Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Exploring the active compounds of traditional Mongolian medicine in intervention of novel coronavirus (COVID-19) based on molecular docking method

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ABSTRACT

Objective: This article intends to use molecular docking technology to find potential inhibitors that can respond to COVID-19 from active compounds in Mongolian medicine.

Methods: Mongolian medicine with anti-inflammatory and antiviral effects is selected from Mongolian medicine prescription preparations. TCMSP, ETCM database and document mining methods were used to collect active compounds. Swiss TargetPrediction and SuperPred server were used to find targets of compounds with smiles number. Drugbank and Genecard database were used to collect antiviral drug targets. Then the above targets were compared and analyzed to screen out antiviral targets of Mongolia medicine. Metascape database platform was used to enrich and analyze the GO (Gene ontology) annotation and KEGG pathway of the targets. In view of the high homology of gene sequences between SARS-CoV-2 S-protein RBD domain and SARS virus, as well as their similarities in pathogenesis and clinical manifestations, we established SARS-CoV-2 S-protein model using Swiss-Model. The ZDOCK protein docking software was applied to dock the S-protein with the human angiotensin ACE2 protein to find out the key amino acids of the binding site. Taking ACE2 as the receptor, the molecular docking between the active ingredients and the target protein was studied by AutoDock molecular docking software. The interaction between ligand and receptor is applied to provide a choice for screening anti-COVID-19 drugs.

Results: A total of 253 active components were predicted. Metascape analysis showed that key candidate targets were significantly enriched in multiple pathways related to different toxins. These key candidate targets were mainly derived from phillyrin and chlorogenic acid. Through the protein docking between S-protein and ACE2, it is found that Glu329/Gln325 and Gln42/Asp38 in ACE2 play an important role in the binding process of the two. The results of molecular docking virtual calculation showed that phillyrin and chlorogenic acid could stably combine with Gln325 and Gln42/Asp38 in ACE2, respectively, which hindered the combination between S-protein and ACE2.

Conclusion: Phillyrin and chlorogenic acid can effectively prevent the combination of SARS-CoV-2 S-protein and ACE2 at the molecular level. Phillyrin and chlorogenic acid can be used as potential inhibitors of COVID-19 for further research and development.

1. Background:

Since December 2019, the epidemic situation of novel Coronavirus (COVID-19) infection in China has developed rapidly. Up to now, the number of confirmed infections has exceeded 60,000 and spread to more than 20 countries and regions around the world. On January 31, 2020, the World Health Organization declared the COVID-19 epidemic as a public health emergency of international concern (PHIC) (CDC, 2020; Cotton et al., 2013). However, at present, the research and development of virus vaccine is greatly lagging behind, and there is an extreme lack of effective therapeutic drugs against virus clinically. The world urgently needs to find and develop new therapeutic methods and drugs.

Mongolian medicine has played a unique role in the prevention and treatment of new outbreaks of infectious diseases, especially in the prevention and treatment of SARS, H1N1, H7N9 and other epidemic

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situations, and has achieved very good clinical results. Mongolian medicine calls acute infectious diseases pestilence. Prevention and treatment of pestilence is an important part of Mongolian medicine. COVID-19 belongs to the category of "epidemic fever" and "sticky epidemic" in Mongolian medicine, and is a "pulmonary epidemic fever" caused by virus infection (National Health Committee). Against the spread of SARS-CoV-2, many Mongolian medical experts have proposed different Mongolian medicine prevention and treatment programs (Jianyong, 2019).

In order to provide Mongolian medicine plans with certain clinical basis and objective evidence in the first time, in view of the high homology of gene sequences between SARS-CoV-2 and SARS virus, and the great similarity of their onset characteristics, clinical manifestations and potential therapeutic targets, we intends to use the network pharmacology and molecular docking technology to screen compounds with clear anti-COVID-19 based on the experience and theory of Mongolian medicine in preventing and controlling major epidemics.

Network pharmacology is a research strategy for multi-target and multi-channel interactions of drugs. Starting from the integrity and systematicness of interactions between drug targets and diseases, it uses computer methods to model multi-target activities on the basis of multi-level networks of diseases, genes and drugs. At the same time, it studies the biological basis of drugs acting on the body, which is a powerful tool for Mongolian medicine modernization research (Zheng and Fan, 2017). Molecular docking is a computational tool for predicting the binding ability and binding mode of proteins and ligands. Its principle is based on the "lock key model" of the interaction between proteins and small ligands, calculating and predicting the conformation and orientation of ligands at protein active sites, so as to judge the binding degree and play an important role in the target prediction of drug organisms (Duan et al., 2019). There are many links and factors that affect the infection in SARS-CoV-2. According to previous studies, the infection routes of SARS-CoV-2 and SARS-CoV are the combination of S-protein of the virus and angiotensin converting enzyme (ACE2) in human body, which leads to the invasion of the virus into the body and causes disease (Li et al., 1864). In this study, molecular docking screening was carried out on ACE2, a key target protein in the process of virus infection, to obtain active compounds against coronavirus, providing reference for effective drug screening and new drug development.

2. Materials and methods

2.1. Collection and screening of candidate compounds

This study mainly collected classic antipyretic and antiviral Mongolian medicine prescriptions, and used the involved Mongolian medicine to build a candidate Mongolian medicine database. With the help of the TCMSP database (http://tcmspw.com/), the chemical constituents of candidate Mongolian medicines were retrieved, and animal and mineral medicines such as cicada and gypsum were removed. Using TCMSP database, the pharmacokinetic (absorption, distribution, metabolism, exclusion, ADME) properties of main compound components were evaluated, and chemical components satisfying Oral Bioavailability (OB) $\geq$ 30% and Drug-Likeness property (DL) $\geq$ 0. 18 were selected as candidate active components (Yan et al., 2009). OB is directly related to bioavailability. DL refers to the similarity between the molecule to be tested and the drug molecule, i.e. the possibility of becoming a drug. The molecular structure of each active compound was confirmed by literature mining and PubChem (https://pubchem.ncbi.nlm.nih.gov/).

2.2. Prediction of potential targets

Using PubChem database (https://pubchem.ncbi.nlm.nih.gov/), all compounds were converted into standard Canonical SMILES format. The SMILES format file is imported into Swiss TargetPrediction (http://www.swisstargetprediction.ch/) and SuperPred website (http://prediction.charite.de/), and the attribute is set to "homo sapiens" to predict the targets of the compounds. Swiss TargetPrediction selects targets with parameter Probability $\geq$ 0.6 in prediction results for further analysis (Arun, 2015). SuperPred can predict the potential targets of unknown molecules by calculating the Tanimoto similarity between molecules and more than 300,000 known compounds in the server (Gfeller et al., 2014). The prediction results of Swiss and SuperPred databases are summarized and de-weighted, which can be used as prediction targets of compounds for further analysis.

2.3. Collection of antiviral drug related targets

Antiviral drug targets were collected from DurgBank (https://www.drugBank.ca/) and Genecards database (https://www.genecards.org/), and a database of antiviral drug-related targets was established by combining the methods of literature mining. By comparing and analyzing the collected chemical component targets of traditional Chinese medicine and antiviral drug-related targets, the prediction targets with definite antiviral effect are summarized.

2.4. Gene analysis and pathway annotation

Metascape (http://metascape.org) platform is a gene annotation analysis database, which performs enrichment analysis on biological processes and pathways of input genes (Robert et al., 2008). The targets of antiviral action were inputted to the Metascape platform. After submitting, the attributes were set to "home sapiens" and P less than 0.01. GO annotation analysis and KEGG pathway analysis on the targets were performed and the results were saved and sorted by the number of targets involved in each entry to screen top biological processes and pathways.

