Tropical Journal of Pharmaceutical Research June 2021; 20 (6): 1241-1249
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i6.21

Original Research Article

Identification of the anti-COVID-19 mechanism of action of Han-Shi Blocking Lung using network pharmacology-integrated molecular docking

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Sent for review: 30 June 2020 *Revised accepted: 16 May 2021*

Abstract

Purpose: *To investigate the bio-active components and the potential mechanism of the prescription remedy, Han-Shi blocking lung, with network pharmacology with a view to expanding its application.*
Methods: *Chemical components were first collected from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). Pharmmapper database and GeneCards were used to predict the targets related to active components and COVID-19. Using DAVIDE and KOBAS 3.0 databases, Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were enriched. A "components-targets-pathways" (C-T-P) network was conducted by Cytoscape 3.7.1 software. With the aid of Discovery Studio 2016 software, bio-active components were selected to dock with SARS-COV-2 3CL and ACE2.*
Results: *From the prescription, 47 bio-active components, 83 targets and 103 signaling pathways were obtained in total ($p < 0.05$). 126 GO entries ($p < 0.05$) were screened by GO enrichment analysis. Molecular docking results showed that procyanidin B1 eriodictyol, (4E, 6E)-1, 7-bis(4-hydroxyphenyl)hepta-4, 6-dien-3-one, and quercetin had higher docking scores with SARS-COV-2 3CL and ACE2.*
Conclusion: *With network pharmacology and molecular docking, the bio-active components and targets of this prescription, Han-Shi blocking lung, against COVID-19 were identified. Taken together, this study provided a basis for the treatment of COVID-19 and further promotion of this prescription.*

Keywords: *Prescription, Han-Shi blocking lung, COVID-19, Mechanism of action, Bioactive components, Network pharmacology, Molecular docking*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

COVID-19 has swept the world from the end of 2019 to now, with hundreds of thousands of people infected. As at March 17, 2021, the cumulative number of cases worldwide has exceeded 121,076,777, and the number of deaths has exceeded 2,673,579, and this has attracted special attention all over the world. COVID-19 is often accompanied by clinical

manifestations such as fever and dry cough at the early stage. Ill patients may have dyspnea, hypoxemia, or even acute respiratory distress syndrome [1-2]. The world is in urgent need of effective tests and drugs to help fight the epidemic.

In Traditional Chinese Medicine (TCM) theory, *Han-Shi blocking lung* is often manifested as low fever, nausea, dry cough with little phlegm or fatigue. The people's Republic of China published the COVID-19 Clinical Protocols: Seventh Edition, which gives corresponding treatment prescription consisting of Cangzhu (ATRACTYLODIS RHIZOMA) [3], Chenpi (*CITRI RETICULATAE PERICARPIUM*) [4], Houpo (M*AGNOLIAE OFFICINALIS CORTEX*) [5], Huo xiang (*POGOSTEMONIS HERBA*) [6], Caoguo (*TSAOKO FRUCTUS*), Shengmahuang (*EPHEDRAE HERBA*) [7] Qianghuo (NOTOPTERYGII RHIZOMA ET RADIX) [8], Shengjiang (ZINGIBERIS RHIZOMA RECENS) [9], and Binglang (ARECAESEMEN) [10]. At present, the efficacy of this prescription has been verified in China. Therefore, it is necessary to further investigate its bio-active components and mechanism of action, so as to increase the popularity and use of this prescription.

Network pharmacology method integrates multi-direction pharmacology, systems biology, and computer analysis technology. It establishes a "C-T-P" network to study the interaction between bio-active components and candidate targets, so as to systematically and comprehensively correlate drugs and diseases [11], which is consistent with the holistic and systematic characteristics of TCM treatment. The high molecular docking technique simulates the interaction between the receptors and bio-active components, and demonstrates the docking activity of the drug molecules. In this study, using network pharmacology and molecular docking technology, the "C-T-P" network was conducted to predict the potentially bio-active components and mechanism of this prescription against COVID-19, which provided ideas and basis for further research.

METHODS

Collection of prescription components

TCMSP (http://lsp.nwu.edu.cn/tcmsp.php) which provided pharmacokinetic properties of bio-active components, was used to collect all the components of the prescription. With PubChem database (https://pubchem.ncbi.nlm.nih.gov/), a component library was established.

