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 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), FOS (66), 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 TNFα, 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-1β and TNF-α 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-κB 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 [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 [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 [29]. The proliferation and differentiation of cells is an important process of inflammation absorption and tissue regeneration in vivo. Studies confirmed [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

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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	C25H31NO11	522.197	522.1963[M+H]+	-1.34	Lonijaposide E
5	2.824	C27H33NO13	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	C28H35NO13	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-Alpha-methoxvsweroside
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	C26H35NO11	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	Rutinin 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	457.168	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

29	20.646	C10H18O	177.1273	177.1277[M+Na]+	2.26	Linalool
30	26.413	C18H36O2	283.2645	283.2644[M□H]-	-0.35	Trans-linalool oxide
31	29.164	C35H60O6	577.4453	577.4451[M+H]+	-0.35	β-Sitosterol-β-D-glucoside

Note: * is a compound compared with the standard

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

No.	small molecule compound	target ptotein	KEGG pathway	No	small molecule compound	target ptotein	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 imeraction
3	Inositol	MTOR	Insulin signaling pathway	9	Caffeic acid	F2	Neuroactiveligand-receptor imeraction
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 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□10□	Amino acid metabolism	Apoptosis(11)	Physiological processes of homeostasis maintain	insulin signaling pathway(17)	Insulin and glucose metabolism
Starch and sucrose metabolism□8□	Glucose metabolism	Ribosome(11)	Processing of genetic information	phosphatidylinositol signaling system(12)	Processing of environmental information
Vitamin B6 metabolism□4□	Vitamin metabolism	Vascular smooth muscle contraction(16)	Vascular smooth muscle contraction	calcium signaling pathway(27)	Calcium ion transmembrane transportion
Cysteine and methionine metabolism□8□	Amino acid metabolism			neuroactive ligand-receptor interaction(35)	Processing of environmental information
Phenylalanine, tyrosine and tryptophan biosynthesis(4)	Amino acid metabolism				
tyrosine metabolism(10)	Amino acid metabolism				
Alanine, aspartate and glutamate metabolism(9)	Amino acid metabolism				
phenylalanine metabolism(9)	Amino acid metabolism				
nitrogen metabolism(14)	Nitrogen metabolism				
glycine, serine and threonine metabolism(16)	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

