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A network pharmacology approach to explore the mechanism of Kangguan decoction in the treatment of coronavirus disease 2019 with preliminary verification

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Abstract

Objective: To explore the mechanism of Kangguan decoction in the treatment of coronavirus disease 2019 (COVID-19) and then perform preliminary verification. **Methods:** The effective compounds and target genes of Kangguan decoction were obtained from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. COVID-19 related target genes were searched in the GeneCards database. The active target genes of Kangguan decoction acting on COVID-19 were identified to perform GO function enrichment and KEGG pathway enrichment analysis. The compound-target network and protein-protein interaction were constructed; Molecular docking simulations of macromolecular protein target receptors and their corresponding compounds were performed. The clinical data of COVID-19 patients were retrieved from their electronic medical records of Nantong Third People's Hospital. **Results:** We screened out 137 effective compounds and 274 effective target genes of Kangguan decoction from TCMSP. The active target genes of Kangguan decoction were compared with the COVID-19 related target genes, and 63 active target genes for Kangguan decoction acting on COVID-19 were identified. GO function enrichment and KEGG pathway enrichment analysis were performed. The compound-target network and PPI network were constructed and the key compounds and key targets were selected to construct a key compound-target network. Finally, the binding of the target and its corresponding components was verified by molecular docking and two clinical cases with obvious clinical efficacy after Kangguan decoction application were demonstrated. **Conclusion:** The pharmacological mechanism of Kangguan decoction acting on COVID-19 has been explored, and the active compounds and targets of Kangguan decoction acting on COVID-19 and clinical efficacy for Kangguan decoction treating COVID-19 patients have been preliminarily verified.

Key words: Kangguan decoction, Coronavirus disease 2019, Network pharmacology, Molecular docking, Clinical efficacy, Quercetin, PTGS2, Interleukin-6.

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***Abbreviations*:** TCM, traditional Chinese medicine; KGD, Kangguan decoction; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; OB, Oral bioavailability, DL, drug-likeness; BP, biological process; MF, molecular function; CC, cellular component; TNF, tumor necrosis factor; CRP, C-reactive protein; PPI, protein-protein interaction;

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Background

In December 2019, COVID-19, caused by severe acute respiratory SARS-CoV-2, first broke out in Wuhan, China [1, 2]. Soon the global epidemic broke out and no definitive conclusion has been drawn about its origin. Despite efforts have been made to control the global epidemic, some areas are still not optimistic. Update to November 11,2020, there were 50,676,072 confirmed cases, 1,261,075 confirmed deaths, and 219 countries, areas or territories with cases, according to the WHO (https://www.who.int/emergencies/diseases/novel-coronavirus-2019).

There are no special drugs for COVID-19 treatment. How to form a rapid and effective COVID-19 treatment scheme is a major problem. The specific mechanism and efficacy of traditional Chinese medicine (TCM) in the treatment of COVID-19 are not very clear and clinical evidence is needed to evaluate the efficacy of TCM in the future [3, 4]. TCM has been playing an important role in the prevention and treatment of major outbreaks. Chinese herbal formula could be an alternative approach for prevention of COVID-19 in high-risk population and TCM could be considered as an adjunctive therapeutic option in the management of COVID-19 [5−7].

TCM plays a key role in the treatment of viral diseases, especially relieving symptoms [8, 9]. Yinqiao Power and Sangju Yin are two TCM prescriptions, long-term used for respiratory infections, pneumonia and other diseases. Studies have shown their role in anti-virus, anti-inflammatory and antipyretic [10−12].

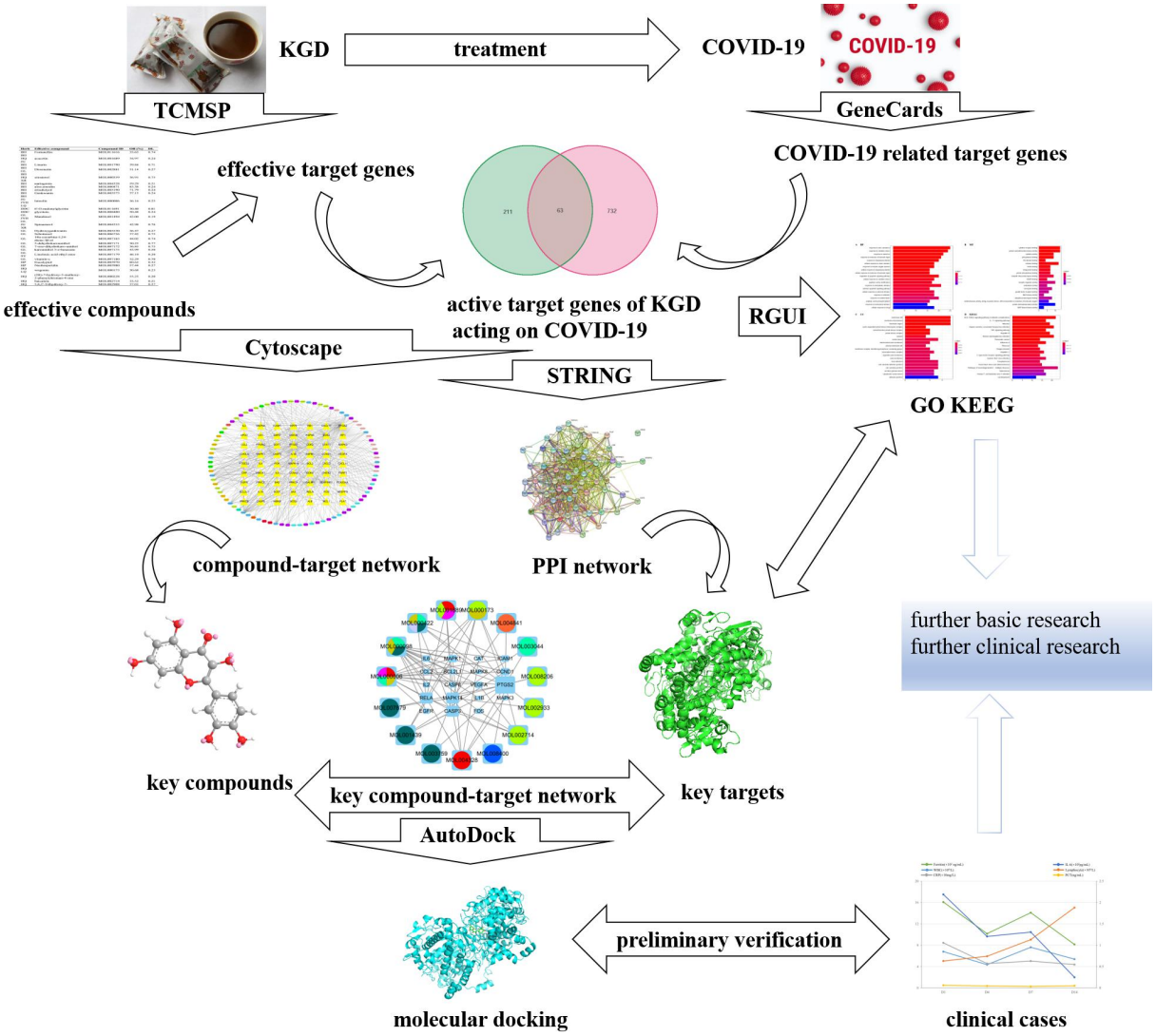

Figure 1 The flowchart of our study. KGD, Kangguan decoction; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform.

Nantong Third People's Hospital is a specialized infectious diseases hospital, which is designated to admit and manage most COVID-19 patients in Nantong city, China [13]. Based on the plan of the State Administration of TCM and traditional experience, Kangguan decoction (KGD) were used to treat of COVID-19 patients in our hospital. KGD was came up based on Yinqiao Power and Sangju Yin, mainly composed of Bohe (*Herba Menthae Haplocalycis*), Dandouchi (*Semen Sojae Preparatum*), Gualou (*Fructus Trichosanthis*), Houpo (*Cortex Magnoliae Officinalis*), Huangqin (*Radix Scutellariae*), Jiegeng (*Radix Platycodi*), Jinyinhua (*Flos Lonicerae*), Xingren (*Semen Armeniacae*), Lianqiao (*Fructus Forsythiae*), Niubangzi (*Fructus Arctii*), and Sangye (*Folium Mori*). From January 23 to now, 34 COVID-19 patients have been diagnosed and all successfully treated by the combined therapy of KGD and Western medicine. Through the application of KGD, we observed certain curative effect in the treatment of KGD on COVID-19 patients. In this study, we used network pharmacology approach to explore the pharmacological mechanism of KGD on COVID-19 and performed preliminary verification using molecular docking and clinical cases. The flowchart of our study is depicted in Figure 1.

Materials and methods

Screening of effective compounds in KGD

The compounds in the herbs of KGD were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com/tcmsp.php) [14]. TCMSP is a unique systems pharmacology platform of Chinese herbal medicine that captures the relationships between drugs, targets and diseases. Oral bioavailability (OB) and drug-likeness (DL) were used to screen for effective compounds. OB is an important index to measure the effectiveness of drugs entering the human circulatory system and good OB is one of the basic prerequisites for the pharmacological activity of compounds. DL is the physical and chemical properties and biological characteristics associated with good clinical efficacy. OB $\geq$ 30% and DL $\geq$ 0.18 are frequently valued in network pharmacology [15].

Screening of the target genes of effective compounds

The target genes of effective compounds were also acquired from TCMSP. The target genes were imported into the UniProt knowledge base, a comprehensive resource for protein sequence and annotation data (http://www.uniprot.org/) [16], and selecting search format as "homo sapiens", the human official gene symbols were charactered, which were considered as the effective target genes of KGD.

