Exploration of molecular targets and mechanisms of Chinese medicinal formula Acacia Catechu -Scutellariae Radix in the treatment of COVID-19 by a systems pharmacology strategy

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Abstract
Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In China, the Acacia catechu (AC)-Scutellariae Radix (SR) formula has been widely used for pulmonary infection in clinical practice for several centuries. However, the potential role and mechanisms of this formula against COVID-19 remains unclear. The present study was designed to dissect the active ingredients, molecular targets, and the therapeutic mechanisms of AC-SR formula in the treatment of COVID-19 based on a systems pharmacology strategy integrated by ADME screening, target prediction, network analysis, GO and KEGG enrichment analysis, molecular docking, and molecular dynamic (MD) simulations. Finally, Quercetin, Fisetin(1-), kaempferol, Wogonin, Beta-sitosterol, Baicalein, Skullcapflavone II, Stigmasterol were primarily screened to be the potentially effective active ingredients against COVID-19. The hub-proteins were TP53, JUN, ESR1, MAPK1, Akt1, HSP90AA1, TNF, IL-6, SRC, and RELA. The potential mechanisms of AC-SR formula in the treatment of COVID-19 were the TNF signaling pathway, PI3K-Akt signaling pathway and IL-17 signaling pathway, etc. Furthermore, virtual docking revealed that baicalein, (+)-catechin and fisetin(1-) exhibited high affinity to SARS-CoV-2 3CLpro, which has validated by the FRET-based enzymatic inhibitory assays with the IC50 of 11.3, 23.8, and 44.1 μM, respectively. And also, a concentration-dependent inhibition of baicalein, quercetin and (+)-catechin against SARS-CoV-2 ACE2 was observed with the IC50 of 138.2, 141.3, and 348.4 μM, respectively. These findings suggested AC-SR formula exerted therapeutic effects involving “multi-compounds and multi-targets.” It might be working through directly inhibiting the virus, improving immune function, and reducing the inflammatory in response to anti-COVID-19. Ultimately, this study would provide new perspective for discovering potential drugs and mechanisms against COVID-19.

KEYWORDS
Acacia Catechu-Scutellariae Radix formula, COVID-19, molecular docking, molecular dynamic simulation, network pharmacology
INTRODUCTION

The number of confirmed infections has climbed to more than 458.5 million and approximately 6.1 million people dead globally since the outbreak of coronavirus disease 2019 (COVID-19) in December 2019 (https://covid19.who.int/), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Lipsitch, Swerdlow, & Finelli, 2020). Infection with the SARS-CoV-2 may result in lung inflammation and infiltration, with the frequent manifestations including fever, coughing, and shortness of breath (Chen et al., 2020; Merza et al., 2021). Severe instances could develop into acute respiratory distress syndrome (ARDS), which results in systemic inflammatory cytokine storms, with unpredictably negative effects for the outcome of the disease (Richardson et al., 2020; Wiersinga, Rhodes, Cheng, Peacock, & Prescott, 2020). The worldwide spread of COVID-19 has posed a profound threat to human health, and the implementation of vaccines is still a major asset in slowing down the pandemic by far (Fiolet, Kherabi, CJ, Ghosn, & Peiffer-Smadja, 2022; Huang, Bai, He, Xie, & Zhou, 2020). However, vaccines are slightly less effective against worrisome variants like Delta and Omicron, while there are remain few specific drugs to cure SARS-CoV-2 infection on the market as of the end of 2021 (Brüssow, 2021; Mohammad, Shayjestehpour, & Mirzaei, 2021). Therefore, the global hunts for potential pharmaceuticals in adequately managing this disease are extremely urgent. In this regard, medicinal plants containing specific phytomoieties may provide a broad range of potential therapeutic applications (Anand et al., 2021; Brendler et al., 2021; Das et al., 2021).

Traditional Chinese Medicine has a history of almost 3,000 years in the prevention and treatment of infectious diseases. It has demonstrated extraordinary benefits in preventing and treating viral respiratory diseases such as MERS, SARS (Hsu et al., 2006; Lau et al., 2005), and H1N1 (Wang et al., 2011) in recent years and provides a number of distinct features, including preventive treatment of disease, therapy based on syndrome differentiation, and multitarget intervention (Xian et al., 2020). During the fight against the COVID-19, TCM has also made great contributions in improving cure rate, shortening illness duration, delaying disease progression, and reducing mortality rate (Ren, Zhang, & Wang, 2020; Shahrajabian, Sun, Soleymani, & Cheng, 2021; Zhang et al., 2021). According to syndrome differentiation-based therapy theory of TCM, the pandemic falls under the classification of “damp epidemic”. The damp epidemic pathogen prefers and is mostly located in the lung, with the sickness affecting the spleen and stomach as well as the liver and kidneys in extreme cases (Shi et al., 2020; Yuan, Xin, Tang, & Cong, 2020). The pathophysiology is centered on a dampness-toxin obstructing the lung and suppressing Qi, which manifests as dampness, heat, poison, blood stasis, and deficiency (Qiu et al., 2020; Ren et al., 2021). As a result, Chinese physicians regard “expelling evil and detoxifying, drying moisture, and removing blood stasis as the most significant aspect” in the face of COVID-19.

Acacia catechu (AC), also called Er-Cha in Chinese, is a traditional medicinal plant having antitussive, antipyretic, hemostatic, and hepatoprotective properties that is widely used in China, India, and Southeast Asia (Khare, 2007). Scutellariae Radix (SR), also known as Huang-Qin in Chinese, is a traditional Chinese herbal medicine that has historically been used to treat respiratory inflammatory or viral infections which cause throat swelling and soreness, pneumonia, and fever (Song et al., 2020). Actually, in clinical practice over the last several centuries, AC and SR have been regularly recommended in combination, famous as Huang-qin Er-cha Decoction, for the prevention or treatment of cough, phlegm, and fever caused by pulmonary infection in China (Wang et al., 2019). The combinational prescript of the two medications was believed to enhance their preventative or therapeutic benefits in the treatment of pulmonary disease. As demonstrated in our previous studies, AC-SR formula exerted a strong anti-inflammatory effect in LPS-induced ALI. AC-SR formula dramatically lowered the wet-to-dry weight ratio of the lungs, ameliorated LPS-induced lung histopathological alterations, decreased inflammation, blunted the production of proinflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). The putative molecular mechanism for the protective effect of AC-SR formula against ALI is responsible for the attenuation of the NF-B activation, blunted the production of proinflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). The application of systems biology methods to the research of SARS-CoV-2 is not only beneficial in deciphering the pathogenesis, as well as the molecular interactions that occur during infection, but also help to develop novel treatment strategies for the COVID-19 pandemic (Banaganapalli et al., 2021; Wynants et al., 2021). Network pharmacology has been offered as a promising method to dissect herbal formulas and predict potential new drugs or targets for the COVID-19 (Hong, Duan, Wu, Yang, & Wu, 2020; Tao et al., 2021; Xia et al., 2020), while molecular docking as well as molecular dynamics simulations represent an unique avenue for structural molecular biology and the computer aided drug design in the development of novel medications (Aljarba, Hasnain, Bin-Merief, & Alkahtani, 2022; Morris & Lim-Wilby, 2008; Saikia & Bordoloi, 2019; Wang et al., 2021). In this study, a systems pharmacology strategy (Figure 1) integrated by network pharmacology, molecular docking, and molecular dynamic simulation were...
adopted to investigate the mechanism of action underlying the effectiveness of AC-SR formula in COVID-19 therapy. It would provide new perspective for discovering potential drugs and mechanisms against COVID-19 and the developed strategy could also be able to serve as role models for the research and development of other natural medicines.
2 | MATERIALS AND METHODS

2.1 | Identification and screening of active compounds

Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmsp-e.com/), Traditional Chinese Medicines Integrated Database (TCMID, http://www.megabionet.org/tcmid/), and Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org/batman-tcm/) were used to authenticate all compounds of the AC-SR formula. We selected compounds of AC-SR formula according to the criterion of oral bioavailability (OB) ≥30% and drug likeness (DL) ≥ 0.18, which are the most important indicators for evaluating the characteristics of absorption, distribution, metabolism, and excretion (ADME) (Xu et al., 2012). In addition, we also search a large-scale text and selected oral absorbable compounds with pharmacological activity, in order to supplement the compounds.

