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ARTICLE

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In silico approach to explore the mechanism of bioactive ingredients from *Belamcandae rhizoma* against COVID-19

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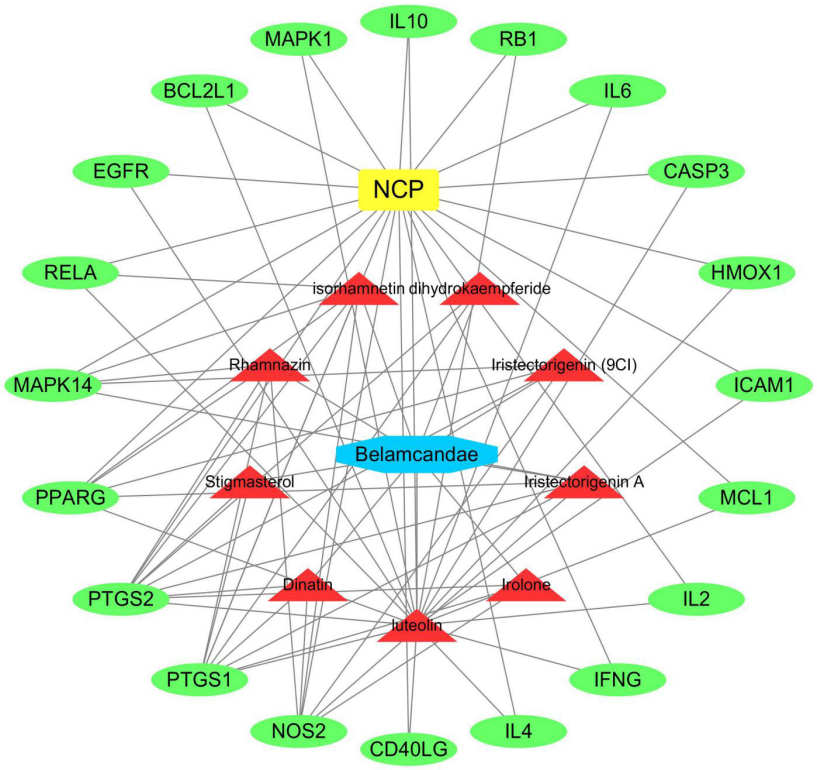
Highlights

Shegan (*Belamcandae rhizoma*), which documented in various Chinese traditional medicine prescription, are broadly applied as antipyretic agents, antiphlogistic analgesics, antidotes have been found to be effective in healing respiratory diseases. Flavonoids are the main active components in *Belamcandae rhizoma*, of which luteolin and dinatin might paly significant roles in anti-coronavirus treatment. *Belamcandae rhizoma* has anti-antiviral effects be bound up with anti-inflammatory. It is noteworthy that IL-17 signaling pathways might be potential mechanism against SARS-CoV-2.



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Abstract

Background: To date, the epidemic of COVID-19, which result from newly discovered virus named SARS-CoV-2, has spawned a heavy economic loss and continued to claim tens of thousands of lives. It's urgent now that develop an ideal agent for suppressing the lifecycle of coronavirus while alleviate collateral severe inflammatory response. *Belamcandae rhizoma*, an herb which documented in various Chinese traditional medicine prescription, was reported to have effects of antiviral, anti-inflammatory and antipyretic. However, the relationship and related molecular mechanism between *Belamcandae rhizoma* and coronavirus are still unknown. **Methods:** Here, we achieved 14 kernel compounds from *Belamcandae rhizoma* and 261 validated "novel coronavirus pneumonia" correlative gene targets using a series of databases. **Results:** Subsequently, the pharmacology network and protein-protein interaction cluster, which constructed by the 20 overlapped genes targets between *Belamcandae rhizoma* and COVID-19, demonstrated that IL10, PTGS2, IL6, MAPK1, MAPK14 and CASP3 were as key targets in the treatment. The potential molecular mechanism involved IL-17, JAK-STAT and MAPK signaling pathways gained from gene enrichment analysis attract our attention. Molecular docking with 3CLpro of SARS-CoV-2 further suggested that luteolin and dinatin were the most bioactive compounds of *Belamcandae rhizoma*, among which luteolin connected with the maximum number of gene targets in above pharmacology network. **Conclusion:** Taken together, these findings provide deep insight into the putative therapeutic targets and underlying mechanism for COVID-19 treatment.

Key words: *Belamcandae rhizoma*, COVID-19, Network pharmacology, Mechanism, Molecular docking

Author contributions:

Zi-Wei Hu contributed to write original draft, review & editing; Xun Song contributed to project administration, write review & edite, fund acquisition; Zhen-Dan He contributed to project administration, supervision, write review & edite, fund acquisition; Jin-Hong Lin contributed to data analysis; Xiao-Jian Li contributed to graphics processing.

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***Abbreviations*:**

COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; PPI, protein-protein interaction; 3CLpro, 3-chymotrypsin-like protease; Mpro, main protease; TCMSP, Traditional Chinese Medicine Pharmacology Database and Analysis Platform; CNKI, the databases of the China National Knowledge Infrastructure; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ADME, absorption, distribution, metabolism, excretion; NCP, novel coronavirus pneumonia.

***Competing interests*:**

The authors declare that they have no conflict of interest.

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Background

Coronavirus disease 2019 (COVID-19), a newly emerging of coronavirus disease, which was formerly known as 2019-nCoV has subsequently developed a global pandemic affected 211 countries with over 10.32 million confirmed cases and half a million deaths due to direct droplet and airborne transmission [1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has now been identified as the highly causative pathogens of COVID-19, ranging from asymptomatic to severe pneumonia resulting in death, being associated with attack the upper respiratory tract. It's now urgently necessary to exploit specific antiviral strategies toward SARS-CoV-2, as there is no approved specific drug to treat the disease at the present.

Medicinal plants can be as valuable sources of new drugs exerted on the remission of severe symptoms which arise from SARS-CoV-2 [2]. *Belamcanda chinensis* (L.) DC. (also known as Shegan or Jiaojiancao in Chinese), is a perennial herb with a pale-brown rhizoma belonging to the genus *Belamcanda* Adans. (Iridaceae), widely distributes throughout the Northeast China [3]. Shegan (*Belamcandae rhizoma*) have been extensively documented in various Chinese folk formula, including Shegan Mahuang decoction, Huangqi Shegan decoction and Qingfeipaidu decoction [4–6]. The rhizomes of *Belamcanda chinensis* (L.) DC. were broadly applied as antipyretic agents, expectorants, antiphlo gistics analgesics, antidotes and have been found to be effective in healing respiratory diseases [7]. The compound of *Belamcandae rhizoma* was reported to have antiviral effects against herpes simplex virus, HIV and influenza virus in vitro [8–10]. However, the bioactivities against coronavirus of *Belamcandae rhizoma* have not been studied previously, and the underlying molecular mechanisms of its antiviral activity remain largely unknown.

