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Research paper

Network pharmacology-based study on the mechanism of "Jiu Wei Zhu Huang San" in respiratory tract infections treatment

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ABSTRACT

Introduction: "Jiu Wei Zhu Huang San" (JWZHS) is a Chinese patent drug that is effective against respiratory tract infections (RTIs). However, its underlying mechanisms are not clear. The objective of the present study was to identify the potential compounds of JWZHS and their therapeutic targets in the context of RTIs; in addition, we investigated the molecular mechanisms by adopting network pharmacology and molecular docking simulation approaches.

Methods: In the present study, the network pharmacology approach mainly comprised of identification of the compounds and their predicted therapeutic targets, network construction, protein-protein interaction (PPI) analysis, gene ontology, and pathway enrichment analysis. In addition, molecular docking simulation was adopted to assess the binding potential of selected target-compound pairs.

Results: The results identified eight potential therapeutic targets of JWZHS in upper respiratory tract infection (URTI) and pneumonia (TNF, IL6, IL1B, IL2, JUN, MAPK1, MAPK8 and EGFR). By clustering the PPI data of compound/disease targets, 2 modules were obtained. Molecular docking simulation revealed good binding affinity of the eight putative targets with the corresponding compounds. Enrichment analysis indicated that the therapeutic effect of JWZHS against URTI and pneumonia was possibly mediated via synergistic regulation of several biological pathways, such as signal transduction, immune system, cell growth, apoptosis, cancer, and other related signaling pathways.

Conclusion: Our results provide a preliminary explanation of the basic pharmacological effects of JWZHS against RTIs and characterize the potential underlying mechanisms. These findings provide a foundation for experimental research and eventual clinical application.

Abbreviations: ADRs, adverse drug reactions; AR, androgen receptor; CASP1, caspase-1; CC, cell component; CCL2, C-C motif chemokine 2; CD14, monocyte differentiation antigen CD14; CLR, cholesterol; CNKI, China National Knowledge Infrastructure Database; CRP, C-reactive protein; CXCL8, interleukin-8; DOCK8, dedicator of cytokinesis protein 8; EGFR, epidermal growth factor receptor; FDR, false discovery rates; BP, biological process; GO, gene ontology; HMOX1, heme oxygenase 1; IL10, interleukin-10; IL1B, interleukin-1 beta; IL2, interleukin-2; IL-4, interleukin-4; IL5, interleukin-5; IL6, interleukin-6; JUN, transcription factor AP-1; JWZHS, Jiu Wei Zhu Huang San; KEGG, kyoto encyclopedia of genes and genomes; LRTI, lower respiratory tract infection; MAPK1, mitogen-activated protein kinase 1; MAPK10, mitogen-activated protein kinase 10; MAPK8, mitogen-activated protein kinase 8; MCODE, Molecular Complex Detection; MF, molecular function; NGAL, neutrophil gelatinase-associated lipocalin; PTGS1, prostaglandin G/H synthase 1; PTGS2, prostaglandin G/H synthase 2; RTIs, respiratory tract infections; SARS, Serine–tRNA ligase, cytoplasmic; TCM, Traditional Chinese Medicine; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRPA1, transient receptor potential cation channel subfamily A member 1; TTD, Therapeutic Target Database; URTI, upper respiratory tract infection

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1. Introduction

Respiratory tract infections (RTIs) are a common reason for pediatric consultations in clinical practice. Indeed, RTIs account for the highest rates of adult and pediatric morbidity and mortality around the world [1]. RTIs are caused by many different bacteria (including mycobacteria) and viruses [2]. Previous studies have shown that host gene expression tests can accurately distinguish between viral and non-viral RTIs, and that these are better than procalcitonin in identifying bacterial/viral co-infection [3,4]. RTIs is classified as upper respiratory tract infection (URTI) and lower respiratory tract infection (LRTI) [5]. Generally, most URTIs in children are caused by viruses [6]. LRTI mainly refers to inflammation of the trachea, bronchi, and lungs [7,8]. In a study by Liu et al., neutrophil gelatinase-associated lipocalin (NGAL) was shown to be a better diagnostic biomarker for LRTI than interleukin-6 (IL-6) [9]. Antibiotics like ribavirin, oseltamivir, cefuroxime sodium and azithromycin are commonly prescribed for treatment of RTIs in clinical settings [10,11]. However, inappropriate and long-term use of antibiotics can have adverse health consequences, such as selection of resistant organisms and adverse drug reactions (ADRs) [12,13]. Therefore, development of more effective and safe treatment alternatives for RTIs is a key imperative.

The theories of Traditional Chinese Medicine (TCM) are based on qi, a vital energy, which is said to flow along channels (referred to as meridians) and helps to maintain the body health. In addition, herbal therapies are believed to work by rebalancing forces known as yin and yang [14]. TCM has been widely used in the treatment and prevention of various diseases, especially in Asian countries such as China, Japan, and South Korea [15]. With the rapid development of bioinformatics and systems biology, network pharmacology has been proposed in the field of TCM [16,17]. Based on the interaction among medicines, active components, target genes and diseases, network pharmacology of TCM facilitates comprehensive investigation of the therapeutic effects of herbs [18–22]. More importantly, it represents a novel strategy for systematic research of the relationship between TCM and diseases.

TCM has long been used for the treatment of URTI and pneumonia [23,24]. JWZHS (composed of Tianzhuhuang, Niuhuang, Honghua, Ligadu, Gancao, Congfu, Tuercao, Tanxiang, and Bangga) is one of the traditional classic prescriptions of Tibetan medicine, which is included in the “Ministry of Health drug standard Tibetan medicine” (Volume 1). The combination of various medicines clears away heat, induces detoxification, reduces phlegm, and relieves cough. JWZHS is a particularly suitable therapeutic option in children because of its clinical efficacy and minimal ADRs. However, the underlying pharmacological mechanisms of JWZHS are not clear.

Although previous studies have demonstrated the efficacy of JWZHS in the treatment of URTI and pneumonia, these studies did not involve investigation of the molecular biology. Therefore, in this study, we adopted network pharmacology method to forecast and analyze the molecular mechanism of JWZHS against URTI and pneumonia. The detailed workflow of the network pharmacology-based study of JWZHS is shown in Fig. 1.

2. Materials and methods

2.1. Identification of active compounds of JWZHS

The active compounds of JWZHS were identified from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform [25] (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php) and the China National Knowledge Infrastructure Database (CNKI, http://www.cnki.net/). After deleting duplicate data and compounds without structural information, a total of 138 candidate compounds were identified.

2.2. Compound targets for JWZHS

In order to identify the targets of the compounds of JWZHS, the PubChem [26] (https://pubchem.ncbi.nlm.nih.gov/) was used to retrieve information pertaining to the structure and biological activity of the compounds. Subsequently, data pertaining to all 138 active compounds were imported into ChEMBL [27] (https://www.ebi.ac.uk/chembl/), an open large-scale biological activity database for prediction of compound targets. Furthermore, the Uniprot [28] database (https://www.uniprot.org/), which contains a large number of protein sequences and detailed annotation information, was used to find the gene name corresponding to the proteins; only human targets were reserved. After discarding duplicate data, 381 predictive targets were retrieved.