2.2 | Identification of protein targets

The protein targets associated with active compounds were retrieved from the TCMSP database (https://old.tcmsp-e.com/index.php), which provided information of 6,511 drug molecules and 3,987 targets as well as the interactions between them (Ru et al., 2014). Then, Universal Protein Knowledgebase (Rolf et al., 2004) (UniProt, http://www.uniprot.org), an authoritative database of protein sequences, which comprised 54,247,468 sequence items was used to extract the targets, including the gene names and gene ID.

2.3 | Predicting the targets of COVID-19

We collected different genes associated with COVID-19 from four resources (Fan et al., 2021). (1) Human Gene Database (GeneCards, https://www.gene_cards.org/), (2) Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/), (3) Comparative Toxicogenomics Database (CTD, http://ctdbase.org/), and (4) DisGeNET database (https://www.disgenet.org/). The search keywords were “Coronavirus Disease 2019,” “SARS-CoV-2,” and “MERS-COV.” The results of the above databases were integrated to obtain the targets of COVID-19 after deleting the repeated genes. To acquire candidate targets of AC-SR formula acting on COVID-19, we integrated the compounds’ predicted targets of AC-SR formula with target genes of COVID-19 and chose those replicate genes.

2.4 | Construction of protein–protein interactions (PPI) network

In order to further analyze the interaction between target proteins, the targets of AC-SR formula and COVID-19 were imported into the STRING database (https://string-db.org/) to build the PPI network interaction (Consortium UP, 2021). Cytoscape V3.7.1 was used to construct and visualize the PPI network (Shannon et al., 2003). “Degree” referred to the number of connections of the node in the whole network, which reflected the interaction information between nodes. The value of ‘Degree’ was used as a reference for the importance of the core target.

2.5 | GO and KEGG pathway enrichment analysis

To investigate the functional annotation and involved pathways of genes. Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were implemented using Org.Hs.eg.db (Version 3.8.2) and ClusterProfiler (Version 3.9) packages in R (Version 3.6.2) (Tao et al., 2021). An adjusted p-value of ≤ 0.05 was considered to identify the enrichAC-SR formula terms.

2.6 | Component-target molecular docking

The three-dimensional (3D) structure of the key target protein was downloaded from the RCSB PDB protein structure database (https://www.rcsb.org/). AutoDock Vina (version 1.5.6) was used to remove the water molecules, isolate proteins, add the nonpolar hydrogen, and calculate Gaussian charges for the structure (Du et al., 2022; Trott & Olson, 2010). Then, the corresponding format components and target proteins were signed in https://www.swissdock.ch/docking website to conduct the molecular docking experiment, and the docking binding energy [Estimated ΔG (kcal mol⁻¹)] was predicted. According to the score of binding energy, the binding ability of active ingredients to certain target proteins was verified. Meanwhile, ritonavir, Nirmatrelvir, a potent SARS-CoV-2 3CLpro protease inhibitor (Reina & Iglesias, 2020), and SSAA09E2, an inhibitor of ACE2 (Bibi, Gul, Ali, & Kamal, 2021), were taken as positive control. The binding energy ≤ 5 kcal/mol demonstrated a very strong binding force (Fan et al., 2021).

2.7 | Molecular dynamic (MD) simulation

The molecular dynamics simulation study was performed using the Discovery Studio (DS) 2019 software package to evaluate the stability and interaction of ACE2 and 3CL receptors with Quercetin and Baicalin. The ligand-receptor complex was placed in an orthorhombic box and solvated using an explicit periodic boundary solvated water model. Then sodium chloride was added to the system with the ionic strength of 0.145 to simulate the physiologic environment. Afterward, we subjected the system to the CHARMM force field (Li et al., 2021) and relaxed it through energy minimization (500 steps of conjugate gradient and 500 steps of steepest descent). And the final root means square gradient was 0.991. Then, the system’s temperature was slowly driven from an initial temperature of 50 K to the aimed temperature of 300 K within 4 ps. The time of equilibration simulations
was 20 ps. Molecular dynamics simulation (production module) lasted for 200 ns with 1 ns time step. We completed the simulations under the normal pressure and the relatively constant temperature of nearly 300 K throughout the procedure. The particle mesh Ewald algorithm was applied for the calculation of long-range electrostatics. And the linear constraint solver algorithm was adapted to identify all bonds involving hydrogen. The MD trajectory was written with 1,000 frames during the entire simulation run but only initial protein backbone frames were aligned to understand the stability of ligand-protein complex. The root means square deviations (RMSD) and root mean square fluctuation (RMSF) were used to understand the stability of complex, which were essential to infer good binding affinities (Doniach & Eastman, 1999; Dubey, Tiwari, & Ojha, 2013).

2.8 | 3Clpro and ACE2 enzyme inhibition test

To further identify the possible inhibitory activities of the screened molecules against SARS-CoV-2 3Clpro, and ACE2, the compounds were tested by two commercial kits (enhanced 2019-nCoV Mpro/3Clpro inhibitor screening kit, P03155, Beyotime Biotech, Shanghai, China; ACE2 inhibitor screening kit, P0320S, Beyotime Biotech, Shanghai, China) based on fluorescence resonance energy transfer (FRET) protease assay (Xu et al., 2021), respectively. According to the operating instructions, ebselen and MLN-4760 were chosen as the positive control, respectively. Enzyme activities were measured with saturated substrate concentration and different inhibitor concentrations. The enhanced fluorescence emission upon substrate cleavage was monitored at the excitation and emission wave lengths of 325 and 393 nm. The 50% inhibitory concentrations (IC 50) were determined by plotting curves of percent inhibition versus compound concentration. Results are reported as IC50 values.

3 | RESULTS

3.1 | Active compounds of AC-SR formula

In total, 126 compounds of AC-SR formula were obtained from the TCMSP, TCMID, BATMAN-TCM database, and relevant documents, including 33 from AC, 93 from SR. Besides, 89 active compounds were screened according to OB ≥30% and DL ≥0.18, including 23 from AC and 66 from SR. After eliminating repeated active compounds (MOL002914, MOL000073), 42 active compounds were retrieved. Information of some active compounds in AC-SR formula is outlined in Table 1.

3.2 | Collection of target information and construction of active component-target network

From the TCMSP and SIB platform, 367 targets of the 42 active compounds were obtained, including 250 from AC and 117 from SR. After eliminating repeated targets, 288 targets and their abbreviations remained. The gene names corresponding to the proteins were found in UniProt. The active ingredient-target network was constructed using the network analysis software Cytoscape (version 3.7.1), and results are depicted in Figure 2. The octagon node represented the active ingredients of AC-SR formula, and the triangle node represented the target genes. Edges represent interaction between compounds and targets. The size of the shape represented the degree of node association, the more connected the edges are, and a higher degree value is obtained. We list the top 8 components in Table 2 according to degree value between the components and the targets. It included MOL000098-quercetin, MOL54758660-Fisetin(1-), MOL000422-kaempferol, MOL000173-Wogonin, MOL00358-Betasitosterol, MOL002714-Baicalin, MOL002927-Skullcapflavone II, and MOL000449-Stigmasterol, with 143, 100, 61, 45, 38, 36, 33, and 31°, respectively.