2.5. Construction of S-protein model of SARS-CoV-2

The sequence of SARS-CoV-2 S-protein fragment in the c-terminal RBD domain has high homology with SARS-CoV (Normile, 2020). It is reported that residues 442, 472, 479, 487 and 491 in SARS-CoV S-protein are located at the receptor complex interface, which is considered to be essential for cross-species and human-to-human transmission (Raj et al., 2013). Compared with the SARS-CoV S-protein, the RBD domain of the SARS-CoV-2 S-protein has been replaced with amino acid residues at positions 442, 472, 479, 487 except for Tyr491 (Fig. 5-A). Substitution of residues at positions 442, 472, 479 and 487 of SARS-CoV-2 S-protein did not change its interface structure. SARS-CoV-2 S-protein and SARS-CoV S-protein have almost the same three-dimensional structure in the RBD domain, thus maintaining similar van der Waals and electrostatic characteristics in the interaction, thus SARS-CoV-2 have significant binding affinity with human ACE2 (Schwede et al., 2003). Since the sequence of SARS-CoV-2 S-protein in the RBD domain has high homology with SARS-CoV, we downloaded the sequence file (FASTA format) (PDB:6ACD) of SARS-COV S-protein using RCSD protein database(www.rcsb.org), replacing Y with L at 442, F with L at 472, C with N at 479, and N with T at 487. The newly obtained SARS-CoV-2 sequence file is imported into Swiss-Model (https://swissmodel.expasy.org/), and thus the structural model of SARS-CoV-2 S- protein is constructed.

2.6. Docking of S-protein with ACE2 protein

ZDOCK Server (http://zdock.umassmed.edu/) was used to rigidly dock SARS-CoV-2 S-protein with ACE2. The crystal structure of ACE2 was obtained from RCSD protein database (PDB: 2AJF). According to molecular composition and literature data, the main parameters of ZDOCK are shown in table 1. After the ligand-receptor complex model is obtained, the

Table 1
ZDOCK parameters of interaction between SARS-CoV-2 S-protein and ACE2 receptor.

Parameter format	Parameter
Angularstepsize	6°
DistanceCutof	2.5 Å
ZRANK	TRUE
ZRANK TopPoses	2000
ClusteringRMSD Cutof	4 Å
ClusteringInterfaceCutof	10 Å
ElectrostaticandDesolvationEnergy	TRUE

ZDOCK results are preliminarily screened using an empirical scoring function to obtain morphological near-natural conformational clusters of the receptor-ligand complex. The general formula of the scoring function is: SPSC + DE + ELEC = RELRPSC + ELEC LPSC + ELEC] + 0.5XLM [RDE LDE], i.e. the scoring takes into account the factors of paired surface fit (PSC), desolvation reaction (DE) and electric potential (ELEC). Finally, PDBePISA database (https://www.ebi.ac.uk/msd-srv/prot_int/pistart.html) was used to optimize and rearrange the conformational clusters, and the model with root mean square deviation (rootmeansquaredeviati0n.RMSD) less than 4A˚was selected as the near natural conformation. The conformation was observed by pyMOL visualization software to obtain the key amino acid residues of the binding site.

2.7. Molecular docking

The docking was mainly completed by AutoDock 4.2.6 with traditional Chinese medicine components as ligands and ACE2 protein as receptors. The ZINC database (http://zinc.docking.org/) is used to download the 3D structure file of the active compound. The ligand and receptor molecules need to be subjected to energy minimization treatment before docking. The water molecules in the receptor molecules (PDB file) are deleted, and polar hydrogen atoms are added to impart electric charge and magnetic field. The position of the original ligand compound is taken as the binding site, and all substructures within a radius of 0.65 nm are taken as the active pocket part of the binding site (Peng et al., 2011). Autodock molecular docking software (version 2.5) is used to dock the active ingredients of traditional Chinese medicine with receptor protein to screen the effective ingredients against coronavirus. The detailed flow chart of this article is shown in Fig. 1.

3. Results

3.1. Collection of effective components

In this study, based on the systematic review and analysis of Mongolian medicine prescriptions during the SARS period, the classic Mongolian medicine prescriptions were analyzed. A total of 13 Mongolian medicine preparations were collected, including 41 Mongolian medicines. By examining the compatibility of prescriptions, 11 Mongolian medicines with clear anti-inflammatory and antiviral effects were included in the analysis. Through TCMSP, 1,597 compounds were found, but no effective compounds were found in Rhizoma Dryopteris Crassirhizomatis. Among the compounds, 150 came from Forsythia, 236 from Honeysuckle, 363 from Herba Ephedrae, 113 from Semen Armeniacae Amarum, 169 from Radix Isatidis, 50 from Herba Houttuyniae, 94 from Herba Pogostemonis, 50 from Radix Rhodiolae, 92 from Radix et Rhizoma Rhei and 280 from Radix Glycyrrhizae. Specific compound information is shown in Schedule 1. With OB ≥ 30% and DL ≥ 0.18, 253 active compounds were screened out, of which 23 came from Forsythia , 23 from Honeysuckle, 23 from Herba Ephedrae, 19 from Semen Armeniacae Amarum, 39 from Radix Isatidis, 7 from Herba Houttuyniae, 11 from Herba Pogostemonis, 14 from Radix Rhodiolae, 16 from Radix et Rhizoma Rhei and 92 from Radix Glycyrrhizae. Specific compound information is shown in Schedule 2. Table 2 is the basic information of some active compounds.

Table 2
basic information of some active compounds.

Chinese medicine	Compound name	OB/%	DL	Molecular Weight
Forsythia	Phillyrin	36.40	0.86	534.61
	Luteolin	36.16	0.25	286.25
Honeysuckle	Kryptoxanthin	47.25	0.57	552.96
	Chlorogenic acid	33.61	0.31	354.34
Herba Ephedrae	Kaempferol	41.88	0.24	286.25
	Herbacetin	36.07	0.27	302.25
Semen Armeniacae Amarum	Liquiritin	65.69	0.74	418.43
	Licochalcone B	76.76	0.19	286.3
Radix Isatidis	Acacetin	34.97	0.24	284.28
	Isaindigodione	60.12	0.41	326.38
Herba Houttuyniae	Isoramanone	39.97	0.51	348.53
	Quercetin	46.43	0.28	302.25
Herba Pogostemonis	Irisolidone	37.78	0.3	314.31
	Acanthoside B	43.35	0.77	580.64
Radix Rhodiolae	Rhodioloside	40.79	0.5	300.30
	7-Hydroxycoumarin	31.29	0.46	162.14
Radix et Rhizoma Rhei	Physciondiglucoside	41.65	0.63	608.60
	Toralactone	46.46	0.24	272.27
Radix Glycyrrhizae	Mairin	55.38	0.78	456.78
	Isorhamnetin	49.60	0.31	316.28

3.2. Target prediction

Target prediction of active compounds is carried out according to Swiss and SuperPred websites. There are 336 putative targets shared by 23 active compounds of Forsythia, 205 putative targets shared by 23 active compounds of Honeysuckle, 121 putative targets shared by 23 compounds of Herba Ephedrae, 56 putative targets shared by 19 active

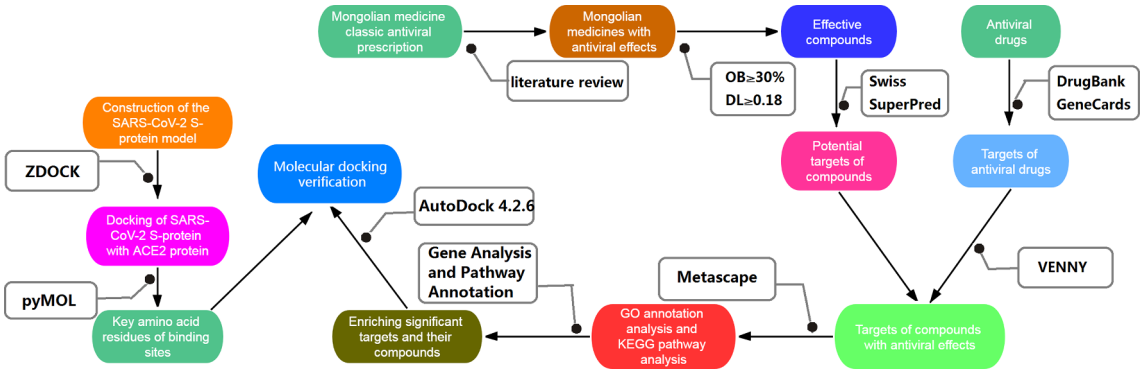

Fig. 1. The flow diagram for screening and verifying Mongolian medicine compounds.

