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Original Article

Network-based pharmacology to predict the mechanism of *Ginger* and *Forsythia* combined treatment of viral pneumonia

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Abstract: Background: Viral pneumonia (VP) is a common inflammatory disease caused by a virus in the upper respiratory tract. However, current treatment options for pneumonia are limited because of the strong infectivity and lack of research. Method: Based on various databases, the mechanisms of *Ginger* and *Forsythia* were predicted by network pharmacology. The possible active ingredients of *Ginger* and *Forsythia* were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and screened by pharmacokinetic parameters. Their possible targets were predicted by the TCMSP database. The VP-related targets were collected from the GeneCards and OMIM databases. The compound-target-disease network was visualized by Cytoscape 3.7.1. In addition, the protein functional annotation and identification of signalling pathways of possible targets were performed with Gene Ontology (GO) and KEGG enrichment analysis. Molecular docking was finally employed for in silico simulation matching between representative *Ginger* and *Forsythia* compounds and their core genes. Results: Twenty-eight active ingredients of *Ginger* and *Forsythia* were found and 30 common targets for the combined treatment of VP were obtained. The enrichment analysis of GO functions and KEGG pathways included 186 GO function entries and 56 KEGG pathways. Molecular docking showed that the main ingredients can closely bind three targets (CASP3, JUN, and ESR1). Thus, *Ginger* and *Forsythia* play significant roles in the prevention and treatment of VP, and this study showed their mechanism was "multicomponent, multitarget, and multipathway" for the prevention and treatment of VP. Conclusion: We successfully predicted the active components and targets of *Ginger* and *Forsythia* for prevention and treatment of VP. This may systematically clarify its mechanism of action and provide a direction for future research.

Keywords: *Ginger*, *Forsythia*, viral pneumonia, network pharmacology, mechanism of action

Introduction

Viral pneumonia (VP) is a serious infection in the lung caused by a virus [1]. Viruses are the most common cause of respiratory tract infection [2]. It is well known that many viruses can cause pneumonia, and the most common are adenovirus, respiratory syncytial virus, metapneumovirus, parainfluenza virus, and coronavirus [3]. Viral infections are more common in children and elderly individuals [4]. The duration and severity of VP are similar to those of lung infections caused by bacteria [5]. Most patients have mild symptoms and a good prognosis, but patients with severe infections also have a risk of dying. VP mainly manifests as some symptoms of acute respiratory infection and 90% of cases are caused by acute respiratory infections. Commonly, patients with viral pneumonia present with fever, cough, headache, and myalgia [6]. There are two treatment methods clinically: symptomatic treatment and antiviral treatment. It is important to pay attention to preventive measures and prevent the spread of respiratory viruses, especially in medical institutions [7]. Viruses can rarely cause pneumonia which are most commonly caused by influenza viruses, followed by parainfluenza viruses and cytomegalovirus . There is a high risk of occurrence in spring and winter, when the spread and outbreak of an epidemic is more likely. Viral infections account for 25% to 50% of

Table 1. Prediction of possible medicinal ingredients of *Ginger* and *Forsythia*

Medicinal herbs	TCMSP MOL ID	Active ingredient	OB (%)	DL (%)
Ginger	MOL006129	6-methylgingediacetate2	48.73	0.32
	MOL008698	Dihydrocapsaicin	47.07	0.19
	MOL000449	Stigmasterol	43.83	0.76
	MOL000358	beta-sitosterol	36.91	0.75
	MOL001771	poriferast-5-en-3beta-ol	36.91	0.75
Forsythia	MOL003330	(-)-Phillygenin	95.04	0.57
	MOL003306	ACon1_001697	85.12	0.57
	MOL003322	FORSYTHINOL	81.25	0.57
	MOL003283	(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	66.51	0.39
	MOL003308	(+)-pinoresinol monomethyl ether-4-D-beta-glucoside_qt	61.2	0.57
	MOL000211	Mairin	55.38	0.78
	MOL003295	(+)-pinoresinol monomethyl ether	53.08	0.57
	MOL003290	(3R,4R)-3,4-bis[(3,4-dimethoxyphenyl)methyl]oxolan-2-one	52.3	0.48
	MOL003347	hyperforin	44.03	0.6
	MOL003348	hyperforin	44.03	0.61
	MOL003344	β-amyrin acetate	42.06	0.74
	MOL003365	Lactucasterol	40.99	0.85
	MOL003281	20(S)-dammar-24-ene-3β,20-diol-3-acetate	40.23	0.82
	MOL003305	PHILLYRIN	36.4	0.86
	MOL003315	3beta-Acetyl-20,25-epoxydammarane-24alpha-ol	33.07	0.79
	MOL000173	wogonin	30.68	0.23

Abbreviations: OB: oral bioavailability value; DL: drug similarity.

nonbacterial pneumonia cases, and compared with adults, children are more likely to develop viral infections due to their incomplete physiolo development.

