Submitted: 26 September, 2022 Accepted: 04 January, 2023 Published: 08 September, 2023 DOI:10.22514/sv.2023.083

Open Access
Signa Vitae

ORIGINAL RESEARCH

Network pharmacology and molecular docking analyses on *Scutellaria barbata* indicate that *JUN* and *RELA* are potential targets to treat and prevent COVID-19 viremia

Xiaoding Song[1,†], Xi Xiao[2], Lingli Liu[1], Xiaobin Wei[3], Feng Li[1,*,†]

[1]Department of Clinical Laboratory, Hainan General Hospital (Hainan Affiliated Hospital of Hainan Medical University), 570311 Haikou, Hainan, China
[2]Department of Clinical Laboratory, Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University), 410000 Changsha, Hunan, China
[3]Department of Clinical Laboratory, Haikou Affiliated Hospital of Central South University Xiangya school of medicine, 570208 Haikou, Hainan, China

***Correspondence**
lifeng1210@hainmc.edu.cn
(Feng Li)

† These authors contributed equally.

Abstract

The coronavirus disease 2019 (COVID-19) pandemic has been ongoing for more than two years and is likely to continue. *Scutellaria barbata* (*S. barbata*) is a traditional Chinese herbal medicine with anti-inflammatory and anti-viral properties and has demonstrated therapeutic effects on patients with COVID-19. Our study aims to shed light on the underlying mechanism and identify possible therapeutic targets. The data on the expression of COVID-19 viremia-associated genes were retrieved from five disease-gene databases. The expression pattern of genes encoding for functional monomer components of *S. barbata* was retrieved from the Traditional Chinese Medicine Systems Pharmacology platform. To determine the potential mechanism, we used "Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses," and the protein-protein interaction (PPI) network was constructed using the STRING online tool. CytoNCA, a plug-in for Cytoscape, was used for screening the hub genes. The AutoDocktools and the "PyMOL" software were used for performing molecular docking between active molecules of drugs and disease-target proteins. We identified the *S. barbata* target and COVID-19 viremia-associated gene sets consisting of 42 genes. GO functional enrichment analysis showed that *S. barbata* can act by the regulation of cytokine activity and the cytokine-mediated signaling pathway. KEGG pathway enrichment analysis showed that these genes were enriched in several pathways like T helper cell 17 differentiation, the Tumor necrosis factor, and Interleukin-17 signaling pathways. In addition, we identified 17 hub genes, including *JUN, RELA, TNF, IL6*, *etc.*, using the PPI network and subnetworks. Molecular docking was performed on two highly significant genes: *JUN* and *RELA*. The former is a transcription factor, regulating activation-induced cell death, Interferon response post-COVID-19 infection, CD95 ligand promoter activity, and the expression of cytokine genes in T-cells. The five active compounds of *S. barbata*, including baicalein, wogonin, quercetin, luteolin, and beta-sitosterol, could enter the active pockets of COVID-19 to exert potential therapeutic effects on COVID-19 viremia. *JUN* and *RELA* could weaken T cell-mediated immune and cytokine-related inflammatory responses. They could be used as therapeutic targets and could aid in reducing COVID-19 viremia.

Keywords

Scutellaria barbata; COVID-19; Viremia; Signaling pathway; Network pharmacology; Molecular docking

1. Introduction

Scutellaria barbata has been historically used in Traditional Chinese Medicine (TCM) to induce diuresis, attenuate edema, promote blood flow, and eliminate heat, toxic materials, and blood stasis. The advancement in technology has helped identify 84 compounds from *S. barbata*. Most compounds derived from *S. barbata* are classified as flavonoids, diterpenoids, polysaccharides, volatile oil, and steroids [1]. Studies have demonstrated that these *S. barbata* compounds exert multiple properties like anti-inflammatory, anti-viral, bacteriostasis, anti-cancer, and anti-oxidative and could boost immunity [2]. On 31st December 2019, China reported the first case of the coronavirus disease 2019 (COVID-19) caused by the new severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [3] and was declared a global pandemic in April 2020 [4]. Airborne droplets and direct contact with infected surfaces are routes of COVID-19 transmission [5, 6]. Most patients with COVID-19 diagnosed using real-time

This is an open access article under the CC BY 4.0 license (https://creativecommons.org/licenses/by/4.0/).
 ©2023 The Author(s). Published by MRE Press.

TABLE 1. Databases, software, and analysis platforms.

Name	URL	Version number
Traditional Chinese Medicine Systems Pharmacology platform (TCMSP)	http://tcmspw.com/tcmsp.php	2.3
UniProt	https://www.uniprot.org	–
DisGeNET database	http://www.disgenet.org/	–
GeneCards database	https://www.genecards.org/	–
Online Mendelian Inheritance in Man (OMIM) database	https://omim.org/	–
Home-Genome-NCBI (National Center for Biotechnology Information)	https://www.ncbi.nlm.nih.gov/genome/	–
Kyoto Encyclopedia of Genes and Genomes (KEGG)-GENE database	https://www.kegg.jp/kegg/genes.html	–
KEGG Orthology-Based Annotation System (KOBAS) web-based analysis platform	http://kobas.cbi.pku.edu.cn/	3.0
Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; protein-protein interaction (PPI) network platform)	https://string-db.org/	–
Database for Annotation, Visualization, and Integrated Discovery (DAVID)	https://david.ncifcrf.gov/	6.8
Cytoscape software	https://download.freedownloadmanager.org/Windows-PC/Cytoscape/FREE.html	3.8.2
Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB)	https://www.rcsb.org/	–
PubChem database	https://pubchem.ncbi.nlm.nih.gov/	–
Open Babel software	http://openbabel.org/	3.1.1
AutoDocktools software	http://autodock.scripps.edu/wiki/AutoDockTools	1.5.6
PyMOL software	www.pymol.org	2.5.0

polymerase chain reaction were asymptomatic, and a very low proportion of patients were symptomatic [7]. Further, the clinical manifestations of COVID-19 vary from asymptomatic to mild acute upper respiratory symptoms like runny nose, cough, fever, sore throat, and severe conditions like multi-organ failure. Approximately 80% of patients with COVID-19 have relatively mild symptoms, 13% of patients suffer from severe illness, and 5% of patients suffer from life-threatening conditions and require treatment in the critical care unit (CCU) [8, 9]. A study has shown that the mortality among patients with COVID-19 treated in the CCU is as high as 40% [10]. In China, the combination of TCM and western medicine was used to treat patients with COVID-19. Further, six TCM ingredients have demonstrated exceptional therapeutic effects in treating patients with COVID-19 [11]. *S. barbata* was one of the six TCM recipes effective against COVID; hence, in this study, we have examined the underlying mechanism of *S. barbata* against COVID-19 viremia.

2. Material and methods

2.1 Table 1 shows the databases, software, and analysis platforms used in this study.

2.2 Obtaining S. barbata target and COVID-19 viremia-associated gene sets

We used the TCMSP [12] to retrieve data on biologically active targets and chemical constituents of *S. barbata*. As per the screening criteria of "oral bioavailability (OB) $\geq$30% and drug-likeness (DL) $\geq$0.18", the monomer chemical components and associated target proteins were filtered and transformed to respective matching gene symbols using UniProt [13].

