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Exploring the possible molecular targeting mechanism of Saussurea involucrata in the treatment of COVID-19 based on bioinformatics and network pharmacology

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ABSTRACT

Objective: Based on bioinformatics and network pharmacology, the treatment of Saussurea involucrata (SAIN) on novel coronavirus (COVID-19) was evaluated by the GEO clinical sample gene difference analysis, compound-target molecular docking, and molecular dynamics simulation. Role in the discovery of new targets for the prevention or treatment of COVID-19, to better serve the discovery and clinical application of new drugs.

Materials and methods: Taking the Traditional Chinese Medicine System Pharmacology Database (TCMSP) as the starting point for the preliminary selection of compounds and targets, we used tools such as Cytoscape 3.8.0, TBtools 1.098, AutoDock vina, R 4.0.2, PyMol, and GROMACS to analyze the compounds of SAIN and targets were initially screened. To further screen the active ingredients and targets, we carried out genetic difference analysis ($n = 72$) through clinical samples of COVID-19 derived from GEO and carried out biological process (BP) analysis on these screened targets ($P \leq 0.05$), gene $= 9$), KEGG pathway analysis (FDR $\leq 0.05$, gene $= 9$), protein interaction network (PPI) analysis (gene $= 9$), and compounds-target-pathway network analysis (gene $= 9$), to obtain the target Point-regulated biological processes, disease pathways, and compounds-target-pathway relationships. Through the precise molecular docking between the compounds and the targets, we further screened SAIN’s active ingredients (Affinity $\leq 7.2$ kcal/mol) targets and visualized the data. After that, we performed molecular dynamics simulations and consulted a large number of related studies to explore the results in the literature.

Results: Through the screening, analysis, and verification of the data, it was finally confirmed that there are five main active ingredients in SAIN, which are Quercitrin, Rutin, Caffeic acid, Jaceosidin, and Beta-sitosterol, and mainly act on five targets. These targets mainly regulate Tuberculosis, TNF signaling pathway, Alzheimer’s disease, Pertussis, Toll-like receptor signaling pathway, Influenza A, Non-alcoholic fatty liver disease (NAFLD), Neuroactive ligand-receptor interaction, Complement and coagulation cascades, Fructose and mannose metabolism, and Metabolic pathways, play a role in preventing or treating COVID-19. Molecular dynamics simulation results show that the four active ingredients of SAIN, Quercitrin, Rutin, Caffeic acid, and Jaceosidin, act on the four target proteins of COVID-19, AKR1B1, C5AR1, GSK3B, and IL1B to form complexes that can be very stable in the human environment. Tertiary structure exists.

Conclusion: Our study successfully explained the effective mechanism of SAIN in improving COVID-19, and at the same time predicted the potential targets of SAIN in the treatment of COVID-19, AKR1B1, IL1B, and GSK3B. It provides a new basis and provides great support for subsequent research on COVID-19.

Data sharing

The datasets generated and/or analyzed during the current study are not publicly available due to the limited scope of data availability, these data are used under the license of this study and are not disclosed, but are available from the author [sedate@stu.shzu.edu.cn (Dongdong Zhang)] on reasonable request.

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1. Introduction

As of January 28, 2022, coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to be at the top of the list of worrisome diseases, partly because it spreads rapidly and is extremely difficult to control [364,191,494 confirmed cases and 5,631,457 deaths from COVID-19] [1]. However, what makes it even more troubling for the world is that COVID-19 can mutate to many types over time, such as the latest Omicron strain [2].

Presently, the research and development of targeted vaccines against COVID-19 in most countries have problems such as complex processes, relatively high market prices (except for a few free vaccination countries), and difficulty in finding targets [3]. Therefore, these restrictions have prevented routine vaccinations in COVID-19 patients. If we can discover an effective traditional Chinese medicine for the treatment of COVID-19, and clarify its effective compounds and targets for the treatment of COVID-19, it can contribute to the research and development of a global COVID-19 vaccine.

In recent years, with the advent of artemisinin [4], berberine [5], and other traditional Chinese medicine extracts, traditional Chinese medicine has played an increasingly important role in the treatment of refractory tumors, including common liver cancer [6], lung cancer [7] and pan-cancer [8]. Thus, we have gradually realized the great utility of traditional Chinese medicine in cancer treatment. In previous research, we identified the effective components and mechanisms of action of traditional Chinese medicine in the treatment of non-small cell lung cancer [9].

Saussurea involucrata (SAIN) is one of many traditional Chinese medicines. It is mainly distributed in high-altitude areas such as Xinjiang and Tibet in China. Research on the pharmacological effects of SAIN began in the 1980s [10]. There are many known pharmacological activities and effects of SAIN, but the most studied are its anti-inflammatory, antioxidant [11], and anti-cancer effects (prostate cancer, gastric cancer, breast cancer, etc.) [12]. Its effects in other diseases (such as COVID-19) have not been studied yet. Therefore, research is urgently needed to evaluate the efficacy of SAIN as a supplement in the treatment of COVID-19.

Previous studies showed that Quercitrin could significantly inhibit the production of reactive oxygen species (ROS) and increase the malondialdehyde content. In the streptozotocin-induced diabetic mouse model, Quercitrin showed strong organ protection characteristics in the pancreas, liver, and kidney by improving antioxidant traits [13], supported by the results of in vitro studies [14]. Studies in the past two years showed that the antioxidant activity of drugs was very important in the treatment of and protection against COVID-19 [15]. In clinical experiments, researchers have also modified it for the treatment of COVID-19 according to the selective antioxidant characteristics of hydrogen therapy [16].

Caffeic acid is a well-known plant nutrient. Through further research, the beneficial effects of Caffeic acid such as antioxidant, antibacterial, and neuroprotective activities were identified and applied [17]. Jaceosidin is a natural flavonoid with anti-inflammatory, antioxidant [18,19], anticancer, and immunosuppressive effects [19]. However, in coronavirus research, evidence on the effects of Caffeic acid (CAF in [20]) and Jaceosidin is still insufficient. Thus, the positive results of our research can be used as powerful evidence to prove that Caffeic acid and Jaceosidin play roles in improving COVID-19.