Taking the network-based method of drug targeted therapy data as an example, network pharmacology is a theory of systems biology [8]. It can be used for drug target and mechanism prediction research and network analysis of biological systems, and network-based proximity allows us to predict novel drug-disease associations [9]. Research on traditional Chinese medicine can effectively use network pharmacology research methods to confirm that multiple active ingredients in traditional Chinese medicine and its compounds are used to treat complex diseases through multiple pathways, multiple links, and multiple targets. In other words, the network-based approach provides alternative tools for drug repurposing, new drug discovery, and systematic pharmacology and systematic toxicology research [10]. There are many similarities between the idea of network pharmacology and traditional Chinese medicine prescriptions that focus on multigroup distribution, multitarget, and integrated regulation. Ginger has been traditionally used to treat fever, bronchial asthma, and cough for thousands of years [11]. Ginger is also a remedy for chronic cough caused by other diseases [12]. Forsythia is traditionally used to treat fever, inflammation, gonorrhea, swelling, and erysipelas [13]. The lignin dimer from *Forsythia* root has strong antiviral effects [14]. At present, there is little research on the compatibility of *Ginger, Forsythia,* or their combination with other herbs to treat diseases, such as cough and fever, and whether the compatibility of these two herbs has changed. There are still few reports on how to determine the effectiveness of combination therapies in drug development [15].

Based on a network pharmacology approach, this study focused on the mechanism governing the effect of *Ginger* and *Forsythia* on viral pneumonia at the molecular level and attempted to elucidate the specific targets and molecular signalling pathways of *Ginger* and *Forsythia* acting on viral pneumonia. We can analyse and predict the active compounds and molecular mechanism of *Ginger* and *Forsythia* combination treatment for viral pneumonia.

Methods

Pharmacokinetic evaluation of Chemical components

The active ingredients of *Ginger* and *Forsythia* respectively were retrieved from Traditional Chinese Medicines for Systems Pharmacology Database and Analysis Platform (TCMSP,

