Network pharmacology and molecular docking analysis on mechanisms of Tibetan Hongjingtian (*Rhodiola crenulata*) in the treatment of COVID-19

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**Abstract**

**Introduction.** Coronavirus disease 2019 (COVID-19) is a highly contagious disease and ravages the world.

**Hypothesis/Gap Statement.** We proposed that *R. crenulata* might have potential value in the treatment of COVID-19 patients by regulating the immune response and inhibiting cytokine storm.

**Aim.** We aimed to explore the potential molecular mechanism for *Rhodiola crenulata* (*R. crenulata*), against the immune regulation of COVID-19, and to provide a referenced candidate Tibetan herb (*R. crenulata*) to overcome COVID-19.

**Methodology.** Components and targets of *R. crenulata* were retrieved from the TCMSP database. GO analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were built by R bioconductor package to explore the potential biological effects for targets of *R. crenulata*. The *R. crenulata*-compound-target network, target pathway network and protein–protein interaction (PPI) network were constructed using Cytoscape 3.3.0. Autodock 4.2 and Discovery Studio software were applied for molecular docking.

**Result.** Four bioactive components (quercetin, kaempferol, kaempferol-3-O-α-L-rhamnoside and tamarixetin) and 159 potential targets of *R. crenulata* were identified from the TCMSP database. The result of GO annotation and KEGG-pathway-enrichment analyses showed that target genes of *R. crenulata* were associated with inflammatory response and immune-related signalling pathways, especially IL-17 signalling pathway, and TNF signalling pathway. Targets-pathway network and PPI network showed that IL-6, IL-1B and TNF-α were considered to be hub genes. Molecular docking showed that core compound (quercetin) had a certain affinity with IL-1β, IL-6 and TNF-α.

**Conclusion.** *R. crenulata* might play an anti-inflammatory and immunoregulatory role in the cytokine storm of COVID-19.

**INTRODUCTION**

Coronavirus disease 2019 (COVID-19) is a highly contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that ravages the world resulting in a pandemic of increasing death [1]. This novel coronavirus has rapid and extensive spread and general susceptibility of the population, which make COVID-19 be a highly contagious disease [2]. Clinical symptoms of COVID-19 patients were acute pneumonia, systemic fever, dry cough, fatigue, myalgia/arthritis, and breathing difficulties etc., and severe cases can lead to acute respiratory distress, multiple-organ failure, and even death [3]. Some studies supported that, during the...
response to SARS-CoV-2, the immune dysregulation and the high level of proinflammatory cytokines could occur in some infected patients, which were called as the cytokine storm [4]. Cytokine storm causes acute respiratory distress syndrome (ARDS) or multiple-organ dysfunction, which may play an important role in the clinical deterioration of COVID-19 [5]. Therefore, effectively suppressing the cytokine storm is the key to preventing the deterioration of COVID-19 and improving the treatment success rate.

Currently, there are few effective medications for treating COVID-19. Some studies have shown that traditional Chinese Medicine (TCM) plays an important role in prevention and treatment of COVID-19. Hongjingtian (Rhodiola), the genus Rhodiola in the family Crassulaceae, is herbaceous perennial plants. There are 96 species of Rhodiola in the world and most are found in different regions of China (73 species), such as Tibet. Rhodiola, also known as 'oriental god grass' and 'plateau ginseng', has great medicinal value. The main chemical components of Rhodiola include salidroside, flavonoids, terpenoids, sterols, tannins and other compounds. The functions of Rhodiola might be involved in promoting blood circulation and removing blood stasis, clearing lung and relieve cough, reducing fatigue and weakness, antiviral infecting and improving immunity [6, 7]. Furthermore, modern research has shown that the comprehensive nourishing effects of the Rhodiola species are largely attributed to its phytochemicals, which exert anti-hypoxic, anti-viral, immune regulatory, anti-tumour, anti-fatigue, anti-depressive, and improvement of learning and memory effects [8–10]. Current investigation reveals that Rhodiola crenulata (R. crenulata) has pharmacological prevention and treatment for many diseases including influenza, sepsis, lung injury and trachea inflammation [11, 12]. R. crenulata might have potential value in the treatment of COVID-19 patients by regulating the immune response and inhibiting cytokine storm.

