Study on Anti-pneumonia Mechanisms of Honeysuckle Based on Network Pharmacology

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Research

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Abstract

**Background:** Honeysuckle is a traditional Chinese herbal medicine. It is widely used in the treatment of respiratory infectious diseases in Asia and even worldwide reported in the literature. However, its mechanism remains unclear. Pneumonia is one of the most common clinical manifestations of respiratory infections. This study is aim to find out the molecular mechanism of honeysuckle in the treatment of pneumonia.

**Methods:** LPS-induced pneumonia rats were administered with honeysuckle decoction and compared its efficacy with dexamethasone. The components in honeysuckle decoction were analyzed by liquid-phase mass spectrometry. The target proteins for those components were obtained by database search, target fishing and text mining methods, and then were matched with the genes of pneumonia in the CTD database. Analyzed interactions of target protein by using String database, enrichment analysis by David database, molecular docking was performed by AUTODOCK and PyRx. Its related network diagrams drew by Cytoscape.

**Results:** Our results indicate that honeysuckle can alleviate the inflammation of rat lung tissue to a certain extent, and has similar therapeutic effects as dexamethasone. There are 31 compounds in honeysuckle, and 366 genes associated with these compounds and pneumonia. Among them, 18 of the top 5%, including GAPDH, TNF, FOS, VEGFA, BDNF, etc., were enriched in pathway closely related to pneumonia: Glycine, serine and threonine metabolism, Nitrogen metabolism, and Neuroactive ligand-receptor interaction, etc. The results of molecular docking confirmed that Inosito binds to the PTEN protein of the Phosphatidylinositol signaling system pathway.

**Conclusions:** This study suggests that honeysuckle can play a role in the treatment of pneumonia by affecting multiple targets and pathways, of which Inosito-PTEN-Phosphatidylinositol signaling system pathway may be the most important mechanism. This reflects the multi-target multi-pathway of traditional Chinese medicine, and has a certain specific therapeutic effect on the disease. By interfering with the signaling pathways of the body cells, honeysuckle can be used as a drug candidate for clinical pneumonia treatment.

Background

Pneumonia refers to inflammation of the lung parenchyma caused by various pathogens such as bacteria, viruses, fungi, parasites, etc., including terminal airways, alveolar spaces and interstitial lung. According to the location and time of pneumonia, it is divided into community acquired pneumonia (CAP) and hospital acquired pneumonia (HAP). The CAP incidence of adult in European and North American countries is 5~11/ (1000 person-years) [1], and the mortality rate is closely related to the age and severity of illness of patients. It has been pointed out in literature that pneumonia is one of the leading causes of death in Chinese adults and children [2]. Clinical manifestations of children and the elderly are atypical, often with non-specific manifestations and insidious onset, with many complications and poor prognosis.

For the treatment of community acquired pneumonia, routine clinical guidelines [3-4] recommend anti-infective treatment based on empirical anti-infective and laboratory evidence, including antibiotics and antiviral drugs, at the onset or at the beginning of the visiting. For severe cases, hormone-assisted treatment is given when evidence supported. Such as the abuse of antibiotics, the resistance of pathogenic microorganisms, the side effects of hormones and so on. A study [5] pointed out that the abuse of antibiotics such as amoxicillin caused neurotoxicity changes and even death in experimental animals. Therefore, it is of great significance to find clinically effective drugs as candidates for the treatment of pneumonia.
Honeysuckle is a traditional Chinese herbal medicine. According to the theory of traditional Chinese medicine, its decoction can treat respiratory infections characterized by fever. Experimental studies \[6\] have confirmed that honeysuckle has a protective cytokine that promotes LPS-induced pneumonia release. Therefore, honeysuckle may have an inhibitory effect on inflammation of pneumonia, but its specific mechanisms remain unclear. The purpose of this network pharmacology study is to determine the effective compounds and their target genes for honeysuckle in the treatment of pneumonia, and to explore the potential mechanisms of honeysuckle in the treatment of pneumonia.