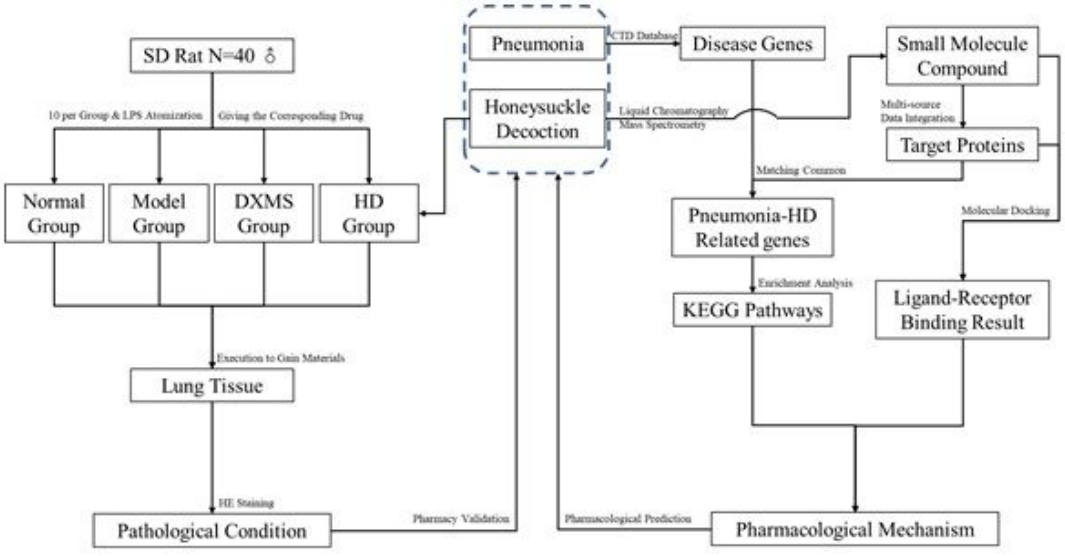

Figure 1

Flow chart of main research methods

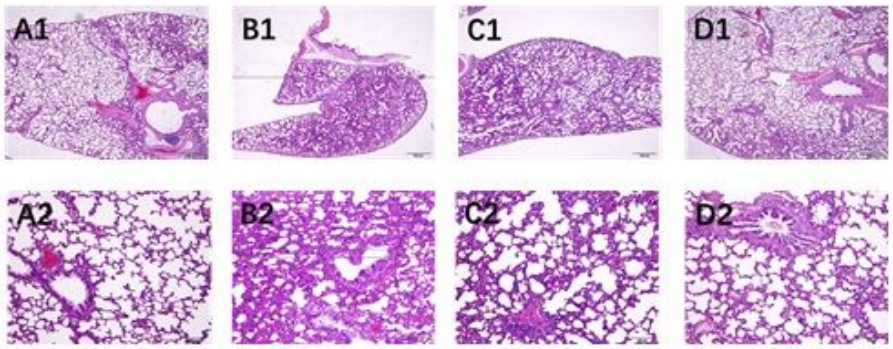

Figure 2

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.

