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Discovering Traditional Chinese Medicine (TCM) Formulas for Complex Diseases Based on a Combination of Reverse Systematic Pharmacology and TCM Meridian Tropism Theory: Taking COVID-19 as an Example

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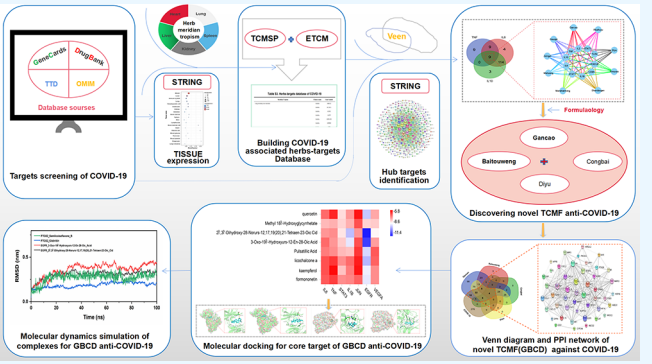
ABSTRACT: Objective: Infections and death have been a part of our daily lives since the COVID-2019 pandemic outbreak in 2019, and the societal and economic consequences have lingered for an unanticipated duration. Novel and effective treatments are still desperately needed around the world to combat the infection. Here, we discovered a novel traditional Chinese medicine formula (TCMF) to potentially combat COVID-19 through reverse systematic pharmacology (disease → targets → TCMF → disease). Methods: Combining Integrative network pharmacology and the traditional Chinese medicine (TCM) theory, a TCMF for COVID-19 was identified. *In silico* physiological interactions between herbs and disease hub targets were validated by molecular docking and dynamics simulation. Results: Based on disease-related gene/pathway targets and a combination of reverse pharmacology and TCM meridian tropism theory, a COVID-19-associated herb database was constructed. A new TCMF, including Gancao, Baitouweng, Congbai, and Diyu (GBCD), was discovered for anti-COVID-19 therapy. The KEGG and GO analyses of 49 intersecting genes suggested that GBCD could combat COVID-19 through antiviral, antiinflammation, immunoregulation, and cytoprotection activities. Moreover, these possible effects were validated through docking and MD simulation. Conclusions: To the best of our knowledge, this study is the first to combine reverse pharmacology and meridian tropism theories for TCMF development, and a novel herbal combination, GBCD, was discovered for anti-COVID-19 therapy.



INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a novel coronavirus pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with common symptoms of fever (98%), which first emerged in Wuhan, Hubei province of China.[1,2] COVID-19 is characterized by its prevalence in elder patients, higher infectivity, and mortality.[3] Globally, despite the fact that a billion doses of vaccines were provided globally, there were still over 598 million confirmed cases and 6.4 million deaths because of a lack of effective medications (data from the World Health Organization, updated on 24 August 2022). As a result, the COVID-19 pandemic will persist, necessitating the development of new and effective treatments. Traditional Chinese medicine (TCM), which has been used for pandemic prophylaxis and therapy for thousands of years, has proved to have remarkable effects in combating SARS, H1N1, and H7N9.[4] A meta-analysis confirmed that TCM, combined with modern medical practice, shows outstanding performances and mild adverse drug reactions for treating COVID-19.[5] Therefore, TCM was suggested for the "Diagnosis and Treatment Scheme of New Coronavirus Pneumonia" (Trial edition three to eight) (http://www.nhc.gov.cn/xcs/xxgzbd/gzbd_index.shtml). Statistically, over 91.5% of the infected patients received different TCM therapies in China, and the overall effectivity rate was more than 90%.[6,7] A series of TCMs worked remarkably and were treated as star agents against COVID-19, such as Qing-Fei-Paidu decoction,[8,9] Huashi Baidu granules,[10] Lianhua-Qing-wen capsule,[11] Xuebijing injection,[12] and Toujie Quwen Granules.[13] However, there are thousands of herbs and different TCM practitioners who promote different opinions

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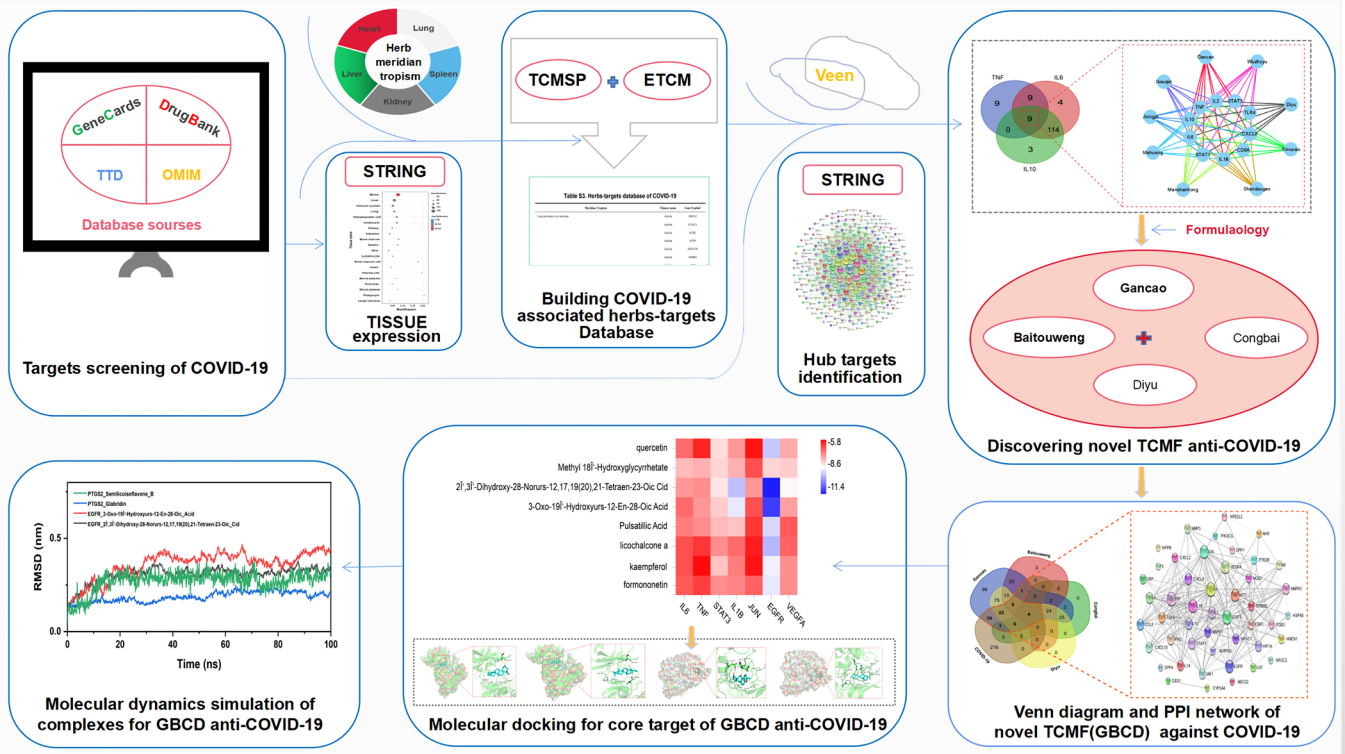

