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Traditional Chinese Medicine

Network pharmacology studies on the effect of Chai-Ling decoction in coronavirus disease 2019

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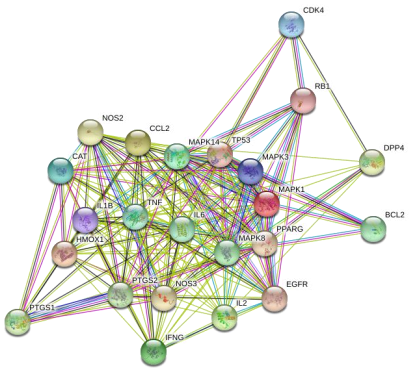
Highlights

The current study applied network pharmacology analysis and molecular docking method to study the potential mechanisms of Chai-Ling decoction (CLD), an empirical formula derived from the classic ancient prescription Xiao-Chai-Hu (XCH) decoction and Wu-Ling-San (WLS), on coronavirus disease 2019 (COVID-19).

Traditionality

The classic ancient prescription XCH and WLS decoctions originated from the ancient book of Chinese medicine Shang Han Za Bing Lun (*Treatise on Cold Damage Disorders,* 200–210 C.E.), written by Zhang Zhongjing. Previous studies have demonstrated that XCH can alleviate fever, cough, and fatigue, which were the primary clinical outcomes of COVID-19. Besides, WLS decoction has shown apparent effects on attenuating gastrointestinal symptoms. CLD, derived from a modification of XCH and WLS decoctions, is used to treat the early-stage of COVID-19 in the *Prevention and Treatment Guidelines of Damp-Heat Syndrome of "Taiyin" Lung* (respiratory system in the theory of traditional Chinese medicine) *Epidemic Disease* (*coronavirus pneumonia*). However, the mechanisms of action of CLD in COVID-19 remain unclear.



Abstract

Background: Chai-Ling decoction (CLD), derived from a modification of Xiao-Chai-Hu (XCH) decoction and Wu-Ling-San (WLS) decoction, has been used to treat the early-stage of coronavirus disease 2019 (COVID-19). However, the mechanisms of CLD in COVID-19 remain unknown. In this study, the potential mechanisms of CLD in COVID-19 were preliminarily investigated based on network pharmacology and molecular docking method. **Methods:** Initially, the active components and targets of CLD were screened based on Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform and PharmMapper database. The targets of COVID-19 were obtained from GeneCards database. The protein-protein interaction network was established using STRING database to analyze the key targets. Gene Oncology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes analysis were also conducted to evaluate the pathways related to the targets of CLD on COVID-19. Moreover, the compound-target-pathway network was established using Cytoscape 3.2.7. Subsequently, the molecular docking method was performed to select the active compounds with high binding affinity on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and angiotensin-converting enzyme 2 (ACE2), which is the key target of SARS-CoV-2 in entering target cells. The possible binding sites were also visualized by a three-dimensional graph. **Results:** Network pharmacology analysis showed that there were 106 active components and 160 targets of CLD. Additionally, 251 targets related to COVID-19 were identified, and 24 candidates of CLD on COVID-19 were selected. A total of 283 GO terms of CLD on COVID-19 were identified, and 181 pathways were screened based on GO and Kyoto Encyclopedia of Genes and Genomes analyses. CLD might alleviate the inflammatory response and improve lung injury to treat COVID-19 through interleukin 17 signaling, T helper cell 17 differentiation, tumor necrosis factor signaling, and hypoxia inducible factor-1 signaling. Besides, molecular docking indicated that beta-sitosterol, kaempferol, and stigmasterol were the top three candidates in CLD with the highest affinity to SARS-CoV-2 and ACE2. **Conclusion:** Our study identifies the potential mechanisms of CLD on COVID-19 and beta-sitosterol, kaempferol, and stigmasterol may be the key compounds that exert antiviral effects against SARS-CoV-2.

Keywords: Chai-Ling decoction, Coronavirus disease 2019, Network pharmacology, Molecular docking, Severe acute respiratory syndrome coronavirus 2, Angiotensin-converting enzyme 2

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***Abbreviations*:**

CLD, Chai-Ling decoction; XCH, Xiao-Chai-Hu; WLS, Wu-Ling-San; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme 2; GO, Gene Oncology; S protein, spike protein; TCM, traditional Chinese medicine; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; OB, oral bioavailability; DL, drug-like; PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; IL, interleukin; TNF, tumor necrosis factor; CCL, C-C motif ligand; MAPK, mitogen-activated protein kinase; CCs: cell components; MFs: molecular functions; BPs: biological processes; HIF-1, hypoxia inducible factor-1; Th17, T helper cell 17.

***Competing interests*:**

There are no conflicts of interest.

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Background

Since December 2019, a number of coronavirus disease 2019 (COVID-19) cases have been detected in various provinces and cities in China, and the disease quickly spread many foreign countries and regions [1]. Following severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome, the worldwide epidemic of COVID-19 has become one of the significant threats to human health and public safety.

Fever, dry cough, and fatigue are major clinical symptoms of COVID-19. Besides, some patients also exhibit myocardial, digestive, and neurological damage. Currently, no effective antiviral drug on COVID-19 has been developed. It remains unclear whether the drugs discovered, such as protease inhibitor indinavir, saquinavir, and kyprolis, have a definite effect on COVID-19. Although remdesivir has been shown to inhibit the replication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), further evidence is still needed. Besides, other possible drugs for COVID-19, including arbidol and darunavir, have shown low bioactivity. Zhou. et al found that SARS-CoV-2 mainly infected target cells by binding the spike protein (S protein) on the envelope to angiotensin-converting enzyme 2 (ACE2) [2], which is the receptor on the surface of target cells, similar to SARS-CoV [3]. ACE2 is a type I transmembrane protein composed of 805 amino acids, mainly distributes in the lung, kidney, testicle, heart, and other tissues. Disrupting the binding of S protein and ACE2 could be a therapeutic target of COVID-19 [4].