3.3 | Potential targets of AC-SR formula in the treatment of COVID-19

A search of the GeneCards, DisGeNET, TTD, and CTD databases identified 7,659 target genes linked with COVID-19. Venn diagrams indicated a total of 209 AC-intersection and 99 SR-intersection targets against COVID-19. Then, a PPI network was constructed to integrate the targets to obtain the intersection. The targets are represented by the circle nodes, while interaction between targets is represented by edges. The degree of node linkage is symbolized by the size and depth of the circle, the more linked the edges are, the greater the degree value. Finally, we obtained 7 key targets from AC and 6 key targets from SR. After eliminating repeated targets, TP53, JUN, ESR1, MAPK1, Akt1, HSP90AA1, TNF, IL-6, SRC, and RELA were considered to be the hub genes.

3.4 | GO functional enrichment and KEGG pathway enrichment analyses

To further analyze the target genes, 209 AC-intersection and 99 SR-intersection targets against COVID-19 were implemented using Org. Hs.e.g.db and ClusterProfiler packages in R for GO analysis and KEGG pathway analysis, respectively. The results showed the top 8 significantly enriched terms in biological processes (BP), cellular components (CC), and molecular functions (MF) (Figure 4a, p < 0.05). BP mainly included response to drug, cellular response to chemical stress, response to oxidative stress, etc. CC mainly included membrane raft, membrane microdomain, protein kinase complex, etc. MF mainly included DNA-binding transcription factor binding, carbonat dehydratase activity, RNA polymerase II-specific DNA-binding transcription factor binding, etc. Similarly, the GO function histogram of SR in COVID-19 treatment was shown in Figure 4c.

The first 20 enriched KEGG pathways of AC in the treatment of COVID-19 were shown in Figure 4b (p < 0.05). The Y-axis represents...
| No. | Mol ID   | Chemical component                                           | OB (%) | DL       | Herb                | Structure                              |
|-----|----------|-------------------------------------------------------------|--------|----------|---------------------|----------------------------------------|
| 1   | MOL008420 | 5-Hydroxy-2-[2-(4-hydroxyphenyl)acetyl]-3-methoxy-benzoic acid | 93.33832 | 0.20887  | Acacia catechu      | ![Structure](image1)                    |
| 2   | MOL000492 | (+)-catechin                                                 | 54.82643 | 0.24164  | Acacia catechu      | ![Structure](image2)                    |
| 3   | MOL008428 | 3,4,8,10-tetrahydroxy-5H-chromeno[3,2-c]isochromen-7-one    | 50.53474 | 0.49886  | Acacia catechu      | ![Structure](image3)                    |
| 4   | MOL008426 | (R)-2,6-dihydroxy-2-(4-hydroxybenzyl)-4-methoxybenzofuran-3(2H)-one | 49.8104  | 0.25786  | Acacia catechu      | ![Structure](image4)                    |
| 5   | MOL008432 | Fisetinidol                                                  | 49.63751 | 0.21042  | Acacia catechu      | ![Structure](image5)                    |
| 6   | MOL008421 | Cis-dihydro quercetin                                        | 47.73094 | 0.26823  | Acacia catechu      | ![Structure](image6)                    |
| 7   | MOL000098 | Quercetin                                                    | 46.43335 | 0.27525  | Acacia catechu      | ![Structure](image7)                    |
| 8   | MOL008430 | 5,7-dihydroxy-2-(4-hydroxyphenyl)-3-methyl-chromone         | 45.04639 | 0.23585  | Acacia catechu      | ![Structure](image8)                    |
| 9   | MOL000422 | Kaempferol                                                   | 41.88225 | 0.24066  | Acacia catechu      | ![Structure](image9)                    |
| 10  | MOL008471 | Isorhyncophylline                                           | 47.31   | 0.57     | Acacia catechu      | ![Structure](image10)                   |
| 11  | MOL002914 | Eriodyctiol (flavanone)                                     | 41.35043 | 0.2436   | Acacia catechu/Scutellariae radix | ![Structure](image11)                   |
| 12  | MOL000073 | Ent-Epicatechin                                              | 48.95984 | 0.24162  | Acacia catechu/Scutellariae radix | ![Structure](image12)                   |
| 13  | MOL002934 | NEOBAICALEIN                                                | 104.3446 | 0.43917  | Scutellariae radix  | ![Structure](image13)                   |
| No. | Mol ID   | Chemical component                                         | OB (%) | DL     | Herb               | Structure |
|-----|----------|------------------------------------------------------------|--------|--------|--------------------|-----------|
| 14  | MOL002932| Panicolin                                                  | 76.25705 | 0.2915 | Scutellariae radix |           |
| 15  | MOL012246| 5,7,4′-trihydroxy-8-methoxyflavanone                      | 74.23522 | 0.26479 | Scutellariae radix |           |
| 16  | MOL002927| Skullcapflavone II                                        | 69.51043 | 0.4379 | Scutellariae radix |           |
| 17  | MOL002937| DIHYDROOROXYLIN                                           | 66.06174 | 0.23057 | Scutellariae radix |           |
| 18  | MOL000228| (2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one            | 55.23317 | 0.20163 | Scutellariae radix |           |
| 19  | MOL002915| Salvigenin                                                | 49.06593 | 0.33279 | Scutellariae radix |           |
| 20  | MOL002917| 5,2′,6′-Trihydroxy-7,8-dimethoxyflavone                   | 45.04743 | 0.33057 | Scutellariae radix |           |
| 21  | MOL008206| Moslosooflavone                                           | 44.08796 | 0.25331 | Scutellariae radix |           |
| 22  | MOL000449| Stigmasterol                                               | 43.82985 | 0.75665 | Scutellariae radix |           |
| 23  | MOL001490| Bis([25]-2-ethylhexyl]benzene-1,2-dicarboxylate           | 43.59333 | 0.34531 | Scutellariae radix |           |
| 24  | MOL002879| Diop                                                       | 43.59333 | 0.39247 | Scutellariae radix |           |
| 25  | MOL002897| Epiberberine                                               | 43.09233 | 0.7761 | Scutellariae radix |           |
| 26  | MOL002928| Oroxylin a                                                 | 41.36757 | 0.23233 | Scutellariae radix |           |
| No. | Mol ID   | Chemical component                          | OB (%) | DL      | Herb                | Structure  |
|-----|----------|---------------------------------------------|--------|---------|---------------------|------------|
| 27  | MOL002910| Carthamidin                                 | 41.15096 | 0.24189 | Scutellariae radix  |            |
| 28  | MOL002913| Dihydrobaicalin, qt                          | 40.03778 | 0.20722 | Scutellariae radix  |            |
| 29  | MOL000525| Norwogonin                                  | 39.40397 | 0.20723 | Scutellariae radix  |            |
| 30  | MOL010415| 11,13-Eicosadienoic acid, methyl ester      | 39.27534 | 0.2289  | Scutellariae radix  |            |
| 31  | MOL012266| Rivularin                                   | 37.94023 | 0.3663  | Scutellariae radix  |            |
| 32  | MOL002925| 5,7,2',6'-Tetrahydroxyflavone               | 37.01349 | 0.24382 | Scutellariae radix  |            |
| 33  | MOL000358| Beta-sitosterol                              | 36.91391 | 0.75123 | Scutellariae radix  |            |
| 34  | MOL000359| Sitosterol                                   | 36.91391 | 0.7512  | Scutellariae radix  |            |
| 35  | MOL012245| 5,7,4'-Trihydroxy-6-methoxyflavanone        | 36.62689 | 0.26833 | Scutellariae radix  |            |
| 36  | MOL002933| 5,7,4'-Trihydroxy-8-methoxyflavone          | 36.562  | 0.26666 | Scutellariae radix  |            |
| 37  | MOL001689| Acacetin                                    | 34.97357 | 0.24082 | Scutellariae radix  |            |
| 38  | MOL002909| 5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone   | 33.81583 | 0.44739 | Scutellariae radix  |            |
| 39  | MOL002714| Baicalein                                   | 33.51892 | 0.20888 | Scutellariae radix  |            |

(Continues)
the name of the pathway, the X-axis represents the ratio of targeted genes to background genes, the size of the dot represents the number of genes concentrated on the modified pathway, and the color of the dot represents the significance of enrichment. AC-KEGG pathway analysis targets mainly involved lipid and atherosclerosis, human cytomegalovirus infection, fluid shear stress and atherosclerosis, IL-17, TNF, etc. signaling pathways. And SR-related pathways were associated with lipid and atherosclerosis, human cytomegalovirus infection, PI3K-Akt, AGE-RAGE, IL-17, TNF, etc. signaling pathways. And SR-related pathways were associated with lipid and atherosclerosis, human cytomegalovirus infection, PI3K-Akt, AGE-RAGE, IL-17, TNF, etc. signaling pathways (Figure 4d), indicating that AC and SR may have synergistic reaction through multiple targets and multiple pathways in the treatment of COVID-19. Figure 5 showed the relevant targets in the key inflammatory pathways of AC-SR formula.

