Exploring the potential pharmacological mechanism of Coix seed on pneumonia based on network pharmacology and molecular docking

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Abstract. To explore the potential target and mechanism of action of Coix seed in the treatment of pneumonia by means of network pharmacology and molecular docking. To construct the potential protein interaction network and “component-target” network diagram, GO and KEGG enrichment analysis were performed, then molecular docking was used for verification. In coix seed, 7 effective components and 144 corresponding potential targets were obtained by screening with OB $\geq$ 30% and DL $\geq$ 0.18% as thresholds. A total of 5014 pneumonia related targets were obtained, and 90 common targets were obtained. 151 nodes and 251 edges were read in the “component-target” visual network diagram. A total of 19 signal pathways with significant differences were obtained by KEGG enrichment analysis. The molecular docking showed that the compounds in Coix seed had higher binding energy with the key proteins that caused pneumonia. This study preliminarily explored the potential action mechanism of coix seed in treating pneumonia by multiple channels and multiple targets, providing scientific basis for clinical application and in-depth study of this decoction piece.

1. Introduction
Pneumonia is one of the common respiratory diseases, which is mainly caused by the patient's low immune function [1], leading to the invasion of viruses, bacteria and mycoplasma into the lungs [2], resulting in respiratory infection, which is clinically manifested as fever, cough, sputum, dyspnea and other symptoms [3, 4]. In recent years, some deadly viruses that cause severe pneumonia have appeared in various countries around the world, seriously threatening people’s lives and health [5]. In particular, COVID-19, which started to wreak havoc around the work at the end of 2019, has caused great losses to mankind [6].

Coix seed, commonly known as semen coicis, is a dry and mature seed of coix seed [7], a grass plant. In 2002, it was listed as a medicinal and edible species by the ministry of health [8]. Its main active substances include esters, sugars, triterpenes and unsaturated fatty acids [9]. Studies have shown that coix seed has anti-tumor, enhanced immunity, hypoglycemic, anti-inflammatory and analgesic effect, etc [10-12], in this prevention and rehabilitation diet program for COVID-19, many traditional Chinese medicines prepared in prepared slices appeared in many prescriptions [13]. However, coix seed still has defects in clinical research and development.
This study explored the potential pharmacological mechanism of coix seed in the treatment of pneumonia based on network pharmacology of Traditional Chinese medicine and molecular docking technology, providing reference and guidance for the later clinical research of coix seed.

2. Materials and methods

2.1. Component information
The TCMSP database (https://tcmsp.com/tcmsp.php) was retrieved by inputting “semen coicis”, screening with drug-likeness (DL) > 0.18 and bioavailability (OB) > 30% as a threshold, and the chemical composition of coix seed was obtained by combining with relevant literature, including sitosterol, coix seed lactone, stigmasterol, sitosterol-α-1, 2-monooleate, etc [14].

2.2. Prediction of composition targets
The obtained chemical composition is queried in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for the SMILES string corresponding to each active component, and the obtained SMILES string is entered into the website (http://www.swisstargetprediction.ch/) to predict its potential target. In the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) obtained chemical components will be searched SMILES strings of each active ingredients, and inputting SMILES strings into Swiss Target Prediction website (http://www.swisstargetprediction.ch/) predicts its potential targets.

2.3. Screening of disease targets
The OMIM database (https://omim.org/) and the Gene Cards database (https://www.genecards.org/) were searched with the keyword “pneumonia (pneumonia)” to obtain the disease targets associated with pneumonia.

2.4. Construction of the network of active components and potential targets
The active component target of coix seed and the related target of pneumonia disease were taken as the potential target of coix seed in the treatment of pneumonia. The relationship between components and potential targets was constructed by Cytoscape 3.7.2 software, and the relationship was topologically analyzed by Cytoscape (NCA) plug-in, and the four components with higher value (degree) were followed by molecular docking. Further verify the relationship between components and targets.

2.5. Construction of protein interaction network
The common targets of drug and disease were uploaded on String platform (https://string-db.org/), and the protein phase action relationship was obtained after screening at the highest confidence=0.900 [15].

2.6. Enrichment analysis
The potential targets were introduced into the Mescape database (https://metascape.org/), and “GO Biological Processes” “GO Cellular Components” “GO Molecular Functions” and “KEGG Pathway” were selected for “Sapiens” to analyze and visualize the results.