Traditional Chinese medicine (TCM) has accumulated abundant experience in the prevention and treatment of infectious diseases and shown beneficial effects on COVID-19. Studies indicate that TCM can decrease the transformation of mild to severe cases [5]. Qing Fei Pai Du decoction, which is a modification of Xiao-Chai-Hu (XCH) decoction, Wu-Ling-San (WLS), Ma Xing Shi Gan decoction, and She Gan Ma Huang decoction in Shang Han Za Bing Lun (*Treatise on Cold Damage Disorders,* 200–210 C.E.), has shown 90% effective rate on COVID-19 [6]. Previous studies have demonstrated that XCH (Chaihu (*Bupleuri Radix*), Banxia (*Pinelliae Rhizoma*), Renshen (*Panax Ginseng* C. A. Mey), Gancao (*Glycyrrhizae Radix et Rhizoma*), Huangqin (*Scutellariae Radix*), Shengjiang (*Zingiberis Rhizoma Recens*), and Dazao (*Jujubae Fructus*)) can alleviate fever [7], cough [8], and fatigue [9], the primary clinical outcomes of COVID-19. According to the *Diagnosis and treatment program of TCM on COVID-19 in Hunan (China)*, XCH can be used to treat patients with COVID-19 with bitter taste, hiccup, and fever [10]. Additionally, WLS decoction (Guizhi (*Cinnamomi Ramulus*), Fuling (*Poria*), Zhuling (*Polyporus*), Zexie (*Alismatis Rhizoma*), and Baizhu (*Atractylodis Macrocephalae Rhizoma*)) has shown apparent effects on attenuating gastrointestinal symptoms [11].

Chai-Ling decoction (CLD) (Chaihu 24 g (*Bupleuri Radix*), Huangqin 9 g (*Scutellariae Radix*), Guizhi 6–9 g (*Cinnamomi Ramulus*), Baizhu 9 g (*Atractylodis Macrocephalae Rhizoma*), Fuling 10 g (*Poria*), Zhuling 10 g (*Polyporus*), Zexie 9 g (*Alismatis Rhizoma*), Gancao 6 g (*Glycyrrhizae Radix et Rhizoma*), Shishangbai 30 g (*Selaginella Doederleinii*), and Lianqiao 30 g (*Forsythiae Fructus*)) was derived from a modification of XCH and WLS decoctions, which originated from Shang Han Za Bing Lun (*Treatise on Cold Damage Disorders,* 200–210 C.E.) written by Zhang Zhongjing . Moreover, CLD has been shown to have anti-inflammatory, anticoagulant, antioxidant, hypoglycemic, immunomodulatory, and diuretic effects and used in the treatment of liver, kidney, and joint diseases [12]. According to the *Prevention and Treatment Guidelines of Damp-Heat Syndrome of "Taiyin" Lung* (respiratory system in the theory of traditional Chinese medicine) *Epidemic Disease* (*coronavirus pneumonia*), CLD could be used to treat the early stage of COVID-19 [13].

Due to the multiple targets and components of TCM, network pharmacology has been used as an essential tool to identify the key targets and mechanisms of TCM [14]. The molecular docking method can be used to analyze the interactions between drug components and targets protein to investigate the binding affinity and predict the possible binding sites of drugs [15]. In this study, the active components of CLD were screened through network pharmacology, and the potential targets and mechanisms of CLD in COVID-19 were predicted. The molecular docking method was used to identify the key compounds in CLD with high binding affinity on the SARS-CoV-2 and ACE2, which is the key target of SARS-CoV-2 in entering target cells.

Materials and methods

Identification of the main active compounds

The main compounds in CLD were screened based on the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com/tcmsp.php) and literatures, and Chaihu (*Bupleuri Radix*), Huangqin (*Scutellariae Radix*), Guizhi (*Cinnamomi Ramulus*), Baizhu (*Atractylodis Macrocephalae Rhizoma*), Fuling (*Poria*), Zhuling (*Polyporus*), Zexie (*Alismatis Rhizoma*), Gancao (*Glycyrrhizae Radix et Rhizoma*), Shishangbai (*Selaginella Doederleinii*), and Lianqiao (*Forsythiae Fructus*) were used as keywords to query the candidate components of CLD. Compounds with an oral bioavailability (OB) ≥ 30% are considered to be absorbed and utilized by the human body [16]. Drug-like (DL) is a necessary condition for the

preparation of compound medicine. The DL value represents the similarity between the composition and known chemical medicine. It is generally considered that the composition with DL value ≥ 0.18 has an important reference value for the activity of the body [17]. The OB and DL values of each compound can be directly obtained in the TCMSP, and the effective components of CLD were obtained by screening for OB ≥ 30% and DL value ≥ 0.18.

Prediction of potential targets and annotation of gene names

Reverse pharmacophore search was used to identify the targets with a high binding affinity with drug components. Initially, the molecular structures of compounds identified from CLD were matched reversely to the pharmacophore database. The chemical composition obtained in 1.1 was imported into the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) by name, and the three-dimensional structure of each component were obtained and stored in sdf format and uploaded to the PharmMapper (http://www.lilab-ecust.cn/pharmmapper/) to obtain human-related protein targets for active ingredients [18]. The UniProt database (https://www.uniprot.org/) was used to obtain the relative gene name of each target.

Potential targets for prediction of the disease

Novel coronavirus pneumonia was used as the keyword and imported to the GeneCards database (https://www.genecards.org) to identify the key targets of COVID-19 [19]. The targets of CLD components and COVID-19 were intersected using Venny (version 2.1, http://bioinfogp.cnb.csic.es/tools/venny/) to obtain the key targets of CLD on COVID-19.

Protein-protein interaction (PPI) analysis and identification of key targets

STRING (https://string-db.org) can be used to study the PPI [20]. The potential targets of CLD in COVID-19 were imported into the STRING database to obtain the PPI among each target. According to the PPI network, proteins with high connectivity showed larger numbers and width of connections. Key targets with the top five connectivity were identified as the key targets.

Pathway enrichment using Gene Oncology (GO) analysis

GO analysis of targets of CLD on COVID-19 was conducted using DAVID database (http://www.david.niaid.nih.gov). DAVID database can provide detailed annotations of pathways, including cell components (CCs), molecular functions (MFs), and biological processes (BPs) [21]. Pathways with $P \leq 0.05$ and $q \leq 0.05$ were obtained.

Pathway enrichment using Kyoto Encyclopedia of Genes and Genomes (KEGG)

Targets of CLD on COVID-19 were imported into the KOBAS 3.0 database (http://kobas.cbi.pku.edu.cn) to obtain the KEGG terms. Pathways that may be involved in COVID-19 with $P \leq 0.05$ and $q \leq 0.05$ were identified, and the top ten KEGG pathways with low *P*-value were selected as the potential pathways of CLD on COVID-19.

Establishment of compound-target-pathway network

Cytoscape 3.7.2 was used to generate the compound-target-pathway network of CLD on COVID-19 [22]. According to the compound-target-pathway network, different active compounds, targets, and pathways were visualized with the nodes in different colors.

