Exploring the Active Compounds of Traditional Mongolian Medicine in Intervention of Novel Coronavirus (2019-nCoV) Based on Molecular Docking Method

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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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Abstract

Objective: This article intends to use molecular docking technology to find potential inhibitors that can respond to 2019-nCoV 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 2019-nCoV S-protein RBD domain and SARS virus, as well as their similarities in pathogenesis and clinical manifestations, we established 2019-nCoV's 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 using AutoDock molecular docking software. The interaction between ligand and receptor is applied to provide a choice for screening anti-2019-nCoV 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 2019-nCoV S-protein and ACE2 at the molecular level. Phillyrin and chlorogenic acid can be used as potential inhibitors of 2019-nCoV for further research and development.

KeyWords: Mongolian medicine; phillyrin; chlorogenic acid; 2019-nCoV; S-protein; ACE2

Introduction:

Since December 2019, the epidemic situation of novel Coronavirus (2019-nCoV) 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 2019-nCoV epidemic as a public health emergency of international concern (PHIC) [1,2]. 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 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. 2019-nCoV pneumonia belongs to the category of "epidemic fever" and "sticky epidemic" in Mongolian medicine, and is a "pulmonary epidemic fever" caused by virus infection[3]. Against the spread of 2019-nCoV, many Mongolian medical experts

have proposed different Mongolian medicine prevention and treatment programs[4].
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 2019-nCoV 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-2019-nCoV 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 [5]. 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[6].There are many links and factors that affect the infection in 2019-nCoV. According to previous studies, the infection routes of 2019-nCoV 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 [7]. 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.

Materials and methods

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) ≥30% and Drug-Likeness property (DL)≥0. 18 were selected as candidate active components[8]. 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/).

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 ≥ 0.6 in prediction results for further analysis[9]. SuperPred can predict the potential targets of unknown molecules by calculating the Tanimoto similarity between molecules and more than 300000 known compounds in the server[10]. 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.

Collection of Antiviral Drug Related Targets

Antiviral drug targets were collected from DurgBank (https://www.drugBank.ca/) and Genecard 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.

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[11]. The targets of antiviral action were inputted to the Metascape platform. After submitting, the attributes were set to "home sapiens" and $P<0.01$. 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.

Construction of S-protein model of 2019-nCoV

The sequence of 2019-nCoV S-protein fragment in the c-terminal RBD domain has high homology with SARS-CoV[12]. 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[13]. Compared with the SARS-CoV S-protein, the RBD domain of the 2019-nCoV S-protein has been replaced with amino acid residues at positions 442, 472, 479, 487 except for Tyr491 (Figure 4A). Substitution of residues at positions 442, 472, 479 and 487 of 2019-nCoV S-protein did not change its interface structure. 2019-nCoV 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 2019-nCoV have significant binding affinity with human ACE2[14]. Since the sequence of 2019-nCoV 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 2019-nCoV sequence file is imported into Swiss-Model (https://swissmodel.expasy.org/), and thus the structural model of 2019-nCoV S- protein is constructed.

Docking of S-protein with ACE2 protein

ZDOCK Server (http://zdock.umassmed.edu/) was used to rigidly dock 2019-nCoV 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 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.

Table 1 ZDOCK parameters of interaction between 2019-nCoV s-protein and ACE2 receptor

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

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 [15]. 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.

Results

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 Fructus Forsythiae, 236 from Flos Lonicerae, 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. With OB≥30% and DL≥0.18, 253 active compounds were screened out, of which 23 came from Fructus Forsythiae, 23 from Flos Lonicerae, 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. Table 2 is the basic information of some active compounds.

Table 2 basic information of some active compounds

Chinese medicine	Number	Compound name	OB/%	DL	Molecular Weight
Fructus Forsythiae	MOL003305	phillyrin	36.40	0.86	534.61
	MOL000006	luteolin	36.16	0.25	286.25
	MOL000522	arctiin	34.45	0.84	534.61
Flos Lonicerae	MOL000009	luteolin-7-o-glucoside	37.29	0.78	448.41
	MOL003059	kryptoxanthin	47.25	0.57	552.96
	MOL003871	Chlorogenic acid	33.61	0.31	354.34
Herba Ephedrae	MOL000422	kaempferol	41.88	0.24	286.25
	MOL002823	Herbacetin	36.07	0.27	302.25

Semen Armeniacae Amarum	MOL010922	Diisooctyl succinate	31.62	0.23	342.58
	MOL010921	estrone	53.56	0.32	270.40
Radix Isatidis	MOL001689	acacetin	34.97	0.24	284.28
	MOL002322	isovitexin	31.29	0.72	432.41
Herba Houttuyniae	MOL003851	Isoramanone	39.97	0.51	348.53
	MOL000098	quercetin	46.43	0.28	302.25
Herba Pogostemonis	MOL005573	Genkwanin	37.13	0.24	284.28
	MOL005922	Acanthoside B	43.35	0.77	580.64
Radix et Rhizoma Rhei	MOL002259	Physciondiglucoside	41.65	0.63	608.60
	MOL002281	Toralactone	46.46	0.24	272.27
Radix Glycyrrhizae	MOL000211	Mairin	55.38	0.78	456.78
	MOL000354	isorhamnetin	49.60	0.31	316.28

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 Fructus Forsythiae, 205 putative targets shared by 23 active compounds of Flos Lonicerae, 121 putative targets shared by 23 compounds of Herba Ephedrae, 56 putative targets shared by 19 active 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 1. For antiviral drugs, we have selected oseltamivir, zanamivir, paramivir, lopinavir, ritonavir, amantadine, rimantadine, chloroquine eight drugs. 1044 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 2.

