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Research paper

Can network pharmacology identify the anti-virus and anti- inflammatory activities of Shuanghuanglian oral liquid used in Chinese medicine for respiratory tract infection?

Zhenjie Zhuang[a,1], Junmao Wen[a,1], Lu Zhang[a], Mingjia Zhang[a], Xiaoying Zhong[a], Huiqi Chen[a], Chuanjin Luo[b,*]

[a] Guangzhou University of Chinese Medicine, Guangzhou, China
[b] The First Affiliated Hospital of Guangdong University of Chinese Medicine, No.12, Airport Road, Baiyun District, Guangzhou 510405, China

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ABSTRACT

Introduction: Shuanghuanglian (SHL) oral liquid is a well-known traditional Chinese medicine preparation administered for respiratory tract infections in China. However, the underlying pharmacological mechanisms remain unclear. The present study aims to determine the potential pharmacological mechanisms of SHL oral liquid based on network pharmacology.

Methods: A network pharmacology-based strategy including collection and analysis of putative compounds and target genes, network construction, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and Gene Ontology (GO) enrichment, identification of key compounds and target genes, and molecule docking was performed in this study.

Results: A total of 82 bioactive compounds and 226 putative target genes of SHL oral liquid were collected. Of note, 28 hub target genes including 4 major hub target genes: estrogen receptor 1 (ESR1), nuclear receptor coactivator 2 (NCOA2), nuclear receptor coactivator 1 (NCOA1), androgen receptor (AR) and 5 key compounds (quercetin, luteolin, baicalein, kaempferol and wogonin) were identified based on network analysis. The hub target genes mainly enriched in pathways including PI3K-Akt signaling pathway, human cytomegalovirus infection, and human papillomavirus infection, which could be the underlying pharmacological mechanisms of SHL oral liquid for treating diseases. Moreover, the key compounds had great molecule docking binding affinity with the major hub target genes.

Conclusion: Using network pharmacology analysis, SHL oral liquid was found to contain anti-virus, anti-inflammatory, and "multi-compounds and multi-targets" with therapeutic actions. These findings may provide a valuable direction for further clinical application and research.

1. Background

SHL oral liquid is a well-known traditional Chinese medicine preparation frequently applied to treat acute upper respiratory tract infection, acute bronchitis, and pneumonia [1]. SHL oral liquid is derived from three Chinese medicinal herbs, namely, *Flos Lonicerae* (Jinyinhua, JYH), *Radix Scutellariae* (Huangqin, HQ), and *Fructus Forsythiae* (Lianqiao, LQ), which were officially recorded in the *Chinese*

Abbreviations: SHL oral liquid, Shuanghuanglian oral liquid; JYH, Jinyinhua, *Flos Lonicerae*; HQ, Huangqin, *Radix Scutellariae*; LQ, Lianqiao, *Fructus Forsythiae*; CFDA, The China Food and Drug Administration; TCM, traditional Chinese medicine; TCMSP, Traditional Chinese Medicine Systems Pharmacology database; CAS, Chemical abstracts service number; SMILES, Simplified molecular input line entry specification; OB, oral bioavailability; DL, drug-likeness; ROS, reactive oxygen species; SARS-CoV, severe acute respiratory syndrome coronavirus; HCV, human cytomegalovirus; NO, nitric oxide; COX, cyclooxygenases; ESR1, estrogen receptor 1; NCOA2, nuclear receptor coactivator 2; NCOA1, nuclear receptor coactivator 1; AR, androgen receptor; AM, alveolar macrophages; RSV, respiratory syncytial virus; HCMV, Human cytomegalovirus; MCP, monocyte chemoattractant protein; HPV, Human papillomavirus; COX-2, cyclooxygenase; PG, prostaglandin; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology

* Corresponding author.
E-mail addresses: 403440273@qq.com (Z. Zhuang), 515301800@qq.com (J. Wen), 734453247@qq.com (L. Zhang), 13726840720@163.com (M. Zhang), 1365077950@qq.com (X. Zhong), chenhuiqi0727@163.com (H. Chen), Gztcm1964@163.com (C. Luo).

[1] These authors contributed equally to this work.

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Pharmacopoeia (2015 Edition) [2], the legal standard for drug use and development in China. SHL oral liquid (approval number Z41020565) was authorized by the China Food and Drug Administration (CFDA) and has been used clinically for over twenty years. SHL oral liquid displays a wide range of biological activities such as antibacterial, antioxidant, and anti-inflammatory [3]. Furthermore, it shows higher cure rates compared with conventional antivirus drugs [4,5]. As a famous Chinese patent medicine based on Traditional Chinese medicine theory, the pharmacological mechanism study of SHL oral liquid is still limited.

TCM, has been extensively used in clinical practice for thousands of years in Asia, and gradually gained popularity in western countries due to the reliable efficacy [6]. As an important part of complementary and alternative medical systems, TCM treats various diseases via potential multiple interactions of herbs [7]. Because of the multichemical components, multi-pharmacological effects, and multi-action targets of TCM in the treatment [8], the pharmacological mechanism of TCM is still difficult to entirely clarify through conventional research [9]. With the rapid progress of systems biology, bioinformatics, and poly-pharmacology [10], network pharmacology is considered to be a novel way to elucidate complex and holistic mechanisms of TCM [11]. Network pharmacology illustrates the complex interactions among the biological systems, drugs, and complex diseases from a network perspective, sharing a similar holistic philosophy as TCM [12]. The combination of TCM and network pharmacology is shifting the conventional "one target, one drug" paradigm to a multilevel network strategy [13]. In many previous studies, network pharmacology has been shown to be effective for exploring mechanisms [14], and has particularly contributed to investigations into the underlying mechanisms of TCM formula [15,16].

In the present study, the approach of network pharmacology was applied to analyze the relationship between drugs, targets, and metabolic pathways of SHL oral liquid (Fig. 1). By unveiling the molecular mechanisms, we expect to provide a relevant theoretical basis for clinical application and research of SHL oral liquid.

2. Methods

2.1. Acquisition of drug-related compounds and target genes

All ingredients in SHL oral liquid including JYH, HQ, and LQ were searched by herb names in Traditional Chinese Medicine Systems Pharmacology database (TCMSP, http://tcmspw.com/tcmsp.php). With the composition of a large number of herbal entries and relevant data, TCMSP is commonly used to identify drug-target networks and drug-disease networks, which contributes to revealing the mechanisms of action of Chinese herbs, uncovering the nature of TCM theory and developing new herb-oriented drugs [17]. All compounds with oral bioavailability (OB) ≥ 30 % and drug-likeness (DL) ≥ 0.18 were retrieved for subsequent research. As one of the most important pharmacokinetic parameters, OB represents the speed of a drug of becoming available to the human body, and the oral dose eventually absorbed, which plays a particularly significant role in drug discovery of TCM for majority of oral Chinese herb formulas. DL is a qualitative characteristic used in drug design to evaluate whether a compound is chemically suitable for drug. The target genes obtained above were verified by the UniProt [18] protein database (https://www.uniprot.org/) and converted into corresponding gene names.