Acquisition of COVID-19 related target genes and identification of active target genes of KGD acting on COVID-19

Using "novel coronavirus pneumonia" and "coronavirus disease" as the key words in the GeneCards database (https://www.genecards.org/) [17], which is a searchable, integrative database providing comprehensive, user-friendly information on all annotated and predicted human genes, COVID-19 related target genes were searched and acquired. Then, the effective target genes of KGD were compared with the COVID-19 related target genes, and the intersecting genes were characterized as the active target genes of KGD acting on COVID-19.

GO function enrichment and KEGG pathway enrichment analysis

RGUI4.0.3 and org.Hs.eg.db package were used to gain the entrezIDs of candidate target genes. Then, RGUI, the clusterProfiler package were applied to operate GO function enrichment analysis, which included biological process (BP), molecular function (MF), cellular component (CC) analysis, and KEGG pathway enrichment analysis [18].

Construction of compound-target network and selection of key compounds

Cytoscape3.6.0 software was used to construct a compound-target network and its NetworkAnalyzer tool function was used to analyze the network [19]. Nodes performed compounds and target genes, and edges represented the relationships between them. On the basis of the degree of connection between the compound and the target gene, the key compounds in KGD acting on COVID-19 were elected.

Construction of PPI network and selection of key targets

A PPI network of KGD acting on COVID-19 was constructed after introducing the active target genes into the STRING database, supporting functional discovery in genome-wide experimental datasets (https://string-db.org/) [20]. Selecting the research species as "homo sapiens" and the lowest interaction score as 0.4, a PPI network was obtained. Then the PPI network was made topology analysis and the key targets of KGD acting on COVID-19 were selected according to the degree values of each target gene using Cytoscape3.6.0 software and its NetworkAnalyzer tool.

Verification of molecular docking

The binding of the target and its corresponding component was verified by molecular docking. The 2D and 3D structures of the small-molecule compounds were obtained from the PubChem Database

(https://pubchem.ncbi.nlm.nih.gov/) and the macromolecular protein target receptors were obtained from the RCSB PDB database (http://www.rcsb.org/). Molecular docking simulations of macromolecular protein target receptors and their corresponding compounds were performed using AutoDockTool 1.5.6 and AutoDock Vina software [21, 22], and further demonstration was using the PyMOL Molecular Graphics System (Version 2.4.0).

Participants of Clinical cases

The clinical data of COVID-19 patients were retrieved from their electronic medical records, including their symptoms, clinical parameters, and treatment and outcomes. The study was approved by Nantong Third Hospital Ethics Committee (E2020003). Written informed consents were obtained from each of the involved patients.

Statistical analysis

Using the bioinformatic tools of the platforms and software mentioned above, some statistical analysis was performed automatically. In GO function enrichment and KEGG pathway enrichment, adj $P < 0.05$ was considered as statistically significant.

Results

Screened effective compounds in KGD

We obtained 164 compounds in *Herba Menthae Haplocalycis*, 15 compounds in *Semen Sojae Preparatum*, 80 compounds in *Fructus Trichosanthis*, 139 compounds in *Cortex Magnoliae Officinalis*, 143 compounds in *Radix Scutellariae*, 102 compounds in *Radix Platycodi*, 236 compounds in *Flos Lonicerae*, 113 compounds in *Semen Armeniacae*, 150 compounds in *Fructus Forsythiae*, 144 compounds in *Fructus Arctii*, and 269 compounds in *Folium Mori* from TCMSP (Supplementary File 1). Setting filter criteria as OB ≥ 30% and DL ≥ 0.18, 10 effective compounds in *Herba Menthae Haplocalycis*, 2 effective compounds in *Semen Sojae Preparatum*, 11 effective compounds in *Fructus Trichosanthis*, 2 effective compounds in *Cortex Magnoliae Officinalis*, 36 effective compounds in *Radix Scutellariae*, 7 effective compounds in *Radix Platycodi*, 23 effective compounds in *Flos Lonicerae*, 19 effective compounds in *Semen Armeniacae*, 23 effective compounds in *Fructus Forsythiae*, 8 effective compounds in *Fructus Arctii*, and 29 effective compounds in *Folium Mori* were selected. Finally, 137 effective compounds in KGD were identified with exclusion of duplications. The basic information on the effective compounds in KGD is shown in Table 1.

Screened effective target genes of KGD

We also obtained the corresponding target genes of the 137 effective compounds from TCMSP, in which 28 compoundsdid not have targets (Supplementary File 2A).

And then, the corresponding gene symbols were screened by setting the format as "homo sapiens" from the UniProt knowledge base, and 3 compounds (MOL001790, MOL007180, and MOL003305) had no gene symbol matching their corresponding target genes (Supplementary File 2B). Finally, 274 effective target genes of 106 effective compounds in KGD were identified (Supplementary File 2C).

Acquired COVID-19 related target genes and identified active target genes of KGD acting on COVID-19

We used "novel coronavirus pneumonia" and "coronavirus disease" as the key words to search in the GeneCards database and 795 COVID-19 related target genes were acquired (Supplementary File 3).

The 274 effective target genes of KGD were compared with the 795 COVID-19 related target genes, and 63 active target genes of KGD acting on COVID-19 were identified (Figure 2, Table 2).

GO function enrichment and KEGG pathway enrichment analysis

The entrezIDs of active target genes of KGD acting on COVID-19 were obtained using RGUI and org.Hs.eg.db (Table 2). Next, GO function enrichment and KEGG pathway enrichment analysis were operated using RGUI and clusterProfiler.

Active target genes of KGD acting on COVID-19 were significantly enriched in response to toxic substance, response to oxidative stress, response to metal ion, response to molecule of bacterial origin, response to lipopolysaccharide, cellular response to biotic stimulus, response to reactive oxygen species, cellular response to lipopolysaccharide, cellular response to molecule of bacterial origin, regulation of apoptotic signaling pathway, and so on (Supplementary File 4A). The top 20 GO BP enrichments ranked by adj *P* value are shown in Figure 3A.

Active target genes of KGD acting on COVID-19 were significantly enriched in cytokine receptor binding, protein serine/threonine kinase activity, cytokine activity, phosphatase binding, BH domain binding, cofactor binding, heme binding, tetrapyrrole binding, protein phosphatase binding, ubiquitin-like protein ligase binding, and so on (Supplementary File 4B). The top 20 GO MF enrichments ranked by adj *P* value are shown in Figure 3B.

Active target genes of KGD acting on COVID-19 were significantly enriched in membrane raft, membrane microdomain, membrane region, cyclin-dependent protein kinase holoenzyme complex, serine/threonine protein kinase complex, protein kinase complex, caveola, vesicle lumen, mitochondrial outer

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Table 1 Basic information on the effective compounds in Kangguan decoction

Herb	Effective compound	Compound ID	OB (%)	DL
BH	Fortunellin	MOL011616	35.65	0.74
BH HQ JG	acacetin	MOL001689	34.97	0.24
BH	Linarin	MOL001790	39.84	0.71
BH GL	Diosmetin	MOL002881	31.14	0.27
BH HQ XR	sitosterol	MOL000359	36.91	0.75
BH	naringenin	MOL004328	59.29	0.21
BH	aloe-emodin	MOL000471	83.38	0.24
BH	eriodictyol	MOL005190	71.79	0.24
BH	Genkwanin	MOL005573	37.13	0.24
BH JG JYH LQ	luteolin	MOL000006	36.16	0.25
DDC	6'-O-malonylglycitin	MOL011691	30.40	0.81
DDC	glycitein	MOL008400	50.48	0.24
GL JYH	Mandenol	MOL001494	42.00	0.19
GL JG XR	Spinasterol	MOL004355	42.98	0.76
GL	Hydroxygenkwanin	MOL005530	36.47	0.27
GL	Schottenol	MOL006756	37.42	0.75
GL	10α-cucurbita-5,24-diene-3β-ol	MOL007165	44.02	0.74
GL	5-dehydrokarounidiol	MOL007171	30.23	0.77
GL	7-oxo-dihydrokaro-unidiol	MOL007172	36.85	0.75
GL	karounidiol 3-o-benzoate	MOL007175	43.99	0.50
GL SY	Linolenic acid ethyl ester	MOL007179	46.10	0.20
GL	vitamin-e	MOL007180	32.29	0.70
HP	Eucalyptol	MOL005970	60.62	0.32
HP	Neohesperidin	MOL005980	57.44	0.27
HQ LQ	wogonin	MOL000173	30.68	0.23
HQ	(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one	MOL000228	55.23	0.20
HQ	baicalein	MOL002714	33.52	0.21

