

Analysis of the Mechanism of *Ophiopogon japonicus* in Treating Pneumonia Based on Network Pharmacology

Abstract: This study explores the mechanism of *Ophiopogon japonicus* in treating pneumonia through network pharmacology, establishes a physiological and pharmacological relationship between the two based on targets, and emphasizes the interaction between signaling pathways and targets. By utilizing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, specific biological processes and signaling pathways are utilized to enhance the understanding of the medicinal properties of traditional Chinese medicine through the use of bioinformatics and biomedical tools. This method aims to identify the key targets and signaling pathways of *Ophiopogon japonicus* affecting pneumonia status, providing insights into its anti-inflammatory effect in the lungs. The active application of network pharmacology methods in the field of pneumonia is a new trend in the modernization of traditional Chinese medicine.

Keywords: Network pharmacology; *Ophiopogon japonicus*; Pneumonia; Signal pathway

Introduction :

Pneumonia, characterized by inflammation in the terminal airways, alveoli, and lung interstitium, can result from various factors including microbial infections, physicochemical triggers, immune dysfunction, allergies, and medications. It affects individuals of all ages, with children, the elderly, and those with underlying health conditions being particularly vulnerable.[25-26]

Different pathogenic factors categorize pneumonia into infectious, physicochemical, allergic, and other types. While treatment options encompass traditional Chinese medicine formulas, chemical drugs, and biological products, the effectiveness of these approaches in improving pneumonia outcomes remains limited. Hence, there's an urgent need to explore more effective therapeutic agents.

Ophiopogon japonicus, a traditional Chinese medicine native to Sichuan, is renowned for its significant anti-inflammatory and antioxidant properties. Traditional Chinese medicine relies on the synergistic interactions among multiple components, forming a cohesive "component structure" that enhances therapeutic effects. However, the synergistic effects and compatibility structures among *Ophiopogon japonicus* components contributing to its anti-inflammatory properties remain underexplored.

This project employs network pharmacology, leveraging its bioinformatics and biomedical advantages, to investigate the therapeutic effects of *Ophiopogon japonicus* on pneumonia. By analyzing its multifaceted components, targets, pathways, and systems, the study aims to uncover the biological mechanisms and signaling pathways underlying *Ophiopogon japonicus*' efficacy in treating pneumonia. Ultimately, this research seeks to provide insights into the interaction between *Ophiopogon japonicus*' active ingredients and pneumonia targets, elucidating relevant signaling pathways and laying the groundwork for advancing traditional Chinese medicine research in pneumonia treatment.

1 Materials and Methods

1.1 Chemical composition and target acquisition of *Ophiopogon japonicus*

We employ the following methodologies to identify the effective ingredients of *Ophiopogon japonicus* unit medicine:

(1) ETCM Database:

- Accessed via <http://www.tcmip.cn/ETCM/>.
- Adhering to drug-like properties criteria, including MW (relative molecular weight) ≤ 500 , ALogP (lipid-water distribution coefficient) ≤ 5 , noHNH (number of H⁺ receptors) ≤ 10 , and noH (number of H⁺ donors) ≤ 5 .
- Effective ingredients are obtained based on these parameters.

(2) PubChem Database:

- Utilizing the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>).
- MW (relative molecular weight) serves as the screening criterion.
- Obtaining the SMILES (Simplified Molecular Input Line Entry System) notation for the chemical formula of *Ophiopogon japonicus* unit medicine.

(3) SWISSTargetPrediction:

- Accessible via <http://swisstargetprediction.ch>.
- Initially, utilizing the SMILES notation of individual ingredients of *Ophiopogon japonicus* to derive their corresponding chemical structural formulas.
- Predicting the target genes associated with each component.
- Conducting screening if the correlation between the components and target genes exceeds 0.

This systematic approach facilitates the identification of effective ingredients and prediction of potential target genes for *Ophiopogon japonicus* unit medicine, enhancing our understanding of its therapeutic mechanisms.

1.2 Acquisition of targets for pneumonia

We utilize GeneCards (<http://www.genecards.org>) to screen for genes associated with pneumonia. Additionally, DisGeNET (<http://www.Disgene.org/home>) is employed to identify genes related to pneumonia (under the name "Pneumonic Plague" with the CUI: C0524688).