2.3. Identification of target proteins related to URTI and pneumonia

The human targets associated with URTI and pneumonia were obtained from two databases. (1) The TTD (Therapeutic Target Database) database [29] (https://db.idrblab.org/ttd/) compiles information on known target diseases, pathway information, and drugs needed to treat diseases. Using "pneumonia", "Upper Respiratory Infections", and "Upper Tract Respiratory Infection" as keywords, five known human targets related to pneumonia were screened and human target genes associated with URTI were not retrieved. (2) The DisGeNet database [30] (http://www.disgenet.org/search) provides information on the genetic basis of human diseases: diseases, genes, and variants. Using "pneumonia", "Upper Respiratory Infections", and "Upper Tract Respiratory Infection" as keywords, 496 known human targets relevant to pneumonia and 28 human targets linked to URTI were acquired.

2.4. Collection of PPI data

The STRING 11.0 database [31] (https://string-db.org/) can be applied to store known or predicted protein interactions. It is straightforward to identify direct or indirect interactions between proteins. What is more, the database defines PPIs with confidence ranges for data scores (low confidence: scores < 0.4; medium confidence: 0.4–0.7; high confidence: > 0.7). Based on these scores, the study reserved PPI data of comprehensive scores > 0.7. In addition, “the 1st shell” and “the 2nd shell” were set to "no more than 50 interactors" in this study.

2.5. Collection of module analysis data

This study adopted the Molecular Complex Detection (MCODE) [32] plug-in in Cytoscape software to analyze the data in the PPI network. The parameters were set to: Degree cutoff = 2; Node score cutoff = 0.2; K-Core = 2; Maximum Depth = 100. Subsequently, the genes contained in the key clustering module were analyzed.

2.6. Network construction

Cytoscape 3.7.0 [33] (https://cytoscape.org/) is an open source bioinformatics analysis software that calculates, visualizes, and analyzes the network using the Network Analyzer tool. Nodes in the network can be used to represent molecules, genes, proteins, and pathways. The edges stand for the interaction between the nodes. “Degree” is regarded as the sum of the number of edges connected to the node.

Five networks were performed as follows: (1) compound-putative target network was built by connecting the herbal compounds and their corresponding targets; (2) PPI network was established by linking the known UTRI and pneumonia common related targets and other human proteins that directly or indirectly interact with them; (3) PPI network of targets for JWZHS against UTRI and pneumonia was constructed by intersecting the two networks of (1) and (2); (4) network of module

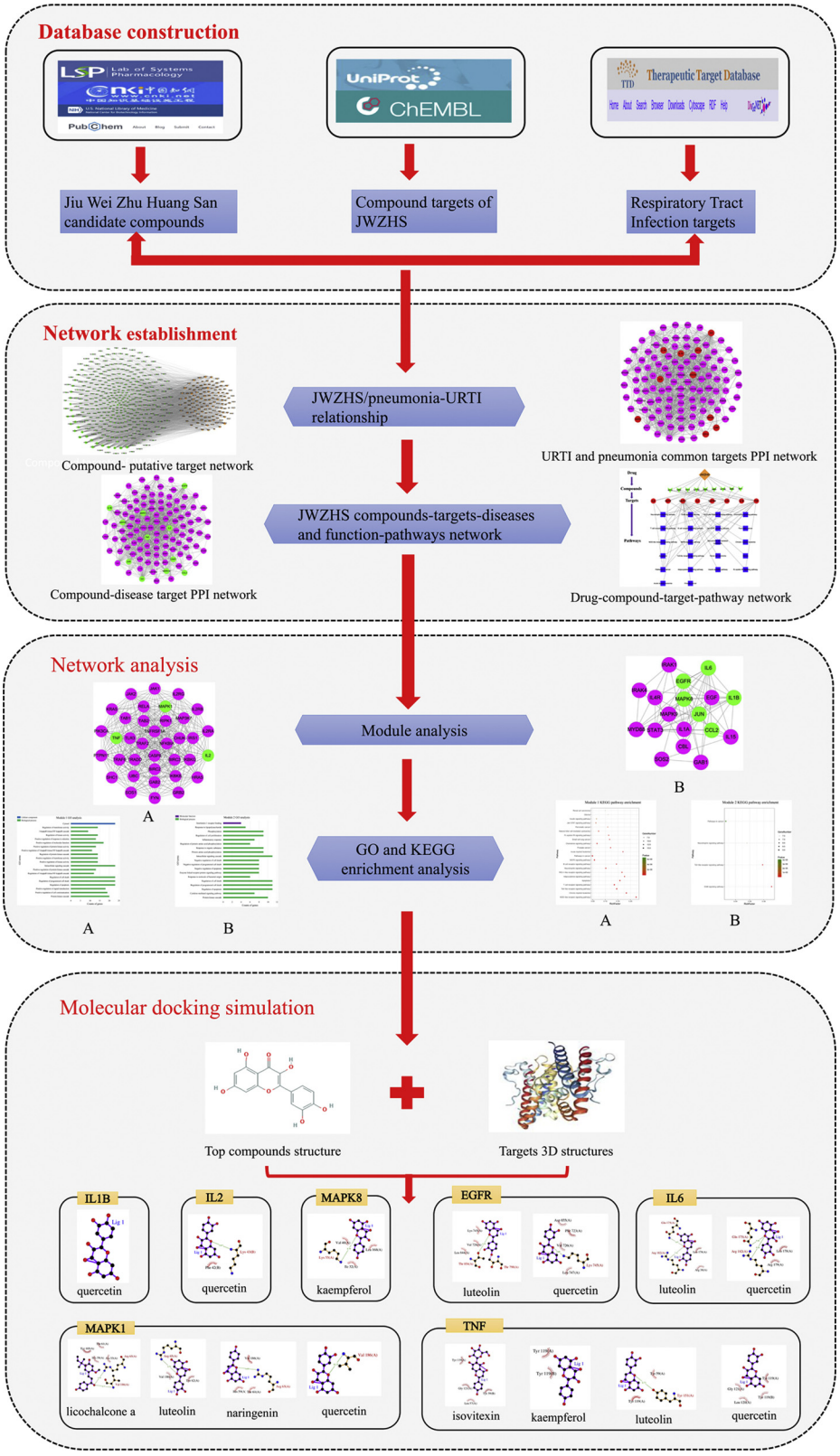


Fig. 1. Schematic illustration of the study methodology and the key results.

analysis by clustering the network of (3); (5) network of drug-compound-target-pathway.