Network pharmacology is a comprehensive strategy combines bioinformatics with traditional pharmacology, which provides a promising approach to uncover the bioactive compounds and latent mechanisms of traditional Chinese medicine formulas from a systemic and holistic perspective based on the "one gene, one drug, one disease" paradigm [11, 12]. Through emphasizing the relationships between nodes and edges, we can directly develop an understanding of drugs and disease targets from massive data and uncover in-depth sophisticated mechanisms and pathways among them.

To lay a foundation for the further research in vitro and in vivo, we aim to conduct a bioinformatics study on preliminary screening the bioactive ingredients of *Belamcandae rhizoma* that may alleviate COVID-19 symptoms via targeting the virus structure and also to find their network and molecular mechanisms. Two main virtual ways will be adopted to study the antiviral effect of *Belamcandae rhizoma*, namely, the network pharmacology and the molecular docking. An integratedly analytical platform based on network pharmacology was built, including target prediction, protein-protein interaction (PPI) network, topology analysis and gene enrichment analysis. Molecular docking is an advanced approach in drug virtual screening. Currently, X-ray crystal structural analyses for the potential targets of SARS-CoV-2 were carried out by multiple research teams [17, 18]. In this study, we will focus on the conserved 3-chymotrypsin-like protease (3CLpro), which also known as main protease (Mpro), mediates proteolytic processing with papain-like protease [19]. The 3CLpro cleaves the poly-protein at 11 distinct sites to generate various non-structural proteins which are vital for coronavirus transcription and replication. It exhibits excessive variability where located the 3' end and unlike structural/accessory protein-encoding genes. Therefore, it is a potential target for anti-coronaviruses inhibitors screening. We have selected 14 drugs, as well as the references remdesivir and ribavirin to investigate their binding interactions with 3CLpro, and to evaluate their potential against coronavirus pneumonia (SARS-CoV-2) infection by means of computational methods using 2 docking tools, Discovery studio and AutodockVina.

Methods and materials

Data preparation and preprocessing

Bioactive compounds of *Belamcandae rhizoma*. Data on the herb relative information in *Belamcandae rhizoma* were primarily acquired from the Traditional Chinese Medicine Pharmacology Database and Analysis Platform (TCMSP, https://tcmspw.com/tcmsp.php, updated on May 31, 2014). To take a supplement of any other omitted ingredients, the databases of the China National Knowledge Infrastructure, SciFinder, and PubMed also serve as powerful engines for efficient searching. Subsequently, we used an in silico integrative model absorption, distribution, metabolism, excretion (ADME) for the next screening, among which the four key ADME-related properties taken together evaluate that chemical components might be potential drugs, namely Caco-2 permeability, oral bioavailability, drug-likeness, and half-life [20–23]. The threshold values indicating effectiveness for these 4 indices were oral bioavailability > 30%, Caco-2 > −0.4, drug-likeness > 0.18 and half-life > 3 h, respectively, as recommended by Hu et al [24]. The values of the 4 indices can be obtained from the TCMSP database. In total, 14 compounds from *Belamcandae rhizoma* were collected (Table 1).

Related targets of COVID-19. Data on

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Table 1 Basic information of 14 bioactive ingredients screened by ADEM-model

Mol ID	Molecule name	Bioavailability (%)	Caco-2	Drug likeness	Half-life
MOL001735	Dinatin	30.97	0.48	0.27	16.44
MOL000449	Stigmasterol	43.83	1.44	0.76	5.57
MOL000351	Rhamnazin	47.14	0.53	0.34	13.54
MOL000354	Isorhamnetin	49.60	0.31	0.31	14.34
MOL003741	Anhydrobelachinal	43.57	0.81	0.78	4.83
MOL003743	Belachinal	31.24	0.09	0.64	5.30
MOL003744	Belamcandal	30.07	0.05	0.67	4.84
MOL003753	Dihydrokaempferide	50.56	0.08	0.27	14.74
MOL003754	Epianhydrobelachinal	43.57	0.84	0.78	5.00
MOL003758	Iristectorigenin	71.55	0.55	0.34	16.32
MOL003759	Iristectorigenin A	63.36	0.54	0.34	16.82
MOL003769	Irolone	46.87	0.57	0.36	19.59
MOL003773	Mangiferolic acid	36.16	0.66	0.84	5.71
MOL000006	Luteolin	36.16	0.19	0.25	15.94

ADEM, absorption, distribution, metabolism, excretion.

COVID-19-related targets were obtained from 2 databases with the following query "Novel Coronavirus Pneumonia", "Severe Acute Respiratory Syndrome": (1) we gained 325 relative targets from the Online Mendelian Inheritance in Man (http://omim.org/, updated on Jan 3, 2018), a seminal medical genetics resource platform provides compendious information for which over 15,500 genes, 26,200 allelic variants, and 7,800 genetic phenotypes [25]; (2) we obtained 265 relevant targets from the GeneCards Database (https://www.genecards.org/, updated on Sep 1, 2014), which closely interrelates with suite knowledgebases and automatically integrates gene-centric information from approximately 125 sources, such as genomic, transcriptomic, clinical and functional [26].

Prediction for gentic targets of *Belamcandae rhizoma*. All the active ingredients were imported into DrugBank database (http://www.drugbank.ca/), which was designed to serve as a fully searchable, comprehensive drug resource that connected sequence, structure and mechanistic data about drug molecules with corresponding drug targets [27]. Since obtained homo species of biological targets, all target names were corrected into official symbols via UniProt websites (http://www.uniprot.org/).

Integration of all targets. Count the intersection between target-disease and drug-target, and achieve the Venn graph. All process were conducted by R languages.

Pharmacology network construction and GO/KEGG enrichment analysis

The obtained intersection targets were uploaded into the STRING online server (http://string-db.org/) to retrieve the PPI network diagram, with the research species was selected as homo sapiens. Then, the parameters analysis of PPI file can be implemented with the CytoNCA plugin in software Cytoscape (version 3.7.2), for instance, degree is to evaluate the importance of medicinal ingredients and targets. The network visualization processing was conducted by Cytoscape 3.7.2 with the data of "node1, node2 and combine score". Additionally, the suite tool network analyzer was used to calculate topological parameters.

To further explore the screened core genes, as well as their relative signaling pathways, gene ontology (GO) analysis with the biological process, cellular component, and molecular function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were carried out by statistical R language. All data source of GO/KEGG enrichment originated from the website (http://bioconductor.org/biocLite.R).