To collect more reliable target genes of SHL oral liquid, all medicinal chemical components of SHL oral liquid in TCMSP were searched in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) one by one to obtain their molecular information including: molecular formula, Chemical abstracts service number (CAS), and Canonical Simplified molecular-input line entry specification (SMILES). PubChem is the world's largest collection of freely accessible chemical information database. PubChem database is constantly adding new data including chemical and physical properties, biological activities, safety and toxicity information, patents, literature citations, and so on [19]. SMILES of the compounds were pasted in the box of Swiss Target Prediction (http://www.swisstargetprediction.ch/). This website allows researchers to estimate the most probable macromolecular target genes of a small molecule. The Swiss Target Prediction is founded on a combination of 2D and 3D similarity with a library of 370,000 known actives on more than 3000 proteins from three different species [20]. The Swiss Target Prediction applied a probability index to evaluate the

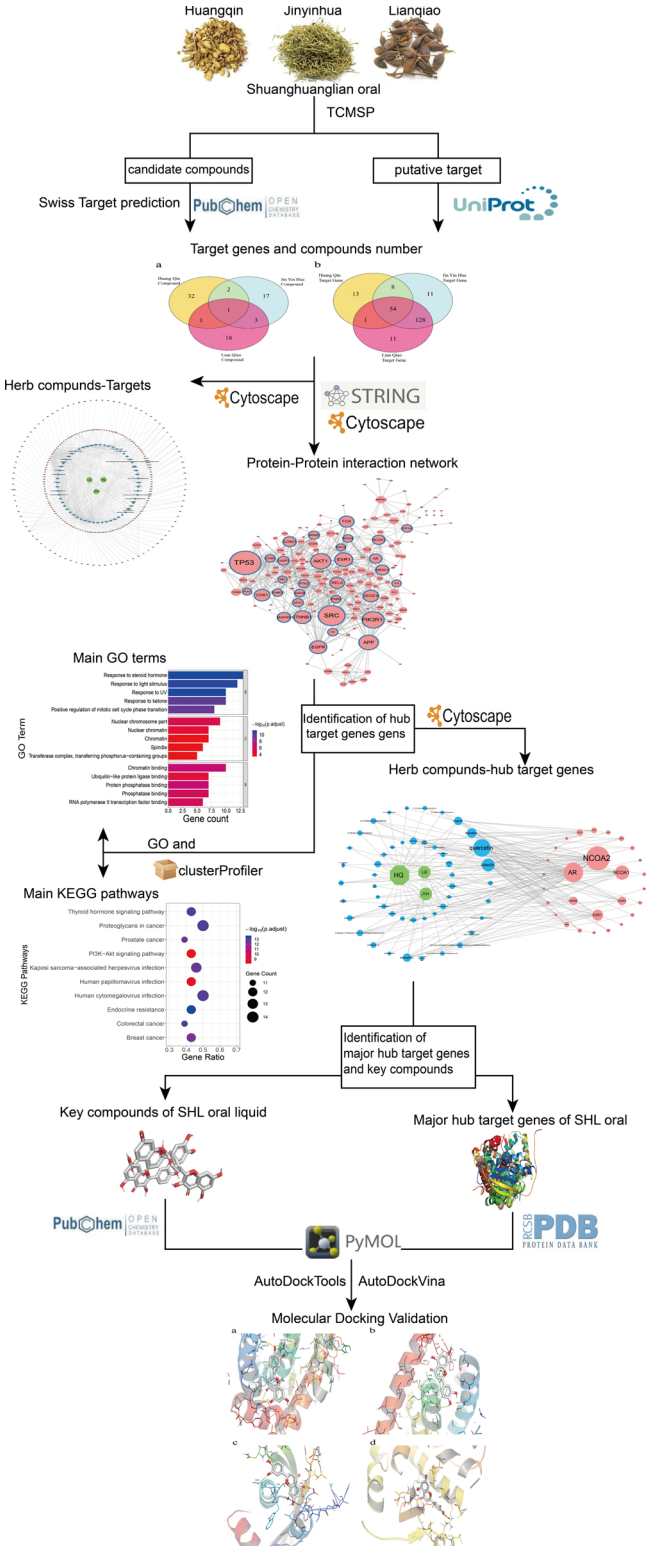


Fig. 1. The flowchart of the present study.

robustness of the prediction results, ranging from 0 to 1. In the present study, we merely included the prediction target with probability equal to 1. Then, the data from TCMSP and Swiss Target Prediction were combined for subsequent analysis.

2.2. Herbs-compounds-target genes network construction

To have a further understanding of the molecular mechanisms, the herb compounds-targets network was constructed by Cytoscape (version 3.7.2), that is, the compounds and target genes retrieved from TCMSP and Swiss Target Prediction were inputted into Cytoscape for constructing the network. Cytoscape is an open-source software platform for visualizing complex biomolecular networks and integrating these with annotations, gene expression profiles, and other state data [21]. During the network construction, users can visually get lots of topology information by different colors, graphics, symbols of every node, making the relationships between each node more understandable and recognizable. Through this network, the pharmacological characteristics of SHL oral liquid which were characterized by multi-component and multi-target would be preliminarily demonstrated. Furthermore, the common compounds and target genes of SHL oral liquid would be analyzed and displayed by Venn diagram.

2.3. Protein-protein interaction (PPI) network construction and identification of hub target genes

To construct a PPI network among target genes of SHL oral liquid, target genes of SHL oral liquid collected from TCMSP and Swiss Target Prediction were inputted into STRING 11.0 (https://string-db.org/, updated on January 19, 2019) for obtaining interactions among proteins expressed by target genes. STRING is one of the major existing regularly updated public PPI databases, which covers the majority of known human protein-protein interactions information [22]. The organism parameter of STRING was set as “has” (Human). In addition, the interaction confidence score was set as greater than or equal to 0.9 to ensure the high confidence and reliability of the outcomes and the disconnected nodes in the network were excluded. Next, the graphical interactions in this network were visualized and analyzed using Cytoscape (version 3.7.2). Besides, ‘Degree’, one of the most important parameters of the topology structure, was used for essentiality assessing each node in the PPI network. ‘Degree’ is regarded as the number of links to a node, which is usually used to describe the topological importance in the PPI network of a protein. Furthermore, only nodes whose ‘Degree’ more than twofold median of the degree of the network were defined as hub target genes according to previous reports [23–25]. Subsequently, the hub target genes were screened out by this way.

2.4. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway enrichment analysis

The GO and KEGG pathway enrichment analyses were performed by *clusterProfiler*. *clusterProfiler* is a functional and regularly updated R package which is widely used for high-throughput data analysis such as bioinformatics analysis based on multiple bioconductor annotation resources and R packages. It could compare gene clusters of any kind of gene–ontology associations including GO and KEGG [26]. GO terms including molecular function (MF), biological process (BP), and cellular components (CC) were identified.

In addition, parameter settings of *clusterProfiler* were as follows: (1) the organism parameter was set as “has” (Human); (2) the analysis cutoff of *P*-value was set as 0.05. Furthermore, the false discovery rate (FDR) served as *P*-value adjustment.

2.5. Herbs-compounds-hub targets network construction

The herb compounds-hub target genes network was generated by Cytoscape (version 3.7.2). In this network, nodes with the more than twofold median of degree value would be defined as major hub target genes, while the compounds with the top five degree value would be regarded as the key compounds of SHL oral liquid that play the most important pharmacological role. By constructing this network, the potential relationship between the compounds and hub targets of SHL oral liquid would be explored to uncover the potential pharmacological mechanisms of SHL oral liquid.