Screening of potential bio-active components

Drugs play an important role in human body through absorption, distribution, metabolism and excretion (ADME). Higher oral bioavailability (OB) and drug likeness (DL) values usually indicate that the drug has better biological activity. In this study, a comprehensive model of OB ≥ 30 %, and DL ≥ 0.18 were constructed for screening potential bio-active components of this prescription [12].

Targets prediction of bio-active components

The potentially bio-active components were imported into the Pharmmapper database (http://lilab.ecust.edu.cn/pharmmapper/index.php) to match the candidate targets, and the plug-in of Uniprot database (limit species to humans) was used to obtain the Gene Official Symbol format.

Screening of COVID-19-related targets

By searching GeneCards database (https://www.genecards.org/) for the keywords "novel coronavirus", the COVID-19-related genes were obtained. The candidate targets of COVID-19-related genes with the bio-active components were compared by Venn diagram.

Gene function and KEGG pathway enrichment

The KOBAS3.0 database (http://kobas.cbi.pku.edu.cn/anno_iden.php) and the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) for a large number of genes provide comprehensive biofunctional annotations. The potential targets were introduced into the two databases. Then, the species and identifier were selected as "Homo sapiens" and "GENE OFFICIAL SYMBOL" respectively for GO enrichment and KEGG pathway annotations. And $p < 0.05$ was set as the threshold to screen meaningful results. Using Omicshare database (http: //www.omicshare.com), the top ten relevant GO enrichment and KEGG pathways were plotted as bubble plots respectively.

Constructing a network

Through Cytoscape 3.7.1 software (https://cytoscape.org/download.html), the "C-T-P" network was conducted. The nodes of different colors represented bio-active components, candidate targets and pathways respectively., and the edges represented the

correlation between different nodes. Topological analysis of the constructed pharmacological network was performed to screen the important bio-active components. It is generally believed that the node's degree is significant when it is greater than twice the median.

Construction of protein-protein interaction (PPI) network

The interaction between proteins was integrated through the STRING database (https://string-db.org/) which collects, scores, and integrates all publicly available PPI information.

Molecular docking

SARS-CoV-2 is a newly discovered β-coronavirus closely related to angiotensin converting enzyme 2 (ACE2) in human cells. Using the Discovery Studio 2016 software, the ligands and non-protein molecules of SARS-CoV-2 3CL (PDB: 6LU7) and ACE2 (PDB: 1R42) were removed. After uploading the sdf format file of the component's 2D structure which was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), their energy was minimized. It is generally believed that the docking ability with protein is stronger when LiDockscore≥90.

RESULTS

Screening bio-active components

The TCMSP database was used to screen the bio-active components of this prescriptions. By OB ≥ 30% and DL ≥ 0.18, 68 chemical components were screened totally. After removing no corresponding target components and repeated chemical components, information of 47 potentially bio-active components were obtained. 17 of them from Ephedrae Herba, which is considered to be the most important Chinese medicine. The information of collected bio-active components is shown in Table 1 and 2.

Table 1: Potential bio-active components of the prescription, *Han-Shi blocking lung*

TCM	PubChem CID	Components	OB%	DL
Atractylodis Rhizoma	70697841	2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydrobenzofuran-5-carboxylic	45.2	0.20
	101689889	Stigmasterol 3-O-beta-D-glucopyranoside_qt	43.8	0.76
	5356634	NSC63551	39.3	0.76
	5742590	Daucosterin_qt	36.9	0.76
	5281703	Wogonin	30.7	0.23
Citri Reticulatae Pericarpium	12303287	Citromitin	86.9	0.51
	72344	Nobiletin	61.7	0.52
	439246	Naringenin	59.3	0.21
	676152	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl) chroman-4-one	47.7	0.27
Magnoliae Officinalis Cortex	442439	Neohesperidin	57.4	0.27
Pogostemonis Herba	5281677	Pachypodol	75.1	0.40
	5282160	Quercetin 7-O-β-D-glucoside	49.6	0.27
	5280343	Quercetin	46.4	0.28
	33934	Diop	43.6	0.39
	443024	Acanthoside B	43.4	0.77
	12314136	Phenanthrone	38.7	0.33
	5281781	Lrisolidone	37.8	0.30
	5281617	Genkwanin	37.1	0.24
Tsaoko Fructus	10613719	(4E,6E)-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one	67.9	0.24
	5280343	Quercetin	46.4	0.28
	5742590	Daucosterin_qt	36.9	0.76
Ephedrae Herba	440735	Eriodictyol	71.8	0.24
	439246	Naringenin	59.3	0.21
	5280343	Quercetin	46.4	0.28
	5280794	Stigmasterol	43.8	0.76
	66540	Truflex OBP	43.7	0.24
	5282184	Mandenol	42.0	0.19
	5280863	Kaempferol	41.9	0.24
	5320438	Pectolinarigenin	41.2	0.30
	68245	Delphinidin	40.6	0.28
	173183	Campest-5-en-3beta-ol	37.6	0.71