Molecular docking of major components in CLD with SARS-CoV-2 and ACE2

The three-dimensional structures of S protein of SARS-CoV-2 (PDB ID, 6LU7) and ACE2 (PDB ID, IR42) were downloaded in the RCSB database (https://www.rcsb.org/) and saved as pdb format. The three-dimensional structures of S protein in SARS-CoV-2 and ACE2 were added with hydrogen, electron, and ROOT using AutoDock software. The top 20 candidate compounds in CLD were selected for molecular docking, and the results of molecular docking were visualized using PyMOL software.

Results

Results of chemical compounds in CLD

A total of 1,068 compounds were obtained from the TCMSP, including 288 from Chaihu (*Bupleuri Radix*), 58 from Huangqin (*Scutellariae Radix*), 280 from Gancao (*Glycyrrhizae Radix et Rhizoma*), 106 from Guizhi (*Cinnamomi Ramulus*), 55 from Baizhu (*Atractylodis Macrocephalae Rhizoma*), 34 from Fuling (*Poria*), 31 from Zhuling (*Polyporus*), 46 from Zexie (*Alismatis Rhizoma*), 20 from Shishangbai (*Selaginella Doederleinii*), and 150 from Lianqiao (*Forsythiae Fructus*). After removing the duplicated compounds, 106 active compounds in CLD with OB ≥ 30% and DL ≥ 0.18 were selected. Based on the literature, compounds that could not be detected by mass spectrum were removed and 106 compounds were identified as the main compounds of CLD, including 12 from Lianqiao (*Forsythiae Fructus*) [23], 6 from Zexie (*Alismatis Rhizoma*) [24], 1 from Shishangbai (*Selaginella Doederleinii*) [25], 5 from Zhuling (*Polyporus*) [26], 9 from Fuling (*Poria*) [27], 4 from Baizhu (*Atractylodis Macrocephalae Rhizoma*) [28], 3 from Guizhi (*Cinnamomi Ramulus*) [29], 14 from Huangqin (*Scutellariae Radix*) [30], 6 from Chaihu (*Bupleuri Radix*) [31], and 46 from Gancao (*Glycyrrhizae Radix et Rhizoma*) [32–34]. The detailed information of selected active compounds is presented in Table 1.

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Table 1 Information of active compounds in CLD

MOL_ID	Molecule_name	OB (%)	DL	Herb	Node
MOL000006	Luteolin	36.16	0.25	FF	M1
MOL000098	Quercetin	46.43	0.28	FF, BR, GRER	M2
MOL000173	Wogonin	30.68	0.23	FF, SR	M3
MOL000358	Beta-sitosterol	36.91	0.75	FF, CR, SR	M4
MOL000422	Kaempferol	41.88	0.24	FF, BR, GRER	M5
MOL003295	(+)-Pinoresinol monomethyl ether	53.08	0.57	FF	M6
MOL003305	Phillyrin	36.4	0.86	FF	M7
MOL003306	ACon1_001697	85.12	0.57	FF	M8
MOL003322	Forsythinol	81.25	0.57	FF	M9
MOL003330	(-)-Phillygenin	95.04	0.57	FF	M10
MOL003347	Hyperforin	44.03	0.6	FF	M11
MOL003348	Adhyperforin	44.03	0.61	FF	M12
MOL000359	Sitosterol	36.91	0.75	SD, AR, CR, SR, GRER	M13
MOL000830	Alisol B	34.47	0.82	AR	M14
MOL000831	Alisol B monoacetate	35.58	0.81	AR	M15
MOL000832	Alisol B 23-acetate	32.52	0.82	AR	M16
MOL000853	Alisol B	36.76	0.82	AR	M17
MOL000854	Alisol C	32.7	0.82	AR	M18
MOL000856	Alisol C monoacetate	33.06	0.83	AR	M19
MOL000816	Ergosta-7,22-dien-3-one	44.88	0.72	PO	M20
MOL000817	Ergosta-5,7,22-trien-3-ol	46.18	0.72	PO	M21
MOL000820	Polyporusterone E	45.71	0.85	PO	M22
MOL000822	Polyporusterone G	33.43	0.81	PO	M23
MOL011169	Peroxyergosterol	44.39	0.82	PO	M24
MOL000276	7,9(11)-Dehydropachymic acid	35.11	0.81	P	M25
MOL000283	Ergosterol peroxide	40.36	0.81	P	M26
MOL000287	3Beta-hydroxy-24-methylene-8-lanostene-21-oic acid	38.7	0.81	P	M27
MOL000289	Pachymic acid	33.63	0.81	P	M28
MOL000290	Poricoic acid A	30.61	0.76	P	M29
MOL000291	Poricoic acid B	30.52	0.75	P	M30
MOL000292	Poricoic acid C	38.15	0.75	P	M31
MOL000296	Hederagenin	36.91	0.75	P	M32
MOL000300	Dehydroeburicoic acid	44.17	0.83	P	M33
MOL000021	14-Acetyl-12-senecioyl-2E,8E,10E-atractylentriol	60.31	0.31	AMR	M34
MOL000022	14-Acetyl-12-senecioyl-2E,8Z,10E-atractylentriol	63.37	0.3	AMR	M35
MOL000049	3β-Acetoxyatractylone	54.07	0.22	AMR	M36
MOL000072	8β-Ethoxy atractylenolide III	35.95	0.21	AMR	M37
MOL000073	ent-Epicatechin	48.96	0.24	CR	M38
MOL000492	(+)-Catechin	54.83	0.24	CR	M39
MOL001736	(-)-Taxifolin	60.51	0.27	CR	M40

Table 1 Information of active compounds in CLD (*Continued*)