**Methods**

**Comparison the efficacy between honeysuckle and dexamethasone in the treatment of pneumonia**

1. **Experimental materials:** Clean grade SD rats, 24, male, 3 weeks old, weighing 110 g ± 10 g. Animal certificate No.: 11401500015429, provided by Sibeifu (Beijing) Biotechnology Co., Ltd., license No.: SCXK (Beijing) 2016-0002. It is kept in animal laboratory of Beijing University of Chinese Medicine. The air conditioning at a room temperature of 22 °C, a humidity of 30-40%, natural light, and free access to water. Ordinary feed is irradiated large/small rats maintenance pellet feed, provided by Sibeifu (Beijing) Biotechnology Co., Ltd. The experimental reagents included honeysuckle aqueous extracts (30g pure water boil for 1 hour, pure water calibration to 393 ml), dexamethasone solution, LPS aqueous solution (0.5 mg/ml), medical saline, medical alcohol, HE staining reagent.

2. **Modeling treatment:** Rats were divided into 4 groups according to body weight: normal group, model group, honeysuckle decoction group (HD group), dexamethasone group (DXMS group). The grouped animals were placed in squirrel cages, 3 per cage, free access to food and water. From the first day, the model group, the HD group and the DXMS group were given LPS for 15 minutes daily, and the normal group was given pure water atomization; each group was intragastrically administered with the corresponding agent 1ml/100g, twice daily. After the end of the third day of intragastric administration, free access to water but not to food. On the fourth morning, the rats were anesthetized by intraperitoneal injection of chloral hydrate. The ice-free lung tissue of rats were washed up by physiological saline. The surface moisture was dried up by absorbent paper, placed in a 4% formaldehyde fixative, and stored at 4 °C.

3. **Detection of indicators:** Intestinal tissue transverse sections were prepared according to the routine procedure for preparing tissue wax block-slice-HE staining.

**Detection and acquisition of honeysuckle components**

The active ingredient in the honeysuckle aqueous extracts was detected by liquid chromatography mass spectrometry.

1. **Analytical conditions:** LC conditions Agilent Proshell 120 EC-C18 column; mobile phase A: 0.1% formic acid-water; mobile phase B: 0.1% formic acid-methanol; detection wavelength: 210, 255, 260, 278, 282, 323, 326,330 nm; column temperature: 35 °C; flow rate 0.6 mL / min; injection volume 3 μl; gradient elution procedure: 0-2 min, 15%-45% B; 2-8 min, 45%-72% B; 8-25 min , 72%-95% B; 25-30 min, 95%-100% B. MS conditions Dual AJS ESI ion source, positive ion and negative ion mode detection respectively; Nebulizer: 35 psig, drying gas temperature: 200 °C, drying gas flow rate: 14 L / min; sheath gas temperature: 350 °C, sheath gas flow rate: 11 L / min; Fragmenter: 385V; scanning range: 50-1500. Reference ions: 121.0508, 149.0233, 322.0481, 922.0098, 1221.9906. Acquisition frequency: 1.5spectra/s; Transients: 3987.
2. Component identification method: peak with reference substance, which is identified by comparing UV absorption, retention time and mass spectrometry information; peak without reference substance, comparison of the retention time and primary mass spectrometry data of each peak with known compounds in the literature.

Acquisition and collection of pneumonia targets by honeysuckle and dexamethasone treatment

According to the obtained components, the corresponding target proteins were obtained by using database matching, text mining and target fishing methods: using the Symmap (http://www.symmap.org)\(^7\) database for search matching; using Polysearch2 (http://polysearch.cs.ualberta.ca/index)\(^8\) Database for text mining; use the ChemDraw 17 software to draw the structure and save it as a “.mol2” file; using ChemMapper (http://lilab-ecust.cn/chemmapper/index.html)\(^9\) Database for target fishing. The Uniport (https://www.uniprot.org/) database was used to retrieve the predicted targets of honeysuckle and dexamethasone. Mark the protein name conversion as gene symbol, and deduplication the corresponding compound and retention unique value. Based on the composition and corresponding targets, the “component-target” network map was constructed by Cytoscape 3.6 software. The target gene corresponding to the disease and the target corresponding to the components are labeled by EXCEL. The common target gene is the prediction target gene of pneumonia treatment by honeysuckle/dexamethasone. Using the OmicShare Cloud Platform tool (http://www.omicshare.com/), the relationship between disease targets and drug targets is presented in Wayne.