Presently, studies to identify SARS-CoV-2 inhibitors have been conducted [21]. Simulation studies showed that Rutin showed the best potential effect in inhibiting SARS-CoV-2. Coupled with its strong solubility and weak lipophilicity, Rutin displayed the potential for becoming a COVID-19-targeted drug.

Network pharmacology is a new research method emerging in recent years. It was first proposed by Professor Hopkins, a pharmacologist from the University of Dundee in the United Kingdom [22]. Based on the theory of systems biology, network pharmacology is a new discipline that selects specific signal nodes for multi-targeted drug molecular design. Network pharmacology emphasizes the multi-way regulation of signaling pathways by targets to improve the therapeutic effect of drugs and reduce their toxicity and side effects, thereby improving the success rate of the clinical trials of new drugs and reducing drug research and development costs. Therefore, we used the network pharmacology method combined with bioinformatics, gene difference analysis, molecular docking, molecular dynamics simulation, and other methods to analyze the mechanism of action of the active ingredients and targets of SAIN active ingredients in the treatment and prevention of COVID-19.

In this study, for the first time, we identified the components of SAIN that could effectively treat or prevent COVID-19, namely Rutin, Jaceosidin, CAFD, and Quercitrin. Moreover, our findings are consistent with the findings of previous research on Rutin and CAFD [23,24], Jaceosidin [25], and Quercitrin [26,27]). However, most of these studies were conducted using the usual basic experimental methods. Different from the research of others, our research was based on a computer deep learning model [28-30] and the use of an improved network pharmacology process to simulate the existing state of these compounds in the standard human environment after binding to their target proteins. Then, the most suitable active ingredients for the treatment or prevention of COVID-19 were selected.

2. Materials and methods

2.1. Screening the active ingredients of SAIN

Traditional Chinese Medicine System Pharmacology Database and Analysis Platform TCMSp (https://tcmsp-e.com/) database [31] is one of the most commonly used databases for the screening of active ingredients in traditional Chinese medicine. OB and drug similarity (DL) parameters, these two parameters have a very important role in the evaluation of drug efficacy, only when these two values exceed a certain value (OB ≥ 25% and DL > 0 or OB>0% and DL ≥ 0.18), can effectively reflect the drug-like properties of certain compounds.

Among them, the calculation of the DL value of this system follows formula (1). To obtain the target drug, only when the lead compound is chemically easy to be synthesized and has the properties of ADMET (absorption, distribution, metabolism, excretion, and toxicity), the DL value will work.

$$T(x,y) = \frac{x - y}{\sqrt{x^2 + y^2} - xy} \geq 0.4$$

X represents the descriptive index of all compounds in SAIN in formula (1), and y represents the average drug similarity index of these compounds from the DrugBank (https://www.drugbank.ca/) database [32].

The lipid-water partition coefficient logP_{O/W} reside used the distribution coefficient of the drug in the n-octanol-water system, which is widely used as a measure of the hydrophobicity of chemical compounds. The main driving force of controls the binding effect of the compounds-target. AlogP_{O/W} is one of the representation methods of logP_{O/W}. Generally, the range of the AlogP_{O/W} value we require is between −0.4 and 5.6. The calculation of logP_{O/W} follows formula (2):

$$\log P_{O/W} = \frac{\log C_{O}}{\log C_{W}}$$

CO represents the equilibrium concentration of the drug in the oil phase, and CW represents the equilibrium concentration of the drug in the water phase in formula (2). The logP_{O/W} value indicates the hydrophobicity of the solute. The larger the logP_{O/W}, the stronger the hydrophobicity, and vice versa.
2.2. Acquisition of SAIN targets and COVID-19 targets

To study the mechanism of SAIN’s active ingredient targeted treatment of COVID-19, first of all, we must get the targets that all active ingredients can act on, we need to get the verified COVID-19 targets then. Only by finding the common part of these two kinds of targets can we continue to study this experiment.

The above-obtained SAIN active ingredients were searched in the TCMSP database to obtain all targets corresponding to SAIN chemical ingredients, which were merged with the COVID-19 targets we obtained in the DisGeNET database (https://www.disgenet.org/) [33], and then take venn coincidence to obtain the final required disease target information.

2.3. Construction of compounds-target-disease network and PPI

To show the corresponding relationship between SAIN and its active components, targets and COVID-19 clearly, we use Cytoscape 3.8.0 software [34] to visualize the above active compounds and targets to construct a compounds-target-disease network (C-T-D network) to obtain the C-T-D network relationship diagram we need. In this network diagram, we use different node shapes and colors to represent each content. So far, we have completed the initial screening of SAIN’s COVID-19-related active ingredients.

To obtain disease targets with interaction relationships, we performed protein interaction analysis (PPI analysis) on all the initially screened targets, we preset the enrichment p value < 1.0e-16 as the analysis condition. After PPI network analysis, we obtained the interaction network of all proteins. According to this network diagram, we got COVID-19 targets that were further screened.

2.4. GEO gene difference analysis

GEO database (https://www.ncbi.nlm.nih.gov/geo/) from NCBI [35], different from TCGA database, GEO database not only contains cancer-related clinical information, but also has many non-cancer clinical information, which makes GEO database more perfect in the provision of clinical case information.

To further screen the active ingredients and their targets that we have preliminarily screened, we obtained enough COVID-19 clinical case samples from the GSE148829 data set of geo database (The data set contains 349 clinical samples, of which 72 samples of human COVID-19 infected persons were selected).

Through the gene difference analysis of GSE clinical samples, we randomly selected samples to draw an intuitive gene difference heat map for target screening again.

2.5. Construction of C-T-P network and gene enrichment analysis

To evaluate the interaction between the targets screened above, a protein interaction network and an active compounds-target-pathway network (C-T-P network) were constructed. To explore the biological processes and pathways involved in each target in vivo, DAVID (https://david.ncifcrf.gov/) database [36] retrieved the data of the target gene, we performed biological process (BP) analysis in GO (Gene Ontology) analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis. Among them, the FDR of GO analysis is less than or equal to 0.05, and the FDR of KEGG enrichment analysis is less than or equal to 0.05, all of which meet the requirements and statistical significance of genes significantly enriched in vivo.