Herb	Effective compound	Compound ID	OB (%)	DL
HQ	5,8,2'-Trihydroxy-7-methoxyflavone	MOL002908	37.01	0.27
HQ	5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone	MOL002909	33.82	0.45
HQ	Carthamidin	MOL002910	41.15	0.24
HQ	2,6,2',4'-tetrahydroxy-6'-methoxychaleone	MOL002911	69.04	0.22
HQ	Dihydrobaicalin_qt	MOL002913	40.04	0.21
HQ JYH	Eriodyctiol (flavanone)	MOL002914	41.35	0.24
HQ	Salvigenin	MOL002915	49.07	0.33
HQ	5,2',6'-Trihydroxy-7,8-dimethoxyflavone	MOL002917	45.05	0.33
HQ	5,7,2',6'-Tetrahydroxyflavone	MOL002925	37.01	0.24
HQ	dihydrooroxylin A	MOL002926	38.72	0.23
HQ	Skullcapflavone II	MOL002927	69.51	0.44
HQ	oroxylin a	MOL002928	41.37	0.23
HQ	Panicolin	MOL002932	76.26	0.29
HQ	5,7,4'-Trihydroxy-8-methoxyflavone	MOL002933	36.56	0.27
HQ	NEOBAICALEIN	MOL002934	104.34	0.44
HQ	DIHYDROOROXYLIN	MOL002937	66.06	0.23
HQ JYH LQ NBZ SY	beta-sitosterol	MOL000358	36.91	0.75
HQ	Norwogonin	MOL000525	39.40	0.21
HQ	5,2'-Dihydroxy-6,7,8-trimethoxyflavone	MOL000552	31.71	0.35
HQ	ent-Epicatechin	MOL000073	48.96	0.24
HQ JYH XR SY	Stigmasterol	MOL000449	43.83	0.76
HQ	coptisine	MOL001458	30.67	0.86
HQ	bis[(2S)-2-ethylhexyl] benzene-1,2-dicarboxylate	MOL001490	43.59	0.35
HQ NBZ SY	Supraene	MOL001506	33.55	0.42
HQ	Diop	MOL002879	43.59	0.39
HQ	epiberberine	MOL002897	43.09	0.78

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Herb	Effective compound	Compound ID	OB (%)	DL
HQ	Moslosooflavone	MOL008206	44.09	0.25
HQ	11,13-Eicosadienoic acid, methyl ester	MOL010415	39.28	0.23
HQ	5,7,4'-trihydroxy-6-methoxyflavanone	MOL012245	36.63	0.27
HQ	5,7,4'-trihydroxy-8-methoxyflavanone	MOL012246	74.24	0.26
HQ	rivularin	MOL012266	37.94	0.37
JG	cis-Dihydroquercetin	MOL004580	66.44	0.27
JG	2-O-methyl-3—O-β-D-glucopyranosyl platycogenate A	MOL005996	45.15	0.25
JG	dimethyl 2-O-methyl-3-O-a-D-glucopyranosyl platycogenate A	MOL006026	39.21	0.25
JG	robinin	MOL006070	39.84	0.71
JYH	Ethyl linolenate	MOL001495	46.10	0.20
JYH	phytofluene	MOL002707	43.18	0.50
JYH	(-)-(3R,8S,9R,9aS,10aS)-9-ethenyl-8-(beta-D-glucopyranosyloxy)-2,3,9,9a,10,10a-hexahydro-5-oxo-5H,8H-pyrano[4,3-d]oxazolo[3,2-a]pyridine-3-carboxylic acid_qt	MOL003006	87.47	0.23
JYH	secologanic dibutylacetal_qt	MOL003014	53.65	0.29
JYH NBZ SY	beta-carotene	MOL002773	37.18	0.58
JYH	ZINC03978781	MOL003036	43.83	0.76
JYH	Chryseriol	MOL003044	35.85	0.27
JYH	kryptoxanthin	MOL003059	47.25	0.57
JYH	4,5'-Retro-.beta.,.beta.-Carotene-3,3'-dione, 4',5'-didehydro-5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl)chromone	MOL003062	31.22	0.55
JYH		MOL003095	51.96	0.41
JYH	7-epi-Vogeloside	MOL003101	46.13	0.58
JYH	Caeruloside C	MOL003108	55.64	0.73
JYH	Centauroside_qt	MOL003111	55.79	0.50
JYH	Ioniceracetalides B_qt	MOL003117	61.19	0.19
JYH	XYLOSTOSIDINE	MOL003124	43.17	0.64

Herb	Effective compound	Compound ID	OB (%)	DL
JYH	dinethylsecologanoside	MOL003128	48.46	0.48
JYH LQ NBZ SY	kaempferol	MOL000422	41.88	0.24
JYH LQ SY	quercetin	MOL000098	46.43	0.28
XR	estrone	MOL010921	53.56	0.32
XR	Diisooctyl succinate	MOL010922	31.62	0.23
XR	11,14-eicosadienoic acid	MOL002211	39.99	0.20
XR	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	MOL002372	33.55	0.42
XR	gondoic acid	MOL005030	30.70	0.20
XR	CLR	MOL000953	37.87	0.68
XR LQ	Mairin	MOL000211	55.38	0.78
XR	(+)-catechin	MOL000492	54.83	0.24
XR	Glycyrol	MOL002311	90.78	0.67
XR	Ziziphin_qt	MOL003410	66.95	0.62
XR	Licochalcone B	MOL004841	76.76	0.19
XR	liquiritin	MOL004903	65.69	0.74
XR	Glabridin	MOL004908	53.25	0.47
XR	Phaseol	MOL005017	78.77	0.58
XR	Machiline	MOL007207	79.64	0.24
XR	l-SPD	MOL012922	87.35	0.54
LQ	20(S)-dammar-24-ene-3β,20-diol-3-acetate	MOL003281	40.23	0.82
LQ	(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	MOL003283	66.51	0.39
LQ NBZ	(3R,4R)-3,4-bis[(3,4-dimethoxyphenyl) methyl]oxolan-2-one	MOL003290	52.30	0.48
LQ	(+)-pinoresinol monomethyl ether	MOL003295	53.08	0.57
LQ	PHILLYRIN	MOL003305	36.40	0.86
LQ	ACon1_001697	MOL003306	85.12	0.57
LQ	(+)-pinoresinol monomethyl ether-4-D-beta-glucoside_qt	MOL003308	61.20	0.57
LQ	3beta-Acetyl-20,25-	MOL003315	33.07	0.79

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Herb	Effective compound	Compound ID	OB (%)	DL
	epoxydammarane-24alpha-ol			
LQ	FORSYTHINOL	MOL003322	81.25	0.57
LQ	(-)-Phillygenin	MOL003330	95.04	0.57
LQ	β-amyrin acetate	MOL003344	42.06	0.74
LQ	hyperforin	MOL003347	44.03	0.60
LQ	adhyperforin	MOL003348	44.03	0.61
LQ	Lactucasterol	MOL003365	40.99	0.85
LQ	Onjixanthone I	MOL003370	79.16	0.30
LQ NBZ	arctiin	MOL000522	34.45	0.84
LQ	bicuculline	MOL000791	69.67	0.88
NBZ	neoarctin A	MOL010868	39.99	0.27
NBZ	Cynarin(e)	MOL007326	31.76	0.68
SY	poriferast-5-en-3beta-ol	MOL001771	36.91	0.75
SY	scopolin	MOL002218	56.45	0.39
SY	Albanol	MOL003842	83.16	0.24
SY	Inophyllum E	MOL003847	38.81	0.85
SY	26-Hydroxy-dammara-20,24-dien-3-one	MOL003850	44.41	0.79
SY	Isoramanone	MOL003851	39.97	0.51
SY	Moracin B	MOL003856	55.85	0.23
SY	Moracin C	MOL003857	82.13	0.29
SY	Moracin D	MOL003858	60.93	0.38
SY	Moracin E	MOL003859	56.08	0.38
SY	Moracin F	MOL003860	53.81	0.23
SY	Moracin G	MOL003861	75.78	0.42
SY	Moracin H	MOL003862	74.35	0.51
SY	4-Prenylresveratrol	MOL003879	40.54	0.21
SY	FA	MOL000433	68.96	0.71
SY	Oxysanguinarine	MOL000729	46.97	0.87
SY	arachidonic acid	MOL001439	45.57	0.20
SY	Iristectorigenin A	MOL003759	63.36	0.34
SY	icosa-11,14,17-trienoic acid methyl ester	MOL003975	44.81	0.23
SY	Norartocarpetin	MOL006630	54.93	0.24
SY	Tetramethoxyluteolin	MOL007879	43.68	0.37
SY	Skimmin (8CI)	MOL013083	38.35	0.32

Abbreviations: BH, *Herba Menthae Haplocalycis*; HQ, *Radix Scutellariae*; JG, *Radix Platycodi*; GL, *Fructus Trichosanthis*; XR, *Semen Armeniacae*; JYH, *Flos Lonicerae*; LQ, *Fructus Forsythiae*; DDC, *Semen Sojae Preparatum*; SY, *Folium Mori*; HP, *Cortex Magnoliae Officinalis*; NBZ, *Fructus Arctii*; OB, oral bioavailability; DL, drug-likeness.

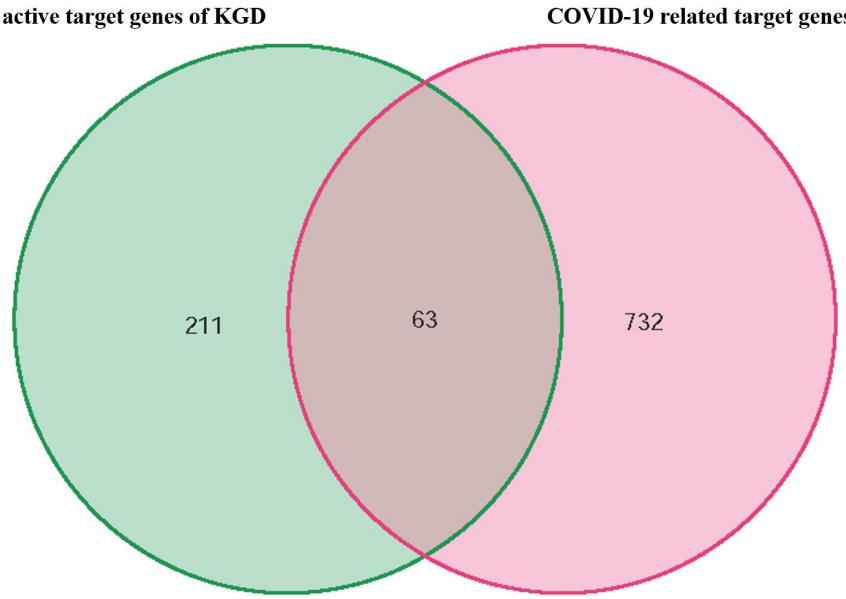

Figure 2 Active target genes of Kangguan decoction acting on COVID-19. The 274 active target genes of Kangguan decoction were compared with the 795 COVID-19 related target genes, and 63 active target genes of Kangguan decoction acting on COVID-19 were identified.