We retrieved the data on COVID-19-associated genes from databases like GeneCards [14], OMIM [15], NCBI-gene [16], and KEGG-gene [17]. Furthermore, the data on viremia-associated genes were retrieved from the DisGeNET [18], OMIM, GeneCards and NCBI-gene databases.

The *S. barbata* target, COVID-19, and the viremia-associated gene sets were intersected to generate the *S. barbata* target and COVID-19 viremia-related gene set.

2.3 Development and enrichment analysis of integrated gene networks

GO enrichment analysis was performed on the *S. barbata* target and COVID-19 viremia-associated gene sets using the "Functional Annotation" module of DAVID [19] as per the following parameters: "Species: *Homo sapiens*, identifier: official gene symbol, gene list; list type: remaining parameters; default values." Next, we performed the KEGG pathway enrichment analysis using the KOBAS database [20]. The pathway with $p < 0.05$ was considered significantly enriched. The "Cytoscape version 3.8.2" software (National Institute of General Medical Sciences (NIGMS), Bethesda, MD, USA) was used for the purpose of constructing the *S. barbata*-target-COVID-19 viremia-signaling pathway relationship network [21], and the steps are as follows: (1) The elements required for constructing the network, such as disease-related genes, drug monomer components-related target molecules, intersection genes-enriched signaling pathways, *etc.*, were summarized to create their tables; (2) The software for grid mapping was imported, and different colors and shapes were assigned to mark them.

2.4 PPI networks and important subnetworks

The STRING online tool was used for constructing the PPI network for the gene sets [22]. The parameters for generating the PPI network were as follows: the protein type was set to "Human," the least interaction cutoff point was >0.9, and the disconnected nodes in the network were concealed. Then, the network was imported into the "Cytoscape version 3.8.2" software. Finally, the CytoNCA [23] plug-in was used to score the PPI network and screen the hub genes based on their degree centrality value.

2.5 Molecular docking technique

Molecular docking was performed on the most significant genes of the key subnetworks. The receptor proteins encoded by these identified genes were retrieved from UniProt. Subsequently, the three-dimensional (3D) structures (PDB file) of the receptor proteins were downloaded from the RCSB PDB [24]. The SDF file (Spatial Data Format file) of the functional monomer component was downloaded from PubChem [25] and converted to Mol2 format using the "Open Babel" software (3.1.1, department of Chemistry, University of Pittsburgh, Pittsburgh, USA) [26]. Next, the "PyMOL" software (1.5.6, http://www.pymol.org; PyMOL by Schrödinger, which is a company headquartered in New York, USA) [27] performed various operations like removing water and hydrogenating from the receptor protein. Finally, AutoDocktools 1.5.6 (the Scripps Research Institute, California, USA) [28] was used for performing molecular docking on the receptor proteins and small molecule ligands, recording binding energy, *etc.* The semi-flexible molecular docking method is widely used for drug designing and virtual screening [29] and was used for subsequent analyses as follows: Step 1: The Mol2 files of small molecules (functional monomer component) and PDB files of large molecules (the receptor protein) were prepared. Step 2: The pdbqt files of small molecules and macromolecules were prepared; Step 3: The GLG files (Grid parameter file) were prepared; Step 4: Docking was performed to obtain the DLG file (Docking parameter file). (The parameters for performing docking include the following: "Genetic Algorithm" was chosen as the parameter for docking search, "use defaults" was chosen as the parameter for docking-docking, and "genetic algorithm" was used for docking-output). Step 5: The docking results were visualized, and the binding energy was recorded; Step 6: The "PyMOL" software was used for visualizing the graphical representation of the docking results.

3. Results

3.1 Determining the potential targets and functional monomer components

We screened 29 functional monomer components and 592 *S. barbata*-related target proteins using TCMSP. Finally, 222 targets were obtained after eliminating the duplicate targets. We identified 4600 COVID-19-associated genes with a standard correlation score $\geq$0.14 from the GeneCards database using "COVID-19" as the keyword. We also identified 338, six, and 232 COVID-19-associated target genes from three databases NCBI-Gene, OMIM and KEGG-gene, respectively. Finally, 4755 COVID-19-associated genes were identified by merging the genes obtained from four databases and eliminating duplicate genes. In addition, we identified 56 viremia-associated genes with a gene-disease association score $\geq$0.01 from the DisGeNET database using "Viremia" as the keyword. A total of 619 viremia-associated genes were identified using the GeneCards database and a threshold correlation score of $\geq$0.14. We also identified 53 viremia-associated genes from NCBI-Gene and 144 viremia-associated genes from OMIM. Finally, 753 viremia-associated genes in total were identified by merging the genes obtained from four databases and eliminating duplicate genes. In addition, we identified the *S. barbata* target and COVID-19 viremia-associated gene sets consisting of 42 genes by intersecting genes encoding for compound-target and diseases-related genes (Fig. 1).

3.2 GO functional enrichment analysis

The intersecting gene sets, *i.e.*, the predicted *S. barbata* target genes acting on COVID-19 viremia. As shown in Table 2, 42 genes matched 15 active *S. barbata* monomer components. GO analysis takes into consideration three different biological aspects: biological processes (BP), molecular functions (MFs), and cellular components (CCs). The results revealed that the overlapping genes were enriched in 483 functions, including 395 BPs, 62 MFs, and 26 CCs. Based on $p < 0.05$ and the total number of significantly enriched genes, the leading ten substantially enriched functions were analyzed and displayed in Fig. 2. GO enrichment analysis showed that the intersecting genes enriched BP terms primarily associated with the cytokine-mediated signaling pathway and the positive

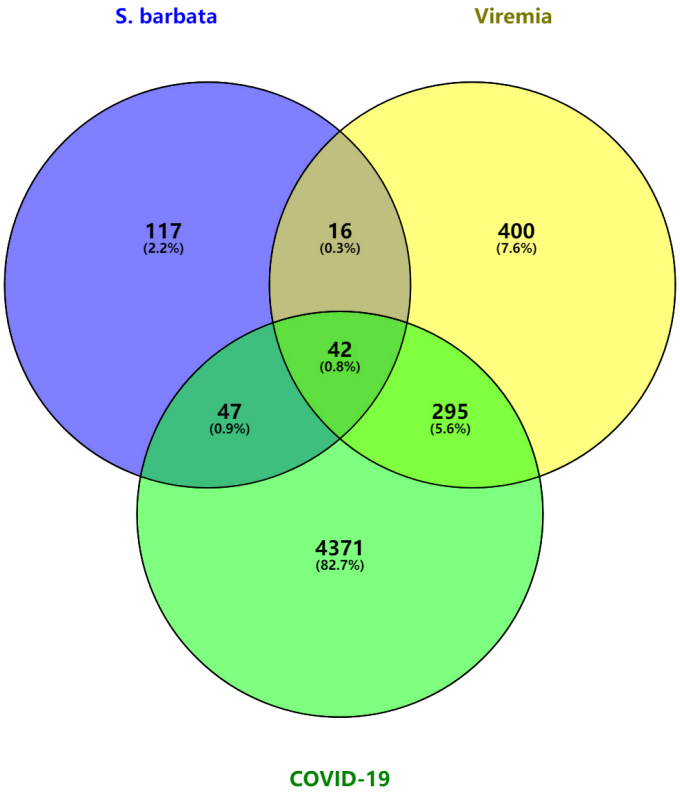


FIGURE 1. Venny diagram shows *S. barbata* target genes and COVID-19 viremia-associated gene. COVID: coronavirus disease.

control of transcription from the RNA polymerase II promoter, *etc.* Furthermore, these intersecting genes primarily enriched CC terms like the extracellular space and region, *etc.*, and significantly enriched MF terms were cytokine activity, protein, and identical in enzyme binding, *etc*.