MOL_ID	Molecule_name	OB (%)	DL	Herb	Node
MOL000228	(2R)-7-Hydroxy-5-methoxy-2-phenylchroman-4-one	55.23	0.2	SR	M41
MOL000525	Norwogonin	39.4	0.21	SR	M42
MOL000552	5,2'-Dihydroxy-6,7,8-trimethoxyflavone	31.71	0.35	SR	M43
MOL001689	Acacetin	34.97	0.24	SR	M44
MOL002714	Baicalein	33.52	0.21	SR	M45
MOL002908	5,8,2'-Trihydroxy-7-methoxyflavone	37.01	0.27	SR	M46
MOL002909	5,7,2,5-Tetrahydroxy-8,6-dimethoxyflavone	33.82	0.45	SR	M47
MOL002917	5,2',6'-Trihydroxy-7,8-dimethoxyflavone	45.05	0.33	SR	M48
MOL002925	5,7,2',6'-Tetrahydroxyflavone	37.01	0.24	SR	M49
MOL002927	Skullcapflavone II	69.51	0.44	SR	M50
MOL002928	Oroxylin a	41.37	0.23	SR	M51
MOL002932	Panicolin	76.26	0.29	SR	M52
MOL002933	5,7,4'-Trihydroxy-8-methoxyflavone	36.56	0.27	SR	M53
MOL002934	Neobaicalein	104.3	0.44	SR	M54
MOL000354	Isorhamnetin	49.6	0.31	BR, GRER	M55
MOL000449	Stigmasterol	43.83	0.76	BR	M56
MOL002776	Baicalin	40.12	0.75	BR	M57
MOL004598	3,5,6,7-Tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone	31.97	0.59	BR	M58
MOL004609	Areapillin	48.96	0.41	BR	M59
MOL004648	Troxerutin	31.6	0.28	BR	M60
MOL000239	Jaranol	50.83	0.29	GRER	M61
MOL000392	Formononetin	69.67	0.21	GRER	M62
MOL000417	Calycosin	47.75	0.24	GRER	M63
MOL000497	Licochalcone a	40.79	0.29	GRER	M64
MOL000500	Vestitol	74.66	0.21	GRER	M65
MOL001484	Inermine	75.18	0.54	GRER	M66
MOL001792	DFV	32.76	0.18	GRER	M67
MOL002311	Glycyrol	90.78	0.67	GRER	M68
MOL002565	Medicarpin	49.22	0.34	GRER	M69
MOL003896	7-Methoxy-2-methyl isoflavone	42.56	0.2	GRER	M70
MOL004328	Naringenin	59.29	0.21	GRER	M71
MOL004808	Glyasperin B	65.22	0.44	GRER	M72
MOL004810	Glyasperin F	75.84	0.54	GRER	M73
MOL004811	Glyasperin C	45.56	0.4	GRER	M74
MOL004815	(E)-1-(2, 4-Dihydroxyphenyl)-3-(2, 2-dimethylchromen-6-yl)prop-2-en-1-one	39.62	0.35	GRER	M75
MOL004827	Semilicoisoflavone B	48.78	0.55	GRER	M76
MOL004828	Glepidotin A	44.72	0.35	GRER	M77
MOL004829	Glepidotin B	64.46	0.34	GRER	M78
MOL004833	Phaseolinisoflavan	32.01	0.45	GRER	M79
MOL004841	Licochalcone B	76.76	0.19	GRER	M80

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Table 1 Information of active compounds in CLD (*Continued*)

MOL_ID	Molecule_name	OB (%)	DL	Herb	Node
MOL004848	Licochalcone G	49.25	0.32	GRER	M81
MOL004855	Licoricone	63.58	0.47	GRER	M82
MOL004856	Gancaonin A	51.08	0.4	GRER	M83
MOL004879	Glycyrin	52.61	0.47	GRER	M84
MOL004883	Licoisoflavone	41.61	0.42	GRER	M85
MOL004884	Licoisoflavone B	38.93	0.55	GRER	M86
MOL004885	Licoisoflavanone	52.47	0.54	GRER	M87
MOL004903	Liquiritin	65.69	0.74	GRER	M88
MOL004908	Glabridin	53.25	0.47	GRER	M89
MOL004910	Glabranin	52.9	0.31	GRER	M90
MOL004911	Glabrene	46.27	0.44	GRER	M91
MOL004912	Glabrone	52.51	0.5	GRER	M92
MOL004915	Eurycarpin A	43.28	0.37	GRER	M93
MOL004917	Glycyroside	37.25	0.79	GRER	M94
MOL004924	(-)-Medicocarpin	40.99	0.95	GRER	M95
MOL004949	Isolicoflavonol	45.17	0.42	GRER	M96
MOL004957	HMO	38.37	0.21	GRER	M97
MOL004959	1-Methoxyphaseollidin	69.98	0.64	GRER	M98
MOL004961	Quercetin der.	46.45	0.33	GRER	M99
MOL005000	Gancaonin G	60.44	0.39	GRER	M100
MOL005001	Gancaonin H	50.1	0.78	GRER	M101
MOL005007	Glyasperins M	72.67	0.59	GRER	M102
MOL005008	Glycyrrhiza flavonol A	41.28	0.6	GRER	M103
MOL005012	Licoagroisoflavone	57.28	0.49	GRER	M104
MOL005013	18α-Hydroxyglycyrrhetic acid	41.16	0.71	GRER	M105
MOL005020	Dehydroglyasperins C	53.82	0.37	GRER	M106

CLD, Chai-Ling decoction; BR, *Bupleuri Radix* (Chaihu); SR, *Scutellariae Radix* (Huangqin); CR, *Cinnamomi Ramulus* (Guizhi); AMR, *Atractylodis Macrocephalae Rhizoma* (Baizhu); P, *Poria* (Fuling); PO, *Polyporus* (Zhuling); AR, *Alismatis Rhizoma* (Zexie); GRER, *Glycyrrhizae Radix et Rhizoma* (Gancao); SD, *Selaginella Doederleinii* (Shishangbai); FF, *Forsythiae Fructus* (Lianqiao).

Identification of the targets of CLD on COVID-19

Initially, 160 active ingredient targets were generated using PubChem and PharmMapper databases. The relative gene names of targets were obtained using UniProt database. Then, 251 targets of COVID-19 were identified according to the GeneCards database. After intersecting the targets of CLD in COVID-19, 24 potential targets of CLD in COVID-19 were obtained (Figure 1).

Establishment and analysis of PPI network

Generally, 24 target protein nodes and 181 interaction edges were obtained using PPI network analysis. The average degree of target protein was 15.1. According to the connectivity of target proteins, IL6, tumor necrosis factor (TNF), C-C motif ligand (CCL) 2, mitogen-activated protein kinase (MAPK) 1, and MAPK3 were identified as the key targets of CLD in COVID-19 (Figure 2).

GO analysis of common targets

Generally, 283 GO terms with $P \leq 0.05$ and $q \leq 0.05$ were generated to be related to the targets of CLD in COVID-19, including 229 BPs terms, 22 CCs terms, and 32 MFs terms. Lipopolysaccharide-mediated signaling pathway and positive regulation of nitric oxide biosynthetic process were the top two GO terms in BPs with low *P*-value. Caveola and extracellular space were the top two GO terms in CCs with low *P*-value. Enzyme binding and MAP kinase activity were the top two GO terms in MF with low *P*-value (Figure 3).

KEGG analysis of targets of CLD in COVID-19

A total of 181 KEGG terms were enriched as the potential pathways of CLD in COVID-19. The top ten pathways with high significance were selected and presented in Table 2. interleukin (IL)-17 signaling pathway, TNF signaling pathway, hypoxia inducible factor-1 (HIF-1) signaling pathway, and the differentiation of T helper cell 17 (Th17) were related to the potential therapeutic pathway of CLD in COVID-19.