Following this, we compile the disease genes obtained from both databases and eliminate duplicates to derive a comprehensive list of pneumonia-related genes.

1.3 Construction of the "Taste Component Target" Network

The filtered ingredient and target data should be saved as a network file, preferably in a format compatible with Cytoscape 3.7.2 Software (<http://www.cytoscape.org/>). Once saved, import the file into Cytoscape to construct a network of medicinal taste components and targets.

1.4 Construction and topological analysis of protein-protein interaction (PPI) networks

Perform an intersection analysis using bioinformatics mapping software available at www.bioinformatics.com.cn. Import the intersection genes of *Ophiopogon japonicus* and pneumonia into the STRING database (<http://string-db.org/>, 10th edition) to establish a Protein-Protein Interaction (PPI) network diagram. Set the threshold to "medium confidence ≥ 0.7 " to obtain pairwise correlation scores.

Import the data from STRING into Cytoscape 3.7.2 for network graph construction and topology analysis. Utilize the Cytoscape plugin "Network Analyzer" to measure six topology parameters: betweenness centrality (BC), closeness centrality (CC), degree centrality (DC), eigenvector centrality (EC), local edge connectivity (LAC), and network centrality (NC). Select nodes that meet the median value of all six indicators as key targets and extract core targets to construct a network.

1.5 GO analysis and KEGG pathway analysis

Perform gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotation analysis on the predicted key target information using DAVID 6 (<https://david.ncifcrf.gov/>) database. Utilize the obtained results to create visual representations using GraphPad Prism 5.

2 Results

2.1 Composition and Target Screening of *Ophiopogon japonicus*

Utilizing the ETCM database, 18 main components were identified from *Ophiopogon japonicus*. To address the requirements for treating pneumonia, a network diagram titled "Pneumonia Major Components Targets" was constructed using Cytoscape 3.7.2 software.

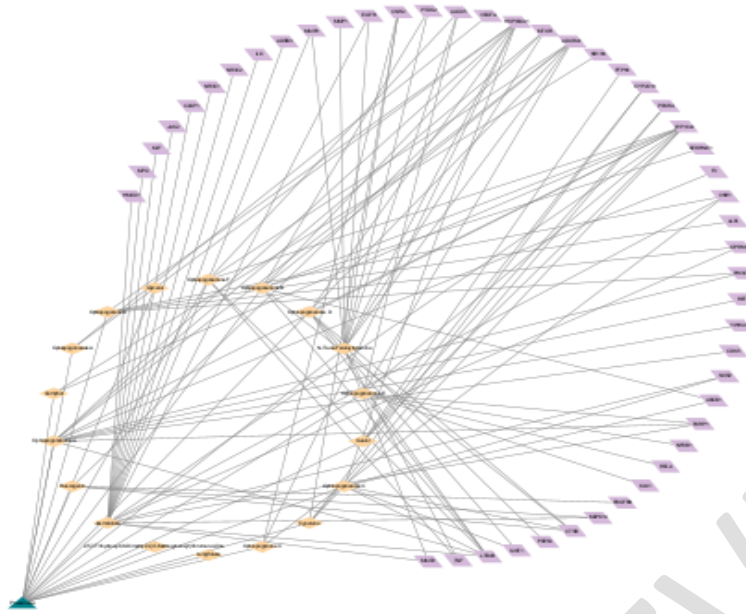


Figure 1 "Pneumonia Component Target" Network

After integrating data from the SwissTargetPrediction database and removing duplicates, 555 prediction targets were identified for the components of *Ophiopogon japonicus*. Utilizing the GeneCards and DisGeNET databases, targets related to pneumonia were selected based on correlation coefficients ≥ 3.24 and a score of gda (database and literature support) ≥ 0.02 . After removing duplicates, 780 pneumonia-related targets were obtained.

Using the online mapping software of Weishengxin, a Venn diagram was generated, revealing a total of 98 shared targets between *Ophiopogon japonicus* and pneumonia (refer to Figure 1).