Table 2: Potential bioactive components of the prescription, *Han-Shi blocking lung* (contd)

TCM	PubChem CID	Components	OB%	DL
	5281617	Genkwanin	37.1	0.24
	222284	beta-Sitosterol	36.9	0.75
	457801	Poriferast-5-en-3beta-ol	36.9	0.75
Ephedrae Herba	5280445	Luteolin	36.2	0.25
	15596633	24-Ethylcholest-4-en-3-one	36.1	0.76
	5280544	Herbacetin	36.1	0.27
	5281612	Diosmetin	31.1	0.27
Notopterygii	134714934	Diversoside_qt	67.6	0.31
Rhizoma Et Radix	68081	Isoimperatorin	45.5	0.23
	5471349	Bergaptin	41.7	0.42
	5316520	Demethylfuropinnarin	41.3	0.21
	6442182	8-geranoxy-5-methoxypsoralen	41.0	0.50
	98608	Phellopterin	40.2	0.28
	222284	beta-Sitosterol	36.9	0.75
	10212	Ammidin	34.6	0.22
	821449	Cnidilin	32.7	0.28
	6439317	6'-Feruloylnodakenin	32.0	0.67
	5281612	Diosmetin	31.1	0.27
Zingiberis Rhizoma	107982	Dihydrocapsaicin	47.1	0.19
Recens	5280794	Stigmasterol	43.8	0.76
	222284	beta-Sitosterol	36.9	0.75
	457801	Poriferast-5-en-3beta-ol	36.9	0.75
Arecaesemen	11250133	Procyanidin B1	67.9	0.66
	446284	EPA	45.7	0.21
	6786	WLN: 6OVR BVO6	43.7	0.24

Screening potential targets

The targets of 47 potentially effective components and related diseases were screened by Venn diagram, as shown in Figure 1. The 83 candidate targets of bio-active components against COVID 19 are shown in Table 3.

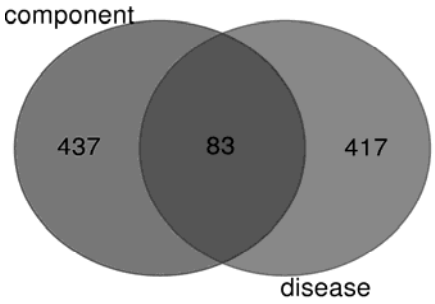

Figure 1: Venn diagram of candidate targets

Functional analysis of potential targets

The KEGG annotation of DAVID and KOBAS 3.0 database involved 103 pathways (P<0.05), and the top ten were PI3K-Akt signaling pathway, Rap1 signaling pathway and HIF-1 signaling pathway (Figure 2A). It is generally believed that the balance control of inflammatory factors and the improvement of human immunity are the main directions for the treatment of COVID-19. Most pathways were involved in the development of inflammation, which was consistent with the generally accepted view.GO functional enrichment analysis resulted in 126 GO items, including 95 (75.4%) biological process (BP) items, 14 (11.1%) items for cell composition (CC), and 17 (13.5%) items for molecular function (MF). The top ten BP, CC, MF are shown in Figure 2B, Figure 2C and Figure 2D respectively.