According to Flora of China, Rhodiola crenulata is mainly distributed in Tibet (also named Xizang), PR China [13]. Rhodiola grows on alpine grasslands, valley rocks or glaciers at an altitude range of 1800–5600 m and can adapt to extremely high altitude adversities, including a low temperature, hypoxia, intensive ultraviolet radiation, huge diurnal temperature differences, etc. In the present study, the bioinformatics, network pharmacology and molecular docking were used to predict their potential targets and signal pathways of Tibetan herb R. crenulata and to analyse the relationship of the active compounds with targets. These results are expected to help understand the potential molecular mechanism for Tibetan herb R. crenulata against the immune regulation of COVID-19, and to provide a referenced candidate TCM Tibetan herb to overcome COVID-19.

### METHODS

#### Identification of bioactive components of R. crenulata

The components of R. crenulata were retrieved from the traditional Chinese medicine systems pharmacology (TCMSP) database (http://tcmspw.com/) and previous studies [14, 15]. Oral bioavailability (OB) represents the ratio of an orally administered dose compared to unchanged drug that reaches the systemic circulation, which is one of the most significant pharmacokinetic parameters [16]. Drug-likeness (DL) is a qualitative concept to estimate the drug-ability of a molecule [17]. Substances with OB ≥30% and DL index ≥0.18 were regarded to have high OB and drug ability. Therefore, bioactive components of candidate herbs with OB ≥30% and DL index ≥0.18 were identified for subsequent analysis in the current study.

#### Construction of R. crenulata-compound-target network

The target protein of bioactive components in R. crenulata was also retrieved from TCMSP database. Afterward, the target proteins corresponding to the compounds screened from the Pharmmapper database and PubMed database were standardized in UniProt (http://www.uniprot.org/). The targets from different databases were merged and the duplicated targets were removed. Finally, Cytoscape 3.3.0 software (http://www.cytoscape.org/) was used to construct the herb-compound-target network, which helps to understand the pharmacological mechanism of R. crenulata.

#### Gene ontology and pathway enrichment analysis for targets of R. crenulata

DAVID (the Database for Annotation, Visualization and Integrated Discovery, http://david.abcc.ncifcrf.gov/) and KOBAS [Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology Based Annotation System, https://www.biostars.org/p/200126/] were utilized for retrieving information about functional annotation of genes. Gene ontology (GO) analysis and KEGG-pathway enrichment were built by R bioconductor
package to explore the potential biological effects for targets of *R. crenulata*. GO terminology was annotated including biological process (BP), cellular component (CC) and molecular function (MF) categories. KEGG-pathway database (https://www.kegg.jp/kegg/) was applied for the targets mapped to the pathway. The target-pathway/function network was constructed using Cytoscape 3.3.0 to identify the relationships of *R. crenulata* targets with the involved pathways obtained through enrichment analysis.

**Protein–protein interaction analysis**

Screening for the immunity and inflammation cytokines in COVID-19 among *R. crenulata* targets were performed. The
STRING database (http://string-db.org/) is a search tool for retrieval of interacting genes/proteins [18]. Obtained cytokines genes were uploaded onto STRING database to obtain the relationships of protein–protein interaction (PPI), such as co-expression and co-localization. Finally, Cytoscape 3.3.0 software was used to construct PPI network.

**Molecular docking**
To obtain a deeper understand about the association of quercetin with TNF-α and IL-1β, molecular docking was applied to evaluate the strength and mode of interactions between quercetin and TNF-α/IL-1β. The crystal structure of TNF-α and IL-1β were obtained from RCSB Protein Data Bank (PDB, http://www.rcsb.org/). ChemDraw software or PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used to prepare the chemical structure of quercetin. Autodock 4.2 (http://mgltools.scripps.edu/downloads) and Discovery Studio software were applied for molecular docking.

**RESULTS**
**Identification of bioactive components of R. crenulata**
We searched for *R. crenulata* by retrieving the TCMSP database and previous studies, and found that four active ingredients of *R. crenulata* with OB ≥30% and DL index ≥0.18 were mainly consisted of quercetin (MOL000098), kaempferol (MOL000422),
kaempferol-3-O-α-L-rhamnoside (MOL012777) and tamarixetin (MOL004083, Table 1).