2.7. Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment

The DAVID 6.8 [34] (https://david.ncifcrf.gov/) database integrates multiple categories of database resources, using an improved Fisher's

exact test algorithm for enrichment analysis of gene sets. In the database, the P-*value* was corrected by the false discovery rate (FDR). GO [35] and KEGG [36] pathway enrichment analysis of protein genes in key clustering modules were performed using the DAVID. Furthermore, the criterion for difference screening was *P-value* < 0.01 and FDR < 0.01. In addition, the KEGG advanced bubble chart was built by using the OmicShare platform [37] (http://www.omicshare.com/).

2.8. Molecular docking simulation

SystemsDock [38] (http://systemsdock.unit.oist.jp/iddp/home/index) is a web server based on network pharmacology prediction and analysis; it can perform molecular docking simulation, comprehensively characterize the ligand selectivity, and interpret ligands on complex molecular networks. Besides, the 3D structures of the forecasted target proteins were gathered from the PDB database [39] (http://www.rcsb.org/). Based on this, the SystemsDock was applied to evaluate the binding potential between the key targets and their corresponding compounds.

3. Results

3.1. Collection of JWZHS active compounds

Through a systematic search of the public databases and the literature, a total of 138 compounds met the screening criteria on JWZHS were retrieved. Detailed information pertaining to the 138 active compounds is presented in Table S1. As shown in Table S1, isorhamnetin is a common ingredient of Gancao and Tanxiang; kaempferol and quercetin are common ingredients of Gancao and Honghua; luteolin is a common ingredient of Honghua, Tanxiang and Tuercao; CLR is a common ingredient of Honghua and Niuhuang.

3.2. Compound- putative target network construction

A total of 381 putative targets were predicted for the 138 active compounds of 9 herbs contained in JWZHS. The compound- putative target network consisted of 519 nodes (138 compound nodes, 381 compound target nodes) and 2225 edges (Fig. 2, Table S2). The size of the nodes is directly proportional to the degree of the nodes, and each edge represents the interaction between the compound molecules and the targets. A single target can be regulated by multiple compounds, which may play an important role in mediating the effect of JWZHS against URTI and pneumonia. For instance, PTGS2 can be regulated by 106 compounds, such as luteolin, quercetin, and isorhamnetin. At the same time, a single compound can act on more than one target. For example, luteolin can interact with 118 targets, such as PTGS1, AR, and PTGS2.

3.3. PPI network construction

Eleven targets shared by URTI and pneumonia (SARS, TRPA1, DOCK8, CD14, IL10, CRP, HMOX1, IL1B, IL5, IL6, and CXCL8) were uploaded to the STRING database for protein-protein interactions (Fig. 3), which comprised of 110 nodes and 1074 edges (Table S3). Furthermore, by intersecting the two networks of Figs. 1 and 2, eleven compound/disease targets were obtained, namely, CASP1, CCL2, EGFR, IL1B, IL2, IL6, JUN, MAPK1, MAPK10, MAPK8, and TNF. Subsequently, these compound/disease targets were inputted to the STRING database for protein-protein interactions (Fig. 4). In Fig. 4, there are 111 nodes and 1167 edges (Table S4); 58 nodes with an average degree value ≥21.02703 were selected as major nodes, such as IL6, mitogen-activated protein kinase 1 (MAPK1), MAPK8, and tumor necrosis factor (TNF). It is speculated that the therapeutic effect of JWZHS in children with URTI and pneumonia may be mediated via regulation of these targets.

3.4. Module analysis and enrichment analysis

MCODE is based on complex algorithms that cluster objects with similar properties. The data of compound-disease target PPI network was clustered. As a result, there were 4 modules in one copolymerization and 2 modules with a score of > 5 (Fig. 5, Table S5) were selected. Moreover, GO and KEGG enrichment analysis of the data in these two modules was performed to clarify the integral regulation of JWZHS against URTI and pneumonia.

The GO enrichment analysis of the integrated differential genes was divided into the following three parts: biological process (BP), cell component (CC), and molecular function (MF). The GO enrichment analysis of module 1 resulted in a total of 417 terms. Eighty-three terms qualified the screening criteria ($P < 0.01$ and FDR < 0.01). Among these, 80 terms were related to BP, 2 terms were related to CC, and 1 term was related to MF. GO enrichment analysis of module 2 resulted in a total of 258 terms. Twenty-two terms related to BP and 1 term related to MF qualified the screening criteria ($P < 0.01$ and FDR < 0.01) (Fig. 6, Table S6).

Module 1 contained 22 pathways according to $P < 0.01$ and FDR < 0.01. For example, NOD-like receptor signaling pathway (hsa04621), Toll-like receptor signaling pathway (hsa04620), T cell receptor signaling pathway (hsa04660), Apoptosis (hsa04210), RIG-I-like receptor signaling pathway (hsa04622), and B cell receptor signaling pathway (hsa04662). In Module 2, 4 pathways were selected according to $P < 0.01$ and FDR < 0.01, i.e., ErbB signaling pathway (hsa04012), Toll-like receptor signaling pathway (hsa04620), Neurotrophin signaling pathway (hsa04722), and Pathways in cancer (hsa05200) (Fig. 7, Table S7).

3.5. Drug-compound-target-pathway network construction

Cytoscape software was used to construct a drug-compound-target-pathway network in order to holistically explain the mechanism of action of JWZHS against URTI and pneumonia (Fig. 8). Consequently, there were a total of 40 nodes and 71 edges in the figure (Table S8). As shown in Fig. 8, these pathways closely interacted with 8 key targets. Consequently, 40 nodes and 71 edges are shown in the picture. Besides, the compound with the highest degree value was M86 (quercetin, degree = 8). The target with the highest degree value was MAPK1 (degree = 21). The toll-like receptor signaling pathway (hsa04620, degree = 6) was the pathway with the highest degree value. More importantly, MAPK1 can be regulated by quercetin and is located in the toll-like receptor signaling pathway.

3.6. Molecular docking simulation

Eight of the potential targets were related to 9 of the JWZHS active components; these 8 targets (EGFR, IL1B, IL2, IL6, MAPK1, MAPK8, TNF, and JUN) were imported into the PDB database to determine their 3D structure. However, the complete structural information of JUN was not available. In addition, 9 of the JWZHS active components were inputted into the PubChem to determine their structure (Table 1). Subsequently, these were introduced into the systemsDock for molecular docking simulation. With the exception of MAPK8 and kaempferol, all docking scores were greater than 5, which show better docking activity. Information pertaining to molecular docking is shown in Table 2. The details of the target-compound interactions of the docking simulation are shown in Fig. 9.