Construction of "C-T-P" network

In order to further elucidate on the relationship between the bio-active components, their candidate targets, and the related KEGG pathways, a "C-T-P" network containing 140 nodes (47 bio-active components, 83 candidate targets and 10 pathways) and 424 lines was constructed by Cytoscape 3.7.1 software, as shown in Figure 3A. Red represents the KEGG pathways, purple represents the candidate targets and green represents the bio-active components. It's not hard to find that each component interacted with multiple targets, and different targets interacted with multiple pathways. This phenomenon is consistent with the concept of multi-components, multi-targets and multi-pathways collaborative treatment of diseases in TCM.

Table 3: Condidate targets that associate bio-active components with COVID-19

Gene	Official Symbol						
1	ABCB1	22	F2	43	MAPK8	64	PLG
2	ACE	23	FGFR1	44	MAPT	65	PRKCD
3	AR	24	G6PD	45	MDM2	66	PTGS2
4	ATM	25	HDAC1	46	MET	67	PTPN11
5	BCL2	26	HIF1A	47	MMP1	68	PTPRC
6	BRAF	27	HRH2	48	MMP12	69	RAF1
7	BTK	28	IDO1	49	MMP2	70	RPS6KB1
8	CASP1	29	IKBKB	50	MMP3	71	SELE
9	CASP3	30	IL2	51	MMP8	72	SERPINE1
10	CCR5	31	IRAK4	52	MMP9	73	SLC2A1
11	CDK1	32	ITGAL	53	MPO	74	SLC6A3
12	CFTR	33	JAK1	54	MTOR	75	SNCA
13	CTNNB1	34	JAK3	55	NOD2	76	SRC
14	CTSB	35	KIT	56	NOS2	77	STAT3
15	CXCR1	36	LCK	57	NRAS	78	SYK
16	CXCR2	37	LYN	58	PIK3CA	79	TERT
17	CYP1A1	38	MAP2K1	59	PIK3CD	80	TLR9
18	DHFR	39	MAPK1	60	PIK3R1	81	TNF
19	EGFR	40	MAPK14	61	PLA2G1B	82	TTR
20	ELANE	41	MAPK3	62	PLAU	83	TYK2
21	ERBB2	42	VEGFA	63	XPO1		

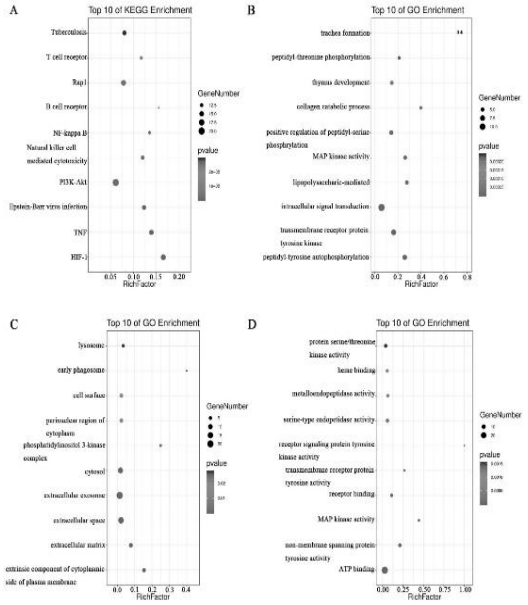

Figure 2: Bubble charts of KEGG pathway enrichment and GO functional enrichment. A: The top 10 KEGG pathways enrichment. B: The top ten significantly-enriched entries in biological process (BP); C: The top ten significantly-enriched entries in cellular component (CC); D: The top ten significantly-enriched entries in molecular function (MF)

According to this criterion, bio-active components were selected based on degree value for molecular docking with the target proteins. In this study, the degree value of key nodes should be greater than twice the median (the median component degree value = 6). By analyzing the degree between components, the top 10 bio-active components were screened, as shown in Table 4.