**Construction of R. crenulata-compound-target network**

The target proteins of the effective components were obtained from the TCMSP database. Finally, 159 potential targets (without repetition) of four bioactive components were collected (Table 2). The pharmacological effect of herbs in preventing and controlling complex diseases might be associated with the synergy between multiple compounds and their targets. Here, R. crenulata-compound-target network was constructed (Fig. 1), which included 164 nodes (one for R. crenulata, four for candidate bioactive components and 159 for potential protein targets) and 165 edges. There were these components associated with targets, namely, quercetin (degree=144), kaempferol (degree=11), kaempferol-3-O-α-L-rhamnoside (degree=2) and tamarixetin (degree=4).

**Gene ontology and pathway-enrichment analysis for targets of R. crenulata**

To recognize the potential biological functions of targets of R. crenulata, the GO annotation and pathway-enrichment analyses were conducted. There were respectively 463 biological process (BP), 47 cellular component (CC) and 98 molecular function (MF) terms in total (count of gene ≥2 and P value <0.05). Top ten significantly enriched BP, CC and MF categories were displayed in Fig. 2, Table 3. The possible BP were related to response to drug, positive regulation of transcription from RNA polymerase II promoter, positive regulation of gene expression, positive regulation of transcription DNA-templated, negative regulation of apoptotic process, cellular response to...
Table 3. GO term for targets of Hongjingtian (top 10)

| Term                              | Description                                                                 | Count | P        | -LOG(P) | Fold Enrichment | Bonferroni | Benjamini | FDR     |
|-----------------------------------|-----------------------------------------------------------------------------|-------|----------|---------|-----------------|------------|-----------|---------|
| **Biological process (BP)**       |                                                                             |       |          |         |                 |            |           |         |
| GO:0042493                        | Response to drug                                                           | 28    | 6.36E-19 | 1.82E+01| 9.727243        | 1.46E-15   | 1.46E-15  | 1.11E-15|
| GO:0045944                        | Positive regulation of transcription from RNA polymerase II promoter         | 44    | 5.15E-18 | 1.73E+01| 4.736843        | 1.19E-14   | 5.93E-15  | 9.01E-15|
| GO:0010628                        | Positive regulation of gene expression                                     | 24    | 3.97E-16 | 1.54E+01| 9.674204        | 1.02E-12   | 3.40E-13  | 7.77E-13|
| GO:0045893                        | Positive regulation of transcription, DNA-templated                        | 31    | 8.32E-16 | 1.51E+01| 6.357111        | 1.79E-12   | 4.47E-13  | 1.35E-12|
| GO:0043066                        | Negative regulation of apoptotic process                                   | 27    | 1.35E-13 | 1.29E+01| 6.266971        | 3.10E-10   | 6.20E-11  | 2.36E-10|
| GO:0071222                        | Cellular response to lipopolysaccharide                                     | 16    | 1.68E-13 | 1.28E+01| 14.95364        | 3.85E-10   | 6.42E-11  | 2.93E-10|
| GO:0032496                        | Response to lipopolysaccharide                                             | 18    | 2.43E-13 | 1.26E+01| 11.59135        | 5.58E-10   | 7.97E-11  | 4.24E-10|
| GO:0071456                        | Cellular response to hypoxia                                               | 15    | 2.93E-13 | 1.25E+01| 16.50157        | 6.74E-10   | 8.42E-11  | 5.12E-10|
| GO:0006954                        | Inflammatory response                                                      | 24    | 1.15E-12 | 1.19E+01| 6.687708        | 2.65E-09   | 2.94E-10  | 2.01E-09|
| GO:0001666                        | Response to hypoxia                                                         | 17    | 6.79E-12 | 1.12E+01| 10.4382         | 1.56E-08   | 1.56E-09  | 1.19E-08|