2.6. Molecular docking between compounds and targets

Molecular docking was performed to further validate the relationship between the key compound and major hub target gene. The 3D structures of key compounds of SHL oral liquid were downloaded from the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) as SDF format and were changed to PDB format by a software called *PyMol* (version 2.2). *PyMol* is an open-source molecular visualization system that can render high-quality 3D images of small molecules and biological macromolecules [27]. Meanwhile, proteins of major hub genes complex with crystallographic structures were obtained from the Protein Data Bank (PDB) database (https://www.rcsb.org/). PDB is one of the most important databases to collect the 2.5D structure of biomacromolecule, including protein, nucleic acid, and sugar. *PyMol* was also applied to removing the additional hydrone and ligand of the hub target gene protein. Next, *AutoDockTools* (version 4.2.6) [28] was used for addition of hydrogen atoms, combination of nonpolar hydrogen atoms, calculation of protein charge number, and detection of the docking site before molecular docking. Subsequently, the key compounds were set as ligand and had their structure torsions and roots detected by *AutoDockTools* as well. At last, all the major hub target proteins and key compounds were changed to *pdbqt* format, and then inputted into *AutoDock Vina* (version 1.1.2) [29] for performing molecular docking. The docking affinity score below -5.0 kcal/mol means a strong binding interaction between the compounds and their target genes [30].

3. Results

3.1. Identification of the active compounds and target genes in SHL oral liquid

A total of 82 compounds of the three herbs in SHL oral liquid were retrieved from TCMSP with the criteria of OB ≥ 30 % and DL ≥ 0.18, including 36 of Huangqin, 23 of Jinyinhua, and 23 of Lianqiao (Table 1). According to the target prediction system in TCMSP and Swiss Target Prediction, totally 471 target genes of SHL oral liquid were retrieved including 76 of Huangqin, 201 of Jinyinhua, and 194 of Lianqiao. After combing all the target genes and removed the duplicated genes, 226 unique target genes were identified in total. (Appendix File 1)

3.2. Herbs-compounds-target genes network analysis

Herbs-compounds-target genes network was constructed via Cytoscape 3.7.2 to show the relationship between the herbs, compounds, and target genes (Fig. 2). In this network, there were 288 nodes and 654 edges in total, showing that SHL oral liquid might exert its therapeutic effect by multiple compounds and various target genes.

In the Venn diagram of compounds (Fig. 3a), Huangqin and Jinyinhua shared 3 compounds in common; Lianqiao and Huangqin shared 2; Jinyinhua and Lianqiao shared 4. In the Venn diagram of target genes (Fig. 3b), Huangqin and Jinyinhua shared 62 target genes in common; Lianqiao and Huangqin shared 55; Jinyinhua and Lianqiao shared 182. These findings showed that the herbs of SHL oral liquid might produce the efficacy by the synergic action of the common compounds and target genes shared by each other.

Table 1
Information for 82 chemical compounds of SHL oral liquid.

Mol ID	Molecule Name	OB (%)	DL	Source
MOL001689	acacetin	34.97	0.24	Huang Qin
MOL000173	wogonin	30.68	0.23	
MOL000228	(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	55.23	0.2	
MOL002714	baicalein	33.52	0.21	
MOL002908	5,8,2'-trihydroxy-7-methoxyflavone	37.01	0.27	
MOL002909	5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone	33.82	0.45	
MOL002910	carthamidin	41.15	0.24	
MOL002911	2,6,2',4'-tetrahydroxy-6'-methoxychaleone	69.04	0.22	
MOL002913	dihydrobaicalin_qt	40.04	0.21	
MOL002914	eriodyctiol	41.35	0.24	
MOL002915	Salvigenin	49.07	0.33	
MOL002917	5,2',6'-trihydroxy-7,8-dimethoxyflavone	45.05	0.33	
MOL002925	5,7,2',6'-Tetrahydroxyflavone	37.01	0.24	
MOL002926	dihydrooroxylin A	38.72	0.23	
MOL002927	skullcapflavone II	69.51	0.44	
MOL002928	oroxylin a	41.37	0.23	
MOL002932	panicolin	76.26	0.29	
MOL002933	5,7,4'-trihydroxy-8-methoxyflavone	36.56	0.27	
MOL002934	neobaicalein	104.34	0.44	
MOL002937	dihydrooroxylin	66.06	0.23	
MOL000358	beta-sitosterol	36.91	0.75	
MOL000359	sitosterol	36.91	0.75	
MOL000525	norwogonin	39.4	0.21	
MOL000552	5,2'-dihydroxy-6,7,8-trimethoxyflavone	31.71	0.35	
MOL000073	ent-epicatechin	48.96	0.24	
MOL000449	stigmasterol	43.83	0.76	
MOL001458	coptisine	30.67	0.86	
MOL001490	bis[(2S)-2-ethylhexyl] benzene-1,2-dicarboxylate	43.59	0.35	
MOL001506	supraene	33.55	0.42	
MOL002879	diop	43.59	0.39	
MOL002897	epiberberine	43.09	0.78	
MOL008206	moslosooflavone	44.09	0.25	
MOL010415	11,13-eicosadienoic acid, methyl ester	39.28	0.23	
MOL012245	5,7,4'-trihydroxy-6-methoxyflavanone	36.63	0.27	
MOL012246	5,7,4'-trihydroxy-8-methoxyflavanone	74.24	0.26	
MOL012266	rivularin	37.94	0.37	
MOL001494	mandenol	42	0.19	Jin Yin Hua
MOL001495	ethyl linolenate	46.1	0.2	
MOL002707	phytofluene	43.18	0.5	
MOL002914	eriodyctiol (flavanone)	41.35	0.24	
MOL003006	(-)-(3R,8S,9R,9aS,10aS)-9-ethenyl-8-(beta-D-glucopyranosyloxy)-2,3,9,9a,10,10a-hexahydro-5-oxo-5H,8H-pyrano[4,3-d]oxazolo[3,2-a]pyridine-3-carboxylic acid_qt	87.47	0.23	
MOL003014	secologanic dibutylacetal_qt	53.65	0.29	
MOL002773	beta-carotene	37.18	0.58	
MOL003036	zinc03978781	43.83	0.76	
MOL003044	chryseriol	35.85	0.27	
MOL003059	kryptoxanthin	47.25	0.57	
MOL003062	4,5'-Retro-.beta,beta.-carotene-3,3'-dione, 4',5'-didehydro	31.22	0.55	
MOL003095	5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl)chromone	51.96	0.41	
MOL003101	7-epi-Vogeloside	46.13	0.58	
MOL003108	caeruloside C	55.64	0.73	
MOL003111	centauroside_qt	55.79	0.5	
MOL003117	Ioniceracetalides B_qt	61.19	0.19	
MOL003124	xylostosidine	43.17	0.64	
MOL003128	dinethylsecologanoside	48.46	0.48	
MOL000358	beta-sitosterol	36.91	0.75	
MOL000422	kaempferol	41.88	0.24	
MOL000449	Stigmasterol	43.83	0.76	
MOL000006	luteolin	36.16	0.25	
MOL000098	quercetin	46.43	0.28	

Table 1 (*continued*)