The FDR value is corrected for the P-value, and the results screened with it are more accurate. Therefore, this step aims to obtain the biological process and in vivo pathway of the target action, which provides a basis for subsequent research.

2.6. Molecular docking

Molecular docking [37] is a method for drug design through the characteristics of receptors and the interaction between receptors and drug molecules. A theoretical simulation method that mainly studies the interactions between molecules (such as ligands and receptors) and predicts their binding modes and affinities. In recent years, molecular docking methods have become an important technology in the field of computer-aided drug research [38].

The precise molecular docking between the active ingredients of SAIN and the COVID-19 target is carried out by tools such as AutoDock Vina software [39] and PyMol software [40]. Whether the effect of 19 is reliable, and further exclude compounds in SAIN that are less effective against COVID-19.

2.7. Gromacs molecular dynamics simulation verification

Based on molecular docking and GSE gene difference analysis, we verified the results by molecular dynamics simulation (MD simulation). MD simulation is a means of simulating the movement of small molecules in the body environment by computer. The MD simulation was performed with Gromacs software [41], the physical conditions were set as constant temperature (310 K), constant pressure (101 kPa), and periodic boundary conditions, and the TIP3P water model was used to simulate the human environment in a neutral sodium chloride solution of 0.145 mol/L, after the state balance of all environments, we use the compounds-target complex system that has been screened to perform 50ns MD simulation, in which, every 10 ps, a confirmation storage calculation is performed, and the RMSD of the MD simulation results (Root mean Square deviation), RMSF (Root Mean Square Fluctuation), Rg value (Radius of gyration) and SASA (Solvent accessible surface area) were analyzed and visualized using the Gromacs embedded program and VMD. We know that the radius of gyration Rg of the protein reflects the protein The volume and structural state of macromolecules, the larger the Rg value of the same system, the expansion of the system occurred during the MD process, and SASSA is an important means to describe the hydrophobicity of proteins, and the hydrophobicity of amino acid residues is an important factor affecting protein folding. Physical action. The formulas of RMSD and RMSF areas (3) and (4).

\[
RMSD = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (R_i - R_{i,ref})^2}
\]  
(3)

\[
RMSF = \sqrt{\frac{1}{T} \sum_{i=1}^{T} (R_i - R_{i,ref})^2}
\]  
(4)

Among them, \(R_i - R_{i,ref}\) represents the position of the t-th atom in a certain frame minus its position ref (position offset) in the reference conformation, N is the number of atoms, and T is each sampling time.

3. Results

3.1. Screening SAIN active ingredients

We collected a total of 55 known active ingredients in SAIN from the TCMSP database, including alkaloids, lipids, flavonoids, and flavonoids. Fifteen effective drug-like compounds were screened from the 55 SAIN compounds under different screening conditions. The screening details of these 15 compounds are shown in Table 1.

3.2. Acquisition of coincident targets and construction of a PPI network

After deduplication screening, we obtained a total of 109 targets in the SAIN active ingredients using the TCMSP database. We also obtained 1843 non-duplicated COVID-19 targets using the latest COVID-19 target data from the DisGeNET database. Then, we generated a Venn diagram...
of the SAIN and COVID-19 coincident targets (Fig. 1). The Venn diagram showed that SAIN and COVID-19 had 29 coincident targets (Table 2).

To obtain disease targets with interactive relationships, we performed a PPI network analysis on the 29 targets that were initially screened and obtained an interactive relationship network diagram of the proteins encoded by the 29 genes (Fig. 2A). The network showed that 5-hydroxytryptamine receptor 3A (HTR3A) had no interaction with the remaining 28 targets, indicating that HTR3A did not play a strong role in multi-targeted therapy for COVID-19. Thus, it was discarded and a new PPI network was constructed using the remaining 28 targets (Fig. 2B), from which we obtained the further screened COVID-19 targets.

Fig. 2B shows that there were multiple targets with strong interactions, such as interleukin-6 (IL6), insulin (INS), interleukin-1 beta (IL1B), transcription factor AP-1 (JUN), glycogen synthase kinase-3 beta (GSK3B), and caspase-3 (CASP3).

Targets such as caspase-8 (CASP8) and peroxisome proliferator-activated receptor-gamma (PPARG) may also play significant roles in COVID-19 symptoms, although some proteins did not clearly appear to interact with others. Important roles for aldo-keto reductase family 1 member B1 (AKR1B1), C5a anaphylatoxin chemotactic receptor 1 (C5AR1), and BID-interacting domain death agonist (BID) targets were also identified.

### 3.3. Results of GSE gene difference analysis and C-T-D network construction

To ensure the accuracy of the experimental data, we further screened the compounds and targets. Through the GEO database, we obtained a total of 57,910 COVID-19-related disease genes from the GSE148829 dataset. All these COVID-19 gene expressions had high reference values. These genes corresponded to 72 COVID-19 clinical infection samples in the GSE148829 dataset. We selected the expression data of the 28 genes we needed from these 72 COVID-19 case samples. A heatmap of gene differences was then drawn using the R 4.0.2 visual programming language (Fig. 3).

According to the gene difference analysis of the GSE clinical samples, we excluded 19 targets with no obvious difference among the remaining 28 targets. Thus, 9 COVID-19 differentially expressed targets remained. The expression levels of these 9 targets ranked from high to low were AKR1B1, C5AR1, BID, CASP3, CASP8, IL1B, IL6, JUN, AKR1B1, C5AR1, and BID in COVID-19 patients was significantly higher than that of the other targets. After mapping these 9 targets to the active ingredients of SAIN, we screened out 6 more accurate SAIN ingredients from the 15 SAIN active ingredients.

To map the 6 effective compounds in SAIN and their one-to-one relationship with COVID-19 intersection targets, we used Cytoscape software to construct a C-T-D network (Fig. 4). Through the C-T-D network, we found that the 6 ranking compounds with strong effects on the target were Rutin, dehydrocostus lactone, beta-sitosterol, Jaceosidin, Quercitrin, and CAFD.