| **Cellular component (CC)**       |                                                                             |       |          |         |                 |            |           |         |
| GO:0005615                        | Extracellular space                                                        | 52    | 2.88E-20 | 1.95E+01| 4.424685        | 7.57E-18   | 7.57E-18  | 3.77E-17|
| GO:0005829                        | Cytosol                                                                    | 66    | 1.33E-11 | 1.09E+01| 2.281955        | 3.50E-09   | 1.75E-09  | 1.74E-08|
| GO:0005576                        | Extracellular region                                                        | 37    | 9.07E-08 | 7.04E+00| 2.63404         | 2.39E-05   | 7.95E-06  | 1.19E-04|
| GO:0005654                        | Nucleoplasm                                                                | 51    | 1.70E-07 | 6.77E+00| 2.099653        | 4.48E-05   | 1.12E-05  | 2.23E-04|
| GO:0045121                        | Membrane raft                                                              | 13    | 2.45E-07 | 6.61E+00| 7.233071        | 6.46E-05   | 1.29E-05  | 3.21E-04|
| GO:0031012                        | Extracellular matrix                                                       | 15    | 3.09E-07 | 6.51E+00| 5.808261        | 8.12E-05   | 1.35E-05  | 4.04E-04|
| GO:0005901                        | Caveola                                                                    | 8     | 1.47E-06 | 5.83E+00| 14.10663        | 3.87E-04   | 5.35E-05  | 0.001927|
| GO:0070062                        | Extracellular exosome                                                      | 49    | 1.50E-06 | 5.82E+00| 1.997937        | 3.95E-04   | 4.94E-05  | 0.001996|
| GO:0005739                        | Mitochondrion                                                              | 30    | 3.47E-06 | 5.46E+00| 2.583389        | 9.13E-04   | 1.02E-04  | 0.00455 |
| GO:0005634                        | Nucleus                                                                    | 75    | 3.91E-06 | 5.41E+00| 1.587484        | 0.001028   | 1.03E-04  | 0.005121|
| **Molecular function (MF)**       |                                                                             |       |          |         |                 |            |           |         |
| GO:0019899                        | Enzyme binding                                                             | 32    | 2.08E-22 | 2.17E+01| 10.26708        | 1.03E-19   | 1.03E-19  | 2.99E-19|
| GO:0042802                        | Identical protein binding                                                  | 33    | 3.25E-13 | 1.25E+01| 4.707314        | 1.62E-10   | 8.09E-11  | 4.68E-10|
| GO:0005515                        | Protein binding                                                            | 125   | 1.99E-12 | 1.17E+01| 1.52023         | 9.90E-10   | 3.30E-10  | 2.87E-09|
| GO:0008134                        | Transcription factor binding                                               | 21    | 2.06E-12 | 1.17E+01| 7.900272        | 1.02E-09   | 2.56E-10  | 2.96E-09|
| GO:0042803                        | Protein homodimerization activity                                          | 30    | 2.90E-11 | 1.05E+01| 4.390758        | 1.44E-08   | 2.88E-09  | 4.17E-08|
| GO:0046982                        | Protein heterodimerization activity                                        | 23    | 3.66E-10 | 9.44E+00| 5.284647        | 1.82E-07   | 3.04E-08  | 5.27E-07|
lipopolysaccharide, response to lipopolysaccharide, cellular response to hypoxia, inflammatory response and response to hypoxia (Fig. 2a). These genes were involved in CC including extracellular space, cytosol, extracellular region, nucleoplasm, membrane raft, extracellular matrix, caveola, extracellular exosome, mitochondrion and nucleus (Fig. 2b). Moreover, the MF of these genes were mainly correlated with enzyme binding, identical protein binding, protein binding, transcription factor

