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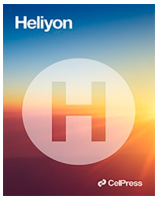

Research article

Utilizing network pharmacology and experimental validation to investigate the underlying mechanism of Denglao Qingguan decoction against HCoV-229E

Yajing Xue [a], Xuejun Cai [b], Yutao Wang [b], Li Ban [d], Manxue Mei [e], Shuqi Chen [f], Qihua Xu [a], Boqian Chen [g], Shuhua Liang [b], Xinhua Wang [b,c,*]

[a] *Artemisinin Research Center, Guangzhou University of Chinese Medicine, Guangzhou, China*
[b] *State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China*
[c] *Institute of Integration of Traditional and Western Medicine, Guangzhou Medical University, Guangzhou, China*
[d] *The Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, China*
[e] *College of Traditional Chinese Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China*
[f] *Guangdong Provincial Hospital of Chinese Medicine, Guangzhou, China*
[g] *Shenzhen Traditional Chinese Medicine Hospital, Shenzhen, Guangdong, China*

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ABSTRACT

Background: Denglao Qingguan decoction (DLQGD) has been extensively utilized for the treatment of colds, demonstrating significant therapeutic efficacy. Human Coronavirus 229E (HCoV-229E) is considered a crucial etiological agent of influenza. However, the specific impact and underlying mechanisms of DLQGD on HCoV-229E remain poorly understood.

Methods: Active ingredients and targets information of DLQGD were collected from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), literature search, and Swiss ADEM database. The Genecard database was used to collect HCoV-229E related targets. We built an "ingredient-target network" through Cytoscape. Protein - Protein interaction (PPI) networks were mapped using the String database. The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) were enriched using the DAVID database. Then, we used molecular docking techniques to verify the binding activity between the core compounds and the core gene targets. Finally, in vitro experiments were conducted to validate DLQGD's antiviral activity against HCoV-229E and assess its anti-inflammatory effects.

Results: In total, we identified 227 active components in DLQGD. 18 key targets involved in its activity against HCoV-229E. Notably, the core active ingredients including quercetin, luteolin, kaempferol, β-sitosterol, and apigenin, and the core therapeutic targets were CXCL8, RELA, MAPK14, NFKB1, and CXCL10, all associated with HCoV-229E. KEGG enrichment results included IL-17 signaling pathway, Toll-like receptor signaling pathway, RIG-I-like receptor signaling pathway and so on. The core active ingredients and the core therapeutic targets and Human Aminopeptidase N (ANPEP) all showed good binding activity by molecular docking verification. In vitro, DLQGD exhibited anti-HCoV-229E activity and anti-inflammatory effects.

Conclusion: Our study suggests that DLQGD has both effects of anti-HCoV-229E and anti-inflammatory. The core active ingredients (quercetin, luteolin, kaempferol, β-sitosterol,

* Corresponding author. Institute of Integration of Traditional and Western Medicine, Guangzhou Medical University, Guangzhou, China.
E-mail address: xinhuaw@gzhmu.edu.cn (X. Wang).

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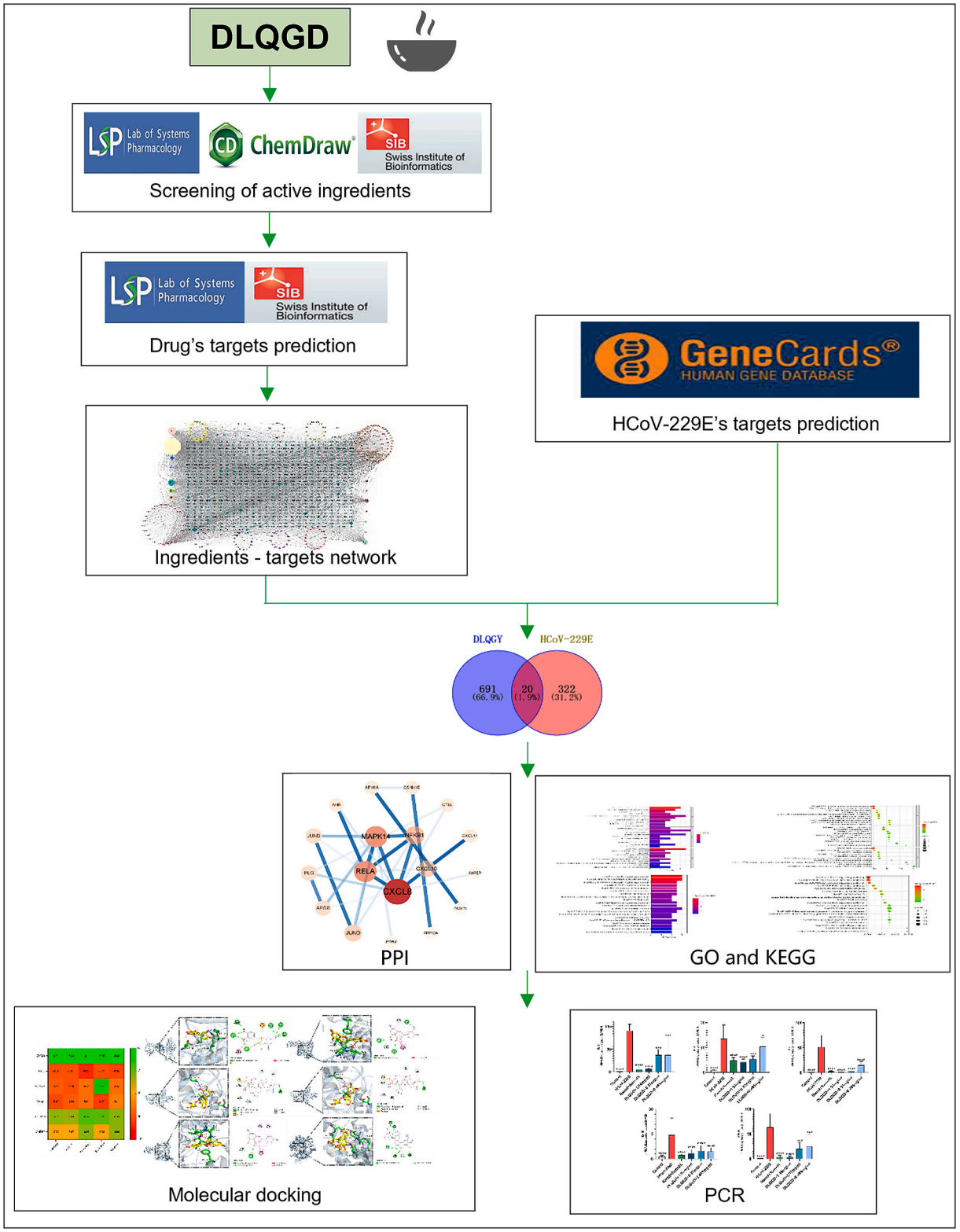

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Fig. 1. Conceptual framework of this study. First, we collected active ingredients and targets information of DLQGD, HCoV-229E related targets. Then we built an "ingredient-target network" through Cytoscape. Second, we used the Venny 2.1 online platform to obtain the intersectional targets of DLQGD in HCoV-229E. Third, we uploaded the common targets to the String 11.5 Database to construct the PPI network. Fourth, we used the KEGG and GO analysis to predict the potential mechanism of DLQGD in the treatment of HCoV-229E. Fifth, we verified the binding activity between the core compounds and the core gene targets by molecular docking techniques. Finally, we validated DLQGD's antiviral and anti-inflammatory activity on HCoV-229E in vitro.

apigenin) and the core therapeutic targets (CXCL8, RELA, MAPK14, NFKB1, CXCL10) may play key roles in the pharmacological action of DLQGD against HCoV-229E.