### 3.4. GO (BP) and KEGG enrichment analysis

From the gene difference analysis of the GSE clinical samples, we used the 9 differentially expressed targets of COVID-19, BID, CASP3, CASP8, IL1B, IL6, JUN, AKR1B1, C5AR1, and GSK3B. Since IL6 levels were significantly elevated in complex COVID-19 cases [42], the researchers assessed the possibility of IL6 as a therapeutic target for COVID-19. A previous study [43] also reported that vitamin D may reduce the mortality of COVID-19 patients by regulating the IL1B

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**Table 1**

| Herb    | Molecule Name   | MW    | AlogP  | OB(%)   | DL     | PubChem CID |
|---------|-----------------|-------|--------|---------|--------|-------------|
| SAIN    | Alloisoimperatorin | 270.3 | 3.282  | 34.80407 | 0.21854 | 5317436     |
| SAIN    | Beta-sitosterol  | 414.79| 3.793  | 36.91391 | 0.75123 | 222284      |
| SAIN    | Caffeic acid     | 180.17| 1.367  | 25.7644  | 0.05089 | 689043      |
| SAIN    | Dehydrocostus lactone | 230.33 | -0.511 | 58.56795 | 0.14095 | 73174       |
| SAIN    | Edulxin         | 386.43| 2.171  | 12.81888 | 0.52011 | 5317013     |
| SAIN    | Isoimipinellin  | 246.23| 6.337  | 25.92866 | 0.17256 | 68079       |
| SAIN    | Jaceosidin      | 330.31| 8.084  | 2.14101  | 0.34229 | 5739096     |
| SAIN    | Lof             | 166.19| 3.503  | 33.27132 | 0.036929| 25245514    |
| SAIN    | Luteolin-7-O-glucoside | 448.41 | -1.446 | 7.292677 | 0.77998 | 5280637     |
| SAIN    | Neperin         | 316.28| 2.051  | 26.75038 | 0.30825 | 5317284     |
| SAIN    | Quercitrin      | 448.41| 0.301  | 4.037653 | 0.73649 | 5280459     |
| SAIN    | Rutin           | 610.57| 0.162  | 3.201533 | 0.68283 | 5280805     |
| SAIN    | Sitogluside     | 576.95| 1.245  | 20.63194 | 0.6241  | 5742590     |
| SAIN    | Syringin        | 372.41| 2.302  | 14.63693 | 0.32496 | 5316860     |
| SAIN    | Xuelanlactone   | 248.35| 2.04   | 56.04411 | 0.16137 | 147111      |

**Table 2**

| Coincident targets |
|--------------------|
| AKR1B1             |
| BCl2               |
| BID                |
| BTK                |
| C5AR1              |
| CASP3              |
| CASP8              |
| CYP3A4             |
| DEP1               |
| DPP4               |
| ESR1               |
| FCER2              |
| GFAF               |
| GSK3B              |
| HTR3A              |
| IL1B               |
| IL6                |
| INS                |
| JUN                |
| KCNMA1             |

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Fig. 1. Intersection of SAIN targets and COVID-19 targets.
Fig. 2. PPI network (A). PPI network with 29 targets (B). PPI network with 28 targets.

Fig. 3. Gene difference heatmap of 28 targets.
Fig. 4. C-T-D network of 6 compounds of SAIN and targets of COVID-19.

Fig. 5. BP analysis of the final screened target.
protein content.

We performed gene ontology (GO) biological process (BP) analysis on the remaining 9 targets and drew a chord diagram representing 21 biological regulatory processes (Fig. 5).

The biological processes in GO enrichment analysis showed that 2 of the 9 COVID-19 targets (IL6 and IL1B) we screened positively regulated interleukin-6 production and nitric oxide (NO) biosynthesis, accelerating NO biosynthesis and IL6 production. In addition, CASP3 and CASP8 in these targets could also kill tumor cells and mediate inflammatory responses by regulating the GO:0034612 process (response to tumor necrosis factor).

To maintain the reliability and statistical significance of the data, we performed a Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis at $P < 0.005$ in the String and DAVID databases, and displayed the 26 pathways in the comprehensively considered the results in the form of a heatmap (Fig. 6).

We performed cluster analysis on the 26 KEGG pathways, which showed that the clusters were divided into 4 types. KEGG enrichment analysis revealed that most of the pathways were related to inflammation, bacterial infection, influenza, tuberculosis, metabolism, and cancer. Nineteen were mainly enriched for non-alcoholic fatty liver disease (NAFLD), neuroactive ligand-receptor interaction, pertussis, the tumor necrosis factor (TNF) signaling pathway, tuberculosis, influenza A, and fructose and mannose metabolism.

### 3.5. Analysis of C-T-P network construction results

Referring to the results of the KEGG enrichment analysis, to make it easy to understand, we linked 6 compounds, 9 targets, and 26 pathways to construct a C-T-P network diagram (Fig. 7) to determine the relationship between the compounds and targets. The relationships between the targets and pathways were visualized, and a total of 42 nodes and 111 edges of the C-T-P network were obtained.

First, from the C-T-P network diagram, according to the number of compounds, targets, and proteins, we ranked the 6 active compounds of SAIN as Rutin, beta-sitosterol, dehydrocostus lactone, Jaceosidin, Quercitrin, and CAFD. According to the number of target regulatory pathways, we ranked the 9 targets of COVID-19 as IL6, IL1B, CASP3, CASP8, JUN, BID, GSK3B, AKR1B1, and C5AR1, and some of the 26 pathways were ranked as NAFLD, tuberculosis, TNF signaling pathway, influenza A, and pertussis, etc. However, although these targets and pathways may contribute to the effects seen in COVID-19 patients, the strength and accuracy of their contributions were still not easily distinguishable. Therefore, further analysis and validation are required.