### Table 3. Continued

| Term                | Description                                                                 | Count | P         | -LOG(P) | Fold Enrichment | Bonferroni | Benjamini | FDR     |
|---------------------|-----------------------------------------------------------------------------|-------|-----------|---------|-----------------|------------|-----------|---------|
| GO:0004879          | RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding | 8     | 3.50E-08  | 7.46E+00 | 23.74262        | 1.74E-05  | 2.49E-06  | 5.04E-05 |
| GO:0043565          | Sequence-specific DNA binding                                               | 20    | 3.54E-07  | 6.45E+00 | 4.125165        | 1.76E-04  | 2.20E-05  | 5.10E-04 |
| GO:0019901          | Protein kinase binding                                                     | 17    | 4.54E-07  | 6.34E+00 | 4.830612        | 2.25E-04  | 2.51E-05  | 6.53E-04 |
| GO:0008144          | Drug binding                                                               | 9     | 5.17E-07  | 6.29E+00 | 12.65232        | 2.57E-04  | 2.57E-05  | 7.43E-04 |

Fig. 3. KEGG enrichment analysis diagram. The diagram of pathway-enrichment analysis the top 20 pathways (a) and immune-related signaling pathways (b) enriched by the KEGG method.
Table 4. Annotation of pathways for targets of Hongjingtian (top 20)

| ID     | Term                                           | Input no. | P-value | -LOG(P) | Corrected P-value |
|--------|------------------------------------------------|-----------|---------|---------|-------------------|
| hsa05200 | Pathways in cancer                              | 60        | 3.31E-67 | 6.65E+01 | 8.15E-65          |
| hsa04933 | AGE-RAGE signalling pathway in diabetic complications | 31        | 3.18E-46 | 4.55E+01 | 3.92E-44          |
| hsa05418 | Fluid shear stress and atherosclerosis          | 33        | 7.04E-46 | 4.52E+01 | 5.78E-44          |
| hsa05161 | Hepatitis B                                     | 32        | 3.83E-42 | 4.14E+01 | 2.35E-40          |
| hsa05215 | Prostate cancer                                 | 27        | 3.27E-39 | 3.85E+01 | 1.61E-37          |
| hsa05167 | Kaposi sarcoma-associated herpesvirus infection | 30        | 3.24E-37 | 3.65E+01 | 1.33E-35          |
| hsa05163 | Human cytomegalovirus infection                | 31        | 1.60E-36 | 3.58E+01 | 5.62E-35          |
| hsa04657 | IL-17 signalling pathway                        | 25        | 5.08E-36 | 3.53E+01 | 1.45E-34          |
| hsa05160 | Hepatitis C                                     | 28        | 5.31E-36 | 3.53E+01 | 1.45E-34          |
| hsa04668 | TNF signalling pathway                          | 25        | 2.99E-34 | 3.35E+01 | 7.36E-33          |
| hsa05169 | Epstein–Barr virus infection                   | 27        | 1.38E-31 | 3.09E+01 | 3.08E-30          |
| hsa04151 | PI3K-Akt signalling pathway                     | 31        | 6.33E-31 | 3.02E+01 | 1.30E-29          |
| hsa05212 | Pancreatic cancer                               | 21        | 1.06E-30 | 3.00E+01 | 2.00E-29          |
| hsa05166 | Human T-cell leukaemia virus 1 infection       | 27        | 1.14E-30 | 2.99E+01 | 2.01E-29          |
| hsa05142 | Chagas disease (American trypanosomiasis)      | 22        | 7.40E-30 | 2.91E+01 | 1.21E-28          |
| hsa05222 | Small cell lung cancer                          | 21        | 5.48E-29 | 2.83E+01 | 8.42E-28          |
| hsa05162 | Measles                                        | 23        | 5.83E-29 | 2.82E+01 | 8.43E-28          |
| hsa04010 | MAPK signalling pathway                         | 28        | 7.20E-29 | 2.81E+01 | 9.84E-28          |
| hsa05225 | Hepatocellular carcinoma                       | 24        | 9.86E-29 | 2.80E+01 | 1.28E-27          |
| hsa05219 | Bladder cancer                                 | 17        | 1.76E-27 | 2.68E+01 | 2.16E-26          |

Fig. 4. Diagram of immune-related signalling pathways. (a) IL-17 signalling pathway, (b) TNF signalling pathway, (c) NF-kappa B signalling pathway, (d) Toll-like receptor signalling pathway, (e) T cell receptor signalling pathway, (f) MAPK signalling pathway.
binding, protein homodimerization activity, protein heterodimerization activity, RNA polymerase II transcription factor activity, sequence-specific DNA binding, protein kinase binding and drug binding (Fig. 2c).

To determine the possible involved pathways of *R. crenulata* targets, KEGG-pathway analysis was performed. Top 20 enriched pathways of *R. crenulata* targets were shown in Fig. 3(a), Table 4. Moreover, KEGG-enrichment analysis showed that many target genes of *R. crenulata* were strongly associated with immune-related signalling pathways (Fig. 3b, Table S1, available in the online version of this article), including IL-17 signalling pathway, TNF signalling pathway, NF-kappa B signalling pathway, Toll-like receptor signalling pathway, T cell receptor signalling pathway and MAPK signalling pathway. KEGG diagram of immune-related signalling pathways were shown in Fig. 4.

The illustrated network that a targets-pathway was established to understand their interaction (Fig. 5). Many targets were simultaneously involved in multiple biological processes. Among these potential targets, IkBα, TNF-α and IL-1β were identified as relatively high-degree targets, which played an essential role in NF-kappa B signalling pathway, Toll-like receptor signalling and IL-17 signalling pathway.

The above results indicate that *R. crenulata* can exert an anti-inflammatory and immunoregulation through multiple targets and pathways.

**Protein–protein interaction analysis**

PPI network for the immunity and inflammation cytokines in COVID-19 among *R. crenulata* targets was displayed as Fig. 6. The results showed that IL10, IL-6, IL-1β, TNF-α, CCL2 and CXCL8 were important nodes in the network (Table S2).