1. Introduction

Human coronaviruses (HCoVs) are a prevalent cause of upper respiratory tract infections in humans, with seven identified strains, namely 229E, NL63, HKU1, OC43, SARS-CoV, MERS-CoV, and SARS-CoV-2. These viruses have become global health concerns due to their varying severity, ranging from mild upper respiratory tract infections or pulmonary infections [1,2] to severe pneumonia, and in some cases, leading to fatalities [3,4]. The vulnerability of certain populations, such as immunocompromised individuals, infants, and the elderly, further highlights the need for effective management and preventive measures [5]. Coronaviruses encompass four subtypes, alpha, beta, gamma, and delta, of which 229E and OC43 are the prototype strains (ancestral strains) of the alpha and beta subtypes, respectively, and are representative strains of the two subtypes, which can cause 15%–29% of common colds [5].

Main drugs in development against coronaviruses are monoclonal antibodies and direct antivirals, including proteases, helicases, polymerase inhibitors, along with the immunomodulatory drugs like interferons and glucocorticoids and the broad-spectrum antiviral nucleoside analogue ribavirin is also being investigated [6]. Remdesivir, a nucleoside analog, has been shown to have inhibitory effects on SARS-CoV and MERS-CoV, but due to its strong toxicity of nucleoside analogs, it cannot be used for the treatment of mild patients [7–9]. Paxlovid (nirmatrelvir plus ritonavir) is a novel oral drug for the treatment of COVID-19. Nirmatrelvir serves as a major viral protease (Mpro) inhibitor targeting SARS-CoV-2. Concurrently, ritonavir as a CYP3A inhibitor, effectively slowing down the metabolism of nirmatrelvir, which consequently enhances its therapeutic efficacy. However, as a new drug, the drug interaction (DDI) [10], adverse reactions [10] and drug resistance [11,12] of Paxlovid have attracted the attention of researchers. Currently, screening potential drugs and providing laboratory data support for their clinical applications is still one of the current research priorities of Traditional Chinese Medicine (TCM) in the treatment of coronavirus pneumonia.

DLQGD is composed of 14 herbs, including Flos Lonicerae, Flo Chrysanthem, Herba Taxilli, Herba Taraxaci, Herba Menthae, Pogostemon Cablin (Blanco) Benth, Radix Glycyrrhizae, Semen Armeniacae Amarum, Semen Juglandis, Radix Platycodonis, Rhizoma Imperatae, Pericarpium Citri Reticulatae, and Fructus Hordei Germinatus, Ficus simplicissima Lour. This recipe comes from Wuwei Disinfection Drink and Ganlu Xiaodu micropills to clear heat and detoxify, also the dampness and heat. It is widely used to prevent and treat the fever, cough, sore throat, and so on caused by influenza. Among them, Flos Lonicerae [13,14], Herba Taraxaci [15], Radix Glycyrrhizae [16–18], Herba Taxilli [19], and Pogostemon Cablin (Blanco) Benth [20,21] have good inhibitory activity against influenza virus.

TCM compounds possess the characteristics of multi-component and multi-target action [22]. Network pharmacology maps drug targets and disease-related targets into biomolecular networks, offering insights into drug action on diseases, aiding drug discovery and development. By analyzing complex and multilayered networks of various kinds, it can screen active pharmaceutical ingredients in TCM compounds, key targets, and their potential mechanisms for treating diseases [23]. In this study, we explored the potential mechanism of DLQGD in the treatment of HCoV-229E based on network pharmacology and molecular docking. In addition, we verified the antiviral activity and anti-inflammatory effect of DLQGD on HCoV-229E in vitro. The workflow is shown in Fig. 1.

2. Materials and methods

2.1. Screening of active ingredients and targets in DLQGD

DLQGD is comprised of a combination of 14 different herbs, and as traditional Chinese herbal medicines possess complex composition and functionalities. So we employed a rigorous screening process for active components and target information using the TCMSP(http://tcmspw.com/tcmsp.php) [24]. It provides essential data such as oral bioavailability (OB) of medicinal chemical components, drug likeness (DL), targets. High OB and DL are the key indicators of bioactive molecules [25,26]. In this study, all the active ingredients we screened met the conditions of OB $\geq$ 30% and DL $\geq$ 0.18.

Herba Taraxaci and Ficus simplicissima Lour are not included in the TCMSP database. We collected the constituents of Herba Taraxaci by conducting a thorough review of the relevant literature, and obtained the SMILES format through Pubchem (https://pubchem.ncbi.nlm.nih.gov/). Jun Cheng separated and identified 70 compounds of Ficus simplicissima Lour by various chromatographic and spectroscopic methods [27], we draw their chemical structures by using ChemDraw 20.0, and save as SMILES format. We uploaded the SMILES format of all the identified compounds to the SwissADME platform (http://www.swissadme.ch/) [28] platform for toxic pharmacokinetic (ADME) analysis. Compounds were considered active if they met both gastrointestinal absorption as "High" and two or more drug-like principles as "Yes." Compounds not meeting these criteria were still included if supported by relevant

literature. Additionally, using the Swiss Target Prediction database [28,29], for "*Homo sapiens*," we screened target information of active compounds with $P > 0$ to explore potential interactions.

Finally, the screened targets are normalized by the UniProt (https://www.uniprot.org/) [30] database for gene names.

2.2. Selection of HCoV-229E related targets

We searched the keyword "HCoV-229E" on the Genecard database (https://www.genecards.org/) [31] to obtain the related targets. Then standardized names are implemented through the UniProt database (https://www.uniprot.org/).

2.3. Construction of the drug-active ingredient-potential target network

Imported the corresponding relationship between the active ingredients and targets of DLQGD into Cytoscape3.8.2 (https://cytoscape.org/) [32]to construct a drug-active ingredient-potential target network. In this network, the degree values of each active ingredient were calculated.

2.4. Obtaining of the key targets of DLQGD in HCoV-229E

The Venny 2.1 online platform (https://bioinfogp.cnb.csic.es/tools/venny/) was used to obtain the intersectional targets of DLQGD in HCoV-229E, as the key therapeutic targets.

2.5. Construction of the protein-protein interaction (PPI) network

We uploaded drug and disease intersection targets to the String 11.5 Database (https://string-db.org/) to construct the PPI network, revealing potential interactions and mechanisms between the drug and the disease. Upload drug and disease intersection targets to STRING 11.5 database. The confidence scores were limited to $\geq$0.4. Set species as "*Homo sapiens*". Hide the disconnected nodes in the network and exported export TSV files. Then we built a PPI network using Cytoscape3.8.2 software. And the degree values of each key targets were calculated.