Construction of core target interaction network

The String database was used as the background network database to introduce the target gene for the detection of pneumonia by the honeysuckle/dexamethasone component. The research species was selected as “Homo sapiens”, and the target protein interaction relationship was obtained and saved as a TSV format file. Import the TSV file into the Cytoscape 3.6 software to draw a network map.

Target Path Visualization

Upload the predicted target gene to the David database (Version 6.8 https://david.ncifcrf.gov/) for GO enrichment analysis (select 3 modules: biological process, molecular function and cellular component) and KEGG pathway analysis. Screen out the pathway with P value < 0.001, and sort in descending order according to the number of enrichment genes, select the top 20 pathways, and use the OmicShare website (http://www.omicshare.com/) to compare the results with the presentation form of advanced bubble maps.

Network construction of the “component-target-path”

The top 20 and not specific disease pathways in the KEGG pathway enrichment analysis of honeysuckle/dexamethasone were selected, matched with targets with higher degree values in the target interaction network, and the pathway, target and active components were uploaded to Cytoscape 3.6 Software respectively to build a “component-target-path” network map to visualize the relationship between the three.

Molecular docking

For the target protein with the highest degree value in the above-mentioned “component-target-path” network, the 3D structure of the human target protein was downloaded in PDB database and saved as PDB format. The protein was dehydrated, hydrotreated, and stored as PDBQT format by AutodockTools software. The 3D structure of the small molecule compound corresponding to the component of honeysuckle/dexamethasone was subjected to energy
minimization treatment, and then stored as PDBQT format. The structure of the small molecule and the corresponding target protein was uploaded into PyRx software, and using Autodock Vina algorithm for molecular docking. Based on the results of the Docking score, assess the potential integration between them. The docking results were exported to PDB files, and PyMol software was used to generate ligand-receptor-bound PDB format files, and LigPlot software was used to generate ligand-receptor two-dimensional structure maps.

Results

Animal experiment results

As shown in Figure 2, according to the pathological HE staining results of lung tissue, the alveolar structure of normal group was intact, the wall was thin, no inflammatory infiltration was observed, and the bronchioles were intact and clear; compared with the normal group, the lung tissue structure in the pneumonia group was damaged; the alveolar septum was different in size; the alveolar wall was thickened and fractured; a large number of neutrophil infiltration and erythrocyte extravasation were seen in the interstitial lung; and vascular endothelial cell proliferation was observed. Compared the two treatment groups with the pneumonia group, the lung tissue and alveoli structure in rats became clear, and the interstitial hyperplasia was alleviated with a small amount of inflammatory infiltration.

Test results of honeysuckle and dexamethasone

According to the qualification conditions, 31 honeysuckle components were obtained, and a total of 3 compounds were identified by comparison with the standard, as shown in Figure 3, Table 1 and supplementary data Figure 1.

Network diagram and analysis of “drug-ingredient-action target”

After removing the repeated targets, 545 honeysuckle aqueous extracts and 34 dexamethasone targets were finally obtained. The drug component and its corresponding target were introduced into the Cytoscape 3.6 software, and the “component-target network map” was drawn (see Figure 4, Figure 5). The honeysuckle network diagram contains 645 nodes and 631 edges; the dexamethasone network diagram contains 35 nodes and 34 edges.

Predictive target of honeysuckle/dexamethasone against pneumonia

A total of 30083 related targets for pneumonia were obtained. After matching the drug component corresponding target, 366 targets common to honeysuckle and 30 targets common to dexamethasone were obtained, that is, the predictive target of honeysuckle/dexamethasone against pneumonia. Among them, there are 8 target proteins common to honeysuckle and dexamethasone against pneumonia, respectively are: CD38, ENPP1, ESR1, IL1B, NR3C2, PGR, PLA2G4A, RPS6KA3. The relationship between the targets is shown in Figure 6, and the common targets are shown in supplementary data table 1.