**Molecular docking**

In COVID-19 patients, a pro-inflammatory status with high levels of IL-1β, IL-6 and TNF-α have been demonstrated [19]. In addition, IL-1β, IL-6 and TNF-α were important nodes in KEGG pathway and PPI network for *R. crenulata*. Here, molecular docking for *R. crenulata* ingredient (quercetin) and IL-1β, IL-6 and TNF-α proteins were analysed, and the results showed that quercetin had strong affinity with IL-1β, IL-6 and TNF-α proteins (Fig. 7). In terms of the interaction point, quercetin mainly interacted with amino acid residues ASN92, LYS148, GLU149, LYS150, TYR153, PRO176 and TYR175 of IL-1β (Fig. 7a). Quercetin and IL-6 formed a stable complex by interacting with the amino acid residues GLU127, ARG141, GLU137
DISCUSSION

In this study, four bioactive components (quercetin, kaempferol, kaempferol-3-O-α-L-rhamnoside and tamarixetin) and 159 potential targets of *R. crenulata* were identified from the TCMSP database. *R. crenulata*-compound–target network diagram displayed the potential synergy between multiple compounds and their targets. Moreover, GO annotation and KEGG-pathway-enrichment analyses were conducted to recognize the potential biological functions of targets of *R. crenulata*. The result showed that target genes of *R. crenulata* were associated with inflammatory response and immune-related signalling pathways, including IL-17 signalling pathway, TNF signalling pathway, NF-kappa B signalling pathway, Toll-like receptor signalling pathway, T cell receptor signalling pathway and MAPK signalling pathway. Targets-pathway network and PPI network showed that IL-6, IL-1B and TNF-α were considered to be hub genes. Molecular docking showed that quercetin (MOL000098) had certain affinity with IL-1β, IL-6 and TNF-α. These results suggested that *R. crenulata* might play an anti-inflammatory and immunoregulatory role in the cytokine storm of COVID-19.

Hongjingtian (*R. crenulata*), a traditional Chinese medicine herb, can be mainly found at high altitudes in PR China such as Tibet, Qinghai. A recent study has revealed that the major bioactive components of *R. crenulata* have anti-inflammatory and antioxidant effects [20]. *R. crenulata* could achieve a certain effect on improvement of pulmonary inflammation of the mice infected with influenza virus and release of inflammatory factors in serum and lung tissue.

![Fig. 6. PPI network for the immunity and inflammation cytokines in COVID-19 among *R. crenulata* targets. The nodes represent proteins, and the connections represent interactions between proteins.](image-url)
R. crenulata has the effect to improve the immunological functions of mice [6]. R. crenulata inhibit activation of NF-κB pathway to reduce acute lung injury caused by sepsis [21]. These studies support that R. crenulata has an important role in the inflammatory response. Recent studies have revealed that R. crenulata possess multiple active ingredients. Here, four active ingredients of R. crenulata with OB ≥30% and DL index ≥0.18 were found including quercetin (MOL000098), kaempferol (MOL000422), kaempferol-3-O-α-L-rhamnoside (MOL012777) and tamarixetin (MOL004083). Moreover, 159 potential targets (without repetition) of four bioactive components were collected. R. crenulata-compound-target network diagram displayed that the potential synergy between multiple compounds and their targets.

GO annotation and pathway-enrichment analyses were conducted to recognize the potential biological functions for targets of R. crenulata. GO enrichment analysis displayed that the major biological processes included response to drug, positive regulation of transcription DNA-templated, negative regulation of apoptotic process, response to lipopolysaccharide, inflammatory response and response to hypoxia. KEGG-enrichment analysis showed that many target genes of R. crenulata were strongly associated with immune-related signalling pathways, including IL-17 signalling pathway, TNF signalling pathway, NF-kappa B signalling pathway, Toll-like receptor signalling pathway, T cell receptor signalling pathway and MAPK signalling pathway. IL-17 signals synergistically with numerous ligands that activate surprisingly diverse signalling pathways, such as TNF-α, IFN-γ, IL-13, or TGF-β [22]. A study reported targeting the IL-17 pathway to prevent acute respiratory distress syndrome associated with SARS-CoV-2 infection [23]. TNF signalling pathway has been identified as an important pathway in inflammatory response [24]. TNF induced the production of IL-6 and other cytokines, involved in the process of cytokine storm in COVID-19 [25]. NF-κB/Nrf2 balance might be associated with the treatment of COVID-19 [26]. Toll-like receptor family members upregulated anti-viral and pro-inflammatory mediators (IL-6 and IL-8 and type I and type III interferons among others), through the activation of Nuclear Factor (NF)-kB in COVID-19 [27]. SARS-CoV infection could activate p38 MAPK and the downstream signalling possibly to increase human coronavirus viral replication leading to cell death [28]. These results indicate that R. crenulata may interfere with COVID-19 through multiple immune-related signalling pathways.