Molecular docking

We performed the docking simulation with the software AutodockVina to investigate interaction of protein and ligands, according to the following procedure. (1) Preparation of template structure of SARS-CoV-2 3CLpro. Downloaded the crystal structure of 3CLpro in complex with an inhibitor O6K (PDB ID: 6Y2G) used in the docking analysis from the Protein Databank (http://www.rcsb.org) [18]. The 3CLpro was processed by removing existing ligands and water molecules while missing polar hydrogen were added utilizing AutoDockTool software. Thereafter, the model saved into a dockable PDBQT format for the follow-up virtual work. (2) Docking ligands preparation. The structure data file format of small molecules and positive inhibitors (remdesivir and ribavirin) were achieved from the PubChem website (https://pubchem.ncbi.nlm.nih.gov) and subsequently converted to PDB format by Discovery Studio. Using program optimized all above small ligands and converted files into PDBQT chemical format. (3) Set docking grid box. Imported PDBQT file of 3CLpro into the software to build up a docking grid box parameter file based on the re-docking results

of original protein and self-existent inhibitor OK6. (4) Molecular docking was carried out by AutodockVina and the final results were analyzed by Pymol and Discovery Studio.

Results

Identification of druggable targets in COVID-19

Based on databases and a set of biomedical literature, we conducted ADME-related model screening for the 40 bioactive ingredients, accepting 14 as the candidates (Table 1). In this study, a total of 229 known gene targets for *Belamcandae rhizomas*' active compounds were collected from DrugBank database, and there are 5 compounds without corresponding gene targets. Above genes were transformed into official annotations using R languages based on UniProt database, which avoid confusion across databases and platforms (Table S1). Virus infection also participate in the polygenic process. Research on gene and environment interaction is beneficial to uncover the pathogenesis of COVID-19. Therefore, we have found 259 and 2 genes acting on novel coronavirus pneumonia from GeneCards and the Online Mendelian Inheritance in Man database, respectively. After filtration of the partially redundant targets, totaling 261 were selected as the potential targets of *Belamcandae rhizoma* for the following analysis, and 20 targets were shown in intersection of *Belamcandae rhizoma* and novel coronavirus pneumonia, which was visualized in Venn graph (Figure 1).

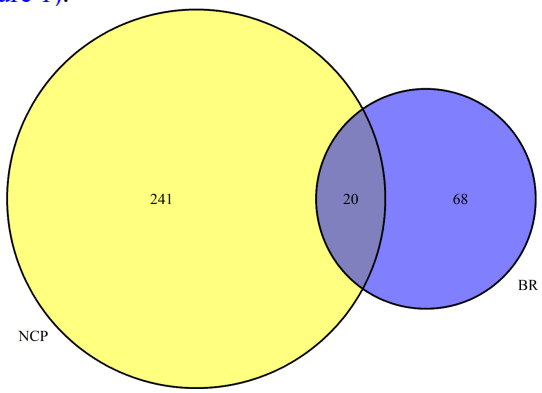

Figure 1 Venn graph showing the numbers of 20 overlapped target genes among BR and NCP. BR, *Belamcandae rhizome;* NCP, novel coronavirus pneumonia.

Protein-protein interaction network and topological analysis

The protein-protein interaction (PPI) network was established using STRING with medium confidence (0.4 by default) (Figure 2). The PPI network contains 19 nodes and 143 edges altogether, of which the line color presents the types of interaction evidence and the thickness indicates strength of data support. The CytoNCA, a plugin of Cytoscape, is used to calculate the interaction between the 20 genes. The results of PPI topological analysis are listed in Table 2. *IL-10*, *PTGS2*, *IL-6*, *MAPK1*, *MAPK14* were the top 6 genes with degree 18 (Table 2). These higher-degree compounds are the potential key targets for the therapeutic effect of COVID-19. The scores of other parameters are also listed in Table 1.

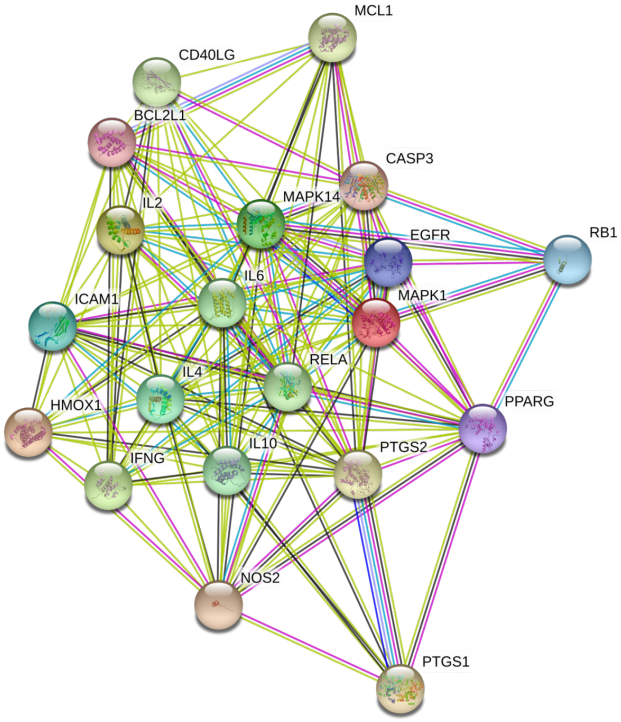

Figure 2 Visualization of the PPI of the 20 target genes using STRING database. PPI, protein-protein interaction.

Integrative network analysis and target selection

To elucidate the molecular mechanism underlying the effects of *Belamcandae rhizoma* against COVID-19, we performed an ingredient-target-disease pharmacology network with Cytoscape 3.7.2 (Figure 3). An intricate network was formed among *Belamcandae rhizoma* and their potential targets regarding COVID-19. Among the 14 ingredients of *Belamcandae rhizoma*, 5 compounds have no corresponding targets, and only 9 compounds were active. The correlation between the 9 active compounds of *Belamcandae rhizoma*, 20 intersection targets and disease are shown in Figure 3. The round rectangle nodes and octagon nodes represent disease and *Belamcandae rhizoma*, respectively. The ellipse nodes represent 20 common gene targets, while the triangle nodes are bioactive ingredients. Using the network analyzer plugin to analyze the network topology parameters, the average number of neighbors is 4.968, the network heterogeneity is 0.907, the network density is 0.166, and the network center network centralization is 0.536. The compounds of *Belamcandae rhizoma* act on multiple targets and disease, reflecting the coordinated and interacted

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Table 2 Topological analysis of the PPI network of the 20 genes shared by *Belamcandae rhizoma* and NCP (data were ranked by degree).