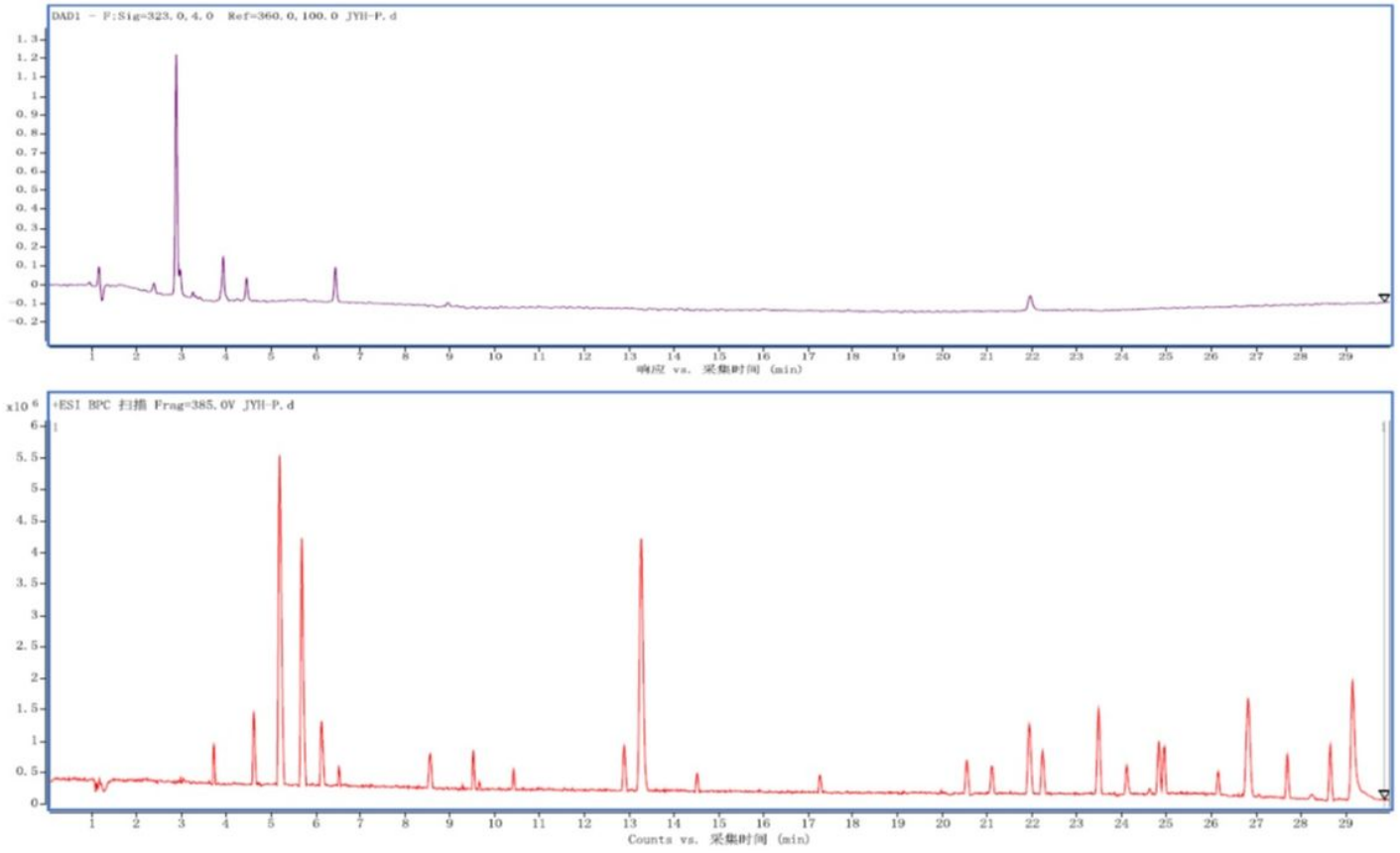

Figure 3

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

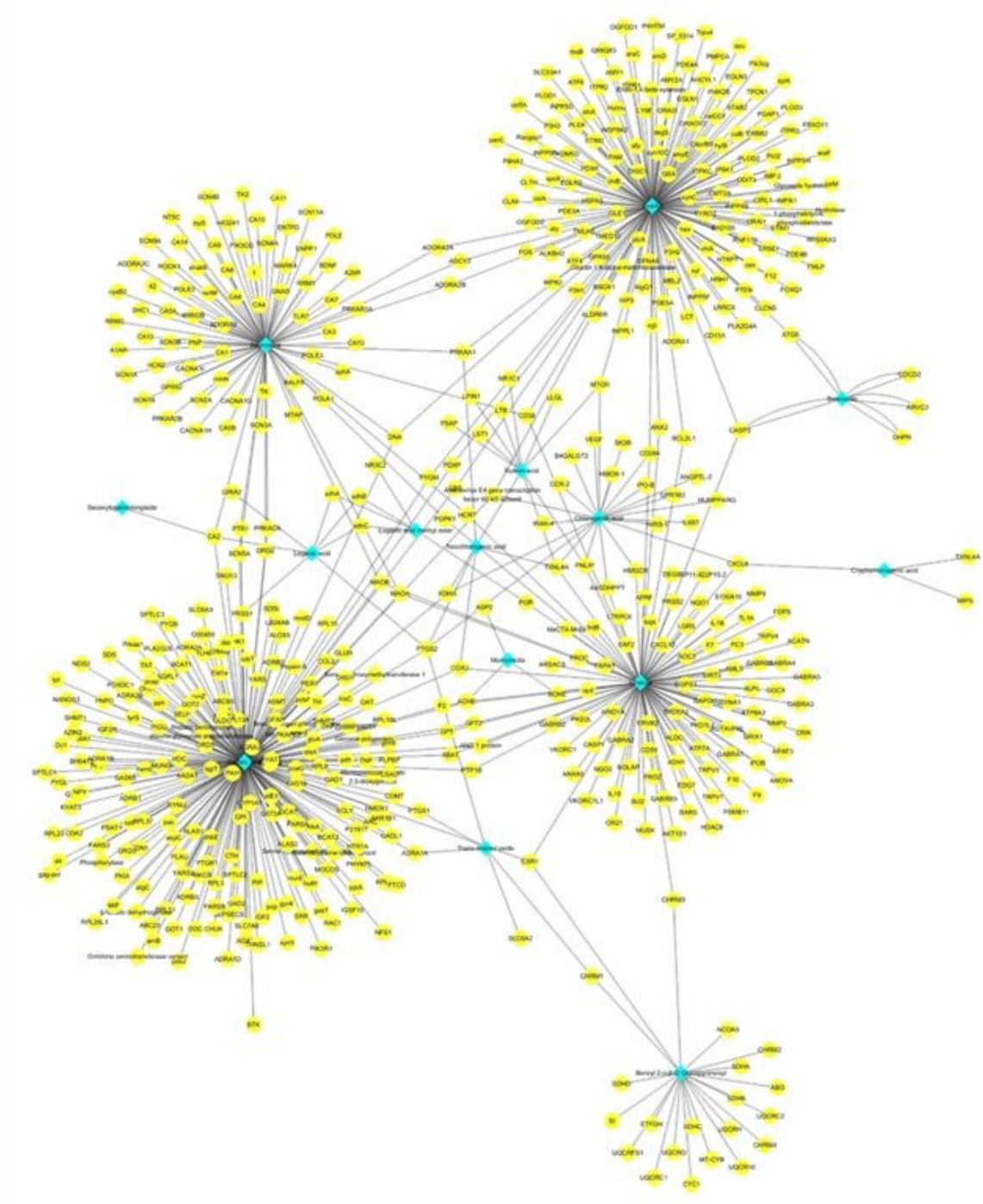

Figure 4

Honeysuckle “component-target” network Note: Blue diamond is chemical, yellow round is target protein