3.3 KEGG pathway enrichment analysis

We searched the KOBAS database to identify the signaling pathway enriched by 42 overlapping genes, and 186 significantly enriched pathways in total were identified. Fig. 3A shows the top 30 signaling pathways enriched based on the total number of genes in each pathway. $p < 0.05$ was the cutoff value for screening and assessing enriched pathways. The overlapped genes enriched several KEGG pathways like the AGE-RAGE (advanced glycosylation end products-receptor of AGEs) signaling pathway associated with diabetic complications, T helper (Th) 17 cell differentiation, the Toll-like receptors, TNF, and interleukin (IL)-17 signaling pathways, *etc.* The IL-17 signaling pathway map is shown in Fig. 3B.

3.4 Establishment and analysis of integrative gene network

The "Cytoscape version 3.8.2" software was used for constructing the *S. barbata* functional monomers-target-disease pathway relationship network (Fig. 4A). In the network, red indicated illness, blue indicated target genes, yellow indicated functional monomer components of drugs, green indicated the signal pathways, and connectivity was used to show the interconnections. The network diagram highlighted the intricate correlation between several pathways and targets shared by COVID-19 viremia and *S. barbata* functional monomers. In most cases, numerous active chemicals could target a single gene, and in some cases, a single compound could target several genes simultaneously. *S. barbata* compounds targeted *DPP4* at a higher frequency compared to the other 42 genes.

3.5 Construction of PPI and critical subnetwork as well as screening hub genes

The STRING online tool was used to construct the PPI network. Next, we imported the PPI network into Cytoscape to perform further analyses. We retrieved one key subnetwork comprising 17 hub genes from CytoNCA based on the Degree centrality (DC) value $\geq$10 (Fig. 4B). This indicates that these genes performed a crucial function in this network and were associated with COVID-19 viremia targeted by functional monomers of *S. barbata*.

3.6 Molecular docking of hub genes encoding proteins and active compounds

Out of the 17 hub genes, we selected two highly significant genes namely, *RELA* and *JUN*, for molecular docking. In particular, molecular docking was performed between the two hub genes encoding proteins and the functional components of the monomer corresponding to all hub genes. In most cases, the binding energy >0 kcal/mol shows the binding activity of molecules. The lower the binding energy, the more stable the conformation [30]. Table 3 shows the docking scores, which indicate strong binding capacities. Next, the "PyMOL"

TABLE 2. Basic information of the functional monomer component of *S. barbata* against COVID-19 viremia.

Mol ID	Molecule Name	MW	OB (%)	DL
MOL000173	Wogonin	284.28	30.68	0.23
MOL002714	Baicalein	270.25	33.52	0.21
MOL000006	Luteolin	286.25	36.16	0.25
MOL000098	Quercetin	302.25	46.43	0.28
MOL000358	Beta-sitosterol	414.79	36.91	0.75
MOL005190	Eriodictyol	288.27	71.79	0.24
MOL012250	7-hydroxy-5,8-dimethoxy-2-phenyl-chromone	298.31	43.72	0.25
MOL000351	Rhamnazin	330.31	47.14	0.34
MOL008206	Moslosooflavone	298.31	44.09	0.25
MOL000449	Stigmasterol	412.77	43.83	0.76
MOL012248	5-hydroxy-7,8-dimethoxy-2-(4-methoxyphenyl) chromone	328.34	65.82	0.33
MOL012251	Chrysin-5-methylether	268.28	37.27	0.20
MOL012266	Rivularin	344.34	37.94	0.37
MOL001735	Dinatin	300.28	30.97	0.27
MOL002915	Salvigenin	328.34	49.07	0.33

Notes: MW: Molecular weight; OB: Oral bioavailability; DL: Drug-like properties.

TABLE 3. Molecular docking: COVID-19 viremia hub genes encoding protein corresponding to active components of *S. barbata*.

Hub Gene	functional monomer component	Binding Energy (kcal/mol)	Intermolecular Energy (kcal/mol)
JUN			
	Luteolin	−3.86	−5.35
	Quercetin	−3.12	−4.91
	Wogonin	−3.88	−5.07
	Beta-sitosterol	−4.77	−6.86
RELA			
	Luteolin	−3.86	−5.35
	Quercetin	−3.98	−5.77
	Wogonin	−4.40	−5.59
	Baicalein	−4.25	−5.85

software was used for visualizing the optimal configuration of molecular docking between the target protein and the active components. Fig. 5 shows luteolin, an example of a docking configuration. The results showed that *JUN* and *RELA* could be viable targets for preventing and treating COVID-19 viremia.

4. Discussion

TCM is a distinctive and mature medical system extensively used for over 1000 years to prevent and treat multiple illnesses in China. Further, the combination of western medicine and TCM was used for treating patients with COVID-19 in China. Approximately 85% of patients with COVID-19 were treated with TCM based on the Chinese Association for Science and Technology's recommendations (China's State Council. 21 February 2020. Press conference on scientific and technological innovation to support epidemic prevention and control. http://www.scio.gov.cn/xwfbh/xwbfbh/wqfbh/42311/42568/index.htm). In this study, we identified five functional monomers of *S. barbata* including luteolin, quercetin, baicalein, wogonin, and beta-sitosterol. The results revealed that these five monomers target important COVID-19 viremia-associated genes like JUN and RELA. Table 3 shows the summary of all aforementioned functional monomers. The molecular docking results revealed that these monomers had strong binding capacity. Lin *et al.* [31] explored the transcriptomic profiles of the colorectal tissues of dextran sodium sulfate (DSS)-induced colitis mice by performing next-generation sequencing. The results revealed that luteolin could significantly attenuate the DSS-activated IL-17 signaling pathway in the colon and also inhibit NOD-like receptor (NLR) family pyrin domain-containing 3 (*NLRP3)* and *NLRP1* expression. Further, the 3D cell co-culture system showed that luteolin could significantly inhibit *NLRP3*