4. Discussion

Based on the theory of TCM and Tibetan medicine, JWZHS clears away the body heat, purges phlegm, and relieves cough. In clinic, JWZHS is commonly used to treat RTIs. Thus, clarifying the mechanism of action of JWZHS against URTI and pneumonia is a key imperative. In

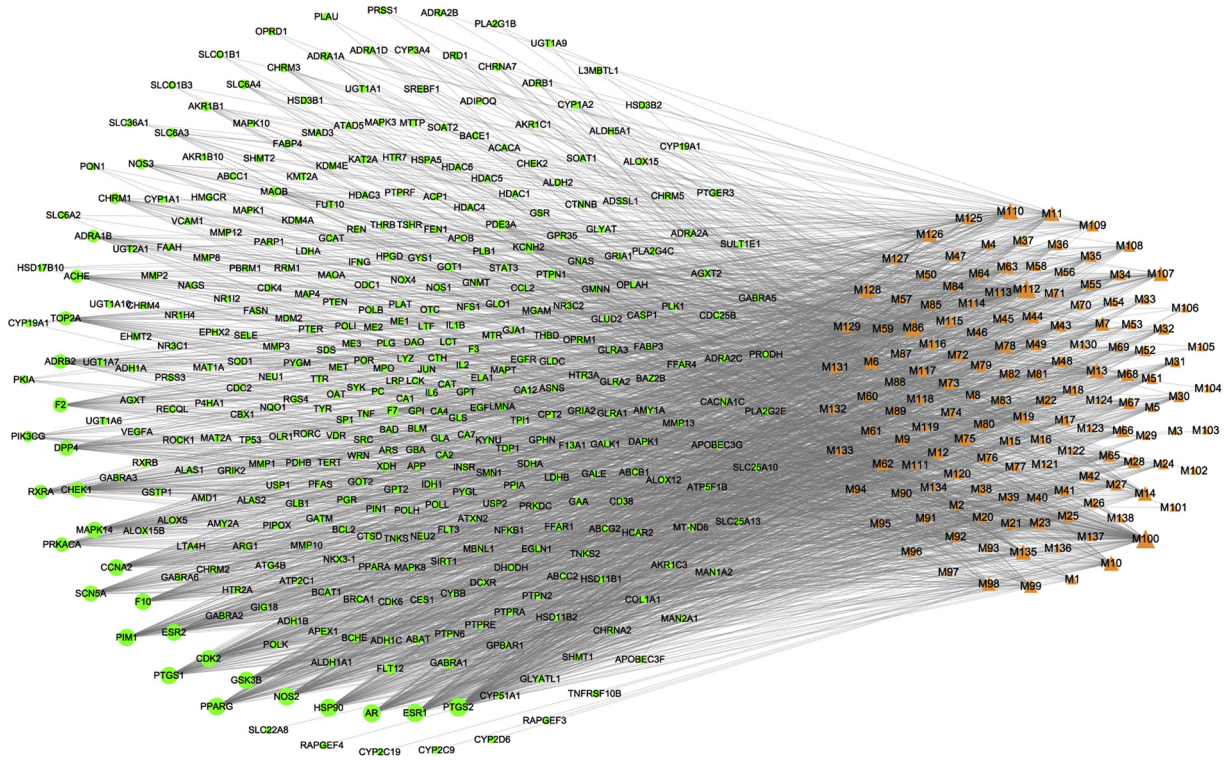


Fig. 2. Compound- putative target network of JWZHS. Green circles represent the targets, and orange triangles represent the compounds. The size of the nodes is directly proportional to the degree of the nodes.

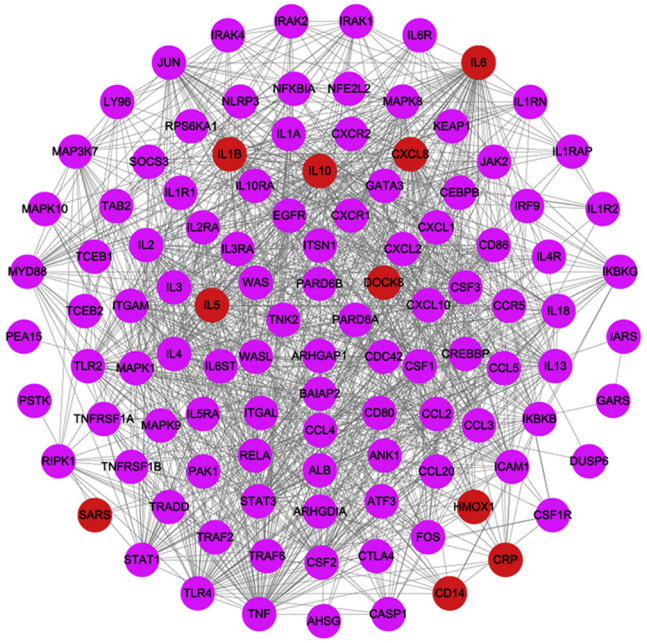


Fig. 3. PPI network with common targets for URTI and pneumonia. Red circles represent common therapeutic targets for URTI and pneumonia. Purple circles represent other human protein targets associated with disease treatment targets.

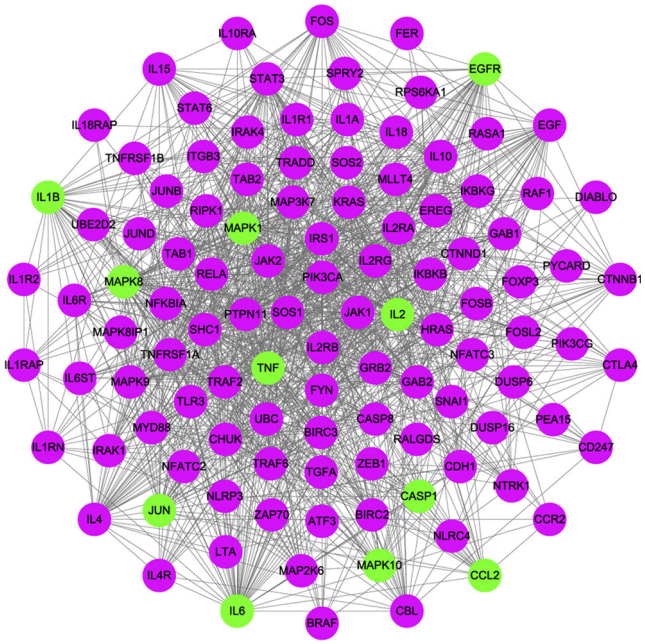


Fig. 4. Compound-disease target PPI network. Green circles represent compound/disease targets and purple circles represent other human protein targets associated with disease treatment targets.

this study, network pharmacology approach was adopted to identify potential compounds, targets, and the pharmacological mechanism of action of complex compounds in JWZHS against URTI and pneumonia. Consequently, the compound-putative target network, PPI network with common targets for URTI and pneumonia, compound-disease target PPI network, and drug-compound-target-pathway network were built to systematically analyze the mechanism of action of JWZHS against RTIs.