COVID-19 patients who succumb to pneumonia and hypoxia had one hallmark feature of the profound...
inflammatory state that marked elevation of serum inflammatory cytokines (IL-6, IFN-γ, IL-1β, TNF-α and TGF-β) and chemokines (CCL2, CCL5, CXCL8 and CXCL10) [29]. PPI network for the immunity and inflammation cytokines in COVID-19 among R. crenulata targets showed that IL-10, IL-6, IL-1B, TNF-α, CCL2 and CXCL8 were important nodes in the network. Combined with targets-pathway network, we found that IL-6, IL-1B and TNF-α were considered to be hub genes. In COVID-19 patients a pro-inflammatory status with high levels of IL-6, IL-1B and TNF-α has been demonstrated [19]. IL-6, a cytokine, has context-dependent pro- and anti-inflammatory properties, and excessive synthesis of IL-6 while fighting environmental stress leads to an acute severe systemic inflammatory response known as cytokine storm [30]. IL-1B, a member of IL-1 cytokine subfamily, has anagelsec, immunomodulatory, anti-hypoxia and anti-inflammatory functions [31]. TNF-α regulates a variety of physiological functions in the body, including immune surveillance, immune response against microbial infections, and induction of cell death [32]. Subsequently, results of molecular docking indicated that quercetin (active ingredients of R. crenulata) could bind with IL-6, IL-1B and TNF-α. These results hinted that R. crenulata could regulate the formation of cytokine storms to reduce excessive inflammation in the body, thereby improving severe systemic damage in COVID-19 patients. However, the exact mechanism requires further validation in biological experiments.