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 Chinese 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 of lianhua qingwen capsule 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. 1B 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 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>0.3), and each node represents an enrichment term. The color of the node in Fig1-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 Fig1-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.

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	response to lipopolysaccharide	42	20.79	-36.61	-32.99

	Processes					
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 1 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.

Functional Attribution of Target Protein

Through the Metascape platform, all genes are connected to the whole protein interaction network(Fig 2B). 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 2C. 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.

Fig 2 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

Construction of 2019-nCoV 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 3. 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 the 2019nCOV S-protein model can be obtained. The result is shown in Fig 3, which is in line with the expected model and indicates the modeling is successful. The 2019-nCoV S-protein model sequence is shown in schedule 3.

Fig 3 The 2019-nCoV S protein model constructed.

Docking of 2019nCOV S protein with ACE2 protein

After 2019-nCoV 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[16]. After comprehensive consideration, complex2 was selected as the near-natural model (Fig 4B). Analysis of the key binding sites of 2019-nCoV and ACE2 showed that 2019- nCoV 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 5), which is consistent with previous research results of Hao Pei team [17].

Table 5 Screening results of 2019-nCoV S-protein and ACE2 binding model

complex	Interface area（Å²）	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

Fig 4 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 coronavirus S-protein and ACE2 protein. C：The combination of coronavirus S-protein and ACE2 protein in pyMOL software view.

Fig 5 Structural simulation of 2019-nCoV 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.

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 Figs. 6 and 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 2019-nCoV 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 2019-nCoV 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 2019-nCoV S-protein RBD domain and ACE2 at Gln42/Asp38. The above results show that the combination of phillyrin and chlorogenic acid will probably block the binding of ACE2 and S-protein more effectively.

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

Fig 6 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.

Discussion

Since the end of December 2019, 2019-nCoV has been ravaging the whole country. Currently, there is still a lack of specific drugs for the treatment of 2019-nCoV pneumonia, 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, 2019-nCoV 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, can save drug development costs, and is suitable for large-scale screening of chemical components of traditional Chinese 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.

The traditional Chinese medicine Fructus Forsythiae has various components such as phenylethanoid glycosides, lignans, pentacyclic triterpenes, volatile oil and the like, and each component has different effects. Phillyrin is the main component of lignan monosaccharide glycoside, and has the effects of antivirus, inhibition of platelet activating factor activity, antioxidation and the like [18]. RT-PCR showed that phillyrin can reduce the copy number of NP gene of influenza A virus. The Jian Sun team believes 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 [19].

Chlorogenic acid is very high in Eucommia ulmoides, honeysuckle, coffee bean, blueberry, apple and potato containing phenolic acids. Chlorogenic acid has antibacterial, anti-inflammatory, antiviral, antioxidant, immunoregulatory, anti-tumor, anti-leukemia, hypolipidemic and hypoglycemic biological activities[20]. Studies such as Wang Xuebing show that chlorogenic acid plays an obvious role in preventing virus infection in the early stage of virus growth, and also has a strong inhibitory effect on porcine parvovirus, showing that chlorogenic acid has a strong antiviral effect [21]. In vitro antiviral experiments by Xiehuang Sheng, Lin Huang, etc. have proved that chlorogenic acid has obvious effect on herpes simplex virus type I (HSV-1) infection, and can effectively prevent viral infection, and the antiviral effect increases with the increase of chlorogenic acid concentration [22]. Lijing Li and others have studied the antiviral effect of chlorogenic acid in Senecio cannabifolius, and the results show that chlorogenic acid extracted from Senecio cannabifolius can

produce better inhibitory effect on adenovirus, respiratory syncytial virus and influenza virus [23]. At present, the specific mechanism and mechanism of chlorogenic acid against 2019-nCoV need further study.

To sum up, the core active compounds phillyrin and chlorogenic acid have been explored to block the binding of 2019-nCoV S-protein and ACE2 by network pharmacology and molecular docking methods. The aim is to provide reference for further development of Mongolian medicine's treatment plan for 2019-nCoV 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.

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 2019-nCoV S-protein and ACE2 at the molecular level. Both can be used as potential inhibitors of 2019-nCoV for further research and development. The relevant research results of this experiment will provide theoretical basis for phillyrin and chlorogenic to resist 2019-nCoV, and also provide methodological reference for the mechanism research of antiviral active ingredients of traditional Mongolia medicine.

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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.

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.

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