This network pharmacology study predicted the following 8 potential targets: MAPK1, TNF, JUN, MAPK8, IL6, EGFR, IL2, and IL1B. These may be the key targets of JWZHS in the treatment of URTI and pneumonia. A recent study has shown a certain association of cytokines with viral respiratory diseases (such as influenza) [40]. Viral infection

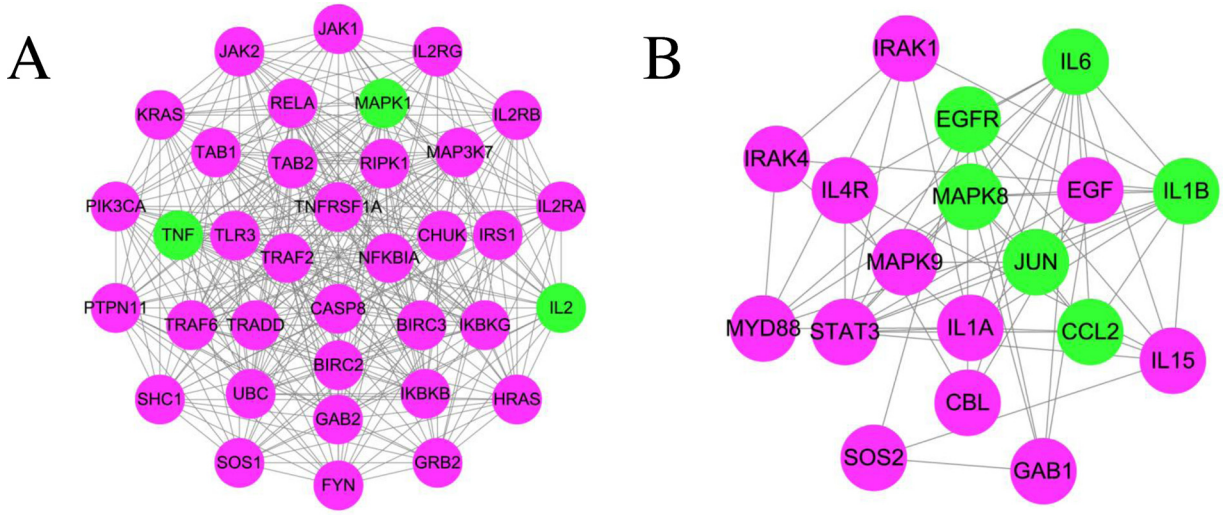


Fig. 5. Clusters of the compound-disease target' PPI network. A: Module 1; B: Module 2; Green circles represent compound/disease targets, and purple circles represent other human protein targets associated with disease treatment targets; Module 1: MCODE score = 18.457; Module 2: MCODE score = 7.412.

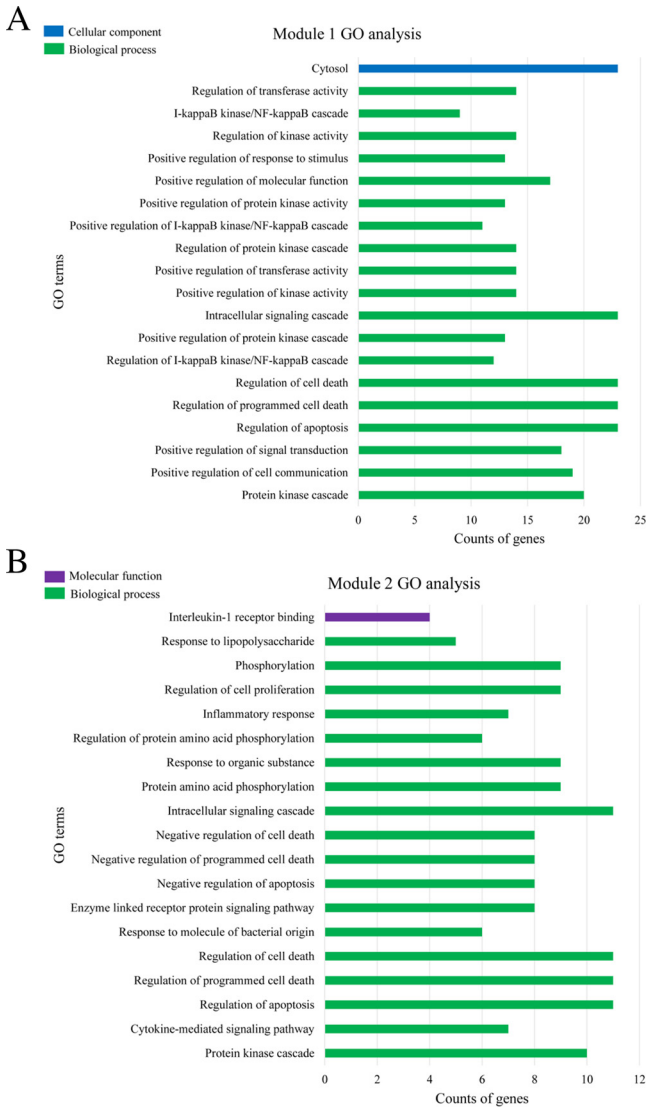


Fig. 6. GO enrichment analysis for each cluster. The top 20 GO terms are shown in the figure; P < 0.01 and FDR < 0.01; A: GO enrichment analysis of Module 1; B: GO enrichment analysis of Module 2.

induces activation of the innate and adaptive immune responses. Excessive or unbalanced immune response may lead to excessive production of cytokines and chemokines, causing inflammation and damage to lung tissues [41,42]. The levels of TNF-α and IL-6 in patients with viral URTI or pneumonia are higher than those in healthy subjects. Additionally, increased levels of IL-6 and TNF-α may be associated with activation of macrophages [43]. TNF-α is a cytokine produced by mononuclear phagocytes and T cells, which is associated with immune regulation, inflammatory response, aggregation of inflammatory cells at the infected site and plays a crucial role in coordinating systemic or local inflammatory response [44]. Besides, Roach et al. performed mouse experiments and found that TNF is critical for the recruitment of early-inducing chemokines and leukocytes to the infected organs, especially macrophages. After activation, TNF-α produces reactive nitrogen intermediates which is essential to clear the infection in mice [45]. IL-6 is a pleiotropic acute proinflammatory cytokine that is related to the host immune response against infection [46,47]. Interleukin-2 (IL-2) is an essential cytokine linked to the normal growth, proliferation and differentiation of T lymphocytes and can down-regulate the immune response [48]. Interleukin-1B (IL1B) is also a potent pro-inflammatory cytokine involved in the activation and proliferation of T and B lymphocytes. Also, both IL1B and IL-6 can induce the synthesis of acute phase proteins and can also increase body temperature during infection [49,50].

