of B. balsamifera. The yellow dots refer to the direct target of inflammation, which had 134 targets. The red triangles was the active chemical component predicted in B. balsamifera, there are 12. Blue dots represented the direct target of active chemical constituents of B. balsamifera, with 329. These purple dots were interactive proteins that link the active chemical constituents of B. balsamifera to disease targets, and there were 724.

3.3 B. balsamifera anti-inflammatory direct target analysis of topological parameters
An interactive network analysis of B. balsamifera was performed by Cytoscape 3.6.1 software, and 221 target proteins associated with inflammation were obtained, and topological parameters were calculated for these target proteins. The median values of the topological parameters Betweenness centrality, Closeness centrality, and Degree were obtained to be 0.0016, 0.2309, and 3.0. Targets with topological parameters greater than 0.0016, 0.2309, and 6.0 were used as potential target proteins for B. balsamifera anti-inflammatory, resulting in a total of 37 eligible targets. The results are shown in Table 1. 37 potential target proteins were used for protein interaction analysis. As shown in Figure 2.

Table. 1 Topological parameters related to the direct anti-inflammatory target of B. balsamifera
| Uniprot ID | Protein names                                      | Closeness Centrality | Degree | Betweeness Centrality |
|-----------|---------------------------------------------------|----------------------|--------|-----------------------|
| P41182    | B cell lymphoma 6 protein                         | 0.2322               | 8      | 0.0044                |
| Q86WV6    | Stimulator of interferon gene protein             | 0.2308               | 8      | 0.0040                |
| P04150    | Glucocorticoid receptor                           | 0.2430               | 8      | 0.0130                |
| O14920    | Inhibitor of nuclear factor kappa-B kinase subunit β | 0.2764               | 15     | 0.0046                |
| Q9Y6K9    | NF-κB essential regulator                        | 0.2822               | 23     | 0.0171                |
| Q99558    | Mitogen-activated protein kinase 14               | 0.2634               | 13     | 0.0109                |
| Q9Y4K3    | TNF receptor related factor 6                     | 0.2560               | 22     | 0.0219                |
| Q99759    | Mitogen-activated protein kinase 3                | 0.2439               | 10     | 0.0101                |
| Q13158    | FAS-associated death domain protein               | 0.2338               | 7      | 0.0041                |
| Q13546    | Receptor-interacting serine/threonine protein kinase 1 | 0.2514               | 7      | 0.0062                |
| P25445    | Tumor necrosis factor receptor superfamily member 6 | 0.2674               | 11     | 0.0143                |
| P23219    | Prostaglandin G / H synthetase 1                  | 0.2957               | 9      | 0.0123                |
| P37231    | Peroxisome proliferators activate receptors γ      | 0.2953               | 12     | 0.0118                |
| P35554    | Prostaglandin G / H synthetase 2                  | 0.3001               | 8      | 0.0099                |
| Q04206    | Transcription factor p65                          | 0.2888               | 22     | 0.0174                |
| P31749    | RAC-α serine/threonine-protein kinase             | 0.3287               | 17     | 0.0348                |
| P15692    | Vascular endothelial growth factor A              | 0.3133               | 15     | 0.0166                |
| P24385    | G1 / S specific cyclin-D1                         | 0.3136               | 13     | 0.0203                |
| P14780    | Matrix metalloproteinase-9                       | 0.2907               | 7      | 0.0047                |
| P01375    | Tumor necrosis factor                            | 0.2733               | 9      | 0.0169                |
| P05412    | Transcription factor AP-1                        | 0.2875               | 15     | 0.0148                |
| P05231    | Interleukin-6                                     | 0.2722               | 8      | 0.0058                |
| P04637    | Cellular tumor antigen p53                       | 0.3198               | 62     | 0.0739                |
| P25963    | NF-kappa-B inhibitor alpha                        | 0.2920               | 20     | 0.0184                |
| Q14790    | Caspase-8                                         | 0.2756               | 7      | 0.0095                |
| Q16665    | Hypoxia-inducible factor 1-alpha                 | 0.2686               | 13     | 0.0167                |
| P13500    | C-C motif chemokine 2                             | 0.2701               | 6      | 0.0229                |
| P01137    | Transforming growth factor beta-1 proprotein      | 0.2663               | 9      | 0.0109                |
| P09874    | Poly[ADP-ribose] polymerase 1                     | 0.3150               | 15     | 0.0282                |
| O15111    | Inhibitor of nuclear factor kappa-B kinase subunit alpha | 0.3036               | 19     | 0.0187                |
| P00533    | Epidermal growth factor receptor                  | 0.2849               | 21     | 0.0220                |
| P05067    | Amyloid-beta precursor protein                    | 0.2688               | 14     | 0.0200                |
| P35968    | Vascular endothelial growth factor receptor 2     | 0.2851               | 13     | 0.0075                |
| O15379    | Histone deacetylase 3                            | 0.2795               | 10     | 0.0089                |
| P49682    | C-X-C chemokine receptor type 3                   | 0.2625               | 8      | 0.0334                |
| P19838    | Nuclear factor NF-kappa-B p105 subunit            | 0.2683               | 22     | 0.0135                |
3.4 Biological function analysis of GO