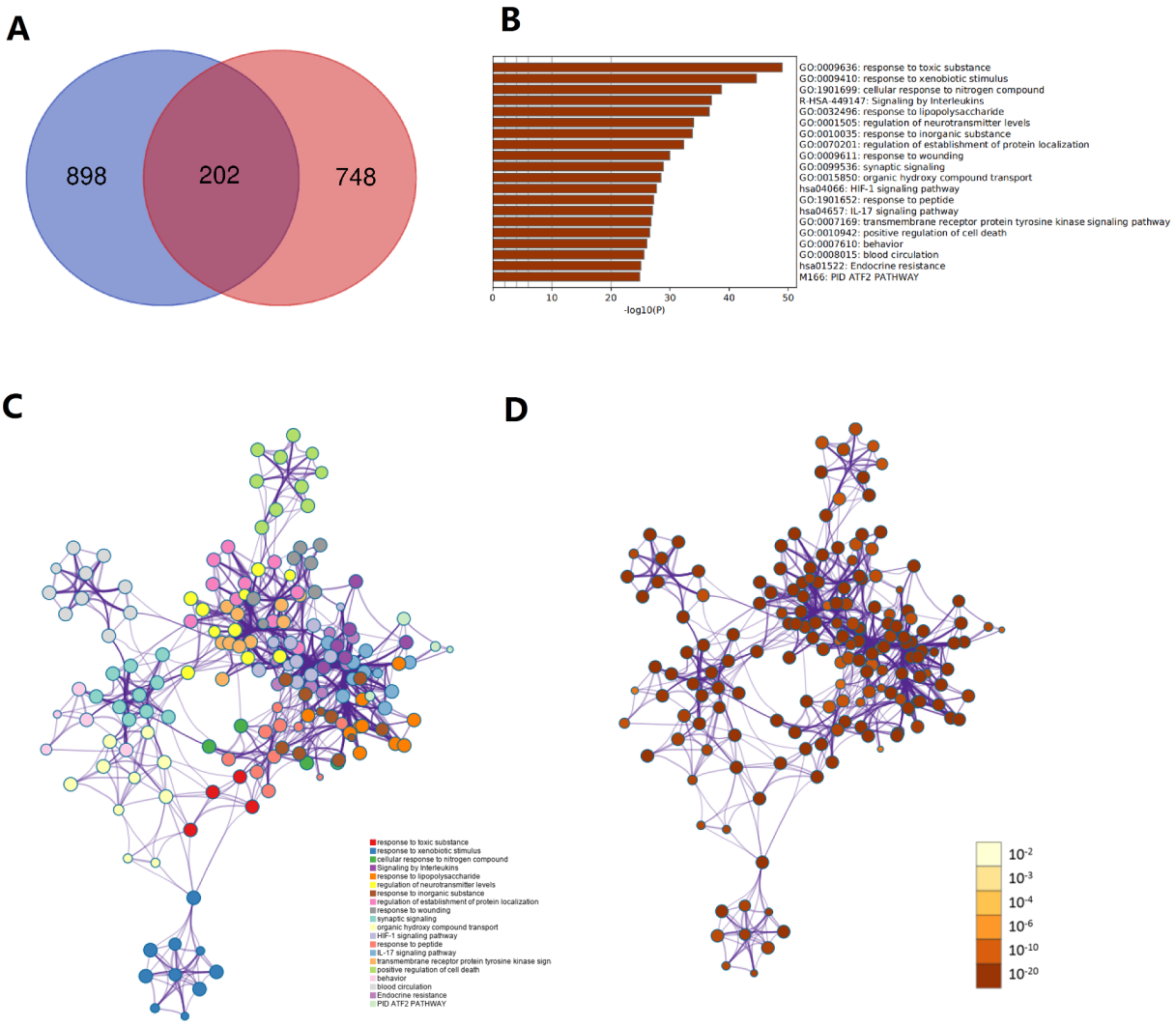

Fig. 2. A:Venn diagram for comparative analysis of compound targets and antiviral drug targets; B: The Metascape platform to select the top GO annotation results and KEGG pathway; C: The map of differential gene enrichment interaction, including 20 groups of enrichment results; D: The network map constructed according to the enrichment degree, the darker the color is, the more genes are enriched into the pathway.

Table 3
TOP20 Bioannotation and Enrichment Analysis Results.

GO	Category	Description	Count	%	Log10(P)	Log10(q)
GO:0009636	GO Biological Processes	response to toxic substance	59	29.21	−48.97	−44.65
GO:0009410	GO Biological Processes	response to xenobiotic stimulus	46	22.77	−44.61	−40.59
GO:1901699	GO Biological Processes	cellular response to nitrogen compound	55	27.23	−38.68	−34.84
R-HSA-449147	Reactome Gene Sets	Signaling by Interleukins	53	26.24	−36.99	–33.28
GO:0032496	GO Biological Processes	response to lipopolysaccharide	42	20.79	−36.61	–32.99
GO:0001505	GO Biological Processes	regulation of neurotransmitter levels	41	20.30	–33.97	−30.55
GO:0010035	GO Biological Processes	response to inorganic substance	48	23.76	–33.77	−30.40
GO:0070201	GO Biological Processes	regulation of establishment of protein localization	52	25.74	–32.27	−29.00
GO:0009611	GO Biological Processes	response to wounding	48	23.76	−29.91	−26.82
GO:0099536	GO Biological Processe	synaptic signaling	48	23.76	−28.84	−25.80
GO:0015850	GO Biological Processes	organic hydroxy compound transport	33	16.34	−28.43	−25.41
hsa04066	KEGG Pathway	HIF-1 signaling pathway	24	11.88	−27.69	−24.76
GO:1901652	GO Biological Processes	response to peptide	41	20.30	−27.18	−24.33
hsa04657	KEGG Pathway	IL-17 signaling pathway	23	11.39	−26.99	−24.19
GO:0007169	GO Biological Processes	transmembrane receptor protein tyrosine kinase signaling pathway	46	22.77	−26.74	–23.97
GO:0010942	GO Biological Processes	positive regulation of cell death	46	22.77	−26.54	–23.80
GO:0007610	GO Biological Processes	behavior	42	20.79	−26.05	–23.35
GO:0008015	GO Biological Processes	blood circulation	40	19.80	−25.56	–22.90
hsa01522	KEGG Pathway	Endocrine resistance	22	10.89	−25.02	–22.42
M166	Canonical Pathways	PID ATF2 PATHWAY	19	9.41	−24.83	–22.26