Mol ID	Molecule Name	OB (%)	DL	Source
MOL000173	wogonin	30.68	0.23	Lian Qiao
MOL003281	20(S)-dammar-24-ene-3β,20-diol-3-acetate	40.23	0.82	
MOL003283	(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	66.51	0.39	
MOL003290	(3R,4R)-3,4-bis[(3,4-dimethoxyphenyl)methyl]oxolan-2-one	52.3	0.48	
MOL003295	(+)-pinoresinol monomethyl ether	53.08	0.57	
MOL003305	phillyrin	36.4	0.86	
MOL003306	acon1_001697	85.12	0.57	
MOL003308	(+)-pinoresinol monomethyl ether-4-D-beta-glucoside_qt	61.2	0.57	
MOL003315	3beta-Acetyl-20,25-epoxydammarane-24alpha-ol	33.07	0.79	
MOL000211	mairin	55.38	0.78	
MOL003322	forsythinol	81.25	0.57	
MOL003330	(-)-Phillygenin	95.04	0.57	
MOL003344	β-amyrin acetate	42.06	0.74	
MOL003347	hyperforin	44.03	0.6	
MOL003348	adhyperforin	44.03	0.61	
MOL003365	lactucasterol	40.99	0.85	
MOL003370	onjixanthone I	79.16	0.3	
MOL000358	beta-sitosterol	36.91	0.75	
MOL000422	kaempferol	41.88	0.24	
MOL000522	arctiin	34.45	0.84	
MOL000006	luteolin	36.16	0.25	
MOL000791	bicuculline	69.67	0.88	
MOL000098	quercetin	46.43	0.28	

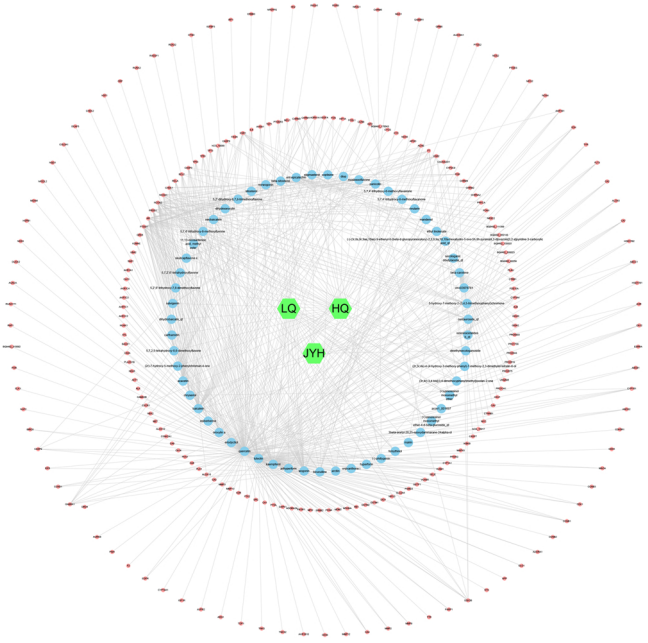


Fig. 2. Herbs-compounds-target genes network of SHL oral liquid.
Note: The green node represents herbs in SHL oral liquid; the blue represents the active compound of SHL oral liquid and the red node represents the target gene.

3.3. Exploration of the target genes in the PPI network

PPI network was constructed after 226 unique target genes being put into STRING software (Fig. 4). The results of the network topology analysis were as follows: mean betweenness: 013; mean degree: 7.317; mean closeness: 0.349; median degree: 6. In this network, there were 167 nodes and 611 edges in total. 59 target genes were excluded from

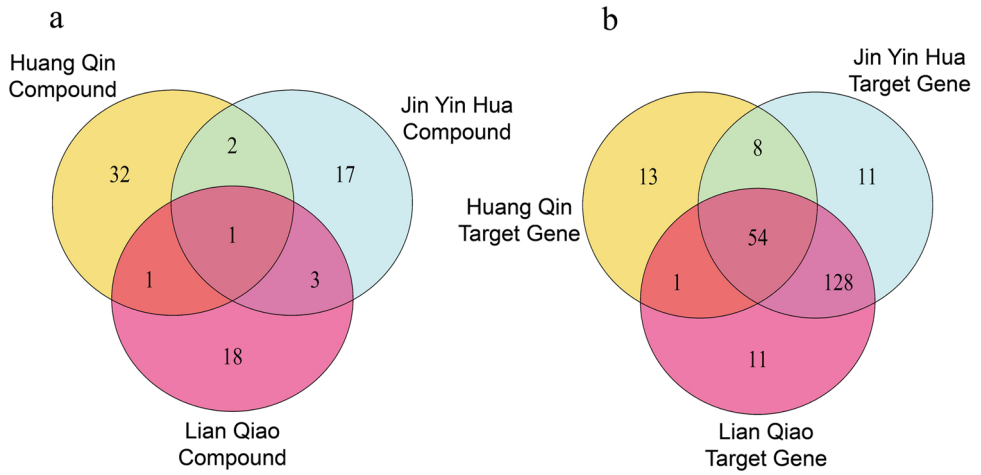


Fig. 3. Venn diagram of compound and target gene relationship in SHL oral liquid.
a: Venn diagram of compound relationship in SHL oral liquid.
b: Venn diagram of target gene relationship in SHL oral liquid.

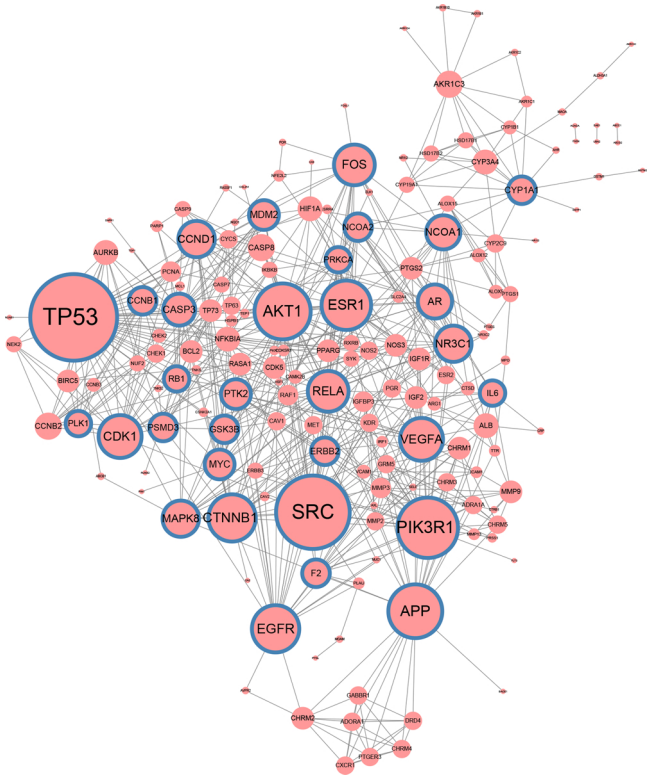


Fig. 4. The PPI network of target genes in SHL oral liquid.
Note: The nodes with blue border represent hub target genes. The node size is positively related to the degree of the node.

Table 2
Information of hub target genes of SHL oral liquid.

