Exploring the Mechanisms of Lian Hua Qing Wen Capsule against COVID-19 by Network Pharmacology and Molecular Docking Approach

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Research

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Abstract

Background The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or COVID-19) disease has led to a wide-spread global pandemic. There is no specific antiviral drug proven effective for the treatment of patients with COVID-19 at present. Combination of western and traditional Chinese medicine (TCM) is recommended, and Lian Hua Qing Wen (LHQP) capsule is a basic prescription and widely used to treat COVID-19 in China. However, the mechanisms of LHQP capsule treating COVID-19 are not clear. The aim of the study is to explore the mechanisms of LHQP capsule treating COVID-19 based on network pharmacy and molecular docking approach.

Methods The active compounds and targets of LHQP capsule were obtained from traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP). COVID-19 related target genes were obtained from GeneCards database and OMIM database. Protein–protein interaction (PPI) networks of LHQP capsule targets and COVID-19-related genes were visualized and merged to identify the candidate targets for LHQP capsule treating COVID-19. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were also performed. The hub genes involved in the gene-related pathways were screened and their corresponding compounds were used for in vitro validation of molecular docking predictions.

Results A total of 185 active compounds of LHQP capsule were screened out, and 263 targets were predicted. Third hundred and fifty-two COVID-19 related target genes were obtained from GeneCards database and OMIM database. GO functional enrichment analysis showed that the biological processes of LHQP capsule treating COVID-19 were closely linked with the regulation of inflammation, immunity, cytokines production, vascular permeability, oxidative stress and apoptosis. KEGG enrichment analysis revealed that the pathways of LHQP capsule treating COVID-19 were significantly enriched in AGE–RAGE signaling pathway in diabetic complications, Kaposi sarcoma–associated herpesvirus infection, TNF, IL–17, and Toll–like receptor (TLR) signaling pathway. The hub targets genes in the gene-related pathways analysis of LHQP capsule treating COVID-19 included MAPK1, MAPK3, RELA, IL-6 and CASP8, which closely associated with inflammation, cytokines storm and apoptosis. Finally, molecular docking showed that top 5 compounds of LHQP capsule also had good binding activities to the important targets in COVID-19.

Conclusions The mechanisms of LHQP capsule treating COVID-19 may involve in inhibiting inflammatory response, cytokine storm and virus infection, and regulating immune reactions, apoptosis and endothelial barrier.

Background

The novel coronavirus 2019 (COVID-19), also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belongs to the genus β , with envelope, round or elliptic and often pleomorphic form, and 60–140 nm in diameter [1]. The ongoing pandemic of SARS-CoV-2 infections has led to more than 4 900

647 cases and 320 107 deaths globally as of May 20, 2020. This pandemic is still ongoing and mortality is increasing, especially in north America and Europe. Therefore, it is very urgent to find new preventive and therapeutic agents as soon as possible. However, there are no specific antiviral drugs or vaccines against COVID-19 at present [2]. According to traditional Chinese medicine (TCM) science, COVID-19 was considered as “plague” and TCM plays important auxiliary roles in the treatment of COVID-19 [3, 4].

Lian Hua Qing Wen (LHQW) capsule was prescribed based on aetiology and symptoms of COVID-19 and has been applied widely in China for treating COVID-19, which has shown satisfactory curative effects [5, 6]. Moreover, LHQW capsule has been listed in many countries for the prevention and treatment of COVID-19. LHQW capsule consists of 13 Chinese medicine extracts and has the effects of clearing away the plague and detoxify, promoting the dispersing function of the lung and discharging heat. The herbs in LHQW capsule include Forsythiae Fructus (FF), Lonicerae Japonicae Flos (LJF), Ephedra Herba (EH), Amygdalus Communis Vas (ACV), Gypsum Fibrosum (GF), Isatidis Radix (IR), Fortunes Bossfern Rhizome (FBR), Houttuyniae Herba (HH), Pogostemon Cablin (Blanco) Benth (PCB), Radix Rhei Et Rhizome (RRER), Rhodiola Rosea L (RRL), Menthae Herba (MH) and licorice (LI). However, the mechanisms of QFPDD treating COVID-19 are not clear. Network pharmacology and molecular docking approach are widely used to clarify the effects of compounds and potential mechanisms of compound-target-disease at the molecular level [7, 8].

In the present study, we performed bioinformatics investigation and molecular docking approach to elucidate an “ingredient-target-pathway” network of LHQW capsule treating COVID-19. The active compounds of LHQW capsule and their targets were firstly identified using the pharmacology analysis platform of the Chinese medicine system (TCMSP). Then, COVID-19 related target genes were obtained from GeneCards database and OMIM database. The mechanisms of LHQW capsule treating COVID-19 were explored by gene ontology (GO) and Kyoto Encyclopedia of gene and genomes (KEGG) pathway enrichment analysis. Molecular docking was used to further verify the mechanisms of herbs intervention in COVID-19. Based on TCM network pharmacology and molecular docking approaches, this study clarified the mechanisms of LHQW capsule treating COVID-19 at molecular and cellular levels.

Methods

The technical strategy of this research is shown in Fig. 1.

Screening the bioactive ingredients

The candidate ingredients of LHQW capsule were obtained from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform [9] (TCMSP, <http://tcmssp.com/tcmssp.php>) and screened with the criteria of oral bioavailability (OB) $\geq 30\%$ and drug-likeness ≥ 0.18 [10]. In total, 230 eligible compounds were obtained, 23 in FF, 23 in LJF, 22 in EH, 19 in ACV, 39 in IR, 28 in FBR, 7 in HH, 11 in PCB, 16 in RRER, 10 in MH, 91 in LI. Eventually, a total of 244 candidate compounds were obtained after removing the duplications.