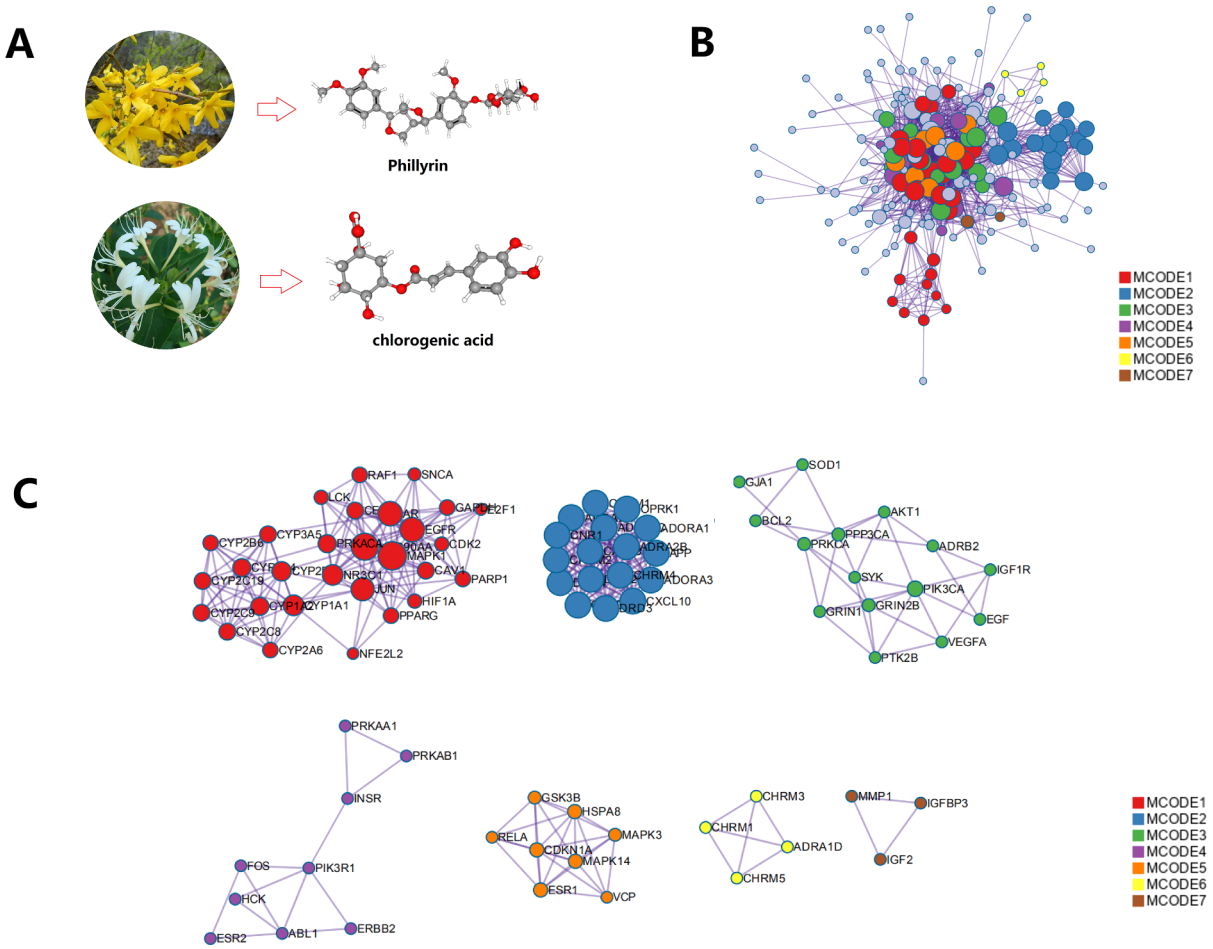

Fig. 3. A:The 3D structure of phillyrin and chlorogenic acid. B: A fully connected interaction network of related proteins of all genes. C: module substructure identified in the interaction network.

Table 4
Functions of Sub-module Proteins.

MCODE	GO	Description	Log10(P)
MCODE_1	R-HSA-211981	Xenobiotics	−23.5
	GO:0009410	response to xenobiotic stimulus	−21.1
	GO:0071466	cellular response to xenobiotic stimulus	−20.1
MCODE_2	R-HSA-373076	Class A/1 (Rhodopsin-like receptors)	−32.0
	R-HSA-418594	G alpha (i) signalling events	−30.3
	R-HSA-500792	GPCR ligand binding	−29.4
MCODE_3	hsa01521	EGFR tyrosine kinase inhibitor resistance	−13.7
	GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	−13.7
	GO:0048871	multicellular organismal homeostasis	−13.6
MCODE_4	hsa04213	Longevity regulating pathway - multiple species	−8.3
	hsa04211	Longevity regulating pathway	−7.7
	hsa01522	Endocrine resistance	−7.5
MCODE_5	R-HSA-2262752	Cellular responses to stress	−11.4
	hsa04917	Prolactin signaling pathway	−11.0
	R-HSA-8953897	Cellular responses to external stimuli	−10.8
MCODE_6	R-HSA-375280	Amine ligand-binding receptors	−11.1
	R-HSA-390648	Muscarinic acetylcholine receptors	−10.8
	GO:0007197	adenylate cyclase-inhibiting G protein-coupled acetylcholine receptor signaling pathway	−10.0
MCODE_7	R-HSA-381426	Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Bi	−6.9

compounds of Semen Armeniacae Amarum, 649 putative targets shared by 39 active compounds of Radix Isatidis and 258 putative targets shared by 7 active compounds of Herba Houttuyniae. There are 15 putative targets for 11 active compounds of Herba Pogostemonis, 225 putative targets for 16 active compounds of Radix et Rhizoma Rhei, 175 putative targets for 14 active compounds of Radix Rhodiolae and 317 putative targets for 92 active compounds of Radix Glycyrrhizae. Specific data was shown in schedule 3. For antiviral drugs, we have selected oseltamivir, zanamivir, paramivir, lopinavir, ritonavir, amantadine, rimantadine, chloroquine, remdesivir and favipiravir ten drugs. 1047 known antiviral targets have been selected through the Drugbank database and Genecard database, and an antiviral target database has been constructed. Specific data was shown in schedule 4.

3.3. Gene function and pathway enrichment analysis

By comparing and analyzing the action targets of the above-mentioned collected active ingredients of traditional Chinese medicine with those related to antiviral drugs, 202 action targets of active ingredients of traditional Mongolia medicine with definite antiviral effects are summarized. The Metascape platform was used to perform GO annotation analysis and KEGG pathway analysis on the potential targets for antivirus. The threshold value $P < 0.01$ was set to screen out the front GO annotation results and KEGG pathway. The results are shown in Fig. 2-B and the specific values are shown in table 3. The results showed that there were 20 signal pathways with high coincidence, and KEGG pathway annotation related to anti-toxic substances was selected to map the genes corresponding to the regulatory target protein directly to

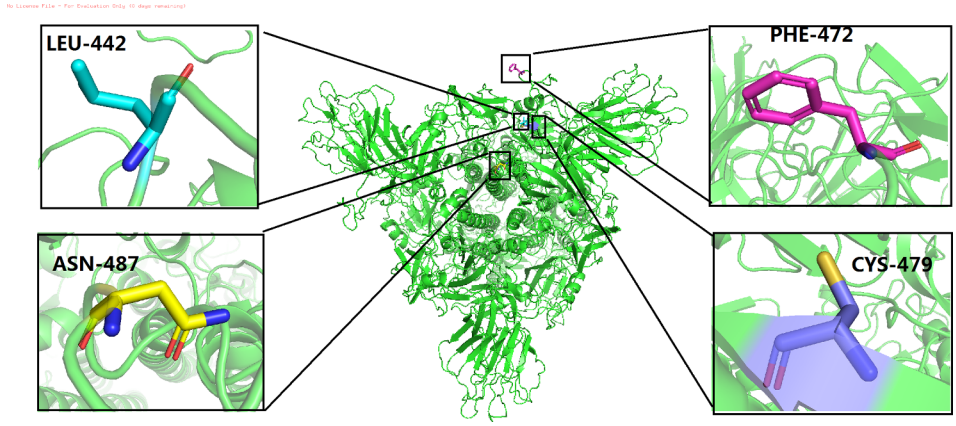

Fig. 4. The SARS-CoV-2 S-protein model constructed.

Table 5
Screening results of SARS-CoV-2 S-protein and ACE2 binding model.

complex	Interface area (Å^2)	ΔG (kcal/mol)	ΔiG (P-value)	Number (Hydrogen bond)
Complex 1	808.2	−5.0	0.491	5
Complex 2	847.3	−0.7	0.616	7
Complex 3	674.9	0.5	0.761	7
Complex 4	632.6	0.6	0.802	7
Complex 5	636.1	−2.0	0.683	5
Complex 6	752.7	−3.9	0.674	8
Complex 7	666.9	−1.8	0.753	8
Complex 8	633.8	0.5	0.803	7

the pathways. The GO annotation has a large amount of data, among which, the top ones in the biological process are response to toxic substance, response to xenobiotic stimulus, Cellular Response to Nitrogen Compound. Through the enrichment analysis of KEGG pathway in Metascape database, two pathways closely related to immunity and anti-allotoxin are mainly IL-17 signaling pathway and PID ATF2 pathway. Kappa Score is an indicator of the extent to which two raters who are examining the same set of categorical data, agree and takes into account agreement occurring by chance. The nodes are connected to form a network by the similarity between terms (Kappa greater than 0.3), and each node represents an enrichment term. The color of the node in Fig. 2-C indicates the cluster to which the node belongs. It can be seen that the terms belonging to the same cluster are closer and more closely related to each other. The color of the nodes in Fig. 2-D indicates the degree of enrichment (P value), and it can be seen that the more the number of genes is included, the more significant the P value is.