Infection of an organism with bacteria or viruses induces activation of the transcription factor AP-1 (JUN), similar to NF-κB; it contains transcriptional regulatory factor binding sites for most inflammatory mediators and mediates inflammation. For instance, release of inflammatory cytokine IL8 can be independently mediated by AP-1 and AP-1 can also regulate angiogenesis induced by pathogen infection [51,52]. AP-1 has also been shown to be associated with cell growth, transformation, and tumorigenesis [53]. More importantly, AP-1 was shown to play a critical role in the treatment of inflammatory diseases (such as atherosclerosis and asthma) caused by chlamydia pneumoniae [54]. MAPK1 and MAPK8 belong to the mitogen-activated protein kinase family and their associated pathway (MAPK pathway) plays an important role in gene transcription, cell growth and apoptosis, cytoskeletal organization and other processes [55]. Previous studies have shown that MAPK family members are also involved in the production of inflammatory mediators [56]. Notably, the MAPK pathway is also associated with the treatment of ischemic heart disease and cancer [57]. Based on the series of results from the database, platform and software, this study preliminarily hypothesized that the therapeutic

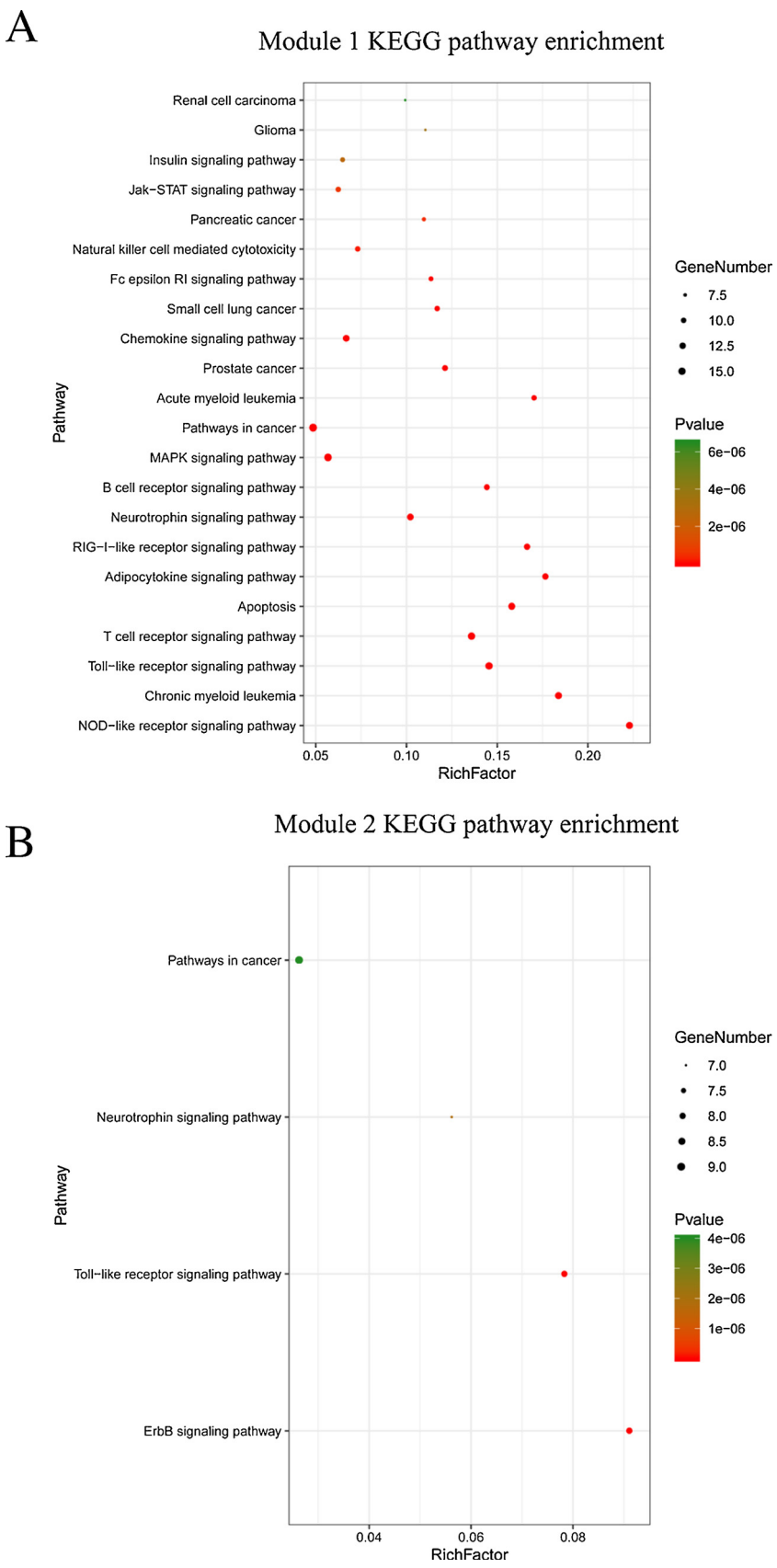


Fig. 7. KEGG pathway enrichment analysis for each cluster. P < 0.01 and FDR < 0.01; A: Pathway analysis of Module 1; B: Pathway analysis of Module 2. The y-axis shows significantly enriched KEGG pathways of Module 1 or Module 2, and x-axis shows the rich factor. Rich factor represents the ratio of the number of target genes belonging to the pathway to the number of all annotated genes located in the pathway. The larger rich factor stands for the higher level of enrichment. The size of the dot denotes the number of target genes in the pathway, and the color shade of the dot indicates the different P-value range.

