Future Integrative Medicine **2024** vol. 3(3) | 160–171
DOI: 10.14218/FIM.2024.00030

Original Article

The Molecular Mechanism of Shufeng Jiedu Capsules in the Treatment of Influenza: A Comprehensive Analysis Based on Network Pharmacology, Bioinformatics, and Molecular Docking

Mengxia Yang[1,2], Chunming Zhao[1,2], Tengfei Chen[1], Xiaolong Xu[1,3*] and Qingquan Liu[1,3*]

[1]Beijing Hospital of Traditional Chinese Medicine of Capital Medical University, Beijing, China; [2]Graduate School of Beijing University of Chinese Medicine, Beijing, China; [3]Clinical Diagnosis, Treatment and Research Center of Sepsis of Traditional Chinese Medicine, Capital Medical University, Beijing, China

Received: June 13, 2024 | **Revised:** July 10, 2024 | **Accepted:** July 31, 2024 | **Published online:** August 30, 2024

Abstract

Background and objectives: Shufeng Jiedu Capsules (SFJD), a traditional Chinese medicine preparation, are widely used in the clinical treatment of influenza, yet their mechanism of action remains unclear. This study aimed to systematically explore the molecular mechanism of SFJD in the treatment of influenza using network pharmacology and bioinformatics techniques.

Methods: The active ingredients of SFJD were retrieved from traditional Chinese medicine databases, and their targets were identified using the Swiss Target Prediction and TCMSP databases. Influenza disease genes were obtained from the GEO, GeneCards, and DisGeNET databases, and a Venn diagram was used to identify potential targets by mapping SFJD targets to influenza disease genes. Network construction and analysis of potential therapeutic targets were performed using the STRING12.0 database and Cytoscape3.9.1 software, leading to the identification of key targets. The expression of potential therapeutic targets in tissues and cells was retrieved using the BioGPS database. Functional enrichment analysis of these targets was conducted using the DAVID database. Molecular docking was then used to assess the interactions between key targets and core active ingredients.

Results: SFJD contains 193 active ingredients and 985 targets. There are 510 influenza disease genes, 97 of which are potential therapeutic targets for SFJD in treating influenza, with 27 key targets identified through network construction and analysis. Tissue/cell-specific analysis revealed that 39 potential therapeutic targets are highly expressed in 37 specific tissues/cells. Functional enrichment analysis highlighted pathways such as the C-type lectin receptor signaling pathway, tumor necrosis factor signaling pathway, and hypoxia-inducible factor-1 signaling pathway. Molecular docking results indicated strong interactions between the core active ingredients and the key targets.

Conclusions: This study systematically reveals that the mechanism of action of SFJD in treating influenza is complex, involving multiple targets and pathways related to antiviral, anti-inflammatory, and immune regulation effects. The findings provide valuable reference information for future clinical treatment and basic research on influenza.

Keywords: Shufeng Jiedu Capsules; Influenza; Network pharmacology; Bioinformatics; Molecular docking; Mechanism.

***Correspondence to:** Xiaolong Xu and Qingquan Liu, Beijing Hospital of Traditional Chinese Medicine of Capital Medical University, Beijing 100010, China. ORCID: https://orcid.org/0000-0003-3333-0906 (XLX); https://orcid.org/0000-0003-0828-0361 (QQL). Tel: +86-18811554937 (XLX); +86-13910055687 (QQL), E-mails: xiaolong_xu3013@126.com (XLX); liuqingquan2003@126.com (QQL)

How to cite this article: Yang M, Zhao C, Chen T, Xu X, Liu Q. The Molecular Mechanism of Shufeng Jiedu Capsules in the Treatment of Influenza: A Comprehensive Analysis Based on Network Pharmacology, Bioinformatics, and Molecular Docking. *Future Integr Med* 2024;3(3):160–171. doi: 10.14218/FIM.2024.00030.

Introduction

Influenza is an acute viral respiratory infection characterized by high infectivity, spreading between individuals through large droplets in the respiratory tract, and causing serious morbidity and mortality worldwide.[1,2] The clinical symptoms of influenza mainly include sudden fever, chills or sweating, muscle aches, headache, discomfort, dry cough, sore throat, and nasal congestion.[3–5] Additionally, gastrointestinal symptoms such as nausea, vomiting, and diarrhea are also common.[6] During clinical treatment, physicians decide whether to use medication by balancing the potential ben-

efits, harms, and costs of antiviral treatment, along with patient preferences. Currently, four antiviral drugs have been approved for the treatment of influenza: the neuraminidase inhibitors oseltamivir, zanamivir, peramivir, and the cap-dependent endonuclease inhibitor baloxavir.[7] However, due to the high mutation rate of the virus, it can easily develop resistance by evading host immune responses through mutations, leading to limitations in the effectiveness of these drugs.[8] Therefore, the development of new drugs and the search for better treatment methods remain active and challenging areas of research in the field of influenza.

Traditional Chinese medicine does not have records of the disease name "influenza", but based on its clinical symptoms, it can be classified under various categories in traditional Chinese medicine, such as "seasonal colds", "cold pathogenic disease", "colds", "wind-warm syndrome", "exogenous fever" and "epidemics".[9] Throughout history, medical practitioners believed that influenza is caused by the invasion of external pathogenic factors and untimely qi. Among the six exogenous pathogenic factors, the wind pathogen is the most prone to invading the human body, often accompanied by cold or heat pathogens. Therefore, clinically, wind-cold and wind-heat are the most common syndromes.[10] Research has found that Shufeng Jiedu Capsules (SFJD) have shown good efficacy in treating wind-heat syndrome associated with influenza.[11] Additionally, in the 2021 version of the "Clinical Practice Guidelines of Traditional Chinese Medicine for the Treatment of Influenza", SFJD was listed as one of the strongly recommended drugs in the D-level category.[12] However, its mechanism remains unclear. Therefore, this study aimed to explore the mechanism of SFJD in the treatment of influenza through network pharmacology and bioinformatics, in order to provide further reference for clinical use.

Materials and methods

Prediction of potential targets of SFJD in the treatment of influenza

Acquisition of active ingredients of SFJD

The active ingredients of SFJD were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwsuaf.edu.cn/tcmsp.php). The screening criteria were set as follows: oral bioavailability ≥30% and drug-likeness index ≥0.18. If the active ingredients of the herbs were not found in the TCMSP database, additional searches were conducted in the Traditional Chinese Medicine Information Database (TCMID, http://www.megabionet.org/tcmid/) and the Traditional Chinese Medicine (TCM) Database@Taiwan (http://tcm.cmu.edu.tw/). The active ingredients retrieved from these sources were then queried in TCMSP to obtain their oral bioavailability and drug-likeness values.