Potential target interaction network and analysis

As shown in Figure 7, the left side is the network diagram of predicted target of honeysuckle in the treatment of pneumonia; the right side is the network diagram of the target of dexamethasone in the treatment of pneumonia. The honeysuckle network diagram contains 365 circular nodes, representing all the predicted targets; 2749 edges, representing the correlation between the targets; the dexamethasone network diagram contains 29 circular nodes and 67 edges; the darker the color of the nodes, it means that the bigger the value of its Degree is. According to the
value of Degree, 18 of the top 5% of honeysuckle were GAPDH (101), TNF (71), VEGFA (65), BDNF (64), CASP3 (59), CXCL8 (56), PTEN (55), MTOR (52), F2 (51), CXCL12 (51), PTGS2 (49), EDN1 (48), TH (47), ESR1 (47), IL1B (45), GPT (44), NPY (43); one of the first 5% of dexamethasone targets is MYC (12). In the brackets is Cytoscape Degree. These targets have the greatest correlation with the treatment of pneumonia, which is the core target.

**GO and KEGG pathway enrichment results and analysis**

The results of GO enrichment analysis of honeysuckle aqueous extracts showed that the high enrichment of biological process (BP) analysis are response to organic substance, response to endogenous stimulus, response to hypoxia, response to oxygen levels, positive regulation of multicellular Organismal process; the high enrichment of cell component (CC) analysis are cell fraction, Cytosol, insoluble fraction, axon, neuron projection, etc.; the high enrichment of molecular function (MF) analysis are pyridoxal phosphate binding, vitamin B6 binding, vitamin binding, cofactor binding, transferase activity, transferring nitrogenous groups and so on. Through KEGG pathway enrichment analysis, Glycine, serine and threonine metabolism, Nitrogen metabolism, Neuroactive ligand-receptor interaction, Calcium signaling pathway, Phenylalanine metabolism pathways, etc. are closely related to the treatment of pneumonia with honeysuckle.

Compared with honeysuckle, the results of GO enrichment analysis of dexamethasone showed that the high enrichment of BP analysis are regulation of apoptosis, regulation of programmed cell death, regulation of cell death, regulation of cell proliferation, response to organic substance, etc.; the high enrichment of CC analysis are cell surface, secretory granule, etc.; the high enrichment of MF analysis are steroid hormone receptor activity, ligand-dependent nuclear receptor activity, sequence-specific DNA binding, steroid binding, transcription factor activity, and so on. The KEGG pathway enrichment analysis speculated that MAPK signaling pathway is closely related to dexamethasone in the treatment of pneumonia.

**Construction and analysis of the “component-target-path” network**

As shown in Figure 9, the mechanisms of the dexamethasone core was predicted to be the dexamethasone-MYC-MAPK signaling pathway. After removing the signaling pathways of Alzheimer's, Parkinson's and prostate cancer's three specific diseases, the mechanisms prediction network of honeysuckle intervention pneumonia, including 4 small molecule compounds, 5 target proteins and 6 signaling pathways, constitutes 12 pathways, see Table 2, of which the most likely mechanism is the Inositol-PTEN-Phosphatidylinositol signaling system.

**Molecular docking results and analysis**

Based on the above results, dexamethasone-MYC and Inositol-PTEN were molecularly docked respectively. The results are shown in supplementary data table 2. The binding energy of DXMS-MYC (6g6j) was -8.1 kcal/mol, and the binding energy of Inositol-PTEN (5bzz) was -5.8 kcal/mol. As can be seen from Figure 10, two molecules were tightly bound to the groove portion of the target protein receptor. Among them, DXMS had hydrogen-bond interaction with two amino acid molecules, and Inositol had hydrogen-bond interacts with 9 amino acid molecules.