2.6. GO enrichment analysis and KEGG pathways enrichment analysis

We conducted GO functional analysis and KEGG pathway enrichment for the key targets using the DAVID 6.8 database (https://david.ncifcrf.gov/). The analysis was performed with "OFFICIAL-GENE-SYMBOL" as the attribute and "*Homo sapiens*" as the species. The threshold for entry screening was set at $P < 0.05$. Subsequently, visual charts were generated using the Weishengxin platform (https://www.bioinformatics.com.cn/) to present the enriched biological processes and pathways associated with the key targets.

2.7. Molecular docking

Human aminopeptidase N (APN/CD13/ANPEP) is the receptor for HCoV-229E, and specific binding to ANPEP is the first step for HCoV-229E to invade the host [33,34]. We used molecular docking to verified the binding activity between core compounds and core targets, ANPNP. The target protein receptor structure was downloaded from RCSB PDB (https://www.rcsb.org/). The structures of active compound components (ligands) were obtained from the Pubchem (https://pubchem.ncbi.nlm.nih.gov/) platform. Auto Dock was used for molecular docking, and calculating the binding energy value [35]. Finally, PyMOL was used for visualization.

2.8. In vitro validation

2.8.1. Cell and virus cultures

The human hepatocellular carcinoma cell line huh-7 was cultured in dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). HcoV-229E was stored at −80 °C in our laboratory for future use. The virus was cultured in huh-7 cells, and the half-infectious dose of the tissue was calculated by the Reed-Muench method.

2.8.2. Cytotoxicity assay

We used to the methyl thiazole tetrazolium (MTT) assay to detect the cytotoxic effect of DLQGD (Guangdong Denglao herbal tea Pharmaceutical Group, China). Huh-7 cells were seeded on the 96-well plate in DMEM (Gibco, USA) culture medium with 10% FBS. After 24 h culture, different concentrations of DLQGD culture medium prepared in DMEM were added to the cells. After incubation at 37 °C for 48 h, the supernatant was removed and incubated with a PBS solution containing 1 mg/mL MTT (Bioss, China). After 37 °C incubation 4 h, the supernatant was removed and 100 μl DMSO (MP, America) was added into each well to dissolve formazan crystals. The absorbance at 490 nm was measured by a Multiskan Spectrum microplate reader (Bio Ttek, America), and the median lethal concentration (TC_{50}) was calculated.

2.8.3. In vitro antiviral activity test

Huh-7 cells was cultured in a 96-well plate and inoculated at 37 °C with 5% CO_2. After 24 h, the cells were infected with 100

TCID50 of HCoV-229E for 2 h at 37 °C. The DLQGD was diluted with DMEM to 7.914, 3.957, 1.979, 0.989, 0.495, 0.247, 0.124, 0.062, 0.031 mg/ml. After 2 h, removed the viral inoculum from cells, and added 100 μl DLQGD solution into cells. After incubation at 37 °C for 48 h, 20 μl of Cell Counting Kit-8 (CCK-8) was added to each culture well. After 37 °C incubation for 0.5–2 h, the absorbance at 490 nm was read by a microplate reader. The concentration that inhibited 50% virus-induced cytopathic effect was determined as IC_{50}.

2.8.4. Determination of inflammatory cytokine expression by real-time PCR

Huh-7 cells was seeded on the 6-well plate and inoculated at 37 °C with 5% CO_2. After 24 h, the cells were infected with 100 TCID50 of HCoV-229E for 2 h at 37 °C. The DLQGD was diluted with DMEM to 1.979, 0.989, 0.495 mg/ml. After 2 h, removed the viral inoculum from cells, and added 100 μl DLQGD solution into cells. After incubation at 37 °C for 48 h. Then, the total RNA of each group was extracted according to the RNA reagent (Invitrogen, USA). The RNA was quantitatively reverse transcribed using HiScript® III RT SuperMix for qPCR kit (Vazyme, China). The cDNA samples were subjected to RT-PCR detection by ChamQ Universal SYBR qPCR Master Mix (Vazyme, China). PCR data were analyzed using the detection system (Analytik Jena AG, Germany). The relative expression of PCR products was calculated using the $2^{-\Delta\Delta Ct}$ method. Primer design was conducted for the GAPDH, IL-8, IP-10, IL-6, IL-1β, and TNF-α genes (Table 1).

2.9. Statistical analysis

GraphPad Prism 8.0 software was conducted for statistical analysis. One-way ANOVA was used for comparison among multiple groups. $P < 0.05$ indicates statistical significance.

3. Results

3.1. Prediction of active ingredients and targets in DLQGD

DLQGD consists of 14 Chinese herbal medicines. After removing duplicates from the active ingredients collected through TCMSP and literature search, a total of 227 unique active ingredients were obtained. Among these, 18 compounds were found to be present in two or more herbal sources, indicating their shared presence in different herbs. In addition, after eliminating duplicates, a total of 711 potential drug targets were identified through data retrieved from TCMSP and the SwissADME database.

3.2. HCoV-229E related targets screening

A total of 342 HCoV-229E related gene targets were retrieved from the Genecard database.

3.3. Construction of a drug-active ingredient-potential target network

The collected drug active ingredients, targets and gene names were sorted and imported into the Catoscape3.8.2, and a network diagram of traditional Chinese medicine ingredients-potential targets was drawn, with 953 nodes and 6584 edges (Fig. 2). The top five active ingredients with potential anti-inflammatory and antiviral effects, based on their degree values, are quercetin, luteolin, kaempferol, β-sitosterol, apigenin (Table 2). Therefore, these compounds are likely to play a pivotal role in the pharmacological action of DLQGD, and we have identified them as the core ingredients.

3.4. Selection of the intersection of the drug targets and disease targets

Import the obtained drug targets and disease targets into the Venny 2.1.0 platform, and the Venn diagram is shown in Fig. 3. There are 20 shared targets between DLQGD and HCoV-229E.

Table 1
Primers.

Target Gene	Direction	Sequence (5′ – 3′)
TNF-α (human)	Forward	AACATCCAACCTTCCCAAACG
	Reverse	GACCCTAAGCCCCCAATTCTC
IL-1β(human)	Forward	GCACGATGCACCTGTACGAT
	Reverse	AGACATCACCAAGCTTTTTTGCT
IL-6 (human)	Forward	CGGGAACGAAAGAGAAGCTCTA
	Reverse	CGCTTGTGGAGAAGGAGTTCA
IP-10 (human)	Forward	GAAATTATTCCTGCAAGCCAATTT
	Reverse	TCACCCTTCTTTTTCAT-TGTAGCA
IL-8 (human)	Forward	CTTGGTTTCTCCTTTATTTCTA
	Reverse	GCACAAATATTTGATGCTTAA