3.4. Functional attribution of target protein

Through the Metascape platform, all genes are connected to the whole protein interaction network(Fig. 3-B). Seven different colors represent the module substructures identified in the interconnection network, and the formed modules are abstracted from the full-connection interconnection network to form Fig. 3-C. The protein functions of specific modules are shown in Table 4. Key genes corresponding to regulatory target proteins are directly mapped onto pathways, and pathways enriched by drug targets are considered as pathways for drug regulation. It can be found that in MCODE 1 and MCODE 5, the protein function annotation is the cell's response to toxin and heterologous biological stimulation, and 36 key targets involved mainly come from two compounds, Phillyrin and chlorogenic acid.

3.5. Construction of the SARS-CoV-2 S-protein model

Using the Swiss model, with 6ACD as the template, the final model was obtained through homology modeling and structural optimization, as shown in Fig. 4. According to previous research, on SARS-CoV RBD domain, TYR442 is replaced by LEU442, LEU472 is replaced by PHE472, ASN479 is replaced by CYS479, THR487 is replaced by ASN487, and SARS-CoV-2 S-protein model can be obtained. The result is shown in Fig. 4, which is in line with the expected model and indicates the modeling is successful. The SARS-CoV-2 S-protein model sequence is shown in schedule 5.

3.6. Docking of SARS-CoV-2 S-protein with ACE2 protein

After SARS-CoV-2 S-protein RBD domain docked with ACE2 via ZDOCK, 8 receptor-ligand complex models were obtained. Energy optimization and model screening were carried out for all conformations using PDBePISA database, and the specific data are shown in Table 5. The best model is the one with the largest surface area, the smallest surface energy and the most hydrogen bonds (Xu et al., xxxx). After comprehensive consideration, complex2 was selected as the near-natural model (Fig. 5-B). Analysis of the key binding sites of SARS-CoV-2 and ACE2 showed that SARS-CoV-2 S-protein Arg426 and Tyr436, respectively, combined with Gln325/Glu329 and Asp38/Gln42 of ACE2 through hydrogen bonds and formed an interaction interface with adjacent residues (Fig. 6), which is consistent with previous research results of Hao Pei team (Zefeng, 2020).

3.7. Molecular docking results

In this study, two key active compounds phillyrin and chlorogenic acid screened by network pharmacology were verified. The 3D structures were imported into AutoDock and docked with ACE2. Their interactions with surrounding key residues and their binding at the active site are shown in Fig. 7, and the energy values of the compounds shown in the docking results are shown in Table 6. Molecular docking results showed that phillyrin and Gln325 of ACE2 were mainly combined in the form of hydrogen bonds and had good binding activity, which indicated that phillyrin could hinder the binding of SARS-CoV-2 S-protein RBD domain and ACE2 at Gln325/Glu329 , and the docking energy value was smaller and the binding was more stable. Chlorogenic acid combines with ACE2 Gln42/Asp38 in the form of hydrogen bonds. Compared with SARS-CoV-2 and ACE2 binding model, the docking energy is smaller and the binding is more stable, which indicates that chlorogenic acid can hinder the binding of SARS-CoV-2 S-protein RBD domain and ACE2 at Gln42/Asp38. The above results show that the combination of phillyrin and chlorogenic acid will probably hinder the binding of ACE2 and S-protein more effectively.

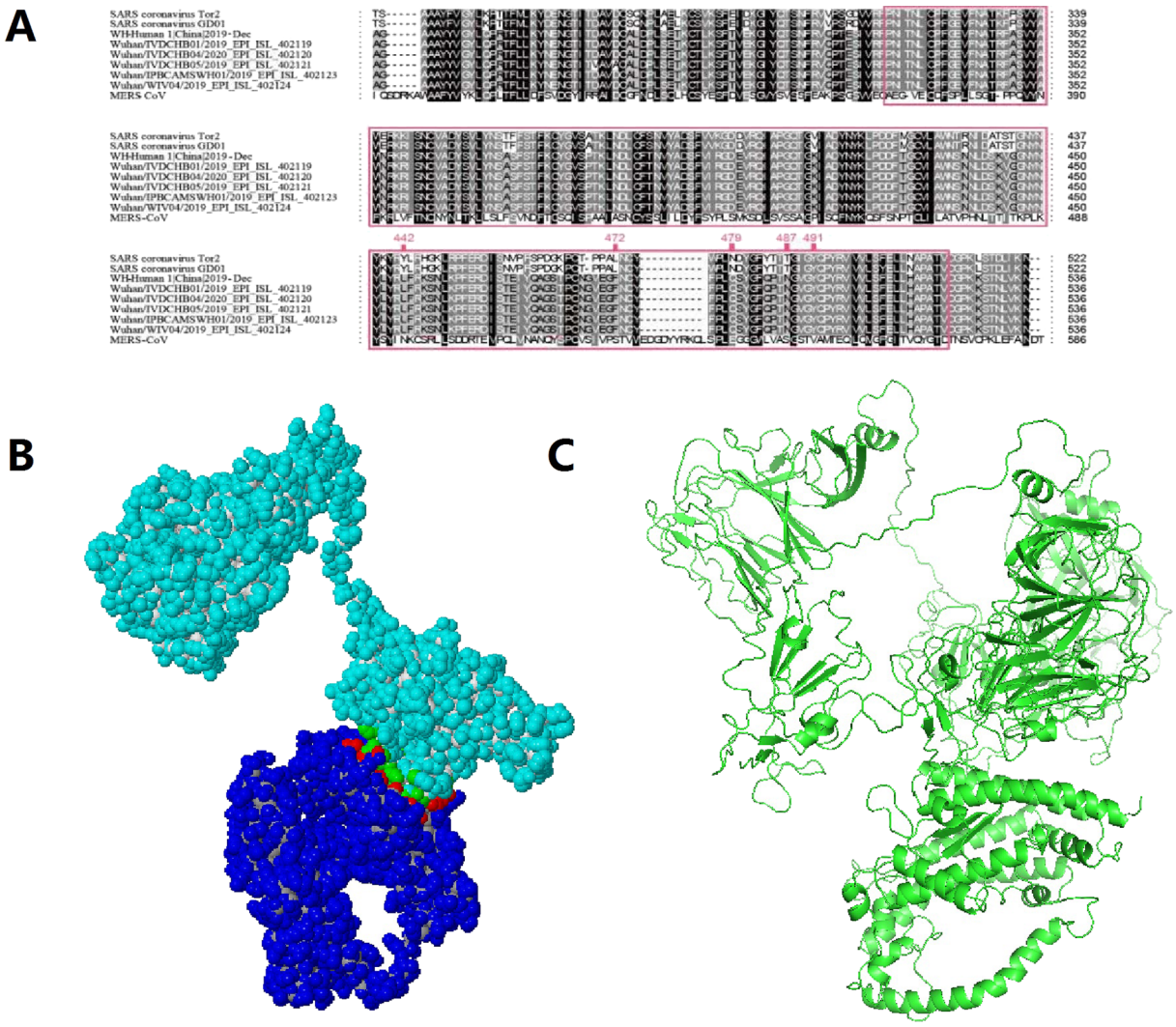

Fig. 5. A: Amino acid sequence alignment of RBD domain of coronavirus S-protein. Residues 442, 472, 479, 487, 491 (numbered according to SARS-CoV S-protein sequence) are important residues interacting with human ACE2 molecules. B: The combination of SARS-CoV-2 S-protein and ACE2 protein. C:The combination of SARS-CoV-2 S-protein and ACE2 protein in pyMOL software view.

4. Discussion

Since the end of December 2019, COVID-19 has been ravaging the whole country. Currently, there is still a lack of specific drugs for the treatment of COVID-19, and the existing chemical drugs can only alleviate some symptoms. The development and research of new drugs based on clinical experiments are long and expensive, which is difficult to apply to clinical treatment in time. Moreover, SARS-CoV-2 has high infectivity and variability. The research requires high-level laboratory conditions, which greatly limits drug screening. Therefore, computer-aided drug design has fast speed, save drug development costs, and is suitable for large-scale screening of chemical components of traditional Mongolia medicine. Molecular docking can simulate the force between the three-dimensional structure of the receptor and the ligand to find a low-energy binding mode between the ligand and the active site of the receptor, which is fast, efficient and low in cost.