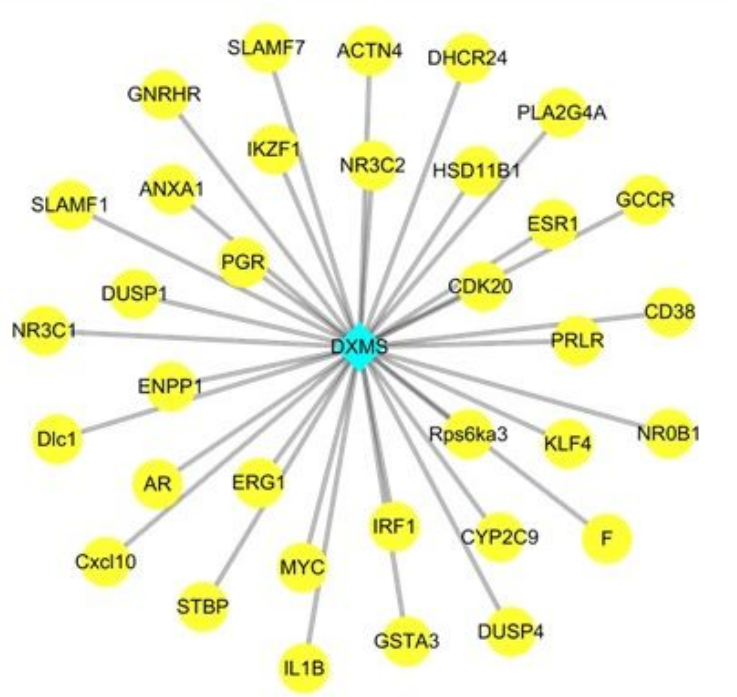

Figure 5

Dexamethasone "Component-Target" Network Note: Blue diamond is chemical, yellow round is the target protein