Subsequently, protein-protein interaction (PPI) analysis was conducted on the common targets of *Ophiopogon japonicus* and pneumonia. Initially, a PPI network was constructed using the STRING database, with a minimum required interaction score ≥ 0.7 . This resulted in 98 nodes (excluding PDE4A and P4HTM, which did not participate in protein interactions) and 561 edges, highlighting the intricate relationship between protein interactions and regulation (refer to Figure 2).

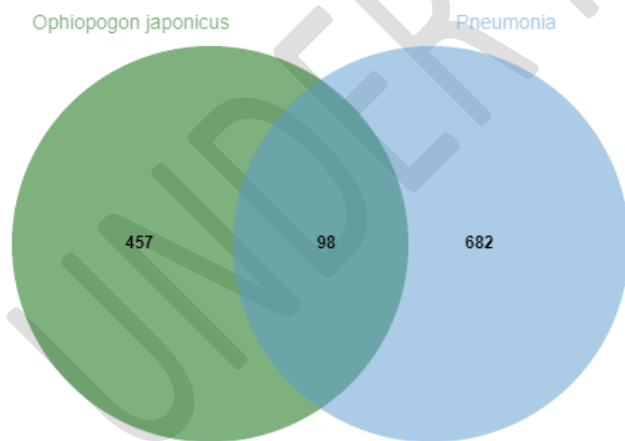


Figure 2 Common targets

Utilizing Cytoscape 3.7.2 software for topology analysis, key target genes for treating pneumonia with *Ophiopogon japonicus* were identified. Targets with values of Degree, Betweenness Centrality (BC), and Closeness Centrality (CC) greater than the median were extracted, resulting in a total of 32 core targets.

From these core targets, the top ten were selected to construct the network, yielding 9 nodes and 28 edges. These targets are deemed to play a crucial role in the treatment of pneumonia with *Ophiopogon japonicus* (refer

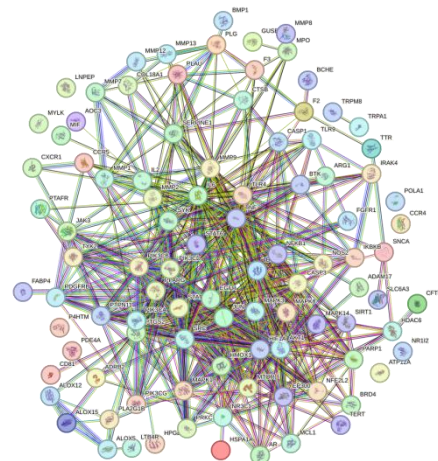


Figure 3 Protein interaction analysis

to Table 1 and Figure 3).

Table 1 Top Ten Target Points

Target Name	Degree Value
IL6	46
TNF	46
STAT3	43
AKT1	38
TLR4	33
EGFR	32
BCL2	31
NFKB1	31
SRC	31
JUN	29

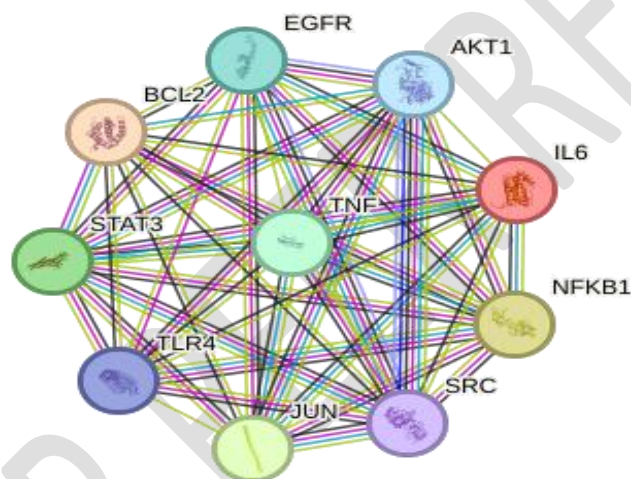


Figure 4 Top Ten Target Protein Interactions

2.2 GO Function Analysis

GO functional analysis was conducted on the 10 targets within the core network using DAVID Bioinformatics Resources 6.7. Following the screening criteria of "Count ≥ 2 " and "EASE scores ≤ 0.05 ", a total of 130 biological processes (BP), 14 cellular components (CC), and 9 molecular functions (MF) were identified.