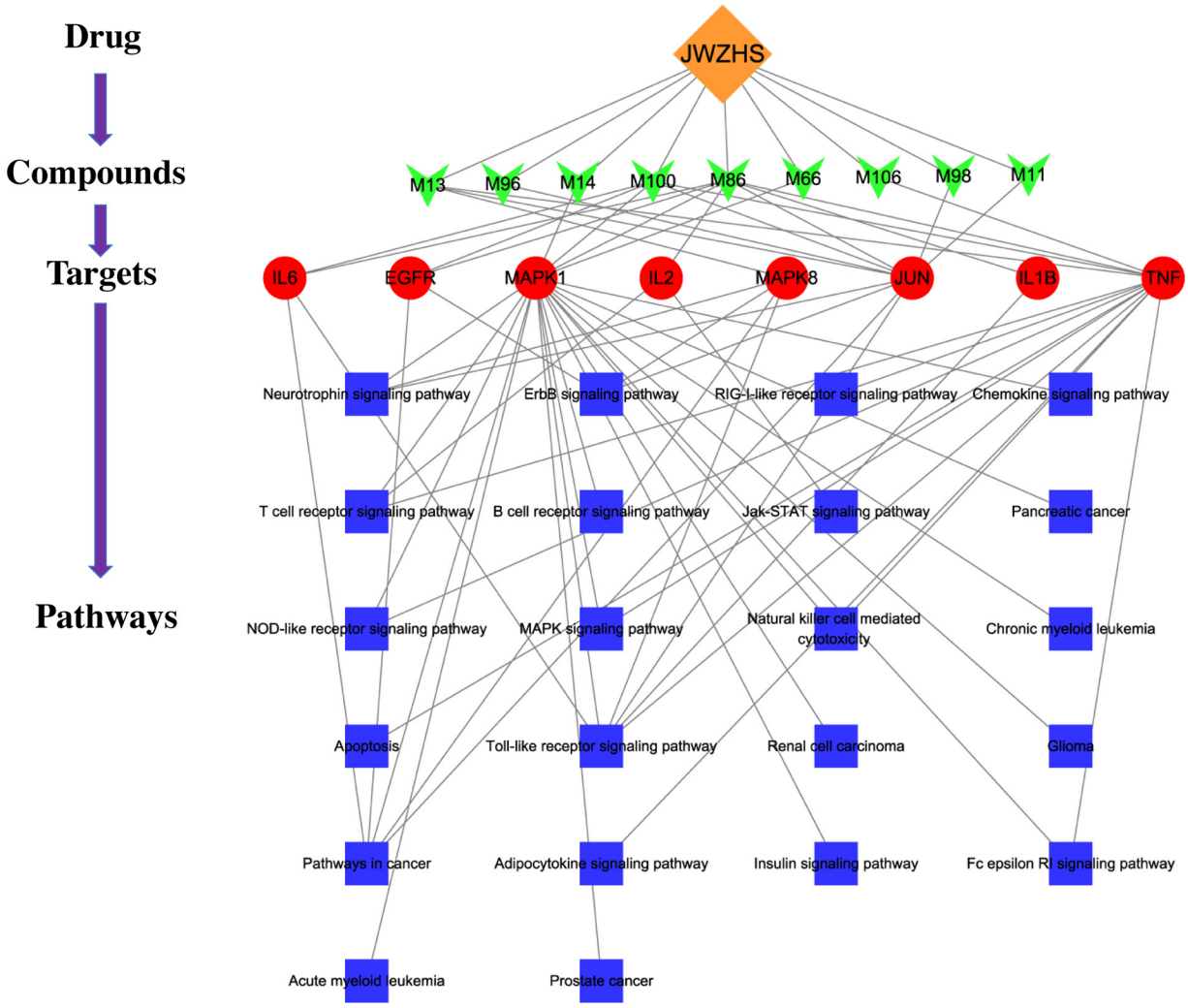


Fig. 8. Drug-compound-target-pathway network. The orange diamond represents the drug JWZHS. Green Vs represent the compounds. Red circles represent compound/disease targets. Blue squares represent the predictive targets KEGG enrichment pathway.

effect of JWZHS against URTI and pneumonia is mediated via regulation of inflammatory cytokines, transcription factor AP-1, and MAPK.

In this study, GO enrichment analysis was performed to statistically analyze the modules. These targets were highly connected with protein kinase cascade, signal transduction, immune response regulation, enzyme activity, cell death and proliferation and inflammatory response. Therefore, the results suggest that JWZHS mainly produces therapeutic effects by participating in these biological processes, cellular components, and molecular functions.

Quercetin, a flavonoid, is a key compound with the highest degree value of JWZHS. It exhibits a wide range of biological actions including immunomodulatory, anti-inflammatory and antiviral activity; in addition, it also shows antioxidant and anti-allergic activity [58–60]. Fortunately, flavonoids have been reported to reduce the incidence and symptoms of URTI through antiviral mechanisms [61]. Furthermore, quercetin also exhibits the characteristics of immune system stimulation, antiviral activity, inhibition of histamine release, reduction of proinflammatory cytokines, leukotrienes, inhibition of IL-4 production; in addition, it also suppresses the level of IL-8, IL-6, and cytosol calcium [62–64]. Crucially, its anti-inflammatory properties have been proven in the treatment of RTIs [59,65]. Luteolin is also one of the key ingredients of JWZHS; it has anti-inflammatory activity and can significantly inhibit the release of pro-inflammatory cytokines (such as IL-6, TNF-α) from macrophages, which may be related to the inhibition of MAK1, ERK1/2, and COX-2 [66]. Moreover, luteolin can alleviate bronchogenic pneumonia by down-regulating miR-132 and attenuating lipopolysaccharide-induced activation of the NF-κB signaling pathway [67]. Moreover, studies have confirmed the relation of NF-κB signaling pathway with influenza pneumonia [68].

By KEGG enrichment analysis, we obtained 23 related items from different modules based on the criteria of $P < 0.01$ and FDR < 0.01. The 23 pathways were mainly related to immune system, signal transduction, and cancer. The pathways directly associated with RTIs were NOD-like receptor signaling pathway (hsa04621), Toll-like receptor (TLR) signaling pathway (hsa04620), MAPK signaling pathway (hsa04010), and RIG-I-like receptor signaling pathway (hsa04622). In addition, TLR signaling pathway was identified as a significant pathway in URTI and pneumonia treatment with the highest number of genes. Studies have shown that viral infection induces activation of the TLR signaling pathway; the intracellular signaling cascades triggered by TLR result in transcriptional upregulation of inflammatory pathways. However, persistent activation of TLR is liable to cause injury to the human body [69,70]. Detection of tissue damage or microbial infection by pattern recognition receptors (PRRs) (such as TLRs or NOD-like receptors) commonly triggers inflammation [71]. Furthermore, multiple bacterial and viral respiratory pathogens can jointly induce apoptosis, TLR, RIG-I-like receptor, and NOD-like receptor signaling pathways [72,73]. Thus, it was speculated that the composition of JWZHS may play a significant role in the treatment of URI through key factors in these signaling pathways. However, the results are only based on

Table 1
Information of active components.

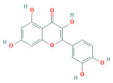
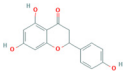
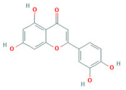
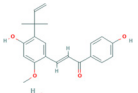
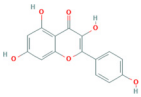
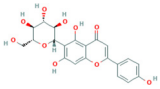
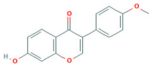
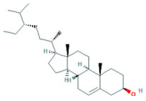
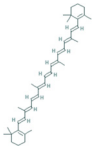
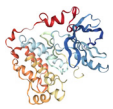
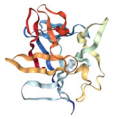
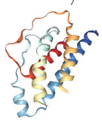
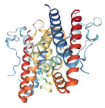
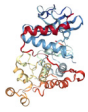
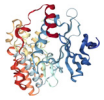
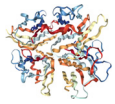
No.	PubChem CID	Compound	Structure
1	5280343	Quercetin	
2	932	Naringenin	
3	5280445	Luteolin	
4	5318998	Licochalcone a	
5	5280863	Kaempferol	
6	162350	Isovitexin	
7	5280378	Formononetin	
8	222284	beta-Sitosterol	
9	5280489	beta-Carotene	

Table 2
Information of molecular docking.