Compound-target-pathway network construction

The compounds, targets, and pathways of CLD in COVID-19 were imported into Cytoscape 3.7.2 to generate the compound-target-pathway network. According to the compound-target-pathway network, red nodes represent the genes, orange nodes represent pathways, light green nodes represent the active ingredients of Chaihu (*Bupleuri Radix*), purple nodes represent active ingredient of Huangqin (*Scutellariae Radix*), light yellow nodes represent active ingredients of Lianqiao (*Forsythiae Fructus*), light blue nodes represent active ingredients of Gancao (*Glycyrrhizae Radix et Rhizoma*), and pink nodes represent the active ingredients of Guizhi (*Cinnamomi Ramulus*), Baizhu (*Atractylodis Macrocephalae Rhizoma*), Fuling (*Poria*), Zhuling (*Polyporus*), Zexie (*Alismatis Rhizoma*), Shishangbai (*Selaginella Doederleinii*) (Figure 4).

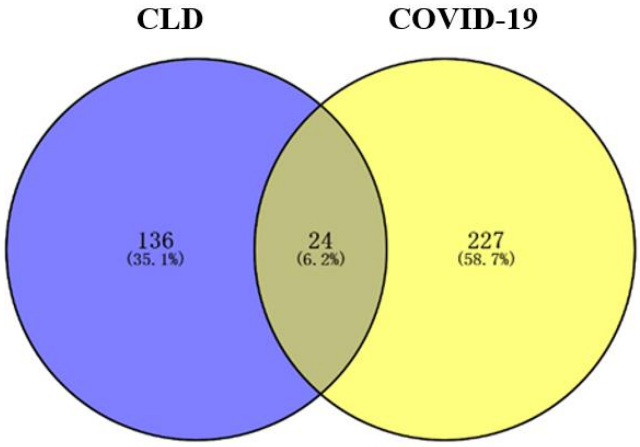

Figure 1 Common targets of CLD in COVID-19. CLD, Chai-Ling decoction; COVID-19, coronavirus disease 2019.

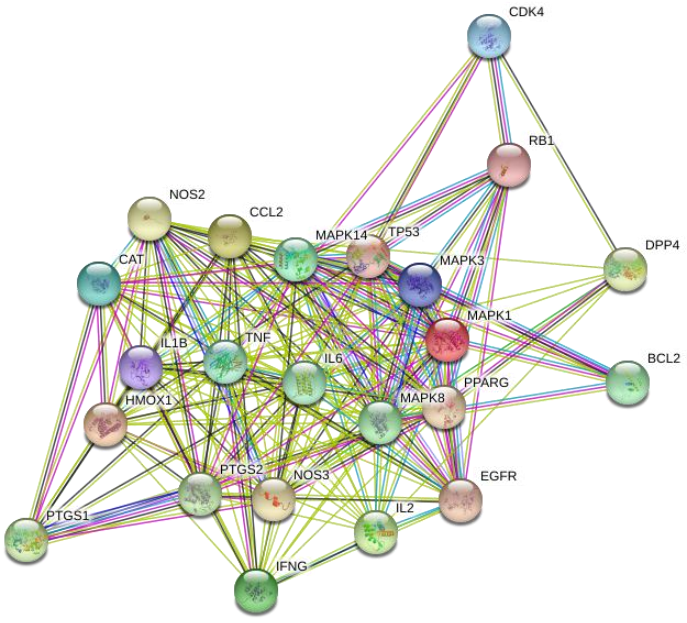

Figure 2 PPI network of target proteins. PPI, protein-protein interaction.

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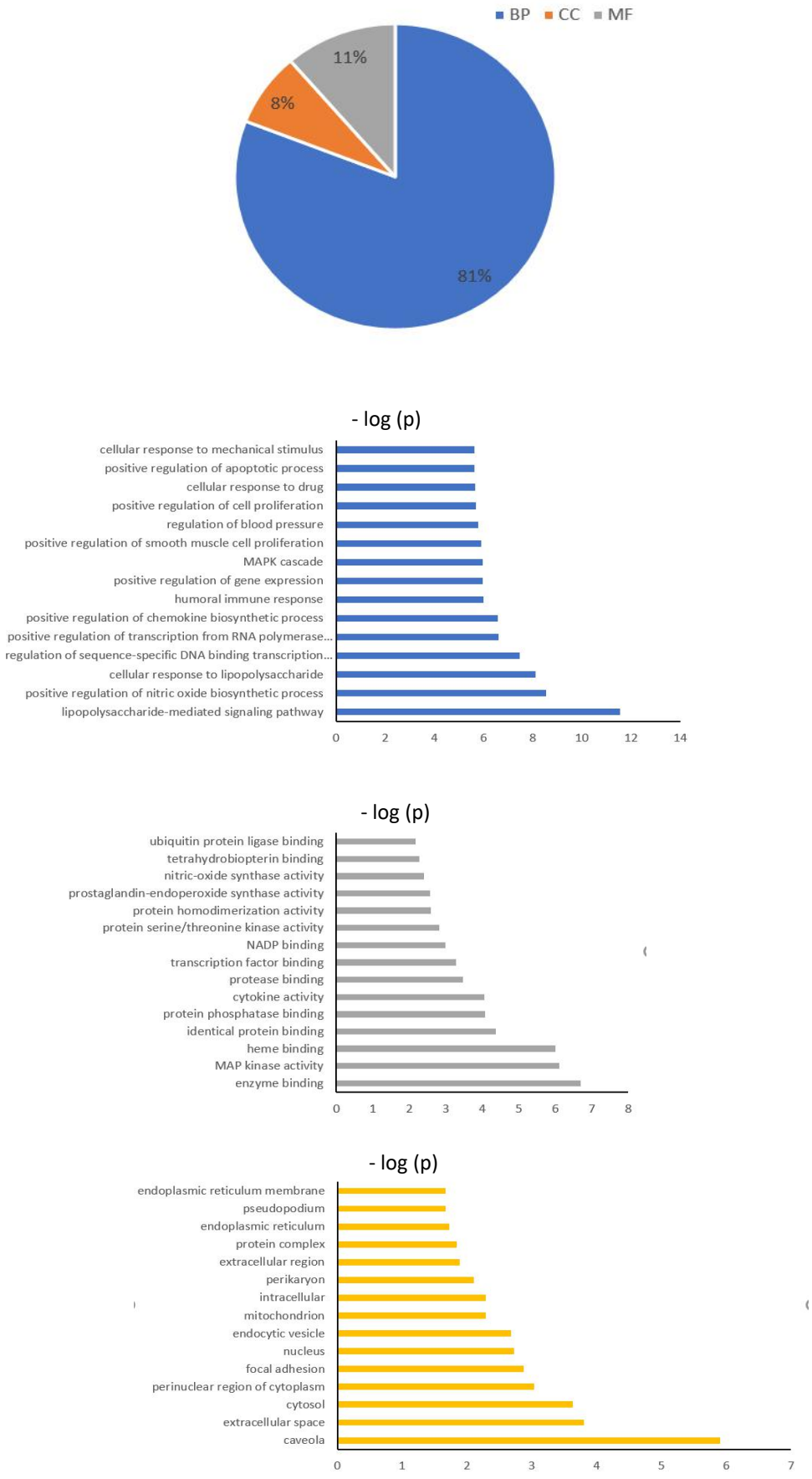