Figure 1. Workflow of the novel TCMF discovery for anti-COVID-19 based on the reverse systematic pharmacology strategy.

and habits regarding COVID-19. Therefore, the identification of a better and more effective TCM combination is very valuable.

Systemic pharmacology is a powerful tool to reveal action mechanisms based on in-hand databases and resources within the context of system-level interactions, especially for the studies of multicomponent TCM combinations.[14−16] For example, network pharmacology was employed, and it was predicted that the Toujie Queen granules might have therapeutic effects on COVID-19 by regulating viral infection and immune- and inflammation-related multitargets.[17] Furthermore, reports suggested that systemic pharmacology was not only applied to screen active ingredients and explain the mechanism of action but also used as a novel approach to design more effective but less toxic therapeutic agents.[18−20] In addition, computer-aided drug design (CADD) assumes an essential role in modern drug research and development; specifically, hierarchical scoring strategies (e.g., molecular docking and scoring, dynamics simulation, etc.) are used to balance the computing efficiency and accuracy respectively in both the lead discovery and optimization phases.[21−23]

Based on the outstanding contribution of the concept of "TCMF → target → disease (target)" of systemic pharmacology in explaining the potential mechanism of TCM, we speculated that the reverse concept of "disease → target → TCMF → disease", which is called reverse systematic pharmacology, combined with the TCM meridian tropism theory, may become a novel and reliable method for drug development in complex diseases (Figure 1). This technique is more efficient and comprehensive than existing animal and clinical-based drug investigations in identifying possible medications for epidemic disease outbreaks such as COVID-19 or other complicated diseases. Therefore, we proposed a rational TCM development strategy, which highlights the discovery of effective TCMF for complex diseases, including but not limited to COVID-19, and lays a foundation for the integration of the TCM theory and modern technology.

METHODS

Screening Potential Gene Targets of COVID-19. Potential COVID-19-associated gene targets were screened from Online Mendelian Inheritance in Man (OMIM) (https://omim.org/), DrugBank (https://go.drugbank.com/), Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd/), and GeneCards (https://www.genecards.org/). After removing duplicates, all targets were input to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (version 11.5) (https://string-db.org/) for building a target−target (T−T) network and enrichment analysis of tissue expression (TISSUES) and KEGG pathways. Network topological analysis was performed on Cytoscape 3.8.2 version,[24] and the resultant hub gene targets were collected by degree values.

Building a COVID-19-Associated Herb−Target Database. According to the TCM theory, diseases are caused by the abnormality of certain *Zang-fu* organs or meridians, and medicines exerting therapeutic effects also are organ- or meridian-selective (called meridian tropism), which may exert almost or even no effects on other organs or meridians (except for certain processing). Based on the TISSUES enrichment results of COVID-19-associated targets, the top three Zang-fu organs (heart, liver, spleen, lung, or kidney) were applied as disease organ selection or major meridian tropism. Meanwhile, an effective herb−compound database was built by online TCMSP[25] (https://tcmsp-e.com/) with parameters of oral bioavailability (OB) ≥40%, drug-likeness (DL) ≥0.18, and the Encyclopedia of Traditional Chinese Medicine (ETCM)[26] (http://www.tcmip.cn/ETCM/) database with a drug-likeness