No.	Proteins	PDB ID	Protein structure	Test compounds	Docking Score (pKd/pKi)
1	EGFR	3W2S		quercetin luteolin	6.829 6.013
2	IL1B	2NVH		quercetin	6.097
3	IL2	1M48		quercetin	6.595
4	IL6	1ALU		quercetin luteolin	6.692 6.625
5	MAPK1	3O71		licochalcone a quercetin luteolin naringenin	7.955 6.708 6.686 6.625
6	MAPK8	3PZE		kaempferol	2.376
7	TNF	2AZ5		luteolin kaempferol quercetin isovitexin	6.629 6.588 6.358 6.614

already known chemical ingredients of JWZHS, related targets, and signaling pathways. Thus, further studies are required for more in-depth characterization of the underlying mechanisms.

Some limitations of our study should be considered while interpreting our results. First, as the bioactive ingredients and target proteins were identified based on the existing databases and literature, some information is liable to be incomplete. Secondly, we identified some core compounds, key therapeutic targets, and biological pathways based on network topology parameters analysis; however, these results need to be verified in animal experiments and clinical studies.

5. Conclusion

In the present study, we identified 138 compounds of JWZHS and predicted their 381 putative targets. Our study demonstrates that JWZHS is a complex preparation with multiple components that have multiple targets. The therapeutic efficacy of JWZHS against URTI and pneumonia is likely mediated via regulation of eight targets, which are mainly connected to EGFR, IL1B, IL2, IL6, JUN, MAPK1, MAPK8, and TNF. Besides, molecular docking simulation demonstrated a good affinity of EGFR, IL1B, IL2, IL6, MAPK1 and TNF with the corresponding compounds. GO enrichment analysis showed that the targets of JWZHS against URTI and pneumonia might be closely associated with protein kinase cascade, signal transduction, immune response regulation, enzyme activity, cell death and proliferation, and inflammatory response. Additionally, the KEGG pathway enrichment analysis suggested that JWZHS may simultaneously act on a variety of immune system, signal transduction, and cancer pathways associated with the pathogenesis of URTI and pneumonia, such as NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and MAPK signaling pathway.

In summary, we employed the network pharmacology method to research the complex relationship between multiple components and multiple targets of JWZHS. Our results provide a preliminarily prediction of the mechanisms related to the therapeutic effect of JWZHS against RTIs and provide a good basis and reference for further exploration. Further studies, such as molecular biological experiments and clinical investigations, should be carried out to verify the mechanism of JWZHS against RTIs.

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Authors' contribution

SYG, WZ and JRW conceived and designed the research; XKL and JHT provided useful suggestions on the methodology; SYL, MWN, JYZ and SSJ collected the information and preprocessed the data of research; SYG wrote the paper. YFL and XMZ reviewed and revised the manuscript. All authors read and approved the final version of the manuscript.

Author's statement

Siyu Guo, Siyu Guo and Jiarui Wu conceived and designed the research.

Ziqi Meng and Jinhui Tian provided useful suggestions on the methodology.

Shuyu Liu, Mengwei Ni, Jingyuan Zhang and Shanshan Jia collected the information and preprocessed the data of research.

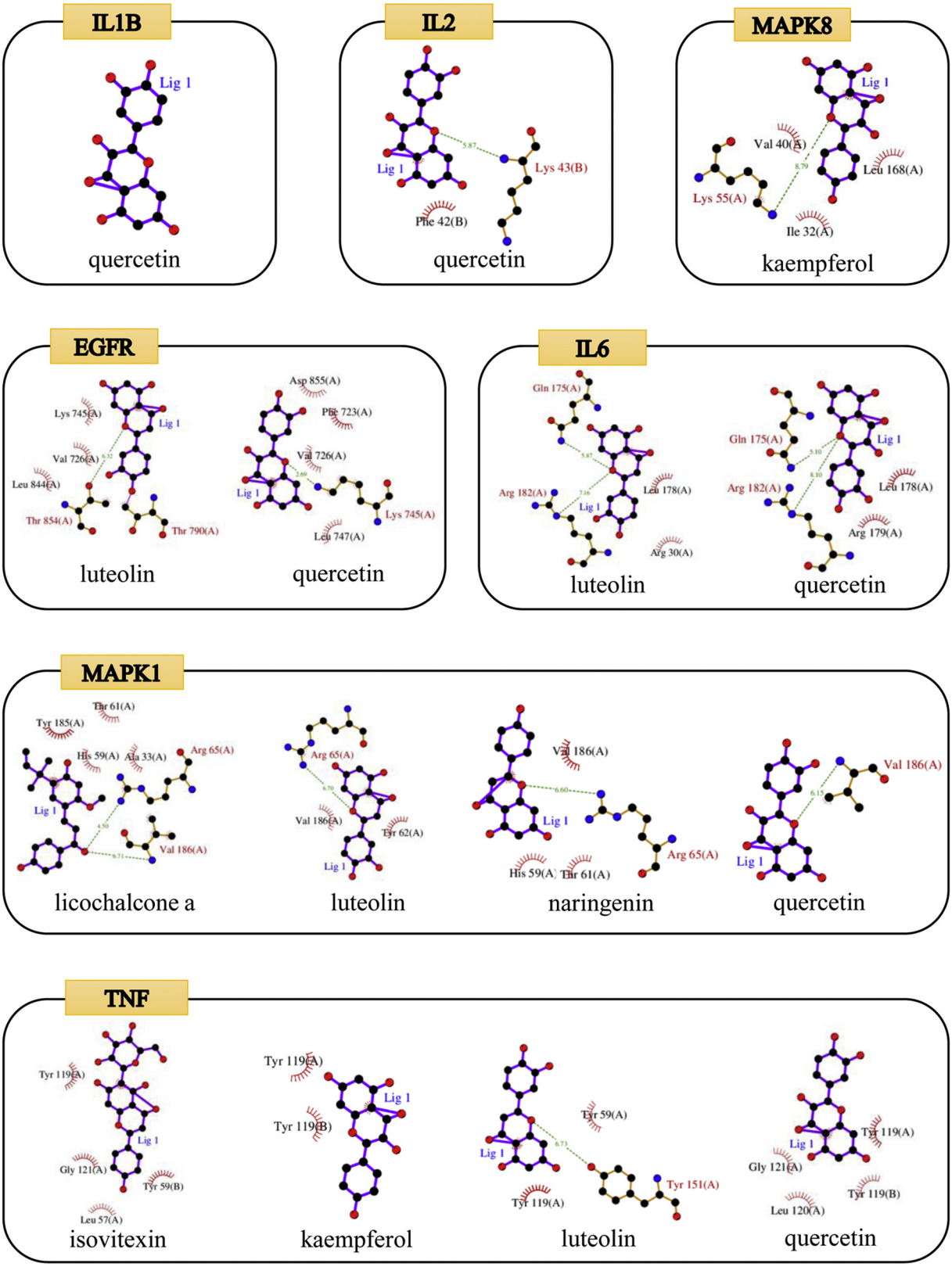

Fig. 9. The detailed target-compound interactions of the docking simulation.

Siyu Guo wrote the paper.

Yingfei Li and Xiaomeng Zhang reviewed and revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflicts of interests regarding the publication of this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eujim.2019.101013.

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