**Discussion**

In this study, dexamethasone was used as a comparison to determine the effectiveness of honeysuckle in the treatment of pneumonia, and to identify small molecule compounds and their associated target proteins of pneumonia. These molecular-protein ligand receptor structures can be involved in the treatment of pneumonia.
Most of the compounds identified in honeysuckle have been shown to have effects on anti-inflammatory, repair body, and promote regeneration to a certain extent.

The compound chlorogenic acid as a standard for the identification of honeysuckle in the Chinese Pharmacopoeia is widely used in a variety of plants. Studies showed that chlorogenic acid has a certain therapeutic effect on inflammation\[^{10-11}\] such as the removal of TNF\(\alpha\), IL6, etc. in peripheral blood\[^{12}\], which may be related to the removal of reactive oxygen species\[^{13}\]. Morroniside is a class of iridoid glycosides that improve antioxidant and anti-apoptotic effects in vitro and neurological recovery in vivo\[^{14-16}\]. Animal experiments showed\[^{17}\] that it can reduce the expression of IL-6, IL-1\(\beta\) and TNF-\(\alpha\) in myocardium of rats with myocardial infarction. Linalool, a natural compound product, exists in fruits and aromatic plants which has a certain anti-inflammatory effect, such as the protective effect of linalool on ovalbumin-induced airway inflammation and excessive mucus secretion. The effect is closely related to the downregulation of inflammatory mediators and MAPKs/NF-\(\kappa\)B signaling.

Among the top 20 pathways of KEGG pathway enrichment, specific diseases such as Alzheimer’s are eliminated. Other pathways can be divided into three categories: material metabolism, cell function and messenger signal (See Table 3). Amino acids, sugars, and lipids, as the three major nutrients in vivo, participate in normal physiological functions, and associated with severity of illness and prognosis in patients with systemic infection as a single metabolite or even more complex metabolomics from different metabolic pathways\[^{18}\]. The messenger signals such as insulin and calcium ions serve as a bridge between cellular homeostasis and external stimuli. Insulin, a protein hormone, secreted by islet beta cells in the pancreas that stimulated by endogenous or exogenous substances. Although insulin is more closely related to endocrine than infectious inflammation, it is reported that inflammatory lung disease is associated with hyperglycemia, even in patients without prior diagnosis of type 2 diabetes. Its mechanisms may be related to the inflammatory hypoxia, which causes an increase in lactic acid in gluconeogenic substrate. A ribosome is a kind of ribonucleoprotein particle in cells. Its main function is to convert the genetic code into an amino acid sequence according to the instructions of the mRNA and construct a protein polymer from the amino acid monomer. The pharmacological mechanisms of many antibiotics, such as macrolides\[^{19}\], inhibits the transcriptional translation of functional proteins by irreversibly binding to bacterial ribosomes.

This study shows that PTEN protein is the core protein in interaction network in the treatment of pneumonia by honeysuckle, and closely bind to Inositol and finally enrich in the phosphatidylinositol signaling pathway. As shown in Figure 11, the mechanisms may be that the Inositol in honeysuckle decoction specifically binds to PTEN, which activated PTEN protein resulting in the conversion of 3, 4, 5-triphosphate phosphatidylinositol (PIP3) to 4, 5.-Phosphatidylinositol diphosphate (PIP2). PIP2, as an important channel on the cell membrane\[^{20}\], involved in the regulation of various cytokines and ions after activation\[^{21-24}\] to interfere with the level of inflammation in vivo. Inositol is a substance that is widely present in food and has a structure similar to glucose. Studies\[^{25}\] pointed out that high concentrations of inositol derivatives in surfactant preparations can interfere with the key pathways of inflammatory lung disease. In addition, according to the Symmap database, the networks of honeysuckle and Inositol contain a number of common clinical manifestations of pneumonia, and also suggest that Inositol of honeysuckle, the small molecule substance, has effect on the treatment of pneumonia, as shown in Table 4.