Forsythia is the dried fruit of Forsythia suspense (Thunb.) Vahl of the osmanthaceae family. It has the functions of clearing away heat and detoxification, reducing swelling and loosening, and is commonly used for treating carbuncle, scrofula, breast abscess, etc (Qian Wei, Zhang, & Wang, 2020). Phillyrin is an active ingredient in Forsythia and a quality control ingredient in Forsythia and its preparations. Its pharmacological effects are extensive, such as clearing away heat and toxic materials, antivirus, antioxidant, antibacterial and anti-inflammatory effects (Pan, Cao, & Li, 2014; Qu, Zhang, & Wang, 2008; Yajun Ren & Qu, 2014). RT-PCR showed that phillyrin can reduce the copy number of NP gene of influenza A virus. The Jian Sun team believed that phillyrin may inhibit NP gene replication by inhibiting the combination of influenza A virus NP and viral RNA to form NP complexes, and may also inhibit the transcription level or post-translational modification of NP. This problem is helpful for further research (Linjian et al., 2012).

Honeysuckle is the dried flower bud of Lonicera japonica Thunb. of Caprifoliaceae and other plants of the same genus, which has the effect of clearing away heat and toxic materials, and belongs to the precious traditional Mongolia medicine under the Strict management in China (Li, 2017; Mao, Li, & Yang, 2018). In April 2018, Honeysuckle was explicitly listed in the “Medicine and food homologous food” catalogue by the Ministry of Health of the People's Republic of China. Chlorogenic acid is the main active ingredient of Mongolian medicine honeysuckle, which is an ester substance formed by caffeic acid and quinic acid. It is easy to isomerize through hydrolysis or ester group migration in the extraction process (Zhang, Hao, & Sun, 2017; Zhao, 2016). Chlorogenic acid is very high in Eucommia ulmoides, honeysuckle, coffee bean, blueberry, apple and potato containing phenolic acids. As the main active ingredient of the Mongolian medicine honeysuckle, chlorogenic acid has been proven to have significant pharmacological effects, such

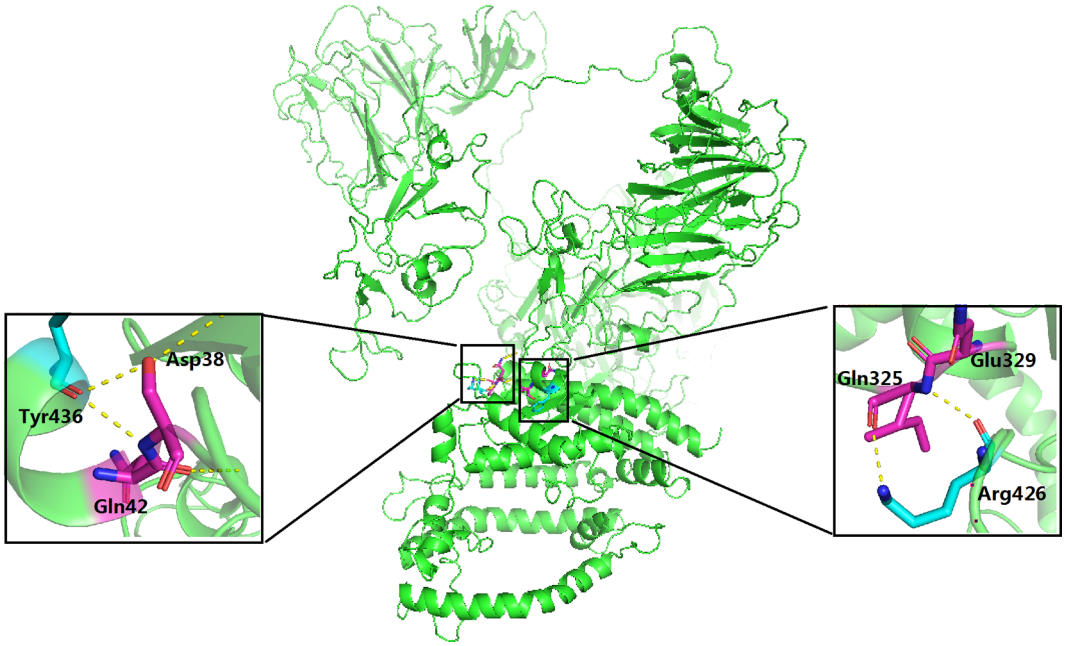

Fig. 6. Structural simulation of SARS-CoV-2 S-protein docking with human ACE2 molecule. Left panel: This area shows the hydrogen bond interaction between Tyr436 in S- protein and Asp38/Gln42 in ACE2. The relevant residues are presented in ball and stick representations. Right panel: This region shows the hydrogen bond interaction between Arg426 in S protein and Gln325/Glu329 in ACE2.

as antioxidant, antitumor, hypoglycemic, anticoagulant, antiviral effects, etc (Zongfu Hu, Yu, & Zhao, 2006; Chen, Wang, & Luo, 2008). Studies by Wang Xuebing and others have shown that chlorogenic acid plays a significant role in preventing viral infection in the early stages of virus growth, and also has a strong inhibitory effect on porcine parvovirus, showing that chlorogenic acid has a strong antiviral effect (Xiehuang et al., 2008). In vitro antiviral experiments by Xiehuang Sheng, Lin Huang, etc. have proved that chlorogenic acid has obvious effect on herpes simplex virus I (HSV-1) infection, and can effectively prevent viral infection, and the antiviral effect increases with the increase of chlorogenic acid concentration (Lin, Juri, & Jing, 2012). Lijing Li and others have studied the antiviral effect of chlorogenic acid in Senecio cannabifolius, and the results showed that chlorogenic acid extracted from Senecio cannabifolius can produce better inhibitory effect on adenovirus, respiratory syncytial virus and influenza virus (Lijing et al., 2005).

Honeysuckle and Forsythia are commonly used as heat-clearing Mongolian medicines, and the combination of the two is a common compatibility form in clinical applications. The combination of the two can enhance the effect of clearing away heat and detoxification, and evacuating wind and heat. It is one of the commonly used drug pairs in the treatment of plague. The representative prescription is Yinqiao Powder contained in Detailed Analysis of Epidemic Warm Diseases. Yinqiao Powder has therapeutic effects on respiratory tract infection, mumps, viral myocarditis and other diseases (Haiqing & Jia, 2020). It also has anti-inflammatory, antipyretic, analgesic, and anti-allergic effects. There are more than 400 Chinese and Mongolian medicine preparations involving honeysuckle-forsythia drug pairs in the existing Chinese patent medicines on the market, such as Lianqiao baidu pill, Yinhua ganmao granule, Zhizi Jinhua pill, etc (Zhang et al.). Meiyi Zhang et al studied the anti-H1N1 influenza virus effect of Jinqiao Tablets in vivo, and found that Jinqiao Tablets can significantly inhibit the increase of lung index in mice, reduce the viral load of lung tissue, and increase the level of γ-IFN, thus having obvious therapeutic effect (Zhang, He, & Yang, 2018) Pan Qu et al. conducted in vitro studies on the anti-influenza virus effects of honeysuckle, forsythia, burdock and their extracts, which are the main drugs of Yinqiao Powder, and found that the single drugs and their compatible extracts have good anti-influenza A virus proliferation effects, and are correlated with drug concentration and duration of action (Pan, Wang, & Nan, 2011). Studies on the effect of honeysuckle-forsythia on respiratory syncytial virus show that Yinqiao powder has the effect of inhibiting respiratory syncytial virus, and the protective effect of drug-containing serum combined with the two drugs on cells is better than that of drug-containing serum of two single drugs (Mo, Lai, & Jiang, 2005). Yinqiao Powder can reduce the lung index of mice (Qin, 2016), reduce the damage of nasal mucosa epithelial cells and lung tissue. Its improvement effect on respiratory system of RSV infected mice is comparable to that of positive control drug ribavirin. In addition, in vitro cell experiments verified the improvement effect of Yinqiao Powder on RSV infection, and its mechanism of action is related to the activation of NALP3 inflammasome. An anti-RSV virus study conducted on Yinhua Ganmao Granules found that the half-toxic concentration of this preparation is 2.3937 mg / mL, the half-therapeutic concentration is 52.03 μg / mL, and the therapeutic index is 46.01, indicating that Yinhua Ganmao Granules have better inhibitory effect on RSV (Wang, 2013). In the study of antipyretic and anti-inflammatory effects of honeysuckle, forsythia single medicine and compatibility, Jiarui Wu et al studied the mechanism of action of honeysuckle-forsythia drug pair by network pharmacology (Jiarui, Jin, & Kaihuan, 2017). The diseases involved are mainly inflammatory diseases, which showed that the compatibility of the two drugs is consistent with the efficacy of clearing away heat and toxic materials. Although water decoction alone has certain anti-inflammatory effect, it has no statistical difference with the model group, and the anti-inflammatory effect is strongest when the two are used in combination at a ratio of 1:1 (Lin, Wang, & Wang, 2008). For antipyretic effect, the research results showed that forsythia alone has no antipyretic effect, and the antipyretic effect of honeysuckle and forsythia 1:1 compatibility is the most obvious, the combination of the two can reduce the level of IL-6 in experimental rats and play a certain antipyretic effect (Duan & Ma, 2009). Animal experiments have verified that in the antipyretic and anti-inflammatory effects, the combination of honeysuckle and forsythia can play a synergistic role.