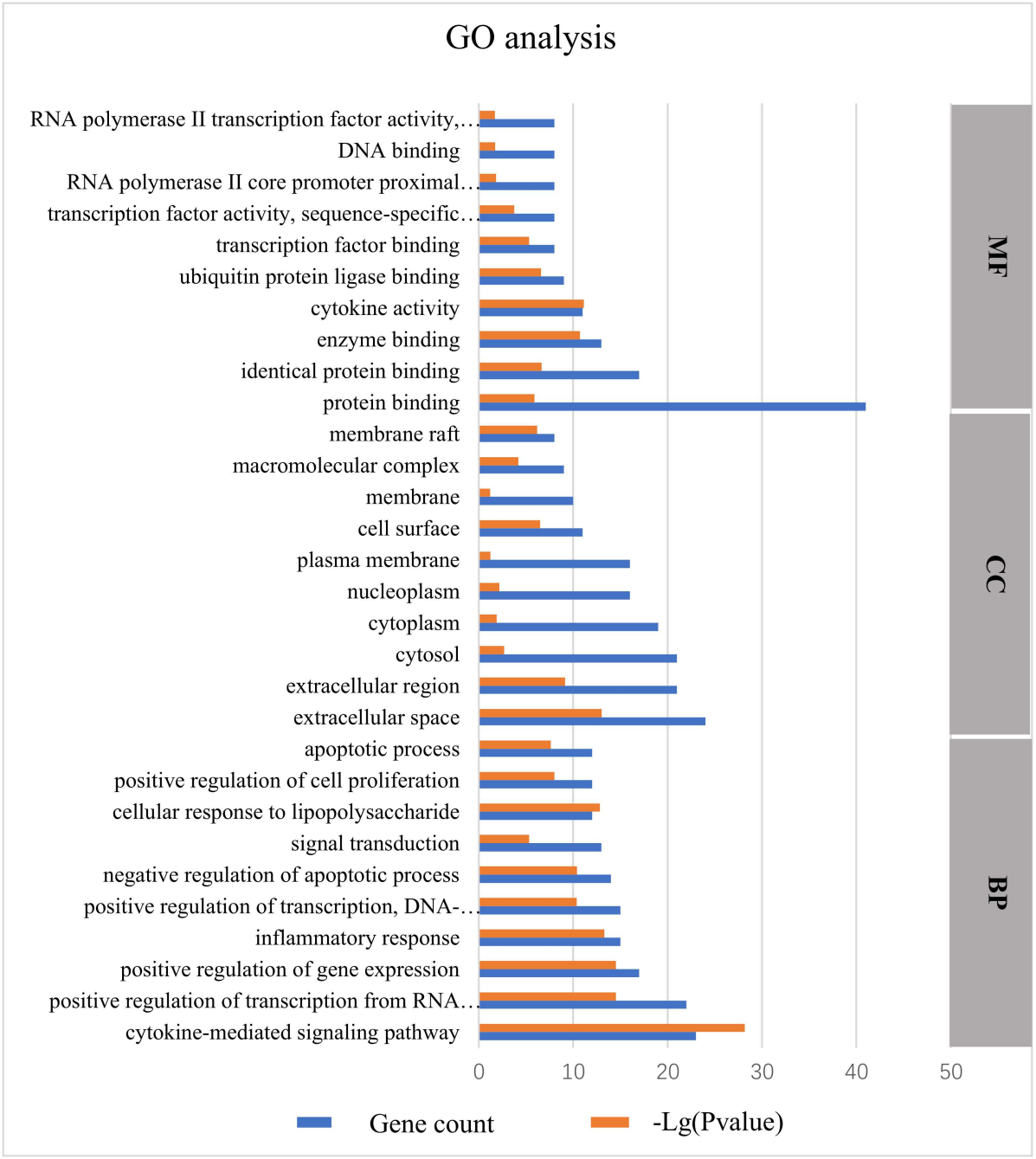
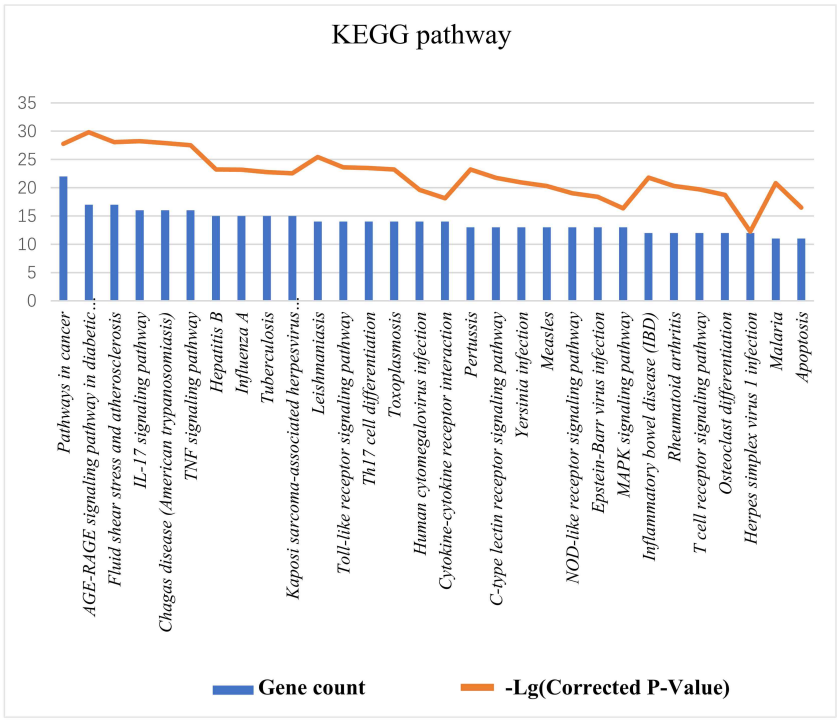


FIGURE 2. GO analysis shows *S. barbata* target genes against COVID-19 viremia. GO: Gene ontology; MF: molecular function; CC: cellular component; BP: biological processes.

expression by disrupting the IL-17A signaling pathway in inflamed colon tissue, thus indicating the therapeutic potential of luteolin for treating patients with inflammatory bowel disease (IBD) [31]. Furthermore, Quercetin alleviates bone destruction and lesions caused by ankle inflammation by reducing *IL-6, IL-17A* and *IL-17F* expression in the IL-17 signaling pathway and regulating the secretion of retinoic acid receptor (RAR)-related orphan receptors like γ-t, IL-17E, IL-1β, IL-6, TNFα, Forkhead box protein P3 (FOXP3), and transforming growth factor β-1 (TGFβ-1). These results indicate that quercetin could be an effective alternate drug for treating patients with gout arthritis [32]. Therefore, these drug monomers mediate therapeutic effects and prevent the onset of diseases via multiple pathways. Currently, the use of these drugs in the treatment and prevention of COVID-19 is still at the preliminary stage. Consequently, the underlying mechanism is still unclear.

JUN is a protein-encoding gene and a transcription factor that recognizes and binds to the activator protein-1 (AP-1) consensus motif 5′-TGA(GC)TCA-3′ [33]. The dimeric transcription factor complex AP-1 modulates the expression of nuclear genes and participates in several functions during T-cell activation [34]. JUN and *FOSB* activate AP-1 transcription by binding to the AP-1 promoter region in Fas ligand/CD95L, thereby activating the T-cell receptor/CD3 signaling pathway and inducing the death of T cells [35]. Patients with COVID-19