CONCLUSION

In summary, bioactive components and potential targets of R. crenulata were identified, and target genes of R. crenulata were associated with immune-related signalling pathways, especially IL-17 signalling pathway and TNF signalling pathway. Moreover, R. crenulata might play an anti-inflammatory and immunoregulatory role in the cytokine storm of COVID-19 by acting on IL-1/β, IL-6 and TNF-α. However, further studies are necessary to elucidate the precise mechanism.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Lai CC, Shih TP, WC K, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. Int J Antimicrob Agents 2020;55:105924.
2. Kannan S, Shaik Syed Ali P, Sheeza A, Hemalatha K. COVID-19 (Novel Coronavirus 2019) - recent trends. Eur Rev Med Pharmacol Sci 2020;24:2006–2011.
3. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, et al. Review of the Clinical Characteristics of Coronavirus Disease 2019 (COVID-19). J Gen Intern Med 2020;35:1545–1549.
4. Henderson LA, Canna SW, Schulert GS, Volpi S, Lee PY, et al. On the alert for cytokine storm: Immunopathology in COVID-19. Arthritis and Rheumatology (Hoboken, NJ) 2020;72:1059–1063.
5. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, et al. Covid-19: Consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033–1034.
6. Wei RB, Wang YY, Yang Y, Cui SY, Shi SZ. Experimental study of immunological function regulated by Fufang Hongjingtian capsule in mice. Zhongguo shi yan xue ye xue za zhi 2012;20:187–191.
7. Zhuang W, Yue L, Dang X, Chen F, Gong Y, et al. Rosenberg (Rhodiola): Potential applications in aging-related diseases. Aging Dis 2019;10:134–146.
8. Nan X, Su S, Ma K, Ma X, Wang X, et al. Bioactive fraction of Rhodiola aligida against chronic hypoxia-induced pulmonary arterial hypertension and its anti-proliferation mechanism in rats. J Ethnopharmacol 2018;216:175–183.
9. Recio MC, Giner RM, Mâñez S. Immunomodulatory and antiproliferative properties of Rhodiola species. Planta Med 2016;82:952–960.
10. Bassa LM, Jacobs C, Gregory K, Henchey E, Ser-Dolansky J, et al. Rhodiola crenulata induces an early estrogenic response and reduces proliferation and tumorsphere formation over time in MCF7 breast cancer cells. Phytomedicine 2016;23:87–94.
11. Lin W, Lu H, Lu P, Zhao Z, Jian R. Influence of salidroside on serum and lung tissue inflammatory factors and immunological indexes of mice infected with influenza virus. Chin J Nosocomiology 2020;30:292–296.
12. Zhang R, Xu T, Wang C, Zhang Y. Effect of Salidroside on antioxidant stress in lung injury induced by swine H9N2 influenza virus in mice. In: Veterinary Pathology Branch of Chinese Society of Animal Husbandry and Veterinary Medicine: 2016, 2016.
13. Mihailovic PM, Lio WM, Yang J, Zhou J, Zhao X, et al. IL-7R blockade reduces post-myocardial infarction-induced atherosclerotic plaque inflammation in ApoE(−/−) mice. Biochim Biophys Acta 2019;1906:9467.
14. Ru J, Li P, Wang J, Zhou W, Li B, et al. TCMP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform 2014;6:13.
15. Ni F, Chen Z, Xu Q, Yang S, Chen D. Chemical constituents from Rhodiola sachalinesis. Chinese Traditional and Herbal Drugs 2013;7:798–802.
16. Xu X, Zhang W, Huang C, Li Y, Hu H, et al. Ling Y. A novel chemometric method for the prediction of human oral bioavailability. Int J Mol Sci 2012;13:6967–6982.
17. Tao W, Xu X, Wang X, Li B, Wang Y, et al. Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix Curcumae formula for application to cardio-vascular disease. J Ethnopharmacol 2013;145:1–10.
18. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, et al. STRING v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019;47:D607–D613.
19. Pedersen SF, Ho Y-C. SARS-CoV-2: a storm is raging. J Clin Invest 2020;130:2202–2205.
20. Choe KI, Kwon JH, Park KH, Oh MH, Kim MH, et al. The antioxidant and anti-inflammatory effects of phenolic compounds isolated from the root of Rhodiola sachalensis A. BOR. Molecules 2012;11:484–11494.
21. Zhang Y, Zang B, Li X, Zhao W. Effect of sofren injection on NF-κB expression in acute lung injury mice with sepsis. Clin Pulm Med 2017;022:2147–2150.
22. Zhou M, Wang H, Zeng X, Yin P, Zhu J, et al. Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: A systematic analysis for the global burden of disease study 2017. Lancet 2019;394:1145–1158.
23. Wiche Salinas TR, Zheng B, Routy JP, Ancuta P. Targeting the interleukin-17 pathway to prevent acute respiratory distress syndrome associated with SARS-CoV-2 infection. In: Respiriology, Vol. 25. Carlton, Vic, 2020. pp. 797–799.

24. Bradley JR. TNF-mediated inflammatory disease. J Pathol 2008;214:149–160.

25. Schett G, Manger B, Simon D, Caporali R. COVID-19 revisiting inflammatory pathways of arthritis. Nat Rev Rheumatol 2020;16:465–470.

26. Martínez-Sánchez G, Schwartz A, Donna VD. Potential cytoprotective activity of ozone therapy in SARS-COV-2/Covid-19. Antioxidants (Basel) 2020;9:389.

27. Sallenave JM, Guillot L. Innate immune signaling and proteolytic pathways in the resolution or exacerbation of SARS-CoV-2 in Covid-19: key therapeutic targets Front Immunol 2020;11:1229.

28. Mizutani T, Fukushima S, Saijo M, Kurane I, Morikawa S. Phosphorylation of p38 MAPK and its downstream targets in SARS coronavirus-infected cells. Biochem Biophys Res Commun 2004;319:1228–1234.

29. Arnaldez FI, O’Day SJ, Drake CG, Fox BA, Fu B, et al. The society for immunotherapy of cancer perspective on regulation of interleukin-6 signaling in covid-19-related systemic inflammatory response. J Immunother Cancer 2020;8:e000930.

30. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. Immunotherapy 2016;8:959–970.

31. Woff Y, Man SM, Aggio-Bruce R, Natoli R, Fernando N. IL-1 family members mediate cell death, inflammation and angiogenesis in retinal degenerative diseases. Front Immunol 2019;10:1618.

32. Zelová H, Hošek J. Tnf-α signalling and inflammation: Interactions between old acquaintances. Inflamm Res 2013;62:641–651.

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