Table 4: Top ten potentially effective components of the, prescription, *Han-Shi blocking lung*

Component	Degree
Procyanidin B1	20
2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene -2,3-dihydrobenzofuran-5-carboxylic	17
Wogonin	17
(4E,6E)-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one	17
Quercetin	16
Delphinidin	16
Genkwanin	15
Pachypodol	15
Diosmetin	14
Eriodictyol	14

Construction of PPI network

After analyzing with the STRING database, the PPI network was imported into the Cytoscape 3.7.1 software and analyzed. And the top candidate targets were screened and analyzed to determine the close interactions amongst them, as shown in Figure 3B. Most importantly, MAPK3 was found to be the link between ACE2 and other targets (Figure 3C). At the same time, it was not hard to find that the top targets were widely exist in the top components, suggesting that the prescription for Han-Shi blocking lung in the treatment of COVID-19 might work through multiple components which regulated genes co-expressed with ACE2 and SARS-CoV-2 3CL.

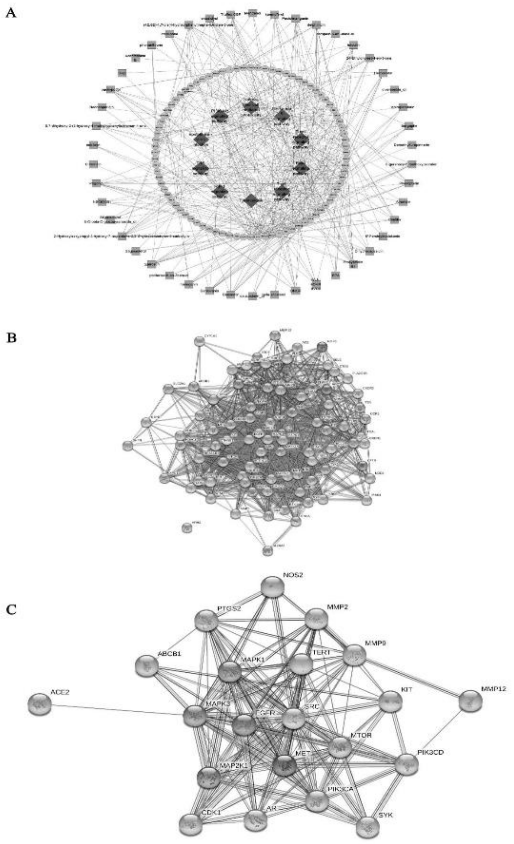

Figure 3: A: "C-T-P" network. B: Components and disease-related targets candidate "PPI" network. C: The top twenty targets for interaction with ACE2

Analysis of molecular docking

The docking results were analyzed with LiDockscore ≥ 90 as the screening criterion. It was obvious that the top ten bio-active components had strong binding affinities to SARS-COV-2 3CL. With the exception of delphinidin and pachypodol, all components had a strong binding ability with ACE2, as shown in Table 5. Obviously, procyanidin B1, eriodictyol, (4E, 6E)-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one and quercetin had higher docking scores with SARS-COV-2 3CL and ACE2. Thus, they were the most important, potentially bio-active components in this prescription. This indirectly confirmed the results of the network pharmacology. These results are shown in Figure 4 and Figure 5.

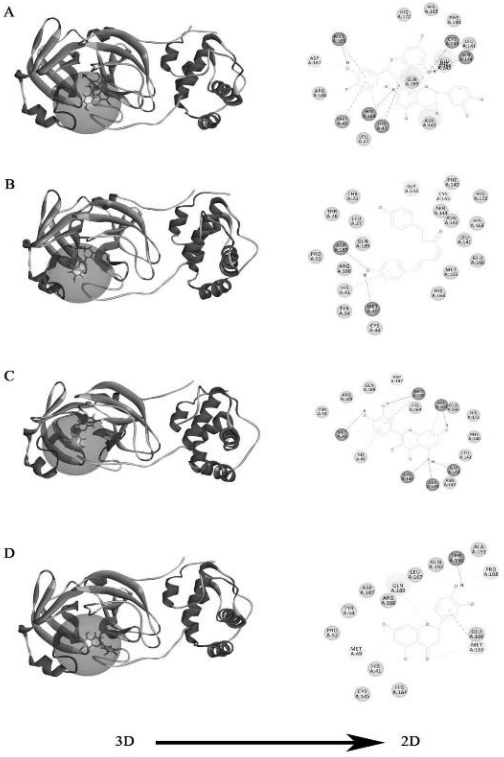

Figure 4: Results of molecular docking of each component with COVID-19 3CL hydrolase. A: Procyanidin B1; B: (4E,6E)-1,7-bis(4-hydroxyphenyl)-hepta-4,6-dien-3-one; C: quercetin; D: eriodictyol