### 3.6. Analysis of molecular docking results

Through the series of analyses, screening, and verification work above, we identified the target sites of 6 SAIN compounds, and these 6 compounds could act on multiple targets in COVID-19. Among them, beta-sitosterol could target CASP8, JUN, and CASP3; dehydrocostus lactone could target CASP8, CASP3, and BID; Rutin could target CASP8, JUN, and CASP3; Jaceosidin could target GSK3B, CASP3, and CAFD; Quercitrin could target AKR1B1; and CAFD, and Quercitrin could only target AKR1B1. Thus, by looking at these correspondences and the known effects of the targets, we inferred that Rutin, dehydrocostus lactone, beta-sitosterol, and Jaceosidin may be effective COVID-19 treatments.
Fig. 7. C-T-P network of SAIN compounds and targets of COVID-19.

Table 3
Molecular docking results between the active ingredients of SAIN and the target of COVID-19.

| PubChem CID | Compounds      | Targets      | Affinity (kcal/mol) | PDB ID | Method               | UniProt ID |
|-------------|----------------|--------------|---------------------|--------|----------------------|------------|
| 5280459     | Quercitrin     | AKR1B1       | −11.5               | 1US0   | X-RAY DIFFRACTION    | P15121     |
| 689043      | Caffeic acid   | AKR1B1       | −9                  | 1US0   | X-RAY DIFFRACTION    | P15121     |
| 5379096     | Jaceosidin     | GSK3B        | −8.7                | 1O6L   | X-RAY DIFFRACTION    | P31751     |
| 5280805     | Rutin          | IL1B         | −7.9                | 5R8Q   | X-RAY DIFFRACTION    | P01584     |
| 5280805     | Rutin          | CASP3        | −7.6                | 5O9H   | X-RAY DIFFRACTION    | P21730     |
| 222284      | Beta-sitosterol| CASP8        | −7.2                | 4JJ7   | X-RAY DIFFRACTION    | Q14790     |
| 5280805     | Rutin          | IL6          | −6.8                | 1ALU   | X-RAY DIFFRACTION    | P95223     |
| 5280805     | Rutin          | CASP3        | −6.5                | 2DKO   | X-RAY DIFFRACTION    | P42574     |
| 5379096     | Jaceosidin     | CASP3        | −6.3                | 2DKO   | X-RAY DIFFRACTION    | P42574     |
| 73174       | Dehydrocostus lactone | CASP8        | −6.2                | 4JJ7   | X-RAY DIFFRACTION    | Q14790     |
| 73174       | Dehydrocostus lactone | CASP3        | −6.1                | 2DKO   | X-RAY DIFFRACTION    | P42574     |
| 73174       | Dehydrocostus lactone | BID          | −6                  | 2BID   | SOLUTION NMR         | P55957     |
| 222284      | Beta-sitosterol| JUN          | −6                  | 5FV8   | X-RAY DIFFRACTION    | P05412     |
| 222284      | Beta-sitosterol| CASP3        | −5.3                | 2DKO   | X-RAY DIFFRACTION    | P42574     |
file were selected, filtered, and ranked by hydrogen bond distance, and the tertiary structures were predicted using X-ray diffraction or solution nuclear magnetic resonance (NMR). Then, we used AutoDock and AutoDock Vina to perform molecular docking between the compounds and targets. The docking results are shown in Table 3 and Fig. 8. We ranked the docking results and selected 6 compound-target complex pairs with binding energy $\leq -7.2$ kcal/mol using the PyMol process and visualized the results (Fig. 9).

To ensure the availability and reference value of the data, we set the coordinates (x, y, z) according to the structural size of the compounds themselves. It is known that the larger this coordinate value is, the greater the docking affinity, indicating that compounds with tighter binding to the target would be more effective. Therefore, the coordinate values we used were the most appropriate for the composition itself. The docking results showed that the best binding energy (kcal/mol) of a compound and target was $-11.5$ kcal/mol, and the worst was $-5.3$ kcal/mol.

The visualization results indicated that the 4 active ingredients of SAIN, Quercitrin, Rutin, CAFD, and Jaceosidin could be well combined with the corresponding targets. Among them, Quercitrin could bind to the amino acid residues TRY-219, ARG-217, TRP-295, ASN-294, and ARG-296 with an average hydrogen bond distance of 3.04 Å. CAFE could bind to the amino acid residues ASN-292, THR191, GLU-193, ASN-294, and ASN-294 of AKR1B1 protein. GLN-192 bound with an average hydrogen bond distance of about 2.4 Å. The binding of Rutin to IL1B was stronger than to CSAR1. Jaceosidin bound to the amino acid residues ALA-232 and LYS181 of GSK3B protein with an average hydrogen bond distance of about 3.15 Å, and beta-sitosterol bound to the amino acid residue ARG-413 of CAP8 protein with a hydrogen bond distance of 2.9 Å.

3.7. Analysis of MD simulation results

MD results such as RMSD, RMSF, Rg value, and SASA provide an important basis for measuring the stability of complex systems of compounds and proteins, and the stability of protein tertiary structures after combining small molecules, the hydrophobicity of amino acid residues, etc. Therefore, with a binding energy of $\leq -7.6$ kcal/mol as the limit, we selected the first 5 molecular docking systems for MD simulations. To make these data more intuitive, we visualized their output data (Fig. 10).

The RMSD results of the MD simulations (shown as a red, broken line in Fig. 10) showed that in the 5 MD systems of compound and target complexes, the overall performance of the CSAR1 and Rutin complex system was not good. The large RMSD fluctuations, large gyration radius, large RMSF fluctuation, and poor SASA performance indicated that the system did not show an effective inhibitory effect against COVID-19.

For the two complex systems (purple and blue broken lines in Fig. 10) formed by AKR1B1 and quercetin and CAFD, we observed that 4 experimental RMSD, Rg, RMSF, and SASSA results in the AKR1B1 and quercetin system were higher than those for AKR1B1 and CAFD. The system formed with acid had better protein hydrophobicity and a more stable tertiary structure, but both systems performed well. The binding energy data of these two systems were also ideal; that is, AKR1B1 was a possible novel therapeutic target for COVID-19. We confirmed that the 2 SAIN compounds Quercitrin and CAFD, could target the AKR1B1 protein well.