3.5 Molecular docking

We selected 8 core target proteins (TP53, JUN, ESR1, MAPK1, Akt1, HSP90AA1, TNF, IL-6) in PPI and 2 of most important targets for treating COVID-19 (3CLpro, ACE2) as the protein receptors via the AutoDock Vina software. We also added (+)-catechin and (-)-Epicatechin, the main active ingredients extracted from AC (Khare, 2007; Xu et al., 2015), and three positive control compounds (ritonavir, Nirmatrelvir and SSAA09E2) as ligands for molecular docking verification. The affinity between these compounds and the targets was lower than $-5.0 \text{ kcal/mol}$, indicating that the core active compounds had a good binding activity with the main target. As shown in Table 3, 43 pairs of compound-target interactions were of good binding affinity. Beta-sitosterol, baicalein, stigmasterol showed strong affinity for Akt1, ESR1, HSP90AA1 with a binding free energy of less than $-7.0 \text{ kcal/mol}$, respectively. The 10 compounds of AC-SR formula were docking with 3CLpro and ACE2 in Table 4. Compared with the positive control drugs for 3CLpro (ritonavir, Nirmatrelvir), Baicalein, (+)-catechin, and Fisetin(1-) showed superior affinity with a binding free energy of less than $-7.4 \text{ kcal/mol}$. Meanwhile, except for Skullcapflavone II and Wogonin, all compounds exhibited more excellent binding affinity for ACE2 with a binding free energy of less than $-8.6 \text{ kcal/mol}$, compared with SSAA09E2. The docking position of

| No. | Mol ID       | Chemical component                                      | OB (%)  | DL   | Herb          | Structure |
|-----|--------------|----------------------------------------------------------|---------|------|---------------|-----------|
| 40  | MOL000552    | 5,2’-Dihydroxy-6,7,8-trimethoxyflavone                    | 31.71246| 0.35462| Scutellariae radix |           |
| 41  | MOL000173    | Wogonin                                                  | 30.68457| 0.22942| Scutellariae radix |           |
| 42  | MOL001458    | Coptisine                                                 | 30.67185| 0.85647| Scutellariae radix |           |

Abbreviations: DL, drug-likeness; OB, oral bioavailability.
the 8 typical compound-target interactions is shown in Figure 6. The 2D image of molecular docking depicts the binding mode between the core target protein and the compound as well as the interaction with the surrounding amino acid residues. The main forces between ligand and protein are hydrophobic force and hydrogen bond, which both are chemical bonds with strong binding force (Fan et al., 2021). For instance, Beta-sitosterol bounded to Akt1, had hydrophobic interactions with Phe150, Tyr184, Pro187, Ile156, Met176, Phe230, Met166, Ala162, His152, Leu110, and Pro147 (Figure 6a). When binding to ACE2, Stigmasterol formed hydrogen bond interactions with residues Tyr202, formed hydrophobic interactions with His401 (Figure 6d). Baicalein formed three hydrogen bonds with the amino acid residues and had hydrophobic interactions with Met49 and Gly143 as well, which make it form a stable complex with 3CLpro (Figure 6h).

### Molecular dynamic simulation

Based on the interactions with the binding pockets and binding energy calculations (Figure 6, Table 4), ACE2-Quercetin and 3CL-Baicalein complexes were selected to run MD simulations. The complex stability was predicted based on the RMSD and RMSF calculation during the entire run of MD trajectories. Figure 7a displayed the RMSD value of these two complexes over 200 ns run, and each trajectory was found with an average RMSD of 2.21–2.99 Å for ACE2-Quercetin complex, 1.25–2.17 Å for 3CL-Baicalein complex, respectively. The complex was stable over the entire 200 ns run. For gaining more insights regarding the stability of the complex binding site, the per-residue RMSF was estimated for each ligand-bound protein. The RMSF value was suggested about the very low fluctuations of molecule form the protein over entire MD trajectories (Figure 7b, c). Further, heatmap of hydrogen bonding interactions for ACE2-Quercetin and 3CL-Baicalein demonstrated the hydrogen-bonding pattern (red blocks) observed during 200 ns simulation in both 2 protein-ligand complexes (Figure 7d, e). These findings all showed that a stable conformation has been achieved in the process of MD simulation.

### FRET-based assay for the SARS-CoV-2 3CLpro and ACE2 enzyme activity inhibition

The in silico docking study and molecular dynamic simulation assay both indicated that the active compounds have certain affinity with 3CLpro and ACE2, the key proteases during SARS-CoV-2 replication.
The anti-COVID-19 property of these compounds may possibly due to its ability to inhibit the enzymatic activity of 3CLpro and ACE2. In this regard, the in vitro enzymatic inhibitory assays based on fluorescence resonance energy transfer (FRET) were applied to determine the median inhibitory concentration (IC50) values. The IC50 for the positive control Ebselen and MLN-4760 have been calculated to be 0.67 μM and 7.5 nM, respectively. Among the top three scored compounds in Table 4, baikalein has the best inhibitory effect on 3CLpro activity with IC50 of 11.3 μM, followed by fisetin(1-) and (−)−catechin with IC50 of 23.8 and 44.1 μM, respectively (Figure 8). A concentration-dependent inhibition of baikalein, quercetin and (−)−catechin against ACE2 was also observed with the IC50 of 138.2, 141.3 and 348.4 μM, respectively. In addition, IC50 values for both beta-sitosterol and stigmasterol were determined to be > 1,000 μM.

FIGURE 3 Targets of AC and SR against COVID-19. (a) Venn diagram and PPI network showed the 209 targets of AC against COVID-19. (b) Venn diagram and PPI network exhibited the 99 targets of SR against COVID-19.
The COVID-19 outbreak has sparked worldwide alarm as an emerging infectious disease. The practice of China in controlling this outbreak has demonstrated the clinical responses and superiorities of TCM (Jiang et al., 2021). Among the drugs recommended by the government and doctors, SR is one of the most frequently utilized herbs (60.84%) for treating COVID-19 (Luo et al., 2020). Indeed, the Chinese medicinal formula AC-SR, also famous as Huang-qin Er-cha Decoction, has been widely used for treating cough, phlegm and fever which caused by pulmonary infection in clinical practice for several centuries. It was reported that the pathophysiology of COVID-19 has been linked to cytokine storms and consequent immunogenic damage. The elevated level of proinflammatory cytokines, such as IL-6, IL-1, and IFN-γ, are related to the influx of leukocytes, which further accelerates the local inflammatory response in the lungs (Zhang et al., 2019). At this point, the disease may quickly progress to severe sickness, manifesting as ARDS, acute lung injury (ALI), multiple organ failure, and septic shock (Kim & Hong, 2016; Yang et al., 2020). Interestingly, our previous studies have demonstrated that AC-SR formula exhibits a strong anti-inflammatory effect against LPS-induced ALI. Thus, to reveal the integrative pharmacological mechanism of AC-SR formula against COVID-19, we applied a unique systems pharmacology strategy which combining network pharmacology, molecular docking analysis, and MD simulations in the present study.