Table 5: Binding affinity of bioactive components and clinical therapeutic drugs with SARS-CoV-2 3CL and ACE2

Component	LiDockScore	
	SARS-CoV-2 3CL	ACE2
procyanidin B1	153.30	138.15
(4E,6E)-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one	122.84	114.32
eriodictyol	122.37	103.28
quercetin	117.34	113.22
diosmetin	123.56	96.98
2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydrobenzofuran-5-carboxylic	118.68	94.04
delphinidin	114.23	85.88
genkwanin	113.75	92.56
pachypodol	111.19	88.45
wogonin	96.83	95.32

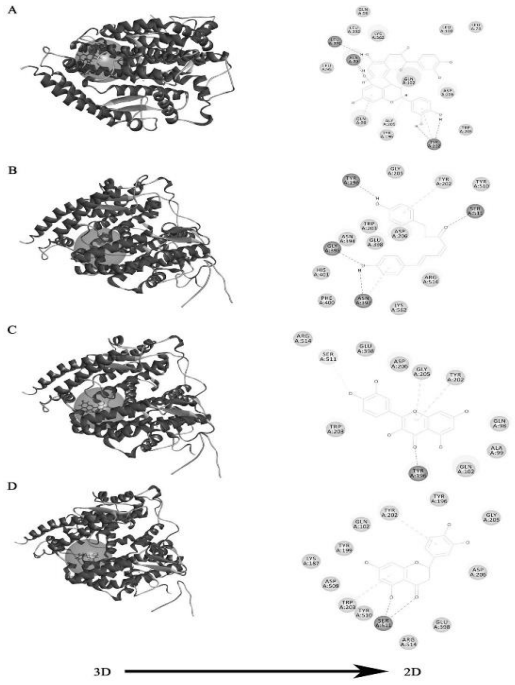

Figure 5: Results of molecular docking of each component with ACE2. A: Procyanidin B1; B: (4E,6E)-1,7-bis(4-hydroxyphenyl)-hepta-4,6-dien-3-one; C: quercetin; D: eriodictyol

DISCUSSION

According to TCM, COVID-19 is a disease caused by the *evil of wet poison*. Because after the *evil of wet poison* attacks the lungs, the patient will have fever, cough, fatigue and other symptoms. At present, China has issued many prescriptions for the treatment of COVID-19, and the clinical results show that they have significant efficacy [13]. The prescription for *Han-Shi blocking lung* is composed of several classic prescriptions. Among them, Chenpi (*CITRI Reticulatae pericarpium*), Houpo (*Magnoliae officinalis cortex*), Caoguo (*Tsaoko fructus*) could dissolve phlegm and relieve asthma, Shengmahuang (*Ephedrae herba*), and Shengjiang (*Zingiberis rhizoma recens*) could diminish inflammation and treat the cough caused by pulmonary inflammation; Cangzhu (Atractylodis rhizoma), Huoxiang (Pogostemonis herba) and Qianghuo (Notopterygii rhizoma et radix) can relieve cold and pain, and treat the sustained low fever caused by cold and dampness; Binglang (Arecaesemen) and Huoxiang (Pogostemonis herba), Qianghuo (Notopterygii rhizoma et radix) have antiviral effect, can reduce the virus activity. Most of the chinese herbal medicines have the effect of *clearing heat* and detoxification, but have no side effects in this prescription. So, they are allowed to be used to prevent COVID-19. It is unclear what mechanism of action makes it not to be recognized abroad.

In order to explore the mechanism of this prescription, the network pharmacology and molecular docking technologies were used for experiments. In the study, 47 bio-active components acted on 83 targets such as MAPK3. MMP2、MAPK14、IL-2 and affected 103 signaling pathways such as HIF-1, PI3K-Akt and Rap1 signaling pathways. The "C-T-P" network was used to screen the bio-active components, potential pathways and targets.