In the 4 aspects of RMSD, Rg, RMSF, and SASA, the MD system (green broken line in Fig. 10) formed by GSK3B and Jaceosidin showed a relatively good state; that is, the system could exist stably without affecting the structure itself or other proteins. The last MD system was IL1B and Rutin. This system had the most stable RMSD and RMSF, and the smallest Rg (indicating that the system would not swell) and SASA values (the amino acid residues were the most hydrophobic). Thus, this system was the best in all aspects.

Therefore, the 4 active ingredients of SAIN, Quercitrin, Rutin, CAFD, and Jaceosidin, could play a role in improving or treating COVID-19 to a certain extent by acting on the 4 target proteins AKR1B1, CSAR1, GSK3B, and IL1B.

4. Discussion

4.1. Non-alcoholic fatty liver disease and COVID-19

NAFLD is a global chronic disease that is part of metabolic syndrome [47]. NAFLD is also a very high-risk factor for contracting the SARS-CoV-2 virus, and there is growing evidence [48-50] that COVID-19 causes liver damage” or “and there is growing evidence [48-50] that some effects of COVID-19 are inseparable from liver damage. There is also evidence [51], that patients infected with COVID-19 are more susceptible to underlying metabolic diseases. A study by Ji et al. [52] of more than 200 patients with COVID-19 and NAFLD reported that approximately 75% of the patients had varying degrees of liver damage during hospitalization, mainly mild hepatocyte damage, manifested as elevated levels of serum C-reactive protein and alanine aminotransferase (ALT) or “manifested as elevated levels of serum alanine aminotransferase (ALT), 3% of the COVID-19 patients in the population presented with ductal or mixed liver injury, and approximately one-third of the patients had persistent, abnormal liver function from admission to the last day of follow-up. The authors also noted an association between COVID-19 disease progression and age (over 60), gender (male), body mass index (higher), and NAFLD, among others. Finally, the authors concluded that NAFLD was an independent risk factor for developing COVID-19.
Our study showed that 2 proteins, IL6 and IL1B, positively regulated 2 biological processes, GO:0032755 and GO:0045429 associated with accelerating NO biosynthesis and IL6 production. Akaberi et al. [53] demonstrated that NO derived from S-nitroso-N-acetyl-penicillamine (SNAP) could delay or completely prevent the development of SARS-CoV-2 virus cytopathic effects in treated cells, and the observed protection was related to the level of viral replication inhibition. Therefore, amid the COVID-19 pandemic, the inhalation of NO gas has emerged as a possible treatment modality, which increases oxygenation in patients and may lead to better clinical outcomes [54]. Rutin, a compound in SAIN, effectively targeted IL1B and IL6 (including MD and MD simulation), and the cytokines IL1B and IL6 were both key targets that could regulate NAFLD. It has been reported [55] that variations in the IL1B and IL6 genes may be associated with disease susceptibility and cytokine storm and/or COVID-19 complications, and rapid and effective vitamin D supplementation may reduce symptoms and mortality in COVID-19 patients by altering serum IL1B levels [56].

4.2. GSK3B and COVID-19

GSK3B, glycogen synthase kinase-3 beta, has two forms in vivo, the phosphorylated and dephosphorylated forms, and when it is phosphorylated, it upregulates the expression of IL6 and TNF. Angiotensin-converting enzyme 2 (ACE2) was highly expressed in tissue samples from 15 human organs, including the heart and kidney, as well as the main target cells of SARS-CoV-2 and alveolar epithelial cells at the main site of injury [57], indicating that ACE2 is a substance that can facilitate the entry of the SARS-CoV-2 virus into the human body. Therefore, we can greatly reduce the risk of SARS-CoV-2 virus infection by reducing the expression of ACE2 in SARS-CoV-2 target cells (gut and alveolar epithelial cells).

Our study showed that Jaceosidin, a compound in SAIN, could target and stabilize GSK3B; that is, Jaceosidin could upregulate the expression of GSK3B to regulate the NAFLD and influenza A pathways. When the expression of GSK3B increases, it will simultaneously increase the expression of IL6 and TNF. Studies showed that the upregulation of inflammatory factors such as IL6, IL1B, and TNF reduced the expression of ACE2 [58], while the latest SARS-CoV-2 virus did not. Therefore, taking oral preparations made from SAIN or the single-use of Jaceosidin can effectively prevent SARS-CoV-2 infection.

Fig. 9. Molecular docking results of 6 compounds of SAIN and 9 proteins of COVID-19, the connection represents a hydrogen bond. (a). Quercitrin- AKR1B1 (b). Rutin- CSAR1 (c). Caffeic acid- AKR1B1 (d). Jaceosidin- GSK3B (e). Rutin- IL1B (f). Beta-sitosterol- CASP8.
4.3. Comprehensive analysis

This study focused on the role of several active ingredients in SAIN, a comprehensive Chinese medicine agent. The 6 SAIN compounds Quercetin, CAFD, Jaceosidin, Rutin, beta-sitosterol, and dehydrocostus lactone could be used as effective substances against the 9 targets AKR1B1, GSK3B, IL1B, C5AR1, CASP8, IL6, CASP3, BID, and JUN. That is, when COVID-19 patients are administered SAIN or these single compounds together, the greatest preventive or therapeutic effects of SAIN can be achieved.

5. Conclusion

The complexity and variability of COVID-19 have prevented us from explaining it in terms of changes in a single gene. Therefore, treatment for COVID-19 cannot be achieved through one approach, but requires multiple approaches, such as combination therapy with multiple targets. The 6 quercetin, CAFD, Jaceosidin, Rutin, beta-sitosterol, and dehydrocostus lactone compounds in SAIN could bind the AKR1B1, GSK3B, IL1B, C5AR1, CASP8, IL6, CASP3, BID, and JUN targets and function as biological processes and pathways by participating in GO:0032755, GO:0045429, NAFLD, neuroactive ligand-receptor interaction, pertussis, the TNF signaling pathway, tuberculosis, and influenza A. However, the role of the inhibitors or activators in the prevention and treatment of COVID-19 under the combined action of these processes is unknown. Therefore, for COVID-19, a complete cure still requires the joint efforts of researchers in various fields.