The biological processes associated with the common targets encompassed positive regulation of smooth muscle cell proliferation, negative regulation of cell apoptosis, positive regulation of peptide serine phosphorylation, positive regulation of RNA polymerase II promoter transcription, positive regulation of transcription, DNA template, positive regulation of cytokine production in inflammatory response, inflammatory response, and cellular response to lipopolysaccharides.

Regarding cellular components, the identified categories included protein binding, transcriptional regulatory region sequence-specific DNA binding, enzyme binding, nitric oxide synthase regulatory activity, ubiquitin-protein ligase binding, among others.

Furthermore, molecular functions encompassed membrane rafts, transcription factor complexes, phagocytic cups, cytoplasm, mitochondria, cell surfaces, macromolecular complexes, cytoplasmic perinuclear regions, folded membranes, among others.

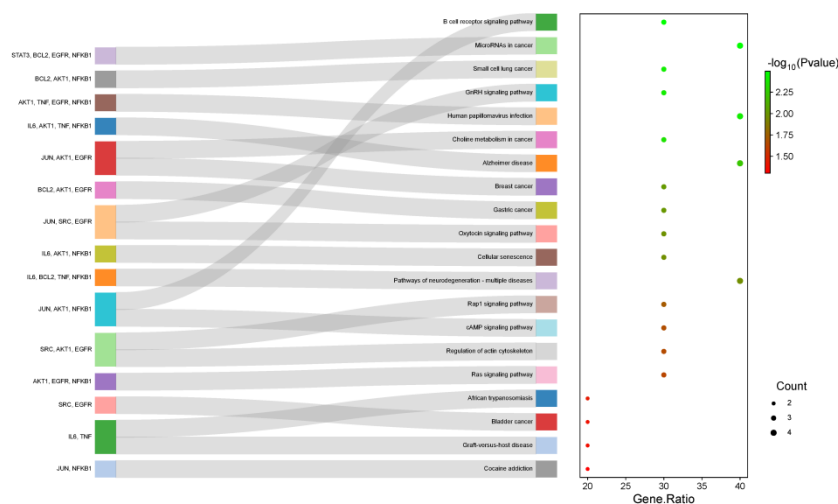


Figure 7 "Target Signal Pathway" Sangi Bubble Diagram

The results indicate that these potential targets exert their effects through multiple pathways, engaging in various regulatory mechanisms, and exhibiting significant potential value. Moreover, the observation of a small P value associated with the Rap1 signaling pathway, along with the presence of numerous enriched targets within it, suggests that *Ophiopogon japonicus* may exert its anti-pneumonia effect primarily through this particular signaling pathway.

3 Discussion

Pneumonia represents a critical medical challenge as an acute respiratory infection affecting the lungs. The quest for more effective and safer treatments for pneumonia is imperative to maintain public health. Leveraging the innovative progress in traditional Chinese medicine, *Ophiopogon japonicus* has emerged as a promising therapeutic agent with anti-inflammatory and antioxidant properties, offering potential in diseases like pneumonia.

This project delves into the principal therapeutic targets and associated signaling pathways of *Ophiopogon japonicus* in pneumonia treatment, employing the advantages of network pharmacology and molecular docking technologies. The primary target of *Ophiopogon japonicus* in pneumonia treatment, along with its potential mode of action primarily through the Rap1 signaling pathway, was identified.

During drug therapy, the upregulation of TLR4 expression levels has been observed to enhance serum levels of IL-2, IL-4, and TNF- α , as well as the content of IgG and IgM, bolstering the body's immune response. Increased expression of STAT3 can mitigate pulmonary fibrosis and alleviate inflammatory response. Regulation to decrease TNF- α , IL-1 β , IL-6 levels and inhibit the expression of iNOS and COX-2 proteins, significantly curtails the inflammatory response. Additionally, downregulating the expression of the IL-6 gene and proteins related to the JNK signaling pathway aids in alleviating the inflammatory response of lung cells. Regulation also reduces the apoptosis rate of pulmonary inflammatory cells, along with the expression of Cleaved caspase-3 protein and TNF- α , IL-6, IL-1 α through horizontal inhibition of AKT1 and EGFR, among others. NF- κ B plays a crucial role in intervening in pneumonia by regulating TNF- α expression and anti-apoptotic gene BCL2.