Hub Gene	Protein names	Degree	UniPort Entry
CYP1A1	Cytochrome P450	13	Q0VHD5
CCNB1	G2/mitotic-specific cyclin-B1	13	H0Y9U8
NCOA2	Nuclear receptor coactivator 2	13	A0A1D5RMT0
ERBB2	Receptor tyrosine-protein kinase erbB-2	13	J3KTI5
PSMD3	26S proteasome non-ATPase regulatory subunit 3	13	F5H8K4
GSK3B	Glycogen synthase kinase-3 beta	13	A0A3B3ITW1
F2	F2 Protein	13	E9PIT3
MDM2	MDM2 protein	14	A0A0A8KA17
PTK2	Focal adhesion kinase 1	14	E7ESA6
MYC	V-myc myelocytomatosis viral oncogene homolog	14	B3CJ87
CASP3	Caspase-3	15	A8MVM1
AR	Androgen receptor splice variant 5	16	C0JKD6
NCOA1	Nuclear receptor coactivator 1	16	B5MCN7
CCND1	G1/S-specific cyclin-D1	17	F5H437
NR3C1	Glucocorticoid nuclear receptor variant 1	17	F1D8N4
MAPK8	Mitogen-activated protein kinase 8	17	A0A3B3IRW7
RELA	Transcription factor p65	19	A0A087WVP0
FOS	Proto-oncogene c-Fos	19	H0YJM3
VEGFA	Vascular endothelial growth factor A	20	H0YBI8
CDK1	Cyclin-dependent kinase 1	20	A0A087WZZ9
CTNNB1	Catenin beta-1	22	A0A2R8Y804
EGFR	Epidermal growth factor receptor	22	A7VN06
ESR1	Estrogen receptor protein	23	K7R989
APP	Amyloid beta A4 protein	25	L7XE61
AKT1	RAC-alpha serine/threonine-protein kinase	26	A0A087WY56
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit alpha	28	E5RK66
SRC	Tyrosine kinase pp60c-src	34	Q71UK5
TP53	Cellular tumor antigen p53	40	J3KP33

the network construction due to not meeting the screening criteria. Finally, 28 hub target genes were screened based on the topological properties of the degree of network nodes (degree > 12). The information of these hub target genes was shown in Table 2.

3.4. Analysis of GO and KEGG pathway analysis result

GO annotation and KEGG pathway enrichment of SHL oral liquid target genes were carried out through *clusterProfier*. GO annotation was performed in three aspects including biological process (BP), cell composition (CC), and molecular function (MF). The enrichment results included 1390 BP terms, 30 CC terms, 109 MF terms. The first five terms with a significant adjusted *P*-value were shown in Fig. 5. Main BP included the response to steroid hormone and response to light stimulus; Main CC involved nuclear chromosome part, nuclear chromatin, and other cell components; Main MF covered chromatin binding, ubiquitin-like protein ligase binding, etc. 168 relevant pathways of SHL oral liquid were obtained by KEGG pathway enrichment. The main KEGG pathways included the PI3K-Akt signaling pathway, human papillomavirus infection, and human cytomegalovirus infection (Fig. 6).

3.5. Herbs-compounds-hub target genes network analysis

Herbs-compounds-hub target genes network was constructed to

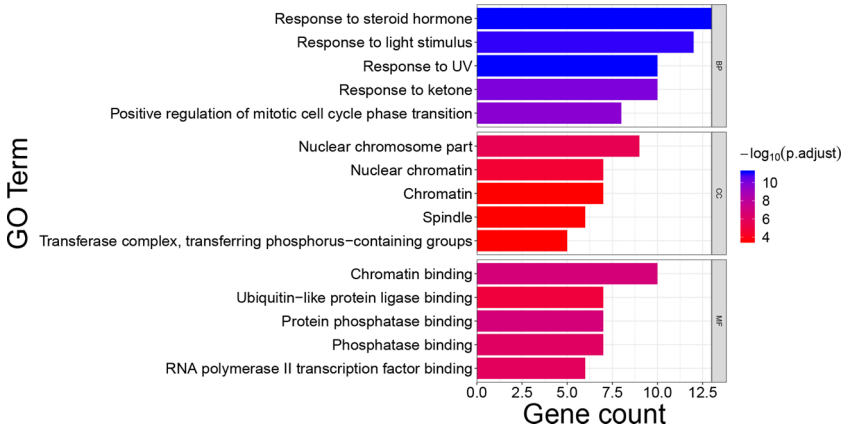


Fig. 5. Main Gene Ontology terms enriched by hub target genes.
Note: The color of terms turned from blue to red. The redder the bar was, the smaller the adjusted *P* value was.
Abbreviations: BP: biological processes; CC: Cellular Component; MF, molecular function.

investigate the relationship between herbs and compounds of SHL oral liquid and the major hub target genes (Fig. 7). The analysis results of the network topology were as follows: mean betweenness (0.021); mean degree (5.367) and mean closeness (0.388). In addition, the information of important nodes in the network was shown in Table 3. In this network, there were 79 nodes and 212 edges in total. The nodes of hub target genes with more than twofold median of degree (degree > 6) including NCOA2, AR, NCOA1, and ESR1, and these hub target genes were regarded as the major hub target genes. In addition, 5 key compounds were screened out as key compounds of SHL oral liquid including quercetin, luteolin, wogonin, baicalein, and kaempferol (Table 4).

3.6. Molecular docking result analysis

With the key compounds and major hub target genes identified, the molecular docking between the key compounds and proteins expressed by major hub target genes was performed. The docking affinity score was calculated by *Autodock Vina*, as shown in Table 5. Moreover, the docking conformations of luteolin and hub target proteins were shown in Fig. 8. Additional docking conformations of compounds and hub target genes were provided in supplementary materials including Fig S1, Fig S2, Fig S3, and Fig S4. As shown in Table 5, baicalein, kaempferol, luteolin, quercetin, and wogonin all had well binding interaction with the hub target protein expressed by major hub target genes. The average docking scores AR, ESR1, NCOA1, and NCOA2 and the key compounds were -8.88, -8.36, -7.54 and -8.74 kcal/mol, respectively, which means great binding interactions between compounds and hub target genes.

4. Discussion

Traditional Chinese Medicine (TCM), which has been assiduously developed for more than two millennia, plays a significant role in clinical practice. SHL oral liquid, is a commonly used Chinese patent medicine, and has been reported to have a good therapeutic effect regarding the clearing of heat and detoxifying [31]. In TCM, heat evil is an important pathogen in infectious diseases. Treatment focuses on the use of compounds with heat-clearing properties, SHL oral liquid has this heat-clearing property and in clinical practice, SHL oral liquid is commonly used for the treatment of infectious diseases such as pneumonia and pharyngitis, etc. [32,33]. In terms of composition, SHL oral liquid is derived from three Chinese medicinal herbs, including *Flos Lonicerae* (Jinyinhua, JYH), *Radix Scutellariae* (Huangqin, HQ), and *Fructus Forsythiae* (Lianqiao, LQ), whose therapeutic effect associated with anti-inflammatory and antiviral [34–37]. Moreover, it was recommended for the treatment of influenza in *Influenza Diagnosis and Treatment Guidelines* (2011 edition) based on its favorable curative effect [38].