Dexamethasone is a synthetic corticosteroid that has been used in the treatment of severe pneumonia and pneumonia complications for a long term due to its inhibition in the release of inflammatory substances in vivo. A study involving more than 2,200 participants\[^{26}\] showed that corticosteroid therapy can reduce the mortality and morbidity of adults with severe CAP and the incidence of non-severe CAP in adults and children, however, it is
related to more adverse events, especially hyperglycemia simultaneously. Studies\textsuperscript{[27]} pointed out that irrational use of glucocorticoids is associated with hyperglycemia, pneumonia, urinary tract infections, gastrointestinal ulcers or increased mortality. Presently, the pharmacological study of dexamethasone mechanism remains unclear. It may\textsuperscript{[28]} be a pleiotropic effect on hormone receptors in various signaling pathways in inflammatory cells. The results of the study indicate that dexamethasone may play its role by the MYC protein of the MAPK signaling pathway. MAPK signaling pathway is involved in a variety of cellular functions; MYC protein is involved in the proliferation and differentiation of cells; and c-MYC in its family has been positively correlated with the high proliferative activity of cells\textsuperscript{[29]}. The proliferation and differentiation of cells is an important process of inflammation absorption and tissue regeneration in vivo. Studies confirmed\textsuperscript{[30-32]} that the drug's intervention in the MAPK signaling pathway has played a positive role in the treatment of pneumonia. The results may explain some of the mechanisms by which dexamethasone is effective against pneumonia, but the mechanisms of action of clinical toxicology has not yet been clarified.

**Conclusion**

In summary, this study built a network based on network pharmacology technology to predict the interaction between compounds and target genes in honeysuckle. The results suggested that honeysuckle may play a role in the treatment of pneumonia through a messenger pathway acting on cells. However, the results obtained by network analysis still need to be verified by pharmacological methods and omics techniques. This study believed that honeysuckle is a candidate for the treatment of pneumonia because honeysuckle is involved in the anti-inflammatory process.

**Abbreviations**

CAP: community acquired pneumonia; HAP: hospital acquired pneumonia; HD group: honeysuckle decoction group; DXMS group: dexamethasone group; BP: biological process; CC: cell component; MF: molecular function; PIP3: 3, 4, 5-triphosphate phosphatidylinositol; PIP2: 4, 5. - Phosphatidylinositol diphosphate.

**Declarations**

**Acknowledgements**

Not applicable.

**Authors' contributions:**

Bai Chen, Ma Xueyan, Liu Tiegang and Gu Xiaohong designed the protocol for this study. Ma Xueyan, Bai Chen and Liu Hui completed animal and drug experiments. Ma Xueyan, Xian Fuyang and Liu Shaoyang completed the bioinformatics analysis. Long Chaojun and Yu He completed the statistics. Bai Chen and Ma Xueyan wrote the draft.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures for animal care and use were approved by the Animal Care Ethics Committee of Beijing University of Chinese Medicine, Beijing, China (No. BUCM-4-2020082602-3132).