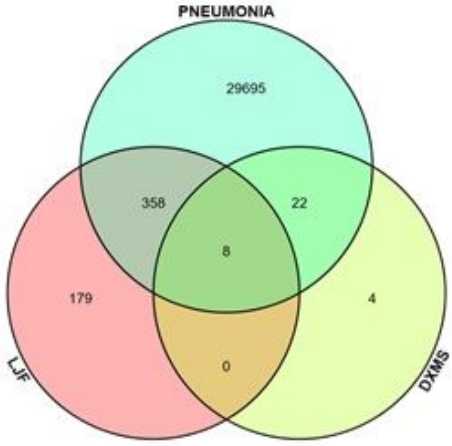

Figure 6

Matching of pneumonia with honeysuckle/dexamethasone target

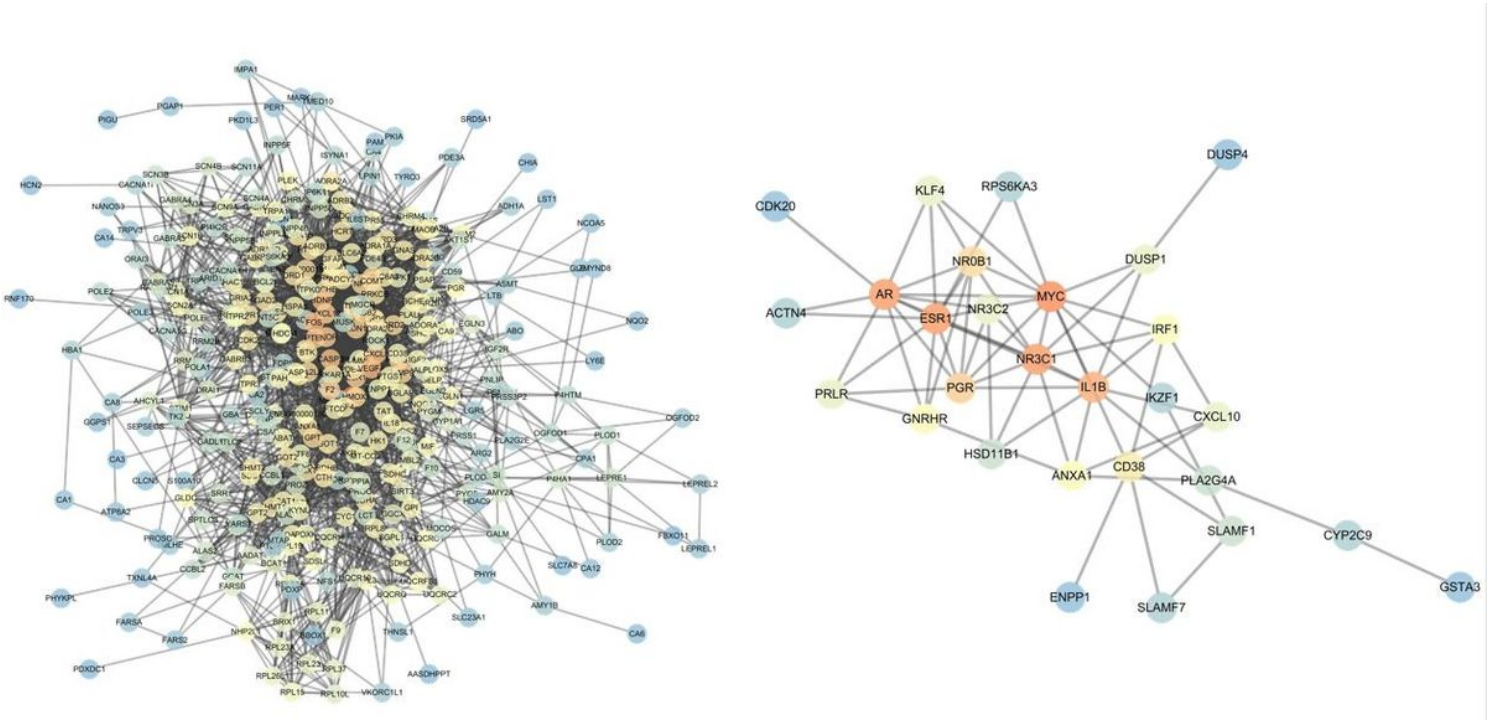

Figure 7

Honeysuckle / dexamethasone for the treatment of potential targets of pneumonia Note: The left picture is honeysuckle and the right picture is dexamethasone.