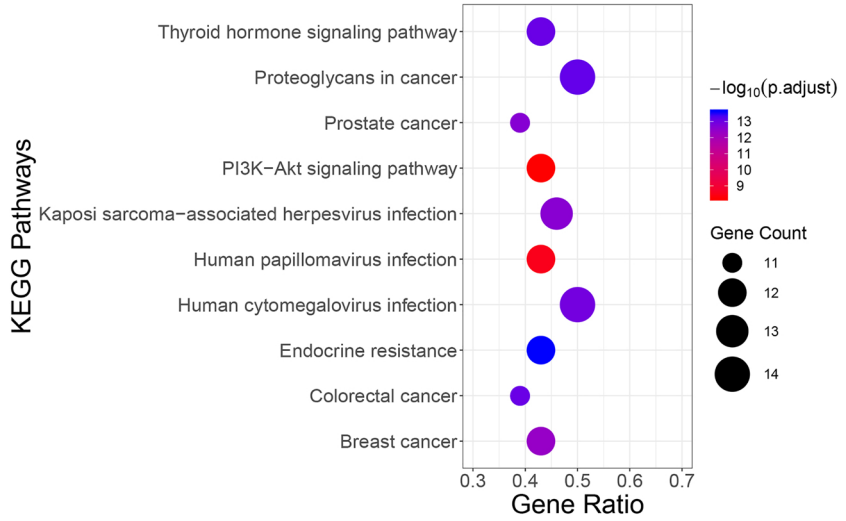


Fig. 6. Main KEGG pathway enriched by hub target genes.
Note: The color of terms turned from blue to red. The redder the bubble was, the smaller the adjusted p value was.

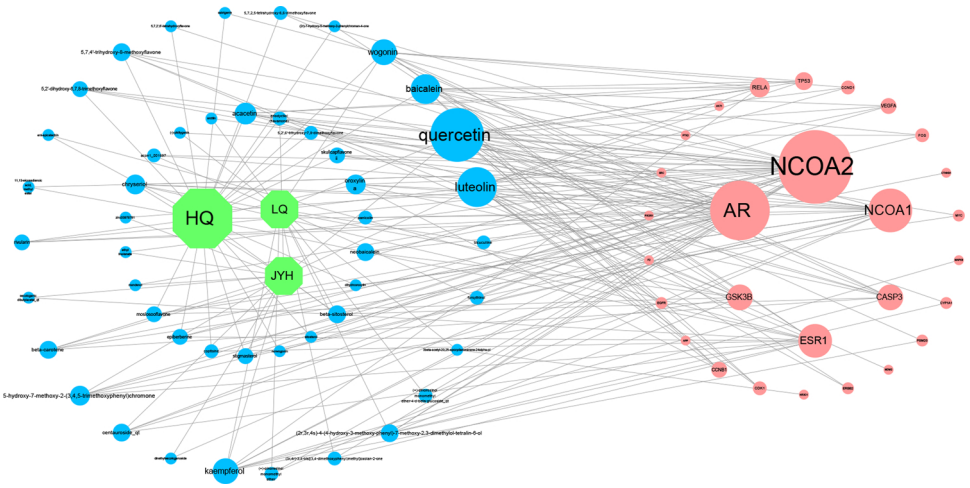


Fig. 7. Herbs-compounds-hub target genes network of SHL oral liquid.
Note: The green node represents herbs in SHL oral liquid; the blue represents the compound of SHL oral liquid and the red node represents the hub target gene. The node size is positively related to the degree of the node.

Though SHL oral liquid was reported to have pharmacologic action of anti-virus and anti-inflammatory, the exact mechanism remains unknown. As such, network pharmacology is in a position to analyze the underlying pharmacological mechanisms of SHL oral liquid. In the present study, network pharmacological analysis of SHL oral liquid identified 5 compounds, 28 hub target genes, and 3 main signaling pathways accounting for the therapeutic effect of SHL oral liquid.

According to the herbs-compounds-hub target genes network, five compounds with high degree values including quercetin, luteolin, baicalein, kaempferol, and wogonin were identified as key compounds of SHL oral liquid. Notably, quercetin, luteolin, baicalein, and kaempferol are all attributed to flavonoids whose biological activities involve anti-inflammatory and anti-virus [39].

Quercetin was reported to have virucidal activity against enveloped viruses including herpes simplex, parainfluenza type, and respiratory syncytium [40]. Quercetin is reported to modulate the transport activities of the multidrug-resistance-associated proteins. Moreover, quercetin could not only influence the bioavailability of anticancer and antiviral drugs in vivo but also alleviate endotoxin-stimulated apoptosis and inflammation by up-regulation of relevant micro-RNA [41,42]. Additionally, quercetin could mitigate the intracellular formation of reactive oxygen species (ROS) to reduce the pro-inflammatory response [43]. Luteolin is proved to have significant protective effects of anti-virus and anti-inflammatory in vivo. As a kind of neuraminidase inhibitors, luteolin could effectively inhibit the replication of the influenza virus and spread in the virus particle surface [44]. Also, luteolin possesses the ability to hinder acute respiratory syndrome coronavirus (SARS-CoV) to enter the host cells by binding avidly to the SARS-CoV inhibited protein [45]. By regulating the inflammatory cytokines and anti-oxidant factors, luteolin is associated with higher clearance of virus and inflammatory lung injury [46].

Baicalein has been found to inhibit the virus in vivo. Baicalein significantly reduces the levels of early and late proteins and DNA synthesis of human cytomegalovirus (HCV) [47]. In a mouse model, baicalein has been found to reduce the titre of influenza A virus leading to inhibition of lung consolidation, and the increase of mean survival time [48]. It's reported that kaempferol could decrease nitric oxide (NO) production to regulate the blood flow and maintain the coordination between vascular endothelial cells, as well as inflammatory cells by suppressing the NO synthetase mRNA expression [49]. Additionally, kaempferol regulates the transcription factor and protein kinase to suppress on virus-induced inflammation [50]. One previous study suggests that kaempferol may be regarded as an effective drug for the potential treatment of influenza virus-induced acute lung injury [51]. Wogonin was attested to suppress the replication of multiple viruses, such as varicella-zoster virus and influenza virus. Wogonin not only inhibits replication of influenza A virus strains but also attenuates the plaque formation of influenza B strains, thus suggesting its broad anti-viral effect on diverse influenza strains [51]. In addition, wogonin effectively reduces virus titer by down-regulating the expressions of viral oncogenes and promoting intrinsic apoptosis in cervical cancer cells [52]. Besides, wogonin can inhibit shingles virus replication through modulation of interferon signal and adenosine monophosphate-activated protein kinase activity [53]. Furthermore, wogonin was verified to down-regulate the expression of inflammation-associated enzymes, such as cyclooxygenases (COX) and lipoxygenases [54].

Taken together, previous studies showed that these key compounds of SHL oral liquid were effective for anti-virus or anti-inflammatory, which might be the scientific interpretations for therapeutic effect of SHL oral liquid for anti-virus and anti-inflammatory activities. In the PPI network, four hub genes including ESR1, NCOA2, NCOA1, and AR with a greater degree (degree > 10) were identified as major hub genes at which SHL oral liquid target to exert therapeutic effect. ESR1 is a transcription activator with growth-suppressive functions [55]. A study shows that the expression of ESR1 could inhibit influenza A virus replication and lower viral titer in primary human nasal epithelial cells by binding to estrogens to regulate the cellular function of respiratory tract cells [56]. Both NCOA1 and NCOA2 are nuclear receptor coactivators that directly bind nuclear receptors and stimulate the transcriptional activities in a hormone-dependent fashion [57,58]. NCOA1 is capable of enhancing the level of retinoic acid receptors, the key regulators of interleukin-17, which play an important role in autoimmunity, chronic inflammation and host protection against extracellular bacteria and fungi [59]. NCOA2 is a transcriptional coactivator for steroid receptors and nuclear receptors. Wei et.al found that NCOA2 promotes the reactivation of herpesvirus by enhancing the expression of the transcription activator [60]. AR, as a member of the nuclear receptor superfamily, regulates a wide range of target gene expression [61]. AR has been shown to modulate immune systems by indirect hormonal modulation of B-cell maturation in stromal cells [62]. By regulating cellar proliferation or survival, AR negatively regulates the number of alveolar macrophages (AM) to reduce the recruitment of inflammatory factors in the lung [63]. Given the AR receptor knocked out, the human body would be more susceptible to microbial, thus the risk of hospitalization for pneumonia would increase [64]. In addition, these major hub genes all had great binding capacity to the key compounds by molecular docking verification. We hypothesized that the efficacy of SHL oral liquid, improving human immunity, anti-inflammatory, and

Table 3
Important node of herbs-compounds-hub target genes network.