To sum up, the active compounds phillyrin and chlorogenic acid have been explored to block the binding of SARS-CoV-2 S-protein and ACE2 by network pharmacology and molecular docking methods. The

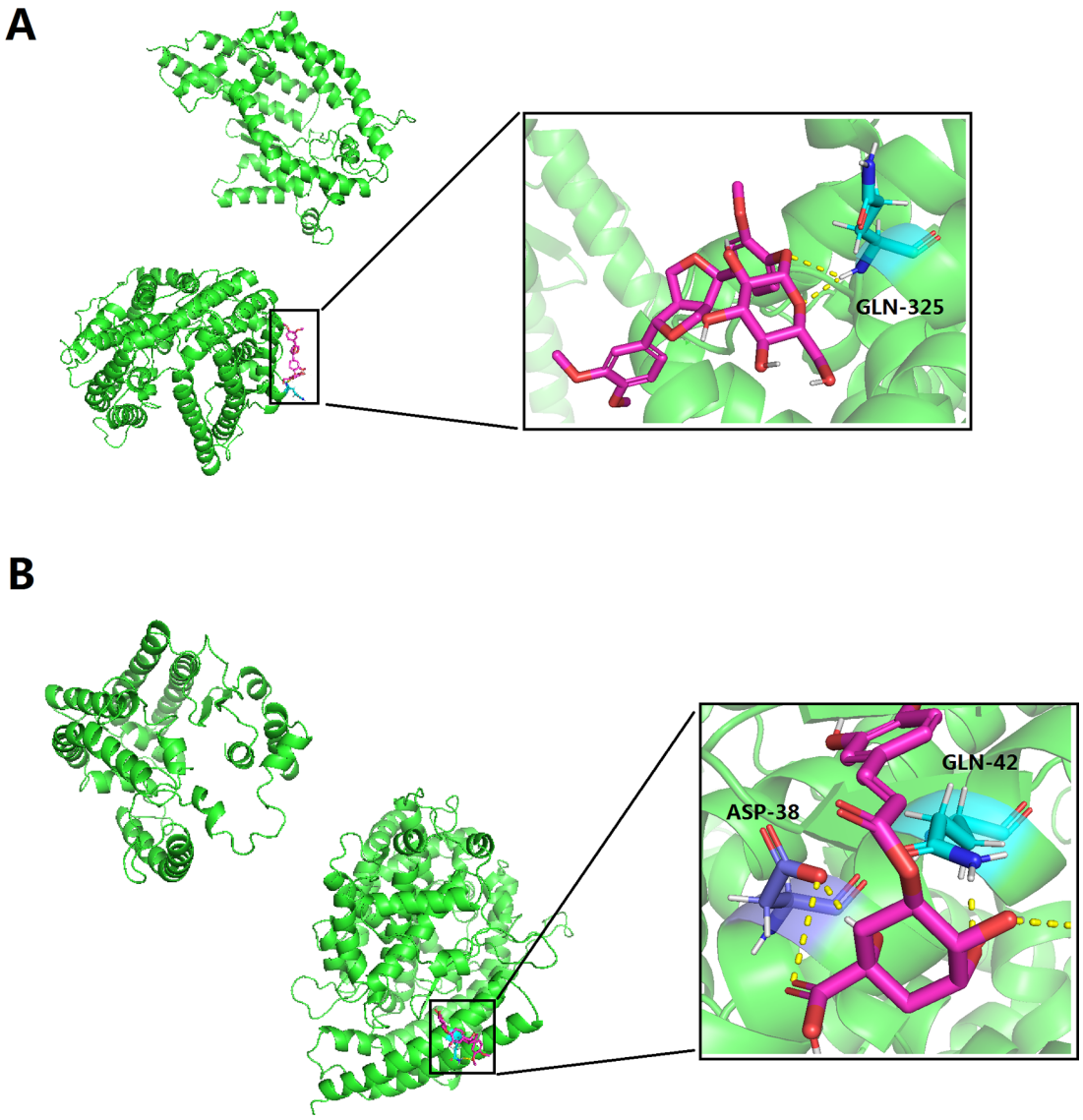

Fig. 7. A:the molecular docking between phillyrin and ACE2. Phillyrin and Gln325 in ACE2 are bonded to each other through hydrogen bonds; B: The binding situation of chlorophenolic acid and ACE2, and the chlorophenolic acid interacts with Asp38/Gln42 in ACE2 through hydrogen bonds.

Table 6
Binding affinity of phillyrin and chlorogenic acid *with ACE2 via* molecular docking.

Chemical compound	Putative target	Binding energy (kcal/mol)
phillyrin	Gln325	−0.29
Chlorogenic acid	Gln42	−0.87
	Asp38	−0.87

aim is to provide reference for further development of Mongolian medicine's treatment plan for COVID-19 epidemic. However, due to the lack of consideration of the content of chemical components, insufficient understanding of viruses and diseases, and limitations of molecular docking itself, the obtained results may have deviations. Later research should also be verified at multiple levels through pharmacodynamic evaluation, metabonomics, single target, etc.

5. Conclusions

In this study, a research strategy combining network pharmacological analysis, protein docking and molecular docking virtual computation was adopted. It was found that phillyrin and chlorogenic acid could block the combination of SARS-CoV-2 S-protein and ACE2 at the molecular level. Both can be used as potential inhibitors of SARS-CoV-2 for further research and development. The relevant research results of this experiment will provide theoretical basis for phillyrin and chlorogenic acid to resist SARS-CoV-2, and also provide methodological reference for the mechanism research of antiviral active ingredients of traditional Mongolia medicine.

6. Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Contributions

JY designed and analysed the data; LB provided technical assistance; LW provided intellectual input; LW Supervised the overall study and advised on study design and data interpretation; JY wrote the manuscript. All authors read and approved the final manuscript.

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Ethics declarations.

Ethics approval and consent to participate.

Not applicable.

Consent for publication.

The manuscript is approved by all authors for publication.

Competing interests.

The authors declare no competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104016.

References

CDC (2020). *2019 COVID-19 (COVID-19)*. China: Wuhan https://www.cdc.gov/coronavirus/novel-coronavirus-2019.html.

Cotton. M., Watson. S.J., Kellam. P., Al-Rabeeah. A.A., Makhdoom. H.Q., Assiri. A., et al. Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study. Lancet 382, 1993–2002.

National Health Committee, state administration of traditional chinese medicine. “Diagnosis and Treatment Plan for Pneumonia Infected in COVID-19 (Trial Fourth Edition)” [EB/OL]. (2020-01-28).

Jianyong, W. (2019). Observation on Clinical Effect of Lianhua Qingwen Capsule in Treating Influenza. *Medical Diet and Health, 16*, 29–31.