Figure 3 GO enrichment analysis of the common targets. GO, Gene oncology.

Table 2 KEGG enrichment analysis of the common targets

No.	Pathways	Numbers of genes	- log (p)
1	Human cytomegalovirus infection	12	22.22
2	IL-17 signaling pathway	10	21.29
3	Tuberculosis	10	18.58
4	Endocrine resistance	9	18.54
5	C-type lectin receptor signaling pathway	9	18.32
6	HIF-1 signaling pathway	9	18.14
7	TNF signaling pathway	9	18.04
8	NOD-like receptor signaling pathway	9	16.30
9	T cell receptor signaling pathway	8	15.90
10	Th17 cell differentiation	8	15.78

KEGG, Kyoto Encyclopedia of Genes and Genomes; IL, interleukin; HIF-1, hypoxia inducible factor-1; TNF, tumor necrosis factor; Th17, T helper cell 17.

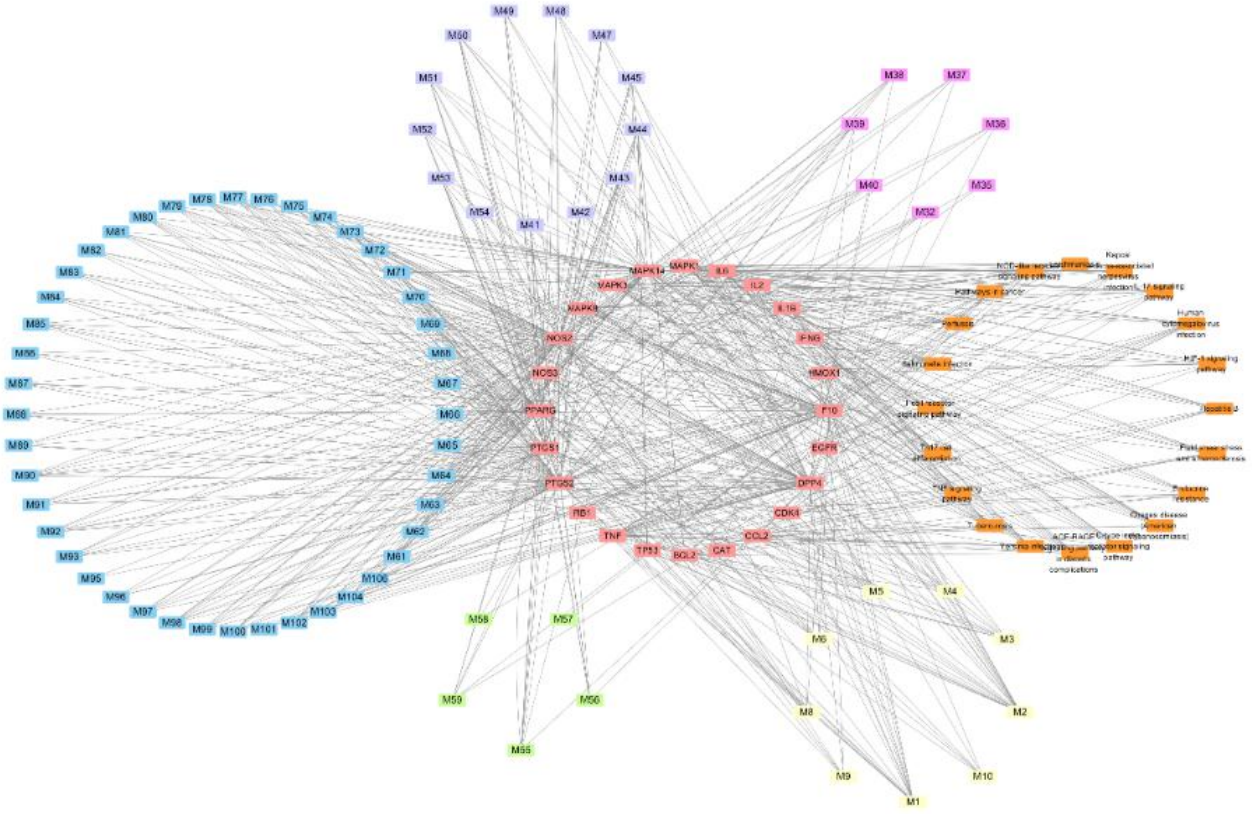

Figure 4 Compound-target-pathway network of CLD in COVID-19. Red nodes represent the genes, orange nodes represent pathways, light green nodes represent the active ingredients of Chaihu (*Bupleuri Radix*), purple nodes represent active ingredient of Huangqin (*Scutellariae radix*), light yellow nodes represent active ingredients of Lianqiao (*Forsythiae Fructus*), light blue nodes represent active ingredients of Gancao (*Glycyrrhizae Radix et Rhizoma*), and pink nodes represent the active ingredients of Guizhi (*Cinnamomi Ramulus*), Baizhu (*Atractylodis Macrocephalae Rhizoma*), Fuling (*Poria*), Zhuling (*Polyporus*), Zexie (*Alismatis Rhizoma*), Shishangbai (*Selaginella Doederleinii*).
CLD, Chai-Ling decoction; COVID-19, coronavirus disease 2019.

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Molecular docking analysis of active components in CLD with SARS-CoV-2 and ACE2

Compounds with high connectivity are associated with more targets. According to the number of related targets, the top 20 active compounds in CLD with high connectivity in compound-target-pathway network were selected for molecular docking. Besides, ten Western medicine compounds (arbidol, atazanavir, darunavir, indinavir, kyprolis, lopinavir, remdesivir, ritonavir, saquinavir, and tipranavir), which have been reported to be possibly used in COVID-19 treatment in Shanghai Institute of Materia Medica, were used as positive control for molecular docking based on the news report (http://www.simm.cas.cn/xwzx/kydt/202001/t20200125_5494417.html). Based on the docking score, a lower docking score indicates a stronger binding affinity to target protein. Beta-sitosterol, kaempferol, and stigmasterol had the strongest affinity with SARS-CoV-2 and ACE2. Moreover, their affinity with SARS-CoV-2 and ACE2 was better than some of these Western medicine compounds (Table 4 and Figures 5–6).