#	GeneSymbol	Degree	Subgragh	Eigenvector	Information	LAC	Betweenness	Closeness	Network
1	IL10	18	410105.56	0.26	8.53	13.89	7.79	0.95	17.68
2	PTGS2	18	410105.47	0.26	8.53	13.89	7.79	0.95	17.68
3	IL6	18	410104.88	0.26	8.53	13.89	7.79	0.95	17.68
4	MAPK1	18	407104.94	0.26	8.53	13.78	8.95	0.95	17.61
5	MAPK14	18	407104.94	0.26	8.53	13.78	8.95	0.95	17.61
6	CASP3	18	407104.94	0.26	8.53	13.78	8.95	0.95	17.61
7	IL4	17	378108.50	0.25	8.35	13.53	5.83	0.90	16.31
8	IFNG	16	361331.20	0.24	8.15	13.75	1.69	0.86	15.35
9	ICAM1	16	361331.20	0.24	8.15	13.75	1.69	0.86	15.35
10	RELA	16	355707.40	0.24	8.15	13.50	2.23	0.86	15.22
11	IL2	16	353954.97	0.24	8.15	13.50	2.22	0.86	15.35
12	BCL2L1	15	317854.75	0.23	7.93	12.93	1.45	0.83	14.40
13	EGFR	15	298801.22	0.22	7.93	11.87	5.42	0.83	13.69
14	PPARG	15	277360.38	0.21	7.93	11.20	9.02	0.83	13.54
15	NOS2	14	268463.34	0.21	7.70	11.57	3.04	0.79	13.15
16	HMOX1	13	252378.11	0.20	7.46	11.54	0.49	0.76	12.50
17	CD40LG	13	250781.48	0.20	7.46	11.54	0.55	0.76	12.65
18	MCL1	11	180094.95	0.17	6.90	9.82	0.15	0.70	10.80
19	PTGS1	6	52490.57	0.09	5.04	5.00	0.00	0.59	6.00
20	RB1	5	36456.65	0.08	4.55	4.00	0.00	0.58	5.00

PPI, protein-protein interaction; NCP, novel coronavirus pneumonia.

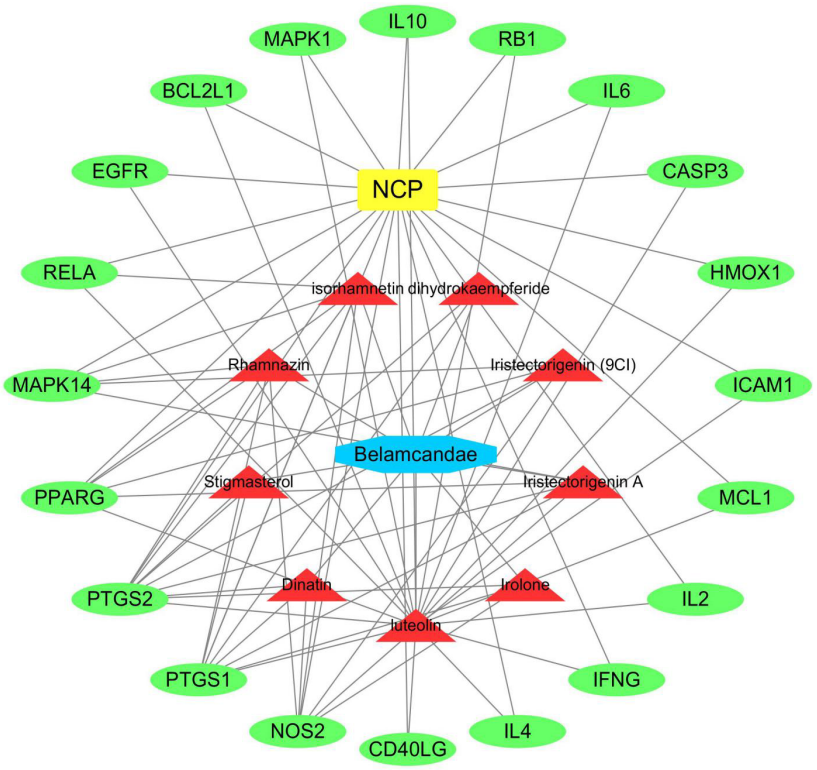


Figure 3 Comprehensive representation of the built network of *Belamcandae rhizoma*, NCP, and gene targets. The round rectangle (yellow) represents disease, the octagon (blue) represents medicinal plant, triangles nodes (red) represent bioactive ingredients, and the ellipse nodes (green) represent common gene targets, respectively. NCP, novel coronavirus pneumonia.

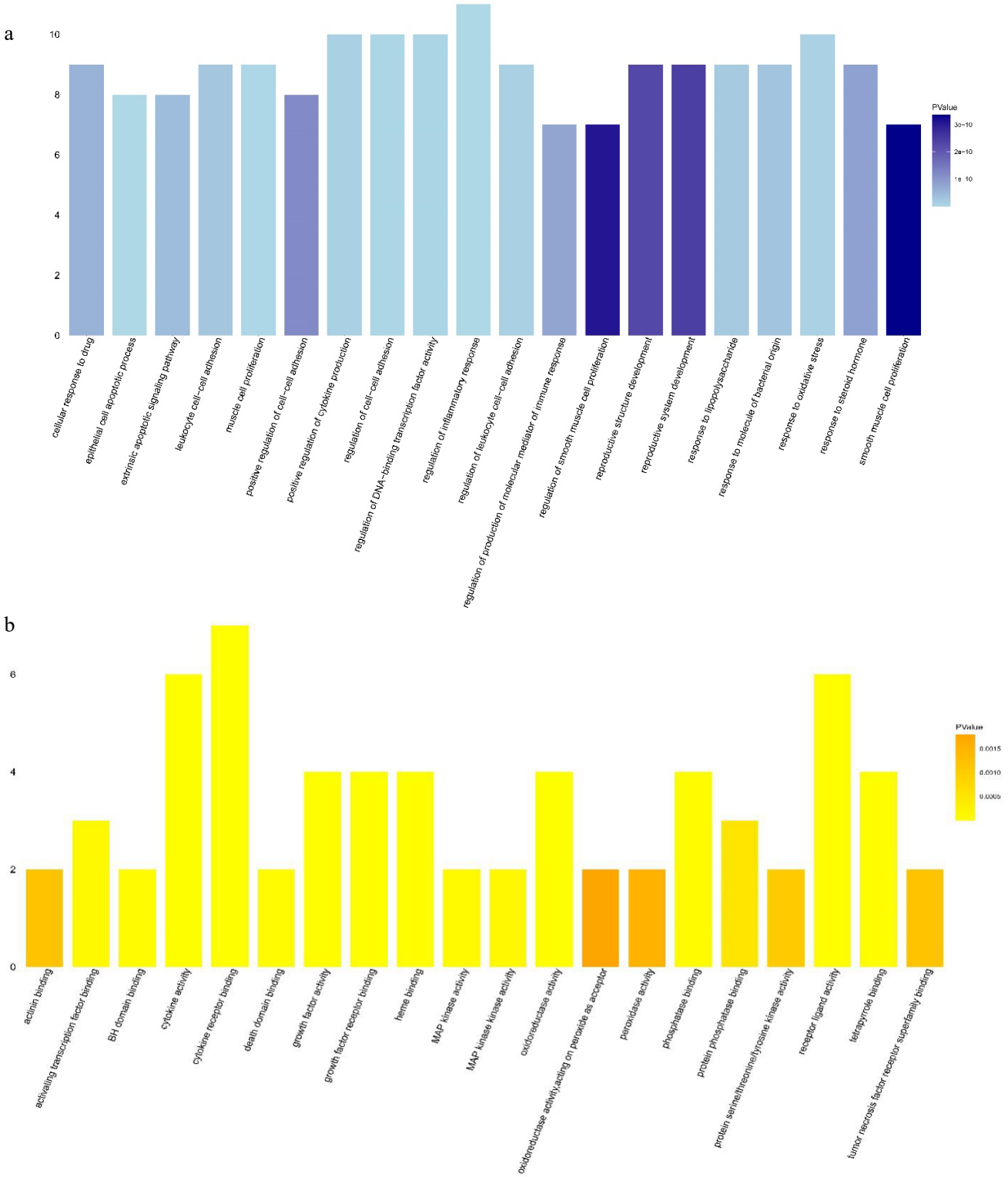
characteristics of pharmacology network.