Obtaining the Potential Targets of LHQW capsule

The potential targets of LHQW capsule were obtained from TCMSP (<http://tcmbspw.com/tcmbsp.php>) [9]. Four thousand targets were collected, 450 in FF, 403 in LJF, 406 in EH, 183 in ACV, 254 in IR, 104 in FBR, 204 in HH, 219 in PCB, 88 in RRER, 165 in MH, 1524 in LI. After deleting duplicates, there were 263 targets in total. The 244 candidate compounds were imported into the DrugBank database (<https://www.drugbank.ca/>) [11] and 185 compounds were obtained after removing 59 compounds which did not link to any targets.

Collecting COVID-19 related targets

The COVID-19 related targets genes were collected from GeneCards database (<https://www.genecards.org>) [12] and OMIM database (<https://omim.org>) [13]. The key word “novel coronavirus 19” was used to obtain COVID-19-related genes. There were altogether 352 COVID-19-related genes received.

PPI Network Construction

The compound-target networks were established to explore the complex interactions between the compounds of QFPDD and the targets of COVID-19 through using STRING and Cytoscape 3.7.2 (<https://cytoscape.org/>) [14]. In the network, nodes separately represented compounds or targets, and edges showed the interactions of the inter-node. The conditions of topological analysis included Betweenness Centrality (BC), Closeness Centrality (CC), Neighborhood Connectivity (NC), Number of Directed Edges (NDE), (Topological Coefficient (TC).

Bioinformatic Analysis

Database for Annotation, Visualization and Integrated Discovery (DAVID, <https://david.ncifcrf.gov>, v6.8) [15] was used to perform GO analysis including biological process, cellular component, and molecular functions. Functional categories were enriched within genes ($FDR < 0.05$) and the top 20 GO functional categories were selected. DAVID database was also used to carry out KEGG pathway analysis [15]. Pathways that had significant changes of $FDR < 0.05$ were identified for further analysis and the top 20 pathways were selected. The gene-pathway network was constructed to screen the key target genes of QFPDD treating COVID-19.

Molecular docking

PubChem and PDB were used to find the chemical and conformational information of the relevant proteins and small-molecule compounds [16, 17]. The AutoTools software was used to remove the

redundant protein chains, ligands and water molecules with hydrogenation before running docking experiments [18]. The AutoGrid software was used to calculate the energy lattice points with the grid box coordinates of $15 \times 15 \times 15$ [18]. AutoDock Vina was used to simulate the docking condition between proteins and small molecules [18]. The Schrodinger software was used to analyse the preferential conformation and map the simulation.

Results

Compound-Target Network of LHQW capsule

Table 1 identified 185 candidate compounds in LHQW capsule. Figure 2 showed the compound-target network of LHQW capsule by using the screened compounds and their targets. The networks contained 448 nodes (185 compounds and 263 targets in LHQW capsule and 4000 edges which indicated the compound-target interactions. One hundred and eighty-five candidate compounds had average degrees of 17.85, indicating that most compounds of LHQW capsule influenced multiple targets. Quercetin, kaempferol, luteolin, naringenin, and beta-sitosterol acted on 140, 55, 54, 34, 28 targets, respectively. And the OB of quercetin, kaempferol, luteolin, naringenin, and beta-sitosterol was 46.43, 41.88, 36.16, 59.29, 36.91%, respectively. Thus, these five top compounds may play important roles in LHQW capsule treating COVID-19.

Table 1
The final selected compounds in LHQW capsule for analysis.

Molecule ID	Molecule name	OB	DL	Source
MOL001689	acacetin	34.97	0.24	IR, MH
MOL002322	isovitexin	31.29	0.72	IR
MOL001721	Isaindigodione	60.12	0.41	IR
MOL001722	2-O-beta-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one	43.62	0.31	IR
MOL001726	pinoresinol-4-O-beta-D-apiosyl-beta-D-glucopyranoside	36.45	0.51	IR
MOL001728	3-[2' -(5' - hydroxymethyl) furyl] -1 (2H) -isoquinolinone-7-O-BETA-D-glucoside_qt	51.74	0.18	IR
MOL001733	EUPATORIN	30.23	0.37	IR
MOL001734	3-[[[(2R,3R,5R,6S)-3,5-dihydroxy-6-(1H-indol-3-yloxy)-4-oxooxan-2-yl]methoxy]-3-oxopropanoic acid	85.87	0.47	IR
MOL001735	Dinatin	30.97	0.27	IR
MOL001736	(-)-taxifolin	60.51	0.27	IR
MOL001749	ZINC03860434	43.59	0.35	IR
MOL001750	glucobrassicin	66.02	0.48	IR
MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76	IR, EH
MOL001756	quindoline	33.17	0.22	IR
MOL001767	hydroxyindirubin	63.37	0.30	IR
MOL001769	beta-sitosterol dodecantate	34.57	0.57	IR
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75	IR, EH
MOL001774	Ineketone	37.14	0.30	IR
MOL001779	Sinoacutine	49.11	0.46	IR
MOL001781	Indigo	38.20	0.26	IR
MOL001782	(2Z)-2-(2-oxoindolin-3-ylidene)indolin-3-one	48.40	0.26	IR
MOL001783	2-(9-((3-methyl-2-oxopent-3-en-1-yl)oxy)-2-oxo-1,2,8,9-tetrahydrofuro[2,3-h]quinolin-8-yl)propan-2-yl acetate	64.00	0.57	IR
MOL001790	Linarin	39.84	0.71	IR

OB, oral bioavailability; DL, drug-likeness; IR, Isatidis Radix; MH, Menthae Herba; RRER, Radix Rhei Et Rhizome; LI, licorice; PCB, Pogostemon Cablin (Blanco) Benth; FBR, Fortunes Bossfern Rhizome; LJF, Lonicerae Japonicae Flos; ACV, Amygdalus Communis Vas; FF, Forsythiae Fructus; EH, Ephedra Herba; HH, Houttuyniae Herba.