Screening of targets of SFJD active ingredients

The active ingredients of SFJD were input into the PubChem database to obtain typical Canonical SMILES structures. These structures were then imported into the Swiss Target Prediction database for target screening. Targets with a probability greater than 0 were retained. Finally, a network diagram of Chinese medicine's active ingredients and their targets was constructed.

Establishment of influenza disease gene library

In the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), we used "influenza" and "normal" as search terms, selected "series" as the entry type, and "Homo sapiens" as the species. We filtered out the dataset GSE68310 (provided by the GPL10558 platform) and employed the online tool GEO2R to compare blood samples from influenza patients with those from normal individuals to identify differentially expressed genes (DEGs).

We also searched for influenza-related genes using the GeneCards and DisGeNET databases. The disease genes retrieved from both databases were intersected and then merged with the DEGs from the GEO analysis. After removing duplicates, this process established the influenza disease gene library.

Venn mapping

We utilized the online tool Venny2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) to map the targets of SFJD against the influenza disease genes, screening for potential therapeutic targets for the treatment of influenza with SFJD.

Construction of potential target network

We imported the potential therapeutic targets into the STRING 12.0 database, selecting "Homo sapiens" as the species and setting the confidence score to 0.4 to obtain a protein-protein interaction (PPI) network. The PPI network data were then imported into Cytoscape 3.9.1 software, where potential core targets were identified based on network topology indicators, including degree centrality (DC) ≥ two times the median, closeness centrality ≥ the median, and betweenness centrality (BC) ≥ the median. Additionally, we used the CytoHubba plugin in Cytoscape 3.9.1 to perform computational analysis using the maximal clique centrality method, selecting the top 10 Hub genes. The MCODE (Molecular Complex Detection) plugin was employed to filter important module genes, with parameters set as follows: degree cutoff = 2, node score cutoff = 0.2, K-core = 2, and max depth = 100.

Expression level of potential therapeutic targets in various tissues/cells

The expression levels of genes in tissues/cells were queried using the BioGPS (http://biogps.org) database. Genes meeting the following criteria were identified as tissue-specific: (1) the expression level of the gene in a single tissue/cell exceeds 10 times the median; (2) the expression level in the second most abundant tissue/cell does not exceed one-third of the expression level in the most abundant tissue/cell.

Functional enrichment analysis of potential therapeutic targets

Potential therapeutic targets were imported into the DAVID database (http://david.ncifcrf.gov) using the following parameters: "Identifier: official_gene_symbol, Species: Homo sapiens". Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted. For GO analysis, the results were categorized into biological process (BP), cellular component (CC), and molecular function (MF). Significant entries were selected based on a false discovery rate (FDR) < 0.05.

Molecular docking

Core active ingredients in SFJD were selected and docked with key target proteins. First, the pdb format files for the 3D structures of proteins were obtained from the RCSB Protein Data Bank (RCSB PDB, https://www.pdb.org/), and the sdf format files for the 2D structures of the active ingredients were downloaded from the PubChem database. The sdf format files of the active ingredients were then converted to pdb format using Open Babel GUI 2.4.1

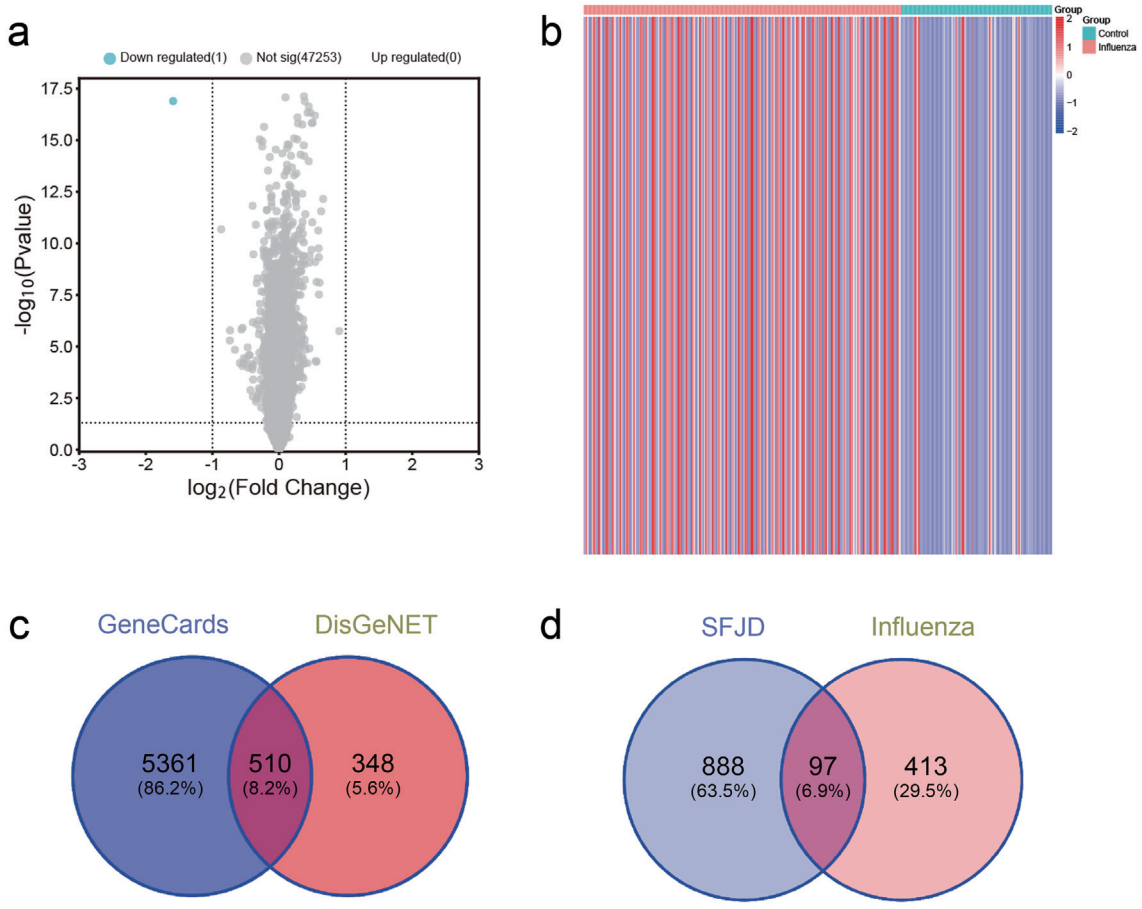

Fig. 1. Screening of disease genes and potential therapeutic targets of SFJD in the treatment of influenza. (a) Volcano map of differential genes in dataset GSE68310. Blue represents downregulated genes, red represents upregulated genes, and gray represents undifferentiated genes. (b) Heat map of DEGs with a multiple of change ≥2 and statistical significance. (c) Intersection genes of influenza in GeneCards and DisGeNET databases. (d) Venn diagram of SFJD targets and influenza disease genes. DEGs, differentially expressed genes; SFJD, Shufeng Jiedu Capsules.

software (https://github.com/openbabel) for further use. Next, AutoDockTools 1.5.6 software (https://autodock.scripps.edu/) was used to add hydrogen atoms to the active ingredients, designating them as ligands; proteins were dehydrated, hydrogenated, and designated as receptors. The docking box was set up, and Autogrid4 and Autodock4 were run to calculate the binding energy. Finally, PyMOL 2.4.1 software (https://pymol.org/2/) was used to visualize the complex structure of the protein-active ingredient complexes. A binding energy < 0 kcal/mol indicates that the active ingredients and proteins can dock in their natural state, while a binding energy < −1.2 kcal/mol indicates good docking results.