Consent for publication

All authors consent to publication of this study in the journal Chinese Medicine.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 31 compounds identified from honeysuckle samples
| No. | tR(min) | Molecular formula | Molecular Mass (m/z) | Product ions (m/z) | Diff(ppm) | Compoundname |
|-----|---------|-------------------|---------------------|-------------------|-----------|--------------|
| 1   | 1.145   | C6H12O6           | 203.0526            | 203.0530[M+Na]+   | -2.16     | Inositol     |
| 2   | 1.256   | C10H13N5O4        | 268.104             | 268.1043[M+H]+    | 1.11      | Adenosine    |
| 3*  | 2.073   | C9H8O4            | 181.0492            | 181.0491[M+H]+    | 0.55      | Caffeic acid |
| 4   | 2.327   | C25H31N011        | 522.197             | 522.1963[M+H]+    | -1.34     | Lonijaposide E |
| 5   | 2.824   | C27H33N013        | 580.2015            | 580.2010[M+H]+    | -0.86     | Lonijaposide H |
| 6*  | 2.946   | C16H18O9          | 377.0843            | 377.0838[M+Na]+   | -1.32     | Chlorogenic acid |
| 7   | 2.947   | C16H18O9          | 353.0878            | 353.0874[M+H]+    | -1.13     | Neochlorogenic acid |
| 8   | 2.957   | C17H26O11         | 429.1363            | 429.1359[M+Na]+   | -0.93     | Morroniside |
| 9   | 3.112   | C16H18O9          | 377.0843            | 377.0838[M+Na]+   | -1.32     | Cryptochlorogenic acid |
| 10  | 3.12    | C16H24O10         | 399.1262            | 399.1260[M+Na]+   | -0.5      | Loganic acid |
| 11  | 3.123   | C28H35N013        | 594.2181            | 594.2176[M+H]+    | -0.84     | Lonijaposide T/U/V |
| 12  | 3.433   | C17H24O10         | 433.1348            | 433.1340[M+COOH]- | -1.85     | 7-Epi-vogeloside |
| 13  | 3.498   | C16H22O9          | 381.1156            | 381.1153[M+Na]+   | -0.79     | Sweroside |
| 14  | 3.831   | C17H26O10         | 435.1508            | 435.1493[M+COOH]- | -3.44     | Loganic acid methyl ester |
| 15  | 3.885   | C17H24O10         | 389.1442            | 389.1440[M+H]+    | -0.51     | Vogeloside |
| 16  | 3.896   | C17H24O10         | 411.1262            | 411.1258[M+Na]+   | -0.97     | Secologanin |
| 17  | 3.897   | C17H24O10         | 433.1348            | 433.1347[M+COOH]- | 0.23      | 7-Aldosecologanin |
| 18  | 3.929   | C17H24O11         | 427.1211            | 427.1208[M+Na]+   | -0.7      | Secoxyloganin/kingiside |
| 19  | 3.93    | C16H22O9          | 403.1245            | 403.1239[M+COOH]- | -1.49     | Isosweroside |
| 20  | 3.996   | C26H35N011        | 538.2279            | 538.2274[M+H]+    | -0.93     | L-phenylalaninosecologanin |
| 21  | 3.996   | C25H24O12         | 515.1192            | 515.1191[M+H]-    | -0.19     | 3,4-Di-o-caffeoyl quinic acid |
| 22* | 4.493   | C27H30O16         | 633.1466            | 633.1457[M+Na]+   | -1.42     | Rutin acid |
| 23  | 4.516   | C25H24O12         | 515.1192            | 515.1186[M+H]-    | -1.16     | 3,5-Dicaffeoylquinic acid |
| 24  | 4.769   | C34H46O19         | 781.2526            | 781.2518[M+Na]+   | -1.02     | Z-Aldosecologanin |
| 25  | 4.77    | C34H46O19         | 803.2605            | 803.2609[M+COOH]- | 0.49      | E-Aldosecologanin |
| 26  | 4.935   | C19H30O11         | 457.1675            | 457.1675[M+Na]+   | -1.09     | Secologanin dimethylacetal |
| 27  | 6.194   | C16H24O8          | 367.1363            | 367.1366[M+Na]+   | 0.82      | Sweroside |
| 28  | 9.409   | C20H22O9          | 429.1186            | 429.1179[M+Na]+   | -1.63     | Benzyl 2-o-β-D-Glucopyranosyl |
| No. | small molecule compound | target protein | KEGG pathway                        | No. | small molecule compound | target protein | KEGG pathway                        |
|-----|-------------------------|----------------|-------------------------------------|-----|-------------------------|----------------|-------------------------------------|
| 1   | Inositol               | PTEN           | Phosphatidylinositol signaling system | 7   | Rutinin acid            | MTOR           | Alanine, aspartate and glutamate metabolism |
| 2   | Inositol               | MTOR           | Chlorogenic acid                     | 8   | Linalool                | F2             | Neuroactiveligand-receptor interaction |
| 3   | Inositol               | MTOR           | Insulin signaling pathway            | 9   | Caffeic acid            | F2             | Neuroactiveligand-receptor interaction |
| 4   | Inositol               | MTOR           | Alanine, aspartate and glutamate metabolism | 10  | Caffeic acid            | TH             | Tyrosine metabolism                  |
| 5   | Rutinin acid           | MTOR           | Chlorogenic acid                     | 11  | Linalool                | GPT            | Alanine, aspartate and glutamate metabolism |
| 6   | Rutinin acid           | MTOR           | Insulin signaling pathway            | 12  | Caffeic acid            | GPT            | Alanine, aspartate and glutamate metabolism |