Node Name	Node Type	Degree
NCOA2	Target Gene	33
AR	Target Gene	26
quercetin	Compound	23
NCOA1	Target Gene	18
luteolin	Compound	16
ESR1	Target Gene	13
baicalein	Compound	11
kaempferol	Compound	9
wogonin	Compound	9
CASP3	Target Gene	9
GSK3B	Target Gene	9
acacetin	Compound	7
5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl) chromone	Compound	6
chryseriol	Compound	6
oroxylin a	Compound	6
RELA	Target Gene	6
(2 r,3 r,4 s)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	Compound	5
centauroside_qt	Compound	5
beta-carotene	Compound	5
beta-sitosterol	Compound	5
neobaicalein	Compound	5
5,7,4'-trihydroxy-8-methoxyflavone	Compound	5
TP53	Target Gene	5
rivularin	Compound	4
moslosooflavone	Compound	4
epiberberine	Compound	4
stigmasterol	Compound	4
5,2'-dihydroxy-6,7,8-trimethoxyflavone	Compound	4
skullcapflavone ii	Compound	4
VEGFA	Target Gene	4
CCNB1	Target Gene	4
bicuculline	Compound	3
forsythinol	Compound	3
(+)-pinoresinol monomethyl ether-4-d-beta-glucoside_qt	Compound	3
acon1_001697	Compound	3
(+)-pinoresinol monomethyl ether	Compound	3
(3 r,4r)-3,4-bis[(3,4-dimethoxyphenyl)methyl]oxolan-2-one	Compound	3
coptisine	Compound	3
panicolin	Compound	3
5,2',6'-trihydroxy-7,8-dimethoxyflavone	Compound	3
eriodyctiol (flavanone)	Compound	3
5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone	Compound	3
FOS	Target Gene	3
CCND1	Target Gene	3
CDK1	Target Gene	3
arctiin	Compound	2
(-)-phillygenin	Compound	2
3beta-acetyl-20,25-epoxydammarane-24alpha-ol	Compound	2
dinethylsecologanoside	Compound	2
zinc03978781	Compound	2
secologanic dibutylacetal_qt	Compound	2
ethyl linolenate	Compound	2
mandenol	Compound	2
11,13-eicosadienoic acid, methyl ester	Compound	2
ent-epicatechin	Compound	2
norwogonin	Compound	2
sitosterol	Compound	2
dihydrooroxylin	Compound	2
5,7,2',6'-tetrahydroxyflavone	Compound	2
salvigenin	Compound	2
(2 r)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	Compound	2
ERBB2	Target Gene	2
PSMD3	Target Gene	2
CYP1A1	Target Gene	2
MYC	Target Gene	2
EGFR	Target Gene	2
NR3C1	Target Gene	1
MDM2	Target Gene	1
MAPK8	Target Gene	1
CTNNB1	Target Gene	1
AKT1	Target Gene	1
PTK2	Target Gene	1
SRC	Target Gene	1
PIK3R1	Target Gene	1
F2	Target Gene	1
APP	Target Gene	1

antiviral effects, stems from these main hub genes.

GO analysis found that hub target genes were mainly associated with the biological processes of response to steroid hormones, response to ketone, and positive regulation of mitotic cell cycle phase transition. As revealed from the KEGG pathway enrichment analysis, SHL oral liquid might produce therapeutic effects primarily by regulating PI3K-Akt signaling pathway, thyroid hormone signaling pathway, human cytomegalovirus infection, and human papillomavirus infection. PI3K/Akt signaling pathway is known to associate with viral replication and inflammatory response [65,66]. Cellular PI3K/Akt signaling pathway may inhibit the lytic infections of both RNA and DNA viruses, thus some inhibitors of PI3K or its downstream signal Akt could significantly block virus entry and replication [66]. The activation of the PI3K/AKT pathway was responsible for the increased levels of adenosine diphosphate and promoted platelet activation, leading to anabatic vascular permeability and pulmonary inflammatory responses [67,68]. Moreover, with the PI3K/Akt pathway inhibited, the activation of negative regulators in respiratory syncytial virus (RSV) would be blocked, resulting the inhibition of RSV [68].

In the present study, the hub targets of SHL oral liquid also enriched in the pathways related to cancer, including colorectal cancer, breast cancer, and thyroid hormone signaling pathway. Thyroid hormone signaling pathway was reported to involve several mechanisms to activate either the tumor cells or cells of the micro-environment, but the mechanisms were unclear [69,70]. These findings showed that the ingredients of SHL oral liquid might have potency for anti-tumor activity.

Besides, the pathway term of human papillomavirus infection and human cytomegalovirus infection might be clues for explaining the mechanisms of SHL oral liquid for treating diseases related to virus infection. Human cytomegalovirus (HCMV), ubiquitous herpes virus, activates the immune system, which produces a specific reaction to combat the virus. HCMV is capable of evading immune detection and various hematopoietic cell monocytes [71]. Besides, HCMV could express homologues of host protein-coupled receptors to promote viral replication and maintain viral persistence. As such, at the initial period of infection, targeting viral proteins may be limited to exacerbation of HCMV infection [72]. Following HCMV infection, monocyte chemoattractant protein (MCP) is over-expressing to accelerate inflammation and tissue injury in vascular tissues [73]. Human papillomavirus (HPV) is characterized by the specific host cells of DNA viruses which are epitheliotropic and mucosotropic [74]. HPV is also believed to stimulate the immune system, induce inflammation, and the body's anti-viral processes. With the pulmonary epithelial cell infected, HPV leads to the abnormal expression of cell cycle regulators in undifferentiated cells creating a replication-competent environment for virus. Hence, the HPV viral genome amplifies and packages into infectious particles [75,76]. Besides, HPV could also upregulate the expression of cyclooxygenase (COX)-2 and prostaglandin (PG) followed by the activation of the COX-PG pathway to cause inflammation [77]. In addition, HPV was also reported to increase the level of epidermal growth factor receptor and activate PI3K-AKT pathway, leading to inflammation [78].

Consequently, SHL oral liquid might act on these signaling pathways to improve immune function and reduce the inflammatory responses, which could explain the apparent effects of SHL oral liquid. These KEGG pathways, associated with related components and hub genes, might interact to exert their combined effects. In summary, the

Table 4
Key compounds of Shuanghuanglian oral liquid.