It has been reported that the virus affects SARS-COV-2 3CL and ACE2, causing immune response disorders in the body, and the "cytokine storm" caused by the overexpression of inflammatory factors can lead to acute lung injury and aggravate the patient's condition. But until now, no clinical therapeutic drug could be used as a positive drug. Therefore, it is very important to find targeted drugs of related genes. It is generally accepted that the lower the docking energy between the binding of the receptors and ligands, the stronger the potential activity of the components. Therefore, high-throughput molecular docking technologies was used to investigate the docking profiles of the bio-active components with SARS-COV-2 3CL and ACE2. The docking results showed that procyanidin B1, eriodictyol, (4E,6E)-1,7-bis(4-hydroxyphenyl) hepta-4,6-dien-3-one, and quercetin had high docking scores with SARS-COV-2 3CL and ACE2. Thus, they were the most important and potentially bio-active components in this prescription. Exploring how they work has become the focus of further research.

It is believed that the main direction of COVID-19 treatment is to control the increase in inflammatory factors and improve the body's immunity. Procyanidin B1 and eriodictyol have already been proven to have anti-inflammatory and anti-viral effects. Quercetin is favored by researchers because of its multiple effects. Related literature has shown that Quercetin can inhibit the NF-κB signaling pathway, reduce the production of NF-κB and ICAM-1, achieve anti-inflammatory effect, and protect LPS-induced acute lung injury mice [14]. It can also significantly reduce the level of IL-4, IL-25, IL-33, TSLP and other inflammatory factors, so as to treat allergic respiratory inflammation in mice [15]. MAPK, PI3K-Akt are important intracellular signaling pathways, which play an important role in *Streptococcus pneumoniae* infection and the activation of macrophage immune response [16].

Streptococcus pneumoniae HSP40 has been proved to be recognized by TLR4 receptor and activated by p38MAPK pathway, resulting in initiate macrophage immune response [17]. IL-2 is an important factor of T lymphocyte response which participates in the regulation of the immune response and its level change is related to respiratory function [18]. Combining PPI network results, multi bio-active components were proven to bind with SARS-COV-2 3CL and ACE2, thereby regulating targets co-expressed with them. Pathways related to inflammation and immunity were the potential mechanisms of this prescription in the treatment of COVID-19. It is reported that chloroquine phosphate and remdesivir inhibit ACE2 and SARS-COV-2 3CL respectively, but they have no significant effect on the treatment of COVID-19. This reminds us that molecular docking is only one of the methods for screening potential active components, and the efficacy and mechanism of components need to be verified by further experiments *in vivo* and *in vitro*. Although MAPK3 is considered as the junction of ACE2 with other genes in this study, its specific role needs to be further clarified.

The widespread use of TCM prescriptions is the main reason the continuous spread of COVID-19 in China should be controlled, which also confirms their efficacy. However, the COVID-19 continues to spread throughout the world, and the situation is still very bad. In the process of developing the COVID-19 vaccine, the whole world should make every effort to find drugs with definite curative effects to rescue patients and alleviate the epidemic. But we have to admit that early protection, early isolation, and early treatment are the best solution for this epidemic at present. Fortunately, TCM can not only treat diseases but also “prevent infections”. Its advantages and potential are demonstrated by the synergistic effect of multi bio-active components, multi-targets and multi-pathways in the treatment of COVID-19. Many countries are asking China for medical assistance with regards to TCM. There is no doubt that under the premise of elucidating the mechanism with TCM, countries all over the world will trust and respect it.

CONCLUSION

As a clinical prescription, *Han-Shi blocking lung* is of great significance to explore its bio-active components and anti-COVID-19 mechanisms. Using network pharmacology and high-throughput molecular docking technologies, this study reveals the potential bio-active components (procyanidin B1, eriodyctyol and (4E,6E)-1,7-bis(4-hydroxyphenyl)-hepta-4,6-dien-3-one, quercetin), targets co-expressed with ACE2, pathways relate to Inflammation and immunity in this prescription. However, these results still need to be verified through experiments *in vitro* and *in vivo*.

DECLARATIONS

Acknowledgement

This study was supported by the National Natural Science Foundation of China (no. 31570343).

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Chong Yuan, Fei Wang and He-Zhen Wu contributed equally to this article.

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