2.7. Molecular docking
2D structure information of four core genes (MAPK3, JAK2, RXRA and SRC) was downloaded from the Pubchem database, and Chem3D software was used to uniformly transform them into 3D structures. The UniProt database (https://www.uniprot.org/) was used to search for the Entry of the obtained protein. The PDB database (http://www.rcsb.org/) was used to enter the Entry and download the 3D structure of the candidate target protein for pneumonia, and Pymol software was used to remove the ligand and water in the obtained target protein. AutoDock_Vina software was used to simulate the docking between the four core components and the protein receptor, taking the lowest binding energy to the best binding position. Pymol software was used to visualize the conformation at the best binding locations.
3. Results

3.1. The establishment of coix seed chemical branch library

In this study, 7 main active ingredients were selected based on OB ≥ 30%, DL ≥ 0.18 and references in TCMSP database, as shown in Table 1.

| Mol ID   | Molecule Name | MW  | OB (%) | DL   |
|----------|---------------|-----|--------|------|
| MOL001323 | sitosterol alpha | 426.8 | 43.28  | 0.78 |
| MOL001494 | mandenol | 308.56 | 42     | 0.19 |
| MOL000359 | sitosterol | 414.79 | 36.91  | 0.75 |
| MOL000449 | stigmasterol | 412.77 | 43.83  | 0.76 |
| MOL002118 | coixenolide | 591.08 | 32.4   | 0.43 |
| MOL008121 | 2-Monoolein | 356.61 | 34.23  | 0.29 |
| MOL000953 | CLR | 386.73 | 37.87  | 0.68 |

3.2. Establishment of component target and disease target libraries

The Target Prediction was carried out by entering the SMILES character with the structural information of the active ingredient of coix seed in The Swiss Target Prediction website, and the Probability was selected with the condition that the Probability was greater than 0.1, so that a total of 144 potential constituent targets were obtained after removing duplication.

Inputting “pneumonia” retrieved to obtain the related target of pneumonia disease in the Gene Cards database and OMIM database respectively, which obtained a total of 5014 pneumonia related disease targets through combining and removing duplication. The potential component targets and disease targets were intersected to obtain 90 common targets, as shown in Fig. 1, and these 90 common targets were taken as the core targets for subsequent enrichment analysis and molecular docking.

![Figure 1. Venn diagram of target of coix seed component and pneumonia disease](image)

3.3. Enrichment analysis

GO (functional) and KEGG (signaling pathway) enrichment analysis was carried out on the 90 potential core targets obtained above, and the possible pharmacological mechanism of coix seed in the treatment of pneumonia was systematically analyzed as a whole. The results suggested that the chemical components in coix seed may be closely related to lipid transport, lipid metabolism regulation, sterol metabolism, fatty acid metabolism regulation, hormone level regulation, nuclear receptor activity, phosphatase binding and other factors in the treatment of pneumonia. The specific results are shown in Fig. 2, and 3. The KEGG signaling analysis showed that the treatment of pneumonia may be closely related to the neural-active ligand receptor interaction pathway, arachidonic acid metabolism pathway,
Th17 cell differentiation pathway, Rap1 signaling pathway, PPAR signaling pathway, Ras signaling pathway, etc., as shown in Fig. 4.

Figure 2. Strip diagram of enrichment analysis

Figure 3. Bubble diagram of GO enrichment analysis
3.4. “Component-Target” network construction and analysis

Cytoscape 3.7.2 software was used to construct the “component-target” network diagram (Fig. 5) and “target-pathway” network diagram (Fig. 6), in which the red nodes represent potential genes for treating pneumonia, the green nodes represent active components, and the yellow nodes represent pathways.

The component-target network diagram shows 151 nodes and 251 edges. According to the target-path network diagram, there are 69 nodes and 108 edges. The obtained access graph was operated twice with greater than the median with the Cytoscape (NCA) plug-in, as shown in Fig. 7.

Figure 4. Bubble diagram of KEGG pathway analysis

Figure 5. Component-target network diagram
3.5. Construction of protein interaction network

The 90 potential targets obtained in “2.2” were uploaded to the STRING protein interaction online analysis platform, and PPI (protein interaction network) of the potential targets for the treatment of pneumonia was obtained by taking highest confidence=0.900 as the screening condition (Fig. 8). Downloading “String Interactions. TSV” and using R language to further calculate and analyze the “Degree”, “LAC”, “Leisenness”, “Schoenness”, “Schoenvector” and “Network” of the 71 core targets mentioned above, and screening the targets whose values are all larger than the median and carrying out list analysis on the first 12. The data shows that the Degree of relationship of these targets in the interaction system is very high (Table 2).
Figure 8. Protein interaction networks with potential targets