A



B

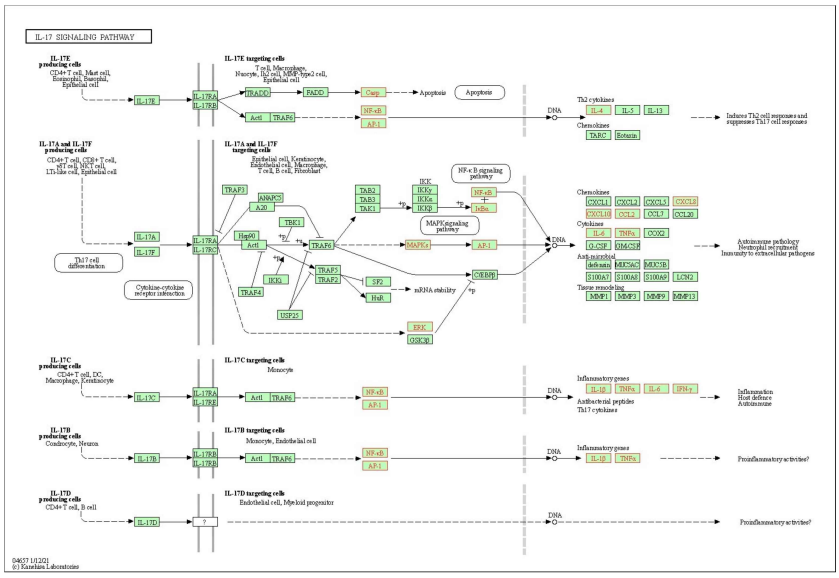


FIGURE 3. KEGG enrichment analysis and pathway map. A, Pathway enrichment analysis of *S. barbata* target genes against COVID-19 viremia using KOBAS. B, Pathway map shows the IL-17 signaling pathway as the most significantly enriched pathway. Notes: IL-17A-F: Interleukin 17A-F; IL-17RA: Interleukin 17 receptor A; IL-17RB: Interleukin 17 receptor B; IL-17RC: Interleukin 17 receptor C; IL-17RE: Interleukin 17 receptor E; TRADD: Tumor necrosis factor receptor type 1-associated DEATH domain protein ; Casp: Caspase 3 Act1: TRAF3 Interacting protein 2; TRAF6: TNF receptor-associated factor 2; TRAF3: TNF receptor-associated factor 3; TRAF5: TNF receptor-associated factor 5; TRAF2: TNF receptor-associated factor 2; TRAF4: TNF receptor-associated factor 4; NF-κb: Nuclear factor kappa B; AP-1: Fos proto-oncogene/AP-1 transcription factor subunit; ANAPC5: Anaphase-promoting complex subunit 5; A20: TNF alpha induced protein 3; TBK1: TANK binding kinase 1; Hsp90: Heat shock protein 90 alpha family class A member 1; IKKi: Inhibitor of nuclear factor kappa B kinase subunit epsilon; USP25: Ubiquitin specific peptidase 25; TAB2: TGF-beta activated kinase 1 binding protein 2; TAB3: TGF-beta activated kinase 1 binding protein 3; TAK1: Mitogen-activated protein kinase kinase kinase 7 (MAP3K7); IKKγ: Inhibitor of nuclear factor kappa B kinase regulatory subunit gamma; IKKα: Inhibitor of nuclear factor kappa B kinase regulatory subunit alpha; IKKβ: Inhibitor of nuclear factor kappa B kinase regulatory subunit beta; IκBα: NFKB inhibitor alpha; MAPKS: Mitogen-activated protein kinase 14; SF2: Serine and arginine rich splicing factor 1; HuR: ELAV-like RNA binding protein 1; C/EBPβ: CCAAT enhancer binding protein beta; ERK: Mitogen-activated protein kinase 1; GSK3β: Glycogen synthase kinase 3 beta.