Molecule ID	Molecule name	OB	DL	Source
MOL001792	DFV	32.76	0.18	IR
MOL001793	(E)-2-[(3-indole)cyanomethylene]-3-indolinone	54.59	0.32	IR
MOL001798	neohesperidin_qt	71.17	0.27	IR
MOL001800	rosasterol	35.87	0.75	IR
MOL001803	Sinensetin	50.56	0.45	IR
MOL001804	Stigmasta-5,22-diene-3beta,7alpha-diol	43.04	0.82	IR
MOL001806	Stigmasta-5,22-diene-3beta,7beta-diol	42.56	0.83	IR
MOL001810	6-(3-oxoindolin-2-ylidene)indolo[2,1-b]quinazolin-12-one	45.28	0.89	IR
MOL001814	(E)-3-(3,5-dimethoxy-4-hydroxy-benzylidene)-2-indolinone	57.18	0.25	IR
MOL001820	(E)-3-(3,5-dimethoxy-4-hydroxyb-enzylidene)-2-indolinone	65.17	0.25	IR
MOL001828	3-[(3,5-dimethoxy-4-oxo-1-cyclohexa-2,5-dienylidene)methyl]-2,4-dihydro-1H-pyrrolo[2,1-b]quinazolin-9-one	51.84	0.56	IR
MOL001833	Glucobrassicin-1-Sulfonate_qt	42.52	0.24	IR
MOL000358	beta-sitosterol	36.91	0.75	IR, EH, RRER, LJF, FF
MOL000359	sitosterol	36.91	0.75	IR, MH, LI, ACV
MOL000449	Stigmasterol	43.83	0.76	IR, LJF, ACV, EH
MOL000953	CLR	37.87	0.68	IR, ACV
MOL011616	Fortunellin	35.65	0.74	MH
MOL001790	Linarin	39.84	0.71	MH
MOL002881	Diosmetin	31.14	0.27	MH, EH
MOL004328	naringenin	59.29	0.21	MH, LI, EH
OB, oral bioavailability; DL, drug-likeness; IR, Isatidis Radix; MH, Menthae Herba; RRER, Radix Rhei Et Rhizome; LI, licorice; PCB, Pogostemon Cablin (Blanco) Benth; FBR, Fortunes Bossfern Rhizome; LJF, Lonicerae Japonicae Flos; ACV, Amygdalus Communis Vas; FF, Forsythiae Fructus; EH, Ephedra Herba; HH, Houttuyniae Herba.				

Molecule ID	Molecule name	OB	DL	Source
MOL000471	aloe-emodin	83.38	0.24	MH, RRER
MOL005190	eriodictyol	71.79	0.24	MH, EH
MOL005573	Genkwanin	37.13	0.24	MH, PCB, EH
MOL000006	luteolin	36.16	0.25	MH, FF, LJF, EH
MOL002235	EUPATIN	50.80	0.41	RRER
MOL002251	Mutatochrome	48.64	0.61	RRER
MOL002259	Physciondiglucoside	41.65	0.63	RRER
MOL002260	Procyanidin B-5,3'-O-gallate	31.99	0.32	RRER
MOL002268	rhein	47.07	0.28	RRER
MOL002276	Sennoside E_qt	50.69	0.61	RRER
MOL002280	Torachrysone-8-O-beta-D-(6'-oxayl)-glucoside	43.02	0.74	RRER
MOL002281	Toralactone	46.46	0.24	RRER
MOL002288	Emodin-1-O-beta-D-glucopyranoside	44.81	0.80	RRER
MOL002293	Sennoside D_qt	61.06	0.61	RRER
MOL002297	Daucosterol_qt	35.89	0.70	RRER
MOL002303	palmidin A	32.45	0.65	RRER
MOL000554	gallic acid-3-O-(6'-O-galloyl)-glucoside	30.25	0.67	RRER
MOL000096	(-)-catechin	49.68	0.24	RRER
MOL001484	Inermine	75.18	0.54	LI
MOL001792	DFV	32.76	0.18	LI
MOL000211	Mairin	55.38	0.78	LI, ACV, FF

OB, oral bioavailability; DL, drug-likeness; IR, Isatidis Radix; MH, Menthae Herba; RRER, Radix Rhei Et Rhizome; LI, licorice; PCB, Pogostemon Cablin (Blanco) Benth; FBR, Fortunes Bossfern Rhizome; LJF, Lonicerae Japonicae Flos; ACV, Amygdalus Communis Vas; FF, Forsythiae Fructus; EH, Ephedra Herba; HH, Houttuyniae Herba.

Molecule ID	Molecule name	OB	DL	Source
MOL002311	Glycyrol	90.78	0.67	LI, ACV
MOL000239	Jaranol	50.83	0.29	LI
MOL002565	Medicarpin	49.22	0.34	LI
MOL000354	isorhamnetin	49.60	0.31	LI
MOL003656	Lupiwighteone	51.64	0.37	LI
MOL003896	7-Methoxy-2-methyl isoflavone	42.56	0.20	LI
MOL000392	formononetin	69.67	0.21	LI
MOL000417	Calycosin	47.75	0.24	LI
MOL000422	kaempferol	41.88	0.24	LI, LJF, FBR, FF, EH, HH
MOL004806	euchrenone	30.29	0.57	LI
MOL004805	(2S)-2-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]-8,8-dimethyl-2,3-dihydropyrano[2,3-f]chromen-4-one	31.79	0.72	LI
MOL004808	glyasperin B	65.22	0.44	LI
MOL004810	glyasperin F	75.84	0.54	LI
MOL004811	Glyasperin C	45.56	0.40	LI
MOL004814	Isotrifoliol	31.94	0.42	LI
MOL004815	(E)-1-(2,4-dihydroxyphenyl)-3-(2,2-dimethylchromen-6-yl)prop-2-en-1-one	39.62	0.35	LI
MOL004820	kanzonols W	50.48	0.52	LI
MOL004824	(2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofuro[3,2-g]chromen-7-one	60.25	0.63	LI
MOL004827	Semilicoisoflavone B	48.78	0.55	LI
MOL004828	Glepidotin A	44.72	0.35	LI
MOL004829	Glepidotin B	64.46	0.34	LI
MOL004833	Phaseolinisoflavan	32.01	0.45	LI