Table 2. PPI network core targets for the treatment of pneumonia

| Target Name                                   | Gene Symbol | Degree |
|-----------------------------------------------|-------------|--------|
| MAP kinase ERK1                               | MAPK3       | 36     |
| Tyrosine-protein kinase SRC                   | SRC         | 34     |
| Retinoid X receptor alpha (by homology)       | RXRA        | 34     |
| Retinoid X receptor gamma (by homology)       | RXRG        | 22     |
| Tyrosine-protein kinase JAK2                  | JAK2        | 20     |
| Protein-tyrosine phosphatase 2C               | PTPN11      | 18     |
| Tyrosine-protein kinase JAK1                  | JAK1        | 16     |
| Protein kinase C alpha                         | PRKCA       | 16     |
| Protein-tyrosine phosphatase 1C               | PTPN6       | 14     |
| Estrogen receptor alpha                       | ESR1        | 14     |
| Protein kinase C delta                         | PRKCD       | 14     |
| Retinoic acid receptor alpha                  | RARA        | 14     |
3.6. Molecular docking
In order to further validate our analysis results, we use molecular docking technology to further explore the possibility of coix seed on the treatment of pneumonia, selecting Fig. 8 obtained core 3 d structure of the gene as ligands including MAP kinase ERK1, tyrosine protein kinase, tyrosine protein kinase JAK2, retinol X receptor alpha (via homologous), respectively, and using PDB database obtained protein molecules as the ligand including 4QTB, 4P7E, 4CN3 and 2SRC. AutoDock_Vina software was used to simulate the docking between the core components and the protein ligand, and size_x, size_y and size_z were uniformly set to 40 in the software; Energy_range is 5; Num_modes is 20, and the specific coordinate parameters are shown in Table 3. The docking analysis results showed that the small molecule ligand had good binding ability with the target protein receptor, and the binding location of the active site was well predicted (Fig. 9).

| Receptor | Ligand | center_x | center_y | center_z |
|----------|--------|----------|----------|----------|
| JAK2     | 4P7E   | 15.682   | 13.435   | 128.851  |
| MAPK3    | 4QTB   | 15.008   | -7.84    | 24.54    |
| RXRA     | 4CN3   | 13.189   | -14.336  | 7.553    |
| SRC      | 2SRC   | 11.865   | -14.412  | 8.54     |

Table 3. Coordinate parameters of ligand and receptor

![Molecular docking diagram](image)

Figure 9. Molecular docking diagram

4. Results
Pneumonia is a common lung disease, usually characterized by cough, fever and dyspnea [16]. Most of them are caused by microorganisms such as bacteria, fungi or viruses, with extremely high morbidity and mortality [17], which is one of the prevention and treatment of four diseases in children [18]. Since SARS in 2003, TCM treatment has always been a participant in the prevention and treatment of various
epidemic situations [19, 20]. However, in the novel Coronavirus pneumonia rehabilitation and treatment in 2019, TCM treatment scheme changed from participation to dominance [21]. In the notice of the Office of the National Administration of Traditional Chinese Medicine and the General Office of the National Health Commission on the publication of the Novel Coronavirus Pneumonia Diagnosis and treatment plan (Trial sixth edition), coix seed is involved in many prescriptions [22]. It can be seen that coix seed has a good effect in the treatment of pneumonia.

The chemical component “sitosterol” in coix seed is an important part of MAP kinase signal transduction pathway, which not only mediates various biological functions such as cell growth, adhesion and differentiation [23], but also plays an important role in the initiation and regulation of cell mitosis, meiosis and post-mitosis functions [24]. In addition, it also participates in the regulation of endoplasmic reticulum dynamics.

Ethyl octadec-6,9-dienoate forms homodimers or heterodimers with retinoic acid receptors (RARS) and binds its ligands to target reaction elements to regulate gene expression in various biological processes [25]. The role played by macrophages in enhancing the immune system in response to viral infection by promoting phagocytosis of myelin fragments and myelin regeneration may be achieved by negatively regulating the transcription of antiviral genes such as IFN genes. By inhibiting the expression of ITTPR2 genes involved in the regulation of calcium signal, the value of virus is controlled, and finally antiviral is realized [26]. Non-receptor protein tyrosine kinases involved in signaling pathways that control multiple biological activities, including gene transcription, immune response, cell adhesion, cell cycle progression, apoptosis, migration and transformation [27].

SRC is one of the major kinases activated after receptor involvement and plays a role in the activation of other protein tyrosine kinase (PTK) families [28]. Receptor aggregation or dimerization leads to SRC recruitment to the receptor complex, which phosphorylates tyrosine residues within the receptor cytoplasmic domain and plays an important role in regulating cytoskeletal tissue not only involved in the transduction of mitotic signals [29], but may also control the progression of the entire cell cycle by interacting with regulatory proteins in the nucleus phosphorylation on multiple tyrosine residues CBLC [30], mediates IL6 signaling through activation of YAP1-NOTCH pathways to induce epithelial regeneration [31].

To sum up, this study explored the potential mechanism of the prevention a treatment of pneumonia by combining the traditional Chinese medicine network pharmacology method with molecular docking technology. The results suggest that coix seed can play a role in the prevention and treatment of pneumonia through multi-pathway and multi-target action, mainly from the aspects of immunomodulation, inflammatory response, cell proliferation and so on. The results provide a theoretical basis for further clinical study of coix seed.

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