Results

SFJD active ingredients and their targets

A total of 193 active ingredients were identified from the TCMSP, TCMID, and TCM Database@Taiwan databases. Among these, there were nine ingredients from Hu Zhang (HZ), 20 from Lian Qiao (LQ), 34 from Ban Lan Gen (BLG), 16 from Chai Hu (CH), 13 from Bai Jiang Cao (BJC), 12 from Ma Bian Cao (MBC), one from Lu Gen (LG), and 88 from Gan Cao (GC) (Table S1). Using the Swiss Target Prediction and TCMSP databases, 985 targets of SFJD active ingredients were identified. These ingredients and their corresponding targets were then imported into Cytoscape 3.9.1 software to construct a network of herbs, active ingredients, and targets, consisting of 1,158 nodes and 8,369 edges (Fig. S1).

Influenza disease genes

The dataset GSE68310 was selected from the GEO database, consisting of 483 samples. Among these, there were 155 normal specimens (84 female and 71 male patients) and 328 influenza specimens (181 female and 147 male patients), with an average patient age of 42 years. Genes meeting the following criteria were considered as DEGs: (1) adj. P. Val < 0.05; (2) |log FC| ≥ 1. The analysis revealed only one differentially expressed gene, *IFI27*, which was downregulated (Fig. 1a and b). Additionally, 5,871 and 858 influenza-related genes were retrieved from the GeneCards and DisGeNET databases, respectively. The intersection of these gene sets yielded 510 common genes (Fig. 1c). After merging with the differentially expressed gene and removing duplicates, a total of 510 disease-related genes were obtained.

Potential therapeutic targets

A Venn analysis was conducted to compare the 985 targets of SFJD active ingredients with the 510 influenza disease genes, resulting

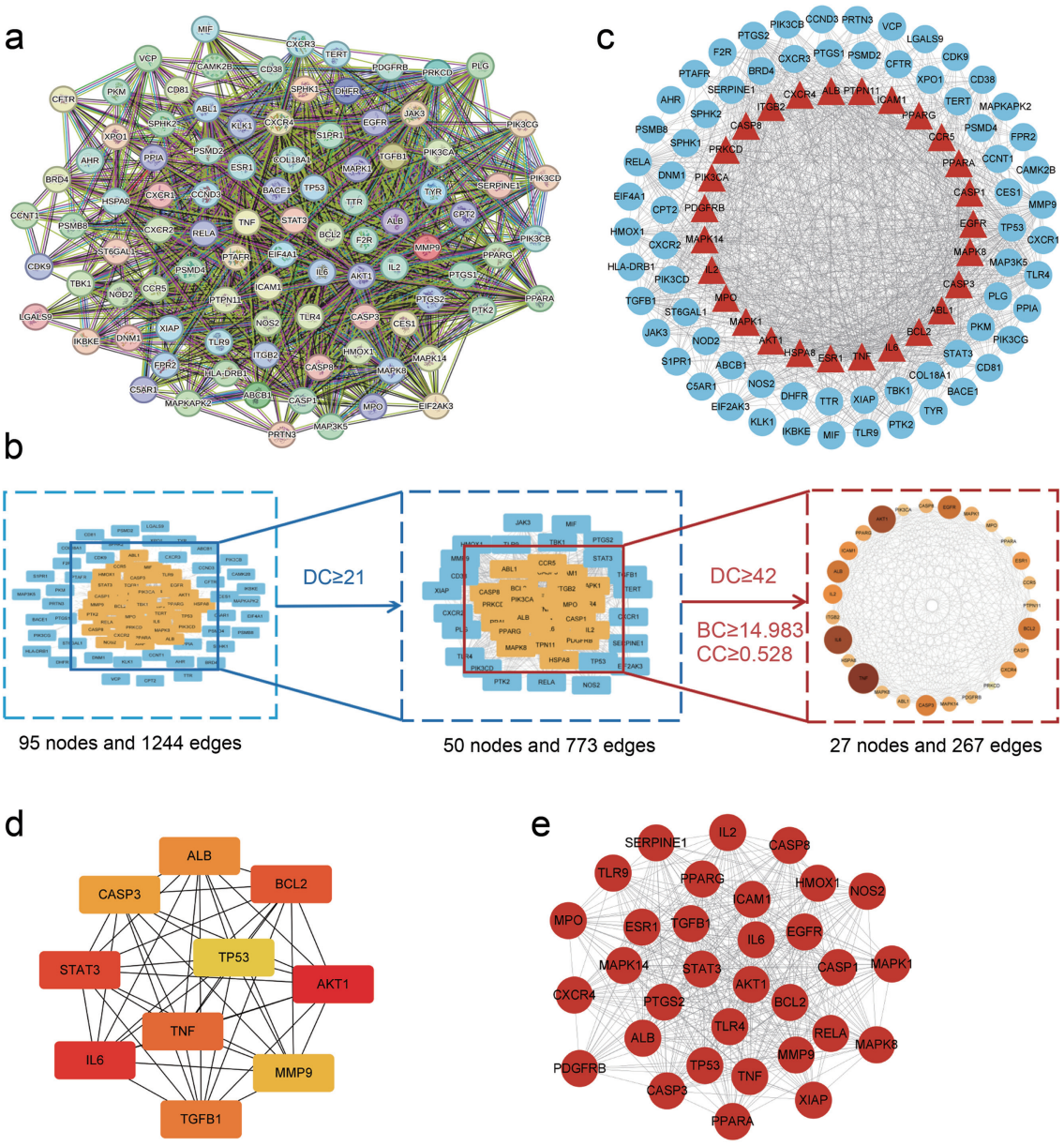

Fig. 2. Interaction network of potential therapeutic targets of SFJD in the treatment of influenza. (a) PPI network of potential therapeutic targets. (b) Screening of key targets. After screening the key targets according to the DC ≥ median, a network consisting of 50 nodes and 773 edges is obtained. Further, according to the DC ≥ twofold median, BC ≥ median, closeness centrality ≥ median, a network diagram of key targets consisting of 27 edges and 267 nodes is obtained. The size of the node is related to the degree value. The larger the node, the greater the degree value, and the smaller the node, the smaller the degree value. (c) Network diagram of key targets and non-key targets. Red represents key targets and light blue represents non key targets. (d) The CytoHubba plug-in in Cytoscape 3.9.1 software was used to screen Hub genes of potential therapeutic targets. The color of node changed from light yellow to red, and the corresponding degree value gradually increased. (e) The MCODE plug-in in Cytoscape 3.9.1 software was used to screen important modules of potential therapeutic targets. BC, betweenness centrality; DC, degree centrality; MCODE, Molecular Complex Detection; PPI, protein-protein interaction; SFJD, Shufeng Jiedu Capsules.

in 97 intersecting genes. These intersecting genes are considered potential therapeutic targets for the treatment of influenza with SFJD (Fig. 1d).