Numerous studies have underscored the significance of the Rap1 signaling pathway in pneumonia development. Rap1, prevalent in animal cells, primarily regulates the MAPK signaling pathway and integrin function, influencing vital biological processes such as cell proliferation, differentiation, migration, and apoptosis. Dysregulation of Rap1 expression can lead to autoimmune diseases. Working in concert with other pathways, the Rap1 signaling pathway activates various cascades, ultimately reducing inflammatory factors' expression, delaying cell degeneration and death, and mitigating pulmonary vascular endothelial injury.

Conclusion: this study offers preliminary insights into the pathway and mechanism of using *Ophiopogon japonicus* to treat pneumonia, leveraging network pharmacology and molecular docking technologies. However,

it is essential to acknowledge the limitations of network pharmacology, emphasizing the necessity for experimental verification of active ingredients, core targets, and pathways to facilitate drug development.

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References

[1] Chang Fangyuan, Xu Dong. Correlation analysis between serum activated protein C expression level and inflammatory response and prognosis in patients with severe pneumonia [J]. Chinese Journal of Health Engineering, 2024, 23 (02): 259-261

[2] Ma Yingxuan, Zhao Xiaoli, Fan Baojun, et al. The value of clinical pulmonary infection scoring in guiding the application of antibiotics in young patients with severe pneumonia [J]. Medical Diet and Health, 2022, 20 (10): 7-9+53

[3] Yin Z ,WenSheng Q .Anti-Inflammatory and Analgesic Effects and Potential Targets of Shenzhu Jiedu Granule Against Novel Coronavirus Pneumonia Based on Network Pharmacology.[J].Alternative therapies in health and medicine,2023

[4] Wan Meixu, Yuan Jing, Zhang Yanxin et al. Research progress on the pharmacological effects of *Ophiopogon japonicus* extract and its active ingredients [J]. Drug Evaluation Research, 2023,46 (08): 1819-1826

[5] Kang Diandian, Zhang Rongrong, Zhan Amber, et al. Exploring the synergistic effect and compatibility structure of anti-inflammatory active ingredients of Duiyiwei based on network pharmacology and component structure theory [J/OL]. Chinese Journal of Traditional Chinese Medicine, 1-13

[6] Zhang Peng, Wu Lan, Li Xiwen, et al. Comparative study on standard decoctions of *Ophiopogon japonicus* and Shanmaidong decoction pieces [J]. Chinese Journal of Traditional Chinese Medicine, 2019, 44 (21): 4612-4620

[7] Huang Yebao, Xiao Qian, Li Hongmei, et al. Research progress on the application of network pharmacology in the treatment of ulcerative colitis with classic formulas [J]. World Journal of Traditional Chinese Medicine, 2023,18 (04): 560-565

[8] Wang Jing. Exploring the mechanism of action of modified Danggui Liu Huang Tang in the treatment of pneumonia based on molecular docking and network pharmacology [J]. Da Dian, 2023,8 (24): 130-134

[9] Liu Nannan, Guo Baihui, Wang Xiaoxi, et al. Effects of *Ophiopogon japonicus* on TRPA1, SP, and CGRP in cough mice infected with *Mycoplasma pneumoniae* [J/OL]. Chinese Journal of Traditional Chinese Medicine, 1-13

[10] Lv Zhiyuan, Song Jianzhong, Qu Zhenzhen, et al. The effect of prickly polysaccharide regulation of Toll like receptor 4 on immunosuppressive activity [J/OL]. Natural product research and development: 1-15

[11] He Cheng, Chen Wei, Zhang Nianzhi, et al. Shenqi Cordyceps Formula improves inflammatory response in pulmonary fibrosis rats through the ASS1/src/STAT3 signaling pathway [J/OL]. Journal of Southern Medical