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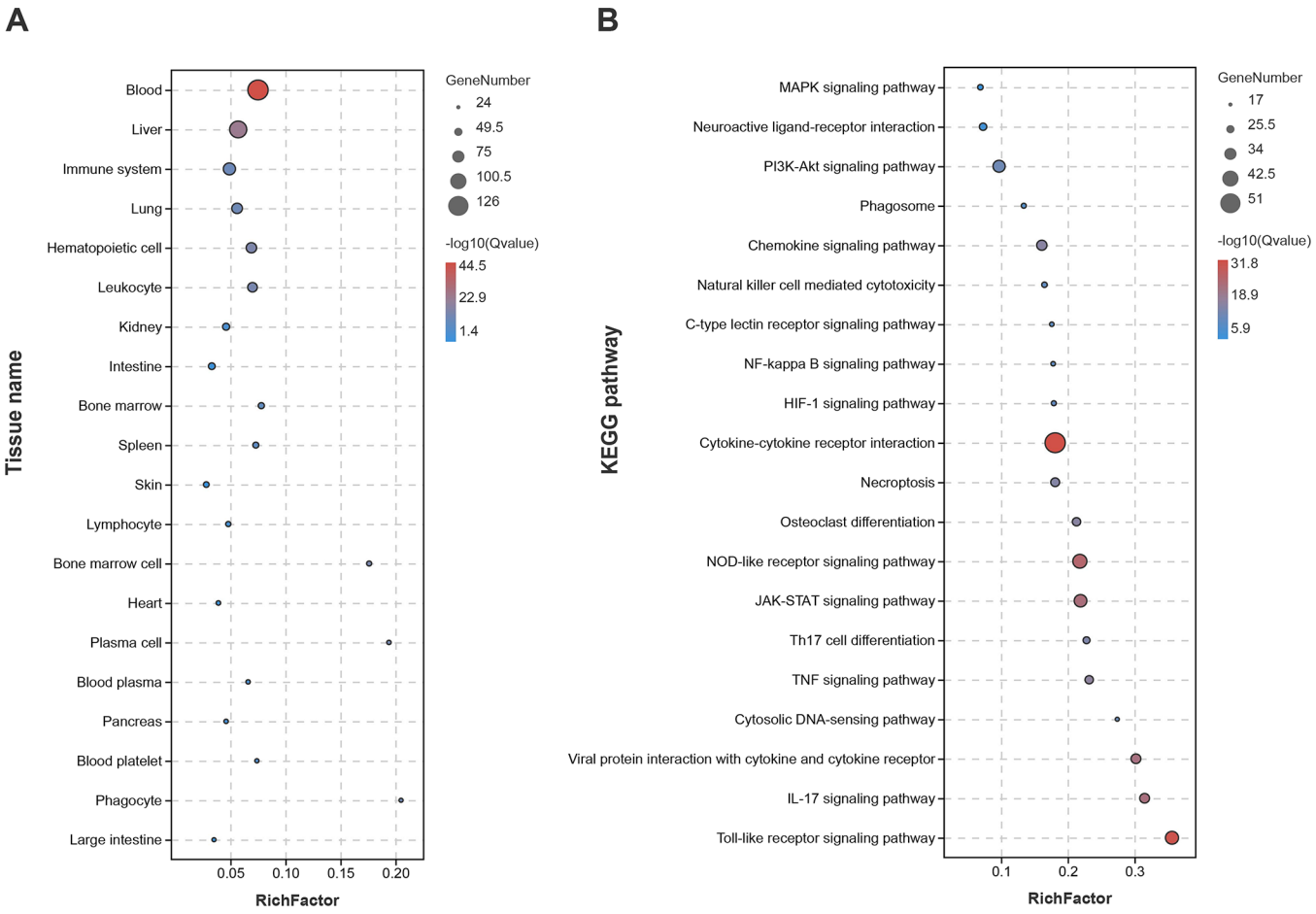

Figure 2. Enrichment analysis of COVID-19-associated genes. (A) Bubble diagram of top 20 enriched TISSUES terms of COVID-19. (B) Top 20 KEGG pathways of COVID-19 (excluding those related to specific diseases) presented by bubble.

weight of over 0.49, in which herbs matched at least one of the COVID-19-related *Zang-fu* meridian tropisms. By excluding the overlapping herbs and compounds, a COVID-19-related herb−compound−target database was constructed based on the TCMSP and ETCM databases. Furthermore, the KEGG pathway analysis for each herb was conducted using the STRING online platform.

Generating a Novel TCMF against COVID-19. Among the herbs in TCMFs, such as JUN-drug, CHEN-drug, ZUO-drug, and SHI-drug, prescriptions exclusively contain JUN-drug(s). JUN-drug, in particular, played a dominant role in the treatment of diseases, while others were auxiliary. Because the hub targets of the T−T network may play critical roles in COVID-19 therapy, we suggest that if the herb can match several hub targets, it should be deemed a JUN-drug. However, if multiple herbs match the same number of core genes, the one that has the most intersections with COVID-19-associated targets will be regarded as the JUN-drug. Common targets and KEGG pathways of JUN-drug and COVID-19 were obtained by Custom Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/) and STRING, respectively.

Previous pharmacological studies of Chinese classical prescriptions suggested that complementary drugs tend to act on similar functional pathways (KEGG pathways) or biological processes that have a synergistic therapeutic effect along with JUN-drug. Therefore, the 10 KEGG signal pathways that were mostly related to both JUN-drug and COVID-19 were employed to filter other complementary herbs in the COVID-19-associated herbal database as parameters for removing specific disease-related pathways. Then, we ranked those having the same observed gene count in parallel, and as a test study, the top four complements were selected to combine the TCMF with JUN-drug.

Potential Effective Analysis of the Novel TCMF against COVID-19. To assess the potential of the novel TCMF against COVID-19, all projected targets from the COVID-19-associated herb−target database were obtained, and Custom Venn diagrams were used to screen the common targets of this new TCMF and COVID-19. Furthermore, Gene Ontology (GO) and KEGG pathway enrichment of these overlaps were carried out by STRING (version 11.5).

Protein−Protein Interaction (PPI) Network Construction and Core Target Identification. Targets of the novel TCMF and COVID-19 were input into STRING (version 11.5) for constructing the PPI network. Network topological parameters were further analyzed by the Network Analyzer in Cytoscape software (version 3.8.2). Core targets were identified based on degree values (i.e., the quantity of linking edges), suggesting that the upper and lower limits were the maximum degree and twice the median degree of freedom, respectively.

Molecular Docking. To understand the binding details of core targets and related small ingredients, molecular docking was performed by AutoDock Vina program. First, the 3D protein structures of COVID-19-associated hub gene targets were obtained from the PDB database (https://www.rcsb.org/

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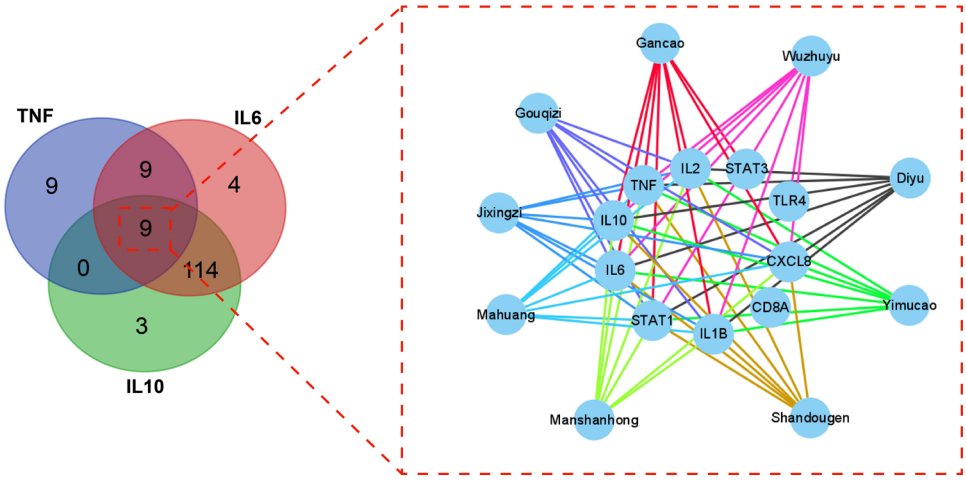

Figure 3. Venn diagram of the top 3 core target-associated herbs and detailed interactions between the 9 intersecting herbs and top 10 hub targets of COVID-19.