Interaction network of potential therapeutic targets

The 97 potential therapeutic targets were imported into the STRING 12.0 database to obtain the PPI network (Fig. 2a). Combined with analysis using Cytoscape 3.9.1 software, the results showed that the network consisted of 95 nodes and 1,244 edges, with median values for DC, BC, and closeness centrality being 21, 14.98, and 0.53, respectively. Using a standard of DC ≥ two times the median value, 27 key targets were selected (Table 1), and these key and non-key targets were visualized (Fig. 2b and c). The top 10 Hub genes were selected using the CytoHubba plugin (Fig. 2d). The MCODE plugin was used to identify important modules among the potential therapeutic targets, revealing that these sites

Table 1. Information on 27 key targets

Uniprot ID	Symbol	Description	Degree
P01375	TNF	Tumor Necrosis Factor	79
P05231	IL6	Interleukin 6	73
P31749	AKT1	AKT Serine/Threonine Kinase 1	71
P00533	EGFR	Epidermal Growth Factor Receptor	60
P02768	ALB	Albumin	58
P10415	BCL2	BCL2 Apoptosis Regulator	57
P42574	CASP3	Caspase 3	54
P60568	IL2	Interleukin 2	50
P05362	ICAM1	Intercellular Adhesion Molecule 1	48
P03372	ESR1	Estrogen Receptor 1	46
P61073	CXCR4	C-X-C Motif Chemokine Receptor 4	43
P37231	PPARG	Peroxisome Proliferator Activated Receptor Gamma	41
P29466	CASP1	Caspase 1	40
Q16539	MAPK14	Mitogen-Activated Protein Kinase 14	39
P28482	MAPK1	Mitogen-Activated Protein Kinase 1	38
Q14790	CASP8	Caspase 8	37
P05107	ITGB2	Integrin Subunit Beta 2	37
P00519	ABL1	ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase	36
P51681	CCR5	C-C Motif Chemokine Receptor 5	34
P11142	HSPA8	Heat Shock Protein Family A (Hsp70) Member 8	34
P45983	MAPK8	Mitogen-Activated Protein Kinase 8	34
P05164	MPO	Myeloperoxidase	33
P09619	PDGFRB	Platelet Derived Growth Factor Receptor Beta	33
Q06124	PTPN11	Protein Tyrosine Phosphatase Non-Receptor Type 11	32
P42336	PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha	31
Q07869	PPARA	Peroxisome Proliferator Activated Receptor Alpha	25
Q05655	PRKCD	Protein Kinase C Delta	21

mainly resided in one module, with a score of 26.303, containing 32 nodes and 434 edges (Fig. 2e).

Tissue/cell-specific expression of potential therapeutic targets

By querying the BioGPS database, we found that most potential therapeutic targets exhibited high expression levels in multiple tissues and cells. We identified 39 potential therapeutic targets with highly specific expression in 37 tissues and cells. These tissues and cells were primarily associated with the lungs, pancreas, and colon, as well as immune cells such as $CD14^+$ monocytes, $CD33^+$ granulocytes, $CD34^+$ T cells, and $CD56^+$ natural killer cells (Fig. 3 and Table S2). These tissues and cells are closely linked to the respiratory and immune systems, suggesting that SFJD may exert therapeutic effects on influenza by modulating systemic immune responses.

GO functional enrichment analysis

The DAVID database was used to conduct GO functional enrichment analysis on the 97 potential therapeutic targets. A total of 76 GO terms were identified, including 52 in the BP category, two in the CC category, and 22 in the MF category (Table S3). Based on the FDR value and gene count, the top 10 entries for BP and MF, as well as two entries for CC, were selected to generate bubble charts and a combined bar chart (Fig. 4). The BP analysis primarily involved the positive regulation of various cytokines such as phosphorylation, oxidative stress, interleukin (IL)-6/8/1β, tumor necrosis factor (TNF), and others. In the CC category, the analysis mainly involved the phosphatidylinositol 3-kinase complex. The MF category primarily included kinase activity, C-C chemokine receptor activity, cytokine activity, and viral receptor activity.

KEGG functional enrichment analysis

KEGG pathway analysis was performed on the 97 potential therapeutic targets, revealing 121 pathways associated with the mechanism of SFJD in treating influenza (Table S4). Based on the FDR value, the top 30 pathways were selected to generate bubble charts and Sankey diagrams and classified. Additionally, a network diagram of herbs, active ingredients, targets, and the top 30 pathways was constructed using Cytoscape 3.9.1 software. This net-