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Molecule ID	Molecule name	OB	DL	Source
MOL004835	Glypallichalcone	61.60	0.19	LI
MOL004838	8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol	58.44	0.38	LI
MOL004841	Licochalcone B	76.76	0.19	LI, ACV
MOL004848	licochalcone G	49.25	0.32	LI
MOL004849	3-(2,4-dihydroxyphenyl)-8-(1,1-dimethylprop-2-enyl)-7-hydroxy-5-methoxy-coumarin	59.62	0.43	LI
MOL004855	Licoricone	63.58	0.47	LI
MOL004856	Gancaonin A	51.08	0.40	LI
MOL004857	Gancaonin B	48.79	0.45	LI
MOL004860	licorice glycoside E	32.89	0.27	LI
MOL004863	3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-enyl)chromone	66.37	0.41	LI
MOL004864	5,7-dihydroxy-3-(4-methoxyphenyl)-8-(3-methylbut-2-enyl)chromone	30.49	0.41	LI
MOL004866	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl)chromone	44.15	0.41	LI
MOL004879	Glycyrin	52.61	0.47	LI
MOL004882	Licocoumarone	33.21	0.36	LI
MOL004883	Licoisoflavone	41.61	0.42	LI
MOL004884	Licoisoflavone B	38.93	0.55	LI
MOL004885	licoisoflavanone	52.47	0.54	LI
MOL004891	shinpterocarpin	80.30	0.73	LI
MOL004898	(E)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl)prop-2-en-1-one	46.27	0.31	LI
MOL004903	liquiritin	65.69	0.74	LI, ACV
MOL004904	licopyranocoumarin	80.36	0.65	LI
MOL004905	3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid	34.32	0.55	LI
OB, oral bioavailability; DL, drug-likeness; IR, Isatidis Radix; MH, Menthae Herba; RRER, Radix Rhei Et Rhizome; LI, licorice; PCB, Pogostemon Cablin (Blanco) Benth; FBR, Fortunes Bossfern Rhizome; LJF, Lonicerae Japonicae Flos; ACV, Amygdalus Communis Vas; FF, Forsythiae Fructus; EH, Ephedra Herba; HH, Houttuyniae Herba.				

Molecule ID	Molecule name	OB	DL	Source
MOL004907	Glyzaglabrin	61.07	0.35	LI
MOL004908	Glabridin	53.25	0.47	LI, ACV
MOL004910	Glabranin	52.90	0.31	LI
MOL004911	Glabrene	46.27	0.44	LI
MOL004912	Glabrone	52.51	0.50	LI
MOL004913	1,3-dihydroxy-9-methoxy-6-benzofurano[3,2-c]chromenone	48.14	0.43	LI
MOL004914	1,3-dihydroxy-8,9-dimethoxy-6-benzofurano[3,2-c]chromenone	62.90	0.53	LI
MOL004915	Eurycarpin A	43.28	0.37	LI
MOL004917	glycyroside	37.25	0.79	LI
MOL004924	(-)-Medicocarpin	40.99	0.95	LI
MOL004935	Sigmoidin-B	34.88	0.41	LI
MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one	71.12	0.18	LI
MOL004945	(2S)-7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)chroman-4-one	36.57	0.32	LI
MOL004948	Isoglycyrol	44.70	0.84	LI
MOL004949	Isolicoflavonol	45.17	0.42	LI
MOL004957	HMO	38.37	0.21	LI
MOL004959	1-Methoxyphaseollidin	69.98	0.64	LI
MOL004961	Quercetin der.	46.45	0.33	LI
MOL004966	3'-Hydroxy-4'-O-Methylglabridin	43.71	0.57	LI
MOL000497	licochalcone a	40.79	0.29	LI
MOL004974	3'-Methoxyglabridin	46.16	0.57	LI
MOL004978	2-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano[6,5-f]chromen-3-yl]-5-methoxyphenol	36.21	0.52	LI
MOL004980	Inflacoumarin A	39.71	0.33	LI

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Molecule ID	Molecule name	OB	DL	Source
MOL004985	icos-5-enoic acid	30.70	0.20	LI
MOL004988	Kanzonol F	32.47	0.89	LI
MOL004989	6-prenylated eriodictyol	39.22	0.41	LI
MOL004990	7,2',4'-trihydroxy-5-methoxy-3-arylcoumarin	83.71	0.27	LI
MOL004991	7-Acetoxy-2-methylisoflavone	38.92	0.26	LI
MOL004993	8-prenylated eriodictyol	53.79	0.40	LI
MOL004996	gadelaidic acid	30.70	0.20	LI
MOL000500	Vestitol	74.66	0.21	LI
MOL005000	Gancaonin G	60.44	0.39	LI
MOL005001	Gancaonin H	50.10	0.78	LI
MOL005003	Licoagrocarpin	58.81	0.58	LI
MOL005007	Glyasperins M	72.67	0.59	LI
MOL005008	Glycyrrhiza flavonol A	41.28	0.60	LI
MOL005012	Licoagroisoflavone	57.28	0.49	LI
MOL005013	18 α -hydroxyglycyrrhetic acid	41.16	0.71	LI
MOL005016	Odoratin	49.95	0.30	LI
MOL005017	Phaseol	78.77	0.58	LI, ACV
MOL005018	Xambioona	54.85	0.87	LI
MOL005020	dehydroglyasperins C	53.82	0.37	LI
MOL000098	quercetin	46.43	0.28	LI, PCB, LJF, FF, EH, HH
MOL002879	Diop	43.59	0.39	PCB
MOL005884	patchoulan 1,12-diol	38.17	0.25	PCB
MOL005890	pachypodol	75.06	0.40	PCB

OB, oral bioavailability; DL, drug-likeness; IR, Isatidis Radix; MH, Menthae Herba; RRER, Radix Rhei Et Rhizome; LI, licorice; PCB, Pogostemon Cablin (Blanco) Benth; FBR, Fortunes Bossfern Rhizome; LJF, Lonicerae Japonicae Flos; ACV, Amygdalus Communis Vas; FF, Forsythiae Fructus; EH, Ephedra Herba; HH, Houttuyniae Herba.