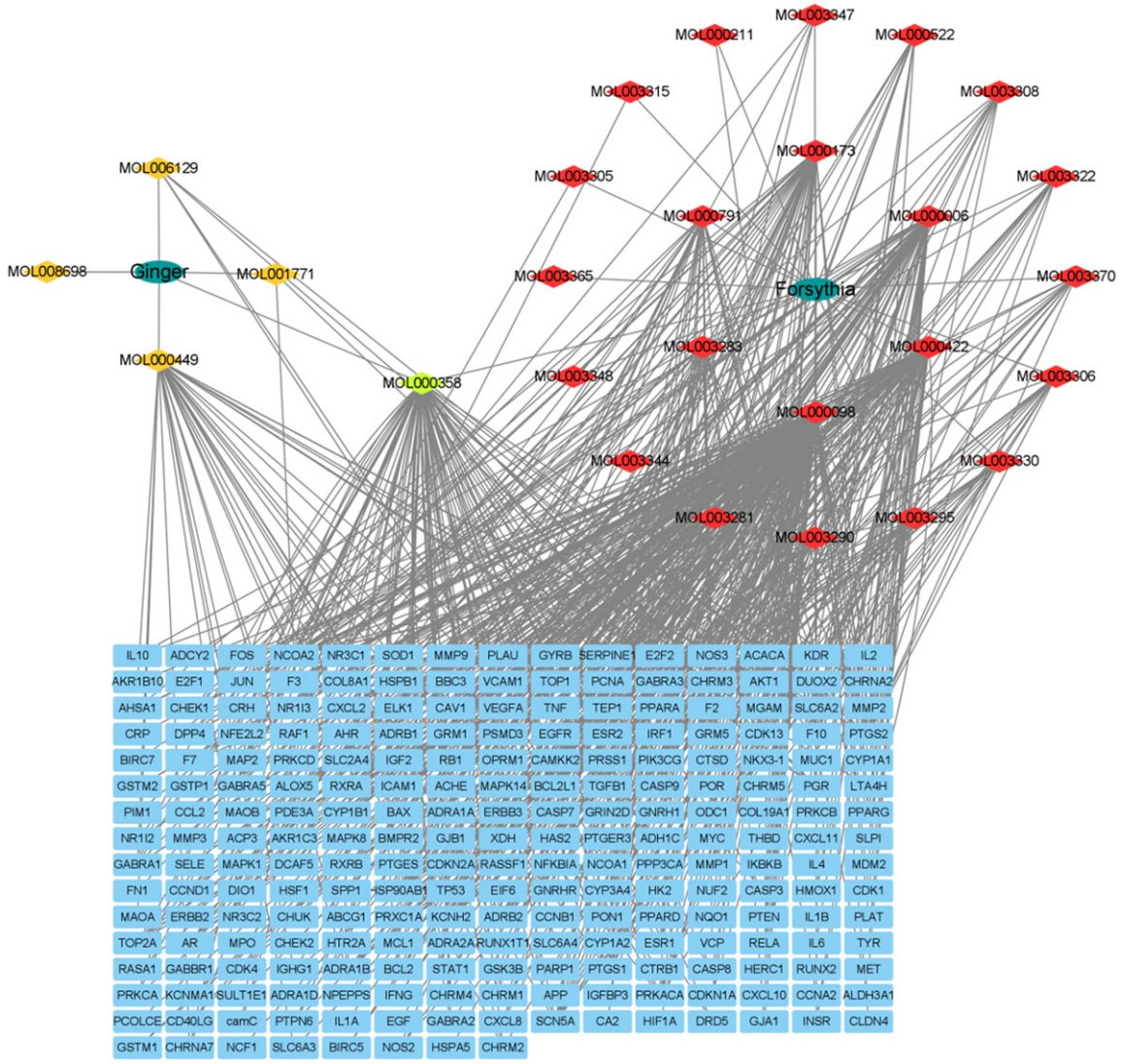

Figure 1. Venn diagram of the targets of *Ginger* and *Forsythia* in the treatment of VP.

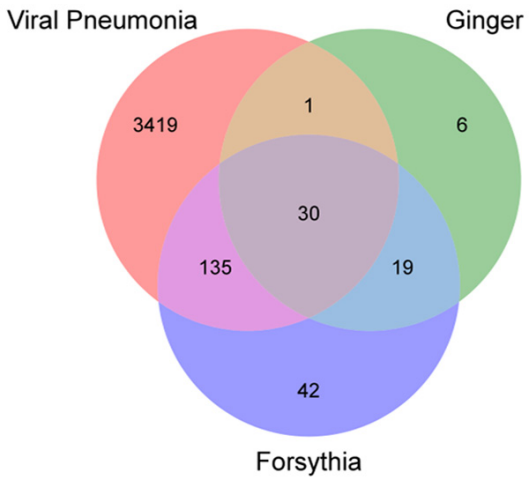

Figure 2. Visualization of the Chinese herbal compound ingredient-target network.

Potential ingredients were retained for OB > 30% and DL > 0.18 in the subsequent studies, and then the ingredients were retained after screening.

Target prediction acting on viral pneumonia

Target genes linked to the active ingredients were further investigated using TCMSP. The related targets of viral pneumonia were extracted from GeneCards (https://www.genecards.org) and OMIM online "Human Mendel Genetics" (OMIM, https://omim.org) with the query "viral pneumonia".

PPI network construction

Protein-protein interaction (PPI) data were filtered with R software and the Venn Diagram