Eight key candidate components (quercetin, fisetin, kaempferol, wogonin, betasitosterol, baicalein, skullcapflavone II, and stigmasterol) from AC-SR formula were screened out based on the degree value of correlation between components and targets. The results of PPI network revealed that TP53, JUN, ESR1, MAPK1, Akt1, HSP90AA1, TNF, IL-6 were considered to be hub genes. These proteins are mainly involved and play important roles in inflammation and immune regulation. JUN, for instance, is an immediate-early gene that plays a crucial role in inflammatory responses (Fahmy et al., 2006). Akt1 activation promotes cell proliferation while inhibiting cell apoptosis. It is a key participant in the immune inflammatory mechanism of COVID-19 (Xia et al., 2020). Furthermore, IL-6 and TNF, which are critical components of the body’s immunomodulatory and proinflammatory effects, have also been well documented in the literature (Yi et al., 2020).
Notably enriched GO biological processes included response to oxidative stress and response to molecule of bacterial origin. AC-KEGG enrichment analysis showed that the key targets were mainly concentrated in lipid and atherosclerosis, human cytomegalovirus infection, fluid shear stress, and atherosclerosis, etc. SR-KEGG enrichment analysis was mainly concentrated in lipid and atherosclerosis.
The affinity of the putative compounds with 8 core targets

| Herb | Mol ID      | Compound     | ESR1 (1gwq) | JUN (1a02) | TNF (6 m95) | HSP90AA1(3o0l) | TP53 (4aggq) | MAPK1 (4fv4) | AKT1 (4gah) | IL-6 (1li6) |
|------|-------------|--------------|-------------|------------|-------------|----------------|--------------|--------------|-------------|-------------|
| AC   | MOL000998   | Quercetin    | −6.2        | −4.4       | −5.1        | −6.5           | −5.1         | −4.9         | −5.1        | −5.5        |
| AC   | /           | Fisetin(1-)  | −6.9        | −5.5       | −5.4        | −6.5           | −4.7         | −4.4         | −5.5        | −5.4        |
| AC   | MOL000422   | Kaempferol   | −6.3        | −6.4       | −5.5        | −6.2           | −5.1         | −5.4         | −4.6        | −5.1        |
| SR   | MOL000173   | Wogonin      | −2.9        | −5.9       | −3.0        | −4.9           | −2.5         | −3.1         | −2.5        | −4.8        |
| SR   | MOL000358   | Beta-sitosterol | −6.1        | −5.7       | −5.1        | −4.9           | −5.1         | −5.1         | −7.5        | −5.4        |
| SR   | MOL002714   | Baicalein    | −7.3        | −4.9       | −6.0        | −6.7           | −6.6         | −5.2         | −6.8        | −5.2        |
| SR   | MOL002927   | Skullcapflavone II | −5.0       | −4.1       | −4.3        | −5.4           | −4.6         | −4.7         | −4.9        | −4.1        |
| SR   | MOL000449   | Stigmasterol | −6.5        | −5.9       | −5.4        | −7.2           | −4.5         | −5.4         | −6.6        | −5.6        |
| /    | Positive drug | Ritonavir | −5.2        | −4.0       | −5.0        | −6.1           | −4.9         | −5.2         | −5.4        | −5.4        |
| /    | Positive drug | Nirmatrelvir | −5.1        | −4.5       | −5.8        | −5.7           | −5.1         | −5.0         | −5.3        | −4.8        |

The binding energy ≤ 0 kcal/mol indicated that the compound could bind and interact with the target, whereas the binding energy < −5 kcal/mol indicated a very strong binding force.

| Pubchem CID | Molecule name | Molecular formula | MW (g/Mol) | SARS-CoV-2 3CLpro (6lu7) docking score (kcal/Mol) | ACE2 (1r4l) docking score (kcal/Mol) |
|-------------|---------------|------------------|-----------|-----------------------------------------------|--------------------------------------|
| 5,280,343   | Quercetin     | C_{15}H_{10}O_{7} | 302.25   | −7.2                                          | −9.1                                 |
| 54,758,660  | Fisetin(1-)   | C_{15}H_{12}O_{6} | 285.23   | −7.5                                          | −8.8                                 |
| 5,280,863   | Kaempferol    | C_{15}H_{10}O_{6} | 286.25   | −7.3                                          | −8.8                                 |
| 5,281,703   | Wogonin       | C_{15}H_{12}O_{5} | 284.26   | −6.7                                          | −8.3                                 |
| 222,284     | Beta-sitosterol | C_{20}H_{10}O | 414.79   | −6.8                                          | −9.5                                 |
| 5,281,605   | Baicalein     | C_{15}H_{10}O_{5} | 270.25   | −7.8                                          | −9.1                                 |
| 124,211     | Skullcapflavone II | C_{35}H_{36}O_{8} | 374.30   | −7.0                                          | −8.2                                 |
| 5,280,794   | Stigmasterol  | C_{20}H_{18}O | 412.77   | −6.8                                          | −9.8                                 |
| 9,064       | (+)–Catechin  | C_{15}H_{14}O_{6} | 290.27   | −7.8                                          | −9.0                                 |
| 72,276      | (−)–Epicatechin | C_{15}H_{14}O_{6} | 290.27   | −7.1                                          | −8.8                                 |
| 392,622     | Ritonavir (positive drug) | C_{29}H_{48}N_{2}O_{8}S_{2} | 720.96   | −6.7                                          | /                                    |
| 155,903,259 | Nirmatrelvir (positive drug) | C_{23}H_{35}F_{3}N_{2}O_{4} | 499.50   | −7.4                                          | /                                    |
| 2,738,575   | SSAA09E2 (positive drug) | C_{14}H_{20}N_{2}O_{2} | 300.36   | /                                             | −8.6                                 |

The binding energy ≤ 0 kcal/mol indicated that the compound could bind and interact with the target, whereas the binding energy < −5 kcal/mol indicated a very strong binding force.

human cytomegalovirus infection, hepatitis B, etc. Moreover, according to our KEGG pathway analysis, the pharmacological mechanisms of AC-SR formula against COVID-19 involved specific modulations of immunological responses, such as human immunodeficiency virus 1 infection, human T-cell leukemia virus 1 infection, and Human cytomegalovirus infection.

The deteriorated clinical presentation of patients with COVID-19 is mainly associated with cytokine release syndrome (Zhang et al., 2020). It has been shown that the consequent inflammatory waterfall factors of host infection are critical for defense against and treatment of new coronavirus infections (Rokni, Ghasemi, & Tavakoli, 2020). Our data implicated TNF signaling pathway, PI3K/Akt signaling pathway, and IL-17 signaling as the key inflammatory signaling pathways of AC-SR formula anti-inflammatory function to treat COVID-19. TNF is a potent cytokine exerting critical functions in the activation and regulation of immune and inflammatory responses (Shivappa et al., 2017). TNF and TNF receptor 2 signaling elicited leukocyte recruitment, activation, and survival of host cells after coronavirus infection (Cheng et al., 2021). The PI3K/Akt signaling pathway regulated the activation of inflammatory response cells and the release of inflammatory transmitters. Targeting PI3K and Akt during the early phases of the immune response may improve effector function and suppress suppressor function, thereby assisting in the elimination of the infection before it progresses to immunological...
dysregulation. When patients develop uncontrolled immune responses, targeting mTOR can also be used to suppress the cytokine storm, inhibit neutrophil recruitment, and enhance suppressor regulatory T cells (Abu-Eid & Ward, 2021). IL-17 family proteins not only mediated the cytokine storm after SARS-CoV-2 infection (Cafarotti, 2020) but also regulated the innate immune responses (Ryzhakov et al., 2011). IL-17 signaling played an underlying immunopathological role to manage COVID-19 patients, particularly those presenting with cytokine storm syndrome (Shibabaw, 2020).