Table 2 Predicted path of honeysuckle intervention in pneumonia mechanisms (sorted by target protein Degree)

Note: * is a compound compared with the standard

Table 3 Pathway analysis of honeysuckle in the treatment of pneumonia
| Material metabolism | Cell function | Messenger signal |
|---------------------|---------------|-----------------|
| Path name(enriched gene number) | Brief description of pathway function | Path name(enriched gene number) | Brief description of pathway function | Path name(enriched gene number) | Brief description of pathway function |
| Tryptophan metabolism | Amino acid metabolism | Apoptosis(11) | Physiological processes of homeostasis maintain | insulin signaling pathway(17) | Insulin and glucose metabolism |
| Starch and sucrose metabolism | Glucose metabolism | Ribosome(11) | Processing of genetic information | phosphatidylinositol signaling system(12) | Processing of environmental information |
| Vitamin B6 metabolism | Vitamin metabolism | Vascular smooth muscle contraction(16) | Vascular smooth muscle contraction | calcium signaling pathway(27) | Calcium ion transmembrane transport |
| Cysteine and methionine metabolism | Amino acid metabolism | | | neuroactive ligand-receptor interaction(35) | Processing of environmental information |
| Phenylalanine, tyrosine and tryptophan biosynthesis | Amino acid metabolism | | | | |
| tyrosine metabolism | Amino acid metabolism | | | | |
| Alanine, aspartate and glutamate metabolism | Amino acid metabolism | | | | |
| phenylalanine metabolism | Amino acid metabolism | | | | |
| nitrogen metabolism | Nitrogen metabolism | | | | |
| glycine, serine and threonine metabolism | Amino acid metabolism | | | | |

Table 4 Respiratory symptoms associated with honeysuckle and Inositol
| TCM symptom id | Symptom pinyin name | MM symptom name           | MM symptom id |
|----------------|---------------------|---------------------------|---------------|
| SMTS00234      | Fa Re               | Chills And Fever          | SMMS00867     |
|                |                     | Fever                     | SMMS00952     |
| SMTS00306      | Gan Mao             | Catarrh                   | SMMS00095     |
|                |                     | Coryza                    | SMMS00626     |
|                |                     | Common Cold               | SMMS00817     |
| SMTS00265      | Feng Re Gan Mao     | Catarrh                   | SMMS00095     |
|                |                     | Coryza                    | SMMS00626     |
|                |                     | Common Cold               | SMMS00817     |
| SMTS00400      | Yan Hou Zhong Tong  | Pharyngolaryngeal Pain    | SMMS00841     |
| SMTS00577      | Kou Ke              | Thirst                    | SMMS00556     |
| SMTS00562      | Ke                  | Thirst                    | SMMS00556     |
|                |                     | Thermal Energy            | SMMS00574     |
|                |                     | Hot Temperature           | SMMS00941     |

**Figures**

**Figure 1**

Flow chart of main research methods
Figure 2

Animal lung tissue pathology Note: A: normal group; B: model group; C: HD group; D: DXMS group. 1:4X light microscope; 2:20X light microscope.

Figure 3

TIC diagram of the honeysuckle sample in UV and positive ion mode
Figure 9

“Component-target-path” network diagram of honeysuckle and dexamethasone Note: The bottom left picture is dexamethasone, and the upper right picture is honeysuckle.
Figure 11

Possible mechanisms of honeysuckle intervention in pneumonia

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