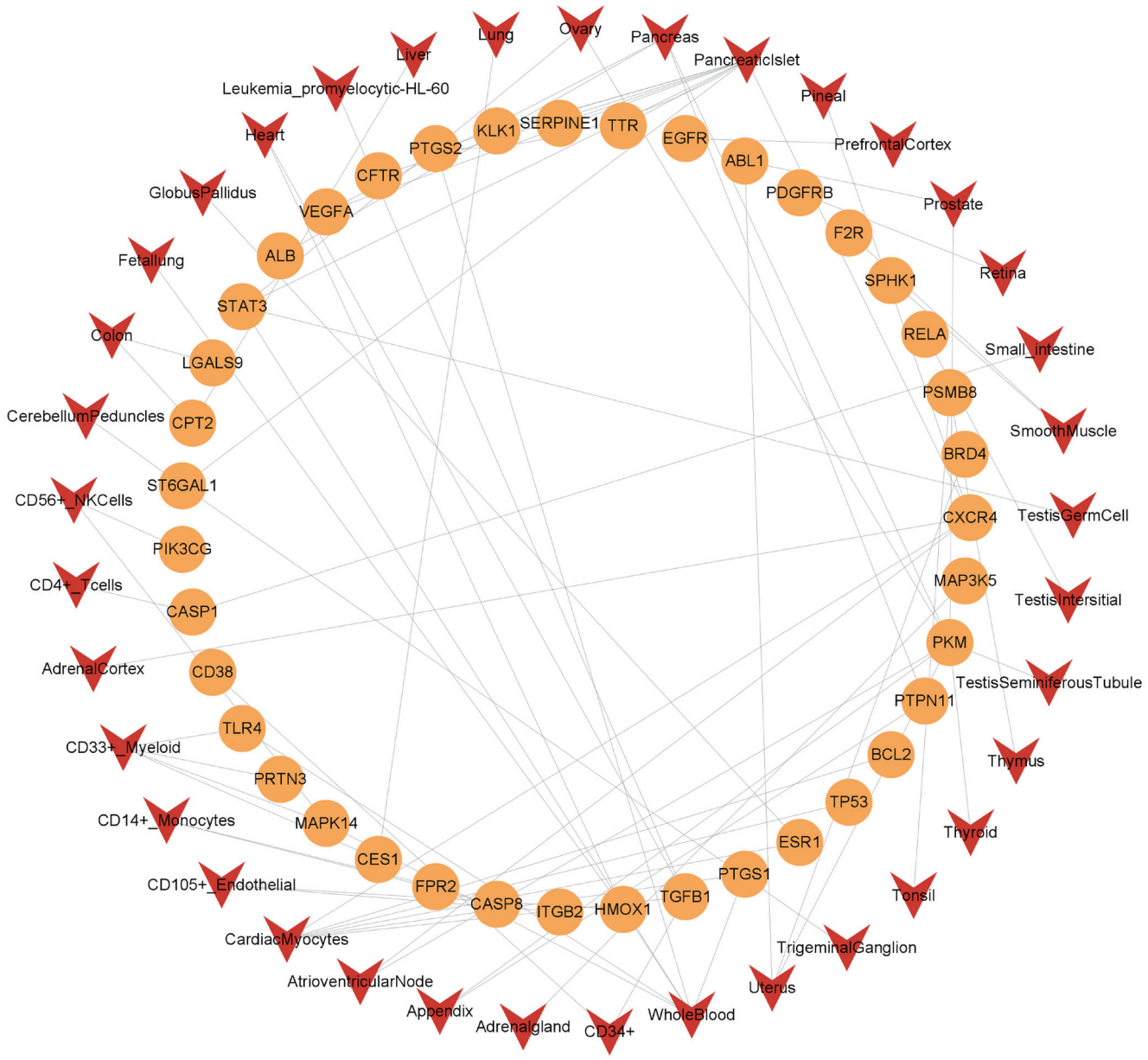

Fig. 3. Network diagram of potential therapeutic targets-tissues/cells. Yellow represents potential therapeutic targets and red represents specific tissues/cells.

work consisted of 245 nodes and 1,575 edges (Fig. 5). The results showed that the C-type lectin receptor signaling pathway, TNF signaling pathway, hypoxia inducible factor-1 (HIF-1) signaling pathway, Toll-like receptor (TLR) signaling pathway, and vascular endothelial growth factor (VEGF) signaling pathway were highly enriched, suggesting that SFJD may exert its therapeutic effects on influenza by modulating these pathways.

Molecular docking visualization

Based on the results of multilevel interaction network analysis and literature retrieval, four core active ingredients—kaempferol, luteolin, isorhamnetin, and beta-sitosterol—were selected to dock with key targets TNF, protein kinase 1 (AKT1), epidermal growth factor receptor (EGFR), and B-cell lymphoma/Leukemia-2 (BCL2), respectively. The results showed that kaempferol had the lowest binding energy with EGFR at −10.74 kcal/mol, while isorhamnetin had the highest binding energy with AKT1 at −6.47 kcal/mol. However, all energies were below −1.2 kcal/mol, indicating satisfactory docking results (Fig. 6 and Table 2). Additionally, PyMOL 2.4.1 software was used to visualize the protein-active ingredient complexes with the lowest binding energies and hydrogen bonds (Fig. 7).

Discussion

Modern medicine identifies influenza as an illness caused by infection with the influenza virus, which is classified into types A,

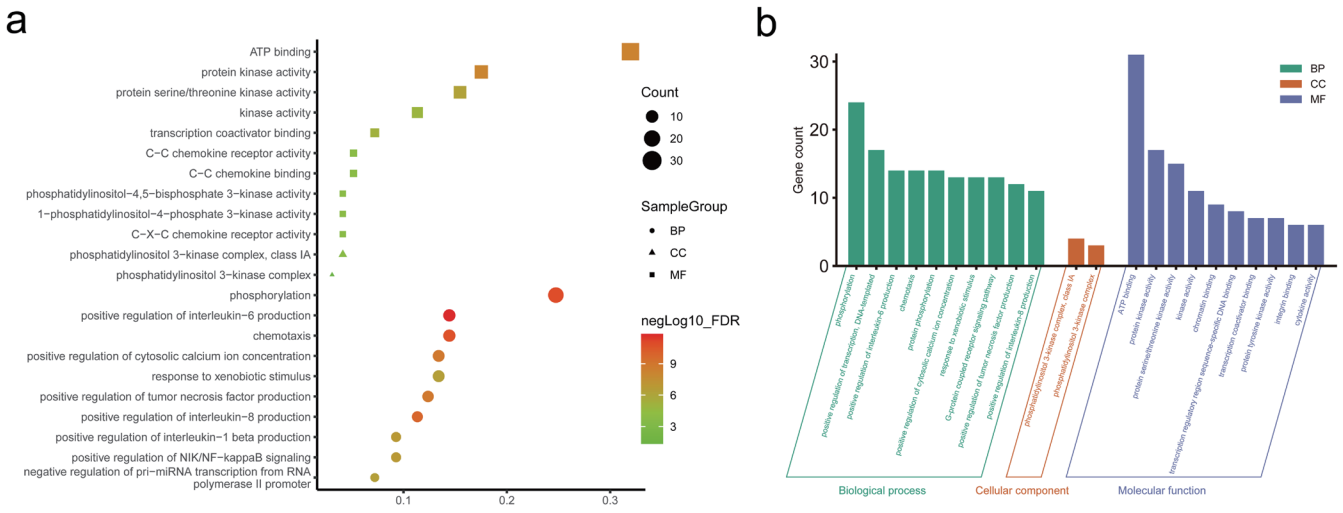

Fig. 4. Go functional enrichment analysis of potential therapeutic targets of SFJD in the treatment of influenza. (a) Bubble chart of the top 10 entries of BP and MF and two entries of CC based on FDR values. (b) Three in one bar chart of the top 10 entries of BP and MF and two entries of CC based on gene count. BP, biological process; CC, cellular component; FDR, false discovery rate; MF, molecular function; SFJD, Shufeng Jiedu Capsules.