GO functional enrichment and KEGG pathways analysis

The list of the 20 screened genes was evaluated by R language for GO and KEGG enrichment analysis. Three terms, namely biological process, cellular component and molecular function, are displayed in

Figure 4. The 20 targets were involved in many biological processes including "epithelial cell apoptotic process", "regulation of inflammatory response", "muscle cell proliferation", "regulation of DNA-binding transcription factor activity" and "regulation of cell-cell adhesion" (Figure 4a). "Cytokine receptor binding", "receptor ligand activity" and "cytokine activity" ranked the highest in the molecular function category (Figure 4b), while "membrane raft", "membrane microdomain", and "membrane region" were the primary cellular component involved (Figure 4c).

To obtain deeply insights into the pharmacological mechanisms of *Belamcandae rhizoma* on COVID-19, we also performed KEGG pathways analysis on the targets, and identified the top 20 pathways of significance (Figure 5). Moreover, the IL-17, T cell receptor, JAK-STAT and C-type lectin receptor signaling pathways were obviously enriched. There were 9 out of the 20 screened genes present in the



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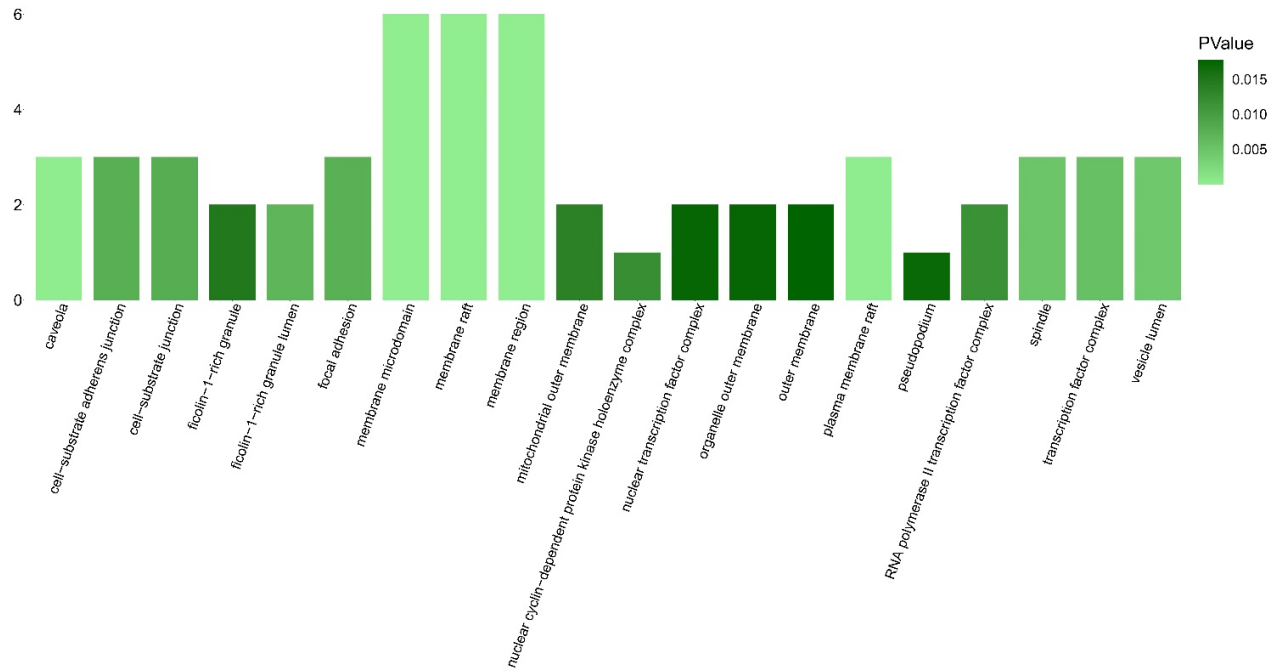

Figure 4 GO analysis of target-disease gene interactions for *Belamcandae rhizoma* to reveal correlative (a) biological process, (b) molecular function and (c) cellular component. Numbers of genes included are marked next to the horizontal bar graph. GO, gene ontology.