University: 1-8

[12] Ulipan Tohuda Ali, Ding Wanting, Sun Yuan, et al. Tannic acid was synthesized by TLR4-SRC/MAPK/NF- κ B pathway inhibits lipopolysaccharide induced inflammatory response of RAW264.7 macrophages [J]. *Zhongnan Pharmaceutical*, 2024,22 (04): 943-949

[13] Zhao Xiaohan, Si Wei, Zhao Qingyu, et al. Glycyrrhetic acid alleviates lipopolysaccharide induced inflammatory response in IPEC-J2 cells through the c-Jun amino terminal protein kinase pathway [J]. *Journal of Animal Nutrition*, 2023,35 (07): 4632-4642

[14] Liu Xiaoxue, Wang Xuefeng, Zhang Xiuying, et al. Qingfei Tongluo Gao on PI3K-AKT-NF in lung tissue of rats infected with respiratory syncytial virus κ The impact of the B signaling pathway [J]. *Chinese Journal of Integrated Traditional Chinese and Western Medicine in Pediatrics*, 2016,8 (02): 136-138+249

[15] Chen Qiang The detection significance of EGFR, hs CRP, and IL-10 in bronchopneumonia [J]. *Jilin Medical Journal*, 2023,44 (11): 3060-3063

[16] Li Chao, Li Jun, Sheng Xiaosheng, etc NFKB1 gene polymorphism and its association with primary hypertension and serum TNF- α Correlation study of [J]. *Zhejiang Medical Journal*, 2022,44 (21): 2274-2277+2282

[17] Hu Jianping, Liu Huiying, Guo Shijie, et al. Study on the expression of anti apoptotic genes bcl-2 and P53 protein in lung cancer [J]. *Journal of Practical Oncology*, 1997 (01): 62-64

[18] Zhang Yunge, Zhang Keyue, Chen Ji, etc The regulatory role of Rap1 in immune cells [J]. *Chinese Modern Doctor*, 2023,61 (31): 120-123

[19] Fan Yuanhe, Yang Yongju, Ma Xiande, et al. Exploring the Delayed Cartilage Degeneration of Right Guiwan in Knee Osteoarthritis Based on RAP1 Signal Pathway [J]. *Chinese Journal of Traditional Chinese Medicine*, 2024,42 (04): 137-142+293-294

[20] Hu Xingrong, Zhou Mingwang, Liu Haiping, etc The expression of Rap1 protein in vascular endothelium of non traumatic osteonecrosis of the femoral head [J]. *Journal of Sichuan University (Medical Edition)*, 2021, 52 (03): 452-457

[21] Gao Yuan, Jie Yuzhen, Ma Tianlong, et al. Study on the Targeted Regulation of RAP1A in Homocysteine Mediated Hepatocellular Autophagy by miR-488-3p [J]. *Chinese Journal of Comparative Medicine*, 2022, 32 (09): 1-9

[22] [1] Zhang Hao, Yu Xiao, Zhu Shen. Expression and significance of SIRT1, NAC1, Rap1 proteins in ovarian serous tumors [J]. *Journal of Practical Cancer*, 2022, 37 (05): 732-735

[23] Liu Bowen, Zhang Chenyan, Zhu Weijie, et al. Network pharmacology analysis and experimental study of Shufeng Huoluo Wan in the treatment of rheumatoid arthritis [J/OL]. *Chinese Journal of Hospital Pharmacy*, 1-12.

[24] Wei C ,Shui-Li Z .Anti-Inflammatory Mechanisms of Total Flavonoids from *Mosla scabra* against Influenza A Virus-Induced Pneumonia by Integrating Network Pharmacology and Experimental Verification[J].*Evidence-Based Complementary and Alternative Medicine*,2022,20222154485-2154485.

25. Xu J, Bai C, Huang L, Liu T, Wan Y, Zheng Z, Ma X, Gao F, Yu H, Gu X. Network pharmacology to dissect the mechanisms of Yinlai Decoction for pneumonia. *BMC Complementary Medicine and Therapies*. 2020 Dec;20:1-5.

26. Yang D, Li Z, Peng Y, Zhu X, Gong J, Chen C. Network pharmacology combined with molecular docking simulations reveal the mechanism of action of *Glycyrrhiza* for treating pneumonia. *Peptide Science*. 2024 Jan 17:e24342.