ACE2 and 3CLpro have been considered as promising COVID-19 drug targets, which play a key role in the replication cycle of the virus. Researches showed that ACE2 and 3CLpro on host epithelial cells affected by its S-protein are considered to be the core targets for inhibiting coronavirus replication (Hall & Ji, 2020; Menachery et al., 2015; Wu et al., 2020). In molecular docking study, the results showed Beta-sitosterol, baicalein, stigmasterol showed strong affinity for Akt1, ESR1, HSP90AA1, respectively. Compared with the positive control drugs for 3CLpro and ACE2, Baicalein, (-)-catechin, Fisetin (1-), beta-sitosterol, stigmasterol, and quercetin showed superior or similar affinity with a lower binding free energy. Beta-sitosterol and stigmasterol are the most frequently used chemical components possibly related to the antiviral signing pathway (Luo et al., 2020). Unfortunately, IC50 values for both beta-sitosterol and stigmasterol were determined to be greater than 1,000 μM in the ACE2 activity inhibition assay, probably due to their poor solubility in water and alcohol, thus further evaluation of the two compounds as potential SARS-CoV-2 ACE2 inhibitors may be warranted. Particularly, we identified that baicalein had good affinity for ACE2 and 3CLpro, mainly through hydrogen bonds and hydrophobic interactions, with measured IC50 values against SARS-CoV-2 3CLpro and ACE2 to be 11.3 and 138.2 μM, respectively, which indicate that baicalein may directly inhibit SARS-CoV-2 replication and transcription. Du et al. (2022) also confirmed that baicalein has good binding activity with ACE2 and that hydrogen bonding plays a key role in the recognition and stability of the active ingredients and proteins. In addition, baicalein is the first identified non-covalent, non-peptidomimetic inhibitors of SARS-CoV-2 3CLpro (Liu et al., 2021; Su et al., 2020). Notably, We found for the first time that fisetin(1-) might have the potential against the novel coronavirus via binding to SARS-CoV-2 3CLpro and ACE2 in silico. This was further confirmed by the enzyme activity inhibition assays. Fisetin(1-) revealed the prominent inhibitory activity against SARS-CoV-2 3CLpro with the IC50 of 23.8 μM. We also found that quercetin and kaempferol, which have multiple pharmacological activities such as anti-inflammatory, immunomodulatory and antiviral (Carullo et al., 2016; Chiow, Phoon, Putti, Tan, & Chew, 2016; Huang, Bai, He, Xie, & Zhou, 2020; Khazdair, Anaieigoudari, & Agbor, 2021; Micek, Jurikova, Skrovankova, & Sochor, 2016), plays crucial roles against COVID-19 via binding to ESR1, HSP90AA1, TP53, TNF, ACE2, and 3CLpro. These findings were in line with several previous studies (Huang et al., 2021; Qiu et al., 2020; Xia et al., 2020).
Catechin, the main antioxidant, anti-inflammatory, and chemoprotective ingredient of AC, exhibits excellent affinity for ACE2 and 3CLpro in the docking test as well. Several previous work have reported that catechin exhibit strong in silico activity against the wild strain of SARS-CoV-2 (Mhatre, Gurav, Shah, & Patravale, 2021), presumably leading to therapeutic efficacies via inhibition of ACE2 and could be potentially explored as a multitargeted agent against COVID-19 (Jena, Kanungo, Nayak, Chainy, & Dandapat, 2021; Mishra et al., 2021). Considering this, quercetin, fisetin(1-), kaempferol, catechin, beta-sitosterol, baicalein, and stigmasterol could be the potential candidates of AC-SR formula against COVID-19. In addition, in order to validate the results of molecular docking as well as evaluate the stability

FIGURE 7  Molecular dynamics simulations. (a) The RMSD plot of ACE2-Quercetin and 3CLpro Baicalein; The RMSF of ACE2 Quercetin (b) and 3CLpro Baicalein (c); Heatmap of hydrogen bonding interactions for ACE2-Quercetin (d) and 3CLpro-Baicalein (e), red indicates hydrogen-bonded, whereas green indicates non-hydrogen-bonded

Catechin, the main antioxidant, anti-inflammatory, and chemoprotective ingredient of AC, exhibits excellent affinity for ACE2 and 3CLpro in the docking test as well. Several previous work have reported that catechin exhibit strong in silico activity against the wild strain of SARS-CoV-2 (Mhatre, Gurav, Shah, & Patravale, 2021), presumably leading to therapeutic efficacies via inhibition of ACE2 and could be potentially explored as a multitargeted agent against COVID-19 (Jena, Kanungo, Nayak, Chainy, & Dandapat, 2021; Mishra et al., 2021). Considering this, quercetin, fisetin(1-), kaempferol, catechin, beta-sitosterol, baicalein, and stigmasterol could be the potential candidates of AC-SR formula against COVID-19. In addition, in order to validate the results of molecular docking as well as evaluate the stability
and interaction of ACE2 and 3CLpro with ligands, ACE2-quercetin and 3CLpro-baicalein complexes were chosen for molecular dynamic simulation test. The binding sites of small molecules did not change considerably after a 200 ns dynamics simulation, suggesting that the results of molecular docking were reliable and the binding of small molecules remained relatively stable. The two complexes also exhibited stable RMSD and RMSF in the simulated test, which demonstrated that quercetin and baicalein may effectively activate the biological pathway in ACE2 and 3CLpro without affecting the conformation of the active site. The amount of hydrogen bonds as well as hydrophobic bond, on the other hand, influences the stability of protein-ligand complexes. The aggregates formed by 3CLpro-baicalein and ACE2-quercetin in the MD test contained 3–5 hydrogen bonds and hydrophobic bond, which suggested that the strong interactions of the key residues (most especially Tyr196, Ser144, Cys145, Leu141, Lys562 and Gln96) with quercetin and baicalein would significantly impede SARS-CoV-2 3CLpro and ACE2 dimerization and substrate binding.