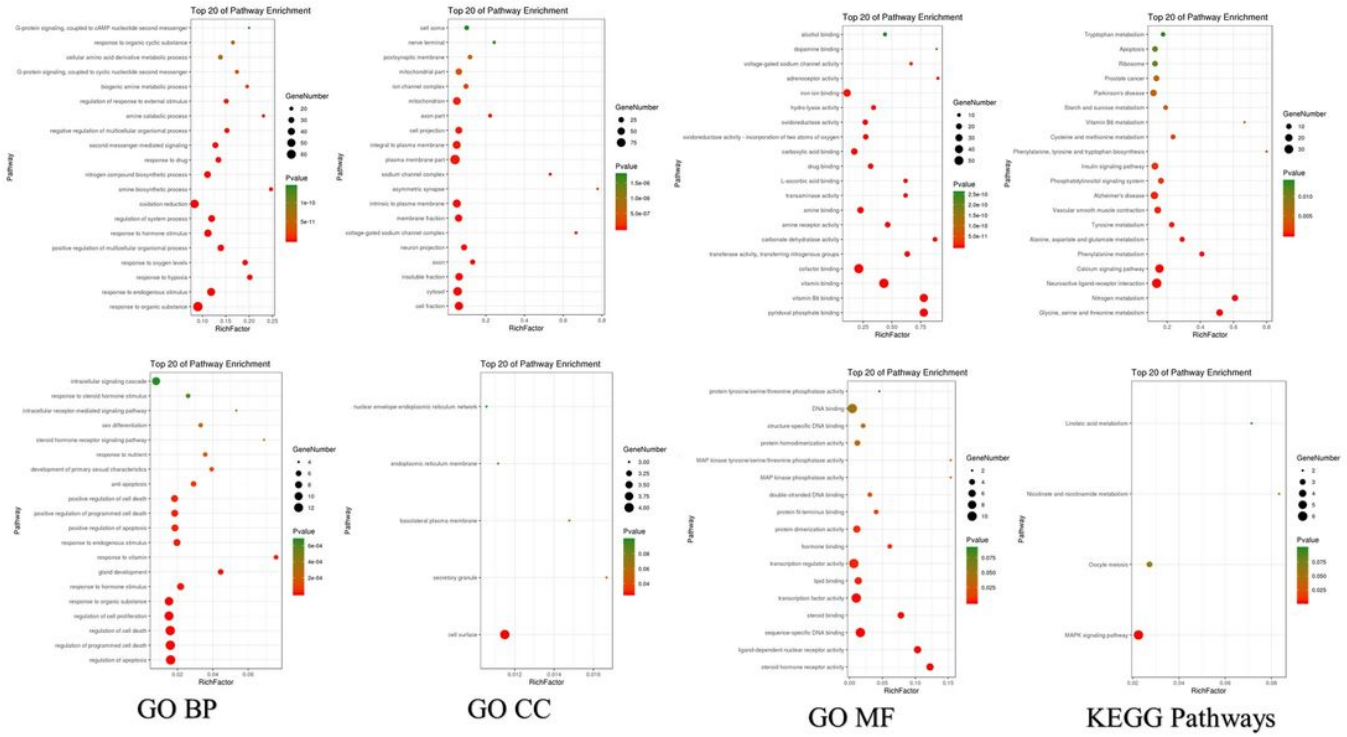

Figure 8

Results of enrichment analysis of honeysuckle/dexamethasone in the treatment of pneumonia Note: The top four pictures are honeysuckle, and the next four pictures are dexamethasone.