B, C, and D. Among these, types A, B, and C can infect humans, with types A and B being the main seasonal strains that lead to mild to severe respiratory tract infections and other complications in humans.[13] TCM holds that there are various types of epidemic colds with diverse symptoms, and the corresponding etiology and pathogenesis are complex. However, these conditions can generally be attributed to external pathogenic factors and a deficiency of righteous qi.[14] TCM treatment emphasizes syndrome differentiation, tailoring the approach according to the specific syndrome presented. SFJD is a traditional Chinese medicine used to treat influenza with wind-heat syndrome, known for its effects in relieving wind, clearing heat, detoxifying, and soothing the throat. To elucidate the mechanism of SFJD in treating influenza, this study employed network pharmacology methods to screen the targets of SFJD, combined with bioinformatics analysis methods to search for influenza-related genes. Through Venn analysis, potential therapeutic targets of SFJD in treating influenza were identified, followed by the construction of PPI networks, functional enrichment analysis, and molecular docking studies.

By screening the active ingredients of SFJD and constructing a network diagram of herbs, active ingredients, and targets, it was confirmed that kaempferol, luteolin, isorhamnetin, and beta-sitosterol are its core active ingredients. Kaempferol, luteolin, and isorhamnetin are flavonoids, which have been proven to possess various pharmacological effects, including anti-inflammatory, antioxidant, antibacterial, immune-regulating, and anticancer properties.[15–20] Additionally, it has been reported that kaempferol and its components can effectively combat DNA viruses (such as hepatitis B virus, herpes simplex virus, and pseudorabies virus) and RNA viruses (such as influenza virus, dengue fever virus, and respiratory syncytial virus) through diverse antiviral mechanisms, such as inhibiting viral polymerase and preventing virus attachment and entry into host cells.[21] An *in vitro* study found that luteolin can target the host protein coat protein I and reduce its expression level, thereby preventing virus replication by blocking virus particles from adhering to the cell surface, obstructing the receptor binding site of viral lectins, or inhibiting the fusion of the viral envelope with the endosome membrane, thus exerting antiviral effects.[22] It has also been suggested that luteolin isolated from herbs can inhibit the activity of neuronal nitric oxide synthase *in vitro*, indicating its potential as an anti-influenza agent.[23] An *in vivo*/*in vitro* study found that isorhamnetin can exert antiviral effects by directly or indirectly inhibiting the expression of viral lectins and neuronal nitric oxide synthase genes, as well as by inhibiting virus-induced autophagy, reactive oxygen species production, and extracellular regulated protein kinases (ERK) phosphorylation. Moreover, isorhamnetin can suppress interferon (IFN)-mediated inflammatory response induced by type A influenza virus infection by blocking the retinoic acid induced gene *I/c-Jun N-terminal kinase (RIG-I/JNK)* and p38 mitogen-activated protein kinase (MAPK) signaling pathways.[24,25] Beta-sitosterol is the most common phytosterol found in plant sterols, with known antioxidant, anti-inflammatory, anticancer, and bronchodilator effects.[26–29] Studies suggest that beta-sitosterol can inhibit the inflammatory response induced by influenza virus through several pathways: by inhibiting RIG-I signal transduction to block the production of IFN and proinflammatory cytokines mediated by type A influenza virus, by disrupting RIG-I-mediated STAT1 activation to weaken the proinflammatory response in sensitized cells, and by blocking the recruitment of toxic T lymphocytes in the lungs during acute lung injury induced by type A influenza virus, significantly improving lung damage and survival rates in mice infected with type A influenza virus.[30]

Through the construction and analysis of the PPI network, 27 key targets have been identified, several of which have been proven to be related to the occurrence and development of influenza. The study found that the excessive release of pro-inflammatory cytokines by infected cells can lead to a "cytokine storm," resulting in a significant increase in serum RNA levels of TNF-α and IL-6 after viral infection.[31] AKT, which includes three isoforms (AKT1, AKT2, and AKT3), plays an important role in regulating various cellular pathways such as cell survival, proliferation, and apoptosis.[32] It is believed that AKT may be involved in the uptake of the influenza virus and in the later stages of its replication cycle.[33] Experiments have shown that apoptosis in canine (MDCK) kidney cells infected with the influenza virus is AKT-dependent, and the