5 | CONCLUSIONS AND PERSPECTIVES

In summary, the present study dissected the possible molecular targets and mechanisms of Chinese medicinal formula AC--SR against COVID-19 from the point of view of a systems pharmacology strategy through network pharmacology approach, molecular docking and molecular dynamic simulation methods. After screening and analysis, 8 active ingredients and 10 core target proteins in AC-SR formula were obtained, involving multiple pathways. AC-SR formula might suppress COVID-19 through their combined antioxidative, antiviral and anti-inflammatory effects, along with immune system activation. The present work provides a rapid and accurate method for facilitate the screening and dissecting of the complex system of TCM and natural medicine. Based on the identified functional processes and pharmacological mechanisms, the Chinese medicinal formulas as well as their ingredients, could be applied to the development of anti-SARS-CoV-2 medications in a more effective and targeted manner. However, for the identification and mechanism study of novel COVID-19 medication combination, we believe that the database-based systems pharmacology should be supplemented by experimental evidence, which will not only decrease the duration of drug development in crisis, but will also assure its dependability. Therefore, in light of our findings, related and more in-depth studies, such as direct evidence for the mechanism of AC-SR formula in a SARS-CoV-2 infection experiment model, remains urgently warranted. The further verification of our present study will be on the way.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES
Abu-Eid, R., & Ward, F. J. (2021). Targeting the PI3K/Akt/mTOR pathway: A therapeutic strategy in COVID-19 patients. Immunology Letters, 240, 1–8. https://doi.org/10.1016/j.imlet.2021.09.005
Aljara, N. H., Hasnain, M. S., Bin-Meferij, M. M., & Alkahtani, S. (2022). Design & discovery of small molecule COVID-19 inhibitor via dual approach based virtual screening and molecular simulation studies. Journal of King Saud University Science, 34(3), 101867. https://doi.org/10.1016/j.jksus.2022.101867
Anand AV., Balamuralikrishnan B., Kaviya M., Bharathi K., Parithathvi A., Arun M., ... Dhamak K. (2021). Medicinal plants, phytochemicals, and herbs to combat viral pathogens including SARS-CoV-2. Molecules, 26(6):1775. https://doi.org/10.3390/molecules26061775
Banaganapalli, B., Al-Rayes, N., Awan, Z. A., Alsaluaimany, F. A., Alamri, A. S., Elango, R., ... Shaik, N. A. (2021). Multilevel systems biology analysis of lung transcriptomics data identifies key miRNAs and potential miRNA target genes for SARS-CoV-2 infection. Computers in Biology Medicine, 135, 104570. https://doi.org/10.1016/j.compbiomed.2021.104570
Bibi, N., Gul, S., Ali, J., & Kamal, M. A. (2021). Viroinformatics approach to explore the inhibitory mechanism of existing drugs repurposed to fight against COVID-19. European Journal of Pharmacology, 885, 173496. https://doi.org/10.1016/j.ejphar.2020.173496
Brendler, T., Al-Harrasi, A., Bauer, R., Gafner, S., Hardy, M. L., Heinrich, M., ... Williamson, E. M. (2021). Botanical drugs and supplements affecting the immune response in the time of COVID-19: Implications for research and clinical practice. Phytotherapy Research, 35(6), 3013–3031. https://doi.org/10.1002/tr.7008
Brüssow, H. (2021). COVID-19: Vaccination problems. Environmental Microbiology, 23(6), 2878–2890. https://doi.org/10.1111/1462-2920.15549
Cafarotti, S. (2020). Severe acute respiratory syndrome-CoV-2 infection and patients with lung cancer: The potential role of Interleukin-17 target therapy. Journal of Thoracic Oncology, 15(7), e101–e103. https://doi.org/10.1016/j.jtoh.2020.04.015
Carullo, G., Cappello, A. R., Frattarulo, L., Badalato, M., Armentano, B., & Aiello, F. (2016). Quercetin and derivatives: Useful tools in the inflammation and pain management. Future Medicinal Chemistry, 9(1), 79–93. https://doi.org/10.4155/fmc-2016-0186
Chen, T., Wu, D., Chen, H., Yan, W. M., Yang, D. L., Chen, G., ... Ning, Q. (2020). Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. BMJ, 368, m1091. https://doi.org/10.1136/bmj.m1091
Cheng, L. C., Kao, T. J., Phan, N. N., Chiao, C. C., Yen, M. C., Chen, C. F., ... Hsu, H. P. (2021). Novel signaling pathways regulate SARS-CoV and SARS-CoV-2 infectious disease. Medicine, 100(7), e24321. https://doi.org/10.1097/md.00000000000024321
Chiow, K. H., Phoon, M. C., Putti, T., Tan, B. K. H., & Chew, V. T. (2016). Evaluation of antiviral activities of Houttuynia cordata Thunb. Extract, quercetin, quercitrin and cinanserin on murine coronavirus and dengue virus infection. Asian Pacific Journal of Tropical Medicine, 9(1), 1–7. https://doi.org/10.1016/j.aptm.2015.12.002
Consortium UP. (2021). UniProt: The universal protein knowledgebase in 2021. Nucleic Acids Research, 49(D1), D480–D489. https://doi.org/10.1093/nar/gkaa1100
Das, A., Pandita, D., Jain, G. K., Agarwal, P., Grewal, A. S., Khar, R. K., & Lather, V. (2021). Role of phytoconstituents in the management of COVID-19. Chemico-Biological Interaction, 341, 109449. https://doi.org/10.1016/j.cbi.2021.109449
Doniach, S., & Eastman, P. (1999). Protein dynamics simulations from nanoseconds to microseconds. Current Opinion in Structural Biology, 9(2), 157–163. https://doi.org/10.1016/S0959-440X(99)80022-0
Du, L., Xiao, Y., Xu, Y., Chen, F., Chu, X., Cao, Y., & Zhang, X. (2022). The potential bioactive components of nine TCM prescriptions against COVID-19 in lung cancer were explored based on network pharmacology and molecular docking. Frontiers in Medicine, 8, 813119. https://doi.org/10.3389/fmed.2021.813119
Dubey, K. D., Tiwari, R. K., & Ohja, R. P. (2013). Recent advances in protein-ligand interactions: Molecular dynamics simulations and binding free energy. Current Computer-Aided Drug Design, 9(4), 518–531. https://doi.org/10.2174/15734099113096660036
Fahmy, R. G., Waldman, A., Zhang, G. S., Mitchell, A., Tedla, N., Cai, H., ... Khachigian, L. M. (2006). Suppression of vascular permeability and inflammation by targeting of the transcription factor c-Jun. Nature Biotechnology, 24(7), 856–863. https://doi.org/10.1038/nbt1225
Fan, Y. H., Liu, W., Jin, Y., Hou, X., Zhang, X. W., Pan, H. D., ... Guo, X. J. (2021). Integrated molecular docking with network pharmacology to reveal the molecular mechanism of Simiao powder in the treatment of acute gouty arthritis. Evidence-Based Complementary and Alternative Medicine, 5570968, 1–15. https://doi.org/10.1155/2021/5570968
Feng, T., Zhou, L. Y., Gai, S. C., Zhai, Y. M., Gou, N., Wang, X. C., ... Wang, S. W. (2019). Acacia catechu (L.f.) Wildt and Scutellaria baikalensis Georgi extracts suppress LPS-induced pro-inflammatory responses through NF-κB, MAPK, and PI3K-Akt signaling pathways in alveolar epithelial type II cells. Phytotherapy Research, 33(12), 3251–3260. https://doi.org/10.1002/ptr.6499
Fiolet, T., Kherabi, Y., Chi, M. D., Ghosn, J., & Peiffer-Smadja, N. (2022). Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: A narrative review. Clinical Microbiology and Infection, 28(2), 202–221. https://doi.org/10.1016/j.cmi.2021.01.005
Hall, D. C., & Ji, H. F. (2020). A search for medications to treat COVID-19 via in silico molecular docking models of the SARS-CoV-2 spike glycoprotein and 3CL protease. Travel Medicine and Infectious Disease, 35, 101646. https://doi.org/10.1016/j.tmaid.2020.101646
Hong, Z. C., Duan, X. Y., Wu, S. T., Yang, Y. F., & Wu, H. Z. (2020). Network pharmacology integrated molecular docking reveals the anti-COVID-19 mechanism of Qing-Fei-Da-Yuan granules. Natural Product Communications, 15(6), 1–15. https://doi.org/10.1177/1934578X20934219
Hsu, C. H., Hwang, K. C., Chao, C. L., Chang, S. G., Ker, C. C., Chien, L. C., ... Chou, P. (2006). The lesson of supplementary treatment with Chinese medicine on severe laboratory-confirmed SARS patients. American Journal of Chinese Medicine, 34(6), 927–935. https://doi.org/10.1142/S0191911X06004405
Huang, Y. F., Bai, C., He, F., Xie, Y., & Zhou, H. (2020). Review on the potential action mechanisms of Chinese medicines in treating coronavirus disease 2019 (COVID-19). Pharmacological Research, 158, 104939. https://doi.org/10.1016/j.phrs.2020.104939
Huang, K., Zhang, P., Zhang, Z. H., Youn, J. Y., Wang, C., Zhang, H. C., & Cai, H. (2021). Traditional Chinese medicine (TCM) in the treatment of viral infections: Efficacies and mechanisms. Pharmacology and...
Mishra, C. B., Pandey, P., Sharma, R. D., Malik, M. Z., Mongre, R. K., Lynn, A. M., … Yang, Z. G. (2020). Traditional Chinese medicine formulation therapy in the treatment of coronavirus disease 2019 (COVID-19). The American Journal of Chinese Medicine, 48(7), 1523–1538. https://doi.org/10.1142/soi192415X20500755
Shibabaw, T. (2020). Inflammatory cytokine: IL-17A signaling pathway in patients present with COVID-19 and current treatment strategy. *Journal of Inflammation Research*, 13, 673–680. https://doi.org/10.2147/JIR.S278335