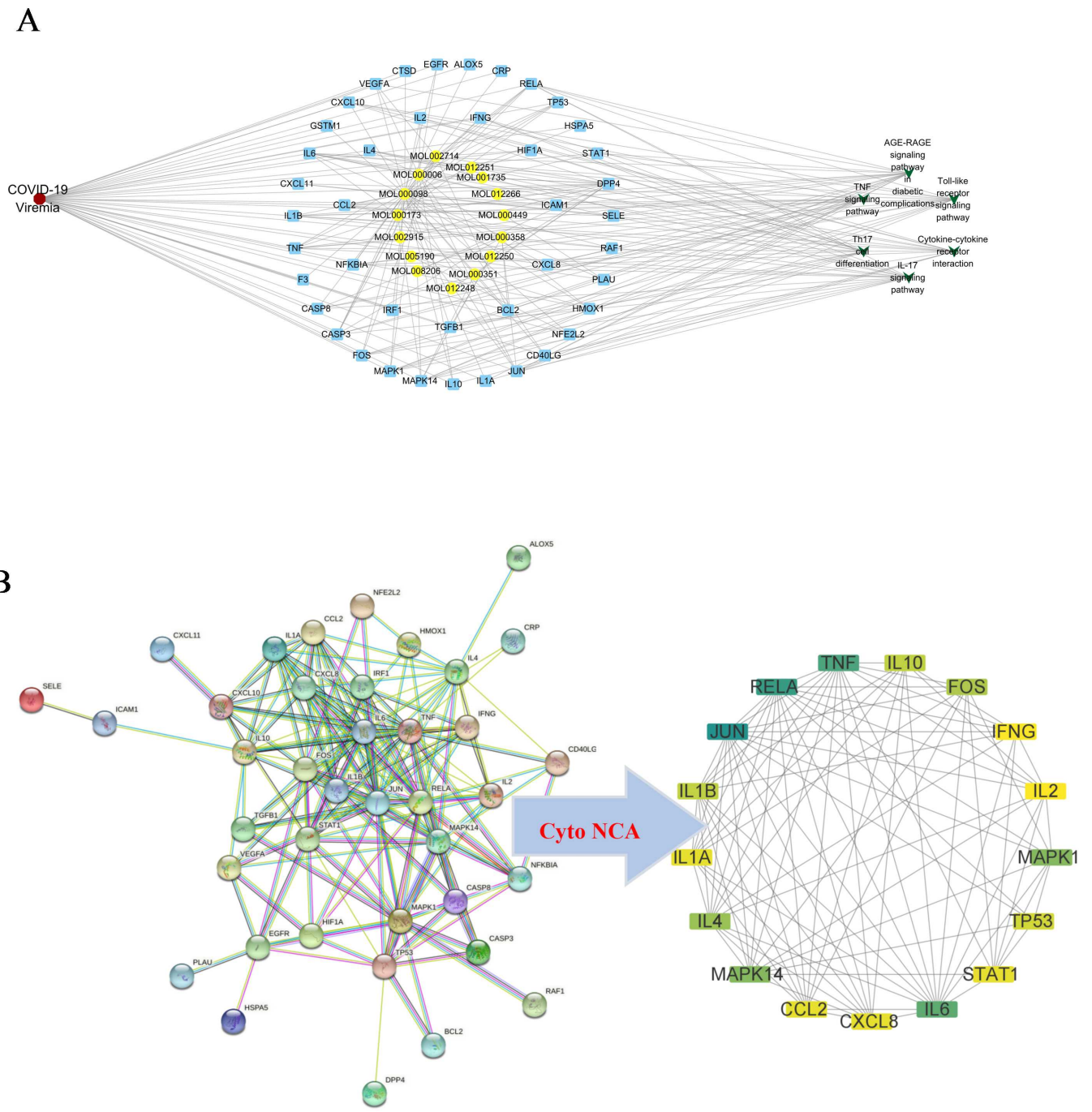


FIGURE 4. Establishment of integrative gene network and screening hub genes. A, The functional monomer components of *S. barbata*-target-COVID-19 viremia-signaling pathway network (Note: Red indicates disease; Yellow shows functional monomer components; Blue represents target genes; Green indicates the signaling pathways). B, The PPI network of anti-*S. barbata* target genes against COVID-19 viremia and subnetwork of hub genes. Notes: TP53: Cellular tumor antigen p53; GSTM1: Glutathione S-transferase Mu 1; IL1B: Interleukin-1 beta; CASP8: Caspase-8; IRF1: Interferon regulatory factor 1; EGFR: Epidermal growth factor receptor; VEGFA: Vascular endothelial growth factor A; IL6: Interleukin-6; TNF: Tumor necrosis factor; TGFB1: Transforming growth factor beta-1 proprotein; BCL2: Apoptosis regulator Bcl-2; CXCL8: Interleukin-8; HIF1A: Hypoxia-inducible factor 1-alpha; CASP3: Caspase-3; MAPK1: Mitogen-activated protein kinase 1; IL10: Interleukin-10; JUN: Transcription factor AP-1; ALOX5: Polyunsaturated fatty acid 5-lipoxygenase; CCL2: C-C motif chemokine 2; CD40LG: CD40 ligand; CRP: C-reactive protein; CTSD: Cathepsin D; CXCL10: C-X-C motif chemokine 10; CXCL11: C-X-C motif chemokine 11; DPP4: Dipeptidyl peptidase 4; F3: Tissue factor; FOS: Proto-oncogene c-Fos; HMOX1: Heme oxygenase 1; HSPA5: Endoplasmic reticulum chaperone BiP; ICAM1: Intercellular adhesion molecule 1; IFNG: Interferon gamma; IL1A: Interleukin-1 alpha; IL2: Interleukin-2; IL4: Interleukin-4; MAPK14: Mitogen-activated protein kinase 14; NFE2L2: Nuclear factor erythroid 2-related factor 2; NFKBIA: NF-kappa-B inhibitor alpha; PLAU: Urokinase-type plasminogen activator; RAF1: RAF proto-oncogene serine/threonine-protein kinase; RELA: Transcription factor p65; SELE: E-selectin; STAT1: Signal transducer and activator of transcription 1-alpha/beta.

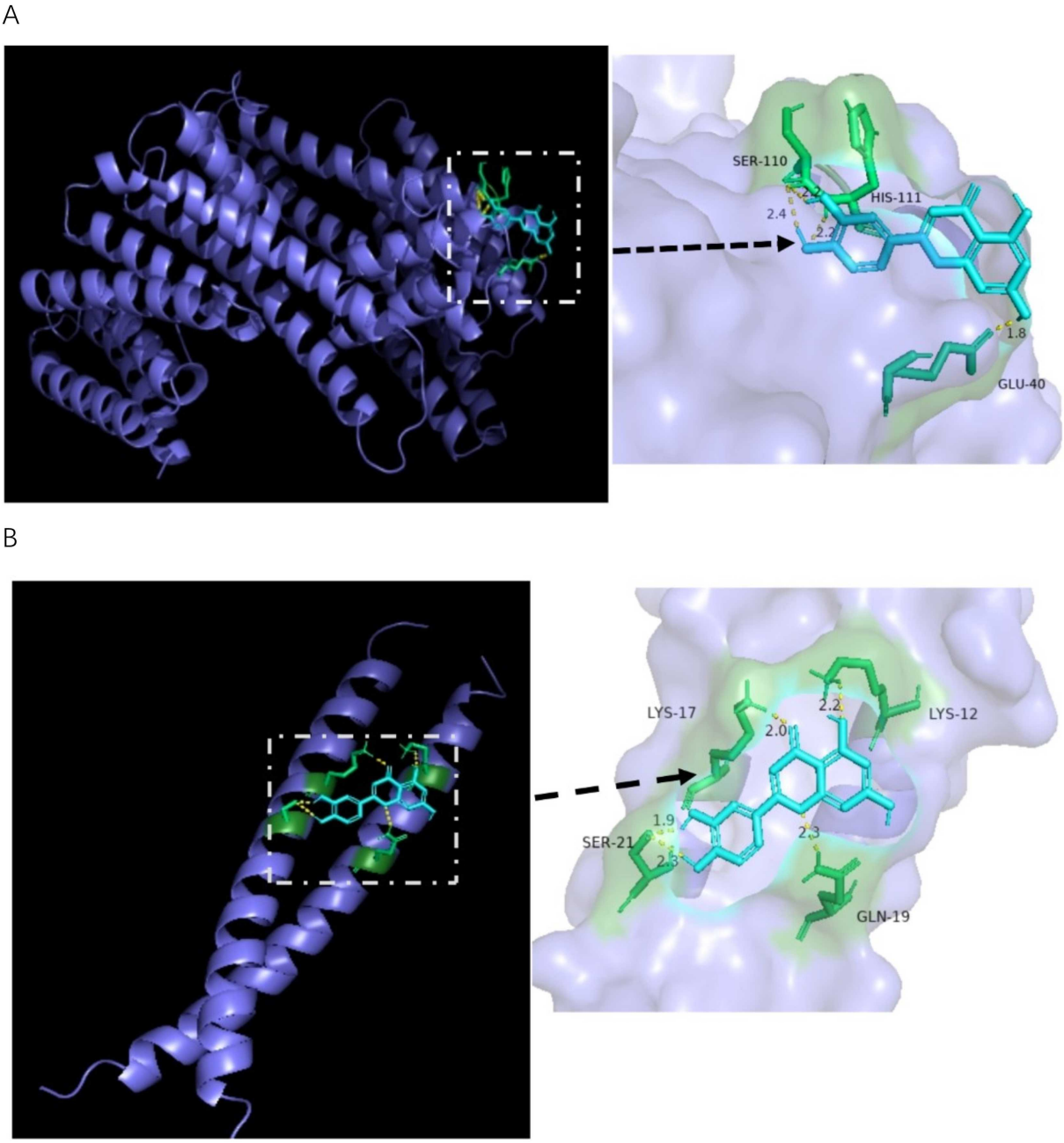


FIGURE 5. Molecular docking. A, Molecular docking between luteolin and RELA. B, Molecular docking between luteolin and JUN. Notes: SER-110/21: Serine-110/21; HIS-111: Histidine-111; GLU-40: Glutamate-40; LYS-12/17: Lysine-12/17; GLN-19: Glutamine-19.

admitted to the CCU as well as deceased patients had significant lymphopenia, neutrophilia, leucocytosis, anemia, and an elevated neutrophil-lymphocyte (N/L) ratio [36]. The inability of the body to eliminate SARS-CoV-2 from infected organs activates the immune system, which triggers the secretion of high levels of pro-inflammatory cytokines to compensate for the loss of cytokines and low lymphocyte counts, thus, leading to cytokine storm syndrome [37]. Hence, *JUN* activation could be a critical component inducing lymphocytopenia during COVID-19 viremia. *RELA* is a transcription factor encoding for the p65 protein. The Nuclear factor-κB complex comprises the Rel-like domain-containing proteins including NFκB2/p52, REL, NFκB1/p50, NFκB1/p105, RELB, and RELA/p65. The NF-κB complex may exist in either a homo- or heterodimeric state, and the heterodimeric RELA-NFKB1 is the most prevalent complex. The heterodimeric NF-κB complexes RELA-REL and RELA-NFκB1 function as transcriptional activators. Furthermore, these complexes alter the accessibility of promoters to transcriptional regulators, thereby indirectly influencing gene expression, which plays a crucial role in the secretion of cytokines by T cells [38]. This could be the underlying cause of the inflammatory storm in critically ill patients with COVID-19. Moreover, the NF-κB homodimeric RELA-RELA complex actively participates in the invasin-mediated IL-8 production and is a major transcription factor regulating IFN response during SARS-CoV-