), and PyMOL software was used to dewater and remove the original ligands (a *pdb format file). After that, AutoDock Tools were used to perform hydrogenation, compute charges, and establish a docking box. The file was saved in the *pdbqt suffix format. Moreover, the 2D structure of herb components was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) with energy minimization performed using the MM2 module in ChemOffice software (version 2015) and saved in the *pdbqt suffix format by AutoDock Tools. In the last step, the molecular docking between COVID-19-associated hub targets and related active compounds of the novel TCMF found in the current study was finished by AutoDock Vina.[27]

Molecular Dynamics Simulation and Binding Free Energy Calculation. To further evaluate the relative stability of the ligand residing in the binding pocket, the general AMBER force field (GAFF)[28] was used for substrates, while the partial atomic charges were obtained from the RESP method by Multiwfn.[29] Na^+ or Cl^- ions were added to the protein surface to neutralize the total charges of the systems. The resulting systems were solvated in a rectangular box of TIP3P[30] waters extending up to a minimum cutoff of 10 Å from the protein boundary. The Amber ff14SB force field[31] was employed for the protein in all of the molecular dynamics (MD) simulations. The initial structures were fully minimized using a combined method of steepest descent and conjugate gradient. The systems were then gently annealed from 10 to 300 K under a canonical ensemble for 0.05 ns with a weak restraint of 15 kcal/mol/Å. 500 ps of density equilibration was performed under the isothermal−isobaric ensemble at a temperature of 300 K and a set pressure of 1.0 atm using the Langevin thermostat[32] and Berendsen barostat[33] with a collision frequency of 0.002 ns and a pressure−relaxation time of 0.001 ns. After proper minimizations and equilibrations, a productive MD run of 100 ns was performed for all of the complex systems. MD simulations were performed with GROMACS 2020.3.[34,35] Finally, the root-mean-square deviation (RMSD) and binding free energy calculated *via* MM/PPBSA[36,37] were used to evaluate the structural stability of each complex.

RESULTS

Screening Potential Gene Targets of COVID-19. A total of 325 genes were associated with COVID-19 according to OMIM, DrugBank, TTD, and GeneCards (with a standard relevance score of over 1.5) database (Table S1). The liver, lung, and kidneys were the top 3 TISSUES enrichments of ZANG organs after putting these genes into STRING. (Figure 2A). 149 KEGG pathways were related to COVID-19 (Table S2), in which the most interesting top 20 were the cytokine−cytokine receptor interaction, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, JAK-STAT signaling pathway, PI3K-Akt signaling pathway, chemokine-signaling pathway, IL-17 signaling pathway, and viral protein interaction with cytokine and cytokine receptor. (Figure 2B). Furthermore, the T−T network was constructed from STRING (minimum required interaction score set at 0.7) and imported to Cytoscape for topological analysis.

Building a COVID-19-Associated Herb−Target Database. Based on the results of tissue expression enrichment, the *Zang* organs, liver, lungs, and kidneys, were chosen as meridian tropism to screen herbs and predict related targets using TCMSP and ETCM platforms. After excluding duplicates and herbs with less than 3 targets, a total of 321 herbs and 1168 targets were obtained (Table S3). The top 10 KEGG pathways of each herb were next enriched by STRING (Table S4).

Generating a Novel TCMF against COVID-19. Topological analysis of the COVID-19-associated target−target network showed the degree-based top 10 core targets, including TNF, IL6, IL10, STAT3, IL1B, STAT1, TLR4, IL2, CD8A, and CXCL8 (Figure S1). For screening the JUN-drug, all COVID-19-related herbs were used to match these core targets in the order of degree from top to bottom. It was found that 9 herbs in the COVID-19 herb−target database with higher core target connections, that is, Gancao, Wuzhuyu, Diyu, Gouqizi, Jixingzi, Mahuang, Manshanhong, Shandougen, and Yimucao, were simultaneously associated with 3 hub targets (Figure 3). Surprisingly, both Gancao and Wuzhuyu match 8 of the top 10 core targets: Gancao targeted TNF, IL6, IL10, STAT3, IL1B, STAT1, IL2, and CXCL8, while Wuzhuyu targeted TNF, IL6, IL10, IL1B, STAT1, TLR4, IL2, and CXCL8 (Figure 3). Thus, Gancao may be the best JUN-drug based on our previous assumption.