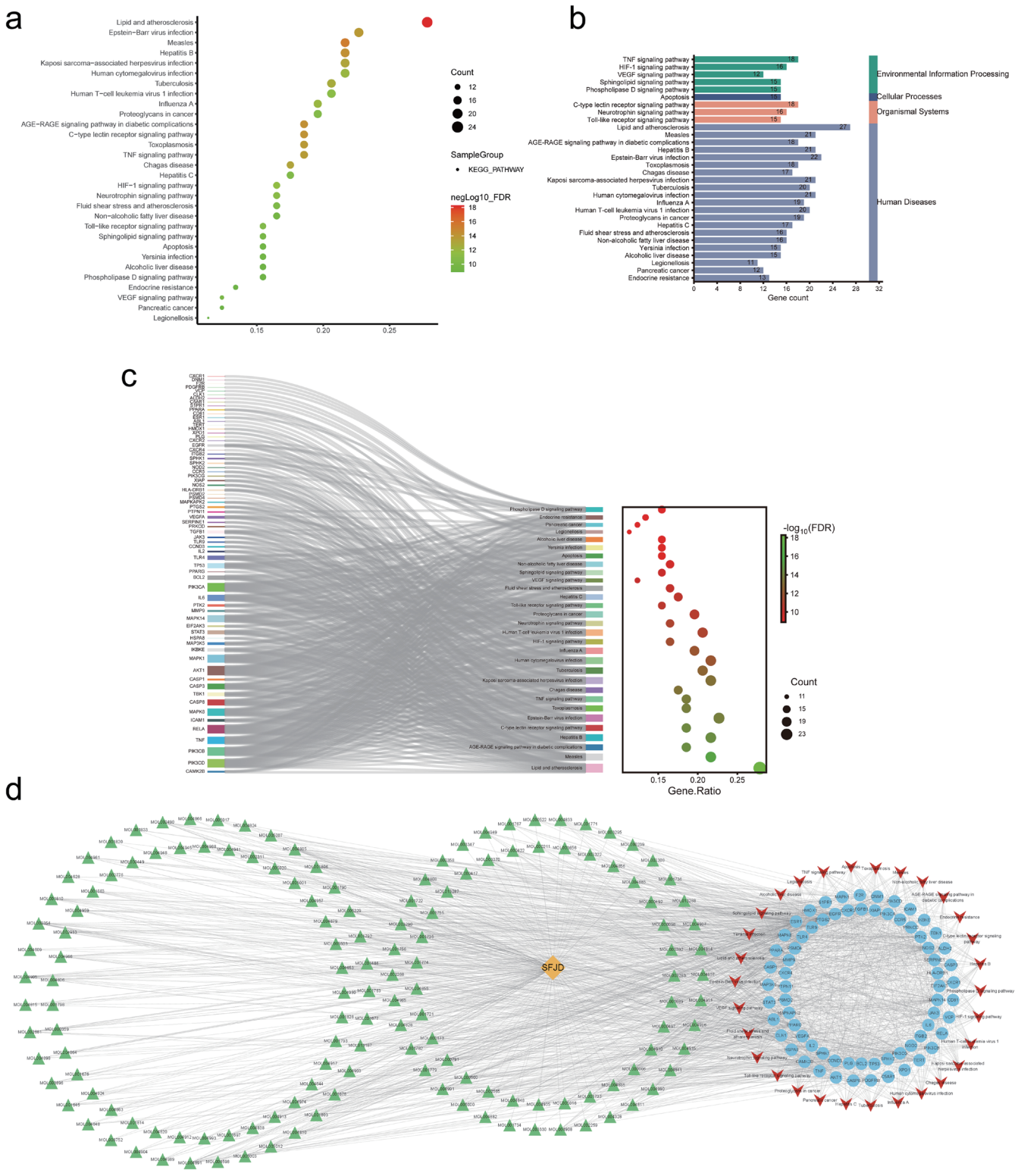

Fig. 5. KEGG functional enrichment analysis of potential therapeutic targets of SFJD in the treatment of influenza and network construction of important pathways. (a) Bubble chart of the top 30 pathways based on FDR values. (b) Classification of the top 30 pathways based on FDR values. (c) Network diagram of sankey dot pathway enrichment of SFJD in the treatment of influenza. (d) Network diagram of SFJD-core active ingredients-targets-important pathways. Yellow represents herbs, green represents core active ingredients, light blue represents targets, and red represents important pathways. FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; SFJD, Shufeng Jiedu Capsules.

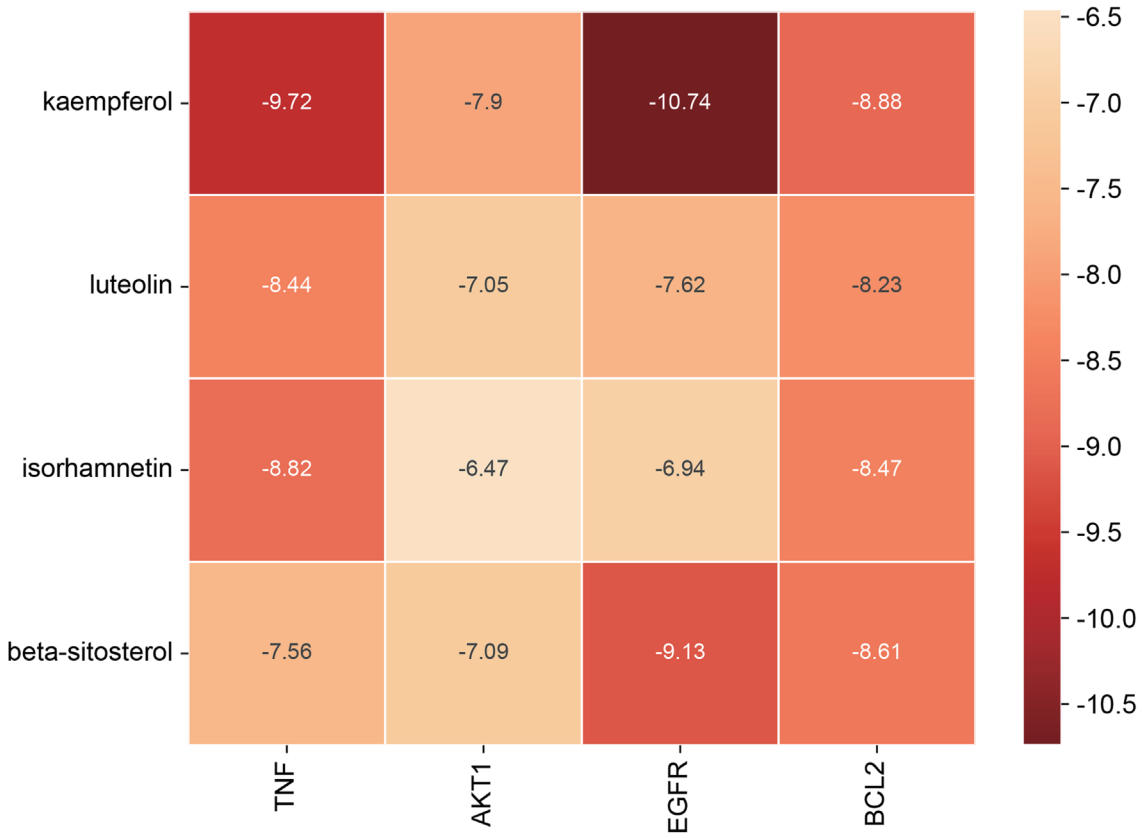

Fig. 6. Heat map of molecular docking binding energy. Binding energy of four core active ingredients (kaempferol, luteolin, isorhamnetin, and beta-sitosterol) and four key targets (TNF, AKT1, EGFR, and BCL2) docking separately. AKT1, protein kinase1; BCL2, B-cell lymphoma/Leukemia-2; EGFR, epidermal growth factor receptor; TNF, tumor necrosis factor.

viral protein nonstructural protein 1, a host AKT inducer, can inhibit this apoptosis.[34] Additionally, research suggests that EGFR can promote the entry of type A influenza virus into host cells, and the phosphatidylinositol 3 kinase/protein kinase B (PI3K/Akt) signaling pathway activated by EGFR can also enhance the uptake of the virus.[35] It has been found that BCL2 can reduce the virus replication rate by inhibiting influenza virus-induced apoptosis, as the activation of CASP3 during apoptosis is crucial for the effective replication of the virus.[36–38] Using the BioGPS database, we found that 37 tissues or cells can specifically overexpress potential therapeutic targets. Based on this, we speculate that SFJD may regulate the systemic immune response through these potential therapeutic targets.