Zheng, C. S., Fan, Z. B., Fu, C. L., Y JX, Wu, G. G., & Liu, X. X. (2017). Study on the material basis, target and characteristics of Rongjin Niantong Decoction in the treatment of Sciatica by computer simulation. *Zhenggu, 29*(10), 20–24.

Duan, H., Zhai, K. F., Jilany, Khan Ghulam, Zhou, J., & Cao, T. Y. (2019). Revealing synergistic mechanism of multiple components in compound Fengshiding capsule for rheumatoid arthritis therapeutics by network pharmacology. *Current molecular medicine, 19*(4), 303–314.

Li, F., Li, W., & Farzan, M. (1864). Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science, 2005*, 309.

Yan, W., Ma, L. Z., & Liu, P. (2019). Feature selection and syndrome prediction for liver cirrhosis in traditional Chinese medicine. *Computer Methods and Programs in Biomedicine, 95*(3), 249–257.

Arun, V., & K.L. (2015). Delivering QOS with security of encrypted tracer and checker model in. *MANET. International journal of advanced intelligence paradigms, 7*(3/4), 342–351.

Gfeller, D., Grosdidier, A., & Wirth, M. (2014). SwissTargetPrediction: A web server for target prediction of bioactive small molecules. *Nucleic Acids Res, 42*, W32–W38.

Robert, Preissner, Stefan, Gconther, & Burghardt, Wittig (2008). SuperPred: Drug classification and target prediction. *Nucleic Acids Research, 36*(2), W55–W59.

Normile, Dennis (2020). Mystery virus found in Wuhan resembles bat viruses but not SARS, Chinese scientist says. *Science*. https://doi.org/10.1126/science:aba8542.

Raj, V. S., Mou, H., Smits, S. L., Dekkers, D. H. W., Müller, M. A., Dijkman, R., et al. (2013). Dipeptidyl peptidase 4 is a functional receptor for the emerginghuman coronavirus-EMC. *Nature, 495*, 251–254.

Schwede, T., Kopp, J., Guex, N., & Peitsch, M. C. (2003). SWISSMODEL: An automated protein homology-modeling server. *Nucleic Acids Res, 31*, 3381–3385.

Peng G, Sun D, Rajashankar KR. Crystalstructureof mousecoronavim sreceptor—binding domain complexed with its murine receptor. ProcNatlAcad SciUSA, 2011, 108 r261:10696—10701.

Xintian Xu, Ping Chen, Jingfang Wang, Jiannan Feng, Hui Zhou, Xuan Li, et al. Evolution of the COVID-19 from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission[J/OL].Science China Life Sciences:1-4[2020-02-19].

Zefeng, Wang (2020). Discussion on the drug pair with forsythia as the core and its compatibility rule in the ancient prescription of warm disease study. *Shanxi Traditional Chinese Medicine, 36*(01), 45–46.

Qian Wei, Zhang Rong, Wang Qin. (2020). Iridoid, phenylethanoid and flavonoid glycosides from Forsythia suspensa. Natural Product Research, 2020, 34(9).

Pan, X., Cao, X., & Li, N. (2014). Forsythin inhibits lipopolysaccharide-induced inflammation by suppressing JAKSTAT and p38 MAPK signalings and ROS production. *Inflammation Res, 63*(7), 597–608.

Yajun Ren, & Qu, Huihua (2014). Synthesis and identification of artificial antigen of forsythin. China. *Journal of Chinese Materia Medical, 39*(12), 2295–2299.

Qu, H., Zhang, Y., & Wang, Y. (2008). Antioxidant and antibacterial activity of two compounds (forsythiaside and forsythin) isolated from Forsythia suspensa. *J Pharm Pharmacol, 60*(2), 261–266.

Linjian, Duan, Qing, Zhang, Nongrong, Wang, Bin, Yang, Shiqin, He, & Jian, Sun (2012). Study on the Effect of Forsythin on Nucleoprotein Gene Expression of Influenza A Virus. *Chinese General Practitioner, 15*(18), 2082–2084.

Li, Haiyan (2017). The medicinal components and pharmacological analysis of honeysuckle. *Strait Pharmaceutical Journal, 29*(4), 46–47.

Mao, Lihua, Li, Shizhou, & Yang, Zhe (2018). Research progress on active components in honeysuckle and development of its products. *Jiangsu Science & Technology Information, 35*(17), 47–49.

Zhang, Haochao, Hao, Baoyan, & Sun, Haoyu (2017). Research progress of chlorogenic acid. *Food and Pharmaceuticals, 19*(3), 222–227.

Zhao, Mengjiao (2016). Advances in research on the active ingredients and pharmacology of honeysuckle. *World Latest Medicine Information, 16*(79), 33–34.

Zongfu Hu, Yu, Wenli, & Zhao, Yaping (2006). Study on the Scavenging of ROS and Anti-lipid Peroxidation by Chlorogenic Acid. *Food Science, 27*(2), 128–130.

Chen, Shaohua, Wang, Yaqin, & Luo, Lixin (2008). Research progress of natural product chlorogenic acid. *Food Science and Technology, 33*(2), 195–199.

Sheng Xiehuang, Liu Wenqian, Xue Xia. Anti-herpes simplex virus effect of chlorogenic acid in vitro. chinese journal of natural medicines, 2008, 5, 6 (3) :232-234.

Lin, Huang, Juri, Tang, & Jing, Huang (2012). Research Progress on Extraction and Application of Chlorogenic Acid Research on Laboratory Work in. *Universities, 9*(3), 114–117.

Li Lijing, Wang Jiyan, yan wang. Study on antiviral effect of Senecio cannabifolius extract and its effective components. Chinese Journal of Basic Medicine of Traditional Chinese Medicine. 2005, 11 (8) :585-587.

Haiqing, Xu., & Jia, Ni (2020). On Modern Clinical Application of Yinqiao Powder. *Journal of Liaoning University of Traditional Chinese Medicine, 22*(02), 164–167.

Tiange Zhang, Yijun Xi, Chaoyuan Huang, Jun Li, Xiaohui Liu, Huiting Huang. Medication Rules of “Feire” Syndrome in Ancient Books of Epidemic Febrile Disease Based on Data Mining [J/OL]. Pharmacology and Clinical Medicine:1-10 [2020-04-22].

Zhang, Meiyi, He, Jiajing, & Yang, Zifeng (2018). Study on Anti-influenza Virus Effect of Jinqiao Tablets. *Journal of Guangzhou University of Traditional Chinese Medicine, 35*(1), 143–148.

Pan, Zhaozhao, Wang, Xuefeng, & Nan, Chunhong (2011). Comparative Study on the Inhibitory Effect of Extracts from Yinqiao Powder on Influenza Virus in Vitro. *Journal of Pediatrics of Traditional Chinese Medicine, 7*(1), 9–12.

Mo, Hongying, Lai, Kefang, & Jiang, Yongnan (2005). Pharmacodynamics Study of Shuanghuanglian and Its Decomposed Prescription on Anti-Respiratory Syncytial Virus. *Chinese Journal of Basic Medicine in Traditional Chinese Medicine, 11*(3), 194–196.

Qin, Likui (2016). *Effect of Yinqiaosan on respiratory system model of respiratory syncytial virus infection in mice.* Guangzhou: Guangzhou University of Chinese Medicine.

Wang, Guanjie (2013). *Preparation of Yinqiao Kanggan Granules.* Jinan: Shandong University of Traditional Chinese Medicine.

Jiarui, Wu., Jin, Yanping, & Wang, Kaihuan (2017). Analysis of Action Mechanism of Honeysuckle-Forsythia Suspensa Based on Network Pharmacology. *Chinese Journal of Experimental Prescription, 23*(5), 179–183.

Lin, Limei, Wang, Zhimin, & Wang, Jinhua (2008). Study on the Anti-inflammatory and Antipyretic Effects of Honeysuckle, Forsythia Suspense and Yinqiao Drugs on Decoction. *Chinese Journal of Traditional Chinese Medicine, 33*(4), 473–475.

Duan, Hongyan, & Ma, Cheng (2009). Experimental Study on the Antipyretic Mechanism of the Compatibility of Honeysuckle and Forsythia. *Journal of Modern Integrative Medicine, 18*(11), 1214–1216.