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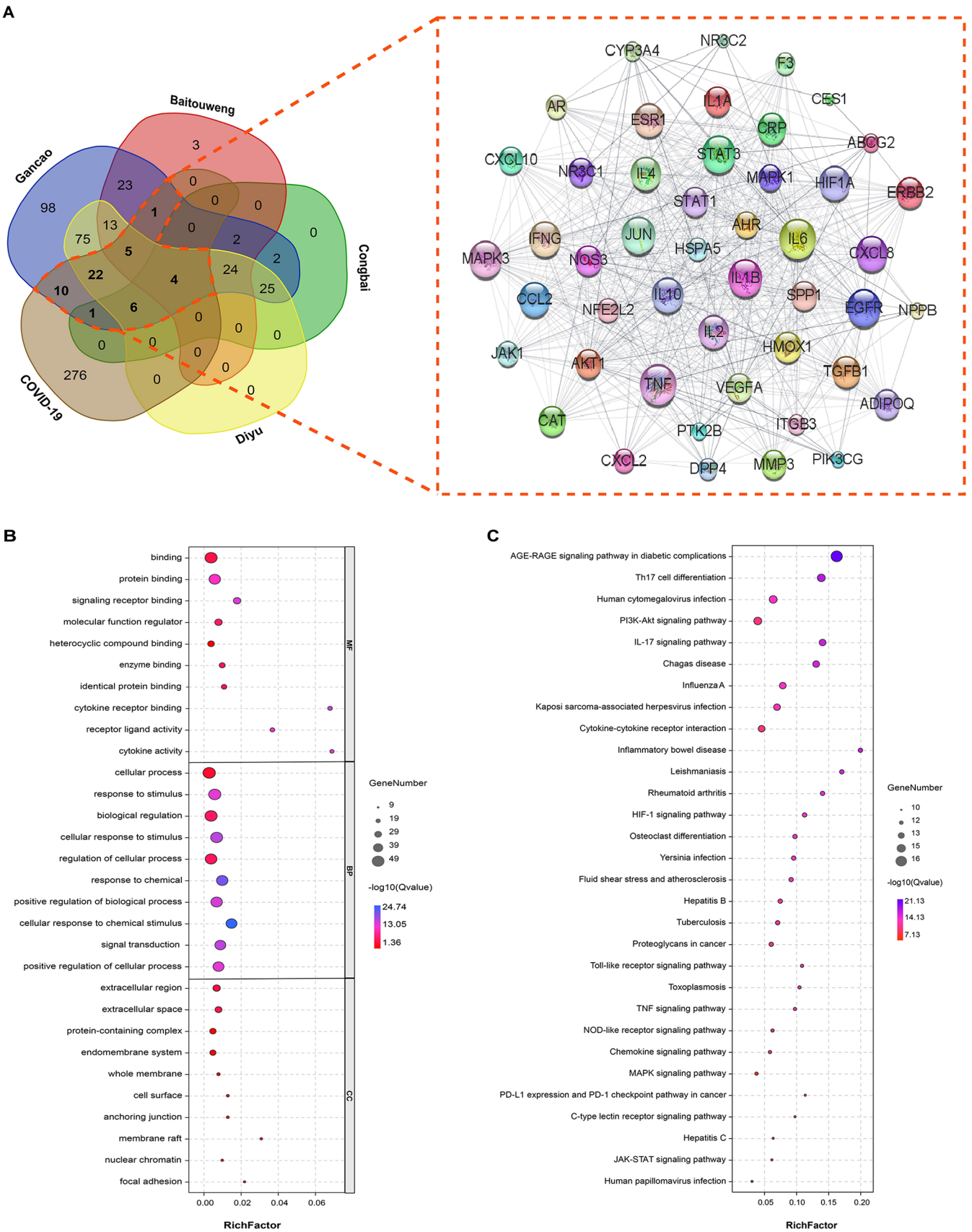

Figure 4. Function characterization of GBCD against COVID-19. (A) Venn diagram and PPI network of the 49 intersecting targets of GBCD and COVID-19. (B) GO enrichment analysis of the 49 common genes. (C) KEGG pathway analysis of the 49 genes.

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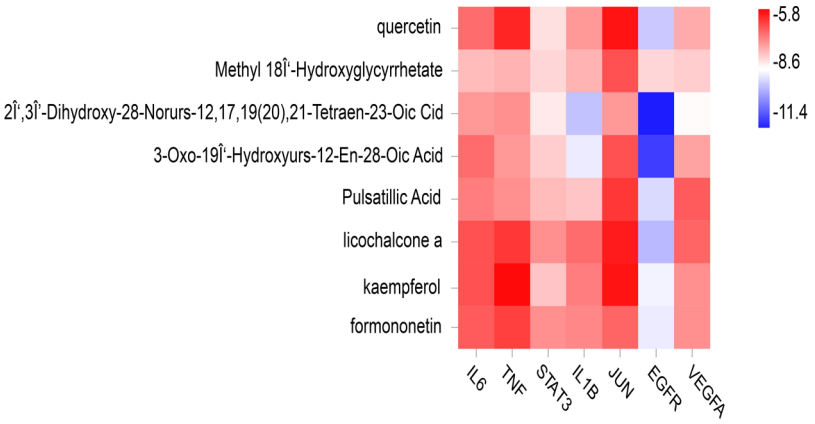

Figure 5. Heat maps presenting the docking results for the 7 core targets of COVID-19 binding with 8 components of GBCD; a deeper color shows lower binding affinity (kcal/mol).

Analysis of the KEGG pathway of Gancao suggested that a total of 142 enriched pathways mainly involved in cancer include the AGE-RAGE signaling pathway in diabetic complications, Th17 cell differentiation, human cytomegalovirus infection, PI3K-Akt signaling pathway, IL-17 signaling pathway, Chagas disease, influenza A, Kaposi sarcoma-associated herpes virus infection, cytokine−cytokine receptor interaction, and so on (Table S5). After excluding pathways related to a specific disease (such as pathways in cancer), the top 10 intersecting signal pathways with COVID-19 were identified: NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway, chemokine signaling pathway, IL-17 signaling pathway, TNF signaling pathway, osteoclast differentiation, neuroactive ligand−receptor interaction, Th17 cell differentiation, and MAPK signaling pathway.

The above-mentioned 10 signal pathways were first curated to recruit auxiliary herbs in the COVID-19-associated herbal database. We found that Baitouweng, Congbai, and Diyu had the highest intersecting pathways (8 overlaps), which were followed by Baishao, Heye, Nvzhenzi, Shandougen, and Xia Ku Cao (7 overlaps). As a case study, we finally chose Baitouweng, Congbai, and Diyu as three complementary herbs to construct the TCMF with Gancao (called GBCD).

Potential Effects of GBCD against COVID-19. A total of 49 targets of COVID-19 could be affected by GBCD (Figure 4A). Surprisingly, the Venn diagram indicated that Gancao could affect all GBCD-intersecting targets against COVID-19, but other complements could only affect some of them, including 37 Diyu intersections (EGFR, AR, ESR1, NR3C1, STAT1, MAPK1, HIF1A, JUN, ERBB2, VEGFA, IL6, AHR, AKT1, IL2, HSPA5, TGFB1, SPP1, NOS3, IL10, DPP4, IFNG, NFE2L2, CXCL2, IL1B, TNF, CXCL10, MMP3, ABCG2, CXCL8, CCL2, ADRA1B, CRP, F3, CYP3A4, HMOX1, NPPB, and IL1A), 11 Congbai intersections (AR, ESR1, STAT1, JUN, AHR, AKT1, DPP4, ADRA1B, CAT, CYP3A4, and HMOX1), and 10 Baitouweng intersections (AR, ESR1, NR3C1, IL6, DPP4, NR3C2, IL1B, TNF, ADRA1B, and NPPB).