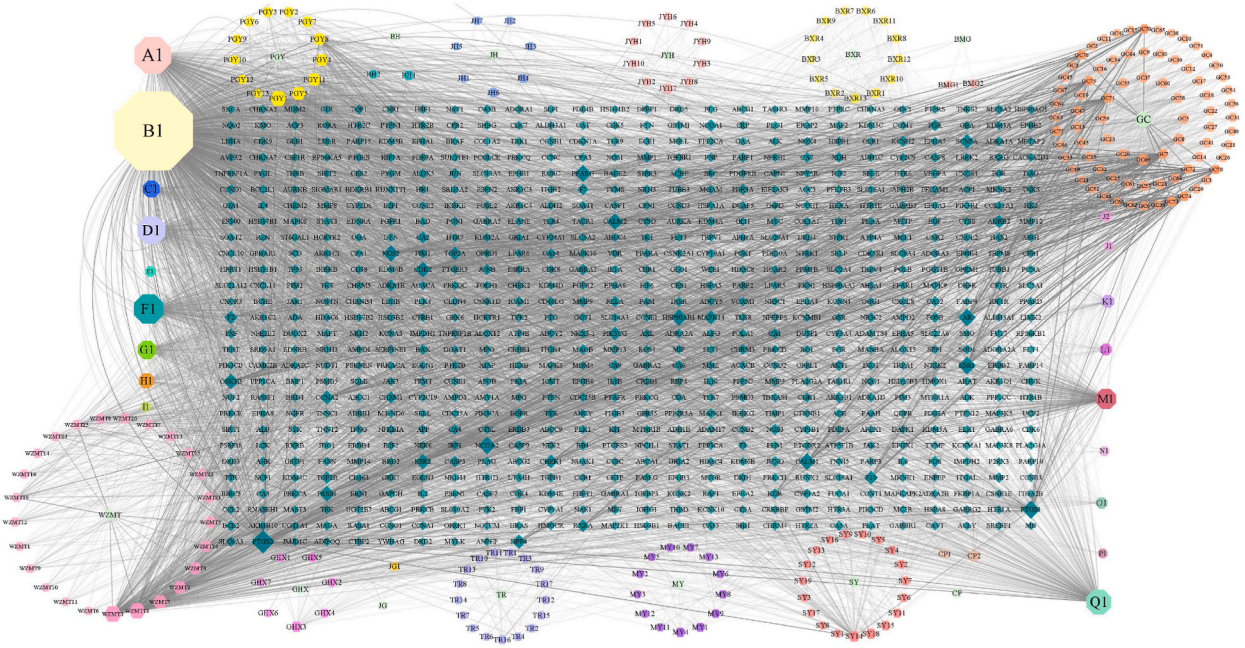

Fig. 2. Ingredient-target network. The elipse represent herbs; the exagon and octagon represent active ingredients, besides octagon represent common components of different herbs; the diamonds represent potential targets; the line between two nodes represents the interaction.

Table 2
The degree value of the core ingredients.

Mol/CAS ID	Compound name	Degree value	Name in the network
MOL000098	Quercetin	924	B1
MOL000006	Luteolin	365	A1
MOL000422	Kaempferol	269	F1
MOL000358	β-sitosterol	246	D1
520-36-5	Apigenin	210	Q1

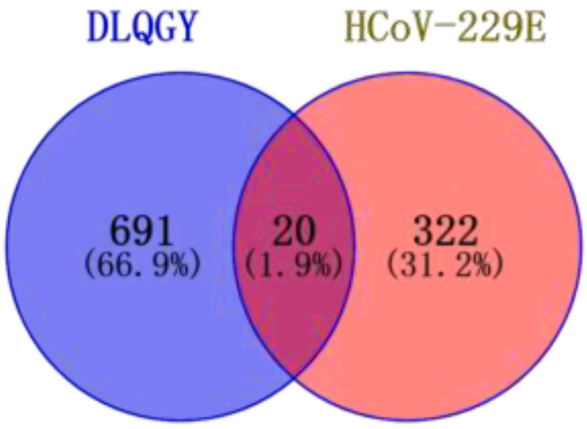

Fig. 3. Venn diagram. A total of 711 and 342 targets of DLQGD and HCoV-229E, respectively, and they shared 20 targets.

3.5. Network topology analysis of potential therapeutic targets

The PPI network was displayed by Cytoscape (Fig. 4), with 18 nodes and 30 edges. These 18 intersecting genes are predicted as the key targets. The color, size and degree of the target points are positively correlated, as are the thickness and degree of the lines. The transparency of points and lines is negatively correlated with the degree value. The topological analysis results revealed that the five

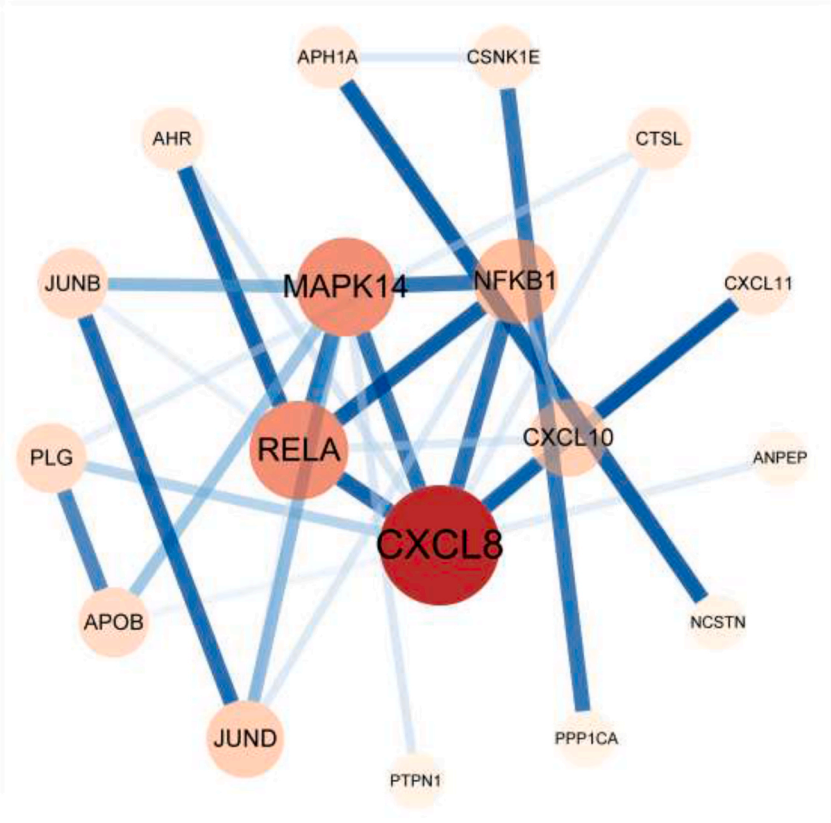

Fig. 4. PPI network of DLQGD-HCoV-229E common targets.

most significant targets were CXCL8 (IL-8), RELA (p65), MAPK14, NFKB1, and CXCL10 (IP-10) (Table 3), identifying them as the core targets.

3.6. GO analysis and KEGG pathway enrichment analysis

Bioinformatic analysis of 18 key targets was performed using DAVID 6.8. GO enrichment analysis yielded a total of 77 results, including 49 BP, 16 CC, and 12 MF. A total of 52 pathways were obtained by KEGG pathway enrichment analysis. The main biological processes (BP) involved cellular response to lipopolysaccharide, inflammatory response, cellular response to tumor necrosis factor, response to muscle stretch, chemotaxis, positive regulation of transcription from RNA polymerase II promoter, inflammatory response, regulation of cell proliferation, chemokine-mediated signaling pathway, circadian regulation of gene expression, neutrophil chemotaxis. The GO functional analysis revealed that CC (Cellular Component) annotations associated with the key targets included transcription factor complex, extracellular region, early endosome, extracellular space, transcription factor AP-1 complex, glutamatergic synapse, NF-kappaB complex, gamma-secretase complex, chromatin, and endocytic vesicle lumen. The molecular functions (MF) involved enzyme binding, CXCR chemokine receptor binding, transcription coactivator binding, chemokine activity, heparin binding, transcription regulatory region sequence-specific DNA binding, CXCR3 chemokine receptor binding, transcription factor activity, sequence-specific DNA binding.