The hierarchy set in this paper provides references and guidance for future research. In recent years, in the field of molecular docking, generally, the binding of a single compound to the target protein has been studied. Although many compounds sometimes bind to the same protein, there will be no additional binding energy. Thus, future research should focus on the calculation program and binding effect of molecular docking. Similarly, in terms of molecular dynamics simulation, although it describes the movement of a compound and protein in the same system, it does not clearly show the interaction between the compound and other proteins (inevitable in the actual environment). Therefore, these problems will be taken into account in future research.

Declarations

We confirm that all methods in the manuscript are carried out in accordance with the relevant guidelines.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due the limited scope of data availability, these data are used under the license of this study and are not disclosed, but are available from the author [sedate@stu.shzu.edu.cn] (Dongdong...
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Authors’ contributions

Dongdong Zhang wrote the main manuscript text and Zhaoye Wang prepared all figures and tables. Jin Li and Jianbo Zhu are corresponding authors. All authors reviewed the manuscript.

Declaration of competing interest

To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

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References

[1] WHO. WHO Coronavirus (COVID-19). Dashboard 28 (1) (2022).
[2] Lowe Scott, Ruijin Xie, Yue Chen, Yifan Shen, Chenye Sun, It is Not the Time to Relax yet: Masks Are Still Needed for the Omicron Variant of SARS-CoV-2, Public Health. 2021, https://doi.org/10.1016/j.puhe.2021.01.050.
[3] O. William, Hahn, Zanthia Wiley, COVID-19 Vaccines, Infectious Disease Clinics of North America, 2022, https://doi.org/10.1053/j.idcm.2022.01.008.
[4] Y. Tu, Artemisinin–A gift from traditional Chinese medicine to the world (Nobel Lecture), Angew Chem. Int. Ed. Engl. 55 (35) (2016 Aug 22) 10216–10226, https://doi.org/10.1002/anie.201601967. Epub 2016 Aug 4. PMID: 27488942.
[5] Y.X. Chen, Q.Y. Gao, T.H. Zou, B.M. Wang, S.D. Liu, J.Q. Sheng, J.L. Ren, X.P. Zou, Z.J. Liu, Y.Y. Song, B. Xiao, X.M. Sun, X.T. Dou, H.L. Cao, X.N. Yang, N. Li, Q. Kang, W. Zhu, H.Z. Xu, H.M. Chen, J.Y. Fang, Berberine versus placebo for the prevention of recurrence of colorectal adenoma: a multicentre, double-blind, randomised controlled study. The Lancet, Gastroenterol. Hepatol. 5 (3) (2020) 267–275, https://doi.org/10.1016/S2468-1253(19)30409-1.
[6] B. Hu, S.S. Wang, Q. Du, Traditional Chinese medicine for prevention and treatment of hepatocarcinoma: from bench to bedside, World J. Hepatol. 7 (9) (2015) 1209–1232, https://doi.org/10.4240/j.wjhe.2019.12.6296.
[7] Li Zhang, Feiyue Zhang, Gaofeng Li, Traditional Chinese medicine and lung cancer - From theory to practice, Biomed. Pharmacother. 137 (2021), https://doi.org/10.1016/j.bjp.2021.1118981.
[8] E.A. Coomes, H. Haghbayan, Interleukin-6 in Covid-19: a systematic review and meta-analysis, Rev. Med. Virol. 30 (6) (2020) 1–18, https://doi.org/10.1002/rmv.1369.
[9] T.A. Collier, T.J. Piggot, J.R. Allison, Molecular dynamics simulation of proteins, Methods Mol. Biol. 2073 (2020) 311–340, https://doi.org/10.1007/978-1-4939-8998-9.
[10] D.C. Wishart, Y.D. Feunang, A.C. Guo, E.J. Lo, A. Marcu, J.R. Grant, T. Sajed, D. Johnson, C. Huang, P. Li, L. Chiu, L. Cisin, P. Maciejewski, N. Gale, A. Wilson, L. Chin, R. Cummings, D. Le, A. Pon, C. Knox, M. Wilson, DrugBank 5.0: a major update to the Drugbank database for 2018, Nucleic Acids Res. 46 (D1) (2018 Jan) D1074–D1082.
[11] J. Piero, J.M. Ramirez-Anguita, J. Saiich-Pitarch, et al., The DisGeNET knowledge platform for disease genomics: 2019 update,(J), Nucleic Acids Res. 48 (D1) (2019).
[12] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, A. Nimm, B. Schwikowski, T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genome Res. 13 (11) (2003) 2498–2504.
[13] T. Barrett, S.E. Wilhite, P. Ledoux, C. Evangelista, I.F. Kim, M. Tomashevsky, K. Binkley, D. King, A. Fridlyand, NCBI GEO: archive for functional genomics data sets–update, Nucleic Acids Res. 41 (Database issue) (2013 Jan), D991–D995.
[14] D. Johnson, C. Huang, P. Li, L. Chiu, L. Cisin, P. Maciejewski, N. Gale, A. Wilson, L. Chin, R. Cummings, D. Le, A. Pon, C. Knox, M. Wilson, DrugBank 5.0: a major update to the Drugbank database for 2018, Nucleic Acids Res. 46 (D1) (2018 Jan) D1074–D1082.
[15] J. Ru, P.I. Li, J. Wang, W. Zhou, B.I. C, H. Huang, P. Lu, G. Wu, T. Yao, Y. Xue, Y. Li, V. Wang, L. Yang, TCMSP: a database of systems pharmacology for drug discovery from herbal medicines, J. Cheminf. 6 (4) (2014) 13.
[16] M. Kandeel, A.H.M. Abdelrahman, K. Ob-Hashi, A. Ibrahim, K.N. Venugopala, M. A. Morsy, M.A.A. Ibrahim, Repurposing of FDA-approved antivirals, antibiotics, anthelmintics, antioxidants, and cell protectives against SARS-CoV-2 papain-like protease, J. Biomed. Struct. Dyn. 39 (4) (2021 Sep) S1292–S1316, https://doi.org/10.1016/j.jbsd.2021.1784939.
[17] S.K. Das, S. Mahanta, B. Tanti, H. Tag, P.K. Hui, Identification of phytochemicals from Houttuynia cordata Thunb. as potential inhibitors for SARS-CoV-2 replication proteins through GC-MS/EC-MS characterization, molecular docking and molecular dynamics simulation, Mol. Divers. 26 (1) (2022) 355–368, https://doi.org/10.1007/s11030-021-10226-2.
[18] A.W. Salehi, P. Baglat, G. Gupta, Review on machine and deep learning models for the detection and prediction of Coronavirus, Mater. Today Proc. 33 (2020) 3896–3901.
[19] A.W. Salehi, G. Gupta, A prospective and comparative study of machine and deep learning techniques for smart healthcare applications, Mobile Health: Advances in Research and Applications (2021) 163–189.
[20] R. Yousef, G. Gupta, C.H. Vanipriya, N. Yousef, A comparative study of different machine learning techniques for brain tumor analysis, Mater. Today Proc. (2021).
[21] J. Ru, P.I. Li, J. Wang, W. Zhou, B.I. C, H. Huang, P. Lu, G. Wu, T. Yao, Y. Xue, Y. Li, V. Wang, L. Yang, TC.MSP: a database of systems pharmacology for drug discovery from herbal medicines, J. Cheminf. 6 (4) (2014) 13.
[22] I. Alaylıo˘, M. Alaylıo˘, C. Alaylıo˘, C. Alaylıo˘, H. Alaylıo˘, Meta-analysis, Rev. Med. Virol. 30 (6) (2020) 1–18, https://doi.org/10.1002/rmv.1369.
[23] K. Ahsan, M. Hossain, M. Alaylıo˘, C. Alaylıo˘, H. Alaylıo˘, Meta-analysis, Rev. Med. Virol. 30 (6) (2020) 1–18, https://doi.org/10.1002/rmv.1369.
[24] H. Refaat, F.M. Mady, H.A. Sarhan, H.S. Rateb, E. Alaaeldin, Optimization and formulation and development, Curr. Drug Discov. Technol. 16 (1) (2019) 30–39.
[25] M. Akdad, S. Moujane, I. Bouadid, M. Benlyas, M. Eddouks, Phytocompounds from Houttuynia staicthaiodes as potential inhibitors for SARS-CoV-2 replication proteins through GC-MS/EC-MS characterization, molecular docking and molecular dynamics simulation, Mol. Divers. 26 (1) (2022) 355–368, https://doi.org/10.1007/s11030-021-10226-2.
[45] L. Andries, L. Masin, M.S. Navarro, S. Zaunz, MMP2 modulates inflammatory response during axonal regeneration in the murine visual system, Cells 10 (7) (2021) 1072.