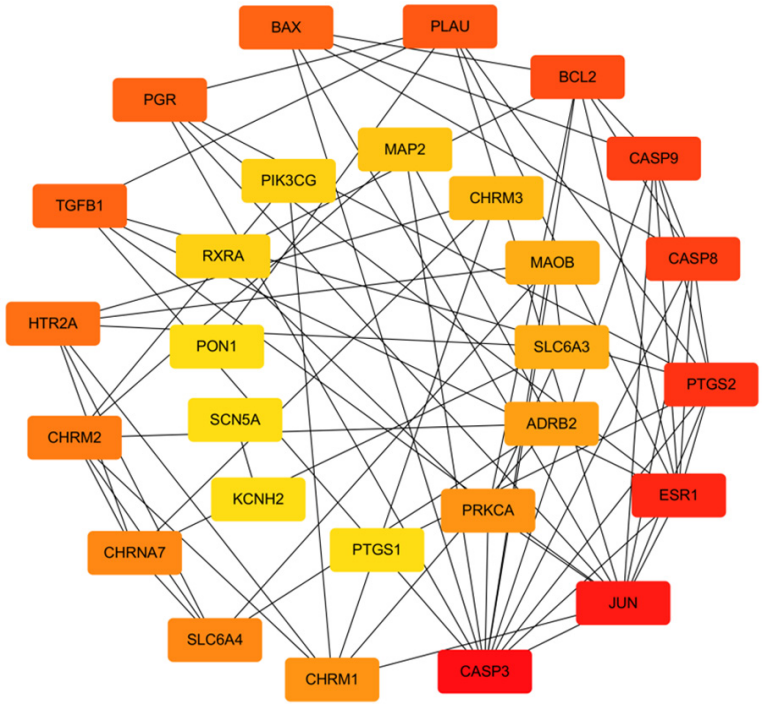

Figure 3. Interaction diagram of target proteins associated with *Ginger* and *Forsythia*.

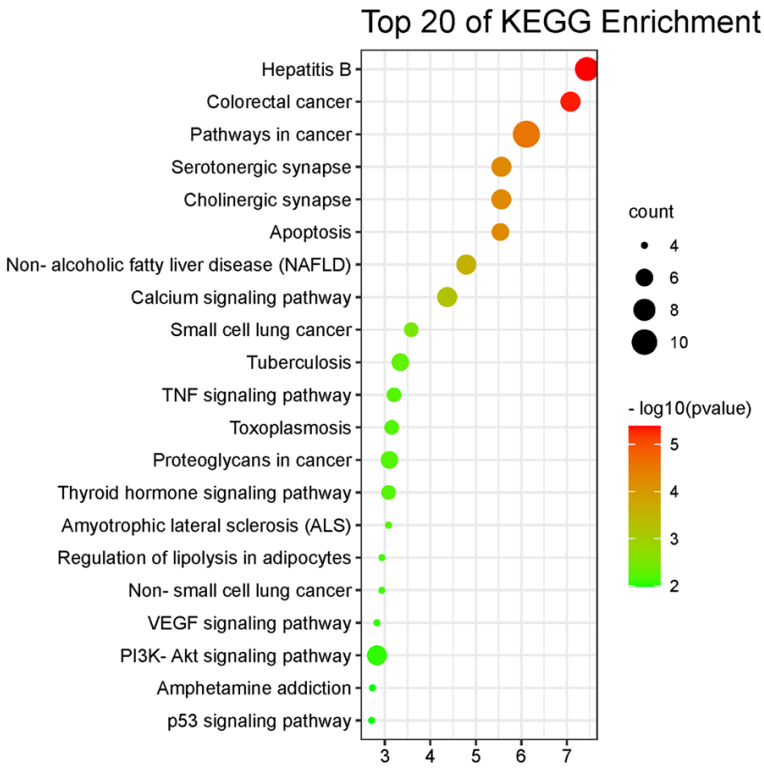

Figure 4. KEGG Pathway enrichment analysis of combined use of *Ginger* and *Forsythia*.

data package. The common target genes were selected in "Venn Diagram network construction" and were identified with the STRING database and the database was employed to construct a PPI network model with the species set to "Homo sapiens" and a confidence score of > 0.4. The complex relationships between viral pneumonia, *Ginger*, *Forsythia*, active compounds, and target genes were visualized using Cytoscape software and a target protein interaction map of *Forsythia* suspensa combination was generated.

Enrichment analysis of GO function and KEGG pathway

The crucial targets screened in "PPI network construction" were used for GO and KEGG pathway analyses and analysed with the DAVID database (https://david.ncifcrf.gov). After sorting, screening, and importing (http://www.bioinformatics.com.cn/), GO functional enrichment analysis and KEGG channel enrichment analysis were performed.

Molecular docking

Molecular docking was used to predict the interaction of the targets with the compounds. The 3D structure of the protein was downloaded from the RCSB PDB database (https://www.rcsb.org/). The 2D structure of the molecular ligands was downloaded from the TCMSP database. The protein structure was constructed using AutoDock software matched the 3D structure of the compound, and the results were visualized using PyMol 2.2.0 software.