Table 2 Gene symbol and entrezID of active target genes

Gene symbol	EntrezID	Gene symbol	EntrezID	Gene symbol	EntrezID
NOS2	4843	HMOX1	3162	MAPK8	5599
PTGS1	5742	EGFR	1956	STAT1	6772
PTGS2	5743	VEGFA	7422	POR	5447
DPP4	1803	CCND1	595	HSPA5	3309
CDK2	1017	BCL2L1	598	PRKCB	5579
CALM1	801	RB1	5925	NOS3	4846
RELA	5970	CDK4	1019	HSPB1	3315
BCL2	596	IL6	3569	PLAT	5327
BAX	581	ICAM1	3383	SERPINE1	5054
CASP3	836	MCL1	4170	IL1A	3552
CASP8	841	IL2	3558	PARP1	142
MAPK3	5595	IL4	3565	CXCL11	6373
MAPK1	5594	CD40LG	959	CXCL2	2920
BAD	572	MAPK14	1432	CHEK2	11200
SOD1	6647	GSK3B	2932	CRP	1401
CAT	847	CCNA2	890	CXCL10	3627
PPARG	5468	CCL2	6347	IRF1	3659
GOT1	2805	FOS	2353	NPEPPS	9520
PRKCA	5578	APOD	347	G6PD	2539
PRKCE	5581	ALB	213	PLA2G4A	5321
IL1B	3553	IKBKB	3551	PTGES2	80142

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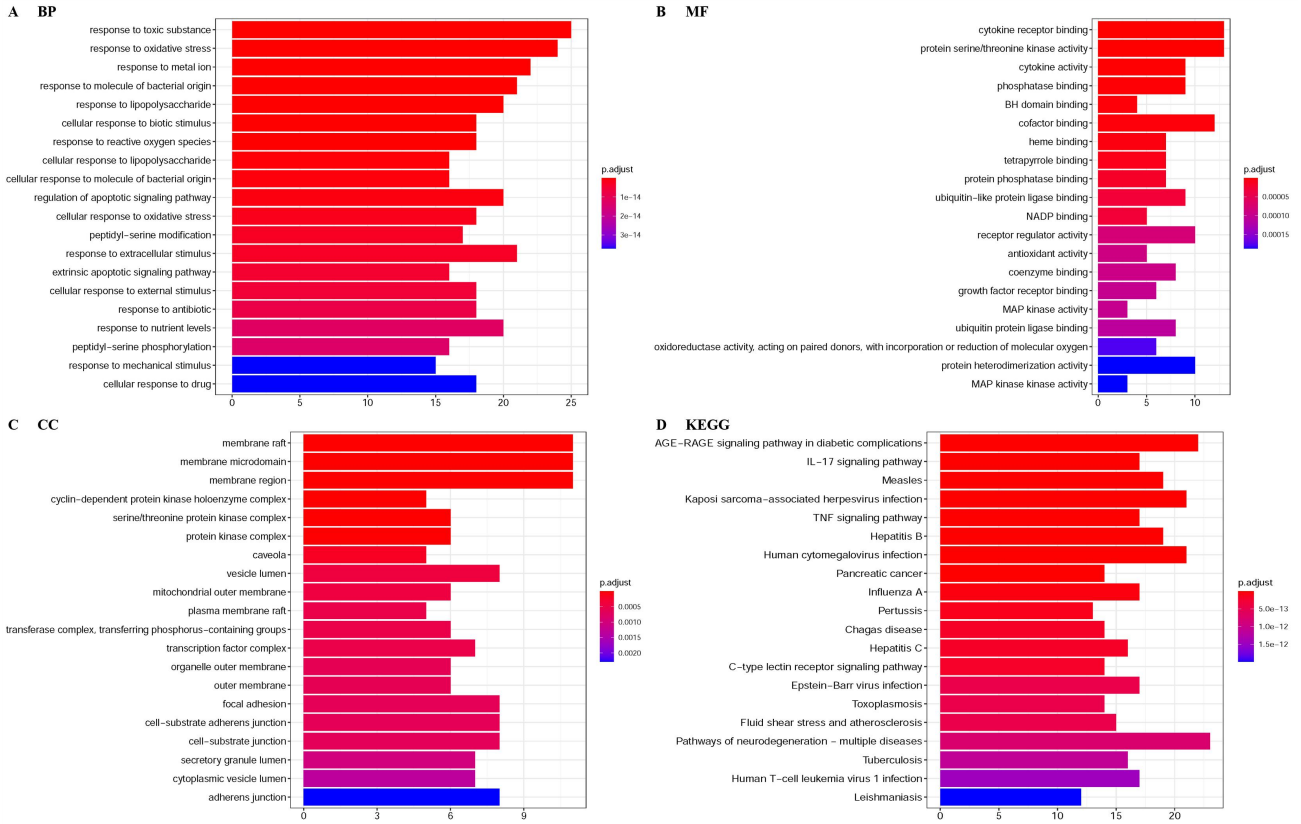


Figure 3 GO function and KEGG pathway enrichments. A, Enriched biological process functions of active target genes. B, Enriched molecular function activities of active target genes. C, Enriched cellular component regions of active target genes. D, KEGG pathway enrichments.

Abbreviations: BP, biological process; MF, molecular function; CC, cellular component.

membrane, plasma membrane raft, and so on (Supplementary File 4C). The top 20 GO CC enrichments ranked by adj *P* value are shown in Figure 3C.

KEGG pathway enrichment analysis showed that active target genes of KGD acting on COVID-19 were significantly enriched in advanced glycation end products-(AGE-) receptor for the AGE signaling pathway in diabetic complications, interleukin-(IL-)17 signaling pathway, tumor necrosis factor (TNF) signaling pathway, and so on (Supplementary File 4D). The top 20 KEGG pathway enrichments ranked by adj *P* value are shown in Figure 3D.

Constructed compound-target network and selected key compounds

As MOL000359, MOL004355, MOL006756, MOL007165, MOL007171, MOL007172, MOL001490, MOL002879, MOL010415, MOL003036, MOL003128, MOL002211, MOL000953, MOL000211, MOL003315, MOL001771, MOL003851, MOL003856, and MOL003860 had no association with an overlapping active target gene, the 63 overlapping active target genes correlate with 87 active compounds (Table 3).

Then, we constructed a network of compound-target using Cytoscape software and analyzed by the NetworkAnalyzer tool. There were 150 nodes (87 compound nodes and 63 target gene nodes) and 460 edges in the network (Supplementary File 5, Figure 4). The top 15 compounds listed by degree in the network were quercetin, luteolin, wogonin, kaempferol, arachidonic acid, naringenin, acacetin, baicalein, iristectorigenin A, glycitein, 5,7,4'-Trihydroxy-8-methoxyflavone, moslosooflavone, chryseriol, licochalcone B, and tetramethoxyluteolin, which can be acknowledged the key compounds in KGD acting on COVID-19. The basic information of these key compounds is shown in Table 4.

Constructed PPI network and selected key targets

We mapped 63 overlapping active target genes into the STRING database and obtained the PPI network. In which, 62 target proteins had interactions (APOD had no interaction) and 791 edges represented the interactions between the proteins, when lowest interaction score was set to 0.40 (Supplementary File 6, Figure 5).

Table 3 Active compounds correlated with overlapping active target genes

Compound	Herb	Compound	Herb	Compound	Herb
MOL001689	BH HQ JG	MOL000358	HQ JYH LQ NBZ SY	MOL005017	XR
MOL002881	BH GL	MOL000525	HQ	MOL007207	XR
MOL004328	BH	MOL000552	HQ	MOL012922	XR
MOL000471	BH	MOL000073	HQ	MOL003283	LQ
MOL005190	BH	MOL000449	HQ JYH XR SY	MOL003290	LQ NBZ
MOL005573	BH	MOL001458	HQ	MOL003295	LQ
MOL000006	BH JG JYH LQ	MOL002897	HQ	MOL003306	LQ
MOL008400	DDC	MOL008206	HQ	MOL003308	LQ
MOL001494	GL JYH	MOL012245	HQ	MOL003322	LQ
MOL005530	GL	MOL012246	HQ	MOL003330	LQ
MOL007179	GL SY	MOL012266	HQ	MOL003347	LQ
MOL005970	HP	MOL004580	JG	MOL003370	LQ
MOL005980	HP	MOL001495	JYH	MOL000522	LQ NBZ
MOL000173	HQ LQ	MOL003006	JYH	MOL000791	LQ
MOL000228	HQ	MOL003014	JYH	MOL002218	SY
MOL002714	HQ	MOL002773	JYH NBZ SY	MOL003847	SY
MOL002909	HQ	MOL003044	JYH	MOL003857	SY
MOL002910	HQ	MOL003095	JYH	MOL003858	SY
MOL002913	HQ	MOL003111	JYH	MOL003859	SY
MOL002914	HQ JYH	MOL003117	JYH	MOL003861	SY