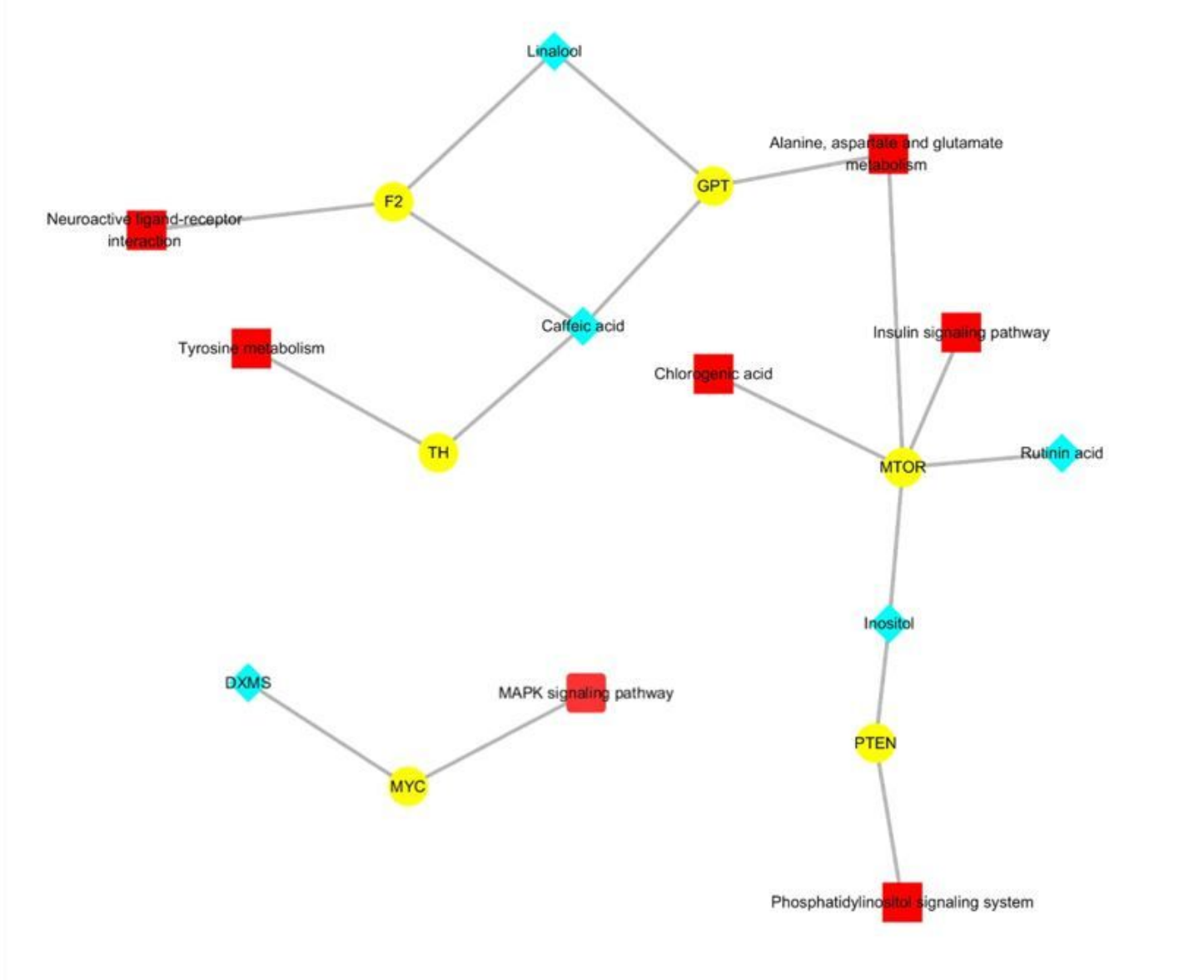

Figure 9

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

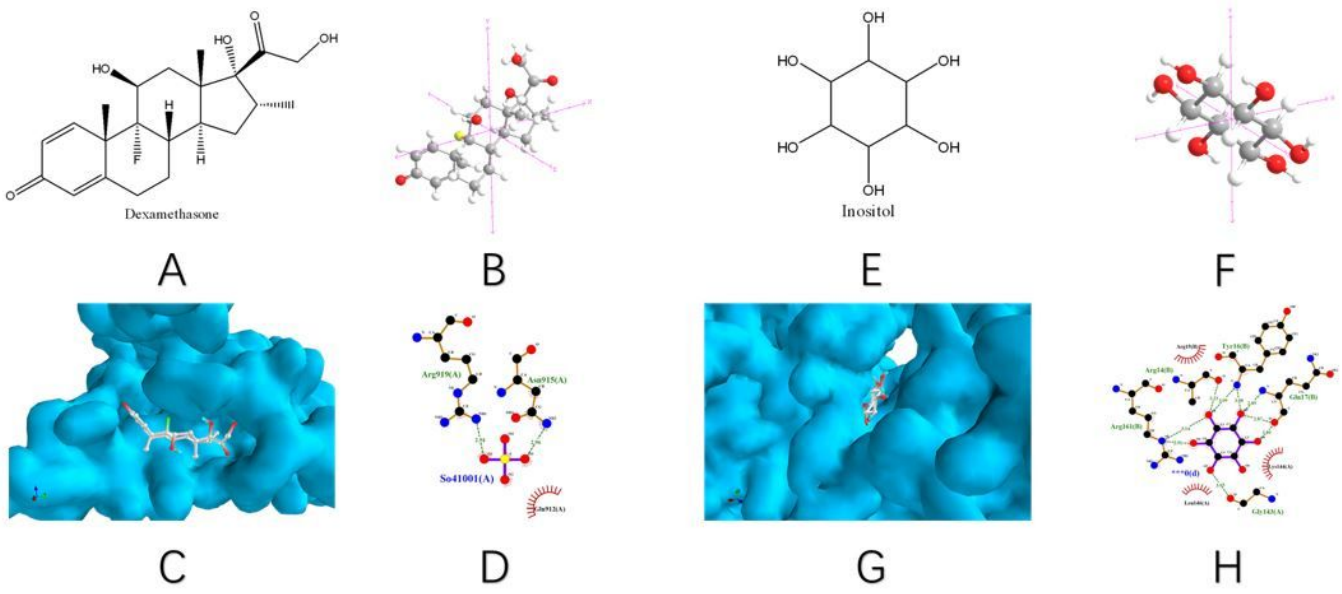

Figure 10

Molecular structure and docking results Note: A. 2D molecular structure of dexamethasone; B. 3D molecular structure of dexamethasone; C. docking result of dexamethasone-MYC; D. two-dimensional structure of dexamethasone-MYC docking result; E. Inositol 2D molecular structure; B. Inositol 3D molecular structure; C. Inositol-PTEN docking results; D. Inositol-PTEN docking results two-dimensional structure;