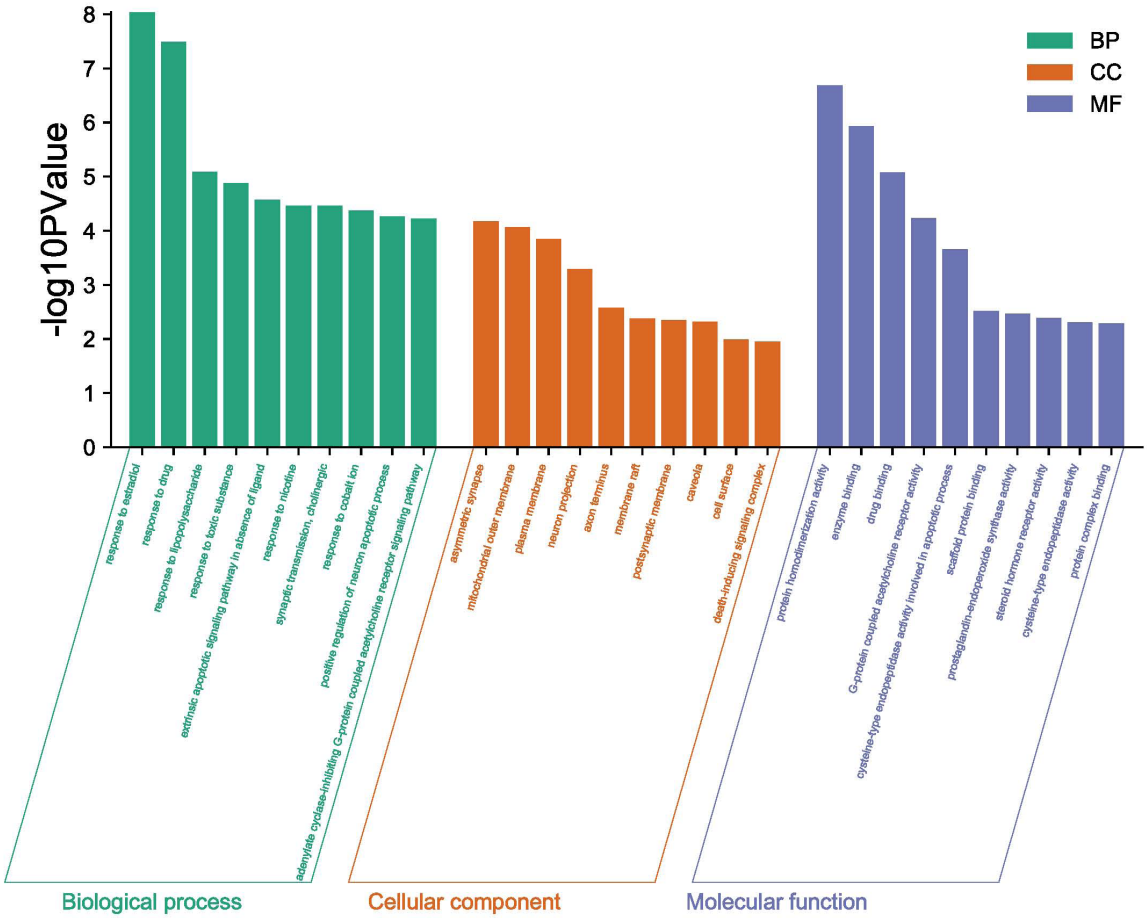

Figure 5. GO molecular functional enrichment analysis of combined use of *Ginger* and *Forsythia.*

Results

Selection of ingredients and targets

In total, 75 possible active targets in *Ginger* were retrieved and 510 possible active targets in *Forsythia* were retrieved. After eliminating redundancies, 56 unique values and 226 unique values were retained respectively. Ultimately, 28 kinds of effective ingredients in *Ginger* and *Forsythia* were collected after retrieval in the TCMSP database and screening with the criteria of OB > 30% and DL > 0.18 (**Table 1**).

Retrieving disease target information

Viral pneumonia-associated targets were obtained from the GeneCards and OMIM databases. There were 3617 targeted parameters related to diseases. Duplicates were deleted, and 3585 disease targets related to viral pneumonia were retained.