GO analysis of the 49 intersecting genes yielded 1129 terms, including 53 molecular functions (MF), 1063 biological processes (BP), and 13 cellular components (CC). The top 10 MF, BP, and CC enrichment results are shown in Figure 4B. KEGG pathway analysis suggested that 142 signaling pathways were enriched and mostly linked to pathways in cancer, the AGE-RAGE signaling pathway in diabetic complications, Th17 cell differentiation, human cytomegalovirus infection, PI3K-Akt signaling pathway, IL-17 signaling pathway, Chagas disease, Influenza A, Kaposi sarcoma-associated herpes virus infection, and cytokine−cytokine receptor interaction (Figure 4C).

Core Target Identification of GBCD against COVID-19. Protein−protein interaction (PPI) networks of the 49 intersecting targets of GBCD against COVID-19 were constructed by the String platform. Topological analysis indicated that the maximum and median degrees of the PPI network were 30 (IL6) and 12 (CRP), respectively. Therefore, the screening criteria of the core targets of GBCD against COVID-19 were set as 24−30, and the identified core targets were IL6, TNF, STAT3, IL1B, JUN, EGFR, and VEGFA. Furthermore, we analyzed the relationships between the ingredients of GBCD and core targets. Quercetin (Gancao and Diyu) was found to bind with IL6, IL1B, JUN, EGFR, and VEGFA. Compounds of 2Î',3Î'-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic acid, and 3-oxo-19Î'-hydroxyurs-12-en-28-oic acid (Baitouweng) were found to bind with TNF. Pulsatillic acid of Baitouweng binds with IL6, IL1B, and TNF. Licochalcone-A (Gancao) binds with STAT3, kaempferol (Gancao, Congbai, and Diyu) binds with JUN, and formononetin from Gancao binds with JUN (Table S6).

Molecular Docking. To look closer at the potential binding of GBCD and COVID-19, crystal structures of 7 identified core targets IL6, TNF, STAT3, IL1B, JUN, EGFR, and VEGFA were obtained from the PDB database with IDs 1ALU, 7KP9, 6NJS, 5R85, 5T01, 5UG9, and 1MKK, respectively. In addition, 2D structures of 8 related components, i.e., methyl 18Î'-hydroxyglycyrrhetate (PubChem CID: 5319680), licochalcone a (PubChem CID: 5318998), formononetin (PubChem CID: 5280378), pulsatillic acid (PubChem CID: 10672073), quercetin (PubChem CID: 5280343), 3-oxo-19Î'-hydroxyurs-12-en-28-oic acid (PubChem CID: 12314450), 2Î',3Î'-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic acid (PubChem CID: 11568667), and kaempferol (PubChem CID: 5280863) were downloaded from the PubChem platform. We not only performed the docking of these core targets with their predicted ingredients, but an intersecting binding with the remaining compounds was also attempted. As shown in Figure 5, all docking results revealed that most of the screened active compounds of GBCD had a significant binding activity with

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Table 1. Top 10 Results of PTGS2 Docked with Its Targeted Compounds in GBCD

rank	compound	PubChem CID	affinity (kcal/mol)
1	glabridin	124052	−11.2
2	semilico isoflavone B	5481948	−11.1
3	kanzonols W	15380912	−10.8
4	glycyrol	5320083	−10.6
5	xambioona	14769500	−10.6
6	glycyrrhiza flavonol A	5317765	−10.5
7	licoisoflavone	5281789	−10.5
8	phaseol	44257530	−10.5
9	(−)-medicocarpin	23724664	−10.4
10	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl)chromone	14604081	−10.3