Molecule ID	Molecule name	OB	DL	Source
MOL005911	5-Hydroxy-7,4'-dimethoxyflavanon	51.54	0.27	PCB
MOL005916	irisolidone	37.78	0.30	PCB
MOL005918	phenanthrone	38.70	0.33	PCB
MOL005921	quercetin 7-O- β -D-glucoside	49.57	0.27	PCB
MOL005922	Acanthoside B	43.35	0.77	PCB
MOL005923	3,23-dihydroxy-12-oleanen-28-oic acid	30.86	0.86	PCB
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21	FBR
MOL002605	11-Hydroxynumantenine	50.79	0.71	FBR
MOL002606	Hopene	6.82	0.77	FBR
MOL002607	Aspidinin	2.13	0.37	FBR
MOL002609	Harmonyl	43.80	0.46	FBR
MOL002610	ZINC00035529	58.39	0.22	FBR
MOL002612	Filixic acid	1.54	0.63	FBR
MOL002613	Flavaspidin	8.49	0.47	FBR
MOL002614	Flavidin	30.10	0.26	FBR
MOL002615	Neohop-13(18)ene solution	6.78	0.77	FBR
MOL013336	Narirutin	8.15	0.75	FBR
MOL002617	Diplopterol	11.25	0.77	FBR
MOL002618	ALBASPIDIN	18.27	0.51	FBR
MOL002619	Albaspidin AA	31.16	0.36	FBR
MOL002620	Dryocrassin	3.01	0.36	FBR
MOL002621	Aspidin	2.40	0.52	FBR
MOL002622	Filicin	6.71	0.63	FBR
MOL002623	Trisalbaspidin ABA	1.46	0.69	FBR
MOL002625	Kaempfe-rol-3,7-dirhamnoside	3.75	0.79	FBR

OB, oral bioavailability; DL, drug-likeness; IR, Isatidis Radix; MH, Menthae Herba; RRER, Radix Rhei Et Rhizome; LI, licorice; PCB, Pogostemon Cablin (Blanco) Benth; FBR, Fortunes Bossfern Rhizome; LJF, Lonicerae Japonicae Flos; ACV, Amygdalus Communis Vas; FF, Forsythiae Fructus; EH, Ephedra Herba; HH, Houttuyniae Herba.

Molecule ID	Molecule name	OB	DL	Source
MOL002626	Adlantone	12.57	0.78	FBR
MOL002627	Filicene	6.81	0.76	FBR
MOL002628	isopentenyaldenosine	29.78	0.34	FBR
MOL002629	7-Fernene	7.03	0.77	FBR
MOL002630	9-(11)-Fernene	17.53	0.77	FBR
MOL002631	9-(11)-Fernene	6.67	0.78	FBR
MOL002632	Adiantone	13.91	0.78	FBR
MOL002633	Albaspidin AP	24.44	0.42	FBR
MOL001494	Mandenol	42.00	0.19	LJF, EH
MOL001495	Ethyl linolenate	46.10	0.20	LJF
MOL002707	phytofluene	43.18	0.50	LJF
MOL002914	Eriodyctiol (flavanone)	41.35	0.24	LJF
MOL003006	(-)-(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	87.47	0.23	LJF
MOL003014	secologanic dibutylacetal_qt	53.65	0.29	LJF
MOL002773	beta-carotene	37.18	0.58	LJF
MOL003036	ZINC03978781	43.83	0.76	LJF
MOL003044	Chryseriol	35.85	0.27	LJF
MOL003059	kryptoxanthin	47.25	0.57	LJF
MOL003062	4,5'-Retro-.beta.,.beta.-Carotene-3,3'-dione, 4',5'-didehydro-	31.22	0.55	LJF
MOL003095	5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl) chromone	51.96	0.41	LJF
MOL003101	7-epi-Vogeloside	46.13	0.58	LJF
MOL003108	Caeruloside C	55.64	0.73	LJF
MOL003111	Centauroside_qt	55.79	0.50	LJF

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Molecule ID	Molecule name	OB	DL	Source
MOL003117	loniceracetalides B_qt	61.19	0.19	LJF
MOL003124	XYLOSTOSIDINE	43.17	0.64	LJF
MOL003128	dinethylsecologanoside	48.46	0.48	LJF
MOL010921	estrone	53.56	0.32	ACV
MOL010922	Diisooctyl succinate	31.62	0.23	ACV
MOL002211	11,14-eicosadienoic acid	39.99	0.20	ACV
MOL002372	(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene	33.55	0.42	ACV
MOL005030	gondoic acid	30.70	0.20	ACV
MOL000492	(+)-catechin	54.83	0.24	ACV, EH
MOL003410	Ziziphin_qt	66.95	0.62	ACV
MOL004355	Spinasterol	42.98	0.76	ACV, HH
MOL007207	Machiline	79.64	0.24	ACV
MOL012922	I-SPD	87.35	0.54	ACV
MOL000173	wogonin	30.68	0.23	FF
MOL003281	20(S)-dammar-24-ene-3 β ,20-diol-3-acetate	40.23	0.82	FF
MOL003283	(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	66.51	0.39	FF
MOL003290	(3R,4R)-3,4-bis[(3,4-dimethoxyphenyl) methyl] oxolan-2-one	52.30	0.48	FF
MOL003295	(+)-pinoresinol monomethyl ether	53.08	0.57	FF
MOL003305	PHILLYRIN	36.40	0.86	FF
MOL003306	ACon1_001697	85.12	0.57	FF
MOL003308	(+)-pinoresinol monomethyl ether-4-D-beta-glucoside_qt	61.20	0.57	FF
MOL003315	3beta-Acetyl-20,25-epoxydammarane-24alpha-ol	33.07	0.79	FF
MOL003322	FORSYTHINOL	81.25	0.57	FF