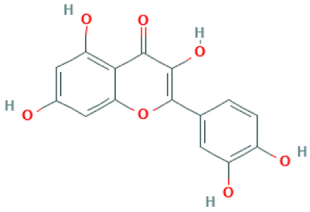
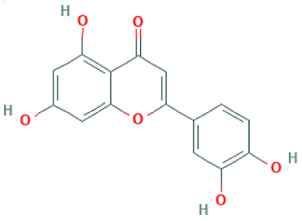
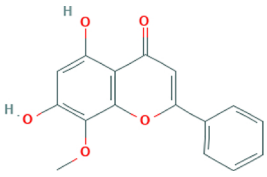
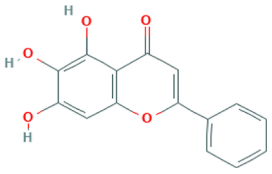
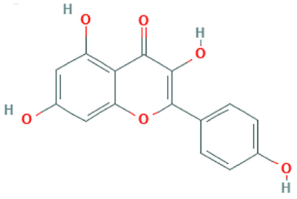
Drug	Molecule Name	Molecular Structural Formula	OB(%)	DL	CAS Code
LQ JYH	Quercetin	 $C_{15}H_{10}O_7$	46.43	0.28	117-39-5
JYH	Luteolin	 $C_{15}H_{10}O_6$	36.16	0.25	491-70-3
LQ HQ	Wogonin	 $C_{16}H_{12}O_5$	30.68	0.23	632-85-9
HQ	Baicalein	 $C_{15}H_{10}O_5$	33.52	0.21	491-67-8
LQ JYH	Kaempferol	 $C_{15}H_{10}O_6$	41.88	0.24	520-18-3

Table 5
Molecular docking result between key compounds and hub target proteins.

Hub target proteins	Docking Affinity with hub target proteins(kcal/mol)			
Key compounds	AR	ESR1	NCOA1	NCOA2
Baicalein	−8.9	−8.8	−7.5	−9
Kaempferol	−8.8	−8.4	−7.7	−8.5
Luteolin	−9.2	−9	−7.6	−8.9
Quercetin	−8.8	−9.1	−7.4	−8.5
Wogonin	−8.7	−6.5	−7.5	−8.8

findings of the present work provided a holistic view of the potential pharmacological mechanisms for SHL oral liquid, which may contribute to its further research and clinical application. However, there are some limitations in the present work. Firstly, the anti-virus and anti-inflammatory effect of key compounds and major hub target genes identified were mainly found in recent cell or animal experiments. Secondly, though the hub target proteins expressed by the hub target genes have good affinity with the key compounds of SHL oral liquid, the interaction between them still needs experimental validation. In summary, the present study provided a new view of pharmacological mechanisms for SHL oral liquid, but further experimental or clinical verification of the findings of the present study is still needed.

5. Conclusion

As an important part of alternative and complementary medicine, TCM has made greater contributions to the treatment of diseases. Using a network pharmacology-based strategy, SHL oral liquid was found to have anti-inflammatory and antiviral effects based on its key compounds, hub target genes, and other relevant pathways. To enhance the reliability of the findings, further experimental and clinical research is needed.

Authors' contributions

Data cleaning and analysis: Zhenjie Zhuang, Junmao Wen, Chuanjin Luo

Figures drawing and table design: Zhenjie Zhuang, Huiqi Chen

Writing - review & editing: Lu Zhang, Mingjia Zhang, Xiaoying Zhong, Chuanjin Luo

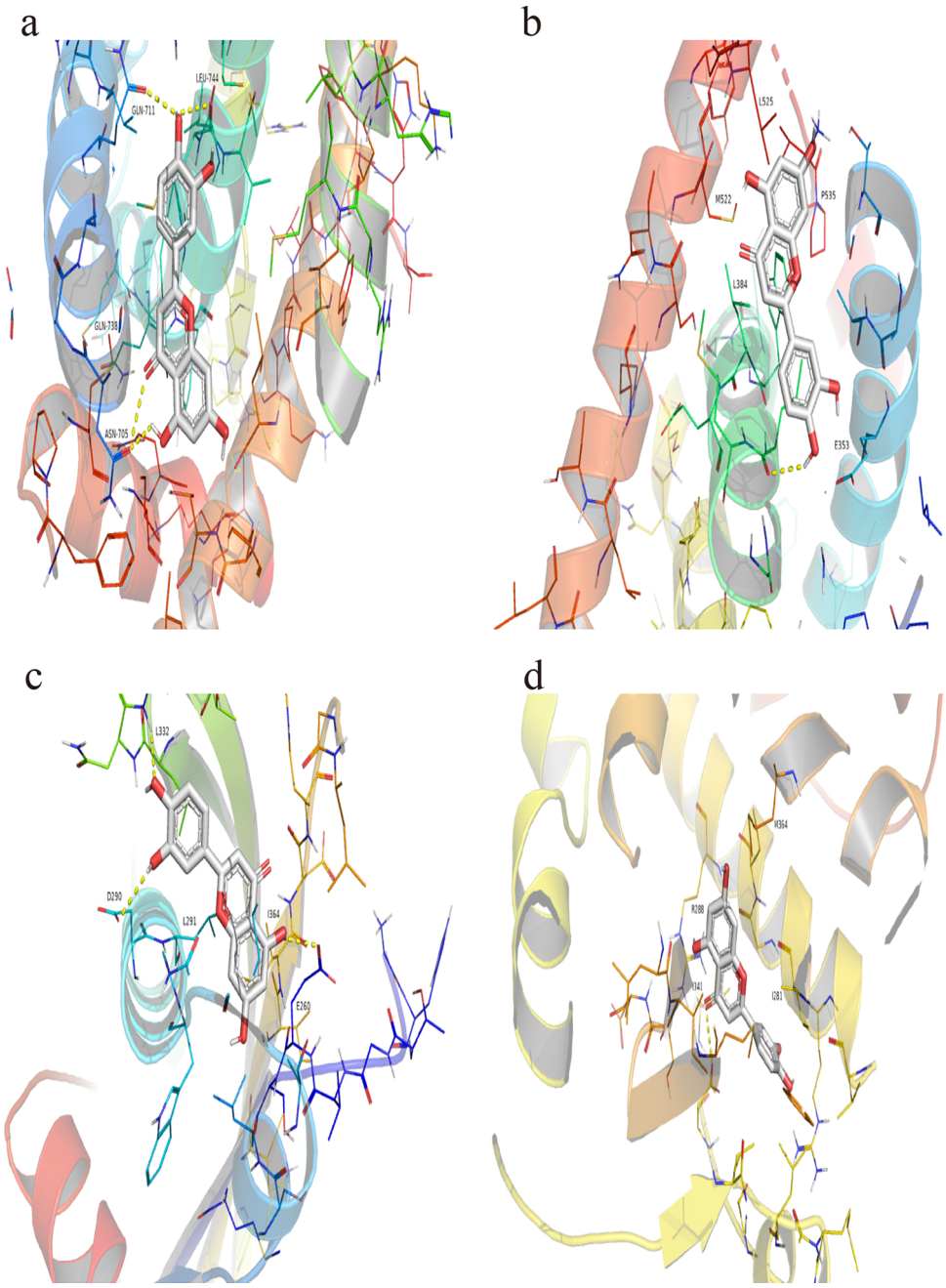


Fig. 8. The docking conformation between luteolin and major hub genes.
a: luteolin-AR; b: luteolin-ESR1; c: luteolin-NCOA1; d: luteolin-NCOA2.

All authors have read and approved the final manuscript.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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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.2020.101139.

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