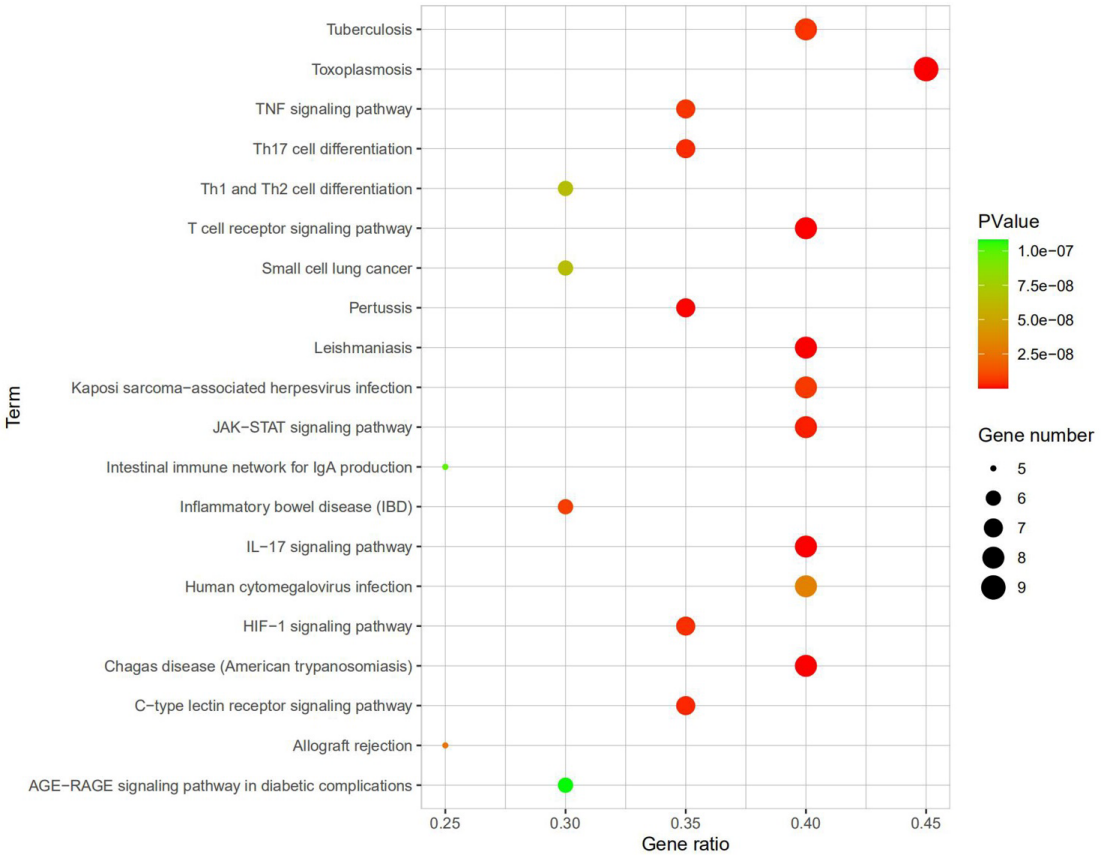

Figure 5 The signaling pathways that include the 20 gene targets analyzed using R languages with bioconductor database. The results of bubble graph demonstrating that "IL-17 signaling pathway" and "JAK-STAT siganling pathway" are 2 compelling signaling pathways regarding *Belamcandae rhizoma*, coronavirus disease and inflammatory response.

IL-17 signaling pathway. It showed that the IL-17 pathway involved several downstream pathways, including NF-κB, MAPKs and Casp signaling pathways (Figure 6). More detailed information of *Belamcandae rhizoma* interrelated genes are highlighted in Figure 6.

Docking between selected compounds and their reported targets

To perform the docking analysis, the 3D structure files of SARS-CoV-2 3CLpro were built based on the corresponding templates, PDB 6Y2G. The best energy ranked result of the binding mode between each component and 3CLpro of SARS-CoV-2 was shown in Table 3, while the stereo view of the top ranked luteotin and dinatin with the amino acids of 3CLpro were represented in Figure 6a and 6b, respectively. From our docking results, dinatin enters into active cavity via 6 conventional hydrogen bonds to Cys44, Gly143, Cys145, Ser144 and Leu141. It further interacted with Thr25 by a pi-sigma bond, while bind with Met49 via a pi-sulfur bond (Figure 7a). Luteolin with a considerable higher binding energy (−7.6kcal/mol), which was closer to the docking result of remdesivir (−7.7kcal/mol), also showed significant binding to Cys145, Gly143 and Cys44 (Figure 7b). We speculate that these kinds of three residues are very important for the binding of flavones to the target proteases.

Discussion

As an emerging human viral epidemic, COVID-19 epidemics have raised global vigilance with the threat that the coronavirus mutation led up to human-to-human spread can be explosive in growth and cause significant impact on the health care and economy of affected areas. It was reported that common antiviral drugs like remdesivir, ribavirin and lopinavir-ritonavir (kaletra) applied in clinical therapy but the therapeutic effects of some drugs remains controversial [28–30]. Thus, the global concern enhances the development for highly effective antiviral agents.

Herbal remedies play critical roles in health care and are capable of acting therapeutically in various viral infections have increasingly drawn attention about the prospect of phyto-antiviral agents. Since the beginning of the outbreak in December 2019, there is an upsurge in the use of herbal preparations and the active components isolated from medicinal plants in China. Up to now, variety of herbal medicine are administered to alleviate COVID-19 related symptoms, especially severe pneumonia and fever. The herb *Belamcandae rhizoma*, where it is used as a traditional Chinese medicine to treat respiratory infection and lung