Compound	Herb	Compound	Herb	Compound	Herb
MOL002915	HQ	MOL000422	JYH LQ NBZ SY	MOL003862	SY
MOL002917	HQ	MOL000098	JYH LQ SY	MOL003879	SY
MOL002925	HQ	MOL010921	XR	MOL000433	SY
MOL002927	HQ	MOL005030	XR	MOL000729	SY
MOL002928	HQ	MOL000492	XR	MOL001439	SY
MOL002932	HQ	MOL002311	XR	MOL003759	SY
MOL002933	HQ	MOL004841	XR	MOL006630	SY
MOL002934	HQ	MOL004903	XR	MOL007879	SY
MOL002937	HQ	MOL004908	XR	MOL013083	SY

Abbreviations: BH, *Herba Menthae Haplocalycis*; HQ, *Radix Scutellariae*; JG, *Radix Platycodi* ; GL, *Fructus Trichosanthis*; XR, *Semen Armeniacae*; JYH, *Flos Lonicerae*; LQ, *Fructus Forsythiae*; DDC, *Semen Sojae Preparatum*; SY, *Folium Mori*; HP, *Cortex Magnoliae Officinalis*; NBZ, *Fructus Arctii*;

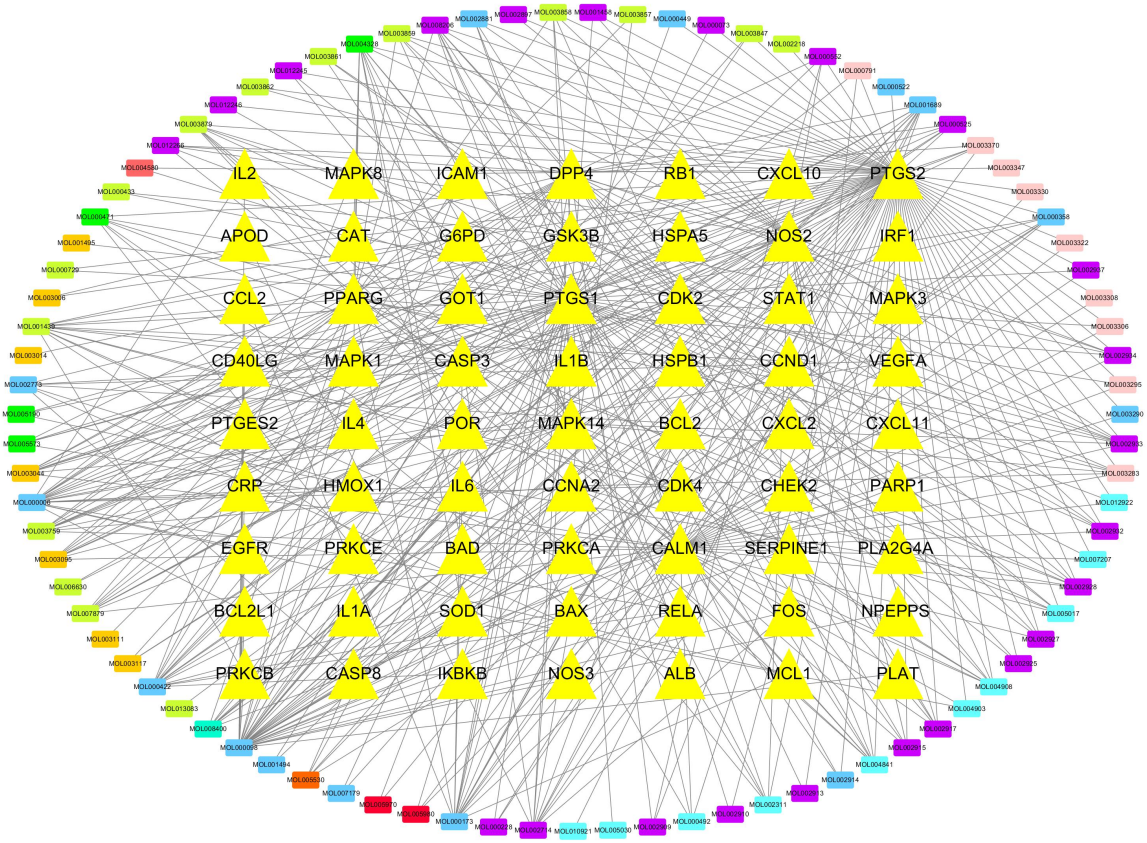
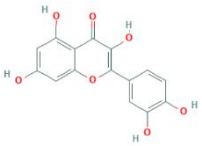
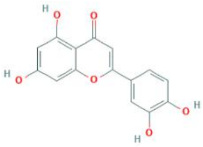
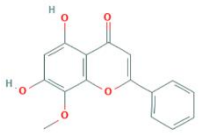
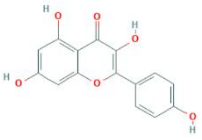
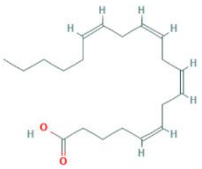
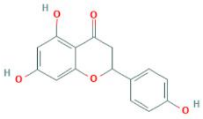
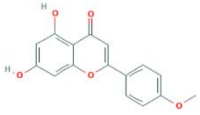
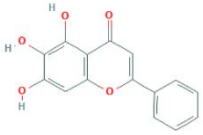
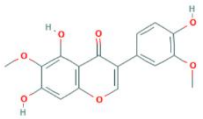
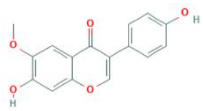
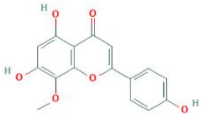
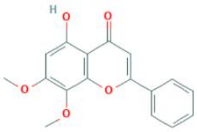
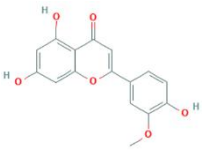
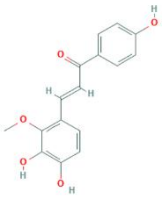
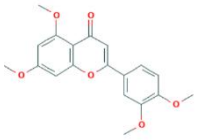


Figure 4 Compound-target network. There were 150 nodes (87 compound nodes and 63 target gene nodes) and 460 edges in the network. Rectangles represent active compounds (different colors represent different compounds), Triangle represent the active target genes, and the edges represent links between the nodes.

Table 4 Key compounds in Kangguan decoction acting on COVID-19.

Compound	PubChem CID	Molecular Formula	2D Structure	Degree	Herb
MOL000098 Quercetin	5280343	$C_{15}H_{10}O_7$		42	JYH LQ SY
MOL000006 Luteolin	5280445	$C_{15}H_{10}O_6$		20	BH JG JYH LQ
MOL000173 Wogonin	5281703	$C_{16}H_{12}O_5$		17	HQ LQ
MOL000422 Kaempferol	5280863	$C_{15}H_{10}O_6$		15	JYH LQ NBZ SY
MOL001439 Arachidonic acid	444899	$C_{20}H_{32}O_2$		13	SY
MOL004328 Naringenin	932	$C_{15}H_{12}O_5$		12	BH
MOL001689 Acacetin	5280442	$C_{16}H_{12}O_5$		11	BH HQ JG
MOL002714 Baicalein	5281605	$C_{15}H_{10}O_5$		11	HQ
MOL003759 Iristectorigenin A	5491637	$C_{17}H_{14}O_7$		10	SY
MOL008400 Glycitein	5317750	$C_{16}H_{12}O_5$		9	DDC
MOL002933 5,7,4'-Trihydroxy-8-methoxyflavone	5322078	$C_{16}H_{12}O_6$		9	HQ

Compound	PubChem CID	Molecular Formula	2D Structure	Degree	Herb
MOL008206 Moslosooflavone	188316	$C_{17}H_{14}O_5$		9	HQ
MOL003044 Chryseriol	5280666	$C_{16}H_{12}O_6$		9	JYH
MOL004841 Licochalcone B	5318999	$C_{16}H_{14}O_5$		9	XR
MOL007879 Tetramethoxyluteolin	631170	$C_{19}H_{18}O_6$		9	SY

Abbreviations: JYH, *Flos Lonicerae*; LQ, *Fructus Forsythiae*; SY, *Folium Mori*; BH, *Herba Menthae Haplocalycis*; JG, *Radix Platycodi*; HQ, *Radix Scutellariae*; NBZ, *Fructus Arctii*; DDC, *Semen Sojae Preparatum*; XR, *Semen Armeniacae*;

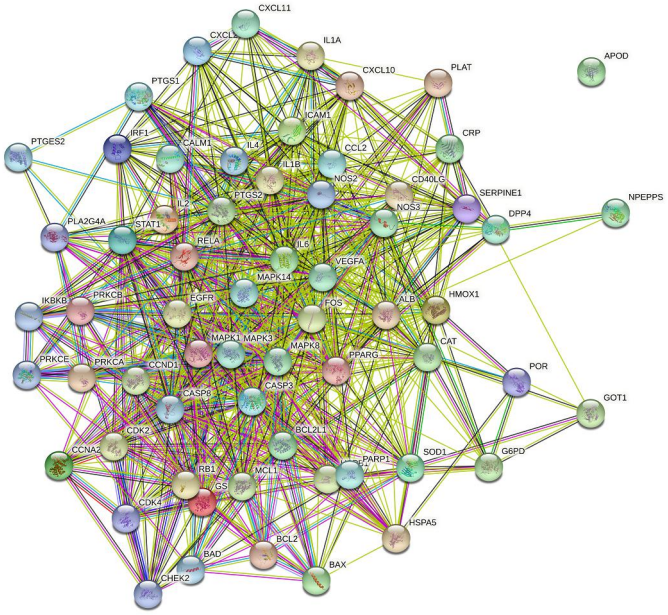


Figure 5 The protein protein interaction network of Kangguan decoction acting on COVID-19. In the network, 62 target proteins had interactions (APOD had no interaction) and 791 edges represented the interactions between the proteins, the lowest interaction score was set to 0.40.