OB, oral bioavailability; DL, drug-likeness; IR, Isatidis Radix; MH, Menthae Herba; RRER, Radix Rhei Et Rhizome; LI, licorice; PCB, Pogostemon Cablin (Blanco) Benth; FBR, Fortunes Bossfern Rhizome; LJF, Loniceræ Japonicæ Flos; ACV, Amygdalus Communis Vas; FF, Forsythiæ Fructus; EH, Ephedra Herba; HH, Houttuyniæ Herba.

Molecule ID	Molecule name	OB	DL	Source
MOL003330	(-)-Phillygenin	95.04	0.57	FF
MOL003344	β -amyrin acetate	42.06	0.74	FF
MOL003347	hyperforin	44.03	0.60	FF
MOL003348	adhyperforin	44.03	0.61	FF
MOL003365	Lactucasterol	40.99	0.85	FF
MOL003370	Onjixanthone I	79.16	0.30	FF
MOL000522	arctiin	34.45	0.84	FF
MOL000791	bicuculline	69.67	0.88	FF
MOL010788	leucopelargonidin	57.97	0.24	EH
MOL002823	Herbacetin	36.07	0.27	EH
MOL010489	Resivit	30.84	0.27	EH
MOL004798	delphinidin	40.63	0.28	EH
MOL001506	Supraene	33.55	0.42	EH
MOL004576	taxifolin	57.84	0.27	EH
MOL005043	campest-5-en-3beta-ol	37.58	0.71	EH
MOL005842	Pectolinarigenin	41.17	0.30	EH
MOL007214	(+)-Leucocyanidin	37.61	0.27	EH
MOL011319	Truflex OBP	43.74	0.24	EH
MOL003851	Isoramanone	39.97	0.51	HH
MOL004345	1-methyl-2-nonacosyl-4-quinolone	31.54	0.50	HH
MOL004350	Ruvoside_qt	36.12	0.76	HH
MOL004351	C09747	37.28	0.25	HH
OB, oral bioavailability; DL, drug-likeness; IR, Isatidis Radix; MH, Menthae Herba; RRER, Radix Rhei Et Rhizome; LI, licorice; PCB, Pogostemon Cablin (Blanco) Benth; FBR, Fortunes Bossfern Rhizome; LJF, Lonicerae Japonicae Flos; ACV, Amygdalus Communis Vas; FF, Forsythiae Fructus; EH, Ephedra Herba; HH, Houttuyniae Herba.				

PPI Networks Analysis of LHQW capsule and COVID-19

To reveal the mechanisms of LHQW capsule against COVID-19, 50 shared target genes were added to STRING and Cytoscape to construct a PPI network for exploring the interaction relationships with each other (Fig. 3). Network Analyzer in cytoscape was used to analyse the degree of each target for further identifying the more important targets in the network. The results and parameters from the PPI topological analysis are listed in Table 2. MAPK3, MAPK8, CASP3, MAPK1, IL6 and RELA were the top 6 genes based on degree.

Table 2
Topological analysis of the PPI network of the 50 genes. Data were ranked by degree.

Gene Symbol	Degree	¹ BC	² CC	³ NC	⁴ NDE	⁵ TC
MAPK3	37	0.053	0.911	22.162	37	0.541
MAPK8	36	0.045	0.891	22.778	36	0.556
CASP3	35	0.041	0.872	22.743	35	0.555
MAPK1	35	0.041	0.872	22.686	35	0.553
IL6	34	0.035	0.854	23.647	34	0.577
RELA	31	0.027	0.804	23.968	31	0.585
CASP8	30	0.025	0.788	23.900	30	0.583
FOS	30	0.028	0.788	23.800	30	0.580
MAPK14	30	0.018	0.788	24.700	30	0.602
EGFR	29	0.024	0.774	23.759	29	0.579
BCL2L1	28	0.017	0.759	24.679	28	0.602
IL1B	28	0.010	0.759	25.893	28	0.632
CCL2	28	0.010	0.759	25.893	28	0.632
IL2	28	0.018	0.759	25.500	28	0.622
PTGS2	28	0.009	0.759	26.214	28	0.639
ICAM1	28	0.013	0.759	25.607	28	0.625
IL4	27	0.009	0.745	26.111	27	0.637
STAT1	27	0.015	0.745	25.889	27	0.631
HMOX1	24	0.008	0.707	26.125	24	0.637
NOS2	23	0.008	0.695	25.565	23	0.624
CXCL10	22	0.004	0.683	26.273	22	0.641
MCL1	21	0.009	0.672	25.333	21	0.618
CD40LG	20	0.003	0.661	28.100	20	0.685
NOS3	19	0.003	0.651	27.000	19	0.659
IRF1	18	0.002	0.641	26.278	18	0.641

¹ BC (Betweenness Centrality); ² CC (Closeness Centrality); ³ NC (Neighborhood Connectivity); ⁴ NDE (Number of Directed Edges); ⁵ TC (Topological Coefficient).