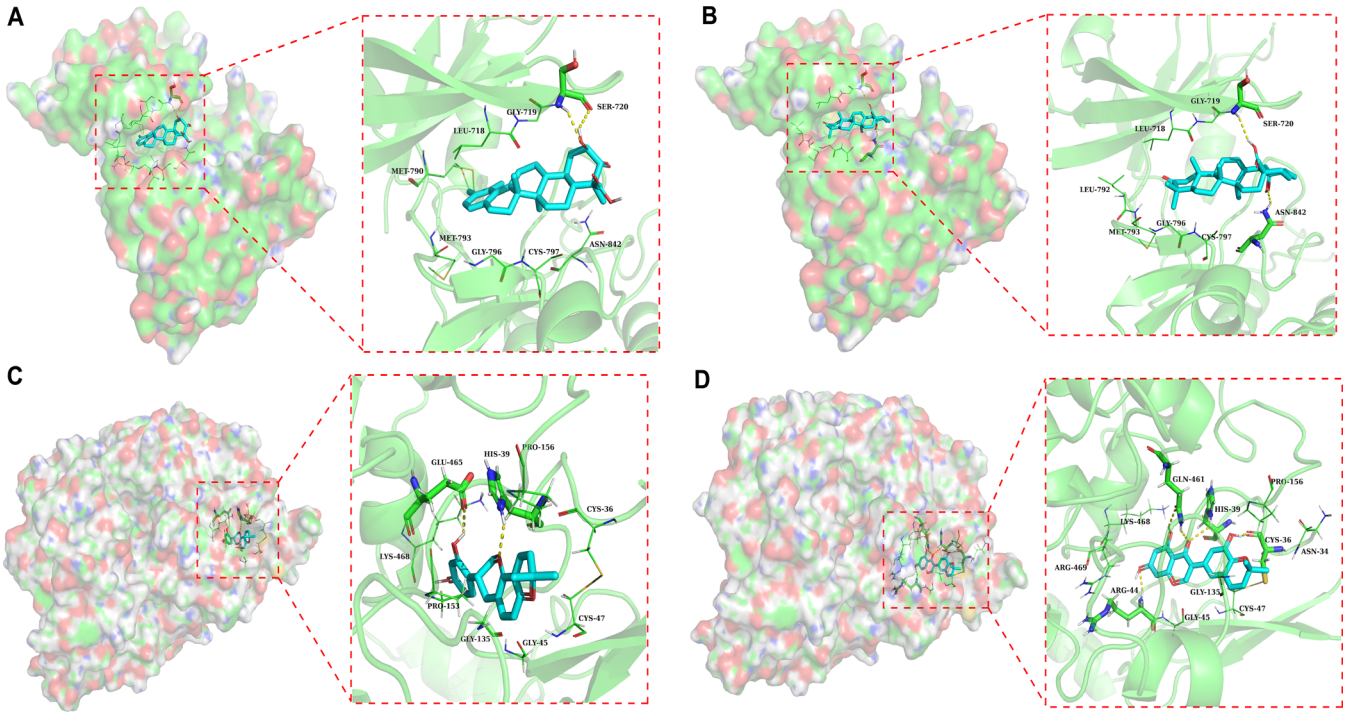

Figure 6. Molecular docking of GBCD with EGFR and PTGS2 of COVID-19. Binding pose of EGFR with (A) 2Î′,3Î′-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic acid and (B) 3-oxo-19Î′-hydroxyurs-12-en-28-oic acid. Hydrogen bond of PTGS2 with (C) glabridin and (D) semilico isoflavone B.

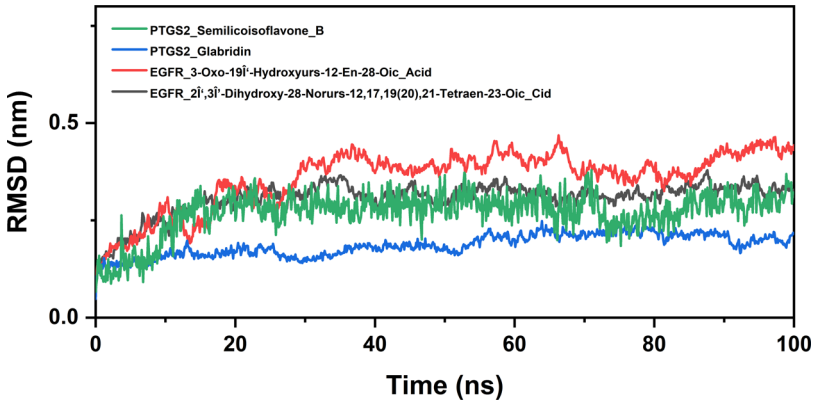

Figure 7. RMSD fluctuations of backbone atoms of the four complexes with MD simulation time.

core targets of COVID-19 (Table S7). Interestingly, the epidermal growth factor receptor (EGFR) and 2Î′,3Î′-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic acid had the highest binding affinities (−11.4 kcal/mol), which was due to a lack of targeting information in TCMSP and ETCM.

Furthermore, we discovered 72 GBCD active components that were targeted with PTGS2, a protein that activates COX2

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Table 2. Binding Free Energies (kcal/mol) of Complexes of GBCD Anti-COVID-19

complex no.	ΔE_{VDW}	EEL	EGB	ESURF	ΔE_{gas}	ΔG_{sol}	ΔE_{total}
PTGS2_glabridin	−48.0019	−23.2499	34.6936	−5.6052	−71.25	29.0884	−42.1616
PTGS2_semilicoisoflavone_B	−44.9241	−10.959	28.6759	−5.7038	−55.8831	22.972	−32.9111
EGFR_3-oxo-19Î′-hydroxyurs-12-en-28-oic_acid	−37.1888	−5.6789	17.7994	−4.345	−42.8677	13.4544	−29.4133
EGFR_2Î′,3Î′-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic_acid	−41.9357	−17.5412	27.7378	−5.6716	−59.4773	22.0662	−37.4112

and stimulates inflammatory cascades in SARS-CoV infections of the lungs.[38,39] The protein was ignored in previous screenings due to a lower relevance score with COVID-19. We found a docking score from −11.2 to −7.5 kcal/mol, and Glabridin (−11.2 kcal/mol) had the peak binding activity with TPGS2, followed by semilico isoflavone B (−11.1 kcal/mol), kanzonols W (−10.8 kcal/mol), glycerol (−10.6 kcal/mol), and xambioona (−10.6 kcal/mol) (Tables 1 and S8). The binding pose and hydrogen bond of EGFR docked with 2Î′,3Î′-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic acid and 3-oxo-19Î′-hydroxyurs-12-en-28-oic acid and of PTGS2 with glabridin and semilico isoflavone B are displayed in Figure 6A−D, respectively.

Molecular Dynamics Simulation. In addition, for each MD trajectory, the RMSD from the average structure of backbone atoms was determined in order to investigate the "position stability" of the initial conformation. The RMSD of the backbone atoms of the complex system is illustrated in Figure 7. During the 100 ns simulation, the conformation of all systems reached a steady state because the RMSD for the original complex structures of EGFR_2Î′,3Î′-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic_acid system and EGFR_3-oxo-19Î′-hydroxyurs-12-en-28-oic_acid systems remained around 3.0 and 4.0 Å, respectively, whereas those of PTGS2_glabridin and PTGS2_semilicoisoflavone_B fluctuated by about 1.8 and 2.7 Å.

In order to derive detailed information about the simulated complexes, we used MM-PBSA to compute the binding free energy (Table 2). The ΔE_{total} (total binding free energy) for PTGS2_glabridin complex, PTGS2_semilicoisoflavone_B complex, EGFR_3-oxo-19Î′-hydroxyurs-12-en-28-oic_acid complex, and EGFR_2Î′,3Î′-dihydroxy-28-norurs-12, 17, 19 (20), 21-tetraen-23-oic_acid complex were −42.1616, −32.9111, −29.4133, and −37.4112 kJ/mol, respectively, demonstrating a strong binding affinity, which was validated by molecular docking studies.