Table 5 Key targets of Kangguan decoction acting on COVID-19

Target	Entry	Entry name	Protein names	Degree
VEGFA	P15692	VEGFA_HUMAN	Vascular endothelial growth factor A	51
MAPK3	P27361	MK03_HUMAN	Mitogen-activated protein kinase 3	48
MAPK1	P28482	MK01_HUMAN	Mitogen-activated protein kinase 1	47
CASP3	P42574	CASP3_HUMAN	Caspase-3	47
IL6	P05231	IL6_HUMAN	Interleukin-6	47
ALB	P02768	ALBU_HUMAN	Albumin	46
MAPK8	P45983	MK08_HUMAN	Mitogen-activated protein kinase 8	44
FOS	P01100	FOS_HUMAN	Proto-oncogene c-Fos	39
PTGS2	P35354	PGH2_HUMAN	Prostaglandin G/H synthase 2	39
CCL2	P13500	CCL2_HUMAN	C-C motif chemokine 2	38
CASP8	Q14790	CASP8_HUMAN	Caspase-8	38
CCND1	P24385	CCND1_HUMAN	G1/S-specific cyclin-D1	37
MAPK14	Q16539	MK14_HUMAN	Mitogen-activated protein kinase 14	37
IL1B	P01584	IL1B_HUMAN	Interleukin-1 beta	37
ICAM1	P05362	ICAM1_HUMAN	Intercellular adhesion molecule 1	37
RELA	Q04206	TF65_HUMAN	Transcription factor p65	37
BCL2L1	Q07817	B2CL1_HUMAN	Bcl-2-like protein 1	37
EGFR	P00533	EGFR_HUMAN	Epidermal growth factor receptor	36
IL2	P60568	IL2_HUMAN	Interleukin-2	35
CAT	P04040	CATA_HUMAN	Catalase	35

The top 20 target genes ranked by degree in the network are shown in Table 5, which can be considered the key targets of KGD acting on COVID-19.

Constructed key compound-target network

Introducing the key compounds (small-molecule compounds) and key targets (macromolecular protein target receptors) and their relationships into Cytoscape3.6.0 software, a key compound-target network was constructed (Supplementary File 7, Figure 6).

Molecular docking

We obtained the 3D structures of the small-molecule compounds from the PubChem Database and the macromolecular protein target receptors from the RCSB PDB database. Then, molecular docking simulations of potential targets and their corresponding compounds were performed using AutoDockTool 1.5.6 and AutoDock Vina software. Finally, the binding of the target and its corresponding component was verified by molecular docking and demonstrated by the PyMOL Molecular Graphics System. We selected several representative to demonstrate. In the molecular docking simulations of PTGS2-quercetin, minimum affinity was -9.5 kcal/mol, grid center was 22.594, 40.999 and 39.56, and distance from best mode was 0.000 rmsd l.b. and 0.000 rmsd u.b. (Figure 7). In the molecular docking simulations of IL6-luteolin, minimum affinity was affinity-7.3 kcal/mol, grid center was -0.585, 0.365 and 0.253, distance from best mode was 0.000 rmsd l.b. and 0.000 rmsd u.b. (Figure 8).

Clinical cases

Case 1. A 58-year-old male was admitted to our hospital on January 31, 2020 due to cough and fever for 4 days. He had an exposure history to SARS-CoV-2 infected persons on January 19, 2020. When he had a fever with body temperature (38.2°C) on January 27, 2020, he received intravenous injection of cefmetazole and peramivir in local community hospital. Fever persisted as a body temperature ranging from 38.2°C

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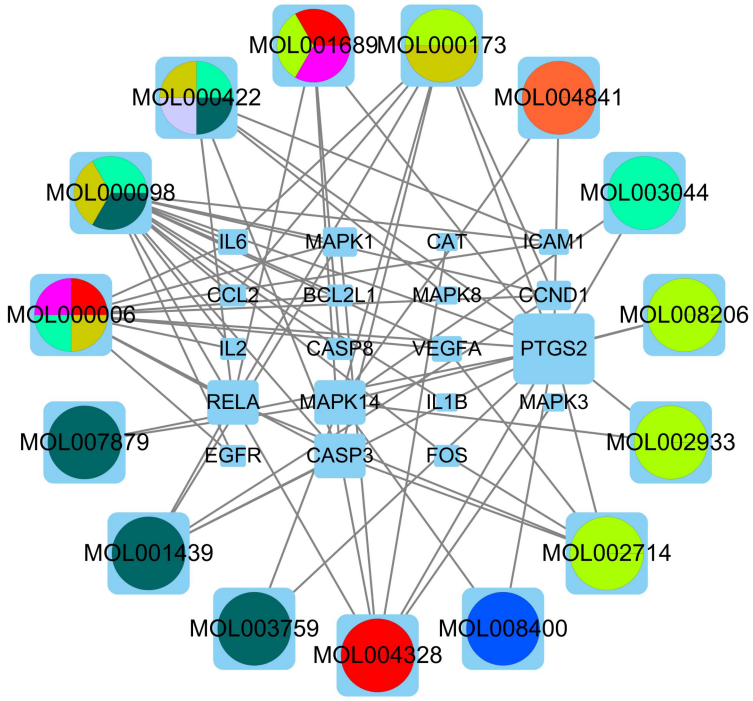

Figure 6 Key compound-target network. Quercetin, luteolin, wogonin, kaempferol, arachidonic acid, naringenin, acacetin, baicalein, iristectorigenin A, glycitein, 5,7,4'-Trihydroxy-8-methoxyflavone, Moslosooflavone, Chryseriol, Licochalcone B and Tetramethoxyluteolin were the key compounds considered as small-molecule compounds, while VEGFA, MAPK3, MAPK1,CASP3, IL6, ALB, MAPK8, FOS, PTGS2, CCL2, CASP8, CCND1, MAPK14, IL1B, ICAM1, RELA, BCL2L1, EGFR, IL2 and CAT were the key targets considered as macromolecular protein target receptors.

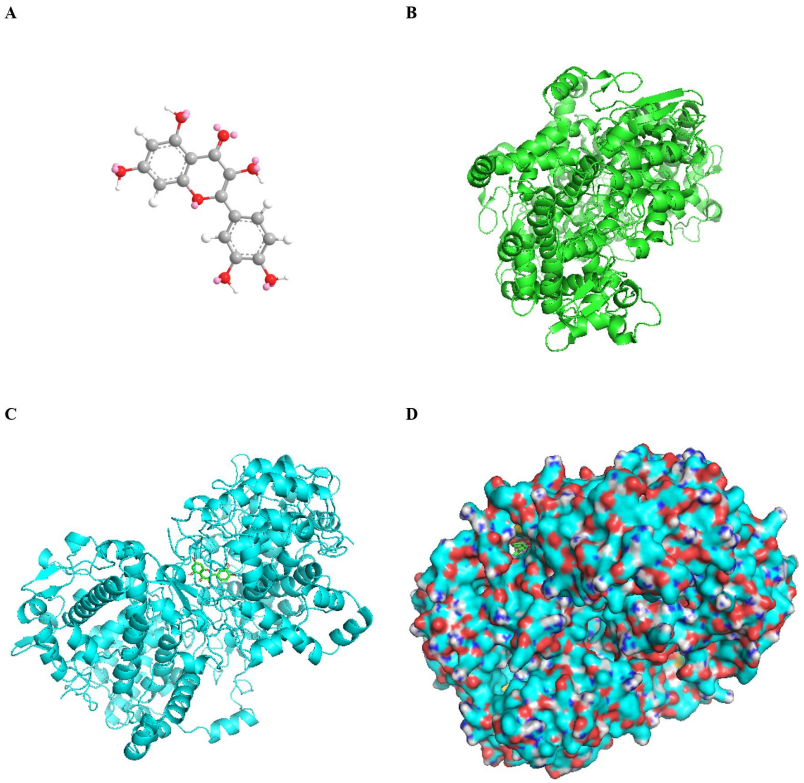

Figure 7 PTGS2-quercetin molecular docking. A, 3D structures of quercetin. B, 3D structures of PTGS2. C, Molecular docking simulation. D, Molecular docking simulation (display protein surface).