Gene Symbol	Degree	¹ BC	² CC	³ NC	⁴ NDE	⁵ TC
CXCL2	17	0.001	0.631	26.588	17	0.648
SOD1	17	0.003	0.631	26.882	17	0.656
SERPINE1	17	0.002	0.631	27.941	17	0.681
IL1A	15	0.001	0.586	24.733	15	0.651
PARP1	15	0.002	0.612	27.933	15	0.681
HSPA5	15	0.002	0.612	27.467	15	0.670
HSPB1	14	0.000	0.603	28.500	14	0.695
BCL2	13	0.003	0.577	24.231	13	0.621
CXCL11	12	0.000	0.554	24.333	12	0.658
BAX	11	0.002	0.562	23.545	11	0.604
PRKCB	11	0.001	0.577	26.455	11	0.645
RB1	10	0.001	0.562	28.300	10	0.708
CDK4	10	0.001	0.569	26.100	10	0.637
BAD	10	0.001	0.554	25.600	10	0.656
PRKCA	10	0.002	0.569	26.500	10	0.646
PRKCE	10	0.003	0.569	23.400	10	0.571
CALM1	9	0.000	0.562	29.111	9	0.710
¹ BC (Betweenness Centrality); ² CC (Closeness Centrality); ³ NC (Neighborhood Connectivity); ⁴ NDE (Number of Directed Edges); ⁵ TC (Topological Coefficient).						

GO and KEGG Pathway Enrichment analysis

Performing GO and KEGG pathway enrichment analysis to expound the biological functions of the 50 core targets in the networks of LHQW capsule and COVID-19. The top 20 significantly enriched terms with a greater number of involved targets in biological process (BP), cellular component (CC), and molecular function (MF) categories are shown in Fig. 4A-C, which indicated that LHQW capsule may regulate virus infection, oxidative stress, endothelial barrier and cytokine storm via response to molecule of bacterial origin, response to oxidative stress, membrane raft, membrane microdomain, cytokine receptor binding and cytokine activity to exert its therapeutic effects against COVID-19. The data of GO analysis were provided in supplementary table 1.

Figure 4D indicated the top 20 significantly enriched pathways according to the analysis of KEGG signaling pathway data. Among these potential pathways, AGE-RAGE signaling pathway in diabetic complications was the most prominently enriched pathway. Others important pathways involved in Kaposi sarcoma-associated herpesvirus infection, TNF, IL-17, and Toll-like receptor signaling pathway, which were categorized for inflammation, virus infection and immune response. The data of KEGG pathway analysis were provided in supplementary table 2.

Gene-pathway network analysis

As genes cannot directly exhibit their biological and pharmacological activities independently, a gene-pathway network was established to further elucidate the molecular mechanisms of LHQW capsule treating COVID. According to the degree of the pathways, the study selected the top 30 pathways and 42 genes to perform topological analysis with BC. Figure 5 identified MAPK3, MAPK1, RELA, IL-6 and CASP8 as relatively high-relevant target genes in virus infection, inflammation, and apoptosis. Moreover, these top five target genes were considered as the key markers for LHQW capsule treating COVID-19.

Molecular docking analysis

Discovery Studio software [18] was used to validate network pharmacology by molecular docking, including the respective top 5 targets in PPI network and the top 5 compounds in LHQW capsule. The LibDock score represented the degree of docking coincidence of molecules. If the score was higher, the binding of ligands to receptor proteins was better. Molecular docking results found that quercetin had good affinity to bind MAPK3, which was the key target protein molecules in COVID-19. Besides, these results also showed that other important compounds (such as, kaempferol, luteolin, naringenin, and beta-sitosterol) in LHQW capsule had regulatory effects of other targets in COVID-19, indicating that the molecular docking results were consistent with the network pharmacology screening results. The partial docking results were showed in Fig. 6 and other results were provided in supplementary table 3.

Discussion

COVID-19 has brought a tremendous threat to public health, especially the elderly over 60 years old being more likely to die after infection [1]. The clinical symptoms of COVID-19 have been reportedly ranged from mild to severe, ultimately leading to death. The mild symptoms included fever, cough, shortness of breath and pneumonia after viral infection, while the severe cases would rapidly developed to acute respiratory distress syndrome, septic shock, metabolic acidosis, coagulation dysfunction and multiple organ failure that eventually brought about mortality [19, 20]. There has been no effective antiviral drug to treat COVID-19 until now, even though significant efforts are used to develop therapeutic interventions against coronavirus infection [2]. Fortunately, the application of TCM in the auxiliary treatment of COVID-19 has achieve satisfactory therapeutic effects in China [21, 22]. LHQW capsule has been recommended for the treatment of patients with mild symptoms in 7th edition of COVID-19 treatment guidelines and

widely used in the clinical treatment of COVID-19 in China [23]. LHQW capsule has been demonstrated to have anti-infection or antiviral effects in vitro or in vivo [24–26]. In the present study, network pharmacological and molecular docking approaches were used to explore the mechanisms of LHQW capsule treating COVID-19.

According to the GO analysis, the LHQW capsule treating COVID-19 is widely involved in regulating inflammation, virus infection, endothelial barrier and cytokine storm via mediating these processes such as response to molecule of bacterial origin, response to oxidative stress, membrane raft, membrane microdomain, cytokine receptor binding and cytokine activity, which were significantly enriched in the GO analysis. Molecule of bacterial origin is involved in Toll-Like Receptor Signaling, which represents a largely evolutionarily conserved pathogen recognition machinery responsible for recognition of bacterial, fungal, protozoan, and viral pathogen associated microbial patterns and initiation of inflammatory response [27]. Oxidative stress is an imbalance between oxidants and antioxidants when the organism exposures to adverse stimuli, playing important roles in virus infection [28]. Membrane raft and membrane microdomain are not only closely associated with inter-endothelial junctions and adhesion of endothelium, but also involved in immune cell adhesion and migration across endothelium [29]. Cytokine storm, emerged from prolonged cytokine/chemokine responses during COVID-19, causes ARDS or multiple-organ dysfunction, ultimately leading to physiological deterioration and death [30]. Quercetin, a typical flavonoid, is one of the major compounds of FF, LIF, EH, HH, PCB, and LI in LHQW capsule and has been reported to have the effects of anti-inflammation, antiviral, strengthening the endothelial barrier integrity and inhibiting the expression of cytokines [31–34]. Kaempferol is flavonoid found in FF, LIF, EH, FBR, HH, and LI of LHQW capsule, and can protect against inflammation, virus infection, endothelial barrier dysfunction, and cytokines release [35–38]. Another important compounds, such as luteolin, naringenin, and beta-sitosterol, in LHQW capsule also have these protective effects [39–42]. Thus, the results of GO analysis indicated that the mechanisms of LHQW capsule treating COVID-19 might be related with suppressing inflammation, virus infection, endothelial barrier dysfunction and cytokine storm.