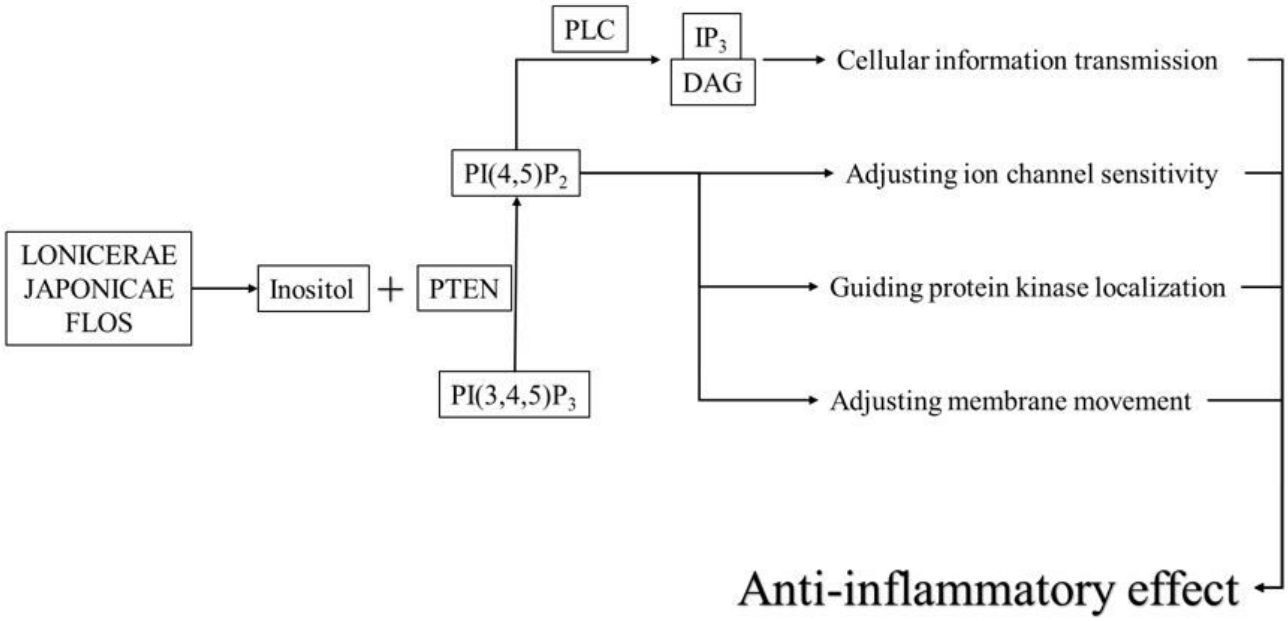

Figure 11

Possible mechanisms of honeysuckle intervention in pneumonia

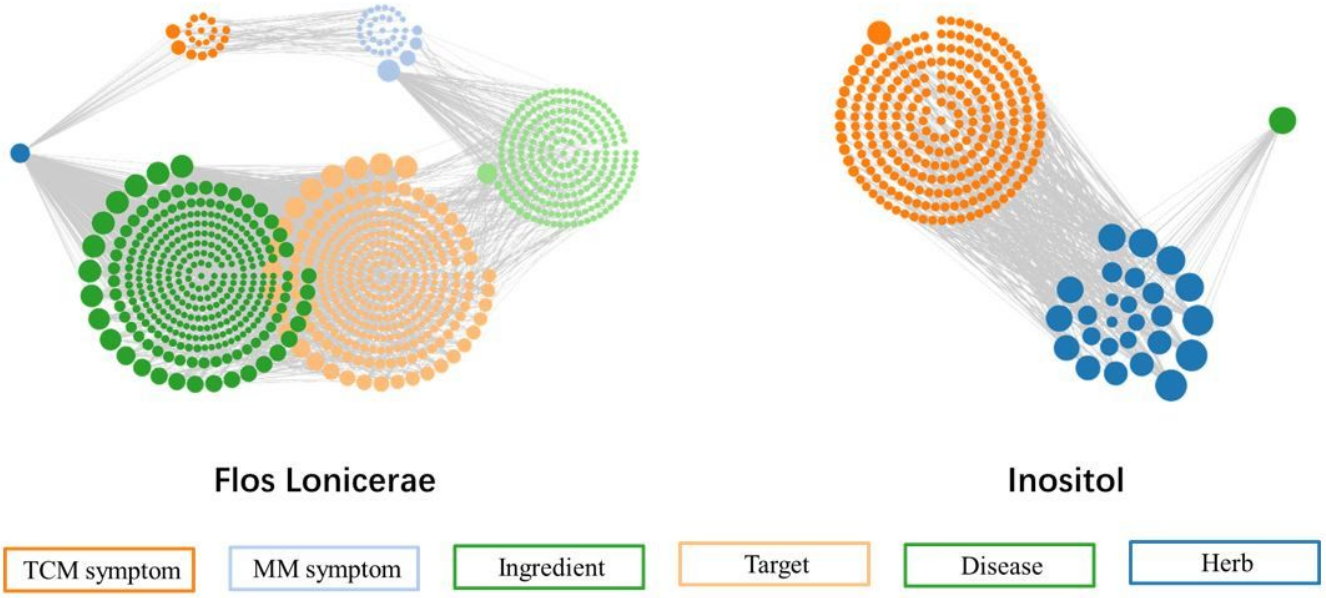

Figure 12

Related networks of honeysuckle and Inositol in SymMap database

Supplementary Files

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- supplementaryfiles.docx