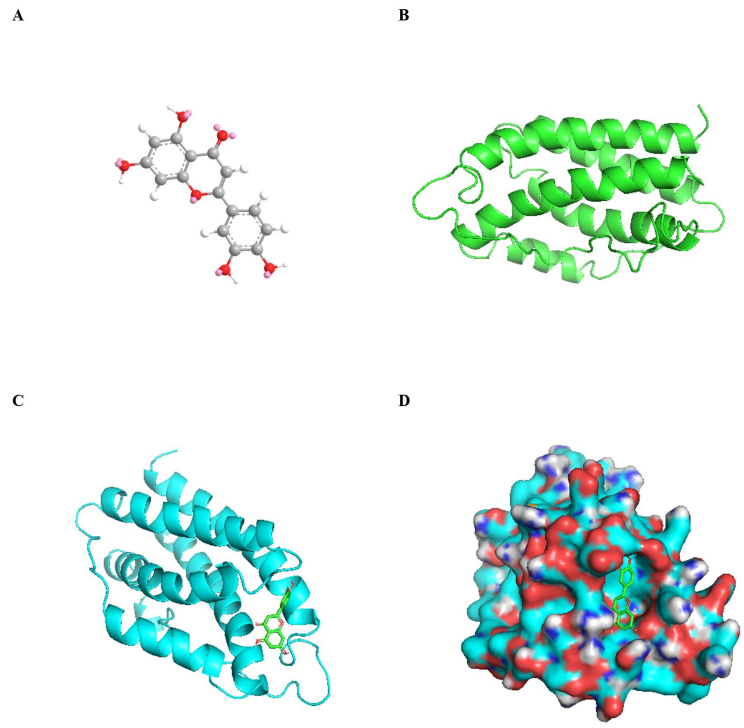

Figure 8 IL6-luteolin molecular docking. A, 3D structures of luteolin. B, 3D structures of IL6. C, Molecular docking simulation. D, Molecular docking simulation (display protein surface).

to 39.8°C, even though there was a temporary decrease after ibuprofen used. On January 31, her chest computed tomography scan showed bilateral distribution of patchy shadows and diagnosis of COVID-19 was confirmed subsequently by positive results of a throat swab for SARS-CoV-2. Laboratory workup included white blood cell, lymphocyte and inflammatory markers C-reactive protein (CRP), erythrocyte sedimentation rate, procalcitonin, interleukin-6 (IL-6) and ferroprotein was shown in Supplementary File 8A. Aerosol inhalation of interferon α-2b (5 million units twice a day), lopinavir plus ritonavir (500 mg twice a day orally), and arbidol (200 mg three times a day orally) was given for antiviral therapy; moxifloxacin (0.4 g once a day, orally) for secondary infection prevention. On the second day after admission, the patient received KGD (one dose a day, take one dose for twice). His symptom of fever continued to improve quickly and his temperature returned to normal on hospital day 5 (Figure 9). Finally, the patient was cured and discharged from our hospital on March 3, 2020.

Case 2. On January 29, 2020, a 45-year-old man was admitted to our hospital, with a 5-day history of fever, and a 2-day history of cough and dyspnea. He had returned to Nantong, China on January 18 after traveling to Wuhan, China. He had persistent severe fever with body temperature from 37.5°C to 39.2°C for 5 days. He started to receive intravenous injection of cefmetazole in local community hospital and took oseltamivir (75 mg, twice a day) for 3 days. On January 29, her chest computed tomography scan showed typical ground-glass lesions and diagnosis of COVID-19 was confirmed subsequently by positive results of a throat swab for SARS-CoV-2. His inflammatory markers such as CRP (84.4 mg/L), IL-6 (218.82 pg/mL) and ferroprotein (1607.87 ng/mL) on admission were significantly higher than other COVID-19 patients in the same period. Aerosol inhalation of interferon α-2b (5 million units twice a day), lopinavir plus ritonavir (500 mg twice a day orally), and arbidol (200 mg three times a day orally) was given for antiviral therapy. On the second day after admission, the patient received KGD (one dose a day, take one dose for twice). With the above combined therapy of KGD and western medicine, his dyspnea was gradually improved, the temperature returned to normal on hospital day 2, and the inflammatory markers decrease gradually (Figure 10, Supplementary File 8B). Finally, the patient was cured and discharged from our hospital on February 12, 2020.

Discussion

COVID-19 belongs to the category of epidemic disease of TCM. As the disease is located in the lung, the basic pathogenesis is characterized by dampness, heat, poison and blood stasis, and Chinese medicine has unique advantages in syndrome differentiation and treatment.

Yinqiao Power and Sangju Yin are from the ancient

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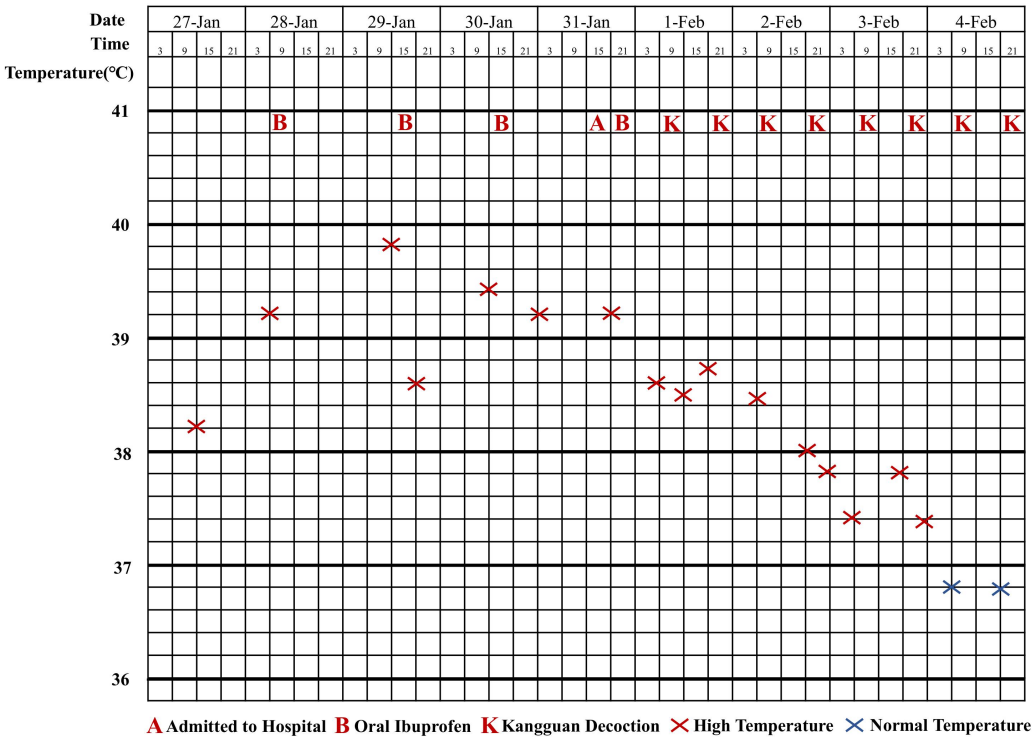

Figure 9 Body temperature of patient in case 1. Body temperature ranging from 38.2°C to 39.8°C, even though there was a temporary decrease after ibuprofen used. On the second day after admission, the patient received Kangguan decoction, body temperature returned to normal on hospital day 3.

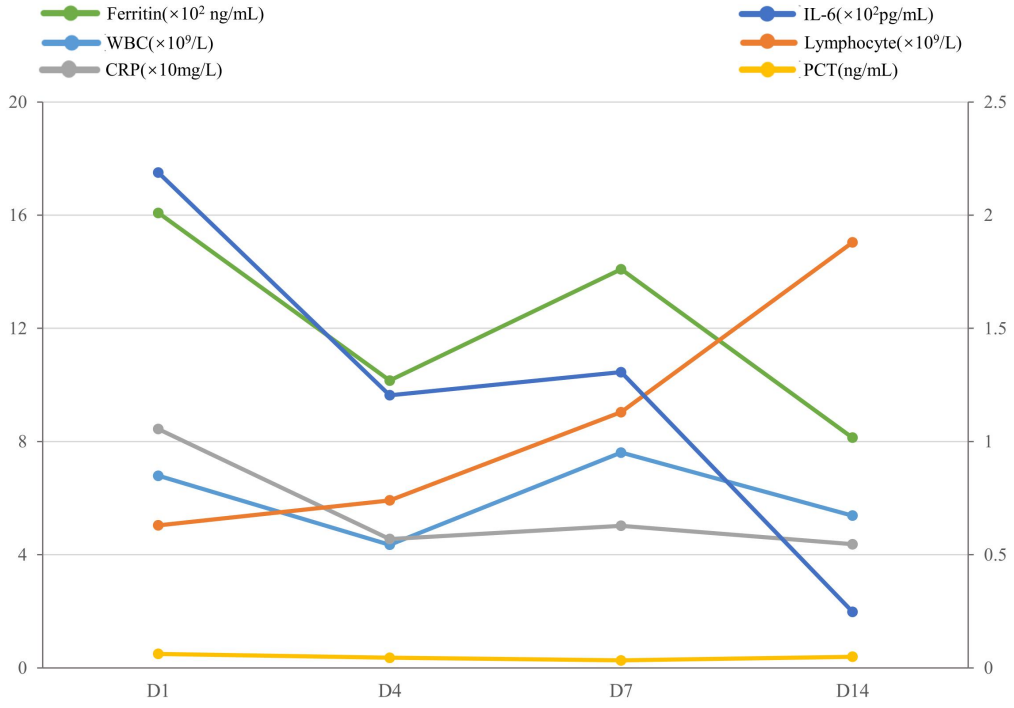

Figure 10 Laboratory workup of patient in case 2. Inflammatory markers such as C-reactive protein (84.4 mg/L), IL-6 (218.82 pg/mL) and ferroprotein (1607.87 ng/mL) on admission were significantly higher than other COVID-19 patients were in the same period. With the above combined therapy of Kangguan decoction and western medicine, the inflammatory markers decrease gradually.

Chinese medicine prescription "Febrile Disease Differentiation". Yinqiao Power has cold-pungent diaphoresis, clearing heat and detoxification effect, often used in the treatment of influenza, acute tonsillitis, measles from the beginning, as well as encephalitis B, meningitis, mumps, pharyngitis, pharynx isthmus herpes and other warm disease from the beginning. Sangju Yin has the effect of clearing away wind and clearing heat, promoting lung and relieving cough. It is often used to treat cold, acute bronchitis, upper respiratory tract infection, pneumonia, acute tonsillitis and so on. Based on the addition and subtraction of Yinqiao Power and Sangju Yin, KGD has been used in our hospital to treat the COVID-19 patients.

At present, network pharmacology is generally applied in the study of TCM. Network pharmacology promotes the research method of network goal and multi-component therapy, which promote the research of TCM from the current model single component and target to a new model of multi-component and network target. TCM network pharmacology can provide a new way for TCM to move from experience-based medicine to evidence-based medicine, and improve the current drug research strategy [23−25].