KEGG pathway analysis results included IL-17 signaling pathway, Toll-like receptor signaling pathway, RIG-I-like receptor signaling pathway, TNF signaling pathway, Chemokine signaling pathway, Osteoclast differentiation, Cellular senescence, Epithelial

Table 3
The degree value of the core targets.

Target name	Uniprot ID	Degree value
CXCL8	P10145	10
RELA	Q04206	7
MAPK14	Q16539	7
NFKB1	P19838	5
CXCL10	P02778	4

cell signaling in *Helicobacter pylori* infection, Influenza A, Pertussis, Chemokine signaling pathway.

Used the Weishengxin platform to draw visual charts to visually analyze the channels in the top 20 of KEGG (Fig. 5–1A, B) and the top 10 of GO (Fig. 5–2A, B). The abscissa length of the bar graph represents the number of genes enriched in the pathway, and the ordinate is the name of the pathway. In the bar graph, the intensity of red color corresponds to the degree of gene enrichment, where a deeper red shade indicates a higher degree of enrichment. Similarly, in the bubble chart, the intensity of the red color represents the degree of enrichment, with a darker red hue indicating a higher level of enrichment. In the visual charts, the size of each bubble corresponds to the number of genes enriched in the pathway. Larger bubbles indicate a higher number of enriched genes, while smaller bubbles signify a lower number of enriched genes.

3.7. Molecular docking

Molecular docking was performed to investigate the interactions between the core compounds (quercetin, luteolin, kaempferol, β-sitosterol, apigenin) and the core gene targets (CXCL8, RELA, CXCL10, NFKB1, MAPK14), as well as the receptor ANPEP of HCoV-229E in the human host. The docking process allowed us to calculate the binding affinity of each ligand (compound) to its respective receptor (protein) (Fig. 6–1). In general, the score is lower than -5.0 kcal·mol^{-1} indicates good binding activity, the score lower than

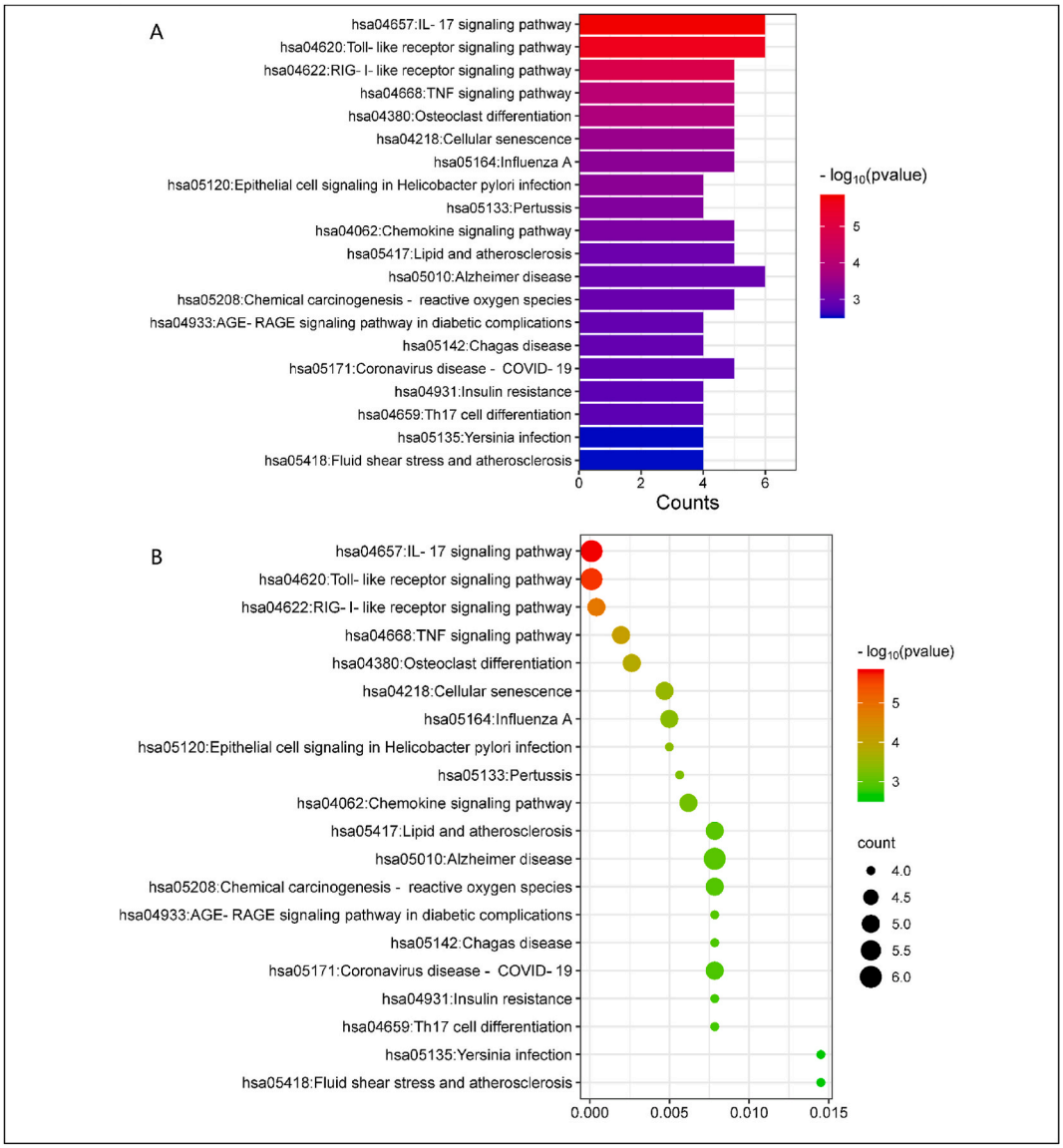

Fig. 5–1. KEGG pathways enrichment analysis of DLQGD-HCoV-229E common targets. A: The bar with color gradient chart of the KEGG pathways enrichment analysis ($P < 0.05$); B: The enrichment dot bubble chart of the KEGG pathways enrichment analysis ($P < 0.05$).