DISCUSSION

Since the outbreak of COVID-19 in 2019, TCM has shown extraordinary achievements in combating this virus in China.[40] According to the TCM theory, COVID-19 is characterized by "dampness, heat, toxin, and stasis," which characterize a wet hot pestilence disease. Early-stage treatment should be dampness-resolving and exterior-releasing, middle-stage should be heat-clearing and detoxifying, and end-stage should strengthen the Qi.[41] Heat-clearing and detoxifying drugs, such as the Lian-Hua-Qing-Wen capsule and Qing-Fei-Pai-Du decoction, show satisfying therapeutic effects.[42,43] However, despite a lot of efforts, the number of infections and deaths due to COVID-19 has increased, and highly transmissible variants, such as Delta, continued to emerge, with yet no magic bullet discovery.[44,45]

In this study, we discovered a new herbal combination named GBCD for treating COVID-19, consisting of Gancao, Baitouweng, Congbai, and Diyu, based on novel systemic pharmacological strategies. According to the Chinese Pharmacopoeia (2020 edition)[46] and Sheng Nong's herbal classic, Gancao helps eliminate heat, resolve toxins, strengthen the spleen, and enhance the Qi, which is balanced by other herbs. Baitouweng, a detoxicating and heat-clearing agent, enters the meridians of the liver, large intestine, and stomach. Congbai can reduce external syndrome through diaphoresis and make expectoration easier by penetrating the lungs and stomach meridians. Lastly, Diyu has properties of blood-cooling and detoxification related to the liver and large intestine meridians.[47] Modern pharmacological studies have also proved that these herbs have significant antiviral, anti-inflammatory, and immune-regulating effects.[48−57] According to a review of their numerous pharmacological actions, Gancao extracts were expected to fight COVID-19 in two clinical studies conducted in Egypt (NCT04487964) and Iran (IRCT20200506047323N2).[58,59] As a result, the present GBCD may have promising COVID-19 effects. Early studies have shown that IL6, TNFα, IL1B, IL10, IL1RA, and IL8 (also known as CXCL8), VEGF, and IL7 of COVID-19 patients were higher than those of their healthy counterparts, among which TNFα, IL2, and IL10 of ICU patients were much higher than those of non-ICU patients.[1] IL6 was also found to be closely related to severe COVID-19, suggesting that IL6 is an important clinical biomarker for early diagnosis as well as clinical severity.[60] This evidence suggested that COVID-19 was strongly associated with cytokine storm. In this study, a total of 325 COVID-19-associated genes were collected from databases. Topological analysis of the target network yielded the top 10 core targets, including TNF, IL6, IL10, STAT3, IL1B, STAT1, TLR4, IL2, CD8A, and CXCL8, which was consistent with previous findings of laboratory characteristics in COVID-19 patients. Based on hub targets and the TCM theory, after a rigorous converse-target screening, a novel herb combination named GBCD was developed. We also found that a total of 314 genes were targeted by GBCD, of which 49 were disease-related, including IL6, TNFα, IL2, IL10, VEGFA, and CXCL8, which may be considered as therapeutic biomarkers of COVID-19. GO and KEGG pathway analyses of the 49 convergent targets suggested that GBCD could exhibit anti-inflammation, anti-viral, immuno-regulation, and cytoprotection activities, which are exemplified by impacting the enzyme binding, cytokine receptor binding, and cytokine activity, as well as regulating diseases associated with signaling pathways, such as the cytokine−cytokine receptor interaction, MAPK, TNF, PI3K-Akt, Th17 cell differentiation, IL17, JAK-STAT signal pathway, etc.

Furthermore, molecular docking research demonstrated that the most active screened GBCD compounds displayed outstanding binding activity with COVID-19 core targets, with the binding affinity ranging from −5.8 to −11.4 kcal/mol. The binding energy predicted by MM-PBSA was modified from −29.4133 to −42.1616 kJ/mol, and the RMSD of the 4 simulated complexes remained generally steady. A previous study also demonstrated that hydrogen bonding is much

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ACS Omega 2023, 8, 26871−26881

stronger than other forces if both surfaces are supportive to form hydrogen bonds.[61] In the current study, there were 2, 2, 2, and 5 hydrogen bonds in the complexes EGFR_3-oxo-19Î′-hydroxyurs-12-en-28-oic_acid, EGFR_2Î′,3Î′-dihydroxy-28-norurs-12,17,19(20),21-tetraen-23-oic_acid, PTGS2_glabridin, and PTGS2_semilicoisoflavone_B, respectively. Overall, the above findings indicated that GBCD might be potent in combating COVID-19.

LIMITATIONS

To begin with, experimental data are required to supplement the current findings, which were based only on database analysis, resource mining, and computer-aided modeling. In view of the novel idea, a potentially more effective TCMF (GBCD) was developed, and we hope our work will be validated *in vivo* in future studies. Moreover, although two commonly used TCM databases were adopted to construct the disease-related herbal database, it is yet difficult to balance the (dis)advantages of existing databases. Therefore, we anticipate that a public and more powerful TCM-related database can be developed. Lastly, the imbalance of research on TCM herbs could limit our understanding of pharmacological mechanisms and information to some extent, which suggests the need for a more precise linkage of the relevant research on the pharmacology of Chinese medicines.

CONCLUSIONS

In conclusion, we provided a notion of reverse pharmacology mixed with the meridian tropism theory for TCMFs against emergencies or complex diseases based on network pharmacology, and we effectively developed a novel herbal combination for treating the COVID-19 infection. Analysis of the TCM theory, modern pharmacological research, and computer simulation suggested that the GBCD developed in this study might have promising effects on COVID-19, for which *in vivo* validations are needed in the future. Our findings may highlight the discovery of an effective TCMF for complex diseases, including but not limited to COVID-19. This work also laid the foundation for integrating the TCM theory and modern technology.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01489.

COVID-19 associated genes; KEGG pathway enrichment of COVID-19-associated genes; herb−target database of COVID-19; top 10 KEGG pathways of COVID-19-associated herbs; protein−protein interaction network of COVID-19-associated targets; Gancao-related KEGG pathways; GBCD-related herbs, ingredients, and targets; docking results for 7 core targets of GBCD anti-COVID-19 with 8 components of GBCD; docking results for PTGS2 with 72 ingredients of GBCD (PDF)

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Author Contributions

H.C., X.Z., and R.C. designed the study. H.C., J.L., Y. Luo, R.L., and Z.X. collected disease-related targets and constructed the COVID-19-related herbs−targets database. H.C. and X.Z. analyzed the data and drafted the manuscript. Y.B. and Y. Liu revised and approved the manuscript. All authors contributed to the article and approved the submitted version.

Notes

The authors declare no competing financial interest.

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