The internal regulation of the body involves a complex regulatory network rather than a single signaling pathway. Therefore, the therapeutic effects of drugs against diseases involve in many pathways. KEGG enrichment analysis showed that LHQW capsule treating COVID-19 included AGE – RAGE signaling pathway in diabetic complications, Kaposi sarcoma – associated herpesvirus infection (KSHV), TNF, IL – 17, and Toll – like receptor (TLR) signaling pathway, which were associated with inflammation, virus infection and immune response. Taking IL – 17 and TLR signaling pathways as examples to explore the mechanisms of LHQW capsule treating COVID-19. IL – 17 is a key T helper cell population and founding member of a novel family of inflammatory cytokines [43]. IL-17 signaling can not only activate downstream pathways that include NF- κ B, MAPKs and C/EBPs to induce the expression of anti-microbial peptides, cytokines and chemokines, but also is critical for protection against a variety of fungal and bacterial infections [44, 45]. TLRs are a well-known family of pattern recognition receptors that play important roles in host immune system and inflammation [46]. TLR signaling activation can result in the production of cytokines, chemokines and interferons and transcription factor NF- κ B [47, 48], thus

inducing inflammatory and immune response. Previous studies have demonstrated that quercetin not only exerted the effect of anti-inflammation by regulating IL-17 signaling [49, 50], but also effectively promoted the immunoregulatory effect by activating the TLR-3 pathway and inhibiting downstream cytokines production [51, 52]. Similarly to quercetin, another important compound also have these effects. As a result, LHQW capsule treating COVID-19 may directly interfere with IL-17 and TLR signaling, and further regulate the downstream signaling pathways, such as the NF- κ B signaling pathway, leading to the inhibition of cytokines production, including TNF- α , IL-1 β , IL-8.

Based on gene-pathway network analysis, MAPK3, MAPK1, RELA, IL-6, and CASP8 are the more important genes in LHQW capsule treating COVID-19. Clinical and laboratory data of COVID-19 showed evidence of inflammatory and immune injury, confirming that cytokine storms could be a crucial factor linked to the severity and mortality of COVID-19. MAPK3 and MAPK1, belong to serine/threonine protein kinase family, and the activation of MAPK promotes virus infection and inflammatory response [53–55]. REL/NF- κ B family of transcription factors plays a central role in initiation and resolution of inflammatory responses [56, 57]. IL-6, promptly and transiently produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses, hematopoiesis, inflammatory and immune reactions [58, 59]. Caspase-8 is the initiator caspase of extrinsic apoptosis and required for the activation of NF- κ B and secretion of cytokines in response to activated antigen receptors [60]. Quercetin, kaempferol, luteolin and naringenin, all can inhibit the activation of MAPK and RELA, decrease the expression of IL-6 and CASP8 [61–65]. Moreover, the most critical targets had good binding activities to main compounds, which indicated that pharmacodynamic mechanisms of LHQW capsule had sufficient material basis through preliminary analysis.

Conclusion

This study investigated the effective active ingredients and molecular mechanisms of LHQW capsule in the treatment of COVID-19 from the perspective of network pharmacology and molecular docking. The therapeutic effects potentially involved in inhibiting inflammatory response, cytokine storm and virus infection, and regulating immune reactions, apoptosis and endothelial barrier.

Abbreviations

COVID-19: novel coronavirus 2019; LHQW: Lian Hua Qing Wen; TCM: traditional Chinese medicine; OB: oral bioavailability; DL: drug-likeness; IR: Isatidis Radix; MH: Menthae Herba; RRER: Radix Rhei Et Rhizome; LI: licorice; PCB: Pogostemon Cablin (Blanco) Benth; FBR: Fortunes Bossfern Rhizome; LJF: Lonicerae Japonicae Flos; ACV: Amygdalus Communis Vas; FF: Forsythiae Fructus; EH: Ephedra Herba; HH: Houttuyniae Herba.

Declarations

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Not applicable.

Authors' contributions

CL contributed to analysis and manuscript preparation, was a major contributor in writing the manuscript; QH contributed to the conception of the study and reviewed the manuscript; TY and WHT helped perform the data analyses; SDZ modified the manuscript and provided funding support; All authors read and approved the final manuscript.

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Availability of data and materials

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

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interests.

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Figures

Figure 1

The technical strategy of this research based on network pharmacology and molecular docking to explore the mechanisms of Lian Hua Qing Wen Capsule against COVID-19.

Figure 2

Compound-Target Network of LHQW capsule. The green prismatic represents targets; the red, light green, yellow, orange, blue, green, grey, violet, dark blue, deep purple and pink represent IR, RRER, LI, PCB, FBR, LJF, ACV, FF, EH, HH, and multiple targets, respectively.

Figure 3

Visualization of the PPI of the target genes using STRING and Cytoscape.

Figure 4

Gene ontology and KEGG pathway enrichment of candidate targets of LHQW capsule against COVID-19. The top 20 GO functional categories with $P < 0.05$ were selected. (A) biological process, (B) cellular component, (C) molecular function, (D) pathways that had significant changes of $P < 0.05$ were identified. Size of the spot represents number of genes and color represents FDR value.

Figure 6

Molecular docking between compounds and targets. (A) quercetin bound to the active pocket of MAPK3, (B) kaempferol bound to the active pocket of MAPK3, (C) quercetin bound to the active pocket of MAPK1, (D) kaempferol bound to the active pocket of MAPK1.