Shivappa, N., Hebert, J. M., Rosato, V., Garavello, W., Serraino, D., & Vecchia, C. L. (2017). Inflammatory potential of diet and risk of oral and pharyngeal cancer in a large case-control study from Italy. *Journal International of Cancer*, 141(3), 471–479. https://doi.org/10.1002/jic.3071

Song, J. W., Long, J. Y., Xie, L., Zhang, L. L., Xie, Q. X., Chen, H. J., ... Li, X. F. (2020). Applications, phytochemistry, pharmacological effects, pharmacokinetics, toxicity of Scutellaria baicalensis Georgi. And its probably potential therapeutic effects on COVID-19: A review. *Chinese Medicine*. 15((1)), 102. https://doi.org/10.1186/s13302-020-00384-0

Su, X. H., Yao, S., Zhao, W. F., Li, M. J., Liu, J., Shang, W. J., ... Xu, X. Y. (2020). Anti-SARS-CoV-2 activities in vitro of Shuanghuanglian preparations and bioactive ingredients. *Acta Pharmacologica Sinica*, 41, 1167–1177. https://doi.org/10.1038/s41401-020-0483-6

Tao, Q. Y., Du, J. X., Li, Y. X., Zeng, J. Y., Tan, B., Xu, J. H., ... Chen, X. L. (2021). Network pharmacology and molecular docking analysis on molecular targets and mechanisms of Huashi Baidu formula in the treatment of COVID-19. *Drug Development and Industrial Pharmacy*, 46(8), 1345–1353. https://doi.org/10.1080/03639045.2020.1788070

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461. https://doi.org/10.1002/jcc.21334

Wang, C., Cao, B., Liu, Q. Q., Zou, Z. Q., Liang, Z. A., Gu, L., ... Jiang, L. D. (2011). Oseitalimvir compared with the Chinese traditional therapy maxingshigan-qingqiao in the treatment of H1N1 influenza: A randomized trial. *Annals of Internal Medicine*, 155(4), 217–225. https://doi.org/10.7326/0003-4819-155-4-201108160-00005

Wang, J., Ge, W., Peng, X., Yuan, L. X., He, S. B., & Fu, X. Y. (2021). Investigating the active compounds and mechanism of Huashi XuanFei formula for prevention and treatment of COVID-19 based on network pharmacology and molecular docking analysis. *Molecular Diversity*, 1–1, 1175–1190. https://doi.org/10.1007/s11030-021-10244-0

Wang, L. B., Shen, X., Mi, L., Jing, J., Gai, S. C., Liu, X. Y., ... Zhang, S. Y. (2019). Simultaneous determinations of four major bioactive components in Acacia catechu (L.f.) Willd and Scutellaria baicalensis Georgi extracts by LC-MS/MS: Application to its herb-herb interactions based on pharmacokinetic, tissue distribution and excretion studies in rats. *Phytomedicine*, 56, 64–73. https://doi.org/10.1016/j.phymed.2018.09.239

Wiersinga, W. J., Rhodes, A., Cheng, A. C., Peacock, S. J., & Prescott, H. C. (2020). Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): A review. *JAMA the Journal of the American Medical Association*, 324(8), 782–793. https://doi.org/10.1001/jama.2020.12839

Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z. G., ... Zhang, Y. Z. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, 579(7798), 265–269. https://doi.org/10.1038/s41586-020-2008-3

Wynants, L., Van Calster, B., Collins, G. S., Riley, R. D., Heinzle, G., Schuit, E., ... Smeden, M. V. (2021). Prediction models for diagnosis and prognosis of COVID-19: Systematic review and critical appraisal. *BMJ*, 369(), m1328. https://doi.org/10.1136/bmj.m1328

Xia, Q. D., Xun, Y. X., Lu, J. L., Lu, Y. C., Yang, Y. Y., Zhou, P., ... Wang, S. G. (2020). Network pharmacology and molecular docking analyses on Lianhua Qingwen capsule indicate Akt1 is a potential target to treat and prevent COVID. *Cell Proliferation*, 53(12), e12949. https://doi.org/10.1111/cpr.12949

Xian, Y. F., Zhang, J., Bian, Z. X., Zhou, H., Zhang, Z. B., Lin, Z. X., & Xu, H. X. (2020). Bioactive natural compounds against human coronaviruses: A review and perspective. *Acta Pharmacuetica Sinica B*, 10(7), 1163–1174. https://doi.org/10.1016/j.apsb.2020.06.002

Xu, H., Li, J., Song, S., Xiao, Z., Chen, X., Huang, B., ... Wang, N. (2021). Effective inhibition of coronavirus replication by Polygonum cuspidatum. *Frontiers in Bioscience*, 26(10), 789–798. https://doi.org/10.2528/4988

Xu, L. W., Liang, Y. H., Chen, X., Chen, B., Han, Y. H., & Zhang, L. (2015). Hyperlipidemia affects the absorption, distribution and excretion of seven catechins in rats following oral administration of tea polyphenols. *RSC Advances*, 5(119), 97988–97994. https://doi.org/10.1039/C5RA19699K

Xu, X., Zhang, W. X., Huang, C., Li, Y., Yu, H., Wang, Y. H., ... Ling, Y. (2012). A novel Chemometric method for the prediction of human Oral bioavailability. *International Journal of Molecular Sciences*, 13(6), 6964–6982. https://doi.org/10.3390/ijms13066964

Yang, L., Liu, S., Han, S., Hu, Y., Wu, Z., Shi, X., ... Jin, J. (2020). The HDL from septic ARDS patients with composition changes exacerbates pulmonary endothelial dysfunction and acute lung injury induced by cecal ligation and puncture (CLP) in mice. *Respiratory Research*, 21(1), 293. https://doi.org/10.1186/s12931-020-01553-3

Yi, H. Y., Zhang, Y., Yang, X. F., Li, M. Y., Hu, H. F., Xiong, J., ... Lian, J. Q. (2020). Hepatitis B Core antigen impairs the polarization while promoting the production of inflammatory cytokines of M2 macrophages via the TLR2 pathway. *Frontiers in Immunology*, 11, 535. https://doi.org/10.3389/immu.2020.00053

Yuan, R., Xin, Q. Q., Tang, S. H., & Cong, W. H. (2020). Treatment of COVID-19 guided by holistic view of traditional Chinese medicine—therapy aimed at both viral and host. *China Journal of Chinese Materia Medica*, 45(7), 1521–1525. https://doi.org/10.19540/j.cnki.cjcmcm.20200304.501

Zhang, X. Y., Lv, L., Zhou, Y. L., Xie, L. D., Xu, Q., Zou, X. F., ... Ye, X. Q. (2021). Efficacy and safety of Xiyanping injection in the treatment of COVID-19: A multicenter, prospective, open-label and randomized controlled trial. *Phytotherapy Research*, 35(8), 4401–4410. https://doi.org/10.1002/ptr.7141

Zhang, L., Zhang, X., Zheng, J., Liu, Y., Wang, J., Wang, G., ... Wang, G. (2019). Depressive symptom-associated IL-1p and TNF-a release correlates with impaired bronchodilator response and neutrophilic airway inflammation in asthma. *Clinical and Experimental Allergy*, 49(6), 770–780. https://doi.org/10.1111/cea.13346

Zhang, W., Zhao, Y., Zhang, F. C., Wang, Q., Li, T. S., Liu, Z. Y., ... Zhang, S. Y. (2020). The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. *Clinical Immunology*, 214, 108393. https://doi.org/10.1016/j.clinim.2020.108393

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