Thirty-seven potential targets were mapped to the DAVID database for GO biological function enrichment analysis. A total of 279 biological processes were enriched, and 28 biological processes with \( P \leq 0.00001 \) were obtained. The results were shown in Table 2.

| Category          | Term                                                      | Count | Count (%) | P-Value       |
|-------------------|-----------------------------------------------------------|-------|-----------|---------------|
| \textsc{GOTERM\_BP\_DIRECT} | positive regulation of transcription from RNA polymerase II promoter | 25    | 69.4      | 4.20E-22      |
| \textsc{GOTERM\_BP\_DIRECT} | inflammatory response | 15    | 41.7      | 1.10E-14      |
| \textsc{GOTERM\_BP\_DIRECT} | positive regulation of NF-kappaB transcription factor activity | 11    | 30.6      | 1.10E-13      |
| \textsc{GOTERM\_BP\_DIRECT} | positive regulation of I-kappaB kinase/NF-kappaB signaling | 11    | 30.6      | 7.40E-13      |
| \textsc{GOTERM\_BP\_DIRECT} | cellular response to mechanical stimulus | 9     | 25        | 1.50E-12      |
| \textsc{GOTERM\_BP\_DIRECT} | negative regulation of apoptotic process | 14    | 38.9      | 3.10E-12      |
| \textsc{GOTERM\_BP\_DIRECT} | positive regulation of protein phosphorylation | 10    | 27.8      | 3.60E-12      |
| \textsc{GOTERM\_BP\_DIRECT} | I-kappaB kinase/NF-kappaB signaling | 8     | 22.2      | 3.20E-11      |
| \textsc{GOTERM\_BP\_DIRECT} | positive regulation of apoptotic process | 11    | 30.6      | 3.50E-10      |
| \textsc{GOTERM\_BP\_DIRECT} | cellular response to organic cyclic compound | 7     | 19.4      | 2.20E-09      |
| \textsc{GOTERM\_BP\_DIRECT} | positive regulation of smooth muscle cell proliferation | 7     | 19.4      | 2.40E-09      |
| \textsc{GOTERM\_BP\_DIRECT} | TRIF-dependent toll-like receptor signaling pathway | 6     | 16.7      | 2.80E-09      |
| \textsc{GOTERM\_BP\_DIRECT} | regulation of tumor necrosis factor-mediated signaling pathway | 6     | 16.7      | 4.00E-09      |
3.5 Enrichment analysis of KEGG pathway

37 potential targets were mapped into the DAVID database for KEGG pathway enrichment analysis. A total of 81 signal pathways were obtained, including 33 signal pathways (P ≤ 0.00001). As shown in Table 3.

| Category         | Term                                           | Count | Count (%) | P-Value     |
|------------------|------------------------------------------------|-------|-----------|-------------|
| KEGG_PATHWAY     | TNF signaling pathway                          | 18    | 50        | 1.70E-22    |
| KEGG_PATHWAY     | Chagas disease (American trypanosomiasis)     | 16    | 44.4      | 4.30E-19    |
| KEGG_PATHWAY     | Apoptosis                                      | 14    | 38.9      | 8.50E-19    |
| KEGG_PATHWAY     | Hepatitis B                                    | 17    | 47.2      | 1.90E-18    |
| KEGG_PATHWAY     | Pathways in cancer                             | 22    | 61.1      | 5.00E-18    |
| KEGG_PATHWAY     | Toll-like receptor signaling pathway           | 14    | 38.9      | 1.40E-15    |
| KEGG_PATHWAY     | RIG-I-like receptor signaling pathway          | 12    | 33.3      | 1.80E-14    |
| KEGG_PATHWAY     | Herpes simplex infection                       | 15    | 41.7      | 7.70E-14    |
| KEGG_PATHWAY     | NOD-like receptor signaling pathway            | 11    | 30.6      | 8.60E-14    |
| KEGG_PATHWAY     | Pancreatic cancer                              | 11    | 30.6      | 4.20E-13    |
| KEGG_PATHWAY     | Osteoclast differentiation                     | 13    | 36.1      | 7.90E-13    |
| KEGG_PATHWAY     | MAPK signaling pathway                         | 15    | 41.7      | 6.70E-12    |
| KEGG_PATHWAY     | Small cell lung cancer                         | 11    | 30.6      | 6.90E-12    |
4. Discuss

*B. balsamifera* is one of the ten major miao medicines in guizhou province. Domestic and foreign studies have shown that it has obvious anti-inflammatory effect, but it is unclear about the mechanism of anti-inflammatory effects of *B. balsamifera*. Therefore, the network pharmacology research method was adopted in this paper, screening 12 active chemical constituents in *B. balsamifera* by this method. Targets for the action of 724 active compounds were searched and a “component-target-disease” interaction network map was constructed. There are 33 signaling pathways and 28 biological processes that are directly or indirectly related to the anti-inflammatory effects of *B. balsamifera*. By KEGG enrichment analysis, the main signaling pathway of *B. balsamifera* anti-inflammatory includes TNF signaling pathway, Hepatitis B, Toll-like receptor signaling pathway, NF-kappa B signaling pathway, and MAPK signaling pathway. According to the predicted results, the NF-kappaB signaling pathway is the closest, and this signaling pathway is involved in the pathogenesis of inflammation. The purpose of this work is to provide a reference for the study of the anti-inflammatory mechanism of the national medicine *B. balsamifera*.

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