[46] K.R. Cousins, Computer review of ChemDraw ultra 12.0, J. Am. Chem. Soc. 133 (21) (2011 Jun 1) 8388.

[47] S. Sachdeva, H. Khandait, J. Kopel, M.M. Aloysius, R. Desai, H. Goyal, NAFLD and COVID-19: a pooled analysis, SN Compr Clin Med (2020) 1–4.

[48] C. Zhang, L. Shi, F.S. Wang, Liver injury in COVID-19: management and challenges, Lancet Gastroenterol Hepatol 5 (2020) 428–430.

[49] C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, B. Cao, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (2020) 497–506.

[50] G. Feng, K.I. Zheng, Q.Q. Yan, R.S. Rios, G. Targher, C.D. Byrne, S.V. Poucke, W.Y. Liu, M.H. Zheng, COVID-19 and liver dysfunction: current insights and emergent therapeutic strategies, J Clin Transl Hepatol 8 (2020) 18–24.

[51] P. Portincasa, M. Krawczyk, W. Smyk, F. Lammert, A. Di Ciaula, COVID-19 and non-alcoholic fatty liver disease: two intersecting pandemics, Eur. J. Clin. Invest. 50 (10) (2020) e13338, https://doi.org/10.1111/eci.13338.

[52] D. Ji, E. Qin, J. Xu, D. Zhang, G. Cheng, Y. Wang, G. Lao, Non-alcoholic fatty liver diseases in patients with COVID-19: a retrospective study, J. Hepatol. 73 (2020) 451–453.

[53] D. Akberi, J. Krambrich, J. Ling, C. Luné, G. Hedenstierna, J.D. Jarhult, J. Lassenstrand, A. Lundkvist, Mitigation of the replication of SARS-CoV-2 by nitric oxide in vitro, Redox Biol. 271 (2020), 01734.

[54] R.A. Alvarez, L. Berra, M.T. Gladwin, Home nitric oxide therapy for COVID, Am. Respir. Crit. Care Med. 202 (2020) 16–20.

[55] I. Friske-Galindo, R. Falfan-Valencia, Genetics insight for COVID-19 susceptibility and severity: a review, Front. Immunol. 12 (2021), 622176, https://doi.org/10.3389/fimmu.2021.622176.

[56] M.S. Gonen, M. Alaylıoğlu, E. Durcan, Y. Özdemir, S. Şahin, D. Konukoğlu, O. K. Nohut, S. Ürkmec, B. Kişlakku, L. Balkan, H.V. Kara, Ş. Börekçi, H. Özkan, Z. Kutlubay, Y. Dikmen, Y. Keskindemirci, S.N. Karras, C. Annweiler, D. Gezen-Ak, E. Dursun, Rapid and effective vitamin D supplementation may present better clinical outcomes in COVID-19 (SARS-CoV-2) patients by altering serum INOS1, IL1B, IFNγ, cathelicidin-LL37, and ICAM1, Nutrients 13 (11) (2021) 4047, https://doi.org/10.3390/nu13114047.

[57] C. Bavishi, T.M. Maddox, F.H. Messerli, Coronavirus disease 2019 (COVID-19) infection and renin angiotensin system blockers[J], JAMA Cardiology 5 (7) (2020), 10.1001/jamacardiol.2020.0948.

[58] D.W. Lambert, M. Yarski, F.J. Warner, P. Thornhill, E.T. Parkin, A.I. Smith, N. M. Hooper, A.J. Turner, Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2), J. Biol. Chem. 280 (2005) 30113–30119.