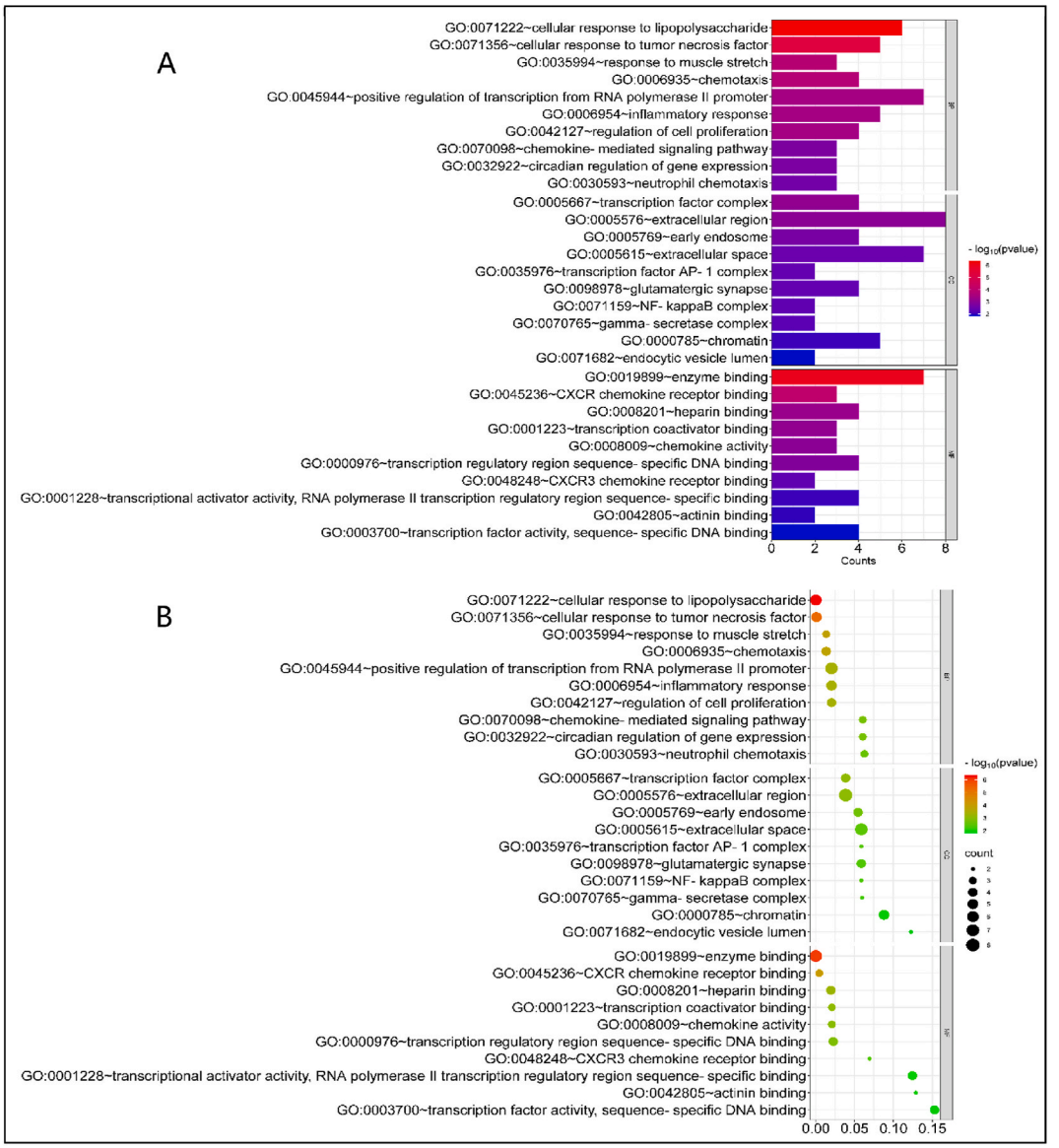

Fig. 5–2. GO enrichment analyses of DLQGD-HCoV-229E common targets. A: The bar with color gradient chart of GO enrichment analysis ($P < 0.05$); B: The enrichment dot bubble chart of the GO enrichment analysis ($P < 0.05$).

-7.0 kcal·mol^{-1} indicates strong binding activity [36]. The results showed that the core ingredients (ligand) determined by network pharmacology method had good binding ability with the protein receptor corresponding to the core targets (receptor) and ANPEP. The visualization results of PyMOL software showed that the drug ligand had strong binding activity with the protein receptor. Quercetin and β-sitosterol had strong interaction with NFKB1 (Fig. 6–2 A, D); luteolin, kaempferol and apigenin had strong interaction with RELA (Fig. 6–2 B, C, E); β-sitosterol had strong binding activity with ANPEP (Fig. 6–2 F). ANPEP is the receptor of HCoV-229E in the human host, and the five core compounds all had good interaction with ANPEP. All the results suggest that the core components of species may be realized through CXCL8, RELA, CXCL10 (IL-10), NFKB1 and MAPK14.

3.8. In vitro validation

3.8.1. In vitro antiviral activities of DLQGD against HCoV-229E

The results of cytotoxicity assay showed that the TC_{50} of DLQGD against Huh7 cells was (8.295 ± 3.110) mg·mL^{-1} in vitro (Fig. 7 A). The results of the antiviral assay showed that the IC_{50} of DLQGD against HCoV-229E in vitro was (0.820 ± 0.166) mg·mL^{-1} (Fig. 7 B). The selection index (SI, SI = TC_{50}/IC_{50}) of the drug calculated by the average of the above two indicators was 10.122, indicating that the antiviral effect of the drug was obvious in vitro.

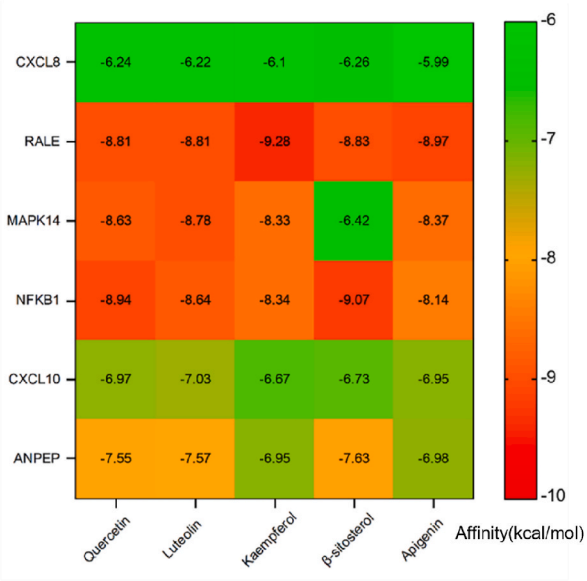

Fig. 6–1. Heat map of molecular docking binging energy between the core active compounds and five core targets protein.

3.8.2. In vitro anti-inflammatory activities of DLQGD against HCoV-229E

The RT-PCR results showed (Fig. 8A–E) that the expression of IL-8, IP-10, IL-6, IL-1β, TNF- α mRNA in the model group was significantly higher than that in the control group. Compared with the model group, the expression of IL-8(CXCL8), IP-10(CXCL10), IL-6, IL-1β, TNF- α mRNA was significantly decreased after DLQGD treatment ($P < 0.05$).

4. Discussion

Network pharmacology can explore complex data interaction. Therefore, we screened core compounds, potential gene targets and pathways through network pharmacology to provide more precise directions for the study of their mechanisms. Then, the binding energy between the core active compound and the core gene target was estimated by molecular docking verification. Finally, the DLQGD's effects of antiviral and anti-inflammatory were validated in vitro.

In the analysis of the DLQGD-ingredient-target network, the top five active ingredients , ranked by degree value, are quercetin, luteolin, kaempferol, β-sitosterol, apigenin. Quercetin has been shown to interfere with viral replication and inhibit viral growth stages by modulating the viral cellular immune system and interacting with viral cellular targets, which includes preventing viral adhesion to cellular surface proteins and inhibiting inflammatory cytokines [37–40]. Lignans can inhibit inflammatory by modulating the NF-κB/AP-1/PI3K-Akt signaling pathway [41]. Kaempferol can suppress the expression and activation of pro-inflammatory cytokines such as IL- 6, IL- 8 and MCP- 1, also can regulate normal T cell expression and secretion [42]. β-sitosterol has anti-inflammatory effect on HAEC, and the mechanism may be mediated by activation of multiple transcription factors [43,44]. The anti-inflammatory effects of apigenin have been demonstrated in numerous studies [45–48]. Apigenin reduces the production of proinflammatory cytokines by inhibiting the activation of COX-2 and NF-kB in a model of lipopolysaccharide (LPS) -induced acute lung injury [49].