2 infection [39]. Previous studies have shown a significant increase in p65 and p65 isoform 5 mRNA levels in patients with COVID-19 compared to healthy individuals. Furthermore, the binding ability of p65 isoform 5 and the wild-type p65 to dexamethasone and their effect on the glucocorticoid response were contrary [40]. Therefore, *RELA* influences inflammatory responses via the mechanism of promoting the synthesis and secretion of pro-inflammatory markers and inhibiting the anti-inflammatory response mediated by glucocorticoids. Mitogen-activated protein kinase (MAPKs), *i.e.*, extracellular signal-regulated kinases 1 and 2 (ERK1/2), are carbon dioxide (CO_2) sensors in humans [41]. CO_2 is a potent inhibitor of pro-inflammatory responses induced by hydrogen peroxide (H_2O_2) or the receptor binding domain (RBD) of the SARS-CoV-2 spike protein in cells. When the CO_2 levels increases in cultured endothelium cells, the MAPKs block ERK1/2 activity. In human bronchial epithelial cells, RBD and some cytokines like TNFα and IFNγ simultaneously activate ERK1/2, involved in enhancing COVID-19 severity, which is inactivated by CO_2 more efficiently compared to dexamethasone or acetylsalicylic acid [42]. Thus, MAPKs could be the most important regulatory gene. Previous studies have shown that in COVID-19, the clinical outcomes of patients with high IL-6 levels were poor. Thus IL-6 inhibition could be a therapeutic target for treating aberrant host responses in patients with COVID-19 [43].

Our results showed that *S barbata* uses multiple mechanisms like the Th17 cell differentiation, the TNF, and the IL-17 pathway for the prevention and treatment of COVID-19. The IL-17 family is a subclass of cytokines consisting of IL-17A-F that mediates chronic and acute inflammatory responses [44]. Few studies have explored the role of IL-17B-F; however, IL-17A, commonly known as IL-17, has been extensively explored for its role as a pro-inflammatory factor in autoimmune disorders. Recent studies have demonstrated that Th17 cells secrete characteristic IL-17A cytokine, which plays a critical role in host defense against external infections and increases inflammation in autoimmune disorders [45]. On the other hand, the host defense system of the nasal mucosa is where IL-17F is most prominently implicated [46]. Th17 cells are characterized by their ability to secrete interleukins like IL-21, IL-22, IL-17A, and IL-17F, which confer protection against pulmonary infection [47]. Furthermore, an increase in levels of IL-17A or IL-17F secreted by Th17 cells during cytokine storm could be the underlying cause of immunopathogenesis of acute respiratory distress syndrome and COVID-19. Thus, for effective treatment of patients with COVID-19 viremia, especially patients with cytokine storm syndrome, pro-inflammatory cytokines like IL-6 and the IL-17 signaling pathway should be targeted [48]. Fig. 3B shows that the IL-17 family transmits signals via their respective receptors and stimulates downstream pathways like the NF-κB, AP-1, MAPKs, and C/EBPs signaling pathways to enhance the production of chemokines, cytokines, and antimicrobial peptides. This indicates that *JUN* and *RELA* could be potential targets. Li *et al*. [49] showed a significant elevation in TNF-α, IL-10, and IL-6 levels in the serum of critically ill patients. Further, the ineffectiveness of NK and T lymphocytes and other cell subsets influence the development and prognosis of individuals suffering from COVID-19. A significant decrease in IL-6, TNF-α, and IL-1β levels in serum and an increase in levels of circulating B and T lymphocytes were observed in critically ill patients treated with baricitinib [50]. The Biomedicine Design at Pfizer Inc study showed that TNF could primarily mediate cytokine storms and destructive effects. Additionally, studies have shown that some drugs disrupt NF-κB the primary signaling molecule involved in the TNF signaling pathway, whereas other drugs inhibit kinases involved in the activation of the downstream pathways [51]. Thus, it is likely that NF-κB could be the core of these signaling pathways, thereby indicating that RELA could be an important target in treating COVID-19 viremia.

5. Conclusions

We integrated pharmacological network and molecular docking results to identify potential mechanisms of action of *S. barbata*. Furthermore, JUN and RELA could be promising targets for treating and preventing COVID-19 viremia.

AVAILABILITY OF DATA AND MATERIALS

As stated in the Materials and Methods section of the publication, the primary data for this study were retrieved from publicly available databases. All data supporting the conclusions of this study are accessible upon valid request from the corresponding author.

AUTHOR CONTRIBUTIONS

All authors contributed to the current work: XDS, FL—designed the study. LLL, XX—acquired the data. XBW—drafted the manuscript. FL—revised the manuscript. The article was reviewed and approved by all authors.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

This study was supported by Hainan Province Clinical Medical Center.

FUNDING

The study was funded by the Science and Technology Project of Hainan Province, Hainan Natural Science Foundation, Grant/Award Number: No.821QN0983.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Chen Q, Rahman K, Wang SJ, Zhou S, Zhang H. Scutellaria barbata: a review on chemical constituents, pharmacological activities and clinical applications. Current Pharmaceutical Design. 2020; 26: 160–175.

[2] Na LI, Ping W, Tie-Feng S, Li H, Ya-Nan HU, Hai-Tao DU, *et al*. Research progress on chemical constituents, pharmacological action and quality control of Scutellaria barbata. Zhongguo Zhong Yao Za Zhi. 2020; 45: 5117–5128.

[3] Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. The Lancet. 2020; 395: 470–473.

[4] Ciotti M, Ciccozzi M, Pieri M, Bernardini S. The COVID-19 pandemic: viral variants and vaccine efficacy. Critical Reviews in Clinical Laboratory Sciences. 2022; 59: 66–75.

[5] Burki TK. Coronavirus in China. The Lancet Respiratory Medicine. 2020; 8: 238.

[6] Araf Y, Akter F, Tang YD, Fatemi R, Parvez SA, Zheng C, *et al*. Omicron variant of SARS-CoV-2: genomics, transmissibility, and responses to current COVID-19 vaccines. Journal of Medical Virology. 2022; 94: 1825–1832.

[7] Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. The Lancet. 2020; 395: 1033–1034.

[8] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020; 395: 497–506.

[9] Shang Y, Pan C, Yang X, Zhong M, Shang X, Wu Z, *et al*. Management of critically ill patients with COVID-19 in ICU: statement from front-line intensive care experts in Wuhan, China. Annals of Intensive Care. 2020; 10: 73.

[10] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. AMA. 2020; 324: 782–793.

[11] Huang K, Zhang P, Zhang Z, Youn JY, Wang C, Zhang HC, *et al*. Traditional Chinese Medicine (TCM) in the treatment of COVID-19 and other viral infections: efficacies and mechanisms. Pharmacology & Therapeutics. 2021; 225: 107843.

[12] Ru J, Li P, Wang J, Zhou W, Li B, Huang C, *et al*. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. Journal of Cheminformatics. 2014; 6: 13.