Through enrichment analysis using the DAVID database, the mechanism of SFJD in treating influenza appears to be complex, involving antiviral, anti-inflammatory, and immunomodulatory effects. TNF signaling can trigger inflammatory cascade reactions by activating the nuclear factor kappa B (NF-κB) pathway.[39] Research studies have shown that dysregulation of NF-κB activation can lead to excessive STAT3 activation, causing lung lesions, while inhibiting STAT3 can significantly reduce lung inflammation in mice infected with the vaccinia virus.[40] HIF-1α, together with TLR4, drives inflammation in innate immune responses and plays an important role in the inflammation that occurs after infection with type A influenza virus.[41] It is believed that HIF-1α can activate angiogenesis by regulating hypoxia-related gene activation.[42] Its overexpression can activate and promote the overexpression of downstream VEGF, thereby promoting endothelial cell proliferation, inducing angiogenesis, increasing vascular permeability, and ultimately leading to hyperosmolar pulmonary edema and inflammatory damage to pulmonary capillary endothelial cells.[43] Studies have shown that cells infected with type A influenza virus

Table 2. Details of key targets and active ingredients for molecular docking

Target	PDB ID	Center Coordinates	Compounds Mol ID	PubChem ID	Compounds Name
AKT1	7MYX	25.4,3.13,8.57	MOL000422	5280863	kaempferol
TNF	5UUI	45.263,52.821,13.638	MOL000006	5280445	luteolin
TNF	5UUI	45.263,52.821,13.638	MOL000354	5281654	isorhamnetin
EGFR	8A27	10.293,-6.702,-16.035	MOL000358	222284	beta-sitosterol

AKT1, AKT serine/threonine kinase 1; EGFR, epidermal growth factor receptor; TNF, tumor necrosis factor.

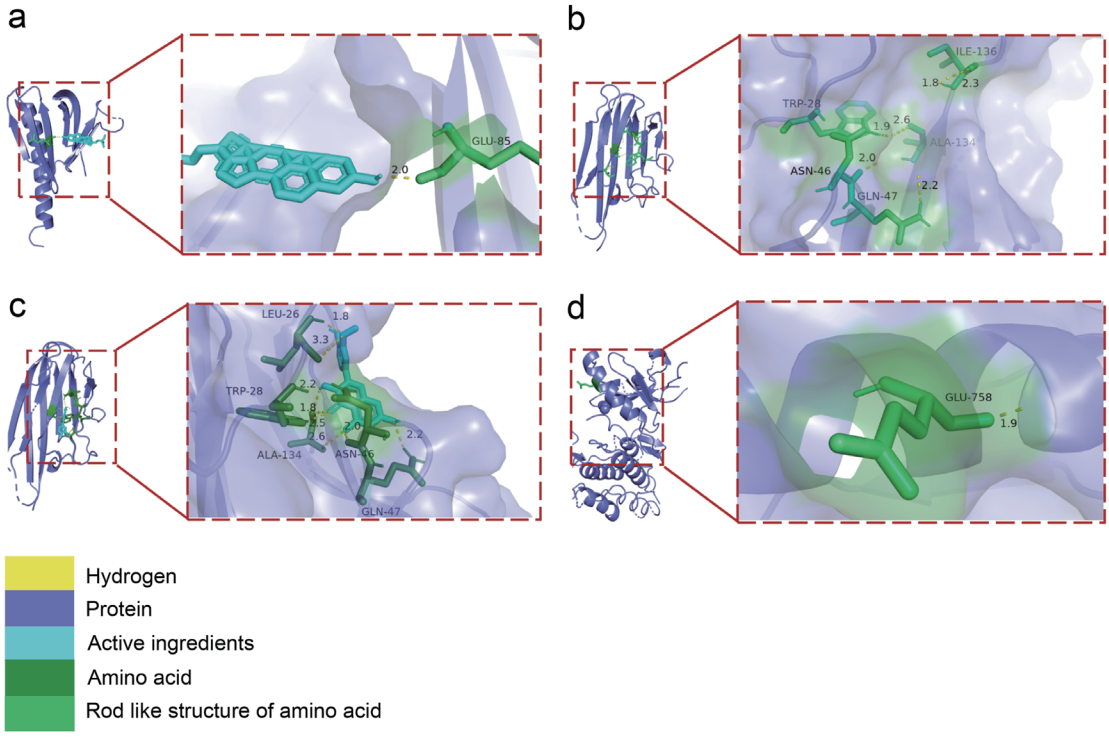

Fig. 7. Docking diagram between core active ingredients and key targets. (a) kaempferol-AKT1. (b) luteolin-TNF. (c) isorhamnetin-TNF. (d) beta-sitosterol-EGFR. AKT1, protein kinase1; EGFR, epidermal growth factor receptor; TNF, tumor necrosis factor.

activate the expression of cellular HIF-1α, promote its translocation to the cell nucleus, and activate the expression of downstream target genes *iNOS* and *VEGF* of HIF-1α, suggesting that inhibiting the HIF-1 signaling pathway may alleviate lung inflammation and damage caused by type A influenza virus infection. It has also been reported that the TLR-mediated signaling pathway plays a crucial role in RNA virus infection.[44] Activation of TLR-7 enhances NADPH oxidase 2 (NOX2) oxidase-dependent oxidative bursts in macrophages, which may underlie acute lung injury in type A influenza virus infection.[45] Furthermore, research has found changes in protein expression in the TLR-7/TLR-8-MyD88 axis in porcine macrophages infected with type H3N2 influenza virus, which may contribute to antiviral responses.[46]

It should be noted that there are still some limitations in this study. We only conducted a comprehensive analysis of the active ingredients of SFJD, disease genes, and mechanisms from the perspective of online public databases. Therefore, the reliability of the results is closely related to the quality of the data uploaded to these databases. Further basic research is needed to verify the mechanism of SFJD in the treatment of influenza.

Conclusions

This study systematically explores the mechanism of SFJD in the treatment of influenza for the first time using network pharmacology, bioinformatics, and molecular docking techniques. Our research results indicate that the core active ingredients of SFJD are kaempferol, luteolin, isorhamnetin, and beta-sitosterol. These ingredients regulate key targets such as TNF, AKT1, and EGFR through pathways such as the C-type lectin receptor signaling pathway, TNF signaling pathway, and HIF-1 signaling pathway, exerting antiviral, anti-inflammatory, and immunomodulatory effects. This study provides valuable reference points for the clinical treatment and basic research of influenza.

Acknowledgments

Not applicable.

Funding

This study was supported by the Key Discipline Construction Project of Traditional Chinese Medicine authorized by the State Administration of Traditional Chinese Medicine (grant no. zyyzdxk-2023001) and the National Multidisciplinary Innovation Team Project of Traditional Chinese Medicine (grant no. ZYYCXTD-D-202201).

Conflict of interest

XLX and QQL have been editorial board members of *Future Integrative Medicine* since November 2021. The authors declare that they have no other competing interests.

Author contributions

Study concept and design (MXY), acquisition of data (CMZ), analysis and interpretation of data (MXY, CMZ, TFC), drafting of the manuscript (MXY), critical revision of the manuscript for important intellectual content (MXY, XLX, QQL), administrative, technical, or material support (XLX, QQL), and study supervision

(XLX, QQL). All authors have made significant contributions to this study and have approved the final manuscript.

Data sharing statement

The data used to support the findings of this study are included in the article.

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