The topology of the PPI network suggested that CXCL8 (IL-8), RELA, MAPK14, NFKB1, CXCL10 (IP-10) are the core targets of DLQGD against HCoV-229E. Research [50] has shown that inflammation-related factors IL-8, IL-6, TNF-α, IP-10 are highly correlated with HCoV-229E infection.

The results of KEGG pathway analysis showed that the major pathways included IL-17 signaling pathway, Toll-like receptor signaling pathway, RIG-I-like receptor signaling pathway, TNF signaling pathway and so on. A number of key pathways have been shown to be involved in the efficacy of herbal medicines, such as Toll-like receptors (TLRs), a specific family of pattern recognition receptors responsible for detecting microbial pathogens and generating innate immune responses. TLR7/MyD88/NF-κB signaling pathway may be an important pathway for compound Yinhuang granule to inhibit the expression of inflammatory cytokines and thus exert anti-influenza viral effects [51]. Xu found that the mechanism by which R Isatidis extract exerts a protective effect in a mouse model of respiratory syncytial virus infection might be through regulation of RIG-I like signaling pathway [52].

Molecular docking verification demonstrated favorable binding affinity between the five core compounds and the predicted core proteins, supporting our predictions. In vitro experiments further corroborated these findings, revealing that DLQGD significantly inhibited the mRNA expression of IL-8, IL-6, IL-1β, TNF-α, and IP-10 compared to the HCoV-229E group. These results are consistent with our molecular docking analysis, reinforcing the potential therapeutic efficacy of DLQGD against HCoV-229E and its anti-inflammatory effects. Moreover, many studies have shown that excessive expression of inflammatory factors by pathogenic micro-organisms in the innate immune system leading to tissue damage is one of the main pathogenic mechanisms of influenza [53,54]. IL-8 is the most important chemokine mediating PMN aggregation and can participate in the inflammatory response by promoting PMN

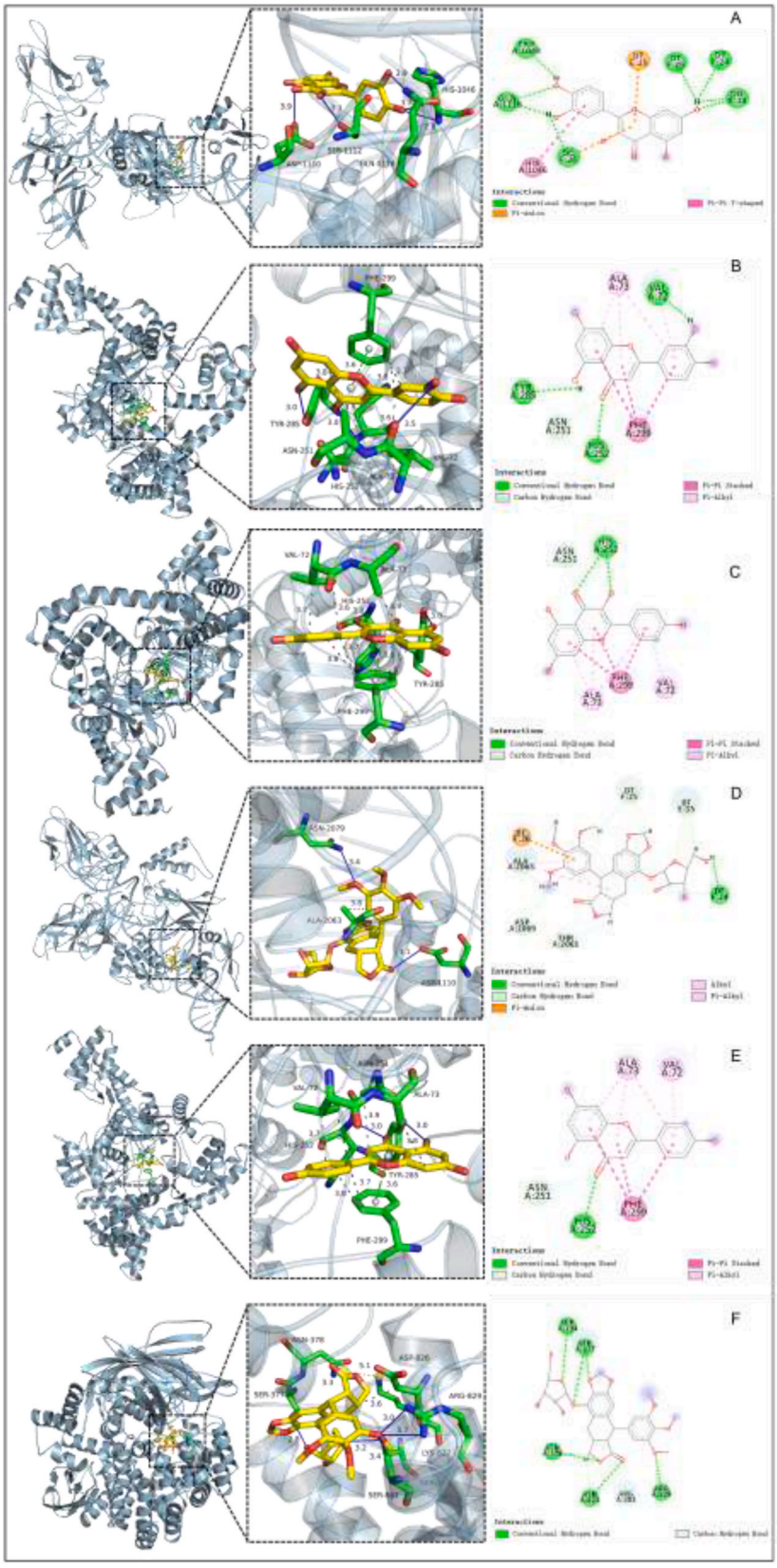

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Fig. 6–2. Result of molecular docking and visualization of the binging between molecules. A: Action mode of Quercetin with target NFKB1. B: Action mode of luteolin with target RELA. C: Action mode of kaempferol with target RELA. D: Action mode of β-sitosterol with target NFKB1. E: Action mode of Apigenin with target RELA. F: Action mode of β-sitosterol with target ANPEP.