[13] The UniProt Consortium. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Research. 2021; 49: D480–D489.

[14] Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, *et al*. The Genecards Suite: from gene data mining to disease genome sequence analyses. Current Protocols in Bioinformatics. 2016; 54: 1.30.1–1.30.33.

[15] Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic Acids Research. 2015; 43: D789–D798.

[16] Brown GR, Hem V, Katz KS, Ovetsky M, Wallin C, Ermolaeva O, *et al*. Gene: a gene-centered information resource at NCBI. Nucleic Acids Research. 2015; 43: D36–D42.

[17] Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Research. 2017; 45: D353–D361.

[18] Piñero J, Ramirez-Anguita JM, Sauch-Pitarch J, Ronzano F, Centeno E, Sanz F, *et al*. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Research. 2020; 48: D845–D855.

[19] The Gene Ontology Consortium. The gene ontology resource: 20 years and still GOing strong. Nucleic Acids Research. 2019; 47: D330–D338.

[20] Wu J, Mao X, Cai T, Luo J, Wei L. KOBAS server: a web-based platform for automated annotation and pathway identification. Nucleic Acids Research. 2006; 34: W720–W724.

[21] Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape StringApp: network analysis and visualization of proteomics data. Journal of Proteome Research. 2019; 18: 623–632.

[22] Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, *et al*. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Research. 2021; 49: D605–D612.

[23] Tang Y, Li M, Wang J, Pan Y, Wu F. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. Biosystems. 2015; 127: 67–72.

[24] Rose Y, Duarte JM, Lowe R, Segura J, Bi C, Bhikadiya C, *et al*. RCSB protein data bank: architectural advances towards integrated searching and efficient access to macromolecular structure data from the PDB archive. Journal of Molecular Biology. 2021; 433: 166704.

[25] Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, *et al*. PubChem substance and compound databases. Nucleic Acids Research. 2016; 44: D1202–D1213.

[26] O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open babel: an open chemical toolbox. Journal of Cheminformatics. 2011; 3: 33.

[27] Mooers BHM. Shortcuts for faster image creation in PyMOL. Protein Science. 2020; 29: 268–276.

[28] Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. Journal of Computer-Aided Molecular Design. 2010; 24: 417–422.

[29] Ferreira LG, Dos SR, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015; 20: 13384–13421.

[30] Hsin KY, Ghosh S, Kitano H. Combining machine learning systems and multiple docking simulation packages to improve docking prediction reliability for network pharmacology. PLoS One. 2013; 8: e0083922.

[31] Lin TJ, Yin SY, Hsiao PW, Yang NS, Wang IJ. Transcriptomic analysis reveals a controlling mechanism for NLRP3 and IL-17A in dextran sulfate sodium (DSS)-induced colitis. Scientific Reports. 2018; 8: 14927.

[32] Feng W, Zhong X, Zheng X, Liu Q, Liu M, Liu X, *et al*. Study on the effect and mechanism of quercetin in treating gout arthritis. International Immunopharmacology. 2022; 111: 109112.

[33] Ji Z, Donaldson IJ, Liu J, Hayes A, Zeef LAH, Sharrocks AD. The forkhead transcription factor FOXK2 promotes AP-1-mediated transcriptional regulation. Molecular and Cellular Biology. 2012; 32: 385–398.

[34] Atsaves V, Leventaki V, Rassidakis GZ, Claret FX. AP-1 transcription factors as regulators of immune responses in cancer. Cancers. 2019; 11: 1037.

[35] Baumann S, Hess J, Eichhorst ST, Krueger A, Angel P, Krammer PH, *et al*. An unexpected role for FosB in activation-induced cell death of T cells. Oncogene. 2003; 22: 1333–1339.

[36] Abd El-Lateef AE, Ismail MM, Thabet G, Cabrido N. Complete blood cells count abnormalities in COVID-19 patients and their prognostic significance: single center study in Makkah, Saudi Arabia. Saudi Medical Journal. 2022; 43: 572–578.

[37] Lo PE, Dieli F, Meraviglia S. Lymphopenia in COVID-19: $\gamma\delta$ T Cells-based therapeutic opportunities. Vaccines. 2021; 9: 562.

[38] Bettelli E, Dastrange M, Oukka M. Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102: 5138–5143.

[39] Yin X, Riva L, Pu Y, Martin-Sancho L, Kanamune J, Yamamoto Y, *et al*. MDA5 governs the innate immune response to SARS-CoV-2 in lung epithelial cells. Cell Reports. 2021; 34: 108628.

[40] Spinelli G, Biddeci G, Artale A, Valentino F, Tarantino G, Gallo G, *et al*. A new p65 isoform that bind the glucocorticoid hormone and is expressed in inflammation liver diseases and COVID-19. Scientific Reports. 2021; 11: 22913.

[41] Gałgańska H, Gałgański A. Mitogen-activated protein kinases are carbon dioxide receptors in plants. bioRχiv. 2020. [Preprint].

[42] Galganska H, Jarmuszkiewicz W, Galganski L. Carbon dioxide inhibits COVID-19-type proinflammatory responses through extracellular signal-regulated kinases 1 and 2, novel carbon dioxide sensors. Cellular and Molecular Life Sciences. 2021; 78: 8229–8242.

[43] Coomes EA, Haghbayan H. Interleukin-6 in Covid-19: a systematic review and meta-analysis. Reviews in Medical Virology. 2020; 30: 1–9.

[44] McGeachy MJ, Cua DJ, Gaffen SL. The IL-17 family of cytokines in health and disease. Immunity. 2019; 50: 892–906.

[45] Miossec P, Kolls JK. Targeting IL-17 and TH17 cells in chronic

inflammation. Nature Reviews Drug Discovery. 2012; 11: 763–776.
[46] Zemke AC, Robinson KM. Right on the nose: IL-17C and nasal host defense. American Journal of Respiratory Cell and Molecular Biology. 2020; 62: 10–11.
[47] Rathore JS, Wang Y. Protective role of Th17 cells in pulmonary infection. Vaccine. 2016; 34: 1504–1514.
[48] Shibabaw T. Inflammatory cytokine: IL-17A signaling pathway in patients present with COVID-19 and current treatment strategy. Journal of Inflammation Research. 2020; 13: 673–680.
[49] Li M, Guo W, Dong Y, Wang X, Dai D, Liu X, *et al*. Elevated exhaustion levels of NK and CD8(+) T cells as indicators for progression and prognosis of COVID-19 disease. Frontiers in Immunology. 2020; 11: 580237.
[50] Bronte V, Ugel S, Tinazzi E, Vella A, De Sanctis F, Canè S, *et al*. Baricitinib restrains the immune dysregulation in patients with severe COVID-19. The Journal of Clinical Investigation. 2020; 130: 6409–6416.
[51] Ablamunits V, Lepsy C. Blocking TNF signaling may save lives in COVID-19 infection. Molecular Biology Reports. 2022; 49: 2303–2309.

How to cite this article: Xiaoding Song , Xi Xiao, Lingli Liu, Xiaobin Wei, Feng Li. Network pharmacology and molecular docking analyses on Scutellaria barbata indicate that JUN and RELA are potential targets to treat and prevent COVID-19 viremia. Signa Vitae. 2023; 19(5): 132-143. doi: 10.22514/sv.2023.083.