Table 4 Molecular docking table of ACE2 and SARS-CoV-2 with 20 active components in CLD and 10 Western medicine compounds

Compound	Molecular formula	Binding energy values (SARS-CoV-2)	Binding energy values (ACE2)
Luteolin	$C_{15}H_{10}O_6$	− 7.4	− 8.8
Quercetin	$C_{15}H_{10}O_7$	− 7.5	− 9.0
Wogonin	$C_{16}H_{12}O_5$	− 6.7	− 8.8
Mairin	$C_{30}H_{48}O_3$	− 7.4	− 9.6
Beta-sitosterol	$C_{30}H_{52}O$	− 8.1	− 10.9
Kaempferol	$C_{15}H_{10}O_6$	− 7.8	− 10.4
Arctiin	$C_{27}H_{34}O_{11}$	− 7.3	− 9.9
Bicuculline	$C_{20}H_{17}NO_6$	− 7.5	− 8.6
(3R,4R)-3,4-bis[(3,4-dimethoxyphenyl)methyl]oxolan-2-one	$C_{13}H_{22}O$	− 6.5	− 8.7
(+)-Pinoresinol monomethyl ether	$C_{27}H_{34}O_{11}$	− 6.9	− 8.9
Phyllyrin	$C_{27}H_{34}O_{11}$	− 7.1	− 9.4
Forsythinol	$C_{21}H_{24}O_6$	− 4.8	− 5.6
(-)-Phillygenin	$C_{21}H_{24}O_6$	− 6.9	− 9.0
β-Amyrin acetate	$C_{32}H_{52}O_2$	− 7.7	− 6.5
Hyperforin	$C_{35}H_{52}O_4$	− 5.9	− 9.1
Onjixanthone I	$C_{16}H_{14}O_6$	− 6.5	− 7.9
Stigmasterol	$C_{29}H_{48}O$	− 7.7	− 9.8
Yangambin	$C_{24}H_{30}O_8$	− 6.3	− 8.2
Alisol b	$C_{28}H_{44}O_4$	− 7.6	− 9.7
Alisol, b, 23-acetate	$C_{31}H_{47}O_6$	− 6.9	− 9.7
Arbidol	$C_{22}H_{26}BrClN_2O_3S$	− 6.5	− 8.2
Atazanavir	$C_{38}H_{52}N_6O_7$	− 7.7	− 12.4
Darunavir	$C_{27}H_{37}N_3O_7S$	− 7.6	− 9.6
Indinavir	$C_{36}H_{47}N_5O_4$	− 8.2	− 10.9
Kyprolis	$C_{40}H_{57}N_5O_7$	− 7.3	− 10.2
Lopinavir	$C_{37}H_{48}N_4O_5$	− 9.1	− 10.8
Remdesivir	$C_{27}H_{35}N_6O_8P$	− 7.5	− 11.0
Ritonavir	$C_{37}H_{48}N_6O_5S_2$	− 8.0	− 9.9
Saquinavir	$C_{38}H_{50}N_6O_5$	− 8.9	− 11.7
Tipranavir	$C_{31}H_{33}F_3N_2O_5S$	− 7.7	− 11.7

ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CLD, Chai-Ling decoction.

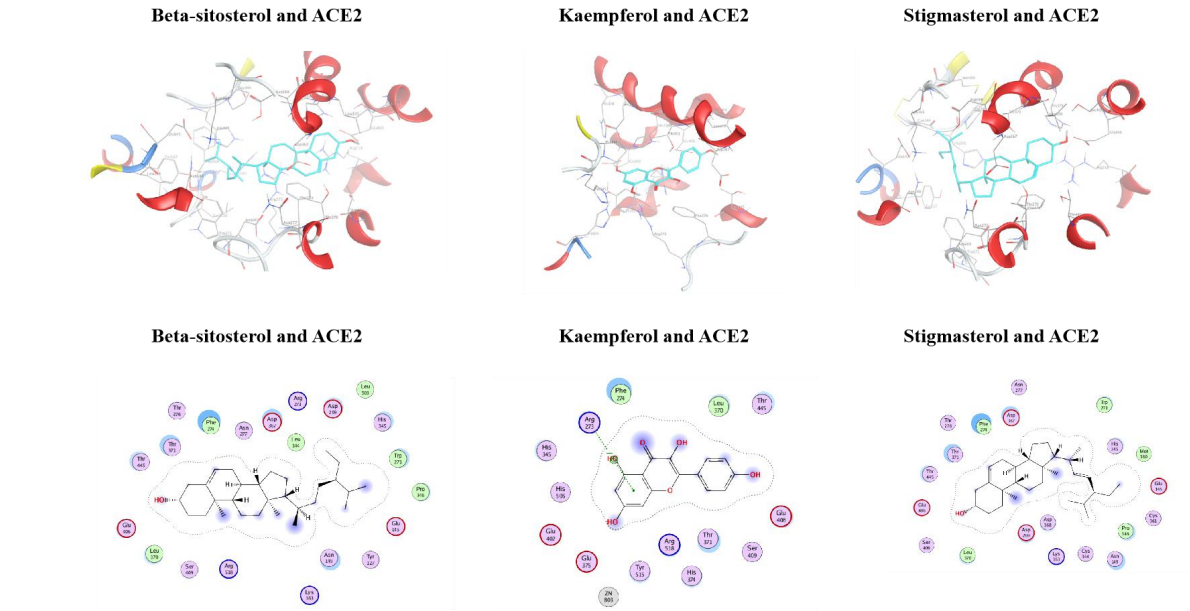

Figure 5 Molecular docking diagram of ACE2 with β-sitosterol, kaempferol, and stigmasterol. ACE2, angiotensin-converting enzyme 2.