Venn diagram network construction

The 282 possible active ingredient targets selected from 2.1 were converted into relative gene names with the UniProt database (https://www.uniprot.org/), and the active ingredient target genes and 30 common target genes of disease target genes were mapped with an online mapping website (http://www.bioinformatics.com.cn/) to obtain the targets of *Ginger* and *Forsythia* for treating viral pneumonia and are shown as a Venn diagram (**Figure 1**). The components and targets of the two traditional Chinese medicines were imported into Cytoscape3.7.1 software to construct the basic adjustment and visualization of the traditional Chinese medicine compound-target network (**Figure 2**).

Table 2. Results of *Ginger* and *Forsythia* molecular docking energy score

Compound		Binding Energy/(kcal·mol^{-1})		
		CASP3	JUN	ESR1
Ginger	6-methylgingediacetate2	-3.83	-3.05	-3.25
	Dihydrocapsaicin	-4.74	-4.75	-5.2
	Stigmasterol	-7.48	-5.5	-8.45
Forsythia	(-)-Phillygenin	-6.47	-5.79	-7.28
	ACon1_001697	-6.64	-5.08	-7.47
	FORSYTHINOL	-6.57	-4.94	-6.09

PPI network construction

There were two genes in the free state when mapping that had been removed (**Figure 2**). With a confidence score of > 0.4 selected, the network of PPI relationships had 30 nodes and 71 edges. With the MCC algorithm in the CytoHubba plug-in, each node in the PPI network was analysed, and the top 5 targets with a high-node degree were considered as key targets with significance and were coloured. The top 5 targets of *Ginger* and *Forsythia* against viral pneumonia were CASP3, JUN, ESR1, PTGS2, and CASP8 (**Figure 3**).

GO function and KEGG enrichment analysis

To clarify the mechanisms of *Ginger* and *Forsythia* aganist viral pneumonia systematically, Gene Ontology (GO) analysis and KEGG pathway enrichment of the 30 target genes and effective components selected in 2.3 were performed in the DAVID database (**Figures 4**, **5**). In total, 186 enriched GO terms and 56 pathways were identified (P<0.05). The top 10 GO functional categories in biologic process, cellular component, and molecular function and 20 significant pathways were selected and presented. In the active ingredients of *Ginger* and *Forsythia*, GO enrichment results showed that the main biologic processes involved were protein phosphatase 2A binding, steroid binding, axon, endoplasmic reticulum, cellular response to dexamethasone stimulus, homeostasis of the number of cells within a tissue, etc. KEGG pathway analysis results showed that *Ginger* and *Forsythia* exerted overall regulatory effects through a variety of pathways, including Regulation of actin cytoskeleton, Prostate cancer, NF-kappa B signaling pathway, ErbB signaling pathway, and Focal adhesion.

Molecular docking of active compounds and core targets

CASP3, JUN, and ESR1 were selected as successful representations of the receptor protein according to the PPI network. The top 3 active components with an OB value were separately selected as ligands. The value of the vina score indicates the binding activity between a compound and a protein. A more negative the vina score indicates that the compound binds to the protein with greater stability (**Table 2**). Stigmasterol (MOL000449) and ACon1_001697 (MOL003306) were the most stable active ingredients in CASP3 binding (**Figure 6A**, **6B**). Meanwhile, Stigmasterol (MOL000449) and (-)-Phillygenin (MOL003330) showed strong associations with JUN (**Figure 6C**, **6D**). Stigmasterol (MOL000449) and ACon1_001697 (MOL003306) showed a compact binding pattern with ESR1 (**Figure 6E**, **6F**).

Discussion

In this study, we aimed to use a network pharmacology strategy to predict the target and mechanism of the combination of *Ginger* and *Forsythia* for the treatment of viral pneumonia. In summary, the combined use of *Ginger* and *Forsythia* can affect various diseases in multiple ways. KEGG pathway enrichment analysis identified many signalling pathways, such as Regulation of actin cytoskeleton, Prostate cancer, NF-kappa B signaling pathway, ErbB signaling pathway, and Focal adhesion. According to relevant reports, substances such as phosphatase can affect chronic obstruction and other lung diseases [16]. BAL fluid ILC2s with elevated TSLP levels in asthmatic patients are associated with steroid resistance [17]. Protein misfolding in the endoplasmic reticulum can affect diseased lung tissue [18]. Related reports indicate that pathogens manipulate actin to promote actin-based movement and coordinate movement and cell-to-cell transmission [19]. Protein phosphatase-2A (PP2A) is the major serine-threonine eukaryotic phosphatase, and accounts for up to 1% of total cellular protein [20, 21]. PP2A activity could be manipulated to prevent the inflammatory and proteolytic responses that occur in the lung [22].