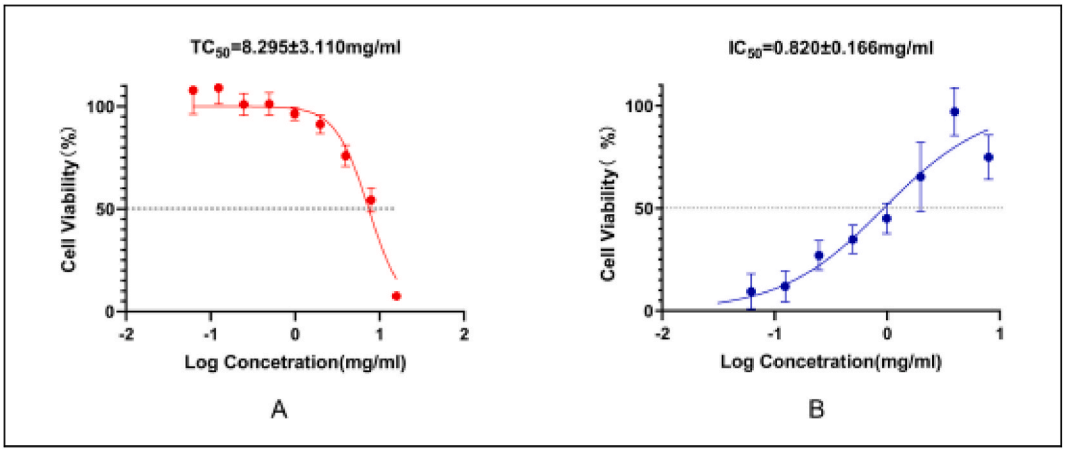

Fig. 7. The results of cytotoxicity assay and antiviral activity test in vitro. A showed the TC_{50} of DLQGD on Huh-7 cells; B showed the IC_{50} of DLQGD against HCoV-229E on Huh-7 cells. Expressed as the mean ± standard error, three independent replicate experiments were performed.

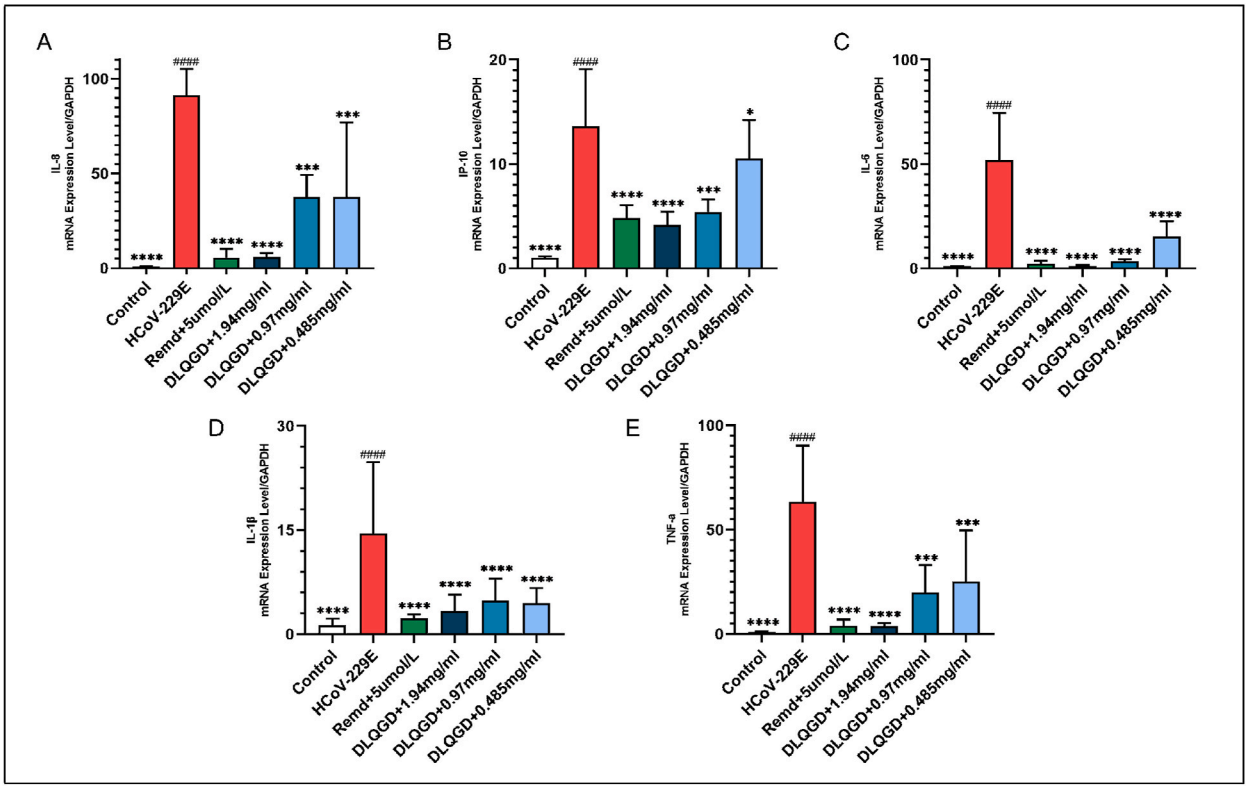

Fig. 8. The expression of IL-8, IP-10, IL-6, IL-1β, TNF- α mRNA in each group. Expressed as the mean ± standard error, three independent replicate experiments were performed, and $P < 0.05$ were considered to indicate statistical significance. Compared with the Control group, $^{\#\#\#\#}P < 0.0001$. Compared with the HCoV-229E group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ $^{****}P < 0.0001$.

chemotaxis, metamorphosis, degranulation and release of lysosomal enzymes [55]. During the inflammatory response of the body, excessive production of pro-inflammatory cytokines like TNF-α, IL-8, and IL-6 occurs. This abnormal cytokine secretion can trigger an uncontrolled activation of vascular endothelial cells, initiating the exogenous coagulation system, and resulting in tissue damage [56, 57].

Therefore, inhibition of inflammation-causing factor expression is one of the main ways to control the occurrence of cytokine storms and reduce mortality in influenza patients. TCM has been widely used to treat infectious diseases with remarkable clinical efficacy, such as Isatidis Radix [58], Lianhua Qingwen [59–62], Gegen soup [63]. With its unique advantage of containing multiple components and targeting multiple pathways, Traditional Chinese Medicine (TCM) offers not only anti-viral and anti-inflammatory effects but also demonstrates the ability to alleviate discomforting symptoms such as fever and respiratory failure in patients [62, 64,65].

Our results show that DLQGD significantly inhibited the expression of IL-6, IL-8, TNF-α, IP-10, IL-1β, suggesting that anti-inflammation may be a potential therapeutic mechanism for DLQGD against HCoV-229E.

5. Conclusion

Using network pharmacology, we successfully predicted the core active ingredients (quercetin, luteolin, kaempferol, β-sitosterol, apigenin) of DLQGD and the core therapeutic targets (CXCL8, RELA, MAPK14, NFKB1, CXCL10) against HCoV-229E. Molecular docking verification confirmed their strong binding activity, suggesting their potential roles in DLQGD's pharmacological action against HCoV-229E. In vitro experiments validated DLQGD's anti-HCoV-229E and anti-inflammatory effects. However, while these findings are promising, further comprehensive studies are warranted to fully elucidate the underlying mechanisms and to explore DLQGD's potential clinical applications for managing HCoV-229E infections.

Data availability statement

The data associated with our study is not deposited into a publicly available repository. However, data will be made available on request.

CRediT authorship contribution statement

Yajing Xue: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Data curation, Conceptualization. **Xuejun Cai:** Methodology, Data curation. **Yutao Wang:** Methodology. **Li Ban:** Methodology. **Manxue Mei:** Writing – original draft, Software. **Shuqi Chen:** Visualization, Software. **Qihua Xu:** Software. **Boqian Chen:** Software. **Shuhua Liang:** Software. **Xinhua Wang:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:XinhuaWang reports financial support was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27829.

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