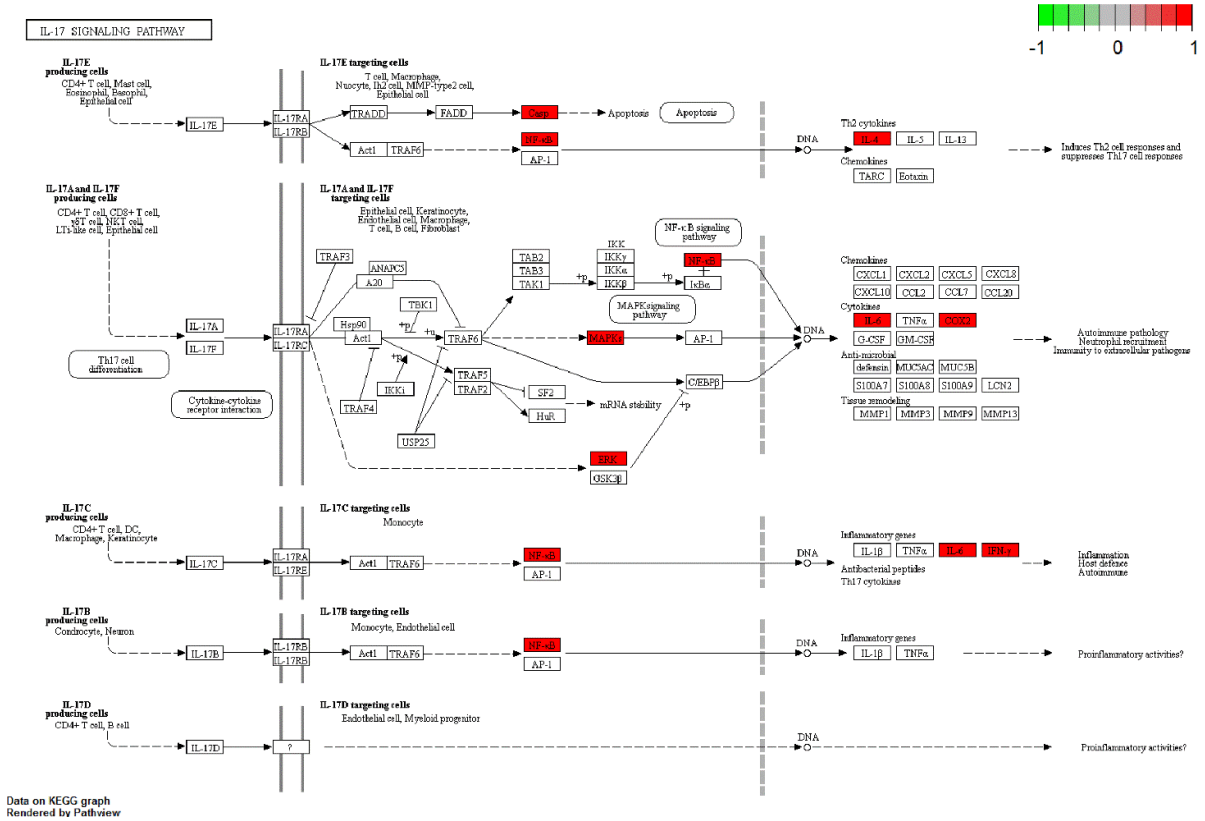

Figure 6 Detailed analysis of the 9 out of the 20 screened genes in "IL-17 signaling pathway (hsa04657)". The genes related to *Belamcandae rhizoma* were highlighted, and the pathway involved the following downstream pathways, including NF-κB, MAPKs and Casp signaling pathways. Color: red, the most related to *Belamcandae rhizoma* compounds targets.

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Table 3 Binding affinities of positive references (remdesivir and ribavirin) and screened bioactive phytochemicals from *Belamcandae rhizoma* to the 3CL^{pro} of coronaviruses.

PubMed ID	Compound	Structure	Binding energy (kcal/mol)
5281628	Dinatin	$C_{16}H_{12}O_6$	−7.5
5280794	Stigmasterol	$C_{29}H_{48}O$	−7.2
5320945	Rhamnazin	$C_{17}H_{14}O_7$	−7.3
5281654	Isorhamnetin	$C_{16}H_{12}O_7$	−7.3
10742927	Anhydrobelachinal	$C_{30}H_{44}O_4$	−7.4
10838654	Belachinal	$C_{30}H_{46}O_5$	−6.8
101615675	Belamcandal	$C_{32}H_{48}O_6$	−6.5
586387	Dihydrokaempferide	$C_{16}H_{14}O_6$	−6.5
10790466	Epianhydrobelachinal	$C_{30}H_{44}O_4$	−7.3
5488781	Iristectorigenin (9 CI)	$C_{17}H_{14}O_7$	−6.8
44257353	Iristectorigenin A	$C_{23}H_{24}O_{12}$	−7.4
5281779	Irolone	$C_{16}H_{10}O_6$	−7.4
45270099	Mangiferolic acid	$C_{30}H_{48}O_3$	−7.4
5280445	luteolin	$C_{15}H_{10}O_6$	−7.6
121304016	Remdesivir	$C_{27}H_{35}N_6O_8P$	−7.7
37542	Ribavirin	$C_8H_{12}N_4O_5$	−6.4

3CL^{pro}, 3-chymotrypsin-like protease.

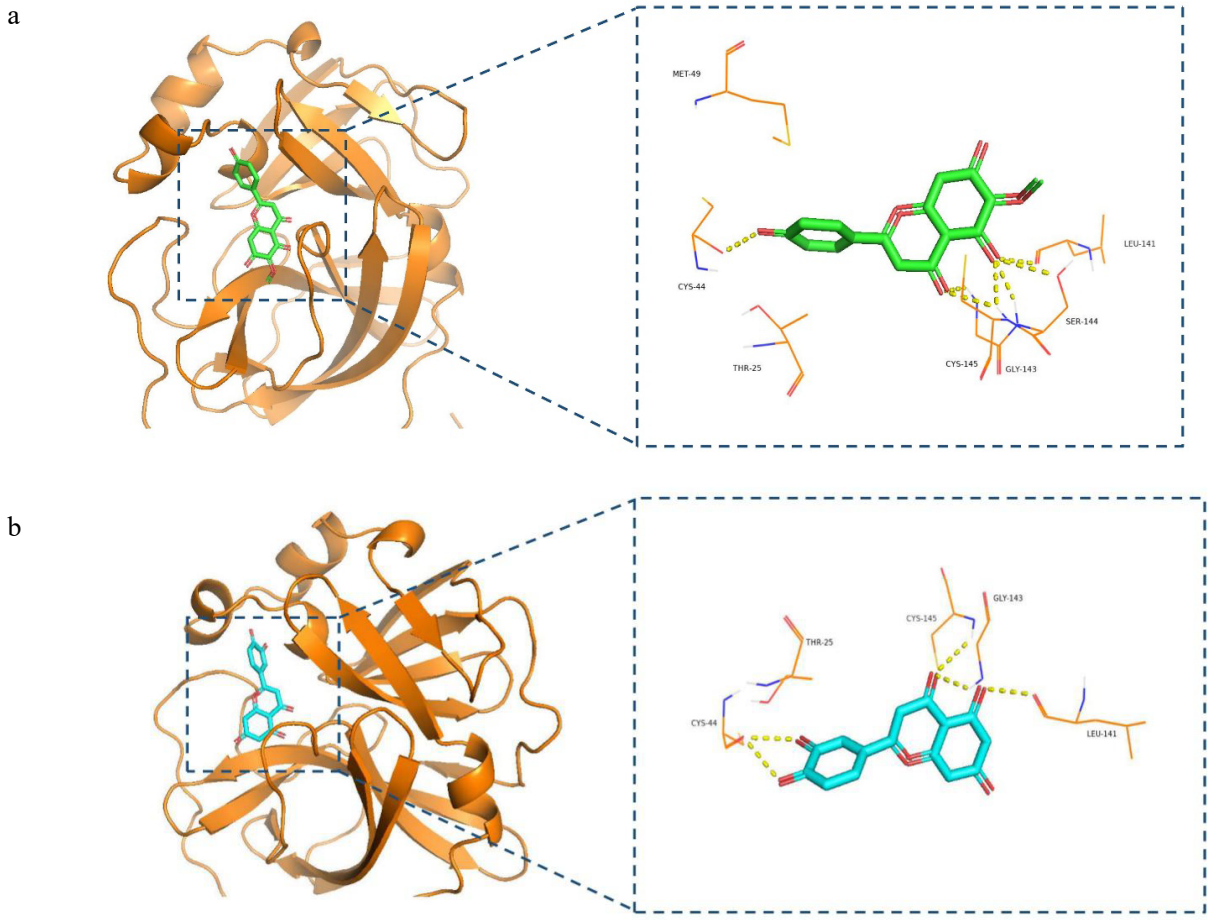

Figure 7 The stereo view of the top ranked 2 small molecules with the amino acids of 3CLpro (PDB ID: 6Y2G). (a) Dinatin is shown with the binding pocket residues and interacting residues with 3CLpro; (b) view of luteolin inside binding pocket.