Compound-target network showed that the active target genes were related to all the 11 herbs in KGD, which indicates that the KGD prescription is reasonable and each single herb plays a role in acting COVID-19 (Figure 3). The key compounds and key targets were taken to construct a key compound-target network. Compound-target network showed that the key target genes were still related to most herbs in KGD (Figure 5). In particular, JYH, LQ, SY and HQ were closely related to key target genes to play a major role. In TCM theory, these herbs are the key herbs, which are known as monarch medicine and minister medicine.

We identified 63 active target genes of KGD acting on COVID-19. GO BP for KGD acting on COVID-19 were enriched in response to toxic substance, oxidative stress, metal ion, molecule of bacterial origin, lipopolysaccharide, reactive oxygen species, cellular response to lipopolysaccharide, biotic stimulus, molecule of bacterial origin and regulation of apoptotic signaling pathway. GO MF analysis indicated that KGD acting on COVID-19 involved in cytokine receptor binding, protein serine/threonine kinase activity, cytokine activity, phosphatase binding, BH domain binding, cofactor binding, heme binding, tetrapyrrole binding, protein phosphatase binding and ubiquitin-like protein ligase binding. GO CC analysis indicated that KGD in the treatment of COVID-19 were enriched in membrane raft, membrane microdomain, membrane region, cyclin-dependent protein kinase holoenzyme complex, serine/threonine protein kinase complex, protein kinase complex, caveola, vesicle lumen, mitochondrial outer membrane and plasma membrane raft. These function enrichments are intimately related to the pathological mechanisms of COVID-19 [26−29].

KEGG enrichment pathway analysis showed that many pathways were closely related to the pathogenesis of COVID-19. The main pathways encompassed IL-17 signaling pathway, measles, TNF signaling pathway, hepatitis B, human cytomegalovirus infection, influenza A and pertussis. Most of these pathways are associated with viruses as well as inflammation. These results illustrated that KGD acts on COVID-19 through multiple pathways [30−34]. These pathways and relevant target genes deserve priority for further study.

We constructed a compound-target network and identified the key compounds of KGD acting on COVID-19. The fundamental key compounds were quercetin, luteolin, wogonin, kaempferol, arachidonic acid, naringenin, acacetin, baicalein, iristectorigenin A and glycitein. Quercetin and vitamin C could be used in the prevention of high-risk populations or in the treatment of COVID-19 patients as adjunctive drugs for promising pharmacological agents such as remisivir or convalescent plasma [35]; a bioavailable form of quercetin should be considered a possible candidate to clinically face COVID-19 [36]. Luteolin could suppress systemic and neuroinflammatory responses in COVID-19 [37]; luteolin is a potential key to designing antiviral therapies for inhibiting viral proteases [38]. Kaempferol 3-O-beta-rutinoside has the potential to be developed as a COVID-19 main protease inhibitor [39]. Arachidonic acid may be useful both to prevent and manage COVID-19 [40]. Naringenin may be a promising treatment strategy against COVID-19 [41]. Baicalein and baicalin have been characterized as the first noncovalent, nonpeptidomimetic inhibitors of SARS-CoV-2 3CLpro and exhibited potent antiviral activities in a cell-based system [42]. These studies are related to the regulation of anti-virus, anti-inflammatory effects. KGD action on COVID-19 should be the result of the interaction of these multiple compounds. However, there are still few related studies about these compounds acting on COVID-19, which can be further studied.

PPI network showed that the main key targets were VEGFA, MAPK3, MAPK1, CASP3, IL6, ALB, MAPK8, FOS, PTGS2, CCL2, CASP8, CCND1, MAPK14, IL1B, ICAM1, RELA, BCL2L1, EGFR, IL2 and CAT. Previous research has indicated that levels of Levels of IP-10, HGF, IL-6, MCP-1, MIP-1alpha, IL-12p70, IL-18, VEGF-A, PDGF-BB and IL-1RA significantly correlate with SARS-CoV-2 infection severity [43]. SARS-CoV-2 ORF3a can efficiently induce apoptosis in cells, activated caspase-3 associate with AORF3a [44]. The serum levels of IL6 and CRP can effectively assess disease

severity and predict outcome in COVID-19 patients [45]; blocking the signal transduction pathway of IL-6 is expected to become a new method for the treatment of severe COVID-19 patients [46]. Immune signatures of COVID-19 relate to shifts in neutrophil to T cell ratio, elevated serum IL-6 and modulation of CD14(+) monocyte phenotype and function and modified features of CD14(+) monocytes included poor induction of the prostaglandin-producing enzyme, COX-2, as well as enhanced expression of the cell cycle marker Ki-67 [47]. COVID-19 patients show profound and sustained T CD4+, CD8+ and B lymphopenia, higher HLA-DR expression on monocytes and higher serum concentrations of EGF, GM-CSF, IL-10, CCL2/MCP-1, CCL3/MIP-1a, CXCL10/IP-10, CCL5/RANTES, and CCL20/MIP-3a [48]. SARS-CoV-2 triggers inflammatory responses and cell death through caspase-8 activation [49]. Critical cases of COVID-19 patients exhibit stronger interactions between epithelial and immune cells, as indicated by ligand-receptor expression profiles, and activated immune cells, including inflammatory macrophages expressing CCL2, CCL3, CCL20, CXCL1, CXCL3, CXCL10, IL8, IL1B and TNF [50]. IL-1-beta, IL-6, TNF-alpha, the cell adhesion molecules ICAM-1, VCAM-1, and E-selectin, and a group of IFN-gamma-induced genes have been specifically identified in the "cytokine storm" observed in fatal cases of COVID-19 [51]. The SARS-CoV-2 infection strongly activates TNF and NF kappaB-signaling pathways through significant upregulation of the TNF, IL1B, IL6, IL8, NFKB1, NFKB2 and RELB genes [52]. The treatment of cytokine storm cytokine release syndrome, characterized by IL-6, IL-2, IL-7, IL-10 has been proposed as a critical part of rescuing severe COVID-19 [53]. The relationship between these genes and COVID-19, some of which already have relevant studies, can be further studied, and possible mechanisms can explore about those genes, which have not been studied before. All of these have explained that the action of KGD on COVID-19 is related to multiple targets.

We also performed molecular docking to verify specific interactions between key compounds and their predicted protein targets, which could improve the accuracy of the network [54]. Preliminary molecular docking results show the key active compounds in KGD have high binding activities with the key gene target proteins. These active compounds may be some important material basis for KGD treating COVID-19 through related signaling pathways.

We have been treating COVID-19 patients since January 23, 2020. KGD has been applied to the patients, and has achieved certain curative effect. We demonstrate two typical clinical cases with obvious efficacy after KGD application.

The antipyretic effect of KGD is especially obvious. The patient in case 1 was characterized of fever. Ibuprofen was used to antipyretic, but the body temperature rose after a period. The application of KGD also played the same rapid antipyretic effect as ibuprofen, while the patient's body temperature is not elevated after KGD continuous used one dose a day. First, we believe that KGD has the same target as ibuprofen and plays an antipyretic role. In fact, ibuprofen is a non-selective COX inhibitor and the inhibition of COX-2 activity decreases the synthesis of prostaglandins involved in mediating inflammation, pain, fever, and swelling; ibuprofen has a specific drug target PTGS2 to play a series of pharmacological effects [55]. Our network pharmacology explains that PTGS2 is one of the key targets of KGD acting on COVID-19, and molecular docking shows that the key active compounds in KGD have high binding activities with PTGS2.

KGD may be involved in anti-inflammatory, anti-virus processes. As discussed above, KGD acting on COVID-19 is through multiple target and multiple pathways, which are related to anti-inflammatory and anti-virus. Major pathways involve a variety of inflammatory factors. The patient in case 2 inflammatory factor elevated. With the combined therapy of KGD and western medicine, his temperature returned to normal rapid in the same way, and the inflammatory markers decrease gradually. Our network pharmacology explains that interleukin series, such as IL6, IL1B, and IL2 are the key targets of KGD acting on COVID-19, and molecular docking shows that the key active compounds in KGD have high binding activities with them.

These curative effects may be strongly associated with the results of our network pharmacology and molecular docking. Around these clinical outcomes, we will pay further attention to obtain a higher level of clinical evidence in order to facilitate the combination of network pharmacology and molecular docking results for further basic research.

The pharmacological mechanisms of KGD acting on COVID-19 have been explored through a network pharmacology approach and preliminary verification has been performed, but there are still some limitations in our study. First, we searched compounds and target genes of KGD from the TCMSP database, the screening criteria for effective compounds were fixed, and COVID-19 related target genes were achieved from the GeneCards database. Some compounds and target genes may still be omitted, although these databases are more comprehensive presently applicable. Other methods, such as literature, may contain more compounds or genes. Second, we performed GO function enrichment and KEGG pathway enrichment analysis and constructed a PPI network to investigate the target genes and pathways of KGD acting on

COVID-19. These potential target genes and pathways need to be further studied by experimental analysis. Third, we only carry out preliminary molecular docking verification and clinical cases demonstration. The research on molecular docking of small-molecule compounds and macromolecular protein target receptors needs further study, while high evidence level of clinical research needs to be conducted.

Conclusion

In conclusion, this study explored the relationships between the compounds, target genes of KGD acting on COVID-19, and screened out the key compounds and key targets of KGD acting on COVID-19. In a word, the pharmacological mechanism of KGD acting on COVID-19 has been explored, and the active compounds and targets of KGD acting on COVID-19 and clinical effects for KGD treating COVID-19 patients have been preliminarily verified.

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