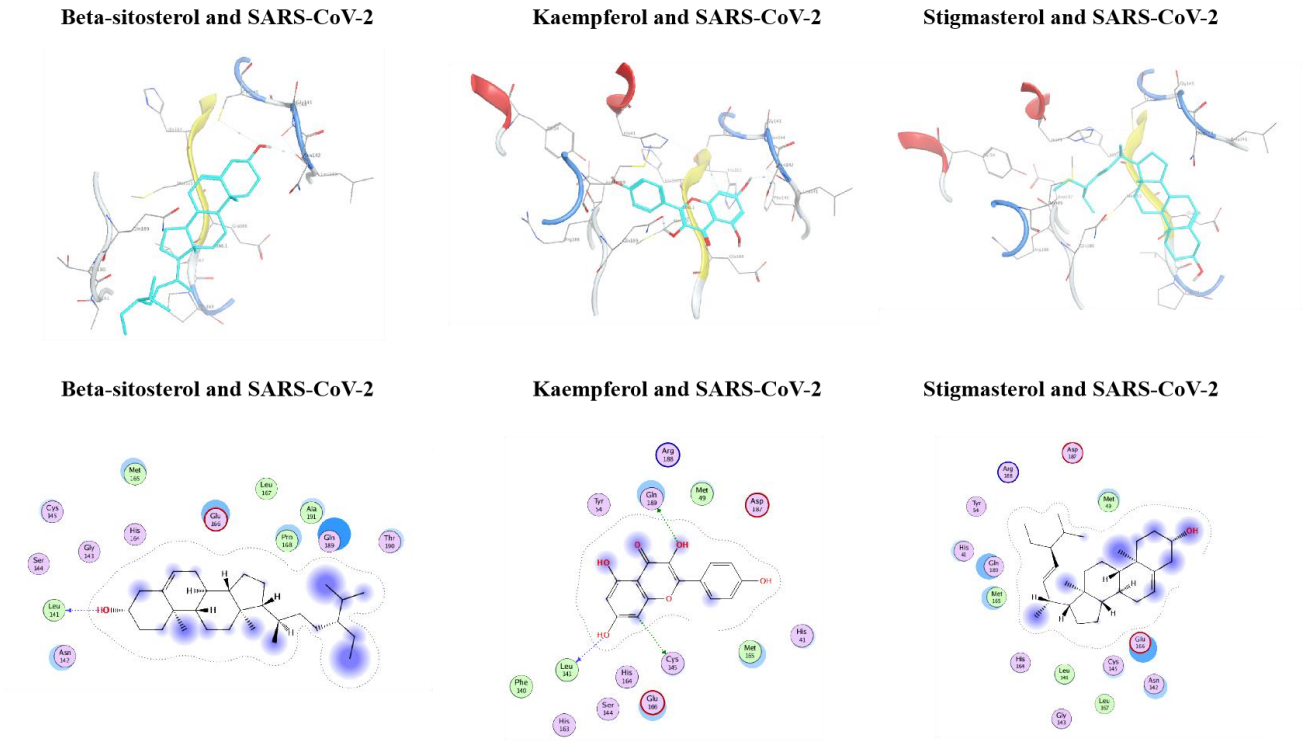

Figure 6 Molecular docking diagram of SARS-CoV-2 with β-sitosterol, kaempferol, and stigmasterol. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Discussion

Network pharmacology is commonly used to analyze the potential targets of TCM in COVID-19. A previous study corroborated that the components of Huoxiang Zhengqi Oral Liquid could be combined with ACE2 binding to prostaglandin endoperoxide synthase 2, heat shock protein 90 AB1, and calmodulin-regulated spectrin-associated protein 2 to modulate multiple signaling pathways, thereby exerting a preventive or therapeutic effect on COVID-19 [35]. Wang et al. indicated that the active compounds of Huanglian Jiedu decoction could bind novel coronavirus 3CL

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hydrolase on a target [36], such as prostaglandin endoperoxide synthase 2, heat shock protein 90 AA1, and estrogen receptor 1, which probably had an antiviral effect on COVID-19. Likewise, the main active components of Qing-Fei-Pai-Du decoction in the treatment of COVID-19 could regulate targets (e.g., MAPK1, MAPK3, MAPK8, MAPK14, and IL6) and pathways (e.g., TNF and nuclear factor kappa-B signaling pathways), thereby inhibiting inflammatory reaction, regulating immune function, reducing lung injury, and protecting nerve function [37].

Currently, according to the clinical investigation, patients with COVID-19 develop an excessive inflammatory response, known as cytokine storm [38, 39]. High levels of cytokines and inflammatory chemokines can overactivate the immune response against viral infections and cause extensive immunopathological damage in vital organs, such as the heart, lung, and kidney [40]. Our results showed that MAPK1, IL-6, TNF, CCL2, and MAPK3 might be the potential targets of CLD in COVID-19. MAPKs are essential transmitters of signals from the cell surface to the nucleus, which regulate many physiological activities, such as inflammation, apoptosis, oncogenesis, invasion, and metastasis of tumor cells [41]. MAPK signaling pathway also plays an essential role in the inflammatory response, which can be activated by some pro-inflammatory factors, such as TNF-α and IL family (e.g., IL-1 and IL-6), thereby aggravating the inflammatory response [42]. GO and KEGG analyses revealed that COVID-19 treatment by CLD involved a variety of BPs, CCs, and MFs. Meanwhile, the potential targets of CLD in COVID-19 were significantly enriched in multiple pathways, including IL-17 signaling pathway, Th17 cell differentiation, TNF signaling pathway, and HIF-1 signaling pathway, indicating that CLD may exhibit significant immunoregulatory effects in COVID-19 treatment. HIF-1, as a hypoxic signaling transcription factor, regulates the occurrence and development of immune inflammation in dendritic cells, macrophages, and T cells by regulating the expression of metabolization-related genes, thereby regulating the expression of immune-related genes and proteins and maturation and differentiation of related cells [43]. CCL can specifically promote chemotaxis and activation of eosinophils, leading to an inflammatory response [44]. Th17 secretes various effectors, such as IL-17, IL-6, and TNF-α, and IL-17 mediates the inflammatory response by inducing chemokines and various pro-inflammatory cytokines, such as IL-6 and TNF-α [45].

Following molecular docking, the active components in CLD, including beta-sitosterol, kaempferol, and stigmasterol, showed high binding affinity with both S protein of SARS-CoV-2 and ACE2, indicating that these compounds may directly inhibit SARS-CoV-2 infection. Interestingly, beta-sitosterol, kaempferol, and stigmasterol showed higher binding affinity to S protein than arbidol, kyprolis, and remdesivir and showed higher binding affinity to ACE2 than arbidol and darunavir.

Conclusion

This study indicates that modulating the inflammatory response can be the potential mechanisms of CLD in COVID-19. Besides, beta-sitosterol, kaempferol, and stigmasterol can be the key compounds that exert antiviral effects against SARS-CoV-2. Our prediction also provides the research fields to further study the mechanisms of CLD in SARS-CoV-2 infection in the future.

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