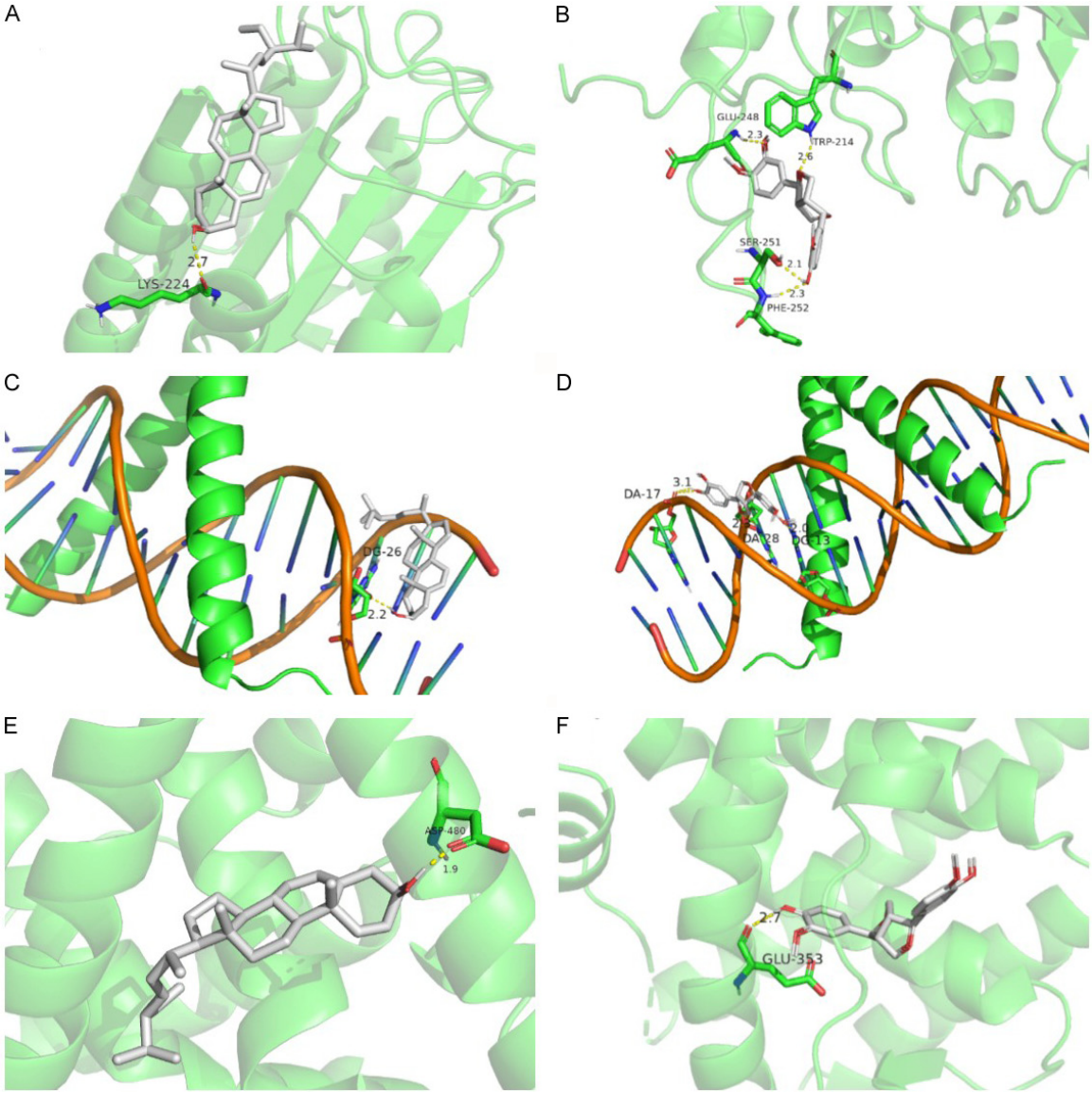

Figure 6. The protein-ligand of the docking simulation. The three core targets (CASP3, JUN, ESR1) are docked with two compounds.

Conclusion

In this study, we predicted that the combination of *Ginger* and *Forsythia* can improve viral pneumonia, which provides ideas and a basis for future experiments.

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Disclosure of conflict of interest

None.

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