diseases, is a cold and bitter material possess efficacy of heat-clearing and detoxification [7, 31]. It is well known that the dried rhizomes of *Belamcanda chinensis* (L.) DC., which harvest in early spring or autumn after cultivation for 2–3 years, are commonly using medicinal parts [3]. Large number of ancient

Chinese pharmacopoeias have suggested that *Belamcandae rhizoma* use in conjunction with other herbs, for instance, Jiegeng (*Platycodonis radix*), Mahuang (*Ephedrae herba*), Shengma (*Cimicifugae rhizoma*) and others. There is no major side effects of *Belamcandae rhizoma* have been reported yet, and the herb would be good drug candidate.

In the past several decades, more than 100 compounds have been isolated from *Belamcandae rhizoma*, including flavonoids, terpenoids, benzoquinonesands and others [32–34]. Flavonoids are the main active components in the *Belamcandae rhizoma*, of which isoflavonoids have been one of the most extensively studied chemical constituents. They are by far the largest class of polyphenols with multitude structures and also broad spectrum of pharmacological properties, such as antibacterial, anti-inflammatory, antioxidant and anticancer effects [35–38]. In spite of many bioactive ingredients of *Belamcandae rhizoma* were performed comparatively deep research, there are pharmacological mechanisms still remain to be elucidated comprehensively and systematically, and virtual screening approaches provide powerful tools to facilitate this process. Therefore, it is necessary to establish useful programs and value assessment of *Belamcandae rhizoma* in further studies.

In this study, we adopted network pharmacology to further explore the mechanisms of *Belamcandae rhizoma* on COVID-19 pneumonia, among which 261 potential targets, 20 biological processes, 20 molecular functions and 20 KEGG pathways were obtained. There are 4 key signaling pathways we retrieved were considered as the key sections of the target herbal drugs on COVID-19 treatment from gene level, namely IL-17 signaling pathway, T cell receptor signaling pathway and JAK-STAT signaling pathway. Above signaling pathways each enriched in total of 8 targets for COVID-19, as well as above *P*-values are far below 0.05. The IL-17 signaling pathways activates downstream pathways that involve NF-κB, MAPKs and C/EBP to up-regulate the expression of pro-inflammatory genes, cytokines and chemokines [39, 40]. The first COVID-19 pathology found bilateral diffuse alveolar injury with cytomyxoid fibroma exudate, and subsequent peripheral flow cytometry analysis found that the increasing of Th17 and high cytotoxicity of CD8 T cells resulting in overactivation of T cells is responsible for part of patient manifested by the immune injury [41]. Under the control of a set of cytokines with mainly IL-6 and IL-23, the naive CD^{4+} T cells differentiate into Th17 cells (helper T cells), and subsequently in turn produce IL-17 to stimulate stromal cells for sustaining NF-κB mediated secretion of IL-6 [42]. IL-17 signaling pathway also relate to JAK-STAT pathways activation, and IL-17 and IL-6 participate in a positive feedback loop [43, 44]. It is suggested that the SARS-CoV-2 invasion would infects alveolar epithelial cells through ACE2 receptor and activate innate and adaptive immune cells to release a large number of inflammatory cytokines, among which IL-6 plays a central role in cytokine storm, thus giving rise to a large number of inflammatory exudates and erythrocytes enter the alveoli that accounts for dyspnea and respiratory failure [45]. Combined with the analysis of "drugs-targets-disease" as mentioned above, we deduce that the object of intense translational research as promising therapeutic targets. As a consequence, our future research will focus on reducing IL-6 production and signaling pathways which are associated with antiviral and inflammatory diseases.

The hot spot where beckons SARS-CoV-2 structure are currently in the spotlight for drugs and vaccines developing. 3CLpro is vital to viral replication of the proteins the viral genome encodes for their peculiarity to cleave the two translated polyproteins at 11 distinct sites into various non-structural proteins, which is considered to be a promising drug target for combating the coronavirus infection [46–48]. Recently, some peptides and small molecules have been reported so far, as inhibitors that target 3CLpro of SARS-CoV-2. As shown by the docking simulation results, we speculate that flavonoids are as dominant components of *Belamcandae rhizoma* for antiviral activity. It can be seen from the results of molecular docking that the binding affinity of luteolin and dinatin come close to reference inhibitor remdesivir with the 3CLpro, thus deduced that both of two are potential SARS-CoV-2 inhibitor and play the putative roles of interactions with other coronaviruses. Previously studies have reported that luteolin has antiviral effects against several viruses, such as Japanese encephalitis virus and influenza A [49, 50]. Luteolin and dinatin share a skeleton of flavone with the presence of double bond between C-2 and C-3, as well as a carbonyl at C-4 in ring C. Comparing their substituents, it was found that luteolin showed the position of hydroxyl at C-3' in ring B, whereas dinatin possessed one methoxyl at C-6 in ring A. The speculation about SARS-CoV-2 inhibitory activity of these two components was that they both have three fixed hydroxyl group, two respectively position at the C-5 and C-7 in ring A, another is at C-4' in ring B.

However, our network pharmacology study lacked in vitro experiments and clinical trials to verify the hypothesis. In the future, we endeavor to verify the relationship between COVID-19 and *Belamcandae rhizoma* from a plurality of experiment levels.

Conclusion

In conclusion, our study has visualized a direct network among *Belamcandae rhizoma* compounds, their potential disease targets of COVID-19 and the related biological processes and signaling pathways.

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These results are consistent with the reported pharmacological effects of extracts, and they demonstrated the efficiency and accuracy of our analytical platform on *Belamcandae rhizoma*. Though more biological validation is required to further verify the current results, for the first time, the possibility of *Belamcandae rhizoma* in treating disease progression has been explored from the perspective of virus targets in a systemic approach. This combination of traditional Chinese medicine and modern analytical methods may introduce a novel strategy to study traditional Chinese medicine and provide a new therapeutic strategy and targets for patients who suffer from SARS-CoV-2.

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